General discussion and future perspectives

Partly based on:

How to position pasireotide LAR treatment in acromegaly?

Coopmans EC, Muhammad A*, van der Lely AJ, Janssen J, Neggers S*

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**Shared first authorship*

INTRODUCTION

The main goal of this thesis was to study the position of pasireotide LAR in the medical management of acromegaly in relation to pegvisomant. To meet this goal we conducted the pasireotide LAR and pegvisomant (PAPE) study with the aim to assess the efficacy and safety of switching to pasireotide LAR with and without PEGV treatment in acromegaly patients controlled with SRL and PEGV combination treatment. The results of the PAPE study were discussed in *Chapters 3 and 4*. Based on the PAPE study we formulated our recommendations on the optimal position of pasireotide LAR in the medical treatment algorithm of acromegaly. In *Chapter 5* we assessed whether the responsiveness to pasireotide LAR treatment was correlated with the responsiveness to first-generation SRL treatment and whether this also correlated with the somatostatin receptor subtype protein expression of the somatotroph adenoma. In *Chapter 6* we investigated acylated and unacylated ghrelin levels in acromegaly patients during combination treatment. Finally, in *Chapter 7* we undertook a pilot study to examine whether Kupffer cells are involved in the clearance of pegvisomant in vivo. This chapter will review the strengths and weakness of the studies presented in this thesis. The section ends with discussion on future developments and perspectives on new medical treatments of acromegaly.

Pegvisomant monotherapy

In **Chapter 2** we aimed to assess the efficacy and safety of withdrawing SRL treatment and switching to weekly PEGV monotherapy in 15 acromegaly patients controlled with combination treatment. We observed that switching to weekly PEGV monotherapy is a feasible treatment strategy without compromising biochemical control. After 12 months of PEGV monotherapy 73% of patients had IGF-I levels within the normal range. The efficacy of switching to PEGV monotherapy depends on the PEGV dose at baseline and the duration of SRL treatment. In this study we deliberately included patients that used low doses of PEGV of ≤ 80 mg/week, as we expected that these patients could maintain control of disease activity in the short term after withdrawal SRL treatment. We observed that IGF-I levels started to increase 4 months after SRL treatment discontinuation. This so-called carry-over effect of SRLs confirms observations in previous studies (1-3). We observed a large variation in PEGV serum levels between controlled and non-controlled subjects which was remains not well understood (4). The major drawback of this study is the small sample size.

Pasireotide and pegvisomant study

The aim of the PAPE study was to assess the real life efficacy and safety of switching to PAS-LAR in acromegaly patients biochemically controlled with SRL and PEGV combination treatment. The patients included in this study all received SRL and PEGV

combination treatment because they were partially to completely resistant to SRL monotherapy. To assess the efficacy of PAS-LAR on IGF-I normalization patients had to be 'uncontrolled' which was achieved by reduction of the PEGV dose by 50% for 3 months. We found that PAS-LAR treatment normalized IGF-I levels in 77% of patients and the PEGV dose could be reduced by 50% at 48 weeks. While the efficacy of PAS-LAR at 24 weeks is partly influenced by the carry-over effect of first-generation SRLs which can take up to 4 months, at 48 weeks this was not the case. We observed a heterogeneous and wide range of clinical response to PAS-LAR treatment. This ranged from patients who were overresponsive to PAS-LAR and could reduce PAS-LAR to the lowest available dose of 20 mg, and patients who were completely unresponsive and could not reduce their high PEGV dose. This heterogeneity is illustrated by the observation that the patients in the PAS-LAR monotherapy group had a lower disease activity at baseline, with lower PEGV dose, IGF-I levels and GH levels compared with patient in the PAS-LAR and PEGV combination group.

An important observation in our study was the potent reduction of IGF-I after the first PAS-LAR injection. Although this early onset of effect of PAS-LAR is to some extent influenced by the carry-over effect of SRL treatment, this rapid reduction is probably caused by suppression of insulin secretion. The concomitant suppression of IGF-I secretion and insulin secretion after the first two PAS-LAR injections suggests that suppression of insulin is an important mechanism which explains the efficacy of PAS-LAR. Another explanation is that unlike octreotide, pasireotide induces less SST_2 internalization which could imply that there is a lower likelihood desensitization to pasireotide treatment.

Safety

Hyperglycemia and diabetes mellitus

The most common and clinically relevant adverse event during the PAPE study was the development of hyperglycemia and diabetes mellitus. At baseline 32.8% of patients had diabetes in our cohort, which increased to 77% at the end of the study. The majority of patients developed mild to moderate hyperglycemia which occurred mainly during the core study and was manageable with a combination of metformin and or vildagliptin. Several reasons account for the higher frequency of hyperglycemia in our study compared with previous clinical studies (5-8). First we included more patients with diabetes, as we used a more liberal HbA1c cut-off (<9%) as inclusion criterion. Secondly, we included older patients and more patients with pre-existent diabetes who were already on antidiabetic medication. Third, the reduction of the PEGV dose during the study progressively worsened glucose metabolism.

As expected, and in line with previous studies (7, 9), the development of severe hyperglycaemia after PAS-LAR treatment was correlated with baseline HbA1c and use of antidiabetic medication (*Chapter 3*). In order to find better predictors for pasireotide-induced hyperglycaemia we performed an oral glucose tolerance test (OGTT) at baseline. We estimated ß-cell function as a measure for insulin secretion based on a model by *Stumvoll et al* (10, 11). An important caveat for the interpretation of insulin secretion during the OGTT is the use of antidiabetic medication. While we excluded the use of insulin as an obvious confounder, the results should be interpreted with caution in patients using sulfonylureas and GLP-I analogues as both antidiabetic drugs increase insulin secretion (12, 13). It was not feasible to discontinue all antidiabetic drugs before the OGTT. A novel finding is that we observed an inverse correlation between both the insulin area under the curve and the (residual) ß-cell function at baseline with the increase in HbA1c between baseline and at 24 weeks (*Chapter 4*). This entails that patients with attenuated (first-phase) insulin secretion had a greater risk of pasireotide-induced hyperglycaemia during the study. These data demonstrate that the baseline ß-cell function is an important independent determinant for hyperglycaemia during PAS-LAR treatment and probably a better marker than single measurements of HbA1c or fasting plasma glucose level. We do not know to what extent GLP-I secretion accounts for the increase in HbA1c levels during the study, as we did not measure GLP-I levels. Of note, the involvement of incretin response was previously suggested by van der Hoek et al (14). They observed that a single injection of short acting pasireotide elicited an acute increase in glucose levels after pasireotide injection in acromegaly patients, which could not be exclusively explained by concomitant insulin suppression, suggesting the involvement of the incretin response (14).

The patients using PAS-LAR and PEGV combination treatment did not have a lower HbA1c level than patients using PAS-LAR monotherapy. This observation suggests that the insulin sensitizing effect of PEGV cannot compensate for the insulin and incretin suppressive effect of PAS-LAR.

Our study was not designed to evaluate which antidiabetic treatment was most effective for pasireotide-induced hyperglycaemia. Previous studies have recommended DPP-4 inhibitors and GLP-I analogues for treatment of pasireotide-induced hyperglycemia. Following the Dutch reimbursement regulations we could only start DPP-4 inhibitors and GLP-I analogues after failure to achieve glycaemic control with maximum doses of metformin and sulfonylurea. However, ideally initiation of incretin-based antidiabetic drugs is the first-line treatment of choice.

Exocrine pancreatic insufficiency

We observed a higher frequency of steatorrhea during PAS-LAR treatment in acromegaly patients than reported previously in clinical trials (5, 6). This adverse event was in general transient and improved with pancreatic enzyme supplementation (*Chapter 3*). Inhibition of exocrine pancreatic function is a well-known (side) effect of SRLs (15). To our knowledge, there are no studies that directly compared the effect of octreotide versus pasireotide on exocrine pancreatic function. But, because of its broader SSTR binding profile, pasireotide has been suggested to be more effective than octreotide in suppressing exocrine pancreatic function (16). For example, pancreatic surgeons have used pasireotide to suppress leakage of exocrine secretions following pancreatectomy (17), with the aim to reduce the risk of pancreatic fistula formation, a major cause of mortality and morbidity for these patients (18).

Costs

We hypothesized that the PEGV sparing effect of PAS-LAR would result in a reduction in treatment costs. Although we have not performed a cost-effectiveness analysis, it is likely that switching to PAS-LAR will not lead to a long-term reduction of overall treatment costs. As mentioned in the discussion of **Chapter 3**, the PEGV sparing effect of PAS-LAR would likely be offset by the diabetes mellitus related medication and healthcare costs.

Current consensus guideline second line medical treatment

In 2018, the Acromegaly Consensus Group published updated consensus guidelines with recommendations on the medical management of acromegaly (19). The consensus statement recommended medical treatment for patients with persistent disease activity despite surgical resection of the adenoma and patients in whom surgery is not advisable (19). In line with the previous consensus statement published in 2014 (20), first-generation SRLs remain the first-line treatment for patients with persistent disease activity after surgery. The new consensus guideline recommends either PAS-LAR or PEGV treatment based on the presence or absence of clinically relevant residual tumour and impaired glucose tolerance. PEGV was recommended for patients with impaired glucose metabolism and/or those who experience worsening hyperglycemia on SRL treatment. PAS-LAR was recommended for patients with a clinically relevant residual tumour unsuitable for resection and without impaired glucose tolerance. However, in patients with a clinically relevant residual tumour and impaired glucose tolerance SRL and PEGV was recommended (19). In the next paragraphs we will outline our expert-opinion based clinical recommendations for the medical management of acromegaly with a focus on the position of PAS-LAR and the differences with the current consensus guideline.

Recommendations for the medical management of acromegaly

In figure 1 we present our recommendations for the second line medical management of acromegaly based on our clinical experience with PEGV and PAS-LAR (*Chapter 3 and 4*) and clinical evidence from previous studies. In accordance with the consensus guideline (19), first-generation SRLs such as lanreotide autogel (ATG) and octreotide LAR are considered the first-line medical treatment of acromegaly (figure 1).

Second-line and alternative treatments

In line with the consensus guideline (19), we recommend PEGV combined with or substituted for first-generation SRL treatment as the second-line treatment of choice in patients with <20% IGF-I reduction (no significant response) during first-generation SRL monotherapy (figure 1).

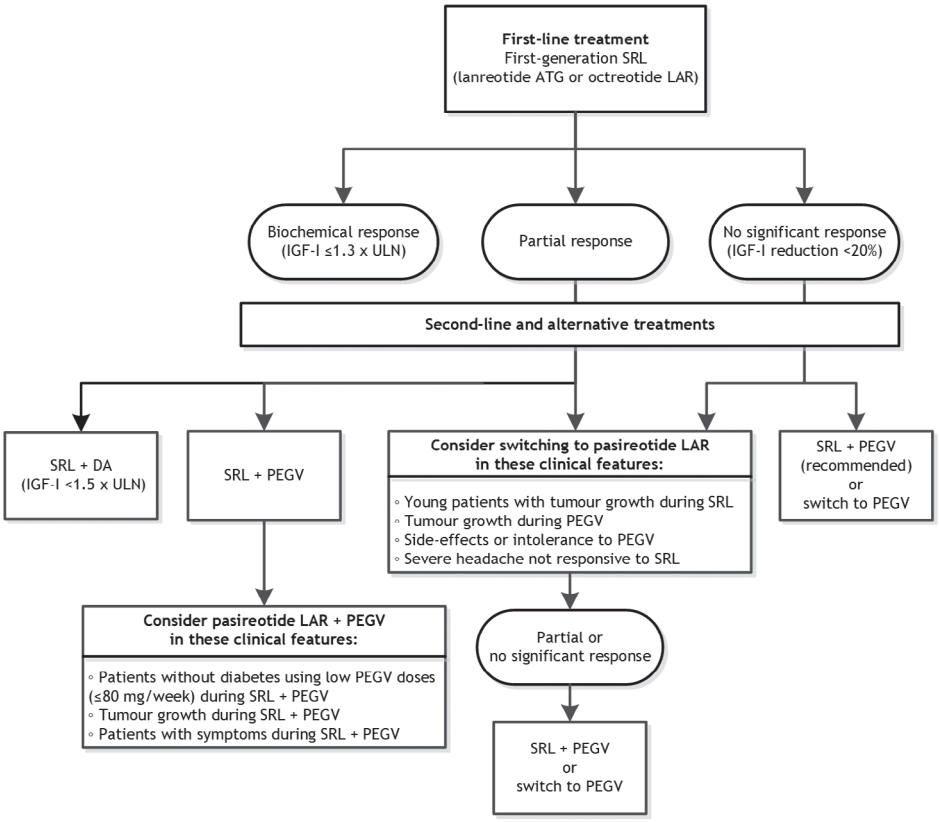

Figure 1. Proposed algorithm for the medical management of acromegaly. Radiation therapy is not mentioned in this algorithm, but it should be considered in patients with biochemically persistent disease and/or tumour growth despite surgery or medical therapy. Abbreviations: ATG, Autogel; DA, dopamine agonist; IGF-I, insulin-like growth factor 1; LAR, long-acting release; SRL, somatostatin receptor ligand; PEGV, pegvisomant; ULN, upper limit of normal.

PEGV, alone or in combination with first-generation SRL, can normalize IGF-I levels in the majority of patients, provided that the PEGV dose is sufficiently increased. In combination treatment the recommended starting dose with either lanreotide ATG or octreotide LAR every 4 weeks is the highest approved dose of respectively 120 mg and 30 mg. To determine the optimal PEGV starting dose to achieve IGF-I normalization, the prediction formulas of *Franck et al* can be used (21).

In contrast to the current consensus guideline (19), we recommend first-generation SRLs and PEGV combination treatment as the second-line treatment of choice in all non-responders (figure 1). Combination treatment has the advantage of a lower required PEGV dose to normalize IGF-I levels compared with PEGV monotherapy (23-25). Due to PEGV dose reduction it may reduce the injection frequency for patients. In a subset of patients, combination treatment might improve QoL compared to SRL monotherapy, including those who are biochemically controlled (26). PEGV monotherapy does not increase tumour size, and combined with SRLs it does control tumour size and may induce tumour shrinkage in a vast majority of patients (25). Another advantage of SRL and PEGV combination treatment is that SRLs are generally effective in resolving headaches through a proposed mechanism of inhibition of nociceptive peptides, which makes it the preferable treatment for patients with headache (27, 28). In general, if patients report improvements in clinical symptoms during SRL monotherapy, we recommend imitating SRL/PEGV combination treatment, except in those patients with poorly controlled or brittle diabetes during SRL treatment. These patients are good candidates for PEGV monotherapy, because PEGV improves glucose metabolism by reducing insulin resistance (29-34). This is in accordance with the current consensus guideline (19), which also recommends PEGV monotherapy in patients without biochemical response to first-generation SRL treatment, but with pre-existing impaired glucose metabolism.

In patients with partial response to first-generation SRLs, defined as a significant reduction in IGF-I levels without normalization, we recommend combination treatment with cabergoline (if IGF-I ≤1.5 x ULN) (35) (figure 1). In contrast to the current consensus guideline, who propose considering co-treatment with cabergoline (if IGF-I <2.5 x ULN) in patients with inadequate control on first-generation SRL treatment, we recommend co-treatment with cabergoline if IGF-I levels remains modestly elevated (IGF-I ≤1.5 x ULN), since IGF-I normalization was observed only in those patients (35). Besides, cabergoline is recommended in patients with no access to PEGV. Although the use of PEGV, combined with or substituted for first-generation SRLs in partial responders, is not covered by the current guidelines (19), we recommend SRL and PEGV combination treatment as the second-line treatment of choice (Figure 1).

Moreover, due to the marginal additional efficacy of dose escalating and shortening of the dosing intervals of SRLs in partial SRL responders (2, 36, 37), we recommend switching these patients to SRL and PEGV combination treatment or to consider pasireotide LAR monotherapy according to the proposed algorithm (figure 1).

Switching to pasireotide LAR monotherapy can be considered as an alternative to PEGV monotherapy or combination therapies in patients with the following baseline clinical characteristics (Figure 1):

- Macroadenomas in young patients (aged <40 years) that show tumour growth during first-generation SRL monotherapy (i.e., clinically aggressive tumours) (38). Pasireotide LAR monotherapy should be considered early in the management of resistant tumours in young patients, and as a treatment step before starting radiotherapy. This is in line with the current consensus guideline (19), which recommends switching from first-generation SRL treatment to pasireotide LAR monotherapy in patients with a clinically relevant residual tumour that is insuitable for resection.
- The same argument can be applied for patients previously not controlled by first-generation SRLs with tumour growth during PEGV monotherapy. Tumour growth may reflect the presence of an aggressive tumour, for that reason pasireotide LAR monotherapy can be considered as a next treatment step before starting radiotherapy.
- Patients previously not controlled by first-generation SRLs who experience side-effects or who are intolerant to PEGV monotherapy, may benefit from switching to pasireotide LAR monotherapy.
- Patients with headache not responsive to first-generation SRL treatment. These patients with severe incapacitating headache have high a likelihood to experience improvement in headache symptoms with pasireotide LAR treatment.

Instead of PEGV, either as monotherapy, or in combination with first-generation SRL, switching to pasireotide LAR therapy combined with PEGV can be considered as third-line treatment option in patients with the following baseline clinical characteristics (Figure 1):

- Patients without diabetes that use low PEGV doses (≤80 mg/week) during combination therapy with first-generation SRL and PEGV. Due to the PEGV sparing effect of pasireotide LAR, the PEGV doses can be reduced or some cases even discontinued.
- Patients with tumour growth during first-generation SRL and PEGV combination therapy. We postulate that pasireotide LAR monotherapy may improve tumour size control or even tumour shrinkage. However, at present there are no data on tumour response to pasireotide LAR and PEGV combination therapy available.
- Patients biochemically controlled during first-generation SRL and PEGV combination therapy, who use first-generation SRL every 3 weeks or have symptoms of active

acromegaly in the fourth week after first-generation SRL administration, may experience symptomatic improvement after switching to pasireotide LAR and PEGV combination therapy.

The package insert recommends starting pasireotide LAR 40 mg every four weeks (39), but we recommend to start with either 40 or 60 mg pasireotide LAR every four weeks. We expect that patients with SRL partial responders will require lower doses of pasireotide LAR, and then we recommend starting with pasireotide LAR 40 mg. For combination treatment with PEGV we recommend initiation of pasireotide LAR at a dose of 60 mg every four weeks while at the same time tapering down the PEGV dose by 33%.

In our experience, IGF-I levels decrease more rapidly with pasireotide LAR than with octreotide LAR or lanreotide ATG after treatment initiation. While it can take up to 6 months for IGF-I levels to normalize with first-generation SRLs therapy, pasireotide LAR induces a significant reduction in IGF-I levels within 2 months (40), and the early response is associated with the more long-lasting response at 48 weeks (41). If biochemical control cannot be achieved by pasireotide LAR monotherapy, then either switching to PEGV monotherapy or combination treatment with first-generation SRLs should be considered (figure 1). The same applies for pasireotide LAR and PEGV combination treatment, but in this case the patients are switched back to their previous PEGV dose, alone or combined with SRL treatment.

Management of pasireotide-induced hyperglycemia

Optimal management of hyperglycemia during pasireotide LAR therapy is essential because of several reasons. First, optimal management is required to initially control diabetes as a long-term risk factor for cardiovascular disease in acromegaly. Secondly, early onset proactive management of hyperglycemia after pasireotide LAR initiation is likely to improve patient long-term compliance with pasireotide LAR. A wider use of pasireotide LAR in the medical management of acromegaly depends on the physician's willingness to try pasireotide LAR and to applicate it in the appropriate patients. The challenge for the endocrinologist is to evaluate in each individual patient whether advantages of pasireotide LAR such as higher likelihood of biochemical control, tumour size control and quality of life outweigh side effects of hyperglycemia and diabetes. We provide recommendations to achieve optimal management of pasireotide-induced hyperglycemia in figure 2. In contrast to previous published recommendations for management of pasireotide-induced hyperglycemia (42-45), we use a more liberal strategy in frequency of monitoring our patients.

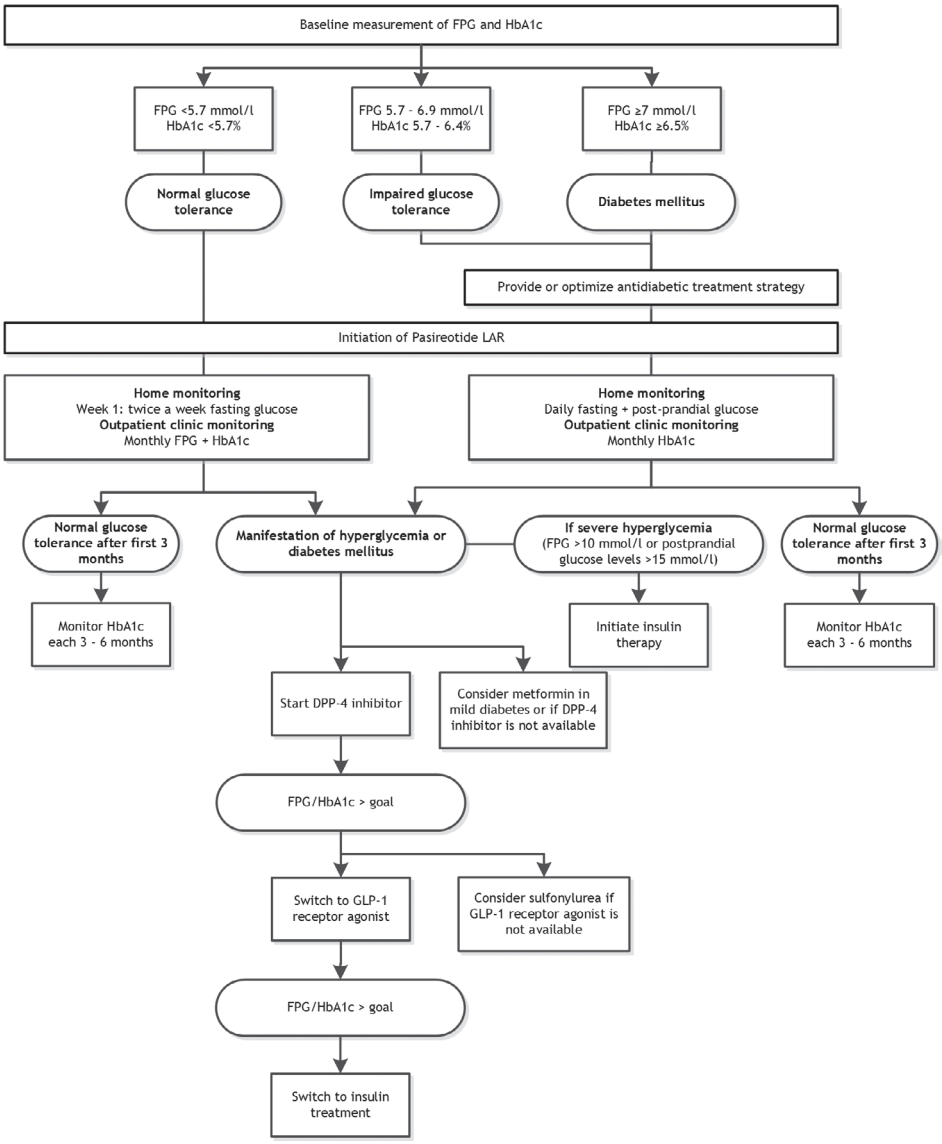

Figure 2. Proposed algorithm for management of pasireotide-induced hyperglycemia. Abbreviations: DPP-4, dipeptidyl peptidase-4; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide 1; HbA1c, glycated haemoglobin, LAR, long-acting release.

Baseline HbA1c is the most important predictor for development of diabetes after pasireotide LAR administration (7, 9, 40, 41). We recommend that baseline FPG and HbA1c levels should be assessed prior to initiating pasireotide LAR treatment. In case of impaired fasting glucose and/or diabetes at baseline, lifestyle management and/or adequate antidiabetic treatment should be started or optimized before initiating pasireotide LAR. Clinical studies have consistently shown that after pasireotide LAR

initiation FPG and HbA1c levels rise the first 3 months, and remain stable without further antidiabetic therapy (7, 9, 39, 46, 47). Therefore, proactive blood glucose monitoring is important, especially in the first 3 months after initiation. After the first 3 months of pasireotide LAR treatment, the intensity of blood glucose monitoring can be decreased. In contrast to *Wildemberg* (44) and *Samson* (43), who propose home blood glucose monitoring twice a week for 3 months for patients with normoglycemia, and we recommend monitoring at the outpatient clinic with monthly FPG and HbA1c the first 3 months. Because of the low likelihood of deterioration of glucose tolerance in young patients (aged <40 years) with normal glucose tolerance at baseline, this latter group can be monitored less intensively and with a lower frequency (figure 2). However, patients with pre-existing impaired glucose tolerance and diabetes, which make up the majority of acromegaly patients, require more extensive monitoring. These patients should receive home blood glucose monitoring the first 3 months by measurement of daily fasting and 2-hours postprandial glucose levels, and monthly HbA1c monitoring at the outpatient clinic. For diabetic patients using insulin, we advise continued home blood glucose monitoring with additional HbA1c monitoring especially at the start of pasireotide LAR treatment, and thereafter every 3 months in accordance with the current American Diabetes Association (ADA) guidelines (48).

If glucose monitoring reveals glucose in the diabetic range per ADA criteria, we recommend first-line treatment with a dipeptidyl peptidase-4 (DPP-4) inhibitor and to consider metformin in patients with mild diabetes or if DPP-4 inhibitors are not available (figure 2). If patients do not achieve their ADA criteria based FPG/HbA1c goal with these interventions, we recommend switching to a GLP-1 receptor analogue once daily. If a GLP-1 receptor agonist is not available, we recommend treatment with sulfonylureas. Treatment with insulin is required in patients intolerant to GLP-1 analogues or if GLP-1 analogues fail to provide glycemic control. In case of severe hyperglycemia (i.e., FPG >10 mmol/l or postprandial glucose levels >15 mmol/l), which may occur even after the first injection, it is important to promptly initiate insulin treatment. In our experience, diabetic patients using insulin treatment or high doses of oral antidiabetic agents have a high risk of severe hyperglycemia after the first injection of pasireotide LAR. On the other hand, in nondiabetic patients the onset of hyperglycemia occurs later during treatment and is less severe. Therefore, it is paramount to be aware of early-onset severe hyperglycemia and promptly initiate antidiabetic therapy, but also to be vigilant about the development of late-onset hyperglycemia and diabetes.

Pasireotide-induced hyperglycemia is reversible after discontinuation of pasireotide LAR (44). Our data indicate that within 8 weeks after discontinuation of pasireotide

LAR and re-initiation of first-generation SRL or PEGV, FPG and HbA1c levels return to baseline (40). To prevent hypoglycemia in patients using insulin or sulfonylureas, it is recommended to gradually taper down these antidiabetic agents with more frequent blood glucose monitoring. Sulfonylureas should be rapidly tapered down within the first 2 weeks to avoid hypoglycemia, whereas GLP-I receptor analogues can be safely continued with a lower dose for a longer period.

Prediction of pasireotide LAR treatment response in acromegaly

We hypothesized that SST_5 protein expression of the somatotroph adenoma was correlated with clinical responsiveness to PAS-LAR in acromegaly. However, in our cohort we observed a positive correlation between the efficacy of PAS-LAR and the efficacy of first-generation SRLs. This was confirmed by the observation that SST_2 protein expression of the somatotroph adenoma, and not of SST_5, correlated with clinical responsiveness to PAS-LAR. Moreover, in the same patients we found a strong correlation between the percentage of IGF-I reduction after first-generation SRL treatment and response to PAS-LAR treatment during the PAPE study. This does not mean that pasireotide and octreotide/lanreotide are equally effective in reducing IGF-I levels. The effect of pasireotide and octreotide on GH suppression is superimposable both in vitro and in vivo, but pasireotide is more effective in reducing IGF-I levels in vivo (5, 49). In other words, overall, patients who responded to first-generation SRLs also seemed to respond to PAS-LAR treatment and vice-versa. The latter is illustrated by our observation that the three patients using the highest doses of PEGV could not reduce their PEGV dose after switching to PAS-LAR (*Chapter 4*). Our observation that SST_2 is the main receptor driving the biochemical responsiveness to PAS-LAR treatment builds on previous preclinical studies in primary cultures of somatotroph adenomas that pasireotide exerts its anti-secretory effects mainly via activation of SST_2 (49-52). Besides its broader binding affinity for the different SST_2 subtypes, pasireotide activates intracellular second messenger pathways and modulates SST internalization and trafficking in a manner different from SRIF and octreotide (53, 54). For example, compared with octreotide, pasireotide induces less SST_2 internalization and quicker recycling of SST_2, which counteracts SST_2-desensitization. These findings indicate that the mere concept of binding affinity is too limited to explain the complex biological activity of pasireotide. It is important to clarify that our results contrasted findings of *Iacovazzo et al* who found that SST_5 expression on somatotroph adenomas correlated with responsiveness to PAS-LAR (55). As discussed in *Chapter 5*, the opposing results between the Iacovazzo study and our study is related to a different selection of patients. In our study we included patients who were mainly partially responsive to SRLs who received PEGV treatment, while *Iacovazzo et al* used only SRL resistant patients.

Our results suggest that SST_2 is the main driver of GH suppression to pasireotide in somatotroph adenomas, but we cannot exclude the involvement of SST_5 in GH suppression (49, 55). In general, in patients that are partial responders to SRLs, pasireotide acts mainly via SST_2. In presence of low SST_2 expression or in adenomas resistant to SST_2 targeting, pasireotide may also act via SST_5, while this is not the case for first-generation SRLs. Therefore, we can understand that in completely resistant patients (55) we observe an effect mainly driven via SST_5. As recently carefully reviewed by *Gatto et al* (56), using the classification of acromegaly proposed by *Cuevas-Ramos et al*, pasireotide mainly acts via SST_2 in type 1 disease (good response to first-generation SRLs, high SST_2 expression), while its effects could also be mediated via SST_5 in type 2 and type 3 acromegaly (unsatisfactory response to first-generation SRLs, low SST_2 expression).

Ghrelin acromegaly

In **Chapter 6** we investigated levels of plasma acylated ghrelin (AG), unacylated ghrelin (UAG), and the AG/UAG ratio in three groups of patients with acromegaly; in patients using SRL and PEGV combination treatment, patients using PEGV monotherapy, and medically naïve patients. We observed that both AG and UAG were suppressed during PEGV and SRL combination treatment and in some patients even undetectable. The underlying mechanism is probably a direct inhibitory effect of somatostatin on ghrelin secretion (57). Interestingly, the positive feedback action of PEGV on ghrelin secretion as observed in our study and by *Roemmler et al* (58) cannot offset the ghrelin suppressive effects of somatostatin. This observation mirrors our observation in the PAPE study that the insulin suppressive effect of pasireotide LAR cannot be compensated by the improvement in insulin sensitivity by treatment with pegvisomant.

We observed that the AG/UAG ratio was similar irrespective of medical treatment. Secondly is that the assessment of the AG/UAG ratio is clinically more relevant than measurement of total ghrelin, because the total ghrelin assay is unspecific as it measures a combination of inactive AG/UAG fragments and the full-length peptide. An advantage of the AG/UAG ratio is that it takes into account the different and sometimes opposing biological actions of AG and UAG. An important limitation of this study was the low number of patients included in the control groups. Also, in general there is a lack of the healthy volunteer data on AG and UAG levels measured with the two-site sandwich ELISA. Future studies should account for this.

Pegvisomant clearance

In the 1970s *Davis et al* described PEGylation as a means to decrease the immunogenicity of non-human proteins (59-61). Since then at least 15 registered PEGylated

drugs are in clinical use, and more are under clinical investigation (62). Although PEGV has been used in clinical practice for over a decade and is one of the most PEGylated drugs available, it is not well understood how PEGV is metabolized and cleared from the body. Understanding PEGV clearance could be important to understand the differences in PEGV serum levels between patients. It is known that the presence of several polyethylene glycol (PEG) molecules in the drug reduce the immunogenicity and improve the clearance of PEGV (63). Because PEG is a non-biodegradable molecule it is assumed that PEG molecules are cleared from the circulation by cells from the mononuclear phagocyte system, such as Kupffer cells (64, 65). Animal studies have shown that repeated administration of PEGV was associated with the presence of vacuolated macrophages (66). However, no studies have demonstrated this in patients with acromegaly treated with PEGV. As reported in *Chapter 7* we undertook this study to examine whether PEGV is expressed in the liver, and if so, whether it co-localizes with Kupffer cells. Using hepatic tissue material obtained from two acromegaly patients that were treated with PEGV, we observed immunohistochemical expression of PEG in the sinusoids where Kupffer cells are located. Immunofluorescence studies confirmed that PEG co-localized with Kupffer cells, but not with hepatocytes. The observation that PEG was not observed in hepatocytes is supported by a fundamental study by Tsoi et al (67). They examined the blood clearance mechanism of PEGylated nanomaterials in relation to hepatic cells of rats. They observed that after injection of PEG-coated quantum dots (PQD), PQD were internalized by cell mononuclear phagocyte system (MPS). The highest uptake was observed in Kupffer cells, but no uptake in hepatocytes. However, these data should be interpreted with caution as quantum dots have a substantially smaller molecular size compared to large molecules like PEGV. Kupffer cell activation in both cases can also be caused by liver diseases, which can impact hepatic metabolism of PEGV. Despite the possible confounding effect of liver diseases which could activate Kupffer cells and change PEG metabolism, both patients showed internalization of PEG in Kupffer cells. This suggests that the uptake of PEGV by Kupffer cells is independent of the underlying liver disease. Unfortunately, it is very difficult to obtain additional liver tissue samples of acromegaly patients using PEGV that do not have liver disease. We could also not study the relation between hypertransaminasemia and PEGV treatment (i.e. PEGV-induced hepatotoxicity), because both patients had normal transaminase levels during PEGV treatment. In this study we highlighted the possible role of Kupffer cells in the clearance of PEGV.

GENERAL CONCLUSION OF THESIS

The findings presented in this thesis represent a step forward in our understanding of the position of PAS-LAR in the modern medical treatment of acromegaly. The results of the PAPE study demonstrated that PAS-LAR has a high efficacy, illustrated by a 50% PEGV sparing effect, and substantial number of patients who could switch to PAS-LAR monotherapy. While PAS-LAR was in general well tolerated, the high efficacy came at the expense of a high frequency of hyperglycemia-related adverse events. With respect to the long-term treatment of acromegaly with PAS-LAR, the benefits of biochemical control and the improvement in quality of life should be weighed against the disadvantages of potential (short- and long-term) complications of diabetes. The main conclusion of our recommendations is that, in general, SRL and PEGV combination treatment remains the second line medical treatment option of choice, and PAS-LAR should be reserved as a third line medical treatment option. However, in specific subgroups of patients PAS-LAR is a viable and preferred treatment option for patients with tumour growth during SRL and/or PEGV treatment, patients with specific symptoms not responsive to SRLs, and patients using low doses of PEGV without diabetes. Besides baseline glycemia and use of antidiabetic drugs, residual beta cell function, as measured with the OGTT, is an important predictor for pasireotide-induced hyperglycemia. In our cohort previous response to SRL treatment and SST_2 protein expression of the somatotroph adenoma was strongly correlated with response to PAS-LAR treatment. In patients well-controlled on SRL and low-dose PEGV combination treatment switching to weekly PEGV monotherapy is a viable treatment strateqy without significant compromise of biochemical control.

FUTURE PERSPECTIVES

In this thesis the results of the PAPE study are presented until 48 weeks. In order to observe whether PAS-LAR provides long-term efficacy and safety longer follow-up studies are needed. The insulin and incretin suppressive effects of pasireotide suggest that it is plausible that pasireotide increases the life time risk of diabetes in patients who do not develop hyperglycemia initially after treatment initiation. Longer follow-up studies are required to evaluate the occurrence of late-onset hyperglycemia and whether diabetes indeed occurs earlier in patients using PAS-LAR. The availability of PAS-LAR has expanded the therapeutic options for patients to achieve biochemical control. However, whether PAS-LAR is indeed more effective van first-generation SRLs in reducing tumour volume has not extensively been studied. It is interesting to analyze whether PAS-LAR induces clinically relevant tumour size reduction in our

cohort, and whether this correlates with PAS-LAR treatment response score and SSTR protein expression of the somatotroph adenoma. Besides biochemical control and tumour size control, quality of life is an important treatment goal. Many acromegaly patients have impaired quality of life despite (long-term) biochemical control. In the PAPE study we systematically assessed quality of life using validated acromegaly health-related quality of life questionnaires. In the future we aim to publish our findings with respect to quality of life, with ultimate goal to predict which subset of patients is likely to experience an improvement in quality of life. Previous studies have indicated that patients with headache not responsive to SRL treatment might benefit from pasireotide treatment (68, 69). There is also a pressing need for robust biomarkers that reliably predict the response to PAS-LAR treatment. Beside SST_2 and SST_5 protein expression, other markers are needed, such as AIP protein expression, ß-arrestin expression, the granulation pattern viewed under an electron microscope, pituitary adenoma signal intensity with MRI. Preferably all these markers should be integrated and validated in multiple cohorts of centers of excellence that treat a high volume of patients with acromegaly. As increased PEGV serum levels are observed during SRL and PEGV combination treatment the question arises whether PAS-LAR elicits the same effect.

FUTURE DEVELOPMENTS IN MEDICAL TREATMENT

Many new drugs are currently under investigation for acromegaly. Given the lack of efficacy of conventional SRLs and the hyperglycemia-related adverse events of PAS-LAR, there is a need for new drugs which provide improved biochemical control with less side effects and less frequent injections. Because PEGV requires frequent high dose injections, efforts have been made to develop a **long-acting GHR antagonist** (GHRA). A novel concept to extend the half-life of a GHRA is by generating a chimeric fusion molecule comprised of a mutated GH linked to GHBP (70). The investigators have developed a technology to link a ligand to the extracellular domain of its receptor, effectively providing the ligand with its own native binding protein within a single molecular entity. This ligand-receptor fusion molecule exists in solution as both a monomer and dimer where the GH moiety of one molecule bound to the receptor portion of another molecule in a head-to-tail reciprocal dimer (70). The pharmacokinetic characteristics suggested this fusion molecule could requires injection every 21–28 days compared to daily GHRA administration. In a proof of concept study, three fusion GHRA molecules have demonstrated delayed clearance and biological activity in vivo (71). An advantage of this technology is that no PEGylation is required to generate the long-acting GHRA, but whether this drug is cheap enough to manufacture

remains to be determined. **Somatoprim** (DG3173) is an investigational SRL with high affinity for SST_2, SST_4, and SST_5 with a pharmacokinetic profile similar to octreotide (72, 73). Somatoprim showed selectivity for GH suppression with minimal inhibition of insulin secretion (72, 74). Recently, a phase II trial of somatoprim in treatment-naïve acromegaly patients was completed (73). **AP102** is a dual SST_2/SST_5-specific SRL that has been designed with the aim to reduce GH secretion without causing hyperglycemia. AP102 is a disulfide-bridged octapeptide SRL containing synthetic iodinated amino acids. Receptor binding studies have demonstrated that AP102 binds with similar subnanomolar affinity to SST_2 and SST_5 as to native somatostatin 14 (75). In contrast to pasireotide, AP102 does not bind to SST_1 and SST_3 (75, 76). In a recent study in rats, the effects of AP102 and pasireotide were investigated on hormonal secretion and glucose metabolism following acute (single dose) and chronic (28-day) administration (77). In contrast to pasireotide, acute and chronic administration of AP102 did not lead to significant hyperglycemia or impaired glucose tolerance. The authors suggested that the neutral effect of AP102 on glucose metabolism was caused by balanced inhibition of insulin and glucagon secretion, and uninhibited GLP-I secretion (77). Despite that both compounds have high binding affinities to SST_2 and SST_5, they produced different effects on glucose metabolism. Several possible mechanisms could account for this discrepancy. In addition to SST_2 and SST_5, pasireotide also binds with high affinity to SST_1 and SST_3, whereas AP102 shows selective binding to only SST_2 and SST_5. The broader SSTR binding profile could therefore also contribute to pasireotide-induced hyperglycemia. Besides SST_5, SST_1 is also expressed on pancreatic ß-cells (78). Recently, knocking down expression of SST_3 through the use of selective SST_3 antagonist mediated glucose stimulated insulin secretion by ß-cells (79, 80). The effects of AP102 on SST_2 and SST_5 phosphorylation patterns has not been studied yet, but may also account for the differences in glucose metabolism compared with pasireotide.

To what extent these upcoming agents will actually benefit acromegaly patients in real life remains to be seen. It is likely that there is no single agent that can be considered the “perfect agent” for all patients.

Besides medical treatment, lifestyle interventions such as diet and exercise may also contribute to biochemical and symptomatic control of acromegaly. Hypothetically, a carbohydrate restricted or ketogenic diet could improve insulin resistance, and therefore reduce the required dose of PEGV in acromegaly.

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SUMMARY

Acromegaly is a chronic systemic condition caused by excessive growth hormone (GH) secretion from a pituitary adenoma. Combination treatment with first-generation somatostatin receptor ligands (SRLs) and pegvisomant (PEGV) is an established effective and safe treatment to normalise IGF-I levels in acromegaly. However, this combination treatment requires chronic monthly SRL injections and daily to weekly PEGV injections which can be troublesome for patients and negatively impact patient adherence. We therefore hypothesized in **chapter 2** that switching patients from SRL and PEGV combination treatment to PEGV monotherapy can reduce the total number of injections, and therefore improve patient adherence. After 12 months treatment with PEGV monotherapy 73% of patients achieved IGF-I normalization, while the median weekly PEGV dose only minimally increased. No significant adverse events occurred during the study. Therefore we concluded that temporary switching from combination treatment to PEGV monotherapy is an effective and feasible treatment strategy. In line with previous studies we confirmed the carry-over effect of SRLs on IGF-I levels of over 4 months. The conclusion of this pilot study is that temporary switching from combination treatment tot PEGV monotherapy is an effective, safe and feasible treatment strategy.

Pasireotide (long-acting release) LAR is a second somatostatin receptor ligand approved for the treatment of patients with acromegaly for whom surgery is not option or not curative. Pasireotide LAR has shown superior efficacy over first-generation somatostatin analogues with respect to IGF-I reduction, but is associated with a higher frequency of hyperglycemia and diabetes mellitus. However, it is unknown what the optimal position is of pasireotide LAR in the modern management of acromegaly in relation to PEGV. We hypothesized that switching from combination treatment to pasireotide LAR could lead to a significant reduction in the required PEGV dose and therefore to a lower number of injections, and possibly lower costs. **Chapters 3 and 4** cover the results of the results of the PAsireotide LAR and PEgvisomant (PAPE) study. The PAPE study assessed the efficacy and safety of switching from combination treatment of first-generation somatostatin analogues and PEGV treatment to pasireotide LAR treatment. The main results show that pasireotide LAR is an effective treatment, as 77% of patients achieved normalisation of IGF-I levels after 48 weeks, and on average 50% of the PEGV dose could be reduced. An important observation is the heterogeneous response to pasireotide LAR in term of IGF-I normalization. This was related to several factors, including the large heterogeneity in the PEGV dose at baseline. An interesting finding was the potent reduction in IGF-I after the first pasireotide LAR injection. This early onset and strong suppression of IGF-I is probably

a direct effect of pasireotide on inhibition of insulin secretion. The concomitant suppression of both IGF-I and insulin is an important mechanism which can explain the efficacy of pasireotide LAR.

Safety

De most common and clinically relevant adverse event during the PAPE study was the occurrence of hyperglycemia and diabetes mellitus. The frequency of diabetes mellitus rose from 32.8% at baseline to 77% at 48 weeks. Most patients had a mild hyperglycemia occurring mainly after initiation of pasireotide LAR treatment and manageable with metformin and vildagliptin. The subgroup of patient with severe hyperglycemia had pre-existent diabetes which was well regulated with multiple antidiabetic drugs such as sulfonylureas, GLP-I analogues and insulin. The development of pasireotide-induced hyperglycemia was correlated with baseline HbA1c and use of antidiabetic drugs. An important novel independent predictor for pasireotide-induced hyperglycemia was the baseline ß-cell function as a measure for insulin secretion based on the oral glucose tolerance test. We observed an inverse correlation between baseline insulin secretion and the increase in HbA1c at 24 weeks.

Predictors of clinical response to pasireotide LAR

As pasireotide exhibits a higher binding affinity to somatostatin receptor subtype 5 (SST_5) compared with SST_2 we assumed that the efficacy of pasireotide LAR would correlate with the SST_5 protein expression of the pituitary adenoma (**chapter 5**). In our cohort we observed a positive correlation between the clinical response of pasireotide LAR on IGF-I reduction and the clinical response of first-generation somatostatin analogues on IGF-I reduction. These clinical results were supported by in vivo observations that SST_2, and not SST_5 protein expression of the pituitary adenoma correlated with clinical response to pasireotide LAR treatment during the PAPE study. Moreover, in the same patients we found a strong correlation between the percentage IGF-I reduction after treatment with pasireotide LAR and after previous treatment with first-generation somatostatin analogues. With other words, patients with good clinical response to first-generation somatostatin analogues also seem to respond well to pasireotide LAR, and vice-versa.

The main conclusion of this translational study is that in our cohort SST_2 is the most important predictor for IGF-I normalisation after treatment with pasireotide LAR.

Ghrelin in acromegaly

The metabolic hormone ghrelin exists in two different isoforms in the circulation, respectively Acylated Ghrelin (AG) and Unacylated Ghrelin (UAG). Both isoforms have

different and (sometimes) opposing biological effects. Earlier studies indicated that measurement of both these isoforms with a validated immunoassay provides more biological information than measurement of total ghrelin. Although ghrelin can stimulate growth hormone secretion, and ghrelin itself can be inhibited growth hormone, the exact physiological role of ghrelin on growth hormone regulation remains not fully elucidated. It is also not well understood what the influence is of different medical treatments on ghrelin levels in acromegaly. In **chapter 6** we therefore measured both AG and UAG in three different acromegaly patient groups. In this study patients biochemically controlled with combination treatment were compared with patients with medically naïve active acromegaly and patients using PEGV monotherapy. We found that both AG and UAG were suppressed during combination treatment compared with the other patient groups. This effect is likely caused by a direct inhibitory effect of somatostatin on ghrelin release. Novel however is the finding that the AG/UAG ratio between the different patient groups is not significantly different. This suggests that combination treatment of SRL and PEGV does not affect the relation between AG and UAG. Another message of this study is that adequate measurement of AG and UAG and calculation of the AG/UAG ratio can yield relevant information on the bioactive state of ghrelin.

Clearance of pegvisomant

Although PEGV is available for the treatment of pegvisomant for over 15 years, it remains unknown how pegvisomant is metabolized and cleared from the circulation. PEGV is a mutated GH molecule attached to multiple polyethylene glycol (PEG) molecules. These PEG molecules extend the half-life of the drug and reduce immunogenicity. We postulated that because PEG molecules are not biodegradable they are cleared by immune cells, such as kupffer cells (liver macrophages) of the mononuclear phagocytic system. Therefore, in the final chapter of this thesis, we performed this proof of concept study to investigate whether PEGV is expressed in the liver, and if so, whether PEGV can be taken up by kupffer cells. We observed that PEG is internalized by kupffer cells and not by hepatocytes using immunofluorescence techniques in hepatic tissue obtained from two patients with acromegaly that were treated with PEGV. This study suggests the involvement of the immune system in the clearance of pegvisomant.

SAMENVATTING

Acromegalie is een chronische systemische aandoening veroorzaakt door overmatige groeihormoonsecretie uit een hypofyseadenoom. Combinatiebehandeling met eerste generatie somatostatine analoga (SRL) en pegvisomant (PEGV) is een effectieve en veilige behandeling om IGF-I te normaliseren in acromegalie. Echter deze combinatiebehandeling vereist langdurige maandelijkse SRL injecties en dagelijks tot wekelijkse PEGV injecties, wat een negatief effect kan hebben op de therapietrouw. Onze hypothese in **hoofdstuk 2** van dit proefschrift was dat tijdelijk switchen van SRL en PEGV combinatiebehandeling naar PEGV monotherapie kan leiden tot een reductie van de totale aantal injecties wat mogelijk kan leiden tot een verbetering van de therapietrouw. Na 12 maanden behandeling met PEGV monotherapie hadden 73% van de patiënten IGF-I waarden binnen de normaalwaarden terwijl hierbij de mediane wekelijks PEGV dosis slechts minimaal steeg. Tijdens de studies traden geen klinisch significante bijwerkingen op. In lijn met voorgaande studies hebben we aangetoond dat somatostatine analoga minstens 4 maanden een doorwerkingseffect hebben op IGF-I waarden. De conclusie van deze pilot studies is dat tijdelijk switchen van combinatiebehandeling naar PEGV monotherapie een effectieve, veilige en praktische haalbare behandelstrategie is.

Pasireotide (langwerkende afgifte) LAR is een tweede generatie somatostatine analoog en is geregistreerd voor acromegaliepatiënten waarbij een operatie niet mogelijk of niet curatief is. Pasireotide LAR is effectiever dan eerste generatie somatostatine analoga op IGF-I reductie, maar is geassocieerd met een hogere frequentie van hyperglycemie en diabetes mellitus. Het is echter niet bekend wat de optimale positie is van pasireotide LAR in relatie tot pegvisomant in de hedendaagse behandeling van acromegalie. Wij postuleerden dat switchen van combinatiebehandeling naar pasireotide LAR kan leiden tot een significante reductie van de pegvisomant dosis en daarmee tot minder injecties en mogelijk minder kosten. **Hoofdstukken 3 en 4** gaan over de resultaten van de PAsireotide LAR and PEgvisomant (PAPE) studie. In de PAPE studie wordt de effectiviteit en veiligheid onderzocht van het switchen van SRL en PEGV combinatiebehandeling naar pasireotide LAR. Uit de resultaten van de PAPE studie blijkt dat pasireotide LAR een effectieve behandeling is, aangezien 77% van de patiënten normalisatie van IGF-I konden bereiken na 48 weken met hierbij een gemiddeld PEGV sparend effect van 50%. Een belangrijke observatie is de grote heterogeniteit in klinische respons op pasireoide LAR. Dit was gerelateerd aan verschillende factoren, waaronder de grote heterogeniteit van de gebruikte PEGV dosis op baseline. Een interessante bevinding was de sterke reductie van IGF-I na de eerste pasireotide LAR injectie. Deze vroegtijdige en sterke reductie van IGF-I is een

waarschijnlijk een direct effect van pasireotide op remming van de insuline secretie. De gelijktijdige suppressie van zowel IGF-I als insuline is een belangrijk mechanisme wat de effectiviteit van pasireotide LAR kan verklaren.

Veiligheid

De meest voorkomende en klinisch relevante ongewenste voorval tijdens de PAPE studie was het optreden van hyperglycemie en diabetes mellitus. De frequentie van diabetes mellitus steeg 32.8% bij aanvang van de studie naar 77% aan het einde. De meeste patiënten hadden hierbij een milde hyperglycemie voornamelijk optredend na het starten van behandeling met pasireotide LAR. Deze milde hyperglycemie kon worden gereguleerd met een combinatie van metformine en vildagliptine. De subgroep van patiënten met ernstige hyperglycemie hadden reeds diabetes mellitus die goed gereguleerd was met meerdere antidiabetische medicatie zoals sulfonylureumderivaten, GLP-I analoga en insuline. Het optreden van pasireotide-geinduceerde hyperglycemie werd voorspeld door de HbA1c waarde bij baseline en het gebruik van antidiabetische medicatie. Een belangrijke nieuwe onafhankelijke voorspeller voor hyperglycemie was de baseline ß-cel functie als een maat voor de insulinesecretie gebaseerd op de orale glucose tolerantie test. Wij vonden een omgekeerde correlatie tussen de baseline insulin secretie en de stijging van het HbA1c op 24 weken.

Voorspellers van klinische respons op pasireotide LAR

Aangezien pasireotide een verhoogde bindingsaffiniteit heeft voor somatostatine receptor subtype 5 (SST_5) vergeleken met SST_2 veronderstelden wij dat de effectiviteit van pasireotide LAR correleert met de mate van SST_5 eiwitexpressie op het hypofyseadenoom (**hoofdstuk 5**). In onze cohort vonden wij echter een positieve correlatie tussen de klinische respons van pasireotide LAR op IGF-I reductie en de klinische response van eerste generatie somatostatine analoga op IGF-I reductie. Deze klinische resultaten werden ondersteund door *in vivo* observaties dat SST_2 en niet SST_5 eiwitexpressie van de hypofyseadenoom correleerde met de klinische response op pasireotide LAR tijdens de PAPE studie. Bovendien vonden wij in dezelfde patiëntengroep een sterke correlatie tussen de percentage IGF-I reductie na behandeling met pasireotide LAR en na (eerdere) behandeling met eerste generatie somatostatine analoga. Met andere woorden, patiënten die eerder goed reageerden op eerste generatie somatostatine analoga leken ook goed te reageren op pasireotide LAR en vice-versa. De belangrijkste conclusie van deze translationele studie is dat in onze cohort SST_2 eiwitexpressie de belangrijkste voorspeller is voor IGF-I normalisatie na behandeling met pasireotide LAR.

Ghreline levels in acromegalie

Het stofwisselingshormoon ghreline bestaat uit twee verschillende verschijningsvormen in de circulatie, respectievelijk Geacyleerd Ghreline (AG) en Ongeacyleerde Ghreline (UAG). Beide verschijningsvormen hebben verschillende en (soms) tegengestelde biologische effecten. Uit eerdere studies is gebleken dat het meten van deze verschijningsvormen van ghreline in een gevalideerde immunoassay meer biologische informatie verschaft dan het louter meten van totale ghreline. Hoewel ghreline groeihormoonsecretie kan stimuleren en ghrelinesecretie geremd kan worden door groeihormoon is de fysiologische rol van ghreline in de regulatie van groeihormoon nog niet opgehelderd. Het is ook nog niet goed bekend wat de invloed is van de verschillende therapieën voor acromegalie op ghreline waarden. In **hoofdstuk 6** is daarom AG en UAG gemeten in drie verschillende acromegalie patiëntengroepen. In deze studie werd patiënten die biochemisch gecontroleerd waren met SRL en PEGV combinatiebehandeling vergeleken met patiënten met medisch naïeve acromegalie en patiënten met PEGV monotherapie. Uit deze studie blijkt dat zowel AG als UAG waarden onderdrukt werden tijdens combinatiebehandeling vergeleken met de andere patiëntengroepen. Dit effect wordt waarschijnlijk veroorzaakt door een direct remmend effect van somatostatine op ghrelineafgifte. Nieuw is echter de bevinding dat de AG/UAG ratio tussen de patiëntengroepen niet significant verschillend is. Dit suggereert dat combinatiebehandeling van SRL en PEGV de relatie tussen AG en UAG niet verandert. Een andere belangrijke boodschap van deze studie is dat adequate meting van AG en UAG en het bepalen van de AG/UAG ratio belangrijke informatie kan verschaffen over de bioactieve status van ghreline.

Klaring van pegvisomant

Hoewel PEGV meer dan 15 jaar beschikbaar is als behandeling van acromegalie is tot op heden nog niet bekend hoe PEGV gemetaboliseerd wordt en geklaard wordt uit de circulatie. Het unieke aan het PEGV is dat het een gemuteerde groeihoormoneiwit is die verbonden is met meerdere polyethylene glycol (PEG) moleculen die ervoor zorgen dat PEGV een verlengde halfwaardetijd heeft en minder immunogeen is. Wij postuleerden dat PEG moleculen, doordat zij niet biologisch afbreekbaar zijn, geklaard worden door immuuncellen, zoals kupffer cellen (lever macrofagen) van het mononucleair fagocytair systeem. Daarom is in dit laatste hoofdstuk van dit proefschrift een proof of concept studie uitgevoerd om te onderzoeken of PEGV tot expressie komt in de lever, en zo ja, of PEGV opgenomen kan worden door kupffer cellen. Door middel van immunofluoresecentietechnieken in leverweefsel van twee acromegaliepatiënten die behandeld werden met PEGV hebben wij geobserveerd dat kupffer cellen PEG moleculen opnemen, terwijl hepatocyten dit niet deden. Deze studie suggereert daarom betrokkenheid van het immuunsysteem in de klaring van pegvisomant.