George Groeneweg

Cold Case:

Vascular dysregulation in the chronic Complex Regional Pain Syndrome

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'Cold Case'
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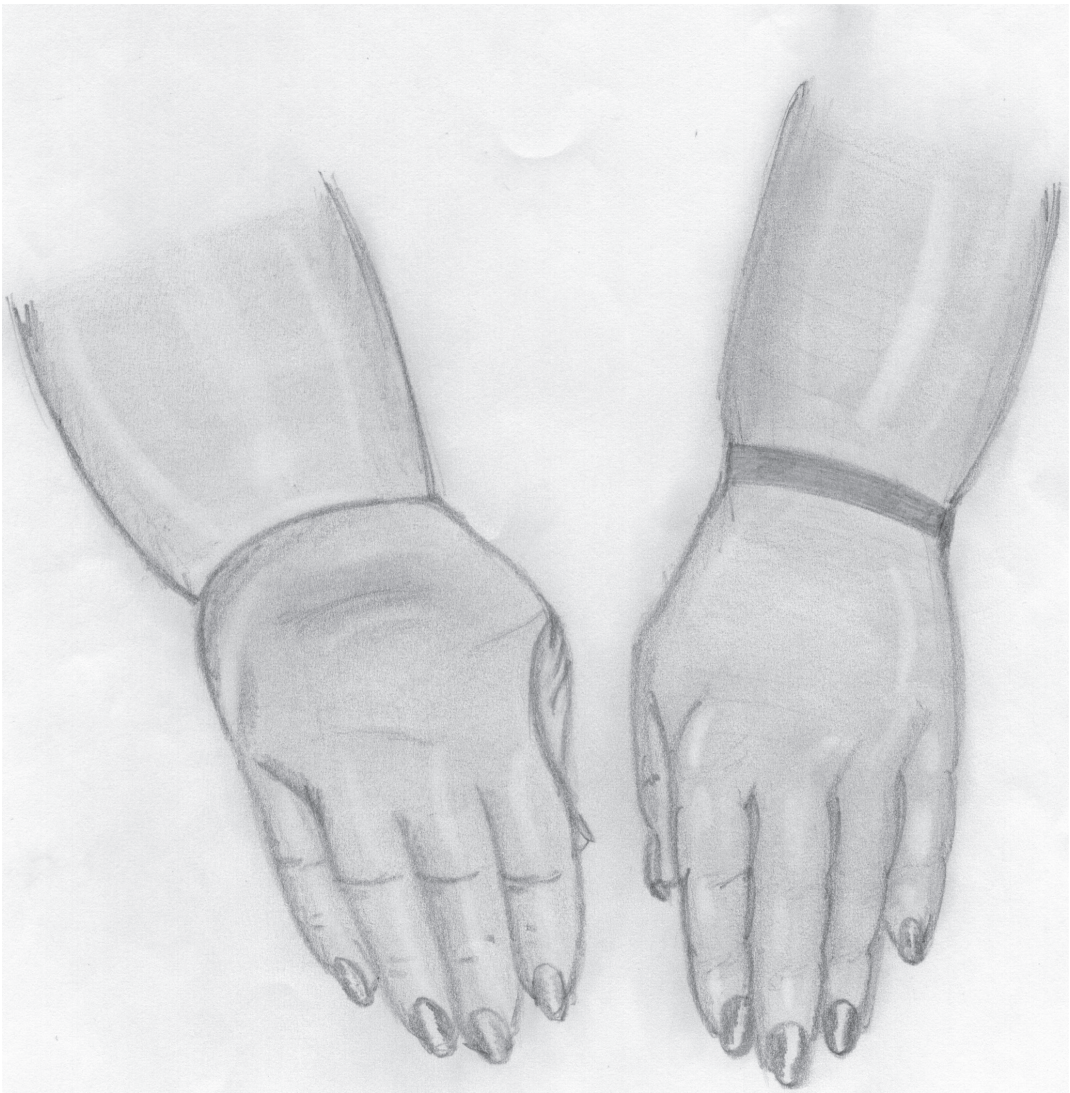


"I got more cross with it, like if you pick up a bottle, I picked up a bottle the other day from the step and it dropped straight out of my hand only because I hadn't gripped it as I thought I'd gripped it."

Ter herinnering aan Tante Joke, die er altijd was voor ons.

Chapter 1
Introduction

Introduction

Although the German physician Paul Sudeck may not have been the first to describe the complex of symptoms we know now as Complex Regional Pain Syndrome (CRPS), his name will always be associated with this devastating disease. In the first years of the last century Sudeck described an injury or herpes zoster infection that was followed by typical radiological changes in combination with trophic skin changes, decreased skin temperature, contractures and muscle atrophy [1, 2]. He called the syndrome 'akute reflektorische Knochenatrophie', but it was soon known as 'Sudecksche Knochenatrophie'.

The French barber-surgeon Amboise Paré (1509 – 1590) was in fact the first to leave us a case description of this disease. Paré, who is considered to be the father of modern surgery because of his many publications on operations in army field hospitals, was royal physician to four French kings, namely Henri II and III, François II and Charles IX, and described how the later King Charles developed symptoms of burning pain and contractures of the muscles in his arm after a lancet wound [3-6].

Since then a vast number of publications has been written on this subject. Mitchell described American Civil War casualties with nerve lesions, which he named 'causalgia' because of the characteristic type of burning pain [5, 7-9]. Leriche (1916) suggested that the sympathetic nervous system was involved, and he successfully introduced a form of blocking the sympathetic innervation of the arteries of the affected extremity, which gradually became the standard treatment [5].

In the Netherlands CRPS research became a major stimulance in the nineties by the traumasurgeon Goris. A decade later the Trauma Related Neuronal Dysfunction (TREND) Consortium was established. Starting from a multidisciplinary approach, TREND combines expertise from several backgrounds in order to make an important contribution to CRPS research. This has already lead to a vast number of publications. The studies described in this thesis were performed under the umbrella of the TREND consortium.

CRPS

CRPS is diagnosed on the basis of the patient's signs and symptoms [10]. In 1994 the International Association for the Study of Pain (IASP) published standardized, consensus based diagnostic criteria for the Complex Regional Pain Syndrome [11]. Due to a lack of specificity, these so-called Orlando or IASP criteria were modified in 1999 [12]. The Bruehl-Harden criteria, as they were soon called, consist of: continuing pain which is disproportionate to any inciting event, and [12, 13]:

	report of at least one symptom in each of the following categories:	**must display at least one sign in two or more of the following categories:**
Sensory:	hyperesthesia	evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch)
Vasomotor:	temperature asymmetry and/or skin colour changes and/or skin colour asymmetry	evidence of temperature asymmetry and/or skin colour changes and/or skin colour asymmetry
Sudomotor/oedema:	oedema and/or sweating changes and/or sweating asymmetry	evidence of oedema and/or sweating changes and/or sweating asymmetry
Motor/trophic:	decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)	evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)

Numerous hypotheses have been suggested to explain the various signs and symptoms of CRPS. It may be clear that whatever the underlying mechanism of CRPS, local vasomotor changes play a crucial role [14].
The mechanism that triggers CRPS is not clear. There is clinical and molecular evidence for a greater susceptibility of some patients to develop CRPS [15, 16], and there may be a familiar predisposition [17], but a relationship between pre-existing psychiatric disorders and CRPS could not be confirmed [18].
The acute phase appears to be a disorder of exaggerated neurogenic inflammation with increased skin temperature, edema, redness, pain and loss of function. These signs and symptoms may be normal after a trauma, but resolve after a while. In CRPS, however, they may even aggravate. This is suggestive of an insufficient remission of inflammation in CRPS, which may be one of the initial pathogenic factors [19].
It has been suggested that oxygen-derived free radicals are involved in the pathophysiology of CRPS [20-22]. In animal models, oxygen-derived free radicals can produce similar symptoms after a slow flow/no-reflow injury of the microvasculature [23-25].

Evidence was found of a proinflammatory profile in CRPS, with an increase in neuropeptides, cytokines and other mediators of inflammation [13, 26-31], which may induce pain and hyperalgesia by direct and indirect mechanisms. Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine, that increases the production and release of CGRP in in-vitro and in-vivo models, indicating a close link between the neuropeptide and cytokine systems. The concept of a pathophysiological role of cytokines in CRPS is further supported by reports of successful anti-TNF-α treatments of CRPS [32].
After a so-called intermediate phase, when skin blood flow and temperature difference appear to alternate between warm and cold, a chronic phase, also called atrophic stage, can develop in a large number of patients in which blood flow and temperature remain decreased [33].
Based on a cluster analysis three distinct CRPS patient subgroups can be identified: 1) a relatively limited syndrome with vasomotor signs predominating, 2) a relatively limited syndrome with neuropathic pain and sensory abnormalities predominating, and 3) a florid CRPS syndrome similar to "classic CRPS" descriptions [34].

The microcirculatory system

The microcirculatory system consists of arterioles, capillaries and venules. The arterioles, also known as resistance vessels, control blood flow through the microcirculatory system by constriction or dilation of the arteriovenous (AV) anastomoses. The diameter of arterioles ranges from 5 to 100 μm, they have a thick smooth muscle layer, a thin adventitial layer, and an endothelial lining. The network of interconnected arterioles gives rise directly to the smaller capillaries with a diameter of 5 to 10 μm, or to AV anastomoses connecting directly to the venule network. Blood flow through the superficial capillaries in the dermal papillae for the exchange of gases and solutes between blood and tissue is called nutritional flow, whereas the flow that bypasses the capillaries is called the nonnutritional or shunt flow. Since the oxygen and nutrients requirements of the skin are relatively small, the primary function of the cutaneous circulation appears to be the maintenance of a constant body temperature, which means that the vasodilation and vasoconstriction in the cutaneous circulation mainly depends on the need for loss or conservation of body heat, and that the responsible mechanisms are activated by changes in ambient and internal body temperatures [35].
The vascular resistance in the skin is under dual control of the sympathetic nervous system and local regulatory factors, and the AV anastomoses are highly sensitive to vasomotor agents such as epinephrine and norepinephrine. While stimulation of sympathetic nerve fibres to skin blood vessels induces vasoconstriction, severance of sympathetic nerves induces vasodilation [36]. After denervation of cutaneous blood vessels, the pre-existing tone is gradually regained over several weeks. [35, 37, 38].

Vascular endothelial cells

The vascular endothelial cells were originally considered to be passive lining cells, forming the barrier between the circulating blood and the surrounding tissue. Now we know that the endothelium plays a crucial role in the regulation of vascular tone by releasing endothelium-derived vasodilators, including nitric oxide (NO), prostacyclin, bradykinin and endothelium-derived hyperpolarising factors, and vasoconstrictors, such as endothelin-1 (ET-1) and angiotensin II, in response to a number of biochemical and physical stimuli [39, 40]. On the luminal surface vascular endothelial cells express several adhesion molecules as well as a variety of receptors including those for histamine, acetylcholine, interleukin-1 etc.. Endothelial function is also involved in angiogenesis, which occurs in repair processes, chronic inflammation and cancer [35].

Nitric oxide

The ability of organic nitrates to relieve angina was already discovered in 1867 by Sir Lauder Brunton [41]. It took more than a century, however, before Nobel prize winners Furchgott and Zawadzki discovered the endothelium derived relaxing factor (EDRF) [42], that was later identified as NO [43, 44]. In the endothelium cells NO is formed in an enzyme-catalysed reaction between molecular oxygen and L-arginine by three major isoforms of NO synthase (NOS), neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) [40]. eNOS, which is the major NOS isoform expressed in the endothelial cells, and nNOS and are both constitutively expressed and activated by intracellular calcium [45]. iNOS is calcium-independent, and its synthesis is induced in inflammatory and other cell types by stimuli such as endotoxin and proinflammatory cytokines [40, 45]. The constitutive enzymes generate small amounts of NO, whereas the activity of iNOS is approximately a thousand times greater [46].

NO is generated and released from the endothelial cells both under basal and agonist-stimulated conditions. Shear stress and pulsatile flow are the stimuli that cause release of NO under basal conditions [47]. This is sensed by endothelial mechanoreceptors and transduced via a serine-threonine protein kinase called Akt or protein kinase B [46].

NO diffuses rapidly out of the endothelial cell into the smooth muscle cell of the vascular wall. Here it activates guanylate cyclase by combining with its haem group, leading to an increase of cyclic guanosine monophosphate (cGMP). cGMP activates protein kinase G, and this leads to a cascade of effects in the smooth muscle, to dephosphorylation of myosin light chains and sequestration of intracellular Ca^{2+} with consequent relaxation of the vascular smooth muscle and subsequent vasodilation [35, 41]. Effects of cGMP are terminated by phosphodiesterase-5 (PDE-5) enzymes [46]. The hydrolysation of cGMP to GMP can be prevented by a PDE-5 inhibitor, which results in local vasodilation [48]. NO acts only locally because it reacts quickly with oxygen to N_2O_4, which combines with water to produce a mixture of nitric and nitrous acids. The half-life time is about 5-10 seconds. In contrast, NO reacts very rapidly with even low concentrations of superoxide anion (O^{2-}) to produce peroxynitrite anion

($ONOO^-$), which is a toxic agent. Many vasodilator substances (e.g. acetylcholine and bradykinin) act via endothelial NO production [46].

Endothelin

Hickey described a vasoconstrictor factor produced by cultured endothelial cells in 1985 [49], that was identified as endothelin, a 21-residue peptide, by Yanagisawa et al. in 1988 [50]. Three different isoforms were found (ET-1, ET-2 and ET-3) [51], that are diversely distributed. ET-1 is the only endothelin present in endothelial cells [46]. Stimuli to endothelin synthesis include many noxious vasoconstrictor mediators released during pathological insults, including activated platelets, endotoxin, thrombin, various cytokines, hypoxia and low shear stress. Inhibitors include NO, natriuretic peptides, PGE_2, PGI_2, heparin and high shear stress [46]. There are at least two types of endothelin receptor, designated ET_A and ET_B. ET-I preferentially activates ET_A-receptors. ET_A-mediated responses include vasoconstriction, bronchoconstriction and aldosterone secretion. ET_A-receptors are coupled to phospholipase C, which stimulates Na^+–H^+ exchange, protein kinase C and mitogenesis as well as causing vasoconstriction through inositol trisphosphate. Endothelin receptor antagonists cause vasodilatation when infused directly into the brachial artery, consistent with tonic ET-I -mediated vasoconstrictor activity in resistance vasculature [46].

Endothelial dysfunction

Endothelial dysfunction is a physiological dysfunction of normal biochemical processes carried out by the endothelium. The vasodilator and platelet-regulatory functions of the endothelium are impared in vascular disorders such as atherosclerosis, coronary artery disease, essential hypertension and diabetes mellitus [40, 52, 53]. The right balance between NO and ET-1 production seems to be crucial in maintaining cardiovascular homeostasis and preventing endothelial dysfunction [54]. This complex interaction between NO and ET-1 has been investigated in many experimental and in vitro models. NO released from endothelial cells inhibits the synthesis and vasoconstrictor effects of ET-1, which in turn stimulates the NO production by means of autocrine interactions with ET_B receptors [55, 56]. In venous plasma of patients with erectile dysfunction, significantly increased ET-1 and decreased NO levels were found [57]. In pathological situations circulating pro-inflammatory cytokines induce the expression of iNOS and ET-1 in smooth muscle cells, also downregulating endothelial eNOS expression. This results in innovated ET-1 production and a subsequent decrease of NO [40, 58].

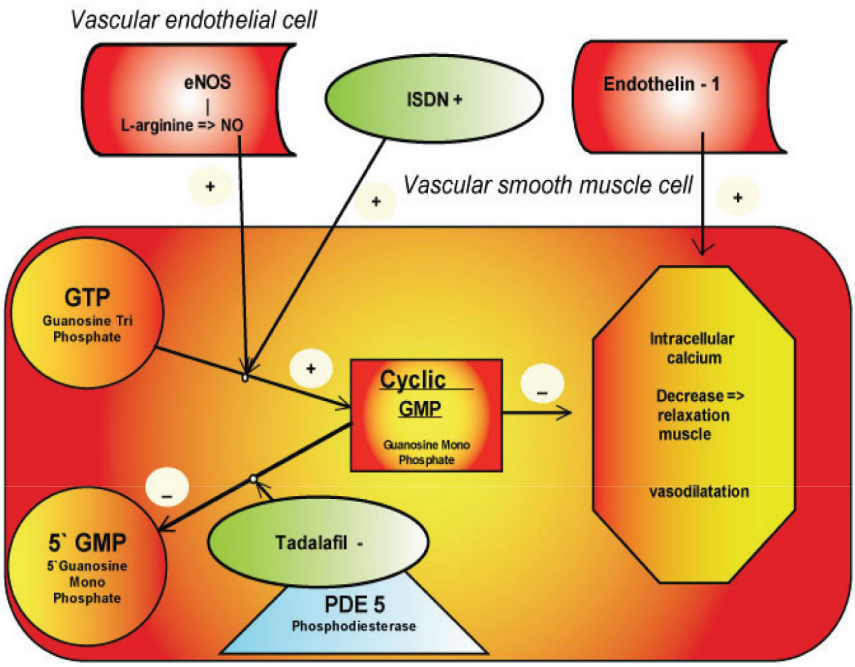


Figure 1.
Overview of the roles of nitric oxide (NO) and endothelin-1 (ET-1) in the regulation of vascular tone. NO is generated from L-arginine by the endothelial cells and activates cyclic Guanosine Mono Phosphate (cGMP) to produce vasodilation. ET-1 is produced by endothelial cells and activates the ET_A receptor on the smooth muscle cell, which leads to vascular vasoconstriction. cGMP is hydrolysed to 5`GMP by Phosphodiesterase (PDE 5).
The NO donor Isosorbide Dinitrate (ISDN) stimulates cGMP. Tadalafil inhibits the hydrolysation of cGMP. In both ways cGMP is stimulated, which leads to a decrease of intracellular calcium and successive vasodilation.

Methods:

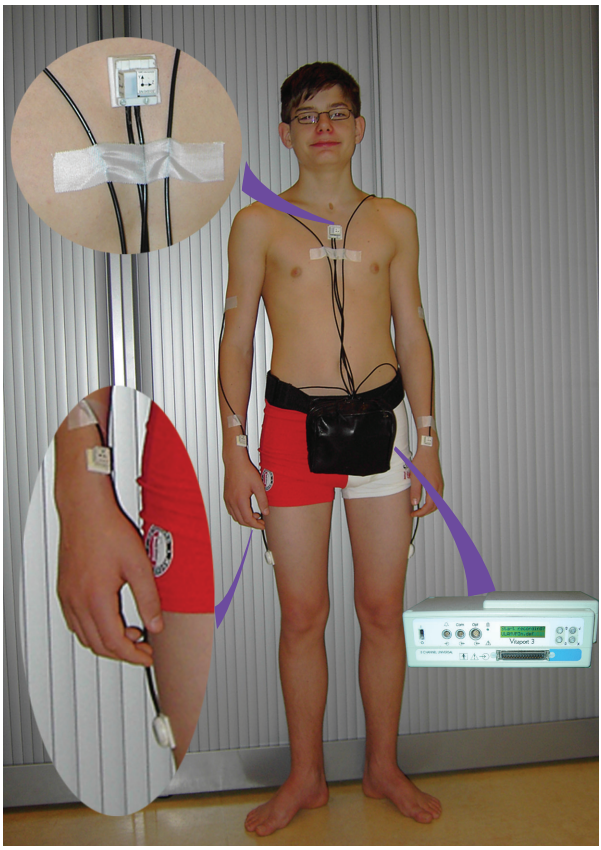
The studies in this thesis focus on the disturbed peripheral blood flow in patients of the subgroup with a relatively limited syndrome with vasomotor signs predominating. In these patients the peripheral vasoconstriction results in tissue hypoxia and tissue acidosis [59, 60], which plays a major role in the pathogenesis of the trophic changes observed in the superficial and the deep tissues [61-63], and may also be involved in the generation of pain by producing tissue hypoxia and acidosis. Lowering of the pH within superficial and deep tissues is followed by sensitization and most likely activation of nociceptive afferents, producing a spontaneous pain sensation. Furthermore, the activity of nociceptive afferents will trigger central sensitization processes, which will support the development of allodynia and hyperalgesia [64]. Pain and disturbed self-perception will lead to fear of pain, fear of movement, and subsequent disuse, resulting in reduced activity [65-70].
Videothermography was used to measure skin temperature, and activity was recorded with the Upper Limb Activity Monitor. Both methods have recently been validated for use in patients with CRPS.

Videothermography
The skin temperatures of both hand or feet were registered with a computer-assisted infrared thermograph (ThermaCAM SC2000, Flir Systems, Berchem, Belgium) following following a standard protocol [71, 72]. The thermographic images of the CRPS side and the contralateral side were compared and the mean temperature (in °C) and the differences in temperature (maximal minus minimal temperature) of the hand or foot were determined [71, 73].
The registration of skin surface temperature by means of computer-assisted videothermography is an objective parameter to study the long-term effects of pharmacotherapeutics [71, 73, 74]. Changes in skin temperature of the extremities are caused either by local inflammation of the tissue or by changes in blood flow in vessels located just underneath the skin. Besides inflammation, vasodilation and enhanced blood distribution also cause a visible increase in skin temperature. In contrast, a decrease in local skin temperature, as normally observed in chronic CRPS, is directly related to diminished tissue blood distribution [75].
Under normal conditions, the thermal asymmetry between opposite sides of the body is very small; in healthy subjects, the degree of thermal asymmetry was less than 1% ($< 0.25^{o}C$) when measured with computerized thermography [73, 76-78]. Therefore, a difference in matched regions of $> 1^{o}C$ is generally considered to be a significant disease-related effect [71, 73].

Upper Limb Activity Monitor

To determine possible changes in activity, the Upper Limb Activity Monitor (ULAM) was used. The ULAM is based on ambulatory accelerometry and enables long-term objective determination of actually performed upper limb activity in different postures and motions during everyday functioning. It is increasingly used in research involving a variety of patient groups [79, 80], including acute [81] and chronic [82-84] CRPS patients.

Figure 2:



Model demonstrating the ULAM with the sensors on the sternum, the sensors on hand and thigh and the Vitaport datarecorder

As figure 2 shows, the ULAM consists of six piezo-resistive accelerometers and a Vitaport2 data recorder. Two sensors are attached to the upper leg, the lower arm, and the sternum, respectively, and the data recorder is worn in a special belt around the waist.

The ULAM was fit to each patient following all measurements in the hospital at the start and end of the study. The patients were instructed to continue their normal activities while wearing the ULAM, with the exception of swimming, bathing, and showering.
The signal analysis and its output have been described by Bussmann and Schasfoort [80, 82, 85, 86]. We calculated ULAM outcome measures for the duration and intensity of activity of the CRPS limb in comparison to the contralateral limb, as well as outcome measures for general activity, like sitting, standing and walking. For these ULAM outcome measures, a higher value indicates a higher level of activity during daily functioning.

Aim and outline of the thesis:

The aim of the research described in this thesis was to study the nature of the vascular alterations in cold chronic CRPS. The sympathetic vascular regulatory system in CRPS has been examined extensively, but this failed to show, why a sympathectomy provides relief in only a minor part of the patients. It seems likely, that other regulation mechanisms are also disturbed in one way or another. We hypothesised, that endothelial dysfunction may in part be responsible for the diminished blood flow in cold chronic CRPS. An improvement on endothelial level would therefore lead to an increased blood flow and a subsequent improvement in pain and activity.

In the second chapter we have described the characteristics of 195 patients with potential CRPS that visited our out-patients clinic. We studied the disease characteristics, the treatment, and the referral patterns, and examined whether these were different in the subgroup of patients with a lower temperature in the affected extremity.
In the third chapter the results of immunohistochemical staining on sections of skin specimens obtained from the amputated limbs (one arm and one leg) of two patients with CRPS are described. The aim of this study was to examine the distribution of eNOS and ET-1 relative to vascular density represented by the endothelial marker CD31-immunoreactivity in the skin tissue of patients with chronic CRPS.
In the fourth chapter we describe the involvement of ET-1 and NO during the early chronic phase. Therefore artificial suction blisters were made on the extremities on the CRPS side and the contralateral side, and the levels of NO, IL-6, TNF-α and ET-1 in the blister fluid were measured.
Since the results indicated that NO levels were decreased on the CRPS side, a pilot study was performed using the NO donor isosorbide dinitrate (ISDN). Five female patients were treated with ISDN ointment 4 times daily during 10 weeks. As a primary objective videothermography was used to monitor changes in blood distribution the involved and the contralateral extremities. This study is described in chapter five.
Encouraged by the positive results of this pilot we initiated two studies. Both studies were randomized placebo-controlled trials, included 24 patients with chronic cold CRPS, and used videothermography and pain as primary outcome measures. The first study, which uses ISDN is described in chapter six. In this study patients were included with CRPS in one hand. In the second study, which is described in chapter seven, the phosphodiesterase inhibitor Tadalafil was used to induce vasodilation in patients with CPRS in one foot. In chapter 8, finally, the main findings are discussed and suggestions are given for future research.

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"It really was very, very hard to do it. when I touched it, in lots of ways it actually reinforced the idea that it wasn't mine at all because it didn't feel like it and I couldn't make it feel like mine and I didn't want to make it feel like mine in a lot of ways because it hurt so much."

Chapter 2

Effectiveness of diagnostic criteria in patients with complex regional pain syndrome assessed in an out-patient pain clinic

submitted

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Abstract

Background:
Specific criteria have been described and accepted worldwide for diagnosing patients with complex regional pain syndrome (CRPS). Nevertheless, a clear-cut diagnosis cannot be confirmed in a number of cases.

Aim:
The objective of this study was to investigate the effectiveness of the described diagnostic criteria used by several clinical disciplines.

Methods:
We included 195 patients that were referred to our pain clinic within a period of one year. Data were collected on patient characteristics, signs, symptoms, disease-related medication, and the background of the referring clinicians.

Results:
The Harden & Bruehl criteria were confirmed in 95 patients (49%). These patients used a higher than average number of analgesics, opiates, and anti-oxidants, and frequently received prescriptions for benzodiazepines instead of anti-depressants. The mean disease duration was 29 ± 4.6 months and the mean visual analogue score for pain was 8.1 ± 0.19. A subgroup of patients had a colder temperature in the affected extremity compared to the unaffected extremity. This subgroup showed longer disease duration and higher visual analogue scale pain. Diagnosis of CRPS was confirmed in 61% of the patients referred by other anaesthesiologists, 63% by rehabilitation doctors, 43% by family doctors, 42% by surgeons, 35% by orthopaedic surgeons, and 13% by neurologists.

Conclusion:
The diagnostic criteria used to determine CRPS should be further improved. Disease-related medication is unrelated to CRPS-specific disease activity. Knowledge of underlying mechanisms is warranted before an adequate pharmaceutical intervention can be considered.

Introduction

Complex regional pain syndrome (CRPS) is a disease characterized by severe chronic pain in an extremity; it usually develops after trauma or surgery. It has been previously termed posttraumatic dystrophy, Sudeck's dystrophy, or reflex sympathetic dystrophy. In addition to pain, patients with CRPS may exhibit other signs and symptoms, including sensory, autonomic, and motor disturbances [1]. Patients may also exhibit changes in skin surface temperature, oedema, hypersensitivity, and allodynia. In the chronic phase of CRPS, patients report continuous pain and a temperature change in the affected extremity that indicates perturbed tissue perfusion. In most cases, CRPS leads to a dramatic loss of function and disability. The subsequent phases and related mechanisms underlying the disease are poorly understood. Therefore, effective treatments are lacking. Some of the prominent signs and symptoms of CRPS (e.g. redness, increased skin temperature, and edema) could be explained by inflammation. CRPS may involve a process of central sensitization that exacerbates pain through neuroimmune activation of cells in the peripheral nervous system [2, 3]. In general, the standard pharmacological and interventional therapy provides insufficient suppression of the spread of CRPS from the distal end through the entire extremity [4].
The diagnostic criteria are still under debate, although during the last decade, provisional criteria have been described for both clinical and research purposes [5-8].
The aim of this study was to investigate whether patients referred to our pain clinic with a diagnosis of CRPS indeed fulfilled these criteria. We evaluated disease characteristics and examined the relationship between the prescribed pharmaceutics and the symptoms of the patients.

Methods

Ethics:
The protocol was approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam as part of the randomized trials regarding nitric oxide related vasodilation (ISRCTN60226869) and infliximab (ISRCTN75765780). Written informed consent was obtained from all patients. Guidelines were followed according to the Declaration of Helsinki (amended version of 2002) and Good Clinical Practice (ICH/GCP version 1996). Data collection was performed according to guidelines for registration of personal data.

Subjects:
Patients diagnosed with CRPS in one extremity were recruited for this study over one year, from October 2006 to September 2007. Patients were referred by clinicians of several disciplines, or visited the pain clinic after reading our invitation on the website. The physical examination was performed by one of our anesthesiologists. Diagnosis followed the modified criteria of Harden & Bruehl [7, 8]: patients were

required to report continuing pain disproportionate to any inciting event; furthermore were they required to report at least one symptom in each of the following categories (sensory, vasomotor, sudomotor/oedema, and motor/trophic); finally, upon visiting the pain clinic, they were required to display at least one sign in two or more of the above categories.

Data collection:
The following information was collected: gender, age, distance from the residence to the pain clinic, duration of CRPS, location and cause of CRPS, name of the medical doctor that referred the patient, disease-related medication, whether the diagnostic criteria conformed to the Harden & Bruehl criteria (both that reported by the patient and that displayed upon visiting the pain clinic), changes in skin temperature of the CRPS affected extremity compared to the unaffected contralateral extremity, measured by videothermograhy as described by Niehof et al. [9].
Statistical analysis was carried out using the SPSS statistical package. The non parametric Kruskal-Wallis test was used to compare different group results.

Results

During the one-year inclusion period, 195 patients (82% females) were diagnosed and included in this descriptive study. All characteristics of the study population are presented in Table 1. There was an equal distribution of the disease in the upper and lower extremities. Slightly more patients were affected on the right side (53%) than the left, and 6% were affected on both sides.

Table 1 Characteristics of the study population

	Not Confirmed (n=84)	Possible (n=16)	Confirmed (n=95)
Gender: male/female	17/67	5/11	13/82
Age in years	48±1.9	45±2.9	47±1.5
Living distance from hospital,	30±4.9, km	30±7.7	48±5.8*
Cause: trauma (fracture, accident)	57(29,28)	11(8,3)	63(39,24)
surgery/spontaneous)	8/19	1/4	7/25
Location: upper/lower extremity	44/40	9/7	46/49
Side: right/left/right+left	34/42/8	6/10/0	40/52/3
Temperature of CRPS extremity: warm/no diff./cold	19/44/21	4/5/7	38/10/47
Duration of CRPS, months	36±6.4	49±22.1	29±4.6
VAS-pain (scale 0-10)	7.6±0.20	7.8±0.33	8.1±0.19

Values are means ± SEM. *P < 0.05 (versus 'not confirmed')

In our pain clinic, 95 patients (49%) were confirmed with CRPS according to the criteria of Harden & Bruehl. About half the patients either did not fulfil the criteria (not confirmed = 43%), or may have had CRPS (possible = 8%), based on the reports of the patients, but did not sufficiently display symptoms during the visit at the pain clinic. The diagnosis of CRPS could not be confirmed for 84 patients. A large proportion (52%) of these patients did not display differences in skin surface temperature. For those patients that were not diagnosed with CRPS, 44 were diagnosed with a myofascial pain syndrome, 31 with a neuropathic pain syndrome, 5 with inflammation, 3 with a vascular disease, and 1 with psychogenic involvement.
No significant differences were found between those confirmed and those not confirmed with CRPS in disease duration and in pain assessment on a visual analogue scale (VAS). However, marked differences were found in the percentage of patients that used disease-related medications (Table 2). In particular, the 'confirmed' patient group had strikingly higher numbers of prescriptions for analgesics, opiates, and anti-oxidants. Furthermore, benzodiazepines had been prescribed more often than anti-depressants. Among the benzodiazepines, equal numbers of anxiolytics and hypnotics were prescribed, and there was no difference in the type of prescriptions written by physicians of all disciplines. A negative correlation between VAS-pain and age was observed in patients with confirmed CRPS ($r_S = -0.42$, $P<0.001$). However, VAS-pain was positively correlated with the disease duration ($r_S = 0.25$, $P=0.017$ and $r_S=0.25$, $P=0.028$ for confirmed and not-confirmed patients, respectively).

Table 2 CRPS-related medications

	Not Confirmed (n=84)	Possible (n=16)	Confirmed (n=95)
Analgesics	18	38	53
Non-steroid anti-inflammatory drugs (NSAIDs)	26	38	39
Opiates	12	6	31
Anti-oxidants	31	50	71
Corticosteroids	8	0	9
Immunosuppressives	2	0	4
Vasodilators	4	19	17
Muscle relaxants	1	0	2
Anti-depressants	20	13	7
Benzodiazepines	14	6	18

Values are the percent of patients in each group

The American Veterans Health Administration defined a VAS pain score ≥ 4 as a substantial level of pain that requires comprehensive assessment and prompt intervention [10]. Substantial pain was reported by 177 (91%) of all 195 referred patients. Of these 177 patients with substantial pain, 59 (33%) received no pain medication whatsoever.

Temperature changes measured by videothermography were investigated for relationships with specific patient characteristics. Physical examination confirmed a difference in temperature between the affected and unaffected limbs in 143 (73%) patients that reported a possible difference in temperature. In 126 patients, a videothermography showed at least a 1°C difference between the extremities (50 extremities were warm and 66 were cold); but in 10 cases no difference was found. As table 3 shows there are large differences between the referring physicians in confirmation rate. The mean age was significantly lower in patients displaying a cold extremity in comparison with a warm extremity (Table 4). Furthermore, both the disease duration and the VAS-pain were increased in patients with cold CRPS-affected limbs.

Table 3 Disciplines of referring physicians and confirmation rate

		DIAGNOSIS IN ERASMUS MC				
	Number of Docters	Total Patients	Not confirmed	Possible CRPS	Confirmed CRPS	Duration of CRPS
	n	n	n	n	n (%)	months
Anaesthesiologist	26	44	11	6	27 (61)	35 ± 9.0
Surgeon	23	43	23	2	18 (42)	14 ± 3.3*
Orthopaedic surgeon	18	31	18	2	11 (35)	26 ± 6.5
Family doctor	27	30	16	1	13 (43)	36 ± 8.6
Neurologist	6	8	5	2	1 (13)	58 ± 27
Rehabilitation doctor	8	8	1	2	5 (63)	103 ± 44*
Others	8	9	6		3 (33)	33 ± 21
Patient's own initiative		22	4	1	17 (77)	40 ± 12

Others: Rheumatologist, traumatologist, internal medicine. Means ± SEM.

* P < 0.05 versus Anaesthesiologist

Of the 75 (39%) patients with a cold extremity, 47 had confirmed CRPS, 7 had possible CRPS, and 21 were not confirmed with CRPS. Within these groups, 4, 2, and 1 patient, respectively, used vasodilative medication.

Table 4 Observed temperature diferences between affected and unaffected limbs related to age, duration of CRPS, and VAS-pain

	Warm extremity	No difference	Cold extremity
	n=38	n=10	n=47
Age, years	54 ± 2.5	52 ± 4.4	41 ± 1.6*†
Duration of CRPS, months	15 ± 4.1	30 ± 10	39 ± 8.0*
VAS-pain, schale 0-10	7.4 ± 0.39	8.1 ± 0.34	8.5 ± 0.18*

Means ± SEM. * P < 0.05 versus 'warm'; † P < 0.05 versus 'no difference'

Discussion

The modified Harden & Bruehl criteria for the diagnosis of CRPS patients are still a matter of debate, especially when diagnosed patients are to be included in clinical research trials. These criteria should either be reported by the patient (symptoms) or displayed and observed by the physician (signs). For diagnostic purposes, the motor/trophic changes are especially important aspects of the criteria.
In this study we confirmed that half of the referred population had CRPS according to these criteria. We found that approximately 62% of the patients referred by anaesthesiologists and rehabilitation doctors were correctly diagnosed. In comparison with anaesthesiologists, the surgeons referred patients earlier in the disease course, and rehabilitation doctors referred patients late in the disease course. In our opinion, it is remarkable that CRPS was confirmed in a large proportion (43%) of the patients referred by family doctors.
We found no difference in temperature between the affected and unaffected limbs in 59 of the 195 referred patients, and in 44 of the 84 patients with unconfirmed CRPS. The use of 1°C as threshold for a significant temperature change is not undebated. We based this threshold on the research of Uematsu et al. who showed that the thermal asymmetry between opposite sides of the body is less than 0.25°C under normal conditions [11, 12]. Therefore this threshold has been used in all studies of our research group [4, 9, 13-16]. A threshold of 1.5°C was used by Schürmann et al. [17], based on a difference of up to 1°C found in patients with distal radial fractures [18]. The use of the larger threshold would have resulted in an increase of the number of patients without temperature difference. Since videothermography has a negative predictive value of 79 – 84%, and a positive predictive value of 17 – 38% [17], when used as a single diagnostic procedure, we used it for the measurement of skin temperature only.
Wasner et al. found 3 types of vascular regulation, termed warm, cold, and intermediate. In the latter, the extremity can be warmer, colder, or show no difference, depending on the state of sympathetic activity [19]. Therefore, these patients may not show a temperature difference despite an actual disturbance in the vascular regulation system. We did not check temperature under different sympathetic conditions; thus, the number of patients with confirmed CRPS might have been higher than reported here. Our videothermography was based on a series of pictures from different angles, taken under the same environmental conditions. A dynamic test would be required to analyse the function of the vascular regulation system under different states of sympathetic activity [9].
We found that the 'confirmed' patient group had increased numbers of prescriptions for analgesics, opiates, and anti-oxidants. These numbers were not related to the number of referring anaesthesiologists. However, the prescriptions were in accordance with guidelines for the treatment of CRPS that were published in the Netherlands in 2006 [20]. We cannot explain the prescription preference of benzodiazepines over anti-depressants in the group with confirmed CRPS.

Patient societies claim that pain associated with CRPS is the highest in comparison with other (chronic) painful diseases. This was confirmed in our study. In general, pain treatment in the referred population was inadequate. A large number of patients experienced substantial pain, and 33% of these did not receive any pain medication. Most patients received one or more disease-related pharmaceutics to suppress pain or affect quality of life. Nevertheless, the mean VAS-pain score of 7.8 was high in this study compared to previous studies where we found mean VAS-pain scores between 5 and 6 [3, 21, 22]. In CRPS-confirmed patients, the mean VAS-pain score was even higher, depending on the warm- or cold-type variant of the disease. Furthermore, patients with a cold CRPS-affected extremity had both increased VAS-pain and prolonged disease durations, suggesting that these parameters were correlated. The mean disease duration found in this study was comparable to those found in previous studies [21, 23, 24]. We also found that the mean age was lower in cold-type CRPS than in warm-type CRPS, and that age was correlated to VAS-pain. In general, it is assumed that inflammatory pain, mainly represented by warm-type CRPS, will be more severe than pain from disturbed tissue perfusion, mainly represented by cold-type CRPS; however, the latter may lead to ischemia and acidosis [3].

Although a large number of patients experienced a cold extremity most likely caused by central or peripheral vasoconstriction, hardly any patients used vasodilative medications. Recent work by our research group showed that the use of a nitric oxide donor might be beneficial in patients with chronic cold CRPS [14].

It is possible that this study population was biased by the fact that the patients were selected by referring clinicians and were willing to cooperate in order to obtain an attractive, new medical treatment (infliximab or nitric oxide-related vasodilation). The mean living distance from the hospital was significantly higher in the confirmed group, indicating that these patients were more willing to travel for the benefits of the treatment. This is not surprising, based on the fact that many of the patients had been living with this disease for over 3 years.

Seventy-seven percent of the patients that visited our website, and thus were not directly referred by a clinician, were diagnosed with CRPS in our centre; however, the disease duration in this group was not different from that in the total population. We do not know how many clinicians had been visited before patients were diagnosed or referred to us.

In conclusion, we have shown that half the CRPS population referred to our pain clinic fulfilled the research criteria of Harden & Bruehl. Dynamic testing of the disturbances in the vascular regulation system might add to the accuracy of these criteria. This population displayed an equal number of affected arms and legs, a normal distribution of warm- and cold-type CRPS, a mean disease duration of 34 months, and a relatively high VAS-pain despite specific analgesia. In general, the patients indicated that their current medication was not sufficent to provide adequate pain relief. Better insight into the processes underlying CRPS could result in more appropriate pharmaceutical interventions. However, a clear-cut diagnosis is warranted to enable clinical research to identify useful drugs for this disabling disease.

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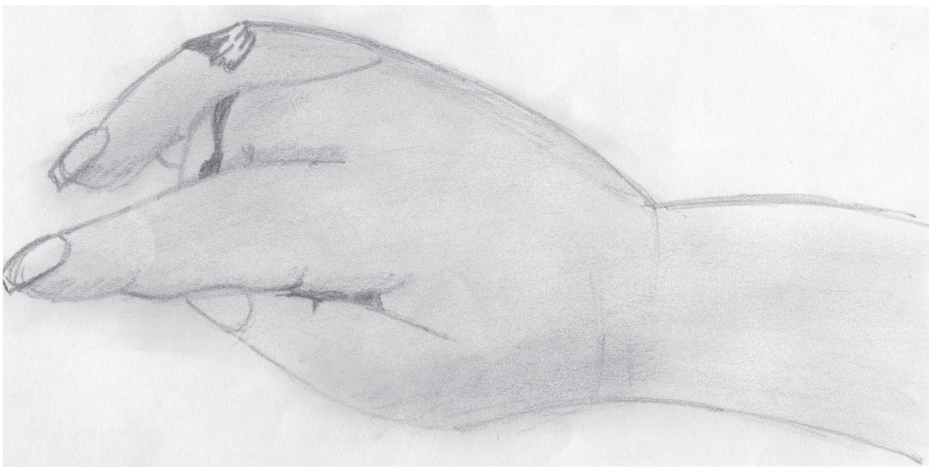

"I've sometimes felt if I could get an axe and chop it off I would do because to me as it stands, at this minute in time sat here it's a useless, it's a useless thing."

Chapter 3

Expression of endothelial nitric oxide synthase and endothelin-1 in skin tissue from amputated limbs of patients with complex regional pain syndrome

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J. George Groeneweg , Claudia Heijmans Antonissen, Frank J.P.M. Huygen and Freek J. Zijlstra

Abstract

Background and Objectives:
Impaired microcirculation during the chronic stage of complex regional pain syndrome (CRPS) is related to increased vasoconstriction, tissue hypoxia, and metabolic tissue acidosis in the affected limb. Endothelial dysfunction is suggested to be the main cause of diminished blood flow. The aim of this study was to examine the distribution of endothelial nitric oxide synthase (eNOS) and endothelin-1(ET-1) relative to vascular density represented by the endothelial marker CD31-immunoreactivity in the skin tissue of patients with chronic CRPS.

Methods:
We performed immunohistochemical staining on sections of skin specimens obtained from the amputated limbs (one arm and one leg) of two patients with CRPS.

Results:
In comparison to proximal specimens we found an increased number of migrated endothelial cells as well as an increase of eNOS activity in distal dermis specimens.

Conclusions:
We found indications that endothelial dysfunction plays a role in chronic CRPS.

Introduction

Complex regional pain syndrome (CRPS) is defined as "a syndrome in which the central nervous system representations of the somatosensory, somatomotor, and sympathetic systems are altered concomitantly with important peripheral changes such as edema, signs of inflammation, sympathetic-afferent coupling, and trophic changes" [1]. The manner in which the peripheral and central changes interact is only partly understood [1]. During the chronic stage of CRPS, increased vasoconstriction [2], tissue hypoxia [3] and metabolic tissue acidosis [4, 5] indicate that microcirculation is impaired, which affects the nutritive blood flow in superficial and deep tissues [6, 7].

The endothelium modulates vascular tone by releasing endothelium-derived vasodilators including nitric oxide (NO), prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor, and vasoconstrictors, such as endothelin-1 (ET-1) and angiotensin II, in response to a number of biochemical and physical stimuli [8]. Growing evidence [9] suggests that endothelial dysfunction is the main cause of diminished blood flow in chronic cold CRPS.

Although numerous papers about CRPS have been published since its first description in 1545 [10], literature regarding the associated pathological alterations in blood vessels is scarce. The few papers on this topic describe clearly visible abnormalities of the entire microvascular system, including an increase in the number of capillaries [11, 12], endothelial swelling, and changes in the vessel laminal wall [13]. The impressive capillary changes range from severely thickened basal membrane with intimal vacuolization, perivascular edema, and debris from pericytes between the basal membrane layers, to necrosis [14, 15]. Greatly thickened multi-laminated walls were found, considerably reducing the inner diameter of the vessel [12, 16]. Endothelial cells with a shrunken appearance and capillaries with only endothelial cell debris in the lumina were observed, while other capillaries could be traced by the thickened basal membrane only, lacking the presence of other cellular remnants [15].

Histochemical procedures [17] and immunofluorescence [16] have been used to investigate the distribution of cutaneous nerve fibers, but there are no reports of using immunohistochemical staining to evaluate endothelial dysfunction in CRPS. Key elements in the function of the endothelium are NO and ET-1. The loss of endothelium dependent NO mediated vasodilation occurs early during endothelial dysfunction [18]. Since NO has a half-life time of only 3-5 s [19], we measured endothelial nitric oxide synthase (eNOS), which is a constitutional endothelial cell enzyme that produces NO from L-arginine. Increased production and/or activity of ET-1 may participate in several pathologic states related to a dysfunctional endothelium [20].

The aim of this study was to examine the distribution of eNOS and ET-1 in relation to vascular density represented by the endothelial marker CD31-immunoreactivity in skin tissue from amputated limbs of patients with CRPS.

Methods

Patients

Human tissue was obtained from the surgically amputated extremities of two patients diagnosed with CRPS, according to the Bruehl diagnostic criteria [21], at the Pain Treatment Centre of the Erasmus MC. Both patients gave written informed consent prior to the amputation. A lower limb was amputated in patient A and an upper limb in patient B. Skin samples, harvested immediately following the amputations, were taken from the dorsal side of the foot of patient A and from the dorsal side of the hand of patient B; both of these samples were categorized as distal tissue samples. Proximal tissue samples were harvested from near the cutting face, taking tissue that appeared to be the least diseased.

Patient A was a 46-year-old female who developed CRPS in her right leg after an electromyography recording in 1996. One year later, symptoms appeared in the left leg, and in 1998, in her right hand. The patients' complaints persisted and despite intense rehabilitation, both legs and the right hand became nonfunctional. There was edema in both legs, and there were severe contractures and pain in the legs and hand. Walking was impossible and she relied on a wheelchair for transport. During a visit to our clinic in 2003, muscle force, reflexes, and coordination could not be tested and there was hyperesthesia in both legs and the hand. She was treated with paracetamol, amitriptyline, durogesic, mannitol, and baclofen, and received an epidural block with marcaine and fentanyl. Due to continuous infections (erisipelas) in both legs in June 2003, antibiotics were prescribed but the infections were resistant to therapy. Therefore, the left leg had to be amputated in October 2003, followed by the right leg one week later. Post-operative healing was complicated by pressure ulcers and she was released from the hospital using paracetamol and tramadol for pain control. A video thermographic recording was not made, but the examining physician described both legs as very cold.

Patient B was a 38-year-old female who developed CRPS in the right hand in December 2002 as a consequence of a metacarpal II fracture. After 10 days in a plaster cast, she showed severe symptoms of CRPS with pain, edema, and contractures. Despite extensive pharmacotherapy to reduce pain and inflammation, improve peripheral blood flow, and relieve the spasticity, the patient developed severe dystonia in the right hand. The dystonia and pain made conventional nail care impossible; therefore, nail clipping was performed under general anesthesia every few months. The patient had contractures in her wrist and fingers, spasms of the musculature, burning pain, allodynia and hyperalgesia, edema, increased transpiration, excessive hair growth, and a darker skin color on the affected limb. A video thermographic recording [22, 23] showed that the CRPS extremity was 6.5°C colder than the contralateral unaffected control. Shoulder functioning was normal. Spinal cord trial stimulation in February 2004 was not successful. At the request of the patient, who could no longer cope with the burden of frequent general anesthesia, upper arm amputation was performed in

May 2006. There were no postoperative complications and the patient experienced a dramatic reduction in pain.

Immunohistochemical staining for CD31 and eNOS
In order to demonstrate the vascular state of the CRPS skin tissues, the endothelial marker CD31 was visualized by immunoreactive (IR) staining.
Frozen skin tissue samples were cut in serial 6-µm sections, transferred to poly-L-lysine-coated microscope slides (Menzel-Glaser, Omnilabo, Breda, the Netherlands), air dried, and stored at ±80°C. For immunohistochemical staining, sections were thawed, fixed in acetone for 10 minutes, and rinsed with phosphate buffer saline (PBS, pH 7.8). The staining procedure was conducted in a half-automatic stainer (Sequenza, Shandon Scientific, Zeist, the Netherlands). The slides were incubated with 10% normal goat serum (NGS; Sanquin, Amsterdam, the Netherlands) for 10 minutes and then with primary mouse anti-human antibodies against CD31 (JC/70A Dako, Glostrup, Denmark) or eNOS (SA-258 BIOMOL International L.P., Exeter, United Kingdom) for 60 minutes. Both antibodies had been diluted in 1% blocking buffer (Blocking Reagent, Roche Diagnostics GmbH, and Mannheim, Germany). After each incubation step, the slides were rinsed with PBS for 5 minutes. After incubation with the primary antibodies, sections were rinsed and incubated with biotinylated goat anti-mouse antibodies (BioGenex, Klinipath, Duiven, the Netherlands) and 10% normal human serum (NHS; Sanquin) for 30 minutes. This step was followed by incubation with alkaline phosphatase-conjugated streptavidin (Biogenex, Klinipath) and 10% NHS for another 30 minutes. Slides were rinsed with both PBS and TRIS buffer (TRIS HCl 0.1 mol/L, pH 8.5), and then incubated with new fuchsine substrate (Chroma, Kongen, Germany) diluted in TRIS buffer. Finally, the sections were washed with PBS, counterstained with Gill's hematoxylin (Merck, Darmstadt, Germany) for 30 seconds, rinsed with tap water, dried, and embedded in VectaMount (Vector, Burlingame, CA).

Immunohistochemical double staining for ET-1 and CD31
After fixing in acetone and washing with PBS, endogenous peroxidase was blocked with 0.1% sodium azide and 0.03% hydrogen peroxide in PBS for 30 minutes. Sections were rinsed and incubated with 10% NGS for 10 minutes, followed by incubation with rat anti-human ET-1 (3G10, R&D systems, Abingdon, United Kingdom) for 60 minutes. The sections were rinsed and incubated with goat anti-rat antibody conjugated with alkaline phosphatase (Sigma, St.Louis, MO, USA.) and 10% NHS for 30 minutes. Thereafter, the slides were rinsed and incubated with mouse anti-human CD31 for 60 minutes. Then sections were rinsed and incubated with biotinylated goat anti-mouse antibodies and 10% normal human serum (NHS) and 10% normal rat serum (Sanquin) for 30 minutes. After washing, the slides were incubated with streptavidin-conjugated peroxidase (Biogenex, Klinipath) and 10% NHS for another 30 minutes. After rinsing with PBS and substrate TRIS buffer, slides were incubated

for 30 minutes in fast blue substrate (Sigma). Finally, sections were rinsed and incubated with peroxidase nova red substrate (Vector) for 5 minutes, rinsed with PBS, and embedded in VectaMount.

Quantification of the immunoreaction

eNOS and CD31 staining was performed on adjacent slides of serial sections. Slides were examined using a Leica microscope fixed with a Leica DC500 camera for digitizing images. Semi-quantitative evaluation of the different markers was performed by counting the number of positive blood vessels in the dermis of two sections each from both the distal and proximal specimens. After measuring the total area of the dermis using the Leica Imaging Analysis System, the number of positive blood vessels per square millimeter was calculated. In the case of ET-1 and CD31 double staining, we determined the number of ET-1 positive cells in the dermis and the percentage of positive ET-1 blood vessels on the total number of blood vessels.

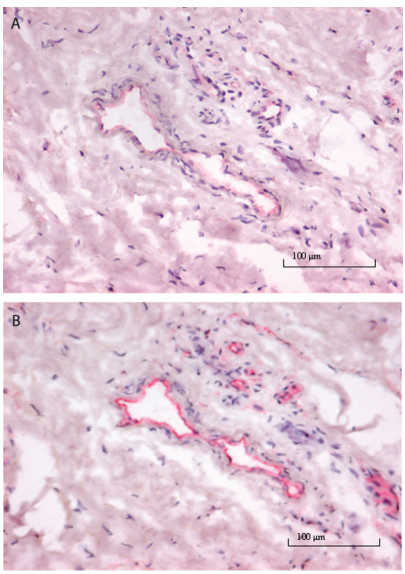

Figure 1
(A) CD31-immunoreactive vessels in skin tissue from the amputated arm of CRPS patient A. CD31-positive blood vessels are stained red. (B) eNOS staining of a serial section. eNOS-positive blood vessels are stained red.

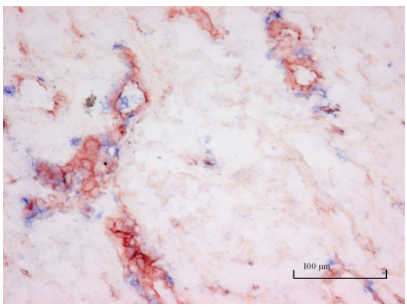

Figure 2
Double staining for CD31 and ET-1 in skin tissue from the amputated leg of CRPS patient B. CD31-positive blood vessels are stained red and ET-1-positive cells are stained blue.

Results

Vascular status in CRPS
The qualitative differences between the distal and proximal specimens are shown in figure 1A. These differences were confirmed by qualitative image analysis of the sections. In both patients, endothelial immunoreactivity was more prominent in distal than in proximal specimens. In patient A, the mean number of CD31-IR capillaries in distal tissue was 43 blood vessels/mm^2 versus 14 blood vessels/mm^2 in proximal tissue; in patient B, the means were 39 and 19 blood vessels/mm^2, respectively.

eNOS immunoreactivity
eNOS immunoreactivity of capillaries and other small-diameter blood vessels was prominent in the distal specimens (Fig. 1B). The measure of eNOS-IR endothelium in patient A was 23 blood vessels/mm^2 in distal tissue versus 7 blood vessels/mm^2 in proximal tissue; in patient B, the means were 20 and 13 blood vessels/mm^2, respectively.

Ratios
The ratios of eNOS/CD31-IR vessels were similar. In patient A, the mean ratios were 53% and 50% in distal and proximal tissue, respectively, whereas in patient B, the mean ratios were 51% and 68%, respectively.

Endothelin-1 positive cells
The mean number of ET-1-positive cells was determined in both the dermis and the blood vessels (Fig. 2). In patient A, there were 81 ET-1-positive dermis cells/mm^2 in distal tissue versus 22 ET-1-positive dermis cells/mm^2 in proximal tissue; in patient B, there were 42 and 12 ET-1-positive dermis cells/mm^2, respectively. The mean percentages ET-1-positive blood vessels were similar; in patient A, there were 70% ET-1-positive blood vessels in the distal tissue and 69% in the proximal tissue; in patient B, the mean values were 78% and 63%, respectively.

Discussion

Because of the increased risk of an overreaction to a skin biopsy [15], we could not take skin tissue samples from CRPS-affected limbs in the usual manner. Therefore, we used specimens taken from amputated limbs, which are seldom available. Proximal and distal specimens from the amputated leg or arm from two different patients were dissected out immediately after the amputation, and deep frozen in liquid nitrogen. As specimens from the contra-lateral side or healthy tissue were not available for comparison; we considered the distal specimens as the most-affected and the proximal specimens as non- or least-affected tissue. This is the first study that investigates the distribution of eNOS and ET-1 in tissue of CRPS patients, therefore we decided to limit the study to skin tissue, following previous observations in skin blister fluid obtained from CRPS patients. Expanding the study to muscle and/or nerve tissue might have provided additional insights in mechanisms underlying CRPS.

Tissue blood distribution is altered in patients with CRPS. It is generally accepted that during the course of this disease, and partly due to disuse, tissue ischemia will occur, leading to chronic pain [24]. However, until there was no evidence suggesting that these patients have endothelial dysfunction or impaired angiogenesis in response to ischemia. We observed regional differences in the expression of endothelial markers in the CRPS-affected limbs. Information about the normal distribution of these markers between proximal and distal upper or lower extremities is not available. Expression of CD31, eNOS, and ET-1 was highest in the distal specimens, representing the most affected samples of the diseased limbs. However, the eNOS/CD31 ratios were similar, ranging from 51-68%. Palatka et al [25] reported an eNOS/CD31 ratio of 92% in healthy mucosal biopsies, whereas this ratio was 8% in tissue samples from patients with Crohn's disease and 82% in samples from patients with ulcerative colitis, which suggests that eNOS activity is diminished in disease-affected endothelial cells. Although values from mucosa may not be comparable to the skin tissue values in our study, the suggestion is made, that eNOS activity was diminished in our patients, but not dramatically. Moreover, we observed no distinct regional differences, coinciding with the observations that during the course of the disease, pain spreads through the entire limb and that CRPS may also occur in the contra lateral limb and/or the other extremities [26]. It has been shown that during hypoxia, expression of both endothelial cell markers CD31 and eNOS increases [27]. In the case of ischemia, eNOS is essential for promoting collateral growth in order to restore blood distribution to the tissues [28, 29]. Therefore, in patients with CRPS, supplementing NO precursors will not provide benefit because eNOS activity is compromised. Treating patients with chronic CRPS with an endothelium-independent NO donor might be a more effective strategy [30].

Our data confirmed the previously reported increase in capillary density in CRPS-affected tissue [11, 12], and showed a higher number of ET-1-positive blood vessels/mm2 in distal versus proximal specimens. There were marked differences (3 to 4–fold)

in the number of ET-1-positive cells between the distal and proximal dermis. ET-1 promotes proliferation and migration of endothelial cells via endothelin-B receptors [31]. These migrated cells are associated with swelling and laminal wall disruptions [13]. The pronounced increase in ET-1 expression in the distal limbs confirms earlier work by our group in which we found elevated levels of ET-1 in the superficial blister fluids harvested from the distal region of CRPS limbs [24]. However, alterations in the number of ET-1-positive cells and skin blister fluid concentrations do not necessarily reflect an increase in the number of endothelin-B receptor-binding CD31-positive endothelial cells [31]. Nevertheless, ET-1 blockers could provide relief.

This study was limited to only two CRPS patients, and lacks appropriate control in the form of contralateral tissue, or a normal distribution of these markers. Both patients were chronic and have been treated with many medical and interventional procedures. Conclusions can therefore only apply to potential mechanisms that maintain chronic CRPS, like severe vasoconstriction, blood supply redistribution due to abnormal blood flow shunting with hypoperfusion in nutritive vessels, hypoxia, lactate increase, and acidosis [1].

In conclusion, we found an indication that endothelial dysfunction plays a role in chronic CRPS. In comparison to proximal specimens we found an increased number of migrated endothelial cells as well as an increase of eNOS activity in distal dermis specimens.

Acknowledgements

We appreciate the skillful assistance of Dr. Joan Holstege (Department of Pathology), Feikje Wesseldijk, and Emmy van Bodegraven. The study was performed as part of the TREND (Trauma-Related Neuronal Dysfunction) knowledge consortium, which integrates research on CRPS. Personnel costs of George Groeneweg were funded by a Dutch government grant (BSIK03016).

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"All the different types of sensations and feelings and everything you get. . . when you explain it to a specialist that doesn't know about this, I mean, they just look at you and think, "Well, you know, you're pretty stupid."

Chapter 4

Increased endothelin-1 and diminished nitric oxide levels in blister fluids of patients with intermediate cold type complex regional pain syndrome

Published in:
BMC Musculoskeletal Disorders 2008

J. George Groeneweg, Frank J.P.M. Huygen, Claudia Heijmans-Antonissen,
Sjoerd Niehof and Freek J. Zijlstra

Abstract

Background
In complex regional pain syndrome (CRPS) pro-inflammatory mediators and vascular changes play an important role in the sustained development and outcome of the disease. The aim of this study was to determine the involvement of vasoactive substances endothelin-1 (ET-1) and nitric oxide (NO) during early chronic CRPS.

Methods
Included were 29 patients with CRPS who were diagnosed during the acute stage of their disease and observed during follow-up visits. Disease activity and impairment were determined and artificial suction blisters were made on the CRPS and the contralateral extremities for measurements of IL-6, TNF-α, ET-1 and nitrate/nitrite (NOx).

Results
The levels of IL-6, TNF-α and ET-1 in blister fluid in the CRPS extremity versus the contralateral extremity were significantly increased and correlated with each other, whereas NOx levels were decreased.

Conclusions
The NOx/ET-1 ratio appears to be disturbed in the intermediate stage of CRPS, resulting in vasoconstriction and consequently in a diminished tissue blood distribution.

Trial registration
Trial registration number is: ISRCTN60226869

Background

Complex regional pain syndrome (CRPS) is a painful disorder which mainly occurs as a complication after surgery or trauma, and the main characteristics are continuous pain, marked changes in tissue blood flow and skin surface temperature, oedema and sweating, movement disorders and trophic changes of the skin [1, 2]. The severity of the symptoms is disproportionate to the initial event. The diagnosis of CRPS is entirely based upon clinical criteria [3, 4]. In the Netherlands, the incidence of CRPS is approximately 2.6% for different fractures, which results in approximately 5100 new patients yearly, of whom a substantial part will not recover completely [5-7]. The female to male ratio is approximately 3:1, with a median age of 52.7 years at onset [7]. The pathophysiology of CRPS is not unravelled yet, but growing evidence indicates the involvement of an exaggerated inflammatory processes. Both central and peripheral mechanisms have been proposed to play a prominent role. Central sensitization leading to exacerbation of pain is thought to result from neuroimmune activation of cells in the peripheral nervous system [5]. During the neurogenic inflammation, neuropeptides [8] and cytokines and other mediators are released [9]. This leads to a so-called 'warm dystrophy' with signs of inflammation such as redness, increased skin temperature, loss of function and pain [4, 5, 10].
During the chronic, disabling stage of the disease, in general the following changes occur: i) signs of extravasation, and oedema changes into atrophy, ii) regional blood flow declines, and iii) increased skin temperatures change into diminished temperatures. Permanent damage of nerve endings can lead to less endothelium-dependent vasodilation and result in a diminished regional blood distribution, the so-called 'cold dystrophy' [11, 12]. Both continuous pain and signs of 'cold dystrophy' can lead to assumed irreversible disuse. The contribution of endothelial derived factors could be crucial; endothelin-1 (ET-1) is a proven potent vasoconstrictive agent that is also believed to affect hyperalgesia [13], muscle weakness and movement disorders [14], and oedema [15].
In contrast to skeletal muscle resistance vessels, ET-1 contributes to the maintenance of skin microvascular tone through both ET_A and ET_B receptor-mediated vasoconstriction [16]. The initially produced pro-inflammatory cytokines tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) also play an important role in the initial and intermediate phases of the disease [9, 17]. TNF-α is known to decrease eNOS mRNA levels by increasing the rate of mRNA degradation [18, 19]. In the human forearm, TNF-α promotes ET-1 production [20]. On the other hand, ET-1 also promotes TNF-α and IL-6 production in skin-derived mast cells [21], one of the cells which seem to play a prominent role in the acute phase of CRPS [22].
The present study aimed to investigate the involvement of the vasoactive substances ET-1 and nitric oxide (NO) in intermediate/cold type CRPS in relation to inflammatory mediators.

Methods

Subjects
For this study 29 patients (6 males, 23 females; mean age 48 ± 11.3 (SD) years) with CRPS in one hand according to the criteria of Bruehl [3], were included. The patients had a mean duration of their disease of 2.8 ± 1.4 (SD) years, all being in the intermediate phase between the acute inflammatory stage and the early atrophic stage.
The mean disease activity of the patients was 35 on a scale of 0-100, calculated using the impairment sum score according to Oerlemans [23], representing a low to medium disease activity.
The Medical Ethics Committee of the Erasmus MC approved the study protocol (MEC 2004-159). The research was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and written informed consent was obtained from all participants.

Blister fluid collection
Artificial blisters were induced by means of a suction method [9, 17, 24-27]. A 3-hole (5 mm diameter per hole) skin suction chamber was positioned on the skin of the upper extremity, on the dorsal side of the involved hand and on the flexor side of the contralateral forearm. The latter site was used in all our previous studies to reduce the inconvenience for the patient and to enable a comparison between our studies [5, 9, 17, 22, 28, 29].
A vacuum of 300 mm Hg negative pressure was applied with an Atmoforte 350A aspirator pump (ATMOS Medizintechnik, Lenzkirch, Germany), which was reduced after 15 minutes to 250 mm Hg and again, 15 minutes later, reduced to 200 mm Hg. This negative pressure was maintained until blisters containing sufficient fluid had been developed, but not longer than 2.5 hours. The contents of the blisters were punctured and pooled from each side into a 1.5 ml Eppendorff conical polypropylene tube and centrifuged for 5 minutes at 1600 xg. The mean recovery of supernatants from control blisters was 144 ± 17 (± S.E.M.) µl fluid, and 106 ± 14 µl blister fluid from the CRPS side. All samples were stored in 1 ml conical polypropylene tubes at −80 °C until analysis.

Measurements of cytokines
In order to determine the contribution of pro-inflammatory cytokines to the disease activity, both IL-6 and TNF-α were determined [9, 17]. Blister fluid samples were diluted 1:4 in appropriate calibrator diluent assay buffer for the direct measurement of cytokines. Cytokine assays were performed following the manufacturer's protocol (Pelikine™ human ELISA compact kits for IL-6 (cat. no. M1906) and TNF-α (cat. no. M1920), Sanquin, Amsterdam, The Netherlands). The standard curve ranges and mean calculated zero signal plus 3 SD for IL-6 were 0-450 pg/ml and 0.2 pg/ml, respectively; and for TNF-α 0-1000 pg/ml and 1 pg/ml, respectively. The requested

solutions were provided with the ELISA compact kits and additional tool kits (Pelikine-ToolTM set (cat. no. M1980), Sanquin, Amsterdam, The Netherlands). Following the manufacturer's step-by-step instructions, at the end of the procedure the absorbance per well was measured at 450 nm with a Medgenix ELISA reader. Sample concentrations were calculated using the appropriate standard calibration lines and the Softmax® software of the reader.

Measurement of endothelin-1

ET-1 concentrations were determined in paired blister fluid samples which were available for 22 patients. Blister fluids were diluted 1:2 in appropriate diluent assay buffer. An ELISA-based commercial assay kit was purchased from R&D Systems (cat. no. QET00, Abingdon, UK). The QuantiGlo ET-1 chemiluminescent immunoassay is a solid phase ELISA designed to measure ET-1 levels in human fluids without extraction procedures. It contains synthetic human ET-1 and antibodies raised against the synthetic factor, which has been shown to accurately quantitate human ET-1. A microplate luminometer was used to measure the intensity of light emitted in RLUs (relative light units). The standard curve ranged from 0-1000 pg/ml. Raw data were transferred to a personal computer in which a log concentration–logit RLU equation was performed after which sample concentrations were calculated. The minimal detectable concentration was 0.16 pg/ml.

Measurement of nitric oxide

Sufficient remaining sample volume was available from 17 paired blister fluid samples to perform the assay procedure for the quantitative determination of nitrate and nitrite concentrations. Samples were diluted 1:1 in appropriate diluent assay buffer. A complete assay kit was obtained from R&D Systems (cat. no. DE1500, Abingdon, UK). This assay determines NO based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by a colorimetric detection of nitrite as an azo dye product of the Griess reaction. At the end of procedure the chromophoric azo derivative which absorbs light at 540 nm is detected in a Medgenix ELISA reader, after which sample concentrations are calculated using the appropriate standard calibration line by means of the Softmax® software of the reader. The standard curve ranges for nitrite and nitrate were respectively 0-200 and 0-100 nmol/ml and the minimal detectable concentrations were respectively 0.22 and 0.54 nmol/ml. Sample concentrations were expressed as total NOx in nmol/ml.

Statistical analysis

The Wilcoxon signed ranks test was used for comparisons between measurements in blister fluid obtained from the CRPS and the contralateral extremity. Correlation coefficients between different parameters were determined by the Spearman's test for untransformed data. SPSS (version 14) for Windows was used and the significance level was set at $p < 0.05$.

Results

Blister fluid.
Pro-inflammatory cytokines IL-6 and TNF-α and vasoactive ET-1 and NOx were determined in blister fluid obtained from the involved CRPS limb and the contralateral limb.
In Figures 1 and 2 the distributions of blister concentrations have been plotted as a correlation between CRPS samples (Y-axis) and contralateral samples (X-axis). Successively (Fig. 1a) IL-6, (Fig. 1b) TNF-α , (Fig. 2a) ET-1 and (Fig. 2b) NOx are displayed. In each plot the straight line indicates per definition a CRPS to contra lateral ratio of 1.00 in case no difference was observed. Both in the IL-6, TNF-α and ET-1 distribution plots, the main number of data-points are located in the upper-left triangle, indicating a CRPS to contralateral ratio above 1.00. On the contrary, in the NOx distribution plot, the main number of data points are located in the lower-right triangle, indicating a CRPS to contralateral ratio less than 1.00.
Box plots of these data, indicating the median, 25-75% interval and the ranges of values, excluding outliers are presented in Figures 1c and 2c.
The Wilcoxon signed ranks test was used to assign differences in paired samples (blister fluid obtained from the CRPS and the contralateral extremity in the same patient). Increments in the paired sample tests of IL-6, TNF-α and ET-1 were significant (p-values of 0.001, 0.003 and 0.002, respectively), whereas NOx in paired samples was significantly decreased (p-value 0.044).
Spearman's correlation coefficients between blister fluid parameters were determined. Significant correlations between IL-6, TNF-α and ET-1 were found (0.79, 0.44 and 0.67 for IL-6 / TNF-α , IL-6 / ET-1 and TNF-α / ET-1, respectively and p-values of $p < 0.001$, $p = 0.039$ and $p = 0.001$, respectively).

Discussion

This is the first study to investigate levels of ET-1 and NOx in blister fluids obtained from CRPS-involved extremities. Apart from a study comparing ET-1 with disease severity in the skin of psoriatic patients [30], only Eisenberg et al. have investigated the possible contribution of ET-1 in CRPS [31]. In that study, ET-1 concentrations were determined in venous plasma from the contralateral limb of CRPS patients and did not differ significantly from values found in patients with other painful conditions and in healthy controls. The ET-1 levels found in controls and patients (mean 2.7 and 3.2 pg/ml) were, however, much higher than those reported in the literature [32, 33]. Eisenberg et al. concluded that ET-1 in plasma can not be regarded as a laboratory marker for CRPS. The mean disease duration of their CRPS patients was 2 years, with a study population of relatively young patients (mean 30 years) and a male/female ratio of 2:1 [31]. These characteristics were not comparable with our study, in which age, duration of CRPS and the male/female ratio were much more comparable with demographic characteristics commonly found in the literature. In general, the main peak in the development of CRPS is seen at the age of 52 years and the male/female ratio is between 1:3 and 1:4.
Kuryliszyn-Moskal et al. found synovial ET-1 levels of 15.5 pg/ml [34] and similar serum levels [35]. Although the ET-1 levels we found in blister fluid (3.2 pg/ml) might be diminished due to interstitial dilution, we believe that interstitial levels better reflect local processes in CRPS than plasma measurements. This is confirmed by the finding of high ET-1 concentrations in blister fluids at wound regions of burn patients [36].

The role of NO in CRPS has not yet been investigated, although increased NO production from interferon-γ stimulated peripheral blood monocytes obtained from CRPS patients has been observed in patients with apparently active disease [37]. We found an inverse relationship between ET-1 and NOx in blister samples. This has also been observed in vascular homeostasis where endothelium dysfunction plays a prominent role [38]. In venous plasma of patients with erectile dysfunction, significantly increased ET-1 and decreased NO levels were found [32]. In pathological situations circulating pro-inflammatory cytokines induce the expression of iNOS and ET-1 in smooth muscle cells, also downregulating endothelial eNOS expression, resulting in an elevated ET-1 production and a decrease of NO [18, 39]. This was observed in our study: a significant increase of IL-6 and TNF-α, which causes a marked increase of ET-1 and a slight but significant decrease of NOx. These results confirm the findings of Munnikes et al. who studied patients with an intermediate duration of CRPS (median 20 months) and also found a significant elevation of IL-6 and TNF-α in the involved extremity compared with the uninvolved extremity [29]. That group consisted of 25 patients who had significantly improved on volume difference, AROM, VAS and McGill Pain Questionnaire, which makes their group comparable to our patients with a Impairment Sum Score [40] of 35.

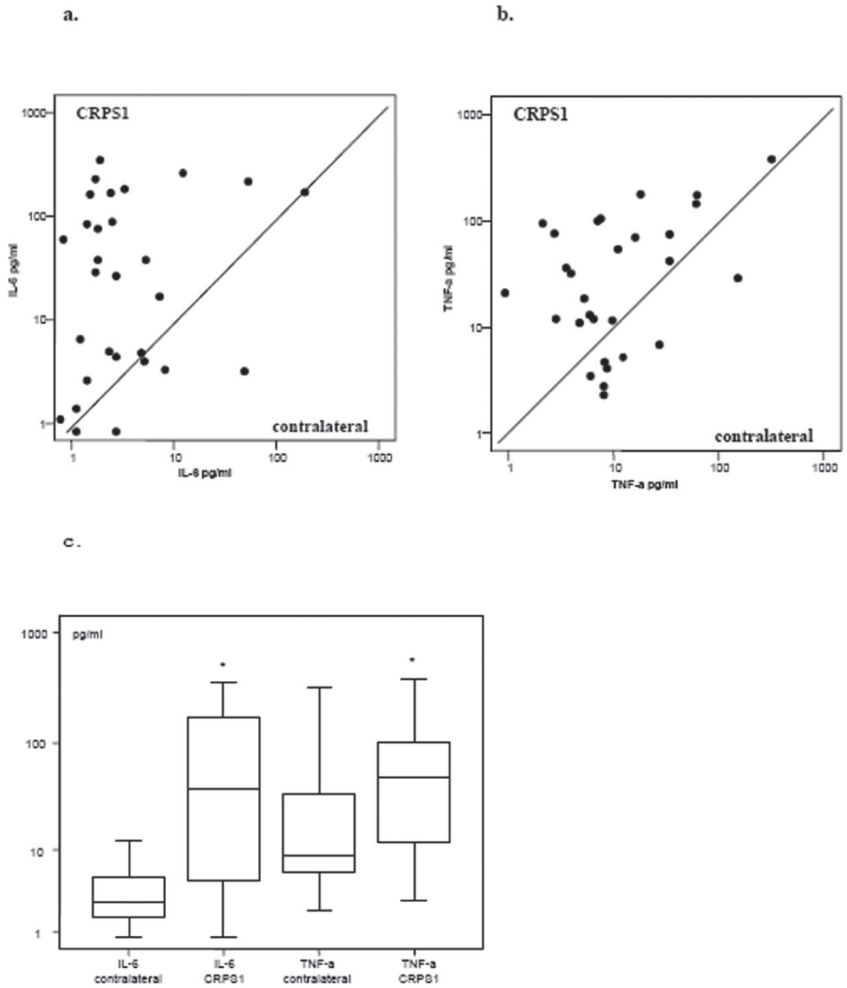

Figure 1: Distribution of IL-6 and TNF-α
Distribution of a) IL-6 data and b) TNF-α data from 29 CRPS patients, from both the involved and the contralateral extremity. In each plot, the straight line indicates per definition a CRPS to contralateral ratio of 1.00 in case no difference was observed.
Values are plotted on logarithmic scales.
c) Box plots of these data, indicating the median, 25-75% interval and the ranges of values, excluding outliers. The Wilcoxon signed ranks test was used to assign differences in paired samples (blister fluid obtained from the CRPS and the contralateral extremity in the same patient).

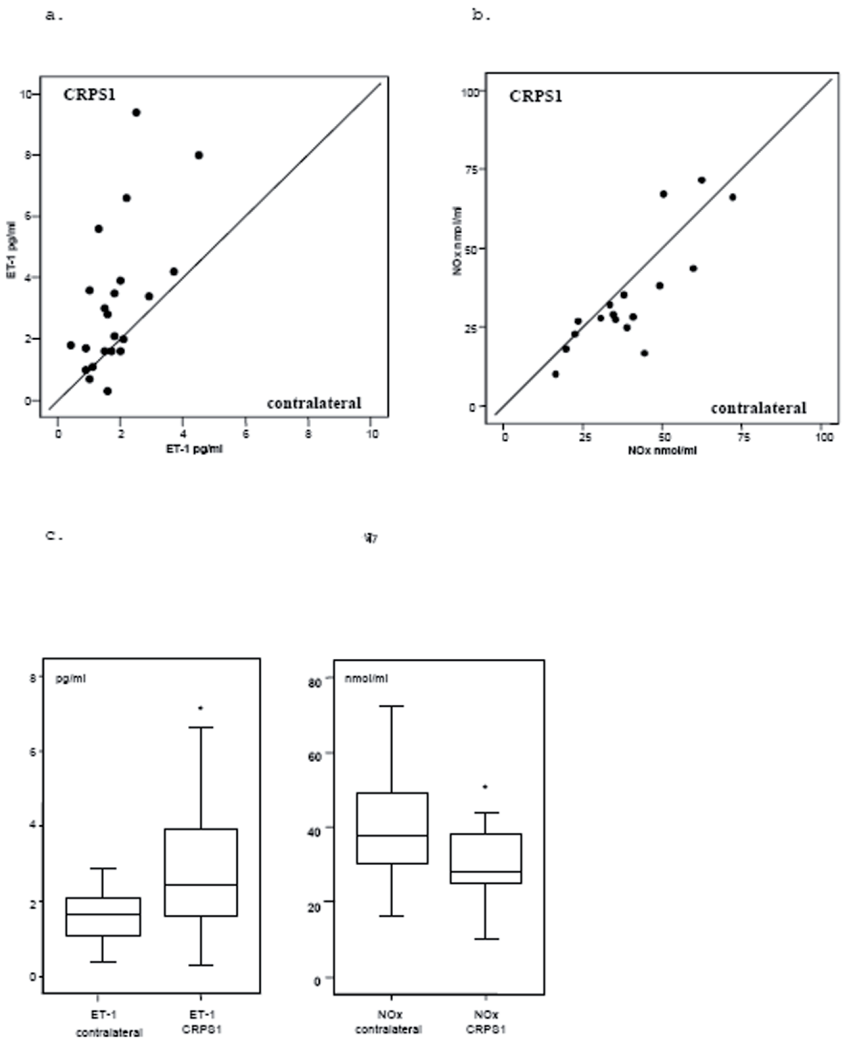

Figure 2: Distribution of Endothelin 1 and Nitric Oxide
Distribution of a) ET-1 data from 22 CRPS patients and b) NOx data from 17 CRPS patients. In each plot, the straight line indicates per definition a CRPS to contralateral ratio of 1.00 in case no difference was observed. Values are plotted on linear scales.
c) Box plots of these data, indicating the median, 25-75% interval and the ranges of values, excluding outliers. The Wilcoxon signed ranks test was used to assign differences in paired samples (blister fluid obtained from the CRPS and the contralateral extremity in the same patient).

Thus there is increasing evidence for the role of proinflammatory cytokines in the initiation and development of CRPS. A longitudinal study is needed that follows selected CRPS patients from the acute to the chronic stage to determine the precise involvement of inflammatory cytokines in the disease activity.

Disuse during chronic CRPS is a common phenomenon. Vasoconstriction is usually followed by local reductions in blood flow [11, 12]. It is unclear what the effect of disuse on the production of ET-1 and NO could be, but the plasma NOx concentration in healthy young humans significantly increases after exercise, with a significant decrease of ET-1 [41]. In healthy subjects, the physiological role of ET-1 in nonworking muscles during exercise has been described [42]. In healthy women, the ET-1 plasma concentration increases with age, which could be significantly counteracted after exercise [43]. Animal studies have shown that endogenous ET-1 participates in the sustained development of the disease in the redistribution of tissue blood flow [44].

In a previous study we observed that treatment with anti-TNF-α initiates recovery during the inflammatory stage of CRPS [17]. The effect of this intervention on the release of ET-1 and NOx is still unclear. Assuming a diminished blood flow, inhibition of the NO synthase is not advisable; on the contrary, NO donors should be supplemented [45]. Besides the smooth muscle constrictive effects of ET-1, hyperalgesia and pain could also be the result of ET receptor stimulation. Therefore, specific ET_A receptor antagonists (such as atrasentan) could provide remission [46]. In pulmonary arterial hypertension, after treatment with the ET receptor antagonist bosentan, the suppression of NO synthesis was abolished and reversed to normal values of controls [47].

During the acute stage of CRPS large amounts of NO will be formed through activation of the inducible NO synthetase (iNOS). In that stage, the blood distribution has been increased which causes an increase of local skin temperature. In the endothelial cell NO will be formed from L-arginine through eNOS activation. Formation of NO could also occur after receptor stimulated activation of constitutive NOS (cNOS) or activation of neuronal NOS (nNOS) in nerve endings. In all cases this will result in increased vasodilation [48]. TNF-α counteracts the activation of eNOS [19], whereas induction of iNOS in smooth muscle cells will be stimulated to generate NO [38]. In the trophic phase of CRPS there will be a decline in the contribution of inflammatory mediators. Consequently, in combination with disuse of the extremity, less NO could be generated, resulting in diminished basal relaxation and retarded blood distribution, after which signs of the 'cold dystrophy' will become apparent.

Conclusion

In the vascular system a clear crosstalk exists between the NO and the ET-1 system. It seems that this interrelationship also exists in CRPS. It is obvious that NO functions as a controlling mechanism against ET-1–induced vasoconstriction. The stage of the disease, the acute or inflammatory phase, or the chronic and/or trophic phase, determines the involvement of inflammatory cytokines which could promote ET-1 production, leading to vasoconstriction and consequently to a diminished tissue blood distribution. A longitudinal study following CRPS patients during the course of the disease is needed to investigate the NOx/ET-1 ratio as an indicator of vascular involvement.

Competing interests:
The authors declare that they have no competing interests.

Authors' contributions
JGG carried out the measurements, was responsible for the coordination of the study and wrote the final version of the manuscript. FJPMH participated in the design of the study, performed the patient selection and revised the definitive article. CHA carried out the immunoassays and assisted in writing. SN participated in the measurements the statistical analysis and corrected the final document. FJZ was responsible for the design of the study, wrote the first draft of the manuscript, performed the statistical analysis and supervised the writing process. All authors read and approved the final manuscript.

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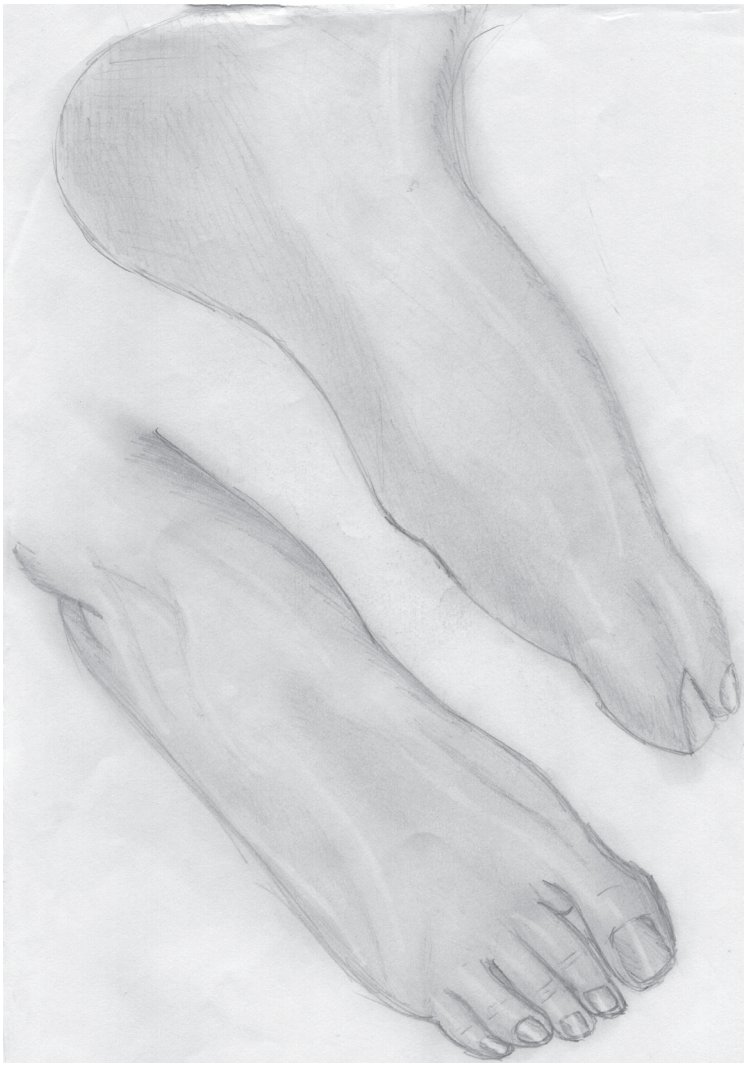

"Although my leg can sometimes feel cold to the touch, to me it's absolutely burning. I can literally feel that my legs are on fire. [um] But if you were to come along and touch them, then they would feel ice cold."

Chapter 5

Vasodilative Effect of Isosorbide Dinitrate Ointment in Complex Regional Pain Syndrome

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Frank J.P.M. Huygen and Freek J. Zijlstra

Abstract

Background:
In complex regional pain syndrome (CRPS) vascular changes occur from the initial, inflammatory event onto the trophic signs during chronicity of the disease, resulting in blood flow disturbances and marked temperature changes. Pharmacotherapeutic treatment is generally inadequate.

Aim:
To determine whether local application of the nitric oxide donor isosorbide dinitrate (ISDN) could cause vasodilation and thereby improve tissue blood distribution in the affected extremity.

Methods:
In a pilot study five female patients with CRPS in one hand were treated with ISDN ointment 4 times daily during 10 weeks. As a primary objective videothermography was used to monitor changes in blood distribution in both the involved and contralateral extremities.

Results:
Patients treated with ISDN showed an increase of 4-6°C in mean skin temperature of the cold CRPS hands, reaching values similar to that of the contralateral extremities within 2-4 weeks time, suggesting normalization of blood distribution. This was confirmed by an improvement in skin colour. In three patients the VAS pain declined, whereas in the other two patients the VAS pain was unchanged over time.

Conclusions:
In this pilot study topical application of ISDN appears to be beneficial to improve symptoms for patients with cold type CRPS, but further study is needed.

Introduction

Complex regional pain syndrome (CRPS) is a painful disorder which usually occurs after an often minor precipitating event or trauma such as a fracture, sprain or after surgery. The main characteristics are continuous pain, marked changes in tissue blood flow and skin surface temperature, edema and sweating, movement disorders and trophic changes of the skin, and the severity of the symptoms is disproportionate to the initial event [1,2]. The diagnosis of CRPS is entirely based upon consensus-derived clinical criteria [3-5]. In the Netherlands, the incidence of CRPS is approximately 2.6% for different fractures, that results in approximately 5100 new patients yearly, of whom a substantial portion will not recover [6,7]. The female to male ratio is approximately 3:1, with a median age of 52.7 years at onset [8]. The hand is affected twice as often as the foot.

Available treatments are limited and insufficient to induce recovery [6]. Analgesics and local anesthetics are used to suppress continuous pain. The long-term prognosis for recovery is poor. Due to pain and severe trophic disturbances, chronic CRPS can even lead to amputation of the dystrophic extremity.

The pathophysiology of CRPS is not unravelled, but growing evidence indicates the involvement of exaggerated inflammatory processes. Both central and peripheral mechanisms have been proposed to play a prominent role [6]. Central sensitization leading to exacerbations of pain is thought to be the result of neuroimmune activation of cells in the peripheral nervous system [9]. During the neurogenic inflammation neuropeptides [10], cytokines, and other mediators are released [11]. This leads to a so-called 'warm dystrophy' with signs of inflammation such as redness, increased skin temperature, loss of function and pain [5,6,12].

During the chronic, disabling stage of the disease signs of extravasation and edema change into atrophy; regional blood flow declines and increased skin temperature changes into diminished temperature [13,14]. These findings point to impaired microcirculation that affects nutritive blood flow in superficial and deep tissues [15,16]. The microcirculation is regulated by neural and endothelial factors and the contribution of the latter could be crucial. Recently we have shown that in patients with an intermediate type of CRPS the endothelin-1 (ET-1) levels in skin blister fluid were increased in affected extremities, whereas the nitric oxide (NO) levels, measured as $NO_2 + NO_3$ (NOx), were decreased [17-19]. As a consequence the ET-1 related vasoconstriction will be overexpressed in comparison with the diminished vasodilative activity of NO.

This pilot study investigates the effects of topical application of the NO donor isosorbide dinitrate (ISDN) on the tissue blood distribution. This vasodilator might be effective in the re-normalization of the disturbed balance between ET-1 and NOx and the diminished microcirculation, thereby reducing tissue acidosis and the resulting pain.

Materials and methods

Patients

Five female patients with mean age 49.6 ± 7.1 years were selected to be treated in an open label study with ISDN. The mean duration of the disease was 39.8 ± 23.9 months, and all patients were diagnosed as stable cold CRPS.

The Medical Ethics Committee of the Erasmus MC approved the study protocol (MEC 2004-159). The research has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and written informed consent was obtained from all participants. The study was internationally registered with the trial registration number ISRCTN60226869.

The patients received 4 times daily (at 8 AM and 12 NOON and at 4 PM and 8 PM) 1 gram ointment containing 1% (10 mg) ISDN during 10 weeks. The medication was supplied by the local pharmacy department.

Thermographic measurements

Registration of skin surface temperature by means of computer-assisted video thermography is an objective parameter to study the long-term effects of pharmacotherapeutics [20,21]. Changes in skin temperature of the extremities are caused either by local inflammation of the tissue or by changes in blood flow in vessels located just underneath the skin. Besides inflammation, vasodilation and enhanced blood distribution also cause a visible increase in skin temperature. In contrast, a decrease in local skin temperature, as normally observed in chronic CRPS, is directly related to diminished tissue blood distribution [22].

Under normal conditions the degree of thermal asymmetry between opposite sides of the body is very small. Using computerized thermography in healthy persons, the skin temperature difference between both sides of the body is less than 1% or <0.25°C [21,23,24]. Therefore, in the present study a difference in matched regions of >1°C was considered to be a significant disease-related effect.

The skin temperature of both hands was registered with a computer-assisted infrared thermographic camera (ThermaCAM SC2000, Flir Systems, Berchem, Belgium) following a standard protocol [19,20]. Thermographic images of the CRPS hand and the contralateral hand were compared and the mean temperature (in °C) and the differences in temperature (maximal minus minimal temperature) of the hand and the fingertips were determined as described before [20,21].

Measurements of pain and muscle force

The patients used a weekly diary to record daily pain (Visual Analogue Scale (VAS), recorded in 0-100 millimetres), co-medication and the occurrence of any adverse events. This pain score was recorded 3 times daily, at 8AM, 12AM and 8 PM.

Pain intensity was assessed with the McGill Pain Questionnaire-Dutch Language Version (MPQ-DLV), measured by counting the total number of words selected [25,26].

The force of the elbow extensor and flexor muscles was measured using a MicroFet 2 dynamometer (Hoggan Health Industries Inc, West Jordan, UT, USA). The elbows were positioned on a table with 45 degrees of flexion and the dynamometer was first placed on the dorsal and then on the ventral side of the distal forearm, just proximal of the processes styloideus ulnae. The patient was asked to resist the movement of the examiner with maximal force, according to the 'break' method [27,28]. The value of three measurements was noted.
The maximal isometric grip force of the hands was measured with a Jamar dynamometer (Sammons Preston Rolyan, Bolingbrook, Il, USA), with the adjustable handle in the second position. The dynamometer was held freely, the forearms rested on a table with both elbows flexed at 90 degrees without touching the trunk. The wrist was held at between 0 and 30 degrees dorsiflexion and between 0 and 15 degrees ulnar deviation. The subjects were asked to exert maximal force to the dynamometer. The value of three trials was noted and the results are expressed as means ± standard deviation [29-32].

Results

A marked and continual increase in skin surface temperature of the CRPS affected hand of more than 4 °C was observed in all patients within two weeks, reflecting improved tissue blood distribution. Although ISDN was applied locally, systemic effects were observed within one week, reflected by a simultaneous increase of temperature in the contralateral side. Thermographic images were taken before and after two weeks of treatment and the calculated mean temperatures and derived data are given in Table 1.

Table 1.
Thermographic data: mean temperature in °Celsius ± SD in 5 patients before treatment, after 2 weeks ISDN and after 10 weeks of treatment

Week	CRPS fingertips[1]	contralateral fingertips	CRPS hand[2]	contralateral hand
0	28 ± 4.5	30 ± 3.8	29 ± 3.7	32 ± 1.9
2	34 ± 1.3	34 ± 1.1	33 ± 1.5	34 ± 1.1
10	33 ± 2.1	31 ± 4.0	33 ± 1.6	31 ± 3.0

[1]The mean fingertip temperature was calculated from 5 fingers

[2]The mean hand temperature was calculated from the whole hand as shown in Figure 1.

Three patients reported headache during the first 2 weeks. Figure 1 shows that the VAS of three patients improved, whereas 2 patients did not report any change in VAS pain. At the end of the 10-week treatment period the mean difference in VAS pain score had decreased from 41 ± 10 to 34 ± 15 mm, and the MPQ was slightly improved from 15 ± 3.4 to 14 ± 6.3 selected words.

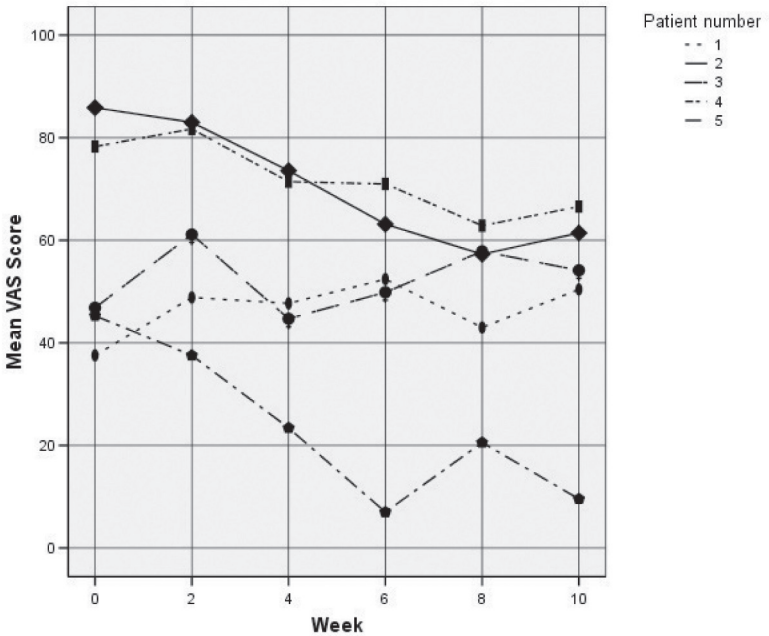

Figure 1:
Visual Analogue Scale (VAS) ranging from 0 to 100 millimetres. The 5 patients registered their pain scores daily at 8 AM, 12 AM. and 8 PM in the week preceding each clinical visit. The figure shows the median pain score before and during 10 weeks of ISDN treatment. In 3 patients the VAS score diminished, but in 2 patients the score remained practically unchanged.

One patient's hand was dystonic without any voluntary movement, this did not change after treatment. The muscle force of the elbow flexors in the remaining 4 patients improved from 63 ± 48 to 103 ± 58 Newton (N), and the extensor muscles from 59 ± 31 to 77 ± 13 N. Although the isometric hand grip force improved in three patients, the mean score in all 4 patients decreased from 87 ± 76 N before treatment to 79 ± 75 N after 10 weeks (difference not significant).

Discussion

This is the first study in which the NO donor ISDN has been used to induce vasodilation in CRPS patients. Although the role of NO in CRPS has not yet been fully elucidated, an in vitro study has shown an increased production of NO from interferon-γ stimulated peripheral blood monocytes obtained from CRPS patients [33]. As indicated above, our previous studies showed an inverse relationship between increased ET-1 and diminished NOx in CRPS compared with contralateral blister samples [17,19]. This inverse relationship has also been observed in vascular homeostasis where endothelium dysfunction plays a prominent role [34]. In male patients with erectile dysfunction, significantly increased venous plasma ET-1 levels and decreased venous NO levels were found [35].

In view of the assumed diminished tissue blood flow, the focus should not be on inhibition of the NO synthase but, on the contrary, NO donors should be supplemented [36].

Transdermal ISDN ointment has been reported to increase the hand vein diameter [37]. The same NO donor has also been successfully used in the treatment of anal fissures [38], chronic painful diabetic neuropathy [39,40] and obstructed hand veins [37].

In the current pilot study we found that local application of ointment improved tissue blood distribution in CRPS. The study does, however, have some limitations. Since only five patients were included, the changes in temperature can be considered to be no more than an indication for the effect of the medication. There was no control group, nor were patients and investigators blinded. To show the effects of a NO donor on the endothelial dysfunction more information is needed on the changes in NO/ET-1 values.

However, an interesting finding was that the VAS scores improved most in those patients reporting "a cold pain deep within". The combined use of descriptive questionnaires like the Neuropathic Pain Scale [41], the McGill Pain Questionnaire and the VAS might reveal more about the nature of these changes. It would also be interesting to investigate whether the changes in muscle force correlate with changes in patient's arm-hand activity level. A larger double-blind randomized controlled trial is needed to address all these questions.

In conclusion, NO seems to function as a controlling mechanism against ET-1–induced vasoconstriction in the chronic, cold stage of CRPS. Prolonged vasodilation induced by NO donors could result in an improved blood distribution as the first step towards remission of this severely invalidating disease.

Acknowledgements

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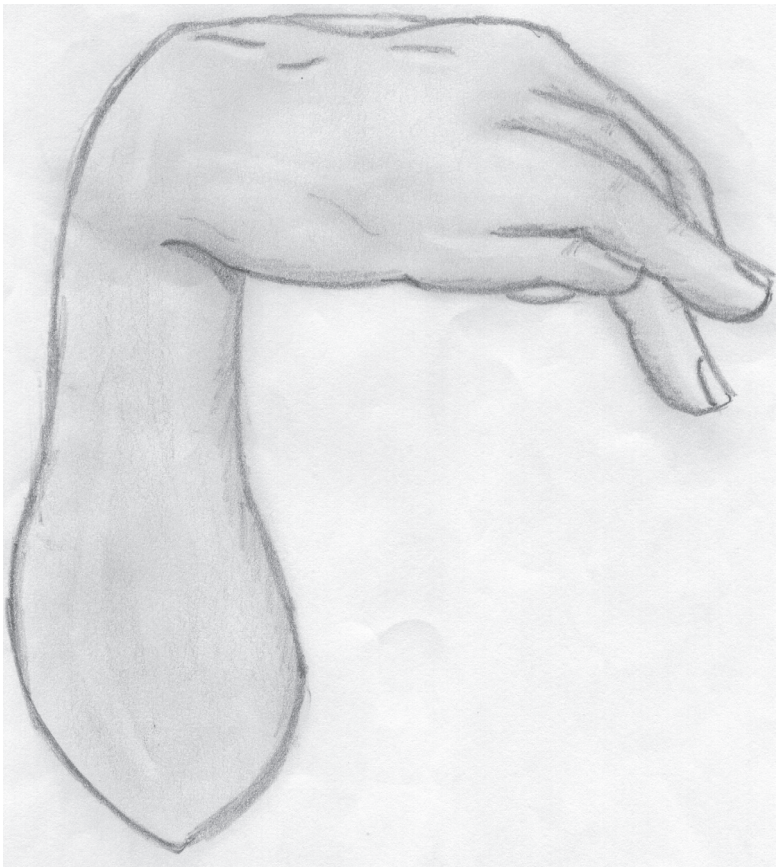

"I feel disgust.

I know it sounds a very strong word to use but I'm disgusted that my arm is this way."

Chapter 6

No Recovery of Cold Complex Regional Pain Syndrome after Transdermal Isosorbide Dinitrate. A Small Controlled Trial.

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Abstract

The microcirculation appears to be impaired in cold chronic Complex Regional Pain Syndrome (CRPS). This double blind randomized controlled trial investigated the effect of the nitric oxide (NO) donor isosorbide dinitrate (ISDN) on the peripheral blood flow in patients with chronic CRPS. Twenty-four patients received 1% ISDN in Vaseline or a placebo ointment applied to the dorsum of the affected hand 4 times daily for 10 weeks. The patients participated in a physical therapy program to improve activity. The primary outcome measure was blood distribution in the affected extremity, which was determined by measuring the skin temperature using videothermography. We also measured NO and endothelin-1 (ET-1) concentrations in blister fluid, pain using the Visual Analogue Scale, and activity limitations using an Upper Limb Activity Monitor (ULAM) and the Disabilities of Arm Shoulder and Hand Questionnaire (DASH). ISDN failed to produce a significant improvement in temperature asymmetry in chronic cold CRPS patients, and it did not result in the expected reduction in pain and increase in activity compared to placebo either. There may be other central or peripheral factors contributing to the disturbed vasodynamics in cold chronic CRPS that are not influenced by NO substitution. This study does not show an improvement of the regional blood distribution by ISDN in the involved extremity of patients with cold-type CRPS.

Introduction

Complex regional pain syndrome (CRPS) is a painful disorder that usually occurs as a complication of surgery or trauma. There are two types. In CRPS type I, the focus of this study, no overt nerve lesion is detectable, whereas in CRPS type II, a nerve lesion is present [1, 2]. Diagnosis is based mainly on consensus-derived clinical criteria [3, 4]. The main characteristics of CRPS are continuous pain, sensory disturbances, marked changes in tissue blood flow and skin surface temperature, edema, sweating, movement disorders, and trophic changes of the skin; the severity of the symptoms is often disproportional to the initial event [5, 6]. Activity limitations are common [7]. The symptoms may be considered an exaggerated local inflammatory response mediated by cytokines [8-11], neurogenic inflammation mediated by neuropeptides [10, 12-14], or both [5]. In the acute stage this leads to a "warm dystrophy" with classic signs of inflammation such as redness, increased skin temperature, edema, loss of function, and pain [15-17].

During the chronic stage of the disease, inflammatory signs are replaced by atrophy, reduced regional blood flow, and, consequently, reduced temperature [18, 19]. These findings indicate impaired microcirculation, which affects temperature and nutritive blood flow in superficial and deep tissues [20-22].

The microcirculation is regulated by neural and endothelial factors [23]. The neural factors were examined by Wasner, who induced whole-body temperature changes to study the sympathetic cutaneous vasoconstrictor activity in CRPS, and identified three vascular regulation patterns: the 'warm', the 'intermediate' and the 'cold' type [24]. It was suggested, that in CRPS unilateral inhibition of sympathetic vasoconstrictor neurons leads to a warmer affected limb in the acute stage, whereas secondary changes in neurovascular transmission would lead to vasoconstriction and cold skin in the chronic stage of the disease [24].

With regard to the endothelial factors, we recently showed that in patients with an intermediate type of CRPS, levels of endothelin-1 (ET-1) are increased in skin blister fluid from the affected extremities, whereas nitric oxide (NO) levels are reduced [25]. As a consequence, ET-1 related vasoconstriction is exaggerated and NO vasodilative activity is suppressed. A NO donor might stimulate NO-related vasodilative function and counteract vasoconstriction by ET-1, thus leading to endothelium-derived vasodilation. In a pilot study with five patients [26], we demonstrated an apparent vasodilative effect of transdermally applied isosorbide dinitrate (ISDN), a NO donor.

The aim of this double blind placebo-controlled randomized clinical trial was to determine whether ISDN ointment improves regional blood distribution in the involved extremity of patients with cold-type CRPS, and if so, whether this improves functioning.

Methods

Study design
This was a double blind randomized placebo-controlled study with 24 patients (12 per group). Patient inclusion took place from June 2005 to December 2006, and the final measurements were obtained in April 2007. Patients were randomized to receive 1% ISDN in Vaseline or a placebo ointment. Three centimeters of ointment, corresponding to approximately 1 g ointment and 10 mg active ingredient, was applied to the dorsum of the affected hand four times daily for 10 weeks. Outcome measures were assessed at the start of the study (start) and after 10 weeks (end).

Patient recruitment
Potential patients were selected from Erasmus Medical Center outpatients, from patients responding to an announcement in the Dutch CRPS Patients Association's magazine and web site, and from patients referred by anesthesiologists at neighboring hospitals. Eligible candidates (n = 195) were invited to visit our outpatient clinic, and FW and FJPMH selected 47 patients with cold CRPS according to the criteria described by Harden and Bruehl [3]. Inclusion criteria were age between 18 and 60 years and CRPS limited to one upper extremity. Patients with cardiovascular or neurovascular disease and patients hypersensitive to nitrates were excluded. Only 30 patients fully complied with these inclusion criteria, of whom 24 agreed to participate in this trial.

Randomization
Randomization was performed by the Erasmus MC pharmacy according to the research policy of the Erasmus MC, using a computerized randomization list. Patients, researchers, and physicians were blind to the intervention administered; only the pharmacist had the allocation code.

Physiotherapy
Patients in both treatment groups received a modified version of a physiotherapy program [27], which consisted of exercises based on a graded activity approach and intended to improve functioning, strength, and mobility of the affected extremity. The patients received one therapy session a week by a local physiotherapist and performed daily exercises at home.

Outcome measures
The primary outcome measure was temperature. The secondary outcome measures were levels of nitric oxide and ET-1 in blister fluid, pain, and level of activity.
Temperature was measured using videothermography. A decrease in local skin temperature, as observed in chronic CRPS, is directly related to diminished tissue blood distribution [28]. Videothermography has been shown to be an effective means to monitor near-surface blood flow in the limbs [29, 30]. A standard protocol was used

to measure the skin temperature of both hands, compare the thermographic images and calculate the mean difference in temperature (°C) [29, 31]. In healthy subjects under normal conditions, the skin temperature difference detected between sides using computerized thermography is less than 1% or 0.25 °C [29, 32, 33].
Artificial blisters were induced using a suction method [34-36], and nitrate and nitrite concentrations in the blister fluid were determined [8, 16, 25, 37, 38]. The sample concentrations were expressed as total NOx (nmol/ml). If sufficient blister fluid remained, ET-1 concentrations were determined (pg/ml).
Pain is often described as the most prominent feature of CRPS [39]. The patients used a diary to record daily pain intensity according to a Visual Analogue Scale (VAS) scale (0-100 mm). The mean of 3 measurements daily during 7 days preceding the hospital visit was used.
To determine possible differences between perceived changes in activity (limitations) and actual activity, the Disabilities of Arm Shoulder and Hand Questionnaire (DASH) and the Upper Limb Activity Monitor (ULAM) were used. The ULAM is based on ambulatory accelerometry and enables long-term objective determination of actually performed upper limb activity in different postures and motions during everyday functioning. It is increasingly used in research involving a variety of patient groups [40, 41], including acute [7] and chronic [42-44] CRPS patients. The signal analysis and its output were described previously [41, 42, 45, 46]. The ULAM was fit to each patient following all measurements in the hospital at the start and end of the study. The patients were instructed to continue their normal activities while wearing the ULAM, with the exception of swimming, bathing, or showering. The following ULAM outcome measures for activity limitations were calculated for a 24-h period: the intensity of activity of the CRPS limb, expressed as the mean motility value (ms^{-2}); the duration of activity of the CRPS limb, expressed as the percentage of time that the activity exceeded predefined motility thresholds; % bimanual, the percentage of time during which both upper limbs were active; and % bimanual/contra, which is the % bimanual divided by the percentage of time during which the contra lateral limb was active. For these ULAM outcome measures, a higher value indicates a higher level of activity during daily functioning.
The DASH was developed to determine perceived changes in activity limitations of the entire upper limb during daily functioning [47, 48]. The DASH Dutch Language Version [49] has been used before in CRPS patients [43]. The total score for 30 items was transformed into one score ranging from 0 to 100, and lower score indicates a higher perceived activity level.

Statistical analysis
Differences in patient characteristics between the treatment groups were analyzed using the Student's t test for independent samples, the Mann Whitney U test, the Chi- square test and Fishers exact test when appropriate. Differences in mean subjective and objective parameters between treatment groups and across time, as well as the

interaction between treatment and time were analyzed using a multivariate repeated-measurements design, with group (ISDN or placebo ointment) and time (start and end) as independent variables.
The multivariate repeated-measurements analysis was used, despite the skewed distribution of the outcome measures, because of the robustness of analysis of variance. Alpha was set at the traditional 0.05 level. Analyses were performed using the Statistical Package for the Social Sciences version 14.02 (SPSS Inc., Chicago, IL, U.S.A.).

Statement of compliance with ethical regulations
The Medical Ethics Committee of the Erasmus MC approved the study protocol (MEC 2004-159). The research was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and written informed consent was obtained from all participants. The trial registration number is ISRCTN60226869.

Results

Twenty-four patients participated; all of them completed the study. The baseline characteristics of the ISDN and placebo groups are shown in table 1. There were no significant differences between the groups, except that the contra lateral hands in the placebo group were significantly warmer. The temperature asymmetry between the CRPS and contra lateral hands at baseline was equivalent for the two groups.

Table 1

Patient characteristics

	ISDN	Placebo	P- value
n	12	12	
Age, years	46.3 ± 8.6	43.5 ± 12.1	P = 0.52 [1]
Gender (female/male)	10/2	10/2	
Duration of CRPS, months	51.5 ± 37.5	45.5 ± 29.6	P = 0.76 [2]
Smoking (yes/ex-smoker/no)	4/1/7	4 /2/6	P = 0.82 [3]
Dominant side affected (yes/no)	5/7	4/8	P = 0.41 [4]
Temperature of CRPS hand	29.1 ± 2.7	30.8 ± 2.6	P = 0.13 [1]
Temperature of contra lateral hand	30.5 ± 2.4	32.3 ± 1.6	P = 0.04 [1*]
Difference in temperature	1.42 ± 1.6	1.56 ± 1.6	P = 0.83 [1]

[1] Independent samples t test; [2] Mann Whitney U test; [3] χ^2 test; [4] Fishers exact test
Values are the mean ± SD unless otherwise indicated.

As shown in table 2, the interaction between treatment and time for temperature asymmetry was not significant.
For the sample as a whole, pain intensity was significantly reduced after treatment, but as table 2 shows, there was no significant improvement in the VAS as a result of ISDN treatment.

Table 2

Temperature difference and pain measurements

	ISDN		Placebo			
	start	end	start	end	P_t	P_{gt}
Temperature difference (°C)	1.51 ± 1.95	0.62 ± 1.25	1.60 ± 1.49	1.23 ± 1.99	0.11	0.44
VAS	45.2 ± 15.7	41.8 ± 22.4	51.7 ± 18.9	43.4 ± 20.7	0.04*	0.38

*P_t = P-value time; P_{gt} = P-value interaction Group*Time*
Values are the mean ± SD.

Although the blistering suction method was applied to all patients, due to a number of reasons (such as too much pain, no blister formation or only small blisters after the maximum suction period), it was not possible to induce blisters with sufficient fluid volume for both measurements on both hands for all patients. Paired samples of nitrate and nitrite concentrations were obtained for 17 patients. Sufficient remaining volume was available from 11 paired blister fluid samples to assay ET-1. In all patients at the baseline there was no significant difference between CRPS and contra lateral NO, (CRPS: 53.4 ± 17.7 pg/ml, contra lateral: 56.6 ± 18.2 pg/ml) or ET-1 concentrations (CRPS: 2.6 ± 1.8 nmol/ml, contra lateral: 2.6 ± 3.1 nmol/ml). Table 3 provides the NO and ET-1 concentrations in the treatment groups. For both groups however, there was a significant increase in the NO concentration over the course of the intervention, but no significant effect of ISDN on the concentration of NO or ET-1.

Table 3

Concentrations of NOx and ET-1 in blister fluid of affected and contra lateral hands

	CRPS		Contra lateral			
	start	end	start	end	P_t	P_{gt}
NO, pg/ml:					0.04*	0.52
ISDN:	54.9 ± 20.6	101.7 ± 92.2	61.3 ± 20.9	110.2 ± 101.5		
Placebo:	51.9 ± 15.3	79.9 ± 48.6	52.8 ± 15.5	65.2 ± 43.5		
ET-1, nmol/ml:					0.32	0.64
ISDN	1.8 ± 1.0	2.1 ± 0.9	2.0 ± 0.8	2.2 ± 0.7		
Placebo	3.2 ± 2.0	3.1 ± 3.1	3.0 ± 4.2	3.4 ± 3.1		

*P_t = P-value time; P_{gt} = P-value interaction Group*Time*
Values are the mean ± SD.

The ULAM outcome measures and the DASH in table 4 show no significant effect of ISDN on activity level.
Some patients in both groups reported strange tickling and itching sensations in the affected hand, an increase of pain during the first few weeks, or a burning sensation. A few patients in the ISDN group complained about headache, which disappeared after the first weeks. There were no severe adverse events.

Table 4

Activity Limitations

	ISDN		Placebo			
	start	end	start	end	P_t	P_{gt}
ULAM:						
Intensity, ms-2	.091 ± .038	.099 ± .044	.076 ± .028	.083 ± .026	0.07	0.85
Duration, %	66.3 ± 13.4	69.0 ± 13.9	59.6 ± 12.5	60.5 ± 11.9	0.39	0.68
% bimanual	60.4 ± 11.6	62.4 ± 12.8	54.9 ± 12.1	52.3 ± 10.4	0.88	0.25
% bimanual/contra	78.7 ± 12.5	81.5 ± 12.4	76.0 ± 7.2	79.1 ± 10.3	0.05	0.93
DASH	54.0 ± 13.2	51.4 ± 20.2	53.5 ± 16.1	50.0 ± 19.4	0.56	0.78

*P_t = P-value time; P_{gt} = P-value interaction Group*Time*

In the ULAM outcome measures duration is expressed as the percentage of time during which the activity exceeded a motility threshold; % bimanual is the percentage of time that activities were performed with both hands and % bimanual/contra is % bimanual divided by the percentage of time that activities were performed with the contra lateral hand. A higher value indicates better performance.

In the Disabilities of Arm Hand Shoulder questionnaire a lower value indicates better performance.

Discussion

This double blind, randomized placebo-controlled study did not show a significant improvement of blood flow in patients with cold chronic CRPS. This may be due to an incorrect hypothesis, due to ineffective medication or compliance of the patients, or it may also be due to the small study, which lacks power to show a real difference, although, in view of the results, the latter seems unlikely.

Although we tried to recruit a homogeneous population, not all patients reacted similarly to the treatment. Most patients responded well to ISDN with a warmer CRPS hand, and fewer periods of deep cold pain, but two patients experienced no improvement at all, with the CRPS side remaining up to 8 °C colder. Lack of compliance might account for these treatment failures, but it seems more likely that other central or peripheral factors contribute to the disturbed vasodynamics in cold chronic CRPS that are not influenced by NO substitution, for example sympathetic dysregulation.

In our previous study [25] we demonstrated an inverse relationship between NOx and ET-1 in patients with the intermediate cold-type CPRS, but in the chronic cold population of our present study, we found no significant differences in NO or ET-1 at baseline. This may indicate a change in the relationship between these mediators in intermediate compared to chronic cold CRPS, thus explaining, why the substitution of NO did not have any beneficial effect on pain.

Although the NO and the ET-1 concentrations were lower than in our previous study [51], the ET-1 concentration was still higher than the concentrations previously reported by others [51, 52]. Apparently some of these chronic cold patients still have active inflammatory components.

For all patients, the mean VAS score was significantly lower after treatment, but there was no significant effect of ISDN. However, most patients reported rapid changes from the moment they began applying the ointment. These changes might have been due to touching and massaging the hand and rubbing in the ointment, as well as to changes in activity elicited by the exercise program. Most patients in the ISDN group indicated that a previous feeling of deep cold pain disappeared steadily.

Results for the ULAM outcome measures intensity and duration of upper limb activity during sitting were comparable to previous findings [44]. In both treatment groups, upper limb activity was clearly reduced compared to healthy subjects [42]. However, there were no significant changes in the upper limb activity, although both treatment groups showed small improvements.

Our patients reported a higher degree of disability on the DASH than a previous study of chronic CRPS [43]; this difference may be due to our specific recruitment of patients with cold chronic CRPS. There was no significant effect of ISDN on the DASH score. The improvements in self-reported limitations of activity are consistent with the small improvements in performed upper limb activity measured using the ULAM. Such changes, although not significant, indicate that a physiotherapy program directed at improving functioning may be effective for patients with cold chronic

CRPS, even for patients who have had the disease for more than 4 years. Since pain-related fear of movement also plays a role in CRPS [53], these small improvements may also have been caused by a reduction in fear-avoidance.
In conclusion, in the present setup ISDN failed to produce a significant improvement in temperature asymmetry in chronic cold CRPS patients. A 10-week treatment with this NO donor did not result in the expected reduction in pain and increase in activity compared to placebo. Future studies investigating the effectivity of ISDN ointment in chronic cold CRPS should focus on patients with endothelial dysfunction and a disturbed NOx/ET-1 ratio. Therefore patients with distinct sympathetic vascular dysregulation should be excluded, which calls for dynamic videothermographic measurements [31], and the studies should also include methods to evaluate the endothelial function, e.g. flow mediated vasodilation.

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"It feels like it's really, really fat. I mean sometimes I actually look at it to, cause I think god my leg's swollen and then I'll look actually look at my leg and I think oh no it's not but it feels like it is."

Chapter 7

Effect of tadalafil on blood flow, pain, and function in chronic cold Complex Regional Pain Syndrome: a randomized controlled trial

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Abstract

Background:
This double-blind, randomized, controlled trial investigated the effect of the phosphodiesterase-5 inhibitor tadalafil on the microcirculation in patients with cold Complex Regional Pain Syndrome (CRPS) in one lower extremity.

Methods:
Twenty-four patients received 20 mg tadalafil or placebo daily for 12 weeks. The patients also participated in a physical therapy program. The primary outcome measure was temperature difference between the CRPS side and the contralateral side, determined by measuring the skin temperature with videothermography. Secondary outcomes were: pain measured on a Visual Analogue Scale, muscle force measured with a MicroFet 2 dynamometer, and level of activity measured with an Activity Monitor (AM) and walking tests.

Results:
At the end of the study period, the temperature asymmetry was not significantly reduced in the tadalafil group compared with the placebo group, but there was a significant and clinically relevant reduction of pain in the tadalafil group. Muscle force improved in both treatment groups and the AM revealed small, non-significant improvements in time spent standing, walking, and the number of short walking periods.

Conclusion:
Tadalafil may be a promising new treatment for patients that have chronic cold CRPS due to endothelial dysfunction, and deserves further investigation.

Trial Registration:
The registration number in the Dutch Trial Register is ISRCTN60226869.

Background

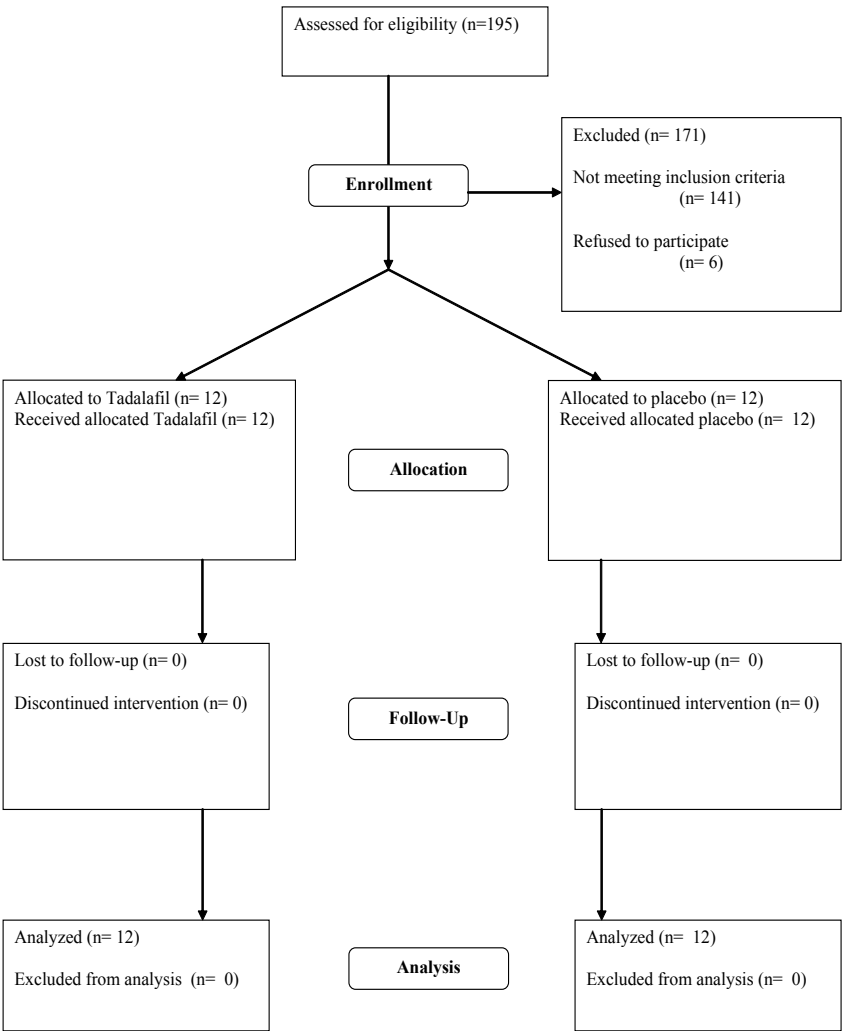
Complex regional pain syndrome (CRPS) is a painful disorder that typically occurs as a complication of surgery or trauma. There are two types of CRPS: in type 1 no overt nerve lesion is detectable, and in type II a nerve lesion is present [1, 2]. Diagnosis is based mainly on consensus-derived clinical criteria [3, 4]. In this study we included only patients with CRPS type 1. The main characteristics of CRPS are continuous pain, sensory disturbances, marked changes in tissue blood flow and skin surface temperature, edema, sweating, movement disorders, and trophic changes of the skin. The severity of the symptoms is often disproportional to the initial event [5, 6].
During the chronic stage of the disease, inflammatory signs give way to atrophy, reduced regional blood flow, and consequently, reduced skin temperature [7, 8]. Impaired microcirculation causes vasoconstriction [9], tissue hypoxia [8], and metabolic tissue acidosis [10, 11] that in turn affect the nutritive blood flow in superficial and deep tissues [6, 12]. A histopathologic study of skin samples in chronic CRPS showed 'numerous abnormal changes in vascular innervation and structure' [13]. The microcirculation is regulated by neural and endothelial factors [14].
The regulatory neural factors were examined by Wasner et al., who induced whole-body temperature changes to study the cutaneous sympathetic vasoconstrictor activity in CRPS. They identified three vascular regulation patterns: the 'warm', the 'intermediate', and the 'cold', distinguished by skin temperature and by the difference in perfusion values between the affected and contralateral limbs [15]. This asymmetry in blood flow and temperature was dynamic and most prominent at a medium to high level of vasoconstrictor activity. It was suggested that in CRPS, unilateral inhibition of sympathetic vasoconstrictor neurons led to a warmer affected limb in the acute stage, but secondary changes in neurovascular transmission, namely supersensitivity to circulating catecholamines and the increase of alpha-1 adrenoceptors, would lead to vasoconstriction and cold skin in the chronic stage of the disease [15].
Schattschneider et al. investigated the abilities of acetylcholine and sodium nitroprusside to induce endothelium-dependent and endothelium-independent vasodilation, respectively, in patients with CRPS. They concluded that endothelial function was impaired in chronic cold CRPS [14].
The endothelium modulates vascular tone by releasing endothelium-derived vasodilators including nitric oxide (NO), prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor. In addition, a number of biochemical and physical stimuli cause the release of vaso-constrictors, including endothelin-1 (ET-1) and angiotensin II [16]. Within the vascular smooth muscle cell, NO activates a soluble guanylyl cyclase that elevates the intracellular concentration of cyclic guanosine monophosphate (cGMP). Cyclic GMP in turn activates a specific protein kinase that phosphorylates proteins and ion channels; this results in the opening of potassium channels and hyperpolarization of the muscle cell membrane, sequestration of intracellular calcium by the endoplasmic reticulum, and blocking of calcium influx by the inhibition of calcium

channels. The consequence is a drop in cytosolic calcium concentrations and relaxation of the smooth muscle that causes vasodilation [17].
Cyclic GMP is hydrolyzed to GMP by phosphodiesterase type 5 (PDE-5). The inhibition of PDE-5 leads to an increase in the intracellular cGMP concentration. The PDE-5-inhibitor tadalafil is an effective treatment for erectile dysfunction (ED) [17], and has also been described in the flow-mediated dilation of the brachial artery [18], the nail fold capillary bed of patients with ED [19], and in pulmonary arterial hypertension [20].
The aim of this double-blind, placebo-controlled, randomized clinical trial was to determine whether the PDE-5 inhibitor tadalafil could improve regional blood flow in the involved extremity of patients with cold type CRPS, and whether this would reduce pain and improve function.

Methods

Study subjects, design, and protocol
This double-blind, randomized, placebo-controlled study included 24 patients with cold CRPS. Patient inclusion took place from June 2005 to June 2007 and the data set was completed in September 2007. Potential patients were selected from outpatients of Erasmus Medical Center (MC), from patients that responded to announcements in the Dutch CRPS Patients Association magazine and website, and from patients referred by anesthesiologists at neighboring hospitals. Eligible candidates were invited to visit our outpatient clinic and the pain clinicians FW, FJPMH, and MVM selected patients with stable cold CRPS according to the criteria described by Harden and Bruehl [3]. Additional inclusion criteria were: age between 18 and 60 years old and CRPS limited to one lower extremity. Patients had to be able to stand on the affected leg and walk at least a few steps. Patients with cardiovascular or neurovascular diseases and patients that were hypersensitive to nitrates were excluded.
The sample size calculation was based on the use of the MANOVA repeated measures design on the primary outcome measure temperature difference. An effect size of 0.6 was used, an α of 0.05, and a power of 0.8 (1-β). The total sample size computed by this method was 24 (12 in each group).
Randomization was performed by the Erasmus MC pharmacy according to the research policy of the Erasmus MC, using a computerized randomization list. Patients, researchers, and physicians were blind to the intervention administered; only the pharmacist had the allocation code.
Patients were randomized (12 per group) to receive either tadalafil or a placebo over a 12-week period. Patients in the tadalafil group started with 10 mg daily during 4 weeks and then took 20 mg daily for another 8 weeks. This titration schedule was advised in order to avoid tolerance effects. Patients in the placebo group also followed this schedule of titration. Because, in the Netherlands, the use of Tadalafil has not yet been approved for CRPS, the study medication was stopped after the study period

Figure 1: Flowchart Tadalafil study



and the patients were seen by FJPMH to discuss further conventional treatment. All patients participated in a modified version of the physiotherapy program described by Kemler et al. [20], which is based on a graded activity approach intended to improve function, strength, and mobility of the affected extremity. The patients performed daily exercises at home, which was instructed and supervised by a local physiotherapist during one therapy session per week. The therapists received written instructions, filled in compliance reports and received feedback by telephone and email by the first author.

Outcome measures

To determine the effects of tadalafil on the microcirculation, the primary outcome measure was the temperature difference between the CRPS affected and the contra lateral limbs. The secondary outcome measures were pain, muscle force, and activity. Outcome measures were assessed at the start and end of the study.

Temperature was measured using video thermography. In chronic cold CRPS, a reduction in local skin temperature is directly related to diminished tissue blood distribution [21]. Video thermography was shown to be an effective method for monitoring near-surface blood flow in the limbs [22, 23]. The skin temperatures of both feet were registered with a computer-assisted infrared thermograph (ThermaCAM SC2000, Flir Systems, Berchem, Belgium) following a standard protocol [24]. To compare thermographic images of the feet, the means and difference in temperature (°C) were calculated as described previously [22]. Under normal conditions, the thermal asymmetry between opposite sides of the body is very small; in healthy subjects, the degree of thermal asymmetry was less than 1% (< 0.25°C) when measured with computerized thermography [22, 25, 26].

Pain is often described as the most prominent feature of CRPS [27]. The patients were instructed to record daily pain intensity, concurrent medication, and adverse events in a diary. The pain intensity was rated according to a Visual Analogue Scale (VAS) scale (0-100 mm). The actual pain score was recorded three times daily (0800, 1200 and 2000 h) during the week before the first and last hospital visits; the average scores for each of those weeks were used in the analyses.

Function was evaluated with muscle force tests, walking capacity tests, and measurements of performance. Muscle force of the flexors and extensors of the knee and foot was measured using a MicroFet 2 dynamometer (Hoggan Health Industries Inc, West Jordan, UT, USA) as described by Bohannon and Andrews [28-30], using the 'break' method [28]. The mean value of three measurements was calculated and given in units of Newtons (N).

A ten meter walking test and a two minute walking test were performed to determine the walking ability [31-33]. The first test was performed three times, at both comfortable and maximum walking speeds, and timed with a stopwatch. The two minute walking test measured the non-stop walking distance in meters.

To determine possible differences between perceived and actual changes in activity levels, patients wore an Activity Monitor (AM) and answered a questionnaire. The AM was used to measure actually performed physical activity [34, 35]. The device is based on ambulatory accelerometry and enables objective determination of activity in different postures (lying, sitting and standing) and motions (walking and general movement) during everyday functions. It is increasingly used in research involving a variety of patient groups, including acute [36] and chronic [37, 38] CRPS patients. The signal analysis and output were described previously [34, 35, 39].

After all other measurements were completed, the AM was fitted on each patient to measure activity over a 24-h period in the hospital at the start and the end of the study.

The patients were instructed to continue their normal everyday activities while wearing the AM with the exception of swimming, bathing, or showering. The following AM outcome measures were calculated: the percentage of time spent standing, walking, or in an upright position, and the number of short walking periods (<10 s). In these measures a higher value represented better performance.
Perceived activity limitations were measured with a questionnaire developed by the Dutch Measuring Mobility Study Group that consisted of 35 questions [40]. In the questionnaire a lower value represented better performance.
Blood pressure and pulse frequency were also recorded at every visit.

Statistical analysis
Differences in patient characteristics between the treatment groups were analyzed with the Mann-Whitney U test, Fisher's Exact test, and the chi square test, as appropriate.
A multivariate repeated-measures design was used to analyze differences in mean parameters between the treatment groups, and over time (between the starting and ending values), with group (tadalafil or placebo) and time (start and end) as independent variables. In addition, we analyzed the interaction between treatment and time. This method of analysis was used despite the skewed distribution of the outcome measures, due to the robustness of the analysis of variance [41]. Alpha was set to the conventional level of 0.05. Analyses were performed with the Statistical Package for the Social Sciences, version 14.02 (SPSS Inc., Chicago, IL, U.S.A).

Statement of compliance with ethical regulations
The Medical Ethics Committee of the Erasmus MC approved the study protocol (MEC 2004-159). The research was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and written informed consent was obtained from all participants.
The registration number in the Dutch Trial Register is ISRCTN60226869.

Results
As the flowchart in figure 1 shows, twenty-four patients participated and completed the study. The baseline characteristics of the patients assigned to the tadalafil and placebo groups are shown in Table 1. There were no significant differences in age, gender, duration of disease, or smoking habit between the two experimental groups.

Table 1

Patient characteristics

	tadalafil	placebo	P-value
n	12	12	
Age, years	39.8 ± 13.1	36.5 ± 10.6	0.56 [1]
Gender (female/male)	9/3	11/1	0.29 [2]
Duration of CRPS, months	37.1 ± 24.5	55.7 ± 52.3	0.60 [1]
Smoking (yes/ex-smoker/no)	6/1/5	4 /2/6	0.66 [3]
Temperature of CRPS foot, °C	26.8 ± 2.8	28.3 ± 4.7	0.60 [1]
Temperature of contra lateral foot, °C	28.5 ± 2.8	29.9 ± 3.3	0.27 [1]
Difference in temperature, °C	1.7 ± 0.77	1.7 ± 2.6	0.41 [1]

[1] Mann Whitney U test; [2] Fisher's Exact test; [3] X^2 test

Values are the mean ± SD unless otherwise indicated

The results of the videothermographic measurements are given in Table 2. Although the temperature difference appeared to be reduced in the tadalafil group and increased in the placebo group, the probability value was only 0.37; thus we cannot exclude that these effects were due to sampling error. However, there was a significant reduction in pain intensity after tadalafil treatment, as indicated by a 15% reduction of the VAS (from 61.3 to 52.3) in the tadalafil group. In contrast, the pain intensity in the placebo group remained unchanged. Since the outcome data were only assessed at the start and end of the study, we cannot provide information on the time course of these changes. We do know, however, from the remarks of the patients, that most changes in temperature and pain appeared to take place after 4 weeks, after the medication had been doubled from 10 to 20 mg daily.

Table 2

Temperature and Pain

The difference in temperature (°C) between the two feet, measured with video thermography. The Visual Analogue Scale (VAS) was used to measure pain intensity three times daily during the week preceding the hospital visits. The start and end times were when treatment started at t=0 and ended at t=12 weeks.

	tadalafil start	end	placebo start	end	P_t	P_{gt}
Temperature difference (in °C)	1.71 ± 0.77	1.09 ± 1.6	1.65 ± 2.6	1.77 ± 1.9	0.54	0.37
Pain intensity VAS (0-100mm)	61.3 ± 14.1	52.3 ± 19.1	57.0 ± 12.1	56.5 ± 10.8	0.03*	0.04*

*Pt = P-value for differences over time; Pgt = P-value for interaction Group*Time*

Table 3 shows the difference in muscle strength between the contralateral and CRPS affected sides. We observed significant improvement over time in both groups for knee extension, but we failed to find significant differences between treatment groups.

Table 3

Differences in muscle strength

Results of the muscle force tests measured with the MicroFet 2 Dynamometer. Values indicate the differences in muscle strength between the CRPS affected and contralateral sides. All values are given in units of Newtons (percent difference between the two sides). The start and end times were when treatment started at t=0 and ended at t=12 weeks.

	tadalafil start	end	placebo start	end	P_t	P_{gt}
Knee *extension*	64.2N (64.8%)	56.7N (70.3%)	97.7N (45.2%)	80.3N (58.9%)	0.01*	0.05
Knee *flexion*	52.0N (66.1%)	45.2N (70.7%)	70.4N (52.9%)	43.5N (71.8%)	0.12	0.08
Foot *dorsal flexion*	79.6N (53.6%)	84.3N (51.0%)	102.5N (39.5%)	81.4N (52.1%)	0.40	0.28
Foot *plantar flexion*	93.0N (56.4%)	100.2N (57.0%)	134.6N (42.4%)	76.1N (64.1%)	0.05	0.34

*Pt = P-value for differences over time; Pgt = P-value for interaction Group*Time*

Table 4 shows the outcome measures for the actually performed activity. The results of the Activity Monitor indicated that the intervention did not significantly affect patient activity levels. This result was corroborated by the lack of changes detected in either the ten meter walking tests or the questionnaire (Table 5). However, there was a significant change over time in both treatment groups in the two minute walking test, though the difference between groups was insignificant.

Systolic blood pressure was 134 (±10) mmHg at the start in the placebo group, and 130 (±16) mmHg at the end of the trial. In the tadalafil group the initial systolic blood pressure was 142 (±18) mmHg and at the end of the study it was 140 (±19) mmHg. There was no significant difference between treatment groups, and no changes were observed in diastolic blood pressure.

Most patients in the tadalafil group reported a warmer affected extremity, sometimes coupled with an itching sensation. Two patients complained about painful muscles in their whole body during the first few weeks of the trial. Several patients in both groups experienced the measurements as very tiresome, and felt exhausted afterwards, however, there were no severe adverse events.

Table 4

Activity limitations determined using the AM

The Activity Monitor measurements were taken over a 24-h period, and are given as mean ± SD. In all these measurements a higher value indicates better performance. The start and end times were when treatment started at t=0 and ended at t=12 weeks.

	tadalafil start	end	placebo start	end	Pt	Pgt
% Time Standing	8.3 ± 2.8	10.7 ± 6.1	10.5 ± 4.3	10.3 ± 3.3	0.25	0.19
% Time Walking	4.5 ± 2.2	5.3 ± 2.8	5.6 ± 2.9	6.5 ± 3.8	0.15	0.88
% Time Upright	12.8 ± 4.5	16.0 ± 8.3	16.1 ± 7.0	16.9 ± 6.9	0.18	0.40
Number of Walking *periods < 10 s*	119.9 ± 50.8	138.7 ± 67.9	141.7 ± 81.8	146.4 ± 58.8	0.40	0.61

*Pt = P-value for differences over time; Pgt = P-value for interaction Group*Time*

Table 5

Walking tests

Results from the walking tests. The patients performed the 10 meter walking test three times at a comfortable speed, and again three times at maximal speed. The mean of the three tests is given± SD. For the two minute walking test the patients had to walk for 2 minutes, and the distance in meters is given. For these three walking tests, a higher value indicates better performance, but for the questionnaire improvement is indicated by a lower value. The start and end times were when treatment started at t=0 and ended at t=12 weeks.

	tadalafil start	end	placebo start	end	Pt	Pgt
10 m, comfortable speed *(time in seconds)*	16.2 ± 13.0	16.3 ± 14.1	15.6 ± 8.2	13.2 ± 5.9	0.36	0.34
10 m, maximal speed *(time in seconds)*	9.8 ± 4.4	9.2 ± 4.5	7.9 ± 1.9	8.6 ± 2.8	0.76	0.06
2 minute walking test *(distance in m)*	106.9 ± 34.9	119.6 ± 46.3	92.5 ± 30.4	111.8 ± 32.6	0.01*	0.49
Walking questionnaire *scores*	27.5 ± 6.9	25.3 ± 10.6	27.6 ± 6.0	28.1 ± 5.7	0.44	0.22

*Pt = P-value for differences over time; Pgt = P-value for interaction Group*Time*

Discussion

Although this double-blind, randomised, placebo-controlled study did not show a significant improvement in blood flow in the extremities as observed by video thermography, we found a significant and clinically relevant reduction of pain in patients with chronic cold CRPS.

It has been repeatedly suggested that the temperature asymmetry between extremities is an important diagnostic measure for CRPS [42, 43]. In this trial, we found a considerable temperature asymmetry in all patients at baseline that was reduced in the tadalafil group, and slightly increased in the placebo group after the treatment period. Although our analysis suggested these treatment effects were insignificant, we recommend caution in interpreting these results because the study had low statistical power as a result of the large variance and relatively low covariance between time points. The observed statistical power of the treatment × time factor was only 0.14 (alpha =0.05). In contrary to the placebo group, most patients in the tadalafil group were very satisfied with the treatment, claiming that the leg was much warmer under almost all circumstances. Therefore, the treatment effects may be clinically relevant. Although the primary indication for PDE-5 inhibitors is erectile dysfunction (ED), prolonged treatment with tadalafil has a vasodilative effect that has also been described for other arteries [18-20]. However, one study in patients with Raynaud's phenomenon (RP) showed that a single dose did not improve digital blood flow at baseline, increased response to heating, or attenuated cold-induced vasoconstriction [44]. However, a review that included several reports found that prolonged treatment with tadalafil was associated with improved microcirculation, symptomatic relief, and ulcer healing in patients with secondary RP [45].

Our results are in accordance with the latter conclusions. We observed a reduction in the temperature asymmetry that was most likely caused by restored microcirculation. The variation in temperature data may be an indication that in some patients other, possibly central, thermo-regulatory mechanisms may have interfered with the peripheral blood flow.

Our patient population is too heavily weighted towards women. The usual ratio is 4:1 [46, 47], and we included 20 women and 4 men. Although it has been suggested, that the influence of hormonal etiological factors may be involved in the pathogeneses of CRPS [47], there are no indications, that these factors still play a role in the vascular alterations in the chronic CRPS.

Pain, which is the most important parameter of CRPS, was significantly reduced compared to placebo. However, in pain studies a 30% increase is usually considered meaningful, and 50% robust. Since we found a mere reduction of 15%, this should be interpreted with care. Pain in CRPS may be due to a neuropathic cause [13, 48], there may be sympathetically maintained pain [49], or it could be caused by local ischemia [8, 50], perhaps even in the same patient. It seems likely, that the present reduction in pain was achieved by an improvement in local ischemia, leaving the other causes

unchanged. On the other hand, an increased physical activity could consequently result in an increased awareness of pain. This needs further investigation.
We also observed a small, but insignificant improvement in muscle force over time. This increase might have resulted from physical therapy and the home exercise program, or it could have been due to a learning effect after repeated muscle force testing. In both groups we observed a slight reduction in the asymmetry in muscle force between the CRPS affected and contralateral sides. The asymmetry was largest in the foot dorsal flexion. However, foot dorsal flexion was also partly influenced by the pain arising from the pressure of the measurement device on the plantar side of the foot; this was the most painful area in most patients. Because these improvements were observed in both groups, muscle force was not apparently influenced by the general pain reduction in the tadalafil group.
During the trial, most patients in the tadalafil group experienced a large improvement in walking ability. Some reported that they were able to leave their crutches at home when walking short distances, even outdoors. However, the results of the walking tests did not reflect this improvement. We found only a small, non-significant progression in the ten meter walking tests. A closer evaluation revealed that some of the improved patients were walking with two crutches at high-speed at the start of the trial, and walked without crutches at the end of the trial, but at the cost of a slower walking speed. Thus, the improvement was not expressed in speed, but in quality of walking. We found significant increases in the two minute walking test in both treatment groups, and an indication of improvement in the walking questionnaire. These findings were supported by the results from the activity monitoring. Although the improvements were insignificant, there were small increases in standing, walking, and the number of short walking periods.
Although we assumed we had recruited a homogeneous study population with chronic cold CRPS, not all patients reacted similarly to the intervention. Most patients responded very well to tadalafil, and reported a warmer extremity with a reduction of pain, but two patients experienced no improvement at all, and the CRPS side remained up to 4 °C colder than the unaffected side. This lack of responce was most likely due to the presence of other central or peripheral factors that contributed to the disturbed vasodynamics in chronic cold CRPS, factors that were not influenced by cGMP stimulation through PDE-5 inhibitors. On the other hand, two patients in the placebo group experienced an almost complete recovery from pain and impairment. This might have been due to the effects of physical therapy, or a combination of the effects of the therapy and extra attention they received while performing tests as a trial participant. In either case, these improvements indicate that a program directed at improving function may be very effective for patients with chronic cold CRPS, even in cases that have lasted more than four years.
This trial was limited to a period of 12 weeks. Little is known about the effects of using PDE-5 inhibitors over a longer period. In patients who experienced reduced pain as a result of tadalafil, a remarkable improvement in function was observed. The use of

crutches was reduced and the number of short walking periods increased. However, the perceived improvements were small, as reported in the walking ability questionnaire. Thus, it may take a longer period of time for patients to acknowledge the extent of improvements. We speculate that over time, tadalafil patients may acquire an activity pattern closer to normal and improvements in function due to the reduction of pain and the subsequent reduction in fear-avoidance, which has been described as one of the limiting factors in CRPS [51].

It has been suggested that CRPS is primarily a disease of the central nervous system [52]. In any case it is a multifaceted disorder, and any effort to treat only one facet is bound to result in failure [53]. However, in this trial we have solely concentrated on the question, whether the inhibition of PDE-5 could improve the blood flow in CRPS. Future investigations should include a larger study group of patients with chronic cold CRPS and an effort should be made to select only patients with endothelial dysfunction. A longer trial period would be required to investigate whether the improvements last. It would also be interesting to investigate whether this newly gained improvement in function, supported by an activity program if necessary, would be sufficient to sustain further progression in activity.

Conclusions

Tadalafil may be a promising new treatment for patients that have cold CRPS due to endothelial dysfunction. The PDE-5 inhibitor produced a clinically relevant reduction in the asymmetry in temperature between the affected and unaffected feet in CRPS, most likely due to the restoration of blood flow in the affected extremity. We also observed a significant reduction of pain compared to placebo, likely due to an increase in vasodilation and the abolishment of ischemia. Future investigations should focus on the long-term effects of tadalafil on improvements in function.

Competing Interests
The authors declare that they have no competing interests.

Authors Contributions
JGG carried out the study, performed measurements and drafted the manuscript, FJPMH assisted in the patient inclusion and helped to draft the manuscript, SPN performed the videothermographic recordings and calculated the temperature values, FW assisted in the measurements and the patient inclusion, JBJB participated in the design of the study and the Activity Monitor measurements, FCS calculated the Activity Monitor data and helped to draft the manuscript, DLS participated in the design of the study and performed the statistical analysis, and FJZ designed and supervised the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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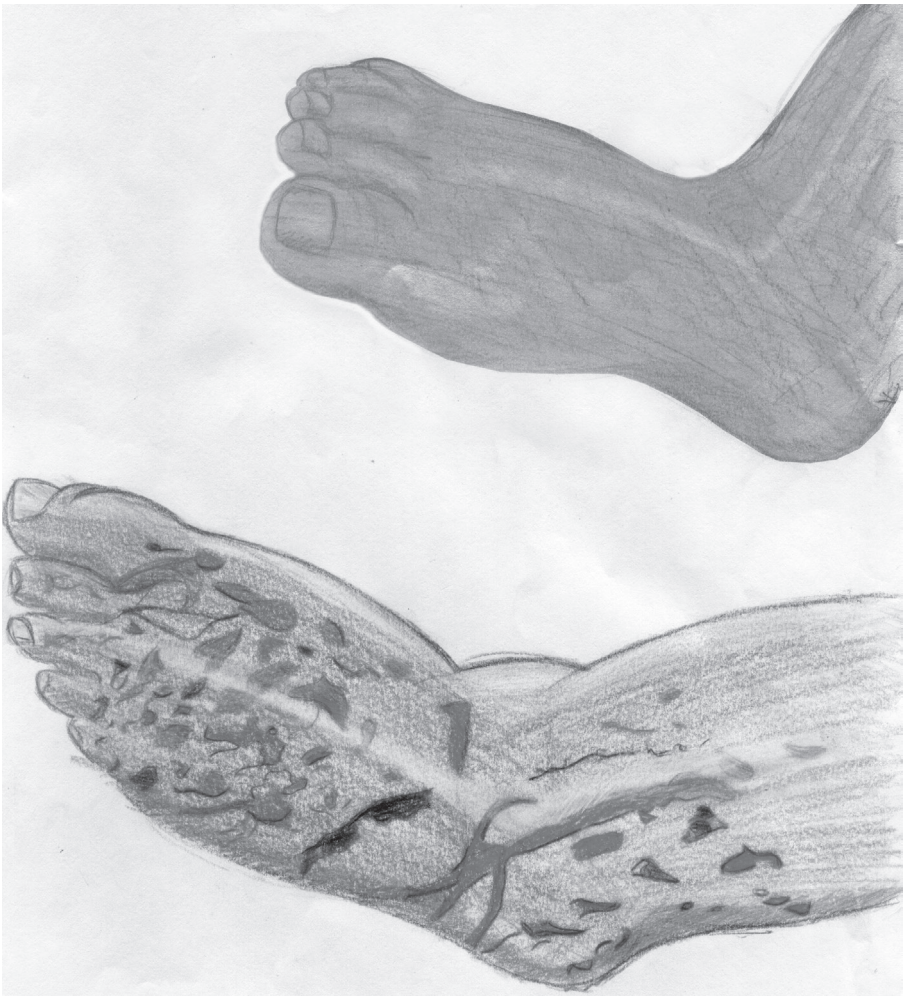

"If I went to move a finger, two or three fingers would move. And, it wasn't like, you know, move that finger and that happened, it was move that finger and, and this went down and you thought, "Well it should be that one." So I felt like my hand wasn't attached to my brain, if you know what I mean. It felt like I really had to concentrate to get that arm going, to get that hand going. And I did have to concentrate. And it was, it was a very hard thing to do."

Chapter 8

Regulation of peripheral blood flow in Complex Regional Pain Syndrome

submitted

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Abstract

During the chronic stage of Complex Regional Pain Syndrome (CRPS), impaired microcirculation is related to increased vasoconstriction, tissue hypoxia, and metabolic tissue acidosis in the affected limb. Several mechanisms may be responsible for the ischemia and pain in chronic cold CPRS. The diminished blood flow may be caused by either sympathetic dysfunction, hypersensitivity to circulating catecholamines, or endothelial dysfunction. The pain may be of neuropathic, inflammatory, nociceptive, or functional nature, or of mixed origin. The origin of the pain should be the basis of the symptomatic therapy.
Since the difference in temperature between both hands fluctuates over time in cold CRPS, when in doubt, the clinician should prioritize the patient's report of a persistent cold extremity over clinical tests that show no difference. Future research should focus on developing easily applied methods for clinical use to differentiate between central and peripheral blood flow regulation disorders in individual patients.

Background

Complex Regional Pain Syndrome (CRPS) is a painful disorder that usually develops as a disproportionate consequence of trauma. The disorder most commonly occurs in the limbs, and is characterized by spontaneous pain, allodynia and mechanical hyperalgesia, abnormal regulation of blood flow and sweating, oedema of skin and subcutaneous tissues, movement disorders, and trophic changes of skin, organs of the skin, and subcutaneous tissues [1, 2].

Growing evidence indicates that CRPS is accompanied by various abnormalities of the microvascular system, including an increase in the number of capillaries [3, 4], endothelial swelling, and changes in the vessel luminal wall [5]. These impressive capillary changes range from severely thickened basal membrane with intimal vacuolization, perivascular edema, and debris from pericytes between the basal membrane layers, to necrosis [6, 7]. Greatly thickened multi-laminated walls are also observed, which considerably reduce the inner diameter of the vessel [4, 8]. Endothelial cells exhibit a shrunken appearance and capillaries with only endothelial cell debris in the lumina have been observed, while other capillaries could be traced by the thickened basal membrane only, lacking the presence of other cellular remnants [7].

In an autopsy study of the affected limbs of two patients, we found an increased number of migrated endothelial cells, as well as an increase of eNOS activity in distal dermis specimens, indicating that endothelial dysfunction may play a role in chronic CRPS [9].

In the CRPS diagnostic criteria [10], a clear distinction is made between two subtypes to reflect the absence or presence of evidence of peripheral nerve injury. However, growing evidence of minor nerve lesions in CRPS [8, 11] indicates that this distinction may be artificial.

Although the debate regarding the pathophysiology is still ongoing, the role of excessive regional inflammation, peripheral sensitization of primary somatosensory afferents, and central sensitization of spinal neurons is becoming clear [1, 2, 12, 13]. Recently, evidence was found for the presence of oxidative stress in CRPS patients since they exhibited increases in salivary and/or serum lipid peroxidation products and antioxidants [14, 15].

The signs and symptoms are related to these mechanisms. Relating the clinical picture to the underlying pathophysiology might help determine the pharmacotherapeutic approach for an individual patient [16].

The clinical picture of CRPS, especially the signs of autonomic dysfunction, and the discovery by Leriche that surgical sympathectomy dramatically improved pain in CRPS supports the important role of the sympathetic nervous system in CPRS etiology [17]. The sympathetic vascular regulatory system in CRPS was extensively examined by Baron et al., who measured differences in blood flow and skin temperature in patients with CRPS after a cold and warm acclimatization period, respectively. The results indicated that differences in skin temperature and blood flow are not static

descriptors, but dynamic values mostly dependent on environmental temperature and likely emotional stress [18]. After sympathectomy, in three out of four patients with cold CRPS, the affected limb was considerably warmer and blood flow was considerably higher compared to the healthy side. After a few weeks, however, skin temperature and perfusion slowly diminished, and the affected hand became cold again. Denervation supersensitivity due to complete sympathectomy was thought to be the underlying mechanism of these alterations [18, 19]. Wasner et al. measured hand temperature in CRPS patients and healthy control groups while changing whole-body thermal stress using a thermal suit [20]. Whole-body cooling appears to be the most effective way to induce massive tonic activation of cutaneous vasoconstrictor neurons [21]. Three distinct vascular regulation patterns were identified, related to the duration of the disorder. Temperature and blood flow differences between the two sides were dynamic and most prominent at a high to medium level of vasoconstrictor activity [20]. As a result, impairments of thermoregulatory responses should be considered only for diagnostic reasons [22, 23].

In the acute phase of CRPS, the affected limb is usually warmer than the contralateral limb due to cutaneous vasodilation, and a functional inhibition of sympathetic vasoconstrictor activity has been shown [20, 24]. In this phase, the thermoregulatory blood flow is increased, but the nutritive skin blood flow is unaltered; these differences may be due to differences in regulatory mechanisms. The smooth muscles in the arterioles of the nutritive capillaries are controlled by local factors, whereas the arterioles in the subpapillary plexus are predominantly sympathetically controlled [25, 26].

After a so-called intermediate phase in which skin blood flow and temperature differences appear to alternate between warm and cold, a large number of patients show a permanent decrease in blood flow and temperature [27] despite the return of sympathetic vasoconstrictor activity with the duration of the disease [24]. This decrease has been attributed to an increased sensitivity to circulating catecholamines, probably due to upregulation of adrenoceptors following the initial period of reduced sympathetic input [20, 25, 28-30]. The decrease in blood flow in the intermediate and cold phase occurs in both the thermoregulatory and the nutritive microcirculation [25].

As demonstrated by simultaneous testing of skin blood flow and sweat in one CRPS patient, vascular abnormalities may not solely be the result of a disturbance of the autonomic nervous system. Although sudomotor activity was greater on the affected side, implying higher sympathetic nerve activity, basal blood flow was also greater on the same side. These results suggest that one or more factors increase basal blood flow despite high sympathetic nerve tone on the affected side [31]. Other mechanisms may also be involved in the pathophysiology of the vascular abnormalities. The demonstrated increase in vasoconstriction, tissue hypoxia, metabolic acidosis [32, 33] and vascular permeability for macromolecules [34] may also indicate endothelial dysfunction.

This paper reviews the central and peripheral mechanisms responsible for pain and vascular abnormalities in CRPS; furthermore, we suggest potential treatment approaches for CPRS.

Discussion

Mediators

The endothelium, the largest organ of the body, releases agents that regulate vasomotor function, trigger inflammatory processes and affect hemostasis in response to shear stress and hormonal stimuli, such as vasoactive substances [35]. Blood pressure and blood flow is regulated by the release of the vasodilators nitric oxide (NO) and prostacyclin (PGI_2), and the vasoconstrictor endothelin (ET) [36].

NO is generated from the amino acid L-arginine by three major isoforms of NO synthase, namely neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) enzymes [37]. nNOS and eNOS are constitutively expressed and activated by calcium entry into the cells; iNOS is calcium independent, and its synthesis is induced in inflammatory and other cell types by stimuli such as endotoxin and proinflammatory cytokines [37]. NO diffuses through the artery wall to the vascular smooth muscle cells in the media, where it increases the activity of guanylate cyclase and the concentration of cyclic guanosin monophosphate (cGMP), thus relaxing the vascular smooth muscle and leading to vasodilation [38]. PGI_2, which only plays a limited role as a vasodilator in most vascular beds, is produced from arachidonic acid by cyclooxygenase in response to shear stress and a number of factors that also increase NO production. PGI_2 activates adenylate cyclase to increase cyclic adenosine monophosphate (cAMP), also leading to vasodilation [39, 40].

The peptide ET, one of the most potent known vasoconstrictors, is generated by cleavage of a large polypeptide within the endothelium. Of the three types of ET, ET-1 is the most important in vascular tissue, and it acts on endothelin-A receptors on the vascular smooth muscle [41]. Evidence suggests a feedback mechanism between ET and NO, as ET inhibits the production of NO, while NO inhibits ET production [37, 40, 42, 43]. The major biological effects of these vasoactive substances depend on their rapid synthesis. The maximal NO stimulation can be reached within seconds, but the maximal vasoconstrictive response of endothelium in vivo takes up to one hour [44]. Vasomotor tone is regulated by ET-1 and NO, depending on the health of the endothelium [44, 45].

Endothelial dysfunction

Endothelial dysfunction was first described by Panza as an impairment of the regulation of endothelium on blood vessels [46]. As a proinflammatory and prothrombic state, endothelial dysfunction has been described in the pathophysiology of different forms of cardiovascular disease, including hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure [35]. Under these conditions, the levels of pro-oxidant reactive oxygen species in the vessel wall are elevated. NO is rapidly degraded by oxidant stress and the vasoinactive and toxic peroxynitrite is produced. In both animal and human studies, this process could be prevented by administration of high concentrations of the antioxidant vitamin C [44, 47, 48].

Flow mediated dilation (FMD) is an important tool for assessment of endothelial function both in the upper [49] and the lower [50] limb. In vivo, endothelial function may be assessed invasively on resistance arteries by measuring blood flow using strain-gauge plethysmography by studying the effects of acetylcholine or metacholine administration through an intra-arterial catheter [51]. Acetylcholine has a direct vasoconstrictive effect on vascular smooth muscles. However, NO from activated healthy endothelium overwhelms the direct effect of acetylcholine, which results in vasodilation. This vasodilation is blocked by inhibitors of NO synthesis, such as monomethylarginine. In endothelial dysfunction, less NO is generated, which may ultimately lead to a vasoconstrictive response to acetylcholine [44, 52, 53]. Shear stress, which stimulates the endothelium to release NO, may be used to non-invasively induce reactive hyperaemia, and the subsequent changes in blood flow can be measured by ultrasound. This has been shown in the brachial artery using FMD [54]. Other non-invasive in vivo techniques include fingertip pulse wave amplitude with peripheral arterial tonometry [55], measurement of intima-media thickness [56], and flow waveform patterns [57]. In vitro, the endothelial dysfunction of isolated resistance arteries dissected from biopsies of gluteal subcutaneous tissue has been studied on a wire or pressurized myograph. A good correlation was found between the endothelial function of small arteries in vitro and FMD of the brachial artery in vivo [35, 58].

Several studies have assessed the microvascular endothelial function in CRPS patients with controversial results. Gorodkin et al. used iontophoresis to test endothelial dependent vasodilation with acetylcholine and endothelial independent vasodilation with sodium nitroprusside in 17 CRPS patients and 16 healthy controls. Among the CRPS patients (IASP criteria) 4 were qualified as warm and 13 as cold; the median duration of the symptoms was 5 years (range 3 months to 20 years). No significant differences were observed between both groups or between affected and unaffected limbs [59].

However, these results could not be reproduced by Schattschneider et al., who compared 14 patients with cold CRPS (disease duration 17.6 ± 2.1 months) with 10 healthy controls. In this study, acetylcholine induced vasodilation was significantly reduced on the affected side compared to the contralateral extremity and controls, whereas no differences were observed after application of sodium nitroprusside [60].

Similar results were found in a study by Duman et al., who used the FMD technique to examine 21 patients in a more acute stage of CRPS (IASP criteria, disease duration 5.9 ± 2.5 months), and compared the results with 15 healthy controls. Upon evaluation of the brachial artery with Doppler ultrasound, significant differences were observed in the waveforms obtained in the affected compared to contralateral limbs; although not significant, there was a trend of larger dilating responses in the affected limbs [57].

In a small study with 9 patients with CRPS in one upper limb and 9 patients with CRPS in a lower limb, Dayan et al. examined FMD and local vascular reflexes [61]. To measure the venoarteriolar reflex (VAR), a cuff is inflated to a steady pressure of 40 mm Hg for 4 minutes to produce venous congestion, which causes a reflexive

regional arteriolar vasoconstriction. To measure the microvascular myogenic reflex (VMR), the subject is placed in supine position to avoid systemic baroreflex changes and the leg or forearm is measured during 40 cm dependency of the leg or forearm below cardiac level for 4 minutes. This activates the myogenic response by increasing the arterial wall pressure, and the venoarteriolar response by gravitationally increasing the venous pressure. The result is local vasoconstriction and, consequently, a reduced blood flow. The duration of the disease was 40 and 46 months for upper and lower limb CRPS, respectively, which was diagnosed using the IASP criteria. In comparing the affected limb to the contralateral side, the resistance artery FMD was impaired on the CRPS side along with exaggerated arteriolar vasoconstriction following activation of the VAR, while the VMR remained unchanged. These changes in vascular reflexes were only significant in the lower limbs. The VAR depends mostly upon intact local autonomic nervous functions, but the VMR is an inherent arteriolar muscle constriction reflex in response to dilation, which appears to be independent of neural transmission. Based on these observations, the authors concluded that the impaired VAR and intact VMR might reflect the adrenergic hypersensitivity in the lower limbs in patients with CRPS [61].
The results from these four studies suggest that changes in vascular response are found locally in only the CRPS side, while there is no difference between the contralateral side and healthy controls. Whether there is a relation between stage of the disease (warm, intermediate or cold) or the duration of the disease, and endothelial dysfunction is not yet clear. In discussing the first three trials, Duman [57] suggested that symptoms of vascular changes, such as hyperemia and edema, may lose their prominence in chronic CRPS, whereas they may be more evident in earlier stages. On the other hand, the study by Dayan in a group of patients with longer disease duration showed clear differences. No significant differences in blood flow were found between CRPS side, unaffected side or healthy controls at baseline. This is another indication that the regulatory mechanisms in particular are affected in vascular alterations in CRPS. The same conclusion was previously made by Wasner [20], who studied the sympathetic regulation mechanism in the hands of CRPS patients by inducing temperature changes to the entire body with a thermal suit; they concluded that the differences in blood flow and temperature were not static. No significant differences were detectable during low or absent sympathetic vasoconstrictor activity, and these differences were most pronounced during periods of intermediate to high sympathetic activity [20].

Pain
Pain is an important symptom in CRPS. Most patients experience an intense, spontaneous, burning pain in the distal part of the affected limb, which is characteristically disproportionate in intensity to the initial event, and increases in a dependent position [1]. Several types of pain can be distinguished: neuropathic, inflammatory, nociceptive and functional [62]. Evidence supports a neuropathic origin of the pain in CRPS [63]. The spatial distribution of pain and sensory abnormalities such as

allodynia to mechanical, cold and heat stimuli, as well as hyperalgesia, indicate that the pathophysiological mechanisms in CRPS involve both the peripheral and central nervous system [1, 64, 65], but the interaction between the peripheral and central changes is only partially understood [63].

Neuropathic pain

The neuropathic pain in peripheral tissues may be generated and maintained by peripheral sensory nerve fibers. Oaklander et al. analyzed the innervation density of the epidermis, and their results suggest that CRPS is especially associated with persistent minimal distal nerve injury affecting nociceptive small fibers, a type of nerve injury that will remain undetected in most clinical settings [11]. Albrecht studied the innervation of CRPS affected skin tissue, and found impressive changes in innervation of different target tissues as well as changes of the target tissue itself (e.g. blood vessels) [8]. Although the results of these two studies must be interpreted with care, as these observations may be the result of secondary tissue changes that may occur in the course of the disease, both studies indicate that CRPS can be associated with peripheral pathological changes of innervation of the skin, thus CRPS may indeed be a neuropathic pain syndrome [8, 11, 63]. Neuropathic pain may also be caused by aberrant activity of the sympathetic nervous system (SNS), in which case the pain is referred to as sympathetically maintained pain (SMP) [64, 66]. In patients with SMP, sympathetic blockade relieves spontaneous pain and mechanical hyperalgesia, but these symptoms may reappear following intracutaneous application of noradrenaline [18, 67, 68] or by stimulation of the SNS by cooling of the body or forehead, or a startle stimulus [69, 70]. SMP is not typically observed in CRPS; only 50% of CRPS patients experience SMP [71-73], and a number of neuropathic pain syndromes might also benefit from sympathetic blocks [29, 64].

Inflammatory Pain

Sudeck was the first to describe the classic signs and symptoms of inflammation, including rubor, calor, dolor, tumor and function laesa, in acute CRPS [74, 75]. Kozin [76] described inflammatory changes in 2 patients with early CRPS, and Oyen [34] found increased vascular permeability for macromolecules, which was thought to be due to the inflammatory response, caused by free oxygen radicals. Increased systemic calcitonin gene-related peptide (CGRP) serum concentrations were found by Birklein [77], suggesting neurogenic inflammation as the pathophysiologic mechanism. In this study, however, pain and hyperalgesia were observed in chronic stages in particular, independent of the increased neuropeptide concentration.

Our research group compared levels of proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) in plasma and fluids of artificially induced blisters on the CRPS side and contralateral side. There was no indication for inflammation in plasma [12], which was confirmed by van de Beek [78]. However, Huygen et al. demonstrated that TNF-α and IL-6 levels were increased in blister fluids

in patients with acute CRPS [12]. This shift in the pro-inflammatory cytokine profile in acute CRPS patients was also found by Uceyler [2].
In three studies of patients with an intermediate disease duration of 2.5, 2.8 and 3.5 years, the levels of these cytokines were still increased [79-81]. Remarkably, a large proportion of these patients no longer displayed any of the above-mentioned signs and symptoms of inflammation. A study of chronic CRPS patients with a disease duration of 6 years showed no differences in TNF-α and IL-6, although some patients still showed signs of inflammation [82]. No relationship between proinflammatory cytokines and disease characteristics, such as pain, changes in temperature, volume, mobility and disease duration, was observed in either study [79, 82].
In rats, during the reperfusion which follows prolonged extremity ischemia, the synthesis of free radicals and pro-inflammatory cytokines leads to inflammatory responses and vasculature injury in the ischemic tissue [83]. This ischemia-reperfusion causes damage to the endothelial cells, resulting in swelling and protrusion of cells in the capillary lumen with impeded passage of red blood cells as consequence, the so-called slow-flow/no-reflow phenomenon. Coderre also showed that ischemia-reperfusion of the rat hind paw induced long-term mechanical and cold hypersensitivity, which was effectively reduced by free radical scavengers [84] as well as classical analgesics, although to a lesser extent [85]. These symptoms are comparable to those described in humans with CRPS. Recently, Coderre and colleagues also showed that mechanical allodynia induced by hind paw ischemia-reperfusion injury is accompanied by increased hind paw muscle malondialdehyde (a product of free radical-induced lipid peroxidation), pro-inflammatory cytokines (IL-1β, IL-6 and TNFα), nuclear factor κ B and lactate; furthermore, mechanical allodynia is reduced by inhibitors of these mediators or an antagonist at acid sensing ion channels (ASICs) [86]. The authors also demonstrated that mechanical allodynia following ischemia-reperfusion injury parallels the development of arterial vasospasms, endothelial cell thickening and capillary slow-flow/no-reflow in hind paw muscle, and is directly correlated with muscle lactate but not with the demonstrated reduction in intraepidermal nerve fibers [86, 87]. Furthermore, similar to CRPS patients, rats with hind paw ischemia-reperfusion injuries exhibit enhanced pain and allodynia following exercise, symptoms that depend on increases in muscle lactate [86].
Free radical infusion in human patients produces CRPS-like symptoms [88], and treatment with free radical scavengers can both reduce the risk of developing CRPS [89-92] and improve the clinical picture [59, 93-95]. This observation was supported by recent evidence of increased levels of saliva and serum antioxidants and serum malondialdehyde in CRPS patients [14], which is almost definitive proof for the involvement of free radicals in the pathophysiology.
Regardless of the initial pathogenesis, one hypothesis has proposed a vicious circle of altered blood flow that leads to hypoxia, production of free radicals, endothelial damage and further reductions in blood flow [59]. The slow-flow/no-reflow injury may even affect the microvasculature of peripheral nerves, thus presenting the cause

of peripheral neuroinflammation in CRPS [15].

Nociceptive pain
One of the characteristics of CRPS is the often observed disproportionate severity of the symptoms with the severity of the trauma, along with a tendency to generalize in the affected distal limb but not confined to the innervation zone of an individual nerve [29]. Nociceptive pain may be caused by tissue damage as a result of the initiating trauma or by secondary tissue changes that occur in the course of the disease, i.e. oedema, changes in the nutritive blood flow, hypoxia, lactate increase and acidosis [32, 33, 63, 96-98].

Functional pain
Functional pain is defined as hypersensitivity to pain resulting from abnormal central processing of normal input [62]. CRPS patients are known to protect their involved limb to minimize pain associated with movement and touching; this protection has been described as a voluntary action, but lately neglect-like behavior has been proposed to play an important role. Two terms have been introduced in describing this behavior: 'cognitive neglect', which suggests that patients perceive their involved limb as feeling foreign to them, and 'motor neglect', which describes situations in which the patients need to focus mental and visual attention to move their limb [99]. In a survey of 224 CRPS patients, 84% of the respondents confirmed the presence of at least one of these neglect symptoms, and 47% indicated they experienced both [99]. Other studies also found a large proportion of CRPS patients with disturbances of self-perception of hand or foot, indicating an alteration in the processing of the higher central nervous system [100-102].
Disuse has often been mentioned in CRPS. In animal studies of immobilization, an increased sensitivity to sensory stimuli as well as changes at the spinal level that could account for this increase have been found [103-105]. In humans, casting produced increased cerebral blood flow in areas associated with sensory processing, motor function, and emotions [104, 106]. These studies indicate that immobility alone may produce many signs and symptoms also found in patients with CRPS [104]. Fear of injury has also been suggested as a potential predictor of disability in CRPS [107], and combined with increased sensitivity for pain, fear of injury may lead to excessive guarding and over-protective behaviors [108].
Rommel et al. found that depressive syndromes frequently develop with chronic CRPS, and psychological treatment can be recommended [109]. Nevertheless, despite the often suggested relationship between CRPS and a psychological predisposition, Van de Laan et al. used the SCL 90 and did not find any specific psychological profiles in CRPS-dystonia [110]. In a large population-based study, De Mos et al. also did not find any relationship between pre-existing psychiatric disorders and CRPS (odds ratio of 1.17) [111].

Peripheral changes

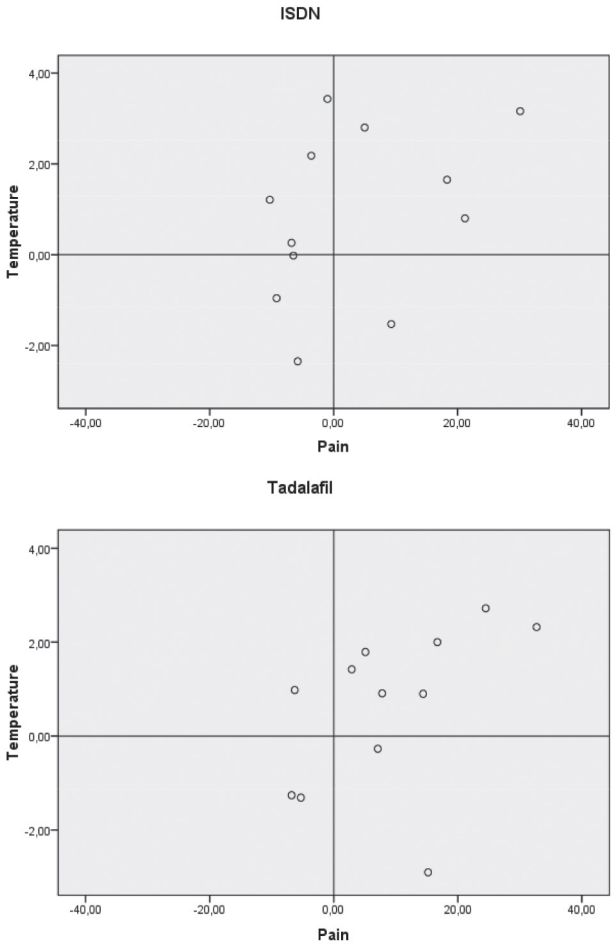
In addition to changes in the somatosensory systems, which process noxious, tactile and thermal information, and changes to the sympathetic systems, which innervate skin and somatomotor systems, peripheral changes are detected in CRPS that cannot be explained by the central changes [1]. These peripheral changes (sympathetic afferent coupling, vascular changes, inflammatory changes, edema, and trophic changes) cannot be seen independent of the central changes [1, 112]. Furthermore, each symptom can also be generated by more than one mechanism, depending on the patient. Therefore, caution must be taken when grouping patients based on symptoms and administering drugs irrespective of their underlying disease [1]. A cold extremity in chronic CRPS could be caused by several factors, such as increased tone of the sympathetic nervous system, pathologic alterations of the vascular wall, changes in small nerve fibers innervating the blood vessels, or endothelial dysfunction. It is also possible that as symptoms of endothelial injury progress, there may be a shift from a predominant oedema associated with plasma extravasation from damaged post-capillary venules (an early consequence of endothelial injury) to chronic ischemia with the development of arterial vasospasms and capillary no-reflow (later consequences of endothelial injury). Furthermore, sympathetic blocks or the use of vasodilatory agents may overcome ischemia that is dependent on arterial vasospasms, which represents a functional reduction in blood flow; this, however, would not be able to overcome ischemia associated with capillary no-reflow, which represents a physical reduction in blood flow. These observations may explain why the results of trials studying medication to improve blood flow in patients with cold chronic CRPS do not show more convincing results.

Pharmalogical interventions

We investigated the involvement of the vasoactive substances ET and NO during early chronic CRPS. Measurement of NO and ET levels in artificial suction blisters in 29 patients showed a significant increase in ET and a decrease of NO on the affected side compared to the unaffected side, indicating an aberrant NO/ET ratio in the intermediate state of CRPS. This altered ratio results in vasoconstriction and consequently in diminished tissue blood distribution [80]. The ratio could be restored by the substitution of NO, substitution of asymmetric dimethylarginine (ADMA) [113], or the blocking of ET_A receptors [42]. As several recent publications indicate that NO substitution might be valuable in treating diabetic neuropathy [114], anal fissures [115, 116], and epicondylitis [117], we propose that the effective treatment of CRPS by NO substitution, which was shown previously [118], occurs by increasing blood flow. In a pilot study, five female patients with cold type CRPS in one hand were treated with NO donor isosorbide dinitrate (ISDN) ointment 4 times daily for 10 weeks; videothermography was used to monitor changes in blood distribution in both the involved and contralateral extremities. The patients treated with ISDN

showed an 4-6°C increase in mean skin temperature of the cold CRPS hands, reaching temperatures similar to that of contralateral extremities within 2-4 weeks, suggesting normalization of blood distribution; normalization was confirmed by an improvement in skin color. In three patients, the VAS pain declined, whereas in the other two patients, the VAS pain remained unchanged. Thus, the topical application of ISDN appeared to be beneficial in improving symptoms for patients with cold type CRPS [119].
Based on these preliminary results, we decided to test the effect of ISDN in a double blind randomized controlled trial. Twenty-four patients with chronic CRPS in one upper extremity received 1% ISDN in Vaseline or a placebo ointment applied to the dorsum of the affected hand 4 times daily for 10 weeks. The patients participated in a physical therapy program to improve activity. The primary outcome measure was blood distribution in the affected extremity, which was determined by measuring the skin temperature using videothermography. We also measured NO and ET-1 concentrations in blister fluid, assessed pain using the VAS, and determined activity limitations using an Upper Limb Activity Monitor (ULAM) and the Disabilities of Arm Shoulder and Hand Questionnaire (DASH). ISDN failed to produce a significant improvement in temperature asymmetry in chronic cold CRPS patients, and also did not generate the predicted reduction in pain and increase in activity compared to placebo. The results of the active treatment group are shown in Figure 1. Together this suggests that other central or peripheral factors may contribute to the disturbed vasodynamics in cold chronic CRPS that are not influenced by NO substitution [120].
To further evaluate the influence of endothelial factors, patients with chronic cold CRPS in one lower extremity were included in a double blind, randomized, controlled trial that investigated the effect of tadalafil on the microcirculation. Tadalafil, a PDE-5-inhibitor known as an effective treatment for erectile dysfunction [121], functions within the vascular smooth muscle cell to inhibit the hydrolyzation of cyclic guanosine monophosphate (cGMP) to GMP. Through the phosphorylation of specific proteins and ion channels, treatment with tadalafil results in the opening of potassium channels and hyperpolarization of the muscle cell membrane, sequestration of intracellular calcium by the endoplasmic reticulum, and block of calcium influx by the inhibition of calcium channels. The consequence is a drop in cytosolic calcium concentrations and relaxation of the smooth muscle that causes vasodilation [121]. In this trial, twenty-four patients received 20 mg tadalafil or placebo daily for 12 weeks, and participated in a physical therapy program. The primary outcome measure was temperature difference between the CRPS and the contralateral sides, as determined by measuring the skin temperature with videothermography. Secondary outcomes were pain measured on a VAS, muscle force measured with a MicroFet 2 dynamometer, and level of activity measured with an Activity Monitor (AM) and walking tests. At the end of the study period, the temperature asymmetry was not significantly reduced in the tadalafil group compared with the placebo group, but there was a significant and clinically relevant reduction of pain in the tadalafil group. Muscle force improved in both treatment groups, and the AM revealed small, non-

Figure 1.
Relationships between the improvements in temperature and pain in the ISDN and Tadalafil studies.



The improvement in pain is shown on the horizontal axis. The Visual Analogue Scale (VAS, score 0-100) is the average of actual pain scores that were recorded three times each day (0800, 1200 and 2000) during one week before the first and the last hospital visits. The improvement in pain is calculated by subtracting the VAS score at the end of the study from the VAS score at the start of the study; thus, a positive value indicates less pain.

The vertical axis shows the changes in temperature calculated by subtracting the temperature difference (CRPS side minus contralateral side) between the dorsal side of both hands at the end of the study from the difference measured at the start of the study. A positive value indicates that the difference in temperature has diminished.

significant improvements in time spent standing and walking, as well as the number of short walking periods. The results of the active treatment group are also shown in Figure 1. Thus, tadalafil may be a promising new treatment for patients with chronic cold CRPS. The use of tadalafil and the role of endothelial dysfunction in CRPS warrants further investigation [122].

In both studies, the results of treatment with study medication was compared to placebo, and, as shown in Figure 1, there were clear responders and non-responders among the patients that received the active medication in both studies.

The results of the static videothermographic measurements should be interpreted with care, due to the dynamic nature of side temperature differences in CRPS [18, 20, 123, 124]. Long-term skin temperature measurements may prove to be a more reliable instrument for determining temperature changes in CRPS [125]. Furthermore, care should be taken when using the clinically unaffected side as a control for studies on thermoregulatory skin blood flow in the CRPS side. One possibility is that the thermoregulatory skin blood flow in both extremities may have resulted from a spinal reflex mechanism initiated by (post-) traumatic excitation of a peripheral nerve on the clinically affected side [126]. Given the pathology of muscle tissue (including lipofuscin deposits, atrophic fibers, and severely thickened capillary basal membranes) observed in the amputated limbs of CRSP patients as described by van der Laan et al. [7], as well as the role of muscle pathology (increased lipid peroxidation products, pro-inflammatory cytokines and lactate) in mechanical allodynia of rats with ischemia-reperfusion injury, alterations in deep tissue blood flow, as well as skin blood flow, may be important in CRPS. Also, since capillary hemoglobin oxygenation (HbO_2) is lowered and skin lactate is increased in CRPS limbs, alterations in nutritive as well as thermoregulatory blood flow may also be important in CRPS.

Recent evidence suggests that both NO and ET also play a role in nociception exhibited in animals with ischemia-reperfusion injuries. The mechanical allodynia that is observed in rats with a hind paw ischemia-reperfusion injury is relieved by systemic administration of the NO donor 3-morpholinylsydnoneimine chloride (SIN-1) [87]. Furthermore, intradermal injections of either norepinephrine or the endothelial NO synthase inhibitor N5-(1-Iminoethyl)-L-ornithine dihydrochloride (L-NIO) (both of which should reduce blood flow) induce sustained nociceptive behaviours in rats with hind paw ischemia-reperfusion injuries; the nociceptive behaviours induced by NE are reduced by local or systemic administration of the NO donors sodium nitroprusside and SIN-1, respectively [87]. This observation raises the possibility that sympathetically maintained pain may depend more on NE-induced vasoconstriction than on sympathetic-afferent coupling. This conclusion is supported by the finding that sustained nociceptive behaviours are also induced in rats with hind paw ischemia reperfusion injuries after intradermal injection of the non-adrenergic vasoconstrictor vasopressin [87]. Coderre and colleagues recently demonstrated that mice with ischemia-reperfusion injury of the hind paw exhibit sustained nociceptive behaviors following intradermal injection of ET-1 or ET-2. These nociceptive behaviours corre-

lated with increased ET_A receptor expression in hind paw muscle, and were reduced by co-administration of an ET_A antagonist [127].
Treatment strategies for endothelial dysfunction in CRPS patients include the substitution of nitric oxide with ISDN, inhibition of PDE5, substitution of ADMA, or blockage of ET_A receptors. Of these strategies, only ISDN and PDE5 inhibition have been tested in randomized placebo-controlled trials with CRPS patients. Because these trials included patients with cold chronic CRPS and did not differentiate between central and peripheral dysfunction, the results may not be conclusive for the treatment of endothelial dysfunction.
Calcium antagonists are used in the treatment of hypertension and angina pectoris, as they bind to the L-type calcium channel in the smooth muscles of the vascular wall, thus reducing the influx of extracellular calcium and resulting in vasodilation. Nifedepine has been examined in two descriptive studies with CRPS patients, and this calcium antagonist was most effective in acute CRPS [128, 129]. In two studies performed by our research group, only a few patients used the calcium antagonist verapamil and none used nifedepine. In addition to peripheral vasodilation, verapamil also causes a reduction of the heart rate and atrioventricular conduction. Since nifedepine is a more potent peripheral vasodilator with only minimal cardial side-effects [130], one of the long-acting dihydropyridines such as nifedipine might prove advantageous in cold chronic CRPS [131]. More research on this subject is warranted.
An interesting development occurred in the tadalafil study, when one patient who had not responded to the active tadalafil treatment with an increase of temperature and showed persistent high pain scores was treated with a lumbal sympathectomy. The leg on the CRPS side became warmer, and the temperature increased until it was more than 1°C warmer than the contralateral side. This improvement in temperature (difference) still persists. We consider this an indication that in cold chronic CRPS, both endothelial dysfunction and sympathetic dysfunction may be responsible for ischemia in the cold extremity. Unfortunately, in this patient, the severe pain was unaffected by the sympathectomy, indicating that in CRPS, pain and ischemia may be related in some cases, but an increase in blood flow will not result in less pain in other cases.
This observation confirms the result of trials performed by Baron et al. in analyzing skin blood flow demonstrating that sympathetic vasoconstrictor reflexes and pain after surgical sympathectomy show no clear relationship of vascular changes and the success of sympathectomy regarding pain relief [18]. Thus, apart from nociceptive and neuropathic pain, pain in chronic cold CRPS may also be due to ischemic pain caused by endothelial dysfunction [60, 65, 84], sympathetic hyperactivity or increased sensibility to circulating catecholamines [19], or sympathetically maintained pain [69].
In a study with intermediate CRPS patients (disease duration 2.8 ± 1.4 years), we found a significant increase in IL-6, TNF-α and ET-1 levels in blister fluid in the CRPS extremity versus the contralateral extremity [80]. ET-1 concentrations in the cold chronic patients in the ISDN study were lower than those in our previous study,

but still higher than levels previously reported by others [120, 132, 133]. Apparently, some of these chronic cold patients still had active inflammatory components, which may explain the case of one of the outpatients who was treated with a PDE5-inhibitor for a very cold painful foot in chronic CRPS. In a few days, the affected foot displayed full-blown warm CRPS. The classical signs of inflammation (rubor, calor, dolor, tumor and functio laesa) depend highly on unimpaired circulation. Similar to the implications of the ET-1 measurements of the ISDN study, this case suggests that there may be patients with chronic cold CRPS with active inflammation who do not show symptoms of inflammation because of impaired vasodilation. Indeed, it has been shown that plasma extravasation does not occur in the later stages of ischemia-reperfusion injury after the development of no-reflow. Thus, oedema only occurs with leakage of plasma from the post-capillary venules of vessels that are adequately perfused [134]. This may account for persistent pain and other therapy-resistant symptoms in some patients.

Current research in the field suggests several possible mechanisms responsible for ischemia and pain in chronic cold CRPS. Because of the fluctuation of temperature difference between both hands, even a patient who reports an extremely cold extremity may present no difference in temperature in the clinic or even a warmer CRPS side. In this case, a static video thermographic recording may not show any difference compared to a manual test. Under circumstances of doubt, the clinician should let the patient's report of a persistent cold extremity prevail over clinical tests that show no difference.

Several tests have been described to separately investigate the sympathetic and the endothelial function. Until now, these tests have only been used for research purposes. Promising results were found using provocative manoeuvres, like the Valsalva manoeuvre, and the cold pressor test [135], or inspiratory gasp and contralateral cooling [18, 136, 137].

Future research should focus on methods to differentiate between sympathetic and endothelial dysfunction for clinical use. Laser Doppler Flow, video thermographic recordings and ultrasound could be used to measure the results of autonomic provocation tests, and flow mediated dilation, in combination with long-term temperature measurements. Investigators should not only focus on blood flow in the skin but also in deep tissues; more information is needed regarding the separate contributions of thermoregulatory and nutritive blood flow.

To differentiate between sympathetically maintained pain (SMP) and sympathetically independent pain (SIP), a sympathetic block has been advised [68], usually indicating an invasive chemical or a surgical block [138-141]. Bolel et al., however, described a non-invasive stellar ganglion blockade using diadynamic current [142] that could provide a simple and easy to perform method to differentiate between SMP and SIP.

Summary

As a result of an impressive amount of research over the last few years, our understanding of CRPS has improved considerably. Although some studies have suggested that CRPS is primarily a disease of the central nervous system, alterations have also been found in the peripheral small nerve fibers innervating skin, blood vessels and sweat glands, as well as aberrations in the vascular wall, muscle fibers and other deep somatic tissues, likely as a result of damage by the initial inflammation. Although this article focused on disturbed peripheral blood flow in patients with cold, chronic CRPS, treatment goals are not necessarily only local. In this respect, we agree with Harden, who proposes that the treatment of CRPS should be a systematic and coordinated interdisciplinary approach with a primary goal of functional restoration [112].

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Summary

Complex Regional Pain Syndrome (CRPS) is a painful disorder that usually develops as a disproportionate consequence of trauma. The disorder is most common in the limbs and is characterised by spontaneous pain, allodynia and mechanical hyperalgesia, movement disorders, abnormal regulation of blood flow and sweating, oedema of skin and subcutaneous tissues, and by trophic changes of the skin, organs of the skin and subcutaneous tissues.

The aim of the research described in this thesis was to study the nature of the vascular alterations in patients with cold chronic CRPS. The sympathetic vascular regulatory system in CRPS has been examined extensively, but this failed to show why a sympathectomy provides relief in only a minor part of the patients. It seems likely, that other regulation mechanisms are also disturbed in one way or another. We hypothesised, that endothelial dysfunction may in part be responsible for the diminished blood flow in cold chronic CRPS. An improvement on endothelial level would therefore lead to an increased blood flow and a subsequent improvement in pain and activity.

A general introduction on CRPS and the microcirculatory system is presented in chapter 1.

In the second chapter we have described the characteristics of 195 patients with potential CRPS that visited our out-patients clinic. We studied the disease characteristics, the treatment, the referral patterns, and examined whether these were different in the subgroup of patients with a lower temperature in the affected extremity.
The Harden & Bruehl criteria were confirmed in 95 patients (49%). These patients used a higher than average number of analgesics, opiates, and anti-oxidants. They frequently received prescriptions for benzodiazepines instead of anti-depressants. The mean disease duration was 29 ± 4.6 months and the mean visual analogue score for pain was 8.1 ± 0.19. A subgroup of patients had a colder temperature in the affected extremity compared to the unaffected extremity. This subgroup showed longer disease duration and higher visual analogue scale pain. Diagnosis of CRPS was confirmed in 61% of the patients referred by other anaesthesiologists, 63% by rehabilitation doctors, 43% by family doctors, 42% by surgeons, 35% by orthopaedic surgeons, and 13% by neurologists. Our results indicate that the diagnostic criteria used to determine CRPS should be further improved. Disease-related medication is unrelated to CRPS-specific disease activity. Knowledge of underlying mechanisms is warranted before an adequate pharmaceutical intervention can be considered.

In the third chapter the results of immunohistochemical staining on sections of skin specimens obtained from the amputated limbs (one arm and one leg) of two patients with CRPS are described. The aim of this study was to examine the distribution of eNOS and ET-1 relative to vascular density represented by the endothelial marker CD31-immunoreactivity in the skin tissue of patients with chronic CRPS. Therefore immu-

nohistochemical staining was performed on sections of skin specimens obtained from the amputated limbs (one arm and one leg) of two patients with CRPS. In comparison to proximal specimens we found an increased number of migrated endothelial cells as well as an increase of eNOS activity in distal dermis specimens. These results indicate that endothelial dysfunction may play a role in chronic CRPS.

In the fourth chapter we describe the involvement of ET-1 and NO during the early chronic phase. Therefore artificial suction blisters were made on the extremities on the CRPS side and the contralateral side. The levels of NO, IL-6, TNF-α and ET-1 in the blister fluid were measured. Included were 29 patients with CRPS who were diagnosed during the acute stage of their disease and observed during follow-up visits. We found that the levels of IL-6, TNF-α and ET-1 in blister fluid in the CRPS extremity versus the contra lateral extremity were significantly increased and correlated with each other, whereas NOx levels were decreased. The conclusion was that the NOx/ET-1 ratio appears to be disturbed in the intermediate stage of CRPS, resulting in vasoconstriction and consequently in a diminished tissue blood distribution.

Since these results indicate that NO levels are decreased on the CRPS side, a pilot study was performed using the NO donor isosorbide dinitrate (ISDN). Five female patients were treated with ISDN ointment 4 times daily during 10 weeks. As a primary objective videothermography was used to monitor changes in blood distribution the involved and the contralateral extremities. This study is described in chapter five.
We found that patients treated with ISDN showed an increase of 4.1°C to 6.1°C in mean skin temperature of the cold CRPS hands, reaching values similar to that of the contralateral extremities within 2 to 4 weeks time, suggesting normalization of blood distribution. This was confirmed by an improvement in skin color. In 3 patients the Visual Analog Scale pain declined, whereas in the other 2 patients the Visual Analog Scale pain was unchanged over time. So, in this pilot study, topical application of ISDN seems to be beneficial to improve symptoms for patients with cold type CRPS.

Encouraged by the positive results of this pilot we initiated two studies. Both studies were randomized placebo-controlled trials, included 24 patients with chronic cold CRPS. Videothermography and pain were used as primary outcome measures. The first study, which uses ISDN, is described in chapter six. In this study patients were included with CRPS in one hand.
Twenty-four patients received 1% ISDN in Vaseline or a placebo ointment applied to the dorsum of the affected hand 4 times daily for 10 weeks. The patients participated in a physical therapy program to improve activity. The primary outcome measure was blood distribution in the affected extremity, which was determined by measuring the skin temperature using videothermography. We also measured NO and endothelin-1 (ET-1) concentrations in blister fluid, pain using the Visual Analogue Scale, activity limitations using an Upper Limb Activity Monitor (ULAM) and the Disabilities

of Arm Shoulder and Hand Questionnaire (DASH). ISDN failed to produce a significant improvement in temperature asymmetry in chronic cold CRPS patients, and it did not result in the expected reduction in pain and increase in activity compared to placebo either. The conclusion was that there may be other central or peripheral factors contributing to the disturbed vasodynamics in cold chronic CRPS that are not influenced by NO substitution. This study did not show an improvement of the regional blood distribution by ISDN in the involved extremity of patients with cold-type CRPS.

In the second study, which is described in chapter seven, the phosphodiesterase inhibitor tadalafil was used to induce vasodilation in patients with CPRS in one foot. Twenty-four patients received 20 mg tadalafil or placebo daily for 12 weeks. The patients also participated in a physical therapy program. The primary outcome measure was temperature difference between the CRPS side and the contralateral side, determined by measuring the skin temperature with videothermography. Secondary outcomes were: pain measured on a Visual Analogue Scale, muscle force measured with a MicroFet 2 dynamometer, level of activity measured with an Activity Monitor (AM) and walking tests.
At the end of the study period the temperature asymmetry was not significantly reduced in the tadalafil group compared with the placebo group, but there was a significant and clinically relevant reduction of pain in the tadalafil group. Muscle force improved in both treatment groups and the AM revealed small, non-significant improvements in time spent standing, walking and the number of short walking periods. We concluded that tadalafil may be a promising new treatment for patients that have chronic cold CRPS due to endothelial dysfunction. This deserves further investigation.

In chapter 8, finally, the main findings are discussed. Several mechanisms appear to be responsible for the ischemia and pain in chronic cold CPRS. The diminished blood flow may be caused by either sympathetic dysfunction, hypersensitivity to circulating catecholamines or endothelial dysfunction. Pain in CRPS may be of neuropathic, inflammatory, nociceptive, functional nature, or of mixed origin. The origin of the pain should be the basis of the symptomatic therapy.
Since the difference in temperature between both hands fluctuates over time in cold CRPS, when in doubt, the clinician should prioritize the patient's report of a persistent cold extremity over clinical tests that show no difference.
Future research should focus on developing easily applied methods for clinical use to differentiate between central and peripheral blood flow regulation disorders in individual patients.

Samenvatting

Samenvatting

Het Complex Regionaal Pijn Syndroom (CRPS) is een ziektebeeld dat gewoonlijk ontstaat als een buitenproportionele reactie op een trauma. Het ziektebeeld komt meestal voor in de extremiteiten en wordt gekarakteriseerd door ernstige, spontane pijn, allodynie en mechanische hyperalgesie Een verstoorde functie van het bewegingsapparaat, veranderde regulatie van de doorbloeding en zweten kunnen eveneens voorkomen, net als oedeem van de huid en onderhuids weefsel door de trofische veranderingen van de huid en onderhuids weefsel.

Het doel van de studies in dit proefschrift was om de veranderingen in de regulatie van de doorbloeding te onderzoeken bij patiënten met koude chronische CRPS. Het sympathische deel van het vasculaire regulatie systeem is bij CRPS uitvoerig onderzocht, maar dit kan niet verklaren, waarom een sympathectomie alleen bij een beperkt deel van de patiënten verbetering brengt. Het lijkt waarschijnlijk, dat ook andere delen van dit regulatie systeem op een of andere manier verstoord zijn. We veronderstellen, dat disfunctie van het endotheel de perifere factor is die mede verantwoordelijk is voor de verminderde doorbloeding in koude chronische CRPS. Een verbetering op dit endotheelnivo zou dan dus leiden tot een verbeterde doorbloeding en aansluitend ook tot verbeteringen van de pijn en op het activiteiteiten- en participatie nivo.

In het eerste hoofdstuk wordt CRPS geïntroduceerd en wordt het systeem van de microcirculatie beschreven.

In hoofdstuk 2 hebben we 195 patiënten beschreven, die vanwege een verdenking op CRPS onze polikliniek bezocht hebben. Van deze patiënten hebben we de verwijzing, de ziektekenmerken en de behandeling vastgelegd en onderzocht in hoeverre deze verschillend waren in de groep patiënten met een chronisch koude CRPS extremiteit.
De CRPS criteria volgens Harden & Bruehl konden bij 95 patiënten (49%) bevestigd worden. Deze patiënten gebruikten meer pijnstillers, opiaten en anti-oxidanten dan gemiddeld en kregen vaker benzodiazepines dan antidepressiva voorgeschreven. De ziekteduur was 29 ± 4.6 maanden en de pijnscore op de visueel analoge schaal (VAS) was 8.1 ± 0.19.
Bij een subgroep van deze patiënten was de temperatuur in de aangedane extremiteit kouder dan in de gezonde extremiteit. Deze groep was langer ziek en had een hogere score op de VAS pijnschaal.
In 61% van de patiënten die door anesthesiologen verwezen waren, kon de diagnose CRPS bevestigd worden, evenals in 63% van de patiënten die door revalidatieartsen verwezen werden, 43% afkomstig van de huisarts, 42% van de algemeen chirurg, 35% van de orthopeden en bij 13 % afkomstig van neurologen. Deze resultaten maken duidelijk dat de CRPS diagnostiek nog verbeterd zou kunnen worden.

Over het algemeen bleek er weinig overeenkomst te zijn tussen de voorgeschreven medicatie en de CRPS ziekte activiteit. Kennis van het onderliggende mechanisme zou toch het uitgangspunt moeten zijn voor een adequate farmaceutische interventie.

In het derde hoofdstuk worden de resultaten beschreven van de immunohistochemische kleuring van huidbiopten, verkregen van de geamputeerde ledematen (een arm en een been) van twee patiënten met CRPS. Het doel van deze studie was om bij patiënten met chronische CPRS de verdeling van eNOS en ET-1 te onderzoeken en te vergelijken met de vaatdichtheid. Deze vaatdichtheid werd zichtbaar gemaakt door een immunologische reactie op CD31, wat een marker is voor endotheelcellen. In vergelijking tot het weefsel wat van proximale locaties van de extremiteit afkomstig was vonden we in het weefsel van distale herkomst een toegenomen aantal endotheelcellen, die vanuit de vaten naar het bindweefsel gemigreerd waren, alsmede een toename van eNOS activiteit. Deze resultaten geven een aanwijzing, dat disfunctie van het endotheel een rol kan spelen in chronische CRPS.

In het vierde hoofdstuk beschrijven we de activiteit van ET-1 en NO in de vroege chronische fase. Om dit te kunnen meten werden met een vacuüm methode kunstmatige blaren gemaakt zowel op de aangedane, als op de contralaterale extremiteit. In de blaarvloeistof werd de hoeveelheid NO, IL-6, TNF- α en ET-1 gemeten. Aan deze studie namen 29 CRPS patiënten uit onze polikliniek deel, bij wie de diagnose reeds in de acute fase van de ziekte gesteld was. De uitkomst van de metingen was dat er significant verhoogde en onderling gecorreleerde concentraties van IL-6, TNF- α en ET-1 gevonden werden in de blaarvloeistof aan de CRPS kant in vergelijking met de andere kant. De concentratie van NOx was daarentegen verlaagd. De conclusie van dit onderzoek was dat de NOx/ET-1 ratio verstoord blijkt te zijn in de vroege chronische fase van CRPS, wat resulteert in vasoconstrictie en als gevolg daarvan in verminderde weefseldoorbloeding.

Omdat uit de resultaten van de ET-1/NO studie bleek dat de NOx concentratie verlaagd was, werd een pilotstudie uitgevoerd met de NO donor Isosorbide Dinitraat (ISDN). Vijf vrouwelijke patiënten gebruikten ISDN zalf 4 maal daags gedurende 10 weken. De primaire uitkomstmaat was de doorbloeding in beide extremiteiten. Dit werd gemeten met videothermografie. Zoals beschreven in hoofdstuk vijf lieten de vrouwelijke patiënten die met ISDN behandeld werden verbeteringen zien in de gemiddelde temperatuur van de koude CRPS handen variërend tussen 4.1°C en 6.1°C. In 2 tot 4 weken was de temperatuur gelijk in beide handen, hetgeen suggereert dat de doorbloeding aan de CRPS zijde genormaliseerd was. Dit werd bevestigd door een verbetering van de kleur van de huid. De VAS voor pijn verbeterde bij 3 patiënten, terwijl 2 patiënten geen verbetering lieten zien.
De conclusie van deze pilotstudie was dat lokale toepassing van ISDN zalf een positief effect lijkt te hebben op patiënten met de koude vorm van chronische CRPS.

De positieve resultaten van deze studie waren de aanleiding om twee gerandomiseerde, placebo gecontroleerde studies op te zetten. In beide studies werden 24 patiënten met koude chronische CRPS geïncludeerd. Doorbloeding, gemeten met videothermografie was samen met pijn, gemeten met de VAS, de primaire uitkomstmaat in beide studies. De eerste studie, waarin ISDN gebruikt werd op de bovenste extremiteit, wordt beschreven in hoofdstuk zes.
De patiënten kregen 1% ISDN in vaseline of een placebo in vaseline om te gebruiken op de dorsale zijde van de aangedane hand, vier maal daags gedurende 10 weken. Tevens werd een standaard fysiotherapie programma door een lokale fysiotherapeut gegeven om de activiteit te bevorderen. Naast videothermografie om de doorbloeding te meten, werd de NO en ET-1 concentratie in blaarvloeistof gemeten en werd pijn gemeten met de VAS. Beperkingen in activiteiten werden vastgelegd met een Upper Limb Activity Monitor (ULAM) en de Disabilities of Arm Shoulder and Hand Questionnaire (DASH).
Er bleek in de ISDN groep in vergelijking met de placebogroep geen significante verbetering te zijn in temperatuur asymmetrie, noch was er de verwachte reductie van pijn en toename in activiteiten. De conclusie was dat er meest waarschijnlijk andere centrale of perifere factoren zijn die mede bijdragen aan de verstoorde regulatie van de doorbloeding in koude chronische CRPS, welke niet beïnvloed worden door substitutie van NO.

In de tweede studie, die beschreven wordt in hoofdstuk 7, wordt de phosphodiesterase remmer tadalafil gebruikt om vaatverwijding te genereren in patiënten met CRPS in 1 onderste extremiteit. De 24 patiënten kregen dagelijks 20 mg tadalafil of placebo gedurende 12 weken en ook zij kregen een standaard fysiotherapie programma. Videothermografie werd gebruikt om verschillen in temperatuur tussen de CRPS kant en contralateraal te meten. De secundaire uitkomst maten waren: Pijn, gemeten met een VAS en spierkracht gemeten met een MicroFet 2 dynamometer. Een activiteitenmonitor, verschillende looptesten en vragenlijsten werden gebruikt om het nivo van activiteiten te meten.
Hoewel er na de twaalf weken geen significante verbetering was in de tadalafil groep in vergelijking met de placebogroep, was er wel een significante en klinisch relevante reductie van pijn. De spierkracht verbeterde in beide groepen en de activiteitenmonitor liet zien, dat er een aantal kleine, maar niet significante verbeteringen waren in het lopen: de tijd staand of lopend doorgebracht en het aantal korte loopperioden waren toegenomen.
De conclusie van het dit onderzoek was dat tadalafil een veelbelovende nieuwe behandeling is voor patiënten met de koude vorm van chronische CRPS ten gevolge van disfunctie van het endotheel. Dit moet echter nog verder onderzocht worden.

De discussie, waarin de resultaten van onze studies worden besproken, staat in hoofdstuk 8. Zoals verwacht blijken verschillende mechanismen verantwoordelijk te zijn voor

de ischemie en de pijn bij de chronische koude vorm van CRPS. De verminderde doorbloeding kan veroorzaakt worden door disfunctie van de sympathicus, door overgevoeligheid voor catecholamines of door disfunctie van het endotheel. De pijn kan neuropathisch, inflammatoir, nociceptief of functioneel zijn, maar ook een mix van verschillende factoren zijn. De basis van de symptomatische therapie moet uiteraard het onderliggende mechanisme zijn.
In CRPS blijkt het verschil in temperatuur tussen de beide extremiteiten te fluctueren in de tijd. Daarom, als de patiënt aangeeft dat de extremiteit overwegend koud is, maar de momentopname met de thermometer of de videothermograaf geen verschil laat zien, moet meer waarde gehecht worden aan de rapportage van de patiënt.
Een belangrijke rol bij toekomstig onderzoek op dit gebied is weggelegd voor het ontwikkelen van een klinisch gemakkelijk toepasbare methode om te differentiëren tussen een centrale en een perifere verstoring van de regulatie van de doorbloeding bij patiënten met de koude chronische vorm van CRPS.

List of publications

List of publications

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Groeneweg JG, Huygen FJPM, Coderre TJ, and Zijlstra FJ: Regulation of peripheral blood flow in Complex Regional Pain Syndrome, submitted.

Dankwoord

Dankwoord

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konden toepassen. En natuurlijk aan Wilmar Antonissen, die opnamen gemaakt heeft en er voor zorgde dat de beelden uitgewerkt werden.

Het bewijs dat de 'inflammatory soup' niet zo heet gegeten wordt als die wordt opgediend, werd geleverd in het proefschrift van Feikje Wesseldijk. Lieve Feikje, het was een eer en groot genoegen om met jou samen te mogen werken. Ik heb veel geleerd van jouw kritisch analystisch denken en ben vol lof over de manier waarop jij met patiënten omgaat.

Beste Dirk, magister Mentos: onze zeer gewaardeerde samenwerking dateert al van heel lang geleden. Het doet mij een groot genoegen dat jij de geschiedenis zal ingaan als degene die de onvolprezen Monte Carlo vergelijkingen geïntroduceerd heeft in mijn verzamelde werken.

Dear Terence Coderre, it was a pleasure discussing the vascular dysregulation in CRPS with you during your last visit to Holland. Thank you for your contribution to the discussion article.

Hans en Fabiënne, dankzij jullie gigantische inzet konden we de Upper Limb Activity Monitor gebruiken om de resultaten van onze trials ook op activiteiten nivo te evalueren.

Fotomodel Juri: bedankt voor het eindeloos poseren voor de ULAM foto's. Geweldig dat je speciaal voor deze foto's een Feijenoord short hebt gekocht.

Marc Mureau en de afdeling Plastische Chirurgie: Bedankt dat jullie me zo geweldig bij de neus hebben genomen! Zonder al die goede zorgen had misschien niet dit boekje, maar in ieder geval wel deze promovendus er heel anders uit gezien.

De medicatie voor het tadalafil onderzoek werd bereid gesteld door Eli Lilly. De apotheek van het Erasmus MC (prof.dr. Arnold G. Vulto en dr. Loes Visser) verzorgde de randomisatie en tevens de medicatie voor de ISDN pilot en trial, waarvoor dank.

Dit onderzoek werd mede ondersteund door het Trauma Related Neuronal Dysfunction (TREND) Consortium. Ik wil van deze grote groep gedreven onderzoekers met name Roberto Perez en Han Marinus bedanken voor de jarenlange geweldige samenwerking.

Talloze patiënten hebben meegewerkt aan dit onderzoek. Een veelvoud van dat aantal heeft screeningsformulieren ingevuld en deze laatste zijn op de poli gekomen voor onderzoek, waarvoor mijn hartelijke dank. Ook de Nederlandse Vereniging voor Posttraumatische Dystrofie Patiënten, en in het bijzonder Ilona Thomasson, heel erg

bedankt voor al jullie ondersteuning.

The ISDN study was sponsored by the American Reflex Sympathetic Dystrophy Syndrome Association. Dear Jim Broach and Peter Moskovitz, it was such a pleasure discussing the research and treatment options for CRPS with you during your last visit, that I almost missed my last train home. Thank you for your support.

Niets is zo heerlijk, als met gezellige mensen muziek maken. Bedankt Prinselijk Dweilorkest BlaoszevandurSokke en Tamboer- en trompetterkorps van het Regiment van Heutsz voor al die heerlijke ontspannen uurtjes. Die vrolijke noot was hard nodig om wat rust te brengen in vijf drukke jaren onderzoek. Hier kon ik nou echt wat je noemt 'uitblazen'.

Lieve Sascha, Wendy en Juri; promoveren is een fantastisch evenement, maar dat valt in het niet bij de impact die jullie op ons leven hebben. Ik was al trots bij de geboorte van ieder van jullie en dat wordt alleen maar meer bij alles wat jullie doen. Bedankt voor jullie hulp om dit promotieonderzoek mogelijk te maken.

Lieve Anneke, in de 24 jaar dat we samen zijn is er heel veel gebeurd. We zijn samen naar Zwitserland vertrokken en met een heel gezin terug gekomen. In de laatste jaren zijn we door van alles uit elkaar gegroeid. Toch hoop ik dat we die kloof weer kunnen dichten. Bedankt voor alles wat geweest is en alles wat nog gaat komen....

Zo kids, de theoretische bergen zijn beklommen, het boekje is klaar, dus nu wordt het tijd om de echte bergen in te gaan! Op naar de Hohe Kasten?

Curriculum Vitae

Curriculum Vitae

George Groeneweg werd op 5 juli 1956 geboren te Dordrecht. Hij groeide op in Rotterdam en Middelburg, en behaalde het havo diploma in 1975 in Middelburg. Hij studeerde in 1979 af aan de Hogeschool voor Fysiotherapie in Vlissingen.
Na een aantal waarnemingen in verschillende fysiotherapie praktijken volgde in 1980 de militaire dienst als fysiotherapeut op de Militair Geneeskundige Dienst van het Provinciaal Militair Commando te Vught. Een waarneming bij het instituut voor geestelijk en visueel gehandicapten "de Blauwe Kamer" te Breda leidde uiteindelijk tot een part time baan op de Mytylschool in Dordrecht.
Gedwongen door de arbeidsmarkt werd in 1984 de blik naar het buitenland gericht, en vertrok hij naar Zwitserland. Hij was eerst werkzaam in de Geriatrische Klinik vom Bürgerspital St. Gallen, en vervolgens tot 1993 op de afdeling Medizinische Physiotherapie vom Kantonsspital St. Gallen. In deze periode werden tal van cursussen gevolgd, waaronder de intensive care opleiding, hartrevalidatie, sportfysiotherapie, McKenzie, Autogene Drainage en Neuro Developmental Therapy. Ook werd een eerste wetenschappelijk onderzoek opgezet naar de effecten van het inhaleren met pressure PEP bij COPD. Daarnaast was hij organisator en instructeur van verschillende cursussen over rugscholing en over ademhalingsproblematiek.
In 1993, inmiddels getrouwd met Anneke, en trotse vader van drie kinderen: Sascha, Wendy en Juri, werd hij aangenomen in de Daniel den Hoed Kliniek in Rotterdam (later AZR-Daniel, en nu Erasmus MC Daniel). Hier was hij onder meer werkzaam aan het Centrum voor Onderzoek naar Palliatieve Zorg. Verschillende cursussen werden gevolgd, nu vooral op het gebied van wetenschappelijk onderzoek, en hij maakte deel uit van de werkgroep peri- en paramedisch onderzoek van het AZR. De cursussen "Scholing in Wetenschap I - III" van het Nederlands Paramedisch Instituut werden afgesloten met het onderzoek: "validiteit en responsiviteit van het meten van spierkracht met behulp van MicroFet dynamometer bij patienten met een partiële dwarslaesie." Tevens werd een artikel geschreven over fysiotherapie in de palliatieve zorg.
Toen Frank Huygen in 2000 een nieuwe opzet maakte voor het Pijnbehandelcentrum, werd hij gevraagd om mee te werken aan het multispreekuur. Tevens kwam hij in dienst van het Pijnkenniscentrum om een netwerk van eerste lijns fysiotherapeuten te vormen. Speciaal voor dit netwerk werd de cursus 'fysiotherapie en chronische benigne pijn" opgezet, en gehouden in 2004 en 2006.
In 2004 is hij begonnen met dit promotie onderzoek naar de effecten van vasoactive substanties in het Complex Regionaal Pijn Syndroom, mogelijk gemaakt door een subsidie van het Ministerie van Economische Zaken aan het Trauma Related Neuronal Dysfunction (TREND) Consortium.

PhD Portfolio

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: J. George Groeneweg	PhD period: 2005 - 2009
Erasmus MC Department:	Promotor: Prof. dr. J. Klein
Anesthesiology	Copromotor: dr. F. J. Zijlstra
	dr. F.J.P.M. Huygen

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- Biomedical English Writing and Communication	2005	4 ECTS
- Research Integrity	2009	2 ECTS
Research skills		
- NIHES course 'Classic methods for Data analysis'	2005	5.7 ECTS
- CPO Minicursus 'Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidieaanvragen'	2005	5 hrs
- Workshop Meta-analysis EPOS	2006	5 hrs
International conferences		
Second International Congress on Neuropathic Pain, Berlin, Germany.	2007	30 hrs
Symposium CRPS vereniging	2008	8 hrs
Seminars and workshops		
Trend Consortium 'Annual meeting'	2006	8 hrs
Trend Consortium 'Annual meeting'	2007	8 hrs
PAOG symposium Nijmegen 'CRPS'	2007	5 hrs
PHD dag Erasmus MC	2008	6 hrs
Nederlandse Vereniging voor Microcirculatie en Vasculaire biologie: 'Annual meeting'	2009	8 hrs
Trend Consortium 'Annual meeting'	2009	8 hrs

2. Teaching activities

	Year	Workload (Hours/ECTS)
Maart symposium Willemstad	2005	8 hrs

'Behandeling van Complex Regionaal Pijn Syndroom (CRPS) door vorming van stikstofoxide'

Mei Regionale CRPS patientenvereniging Gilze Rijen 8 hrs
'CRPS onderzoek Erasmus MC, een voorbeeld: Nitro oxide'

November Regionale CRPS patientenvereniging Dordrecht 3 hrs
'CRPS onderzoek Erasmus MC, een voorbeeld: Nitro oxide'

Organisatie PBC/PKC cursus 2006 200 hrs
'Fysiotherapie en chronische benigne pijn'.

Februari: Organon; Oss 25 hrs
'Vascular Tone in Complex Regional Pain Syndrome'

Maart: Trend Consortium 20 hrs
'Vaatverwijders bij chronische koude CRPS'

April: Regionale patientenvereniging; Middelburg 3 hrs
'CRPS onderzoek Erasmus MC, een voorbeeld: Nitro oxide'

Mei: Landelijke CRPS Patientenvereniging; Lunteren 20 hrs
'Videothermografie bij CRPS'

Juni: afdeling Revalidatie Erasmus MC 18 hrs
'Vascular tone in Complex Regional Pain Syndrome'

Juni: Hogeschool Nijmegen, symposium 30 hrs
'Het effect van vasocactieve medicatie bij chronische koude CRPS'

Juli: Kantonsspital St. Gallen Zwitserland afdeling Physiotherapie Innere Medicin 40 hrs
'Gefässtonus im CRPS'

Oktober: Pan European Pain Specialists; Rotterdam *'Vascular Tone in Complex Regional Pain Syndrome'*		25 hrs
Maart: TREND symposium Hoeven *'Vasodilatatie in de chronische koude fase van CRPS: een update'*	2007	20 hrs
Juni: Second International Congress on Neuropathic Pain, Berlin, Germany: Posterpresentatie: *'Nitric Oxide improves blood flow, pain and function in Complex Regional Pain Syndrome'*		40 hrs
September: Trend Midterm Review Leiden *'Vascular Tone in Complex Regional Pain Syndrome'*		25 hrs
September: RSDSA presentatie: *'Vascular Tone in Complex Regional Pain Syndrome'*		20 hrs
November: Handtherapie opleiding Erasmus MC: *'CRPS'*		15 hrs
Januari: afd fysiotherapie Erasmus MC: *'CRPS'*	2008	15 hrs
Januari : meeting Nederlandse Vereniging voor Microcirculatie en Vasculaire biologie:Biezenmortel presentatie: *'Cold Case: Vascular dysregulation in the chronic Complex Regional Pain Syndrome'*	2009	20 hrs
27 maart: TREND symposium: *'Endothelial dysfunction in cold chronic CRPS: results of the Tadalafil trial'*		20 hrs

Other

- TREND meetdagen (4 per jaar)	2004 – 2009	4 hrs each
- Wekelijkse research meeting afd Revalidatie Erasmus MC	2006 – 2009	1 hr each
- Maandelijks research overleg Pijnbehandelcentrum	2004 – 2009	1 hr each

Mijn dank gaat uit naar de volgende sponsoren,
die mijn promotie mede mogelijk hebben gemaakt:

Biomet Nederland

Boehringer Ingelheim

Eli Lilly Nederland

Medtronic

Merck Sharp & Dohme

Nederlandse Vereniging van Posttraumatische Dystrofie Patiënten

Pfizer

St. Jude Medical