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Chronic Aspirin Treatment Affects Collagen Deposition in Non-infarcted Myocardium During Remodeling after Coronary Artery Ligation in the Rat

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E. A. J. KALKMAN, R. J. VAN SUYLEN, J. P. M. VAN DIJK, P. R. SAXENA AND R. G. SCHOEMAKER. Chronic Aspirin Treatment Affects Collagen Deposition in Non-infarcted Myocardium During Remodeling after Coronary Artery Ligation in the Rat. *Journal of Molecular and Cellular Cardiology* (1995) 27, 2483–2494. Low-dose aspirin (acetylsalicylic acid; ASA), inhibiting platelet thromboxane production in favor of endothelium formation of prostaglandins, is successfully used as primary or secondary prophylaxis against myocardial infarction. Although prognosis may be improved, effects of long-term ASA treatment on wound healing and cardiac remodeling are not well understood. The aim of the present study was to mimic the clinical situation by inducing myocardial infarction in low-dose ASA (25 mg/kg/day, i.p.) pretreated rats, and to determine effects on plasma eicosanoid levels, cardiac hypertrophy and collagen deposition, and left ventricular function during continued ASA treatment. The effects of this dose were verified to selectively inhibit platelet thromboxane production, and lower plasma levels of thromboxane, but did not affect plasma levels of prostacyclin and prostaglandin E_2 during the acute inflammatory stage following myocardial infarction. As measured by heart dry weight/body weight, cardiac hypertrophy was not affected by ASA treatment. However, interstitial fibrosis in the spared myocardium as well as perivascular fibrosis, associated with infarction-induced cardiac remodeling, were affected by ASA treatment. Replacement fibrosis in the infarct itself, considered as representing wound healing, was not significantly influenced by ASA treatment. Wall thinning following infarction was not aggravated, nor did treatment influence left ventricular cavity diameter in a relaxed state. Results from *in vitro* left ventricular function measurements showed no effects on left ventricular peak velocity of contraction or relaxation after ASA treatment. In conclusion, although low-dose ASA may not be expected to have anti-inflammatory action, it did influence post-infarct cardiac remodeling by affecting interstitial and perivascular fibrosis. ASA treatment did not have effects on *in vitro* left ventricular dysfunction. © 1995 Academic Press Limited

KEY WORDS: Aspirin; Collagen; Infarction; Left ventricular function; Prostaglandins; Remodeling.

Introduction

Low-dose aspirin (acetylsalicylic acid, ASA) treatment is used in coronary artery disease as primary or secondary prophylaxis against myocardial infarction, as it reduces platelet production of pro-aggregatory and vasoconstrictor thromboxane in favor of anti-aggregatory and vasodilator prostaglandins (Patrignani *et al.*, 1982; Coller, 1991). ASA improves prognosis and reduces the chance of re-infarction after myocardial infarction (ISIS-2 Collaborative Group, 1988), and this effect can be attributed to its anti-platelet action (Antiplatelet Trialists' Collaboration, 1988).

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At higher doses, ASA can also exert anti-inflammatory activity by interference with the synthesis of prostaglandins. Anti-inflammatory treatment with corticosteroids and non-steroid anti-inflammatory drugs (NSAIDs) has been shown to retard collagen deposition and to cause infarct thinning (Bulkley and Roberts, 1974; Kloner *et al.*, 1978; Brown *et al.*, 1983; Hammerman *et al.*, 1983a,b; Mannisi *et al.*, 1987; Vivaldi *et al.*, 1987; Jugdutt and Basualdo, 1989). These studies focused on the replacement fibrosis that results in scar formation, whereas effects on interstitial fibrosis that occurs in remote, non-infarcted areas (van Krimpen *et al.*, 1991) are still unknown. Moreover, whether scar formation and cardiac fibrosis in spared myocardium would be affected in patients pretreated followed by continued treatment with low-dose ASA, which seems to be devoid of anti-inflammatory action, remains to be determined.

The present study was carried out to address this question in a rat model of myocardial infarction (Fishbein *et al.*, 1978). A dose of ASA that inhibits thromboxane production from platelets but does not interfere with prostacyclin production from endothelial cells, was carefully selected as a treatment reflecting the aims of low-dose aspirin treatment in man. Plasma eicosanoid levels were evaluated at different time points. The effects on interstitial and perivascular fibrosis in spared myocardium, on replacement fibrosis in the infarcted area were investigated, as well as on cardiac remodeling parameters and left ventricular function *in vitro*.

Methods and Materials

Male, Wistar rats (270–320 g, Harlan Zeist, The Netherlands) were used in this study. Saline or ASA 25 mg/kg (lysine-acetylsalicylic acid, Aspégic®, Lorex B.V., Maarssen, The Netherlands) dissolved in saline was administered as daily i.p. injections of 1 ml/kg, starting 2 days before surgery, and the treatment continued until the end of the experiment, at 8, 14 or 21 days after surgery. The injections were administered at the same time each day except on the day of surgery, when the animals were injected immediately after the operation. Rats were housed at a 12 h light/dark cycle with standard rat chow and water available *ad libitum*. The experiments were approved by the University ethics committee for the use of experimental animals.

Validation of the used dose of aspirin

To confirm the selectivity of chronic aspirin treatment as inhibiting platelet versus vascular cyclooxygenase activity, a separate group of rats was treated with either ASA 25 mg/kg body weight ($n = 6$) (based on pilot short-term dose-finding studies) or saline ($n = 6$) as daily i.p. injection. Following 3 weeks of treatment, blood was sampled by heart puncture under pentobarbital (60 mg/kg) anesthesia, 24 h after the last dose of ASA. Native blood (1 ml) was allowed to clot in a Vacutainer® glass tube containing SST® Gel and Clot Activator (Becton Dickinson, Meylan Cedex, France) at room temperature for 30 min. Serum was separated by centrifugation and, after Sep Pak (Millipore, Milford, USA) extraction, stored at $-20°C$ in methanol until assayed for thromboxane B_2 (TxB_2) produced.

A 2 mm-long segment was isolated from the thoracic aorta. The segments were incubated at 37°C for 10 min in 200 μl of Krebs–Henseleit buffer containing 25 μM arachidonic acid (Supelco, Bellefonte). The rings were removed and weighed, and the supernatant passed through Sep Pak filters and stored at $-20°C$ in methanol until assayed for 6-*keto*-$PGF_{1\alpha}$ generation, which was expressed as ng/mg wet tissue of aortic segment.

Surgical preparation

Under pentobarbital (60 mg/kg, i.p.) anesthesia, a PE-10 catheter filled with heparinized saline (50 IU/ml) was introduced into the thoracic aorta via the left carotid artery. The catheter was guided subcutaneously to the neck, where it was fixed and exteriorized. Left anterior descending coronary artery ligation was performed as described in detail elsewhere (Fishbein *et al.*, 1978; Pfeffer *et al.*, 1979; Schoemaker *et al.*, 1991). Briefly, after the trachea was intubated, an incision was made in the skin overlying the fourth intercostal space, while the overlying muscles were separated and kept aside. The animals were put on positive pressure ventilation (frequency 65/min, tidal volume 3 ml), and the thoracic cavity was opened by cutting the intercostal muscles. The heart was left *in situ* and a 6-0 silk suture was looped under the left coronary artery near the origin of the pulmonary artery. The suture was tied except in sham operation. Ribs were pulled together with 3-0 silk. Subsequently, the muscles were returned to their normal position, and the skin was sutured.

Blood sampling and measurement of eicosanoids

On four occasions, 1 ml blood was sampled using syringes filled with 10 μl 0.1 M disodium ethylenedinitrilotetra-acetic acid (EDTA): immediately after implanting the aortic catheter, but before opening the thoracic cavity (day 0), 1 day after surgery, and 8 and 21 days after surgery, when it was obtained by aortic puncture. Day 1 was chosen as a point in time representative of acute inflammation following infarction and day 8 because it represents chronic inflammation, just after peak infiltration of chronic inflammatory cells, and day 21 because inflammation has waned (Fishbein *et al*., 1978).

After centrifugation, the plasma was passed through Sep Pak C18 cartridges (Waters Ass., USA) and eluted with methanol. Samples were stored at -20°C in methanol. Because of the instability of prostacyclin and thromboxane in biological fluids, their stabile metabolites, 6-*keto*-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and TxB_2, respectively, as well as prostaglandin E_2 (PGE_2), were measured using radioimmunoassay (antibodies: Advanced Magnetics, USA; Standards: Sigma, USA) as described in detail by Zijlstra *et al.* (1992). To allow comparison of eicosanoid levels between the three experimental groups, samples from the same time point were assayed together.

Measurement of left ventricular function

Under pentobarbital anesthesia, the heart was rapidly excised and mounted for perfusion with an oxygenated modified Krebs–Henseleit buffer (Composition in mM: NaCl 125, KCl 4.7, $CaCl_2$ 1.35, $NaHCO_3$ 20, NaH_2PO_4 0.4, $MgCl_2$ 1.0, D-glucose 10; pH = 7.4; 37°C) at a constant pressure of 85 mmHg, using the Langendorff technique. Heart rate was kept constant at 300 beats/min by pacing with a Grass stimulator (Grass Medical Instruments, Quincy, MA, USA). A water-filled, latex balloon was inserted into the left ventricle via the left atrium, and connected to a pressure transducer (Viggo-Spectramed, Oxnard, USA). Coronary flow was measured by an in-line flow probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing just before the aorta to monitor the flow of buffer passing through the probe just before entering the coronary arteries. Left ventricular pressure and coronary flow were recorded with a Grass 6B Polygraph (Grass Medical Instruments, MA, USA). Left ventricular end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume and hearts were allowed to stabilize for at least 20 min. Left ventricular pressure tracings were recorded at high paper speed (100 mm/s) for graphical determination of positive and negative dP/dt_{max}. At the end of the experiment, hearts were arrested in diastole with a 1 M KCl injection into the perfusing fluid, and prepared for measurement of infarct size.

Infarct size measurement

Large vessels and atria were removed from the heart, and the right and left ventricles were separated and weighed. The left ventricle was quickly frozen (-80°C) and cut into slices of 1 mm from apex to base. The slices were stained with nitro blue tetrazolium (NBT) according to Leprán *et al.* (1983). Briefly, the slices were incubated in 1 mg/ml NBT in 0.1 M Sörensen phosphate buffer (pH = 7.4) at 37°C for 15 min, and subsequently put in cold saline (0°C). This procedure stains all tissue that was vital at the time of death, so provides no information about the area at risk. Colour pictures were taken from the slices, and infarct size was determined by planimetry, described in detail elsewhere (Schoemaker *et al*., 1991). Infarct size was expressed as a percentage of left ventricular circumference, calculated as the average of infarct size of endocardial and epicardial surfaces of all slices. Minimal scar thickness as well as thickness of the mid-interventricular septum was measured in all slices containing transmural infarction. Left ventricular cavity diameter in an undistended state was estimated from mean endocardial circumference. Thinning ratio was calculated by dividing the scar thickness by the thickness of the interventricular septum. Left ventricular cavity diameter to mean wall thickness ratio was calculated as an index of structural left ventricular dilation (Vogt *et al*., 1987). Dry weights were determined after drying the tissues for 3 days at 37°C.

Measurement of collagen content

At 2 weeks after surgery, when a plateau in collagen content is reached in infarcted hearts (van Krimpen, 1991), the amount of interstitial and perivascular collagen was measured in a separate group of rats, using the method as described previously (Brilla *et al*., 1991; Smits *et al*., 1992). Briefly, the coronary arteries were perfused with saline (Langendorff), followed by perfusion-fixation with 3.6% phosphate-buffered formaldehyde. The atria and large vessels were removed, the ventricles weighed and

cut into four slices from apex to base. These slices were fixed in formaldehyde for at least 24 h. After fixation, the slices were dehydrated and paraffin embedded. Deparaffinized 5 μm thick sections were incubated for 5 min with 0.2% (wt/vol) aqueous phosphomolybdic acid, and subsequently incubated for 45 min with 0.1% Sirius Red F3BA (C.I.35780, Polysciences Inc., Northampton, UK) in saturated aqueous picric acid, washed for 2 min with 0.01 M HCl, dehydrated, and mounted with Entellan (Merck, Darmstadt, Germany). Distribution of collagen fibers in sections examined with normal and with polarized light was similar, validating the quantification of collagen with normal light (Fig. 1). In the interventricular septum, as well as in the right ventricle, interstitial collagen was determined as the picrosirius red positive area in 40 high power fields per ventricle per heart. These areas of myocardium did not show signs of replacement fibrosis following focal necrosis. Thus, we measured interstitial fibrosis as the increase of collagen volume in the interstitium between vital myocytes (Fig. 1). In addition, perivascular collagen of six to 10 septal resistance arteries (diameter approximately 100 μm) was measured (Fig. 2). The perivascular picrosirius red positive area was corrected for luminal area of the vessel (Brilla *et al.*, 1991). In order to evaluate effects on wound healing in infarcted hearts, the picrosirius red positive area was also determined in the central part of the infarct itself.

Data analysis

Results comprise data obtained from six to 12 animals per group. Data are expressed as group means $\pm$ S.E.M. unless indicated otherwise. Rats in the sham group had measured infarct sizes of 0%. Data from rats with measured infarct sizes less than 20% were excluded from analysis, because these infarcts are hemodynamically fully compensated (Schoemaker *et al.*, 1991). Only eicosanoid measurements of animals that survived the complete protocol were used. Because plasma eicosanoid levels did not show a normal distribution, median values $\pm$95% confidence intervals are quoted, and non-parametric data analysis was performed, using the Kruskal–Wallis test. Morphological and functional data were analysed using one-way analysis of variance (ANOVA), followed by a *post hoc* *t*-test (Wallenstein *et al.*, 1980). Differences were considered statistically significant if $P<0.05$.

Results

On average, surgery-related mortality was 32% (shams 21%, saline-treated infarctions 37%, aspirin-treated infarctions 41%), and occurred mainly during the first 24 h after surgery. After exclusion of data from three aspirin-treated and five saline-treated infarcted animals because of infarct sizes smaller than 20%, infarct sizes were comparable in the saline-treated and aspirin-treated infarction groups (Table 1). All infarctions were transmural and were located in the lateral (free) wall of the left ventricle.

Validation of the used dose of aspirin

Generation of 6-keto-$PGF_{1\alpha}$ by aortic endothelium stimulated by arachidonic acid (1.35 ± 0.22 ng/mg wet weight in saline-treated rats) was not affected by 3 weeks of ASA treatment (1.32 ± 0.21 ng/mg wet weight). Production of TxB_2 from platelets during blood clotting was effectively attenuated by ASA therapy (1.91 ± 0.53 ng/ml serum *v* 5.88 ± 0.55 ng/ml serum). Consequently, 6-keto-$PGF_{1\alpha}$ to TxB_2 ratio with ASA treatment (1.07 ± 0.30) was significantly increased compared to saline-treated animals 0.24 ± 0.03).

Plasma eicosanoid levels (Fig. 3)

TxB_2 levels were significantly lowered by ASA treatment at all time points except at day 8, when no statistical significance was reached ($P=0.07$). TxB_2 levels appeared to be higher at 21 days compared to the other time points, but these samples were assayed separately. 6-Keto-$PGF_{1\alpha}$ levels were significantly decreased by ASA treatment at day 0 and 21, but not during inflammatory stimulation, at day 1 and 8. This resulted in a comparable presurgical 6-keto-$PGF_{1\alpha}$ to TxB_2 ratio in ASA- and saline-treated rats (3.5 ± 1.1 and 3.1 ± 1.0, respectively), whereas this ratio was significantly increased in ASA-treated animals, during acute inflammation, at day 1 (16.4 ± 4.9 *v* 5.0 ± 1.7). The ratio returned to presurgical levels at day 8 (3.5 ± 0.7 *v* 2.5 ± 0.6). PGE_2 levels were never affected by ASA treatment, although a tendency towards inhibition might be present after 3 weeks of treatment.

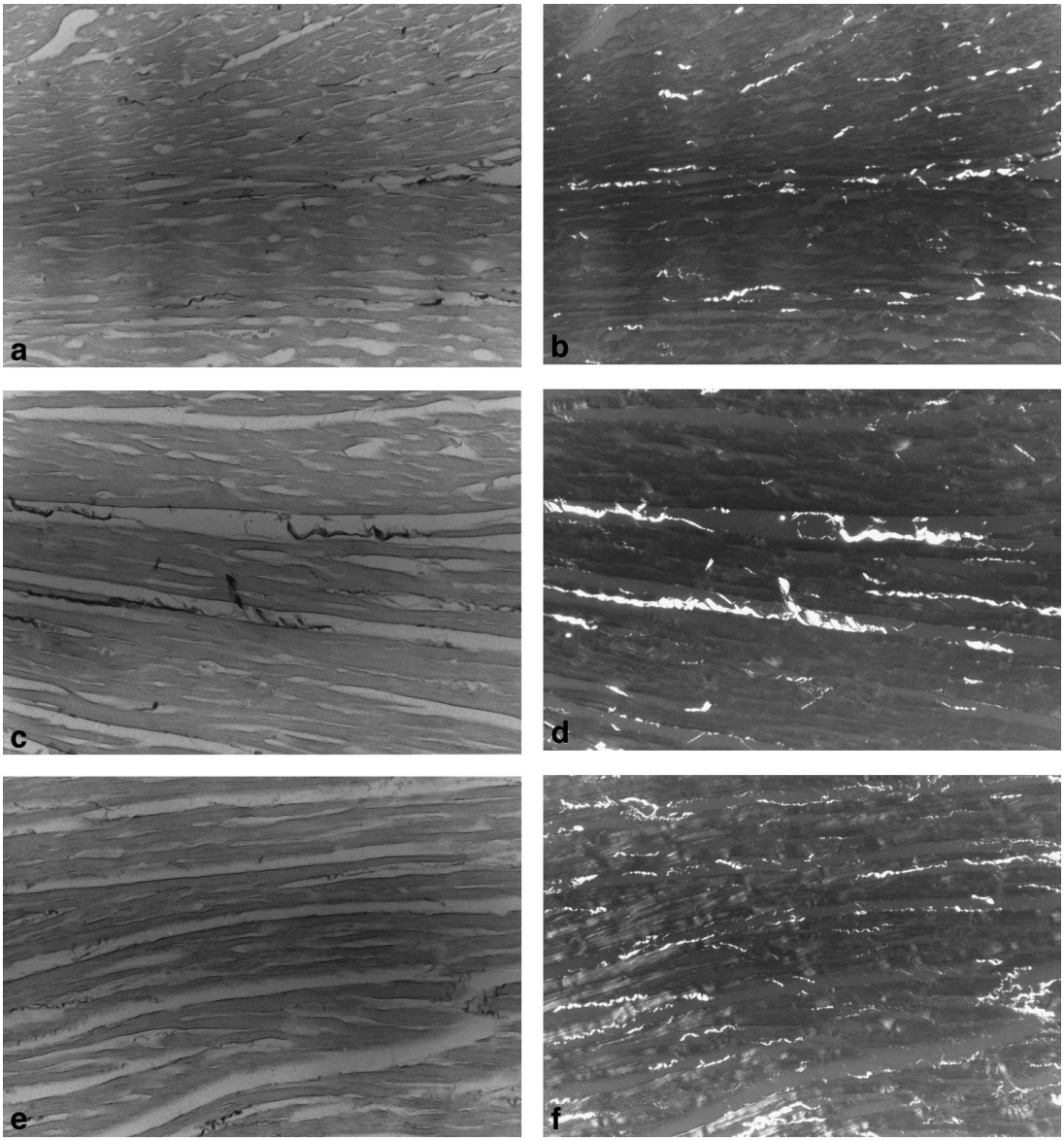

Figure 1 Picrosirius red stained sections of interventricular septum of hearts from a/b: a sham-operated rat (a with normal light and b with polarized light), c/d: a saline-treated rat after infarction of the left ventricular free wall (c with normal light and d with polarized light), e/f: an ASA-treated rat after infarction of the left ventricular free wall (e with normal light and f with polarized light) (original magnification: $\times 62.5$).

Left ventricular morphology and function

Heart wet weight was significantly increased at 8 days after myocardial infarction, which was mainly attributable to the left ventricle. This observation was even more pronounced in infarcted hearts from ASA-treated rats. However, for dry weights no differences between the experimental groups were present. Heart dry weight to body weight ratios did not differ between the groups (Table 1).

The ratio of left ventricular cavity diameter in a relaxed state to wall thickness was significantly increased. This was the result of an increased left ventricular cavity diameter (3.2 ± 0.2 *v* 2.4 ± 0.1 mm) and a decreased wall thickness (3.2 ± 0.1 *v* 3.7 ± 0.1 mm) at an unchanged outer diameter (9.7 ± 0.2 mm for both groups), at 21 days. These parameters were comparable in hearts from saline- and ASA-treated animals. Scar thickness did not differ between untreated and ASA-treated rats (1.1 ± 0.1 *v* 1.2 ± 0.1 mm at 8 days, and 1.3 ± 0.1 *v* 1.5 ± 0.2 mm at 21 days), nor was septal thickness altered by ASA therapy (4.2 ± 0.2 *v* 4.3 ± 0.1 mm at 8 days, and 4.3 ± 0.2 *v* 4.2 ± 0.3 mm at 21 days, in untreated and ASA-

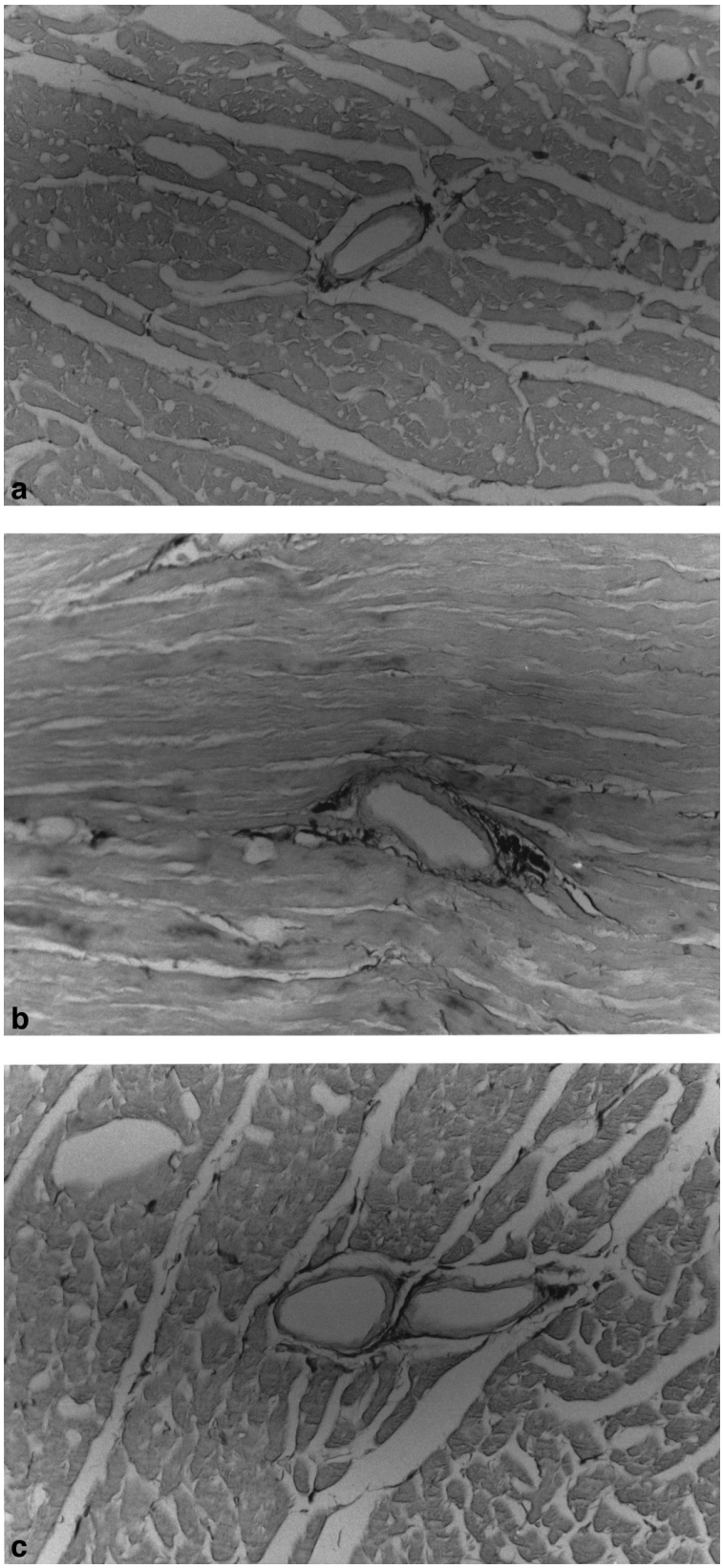

Figure 2 Picrosirius red stained sections of interventricular septum containing resistance arteries, from a: a sham-operated rat, b: a saline-treated rat after infarction of the left ventricular free wall, c: an ASA-treated rat after infarction of the left ventricular free wall (original magnification: $\times 62.5$).

Table 1 Characteristics of hearts 8 days and 3 weeks after surgery

		Sham	MI	MI + ASA
n	Day 8	12	12	11
	Day 21	11	10	8
Infarct size (%)	Day 8	0	41 ± 2	43 ± 3
	Day 21	0	37 ± 1	39 ± 3
Heart wet weight (mg)	Day 8	792 ± 24	954 ± 43*	1046 ± 40*
	Day 21	978 ± 35	928 ± 22	928 ± 23
Wet weight/BW (mg/g)	Day 8	2.81 ± 0.14	3.74 ± 0.30*	4.11 ± 0.24*
	Day 21	2.54 ± 0.10	2.52 ± 0.06	2.60 ± 0.05
Heart dry weight (mg)	Day 8	165 ± 3	154 ± 5	157 ± 4
	Day 21	198 ± 7	179 ± 4	181 ± 5
Dry weight/BW (mg/g)	Day 8	0.58 ± 0.02	0.59 ± 0.03	0.61 ± 0.02
	Day 21	0.51 ± 0.01	0.48 ± 0.01	0.51 ± 0.01
LV thinning ratio	Day 8		0.28 ± 0.02	0.30 ± 0.02
	Day 21		0.31 ± 0.04	0.39 ± 0.10
LV diameter/WT	Day 8	0.46 ± 0.04	1.18 ± 0.09*	1.07 ± 0.07*
	Day 21	0.66 ± 0.04	1.03 ± 0.10*	0.91 ± 0.12*

MI, myocardial infarction; MI + ASA, ASA-treated myocardial infarction; wet weight/BW, heart wet weight/body weight; dry weight/BW, heart dry weight/body weight; LV, left ventricle; thinning ratio, minimal scar thickness to midseptal thickness ratio; LV diameter/WT, left ventricle inner diameter/wall thickness.
*: Significantly different from sham values.

treated rats, respectively). Thus, thinning ratio was not influenced by ASA treatment (Table 1).

In vitro left ventricular dysfunction of infarcted hearts became evident from a depressed peak velocity of contraction and relaxation, demonstrated by a significantly depressed peak $+(dP/dt)$ and $-(dP/dt)$. ASA treatment delayed the development of left chamber dysfunction; contractility and relaxation were not significantly depressed until 3 weeks after infarction. A similar tendency, though less pronounced, could be seen for systolic pressure developed during Langendorff perfusion. Coronary flow in infarcted hearts was not decreased compared to sham values. However, at 8 days after surgery, coronary flow corrected for wet cardiac tissue weight, but not for dry weight, was significantly decreased in infarcted hearts after ASA treatment (Table 2).

Collagen deposition in infarcted and non-infarcted myocardium (Figs 1, 2 and 4)

In the infarcted area itself, $56.0 \pm 3.3\%$ of total tissue area was found picrosirius red positive, which was not significantly influenced by ASA treatment ($49.4 \pm 1.0\%$). Non-infarcted, interventricular septum showed a considerably higher content of picrosirius red positive material after coronary artery ligation than corresponding areas in hearts from sham operated rats, which was almost normalized after ASA treatment (Figs 1 and 4). Also in right ventricular myocardium, infarcted hearts showed a higher interstitial collagen content, which was slightly but insignificantly reduced by ASA treatment (Fig. 4).

Perivascular collagen of resistance arteries in the interventricular septum was significantly increased after infarction (0.96 ± 0.09 *v* 0.63 ± 0.09 picrosirius red positive area/luminal area). ASA treatment significantly affected this collagen deposition (0.71 ± 0.05, $P = 0.04$) (Fig. 2).

Discussion

ASA treatment and eicosanoid profile

In the primary and secondary prophylaxis of myocardial infarction low-dose aspirin is successfully used as anti-platelet therapy because it inhibits platelet production of thromboxane in favor of endothelium formation of prostaglandins. In this study, firstly, we evaluated the effects of ASA treatment in normal healthy animals with regard to platelet and vascular endothelium cyclo-oxygenase potential to generate thromboxane and prostacyclin, respectively. This is important because inter-species differences in sensitivity and metabolism make dose extrapolation from animal to man on a body weight basis questionable. Corresponding to the rationale

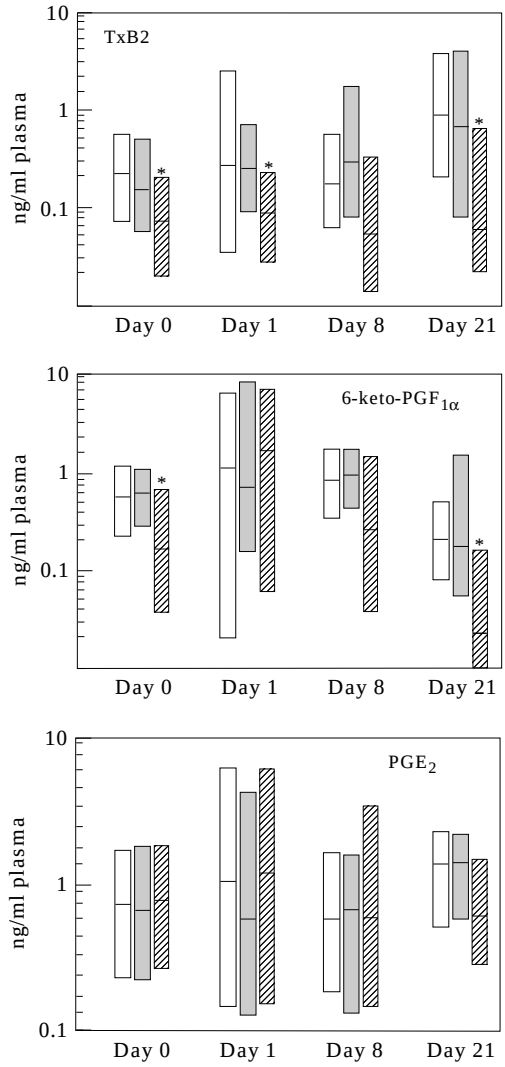

Figure 3 Plasma eicosanoid levels (ng/ml) at different time points following surgery. Median values and 95% confidence intervals are shown. Open bars: sham ($n=9$–12); Gray bars: saline-treated infarction ($n=10$–12); Hatched bars: ASA-treated infarction ($n=8$–11). *:$P<0.05$ *v* saline-treated rats.

behind the clinical use in man, chronic ASA treatment did not affect vascular endothelium cyclooxygenase, while TxB_2 generation by platelets during blood clotting was effectively blocked. However, measurements in plasma during our experiments using the same dose of ASA showed some interference with prostaglandin synthesis as well, as indicated from a decrease of prostacyclin levels at those time points that were not associated with the inflammatory response to infarction. This discrepancy could be explained by the fact that the dose of ASA, that selectively inhibits thromboxane synthesis, was evaluated *in vitro* only in those tissues in which the major production was expected (Gambino *et al*., 1988). The site of production of plasma prostacyclin may not be limited to vascular endothelium (Mehta *et al*., 1985; Vergara-Dauden *et al*., 1985; Weber *et al*., 1989).

Prostacyclin levels during the inflammatory stage (day 1 and 8) were not affected by ASA treatment. Although inflammation did not result in higher plasma concentrations of prostacyclin, there may be a greater proportion of total release into plasma attributable to the inducible cyclooxygenase-2 (COX-2) at the expense of the constitutional cyclooxygenase-1 (COX-1) (Vane *et al*., 1994). The COX-2 isoform is less sensitive to inactivation by ASA (Mitchell *et al*., 1993). Plasma eicosanoid levels during this inflammatory stage showed a high interanimal variability (with no Normal distribution). Since variation of plasma eicosanoid levels rather than absolute values were increased at day 1 compared to day 0, and no differences were observed between sham and infarcted rats, we concluded that the surgical procedure rather than the infarction was responsible for the variability in plasma levels and may represent individual inflammatory responses to surgery. The variation may decline with time, indicative for acute and later waning inflammatory response.

Left ventricle morphology and *in vitro* function

A major finding in the present study is that low-dose ASA therapy affected collagen build-up in spared myocardium after infarction. High-dose ASA treatment, providing plasma levels associated with anti-inflammatory action in humans, has been shown to inhibit both the synthesis and degradation of collagen in rat skin (Solheim *et al*., 1986a), as well as the synthesis of collagen in rat bone (Solheim *et al*., 1986b). Numerous studies have reported on the effects of steroids and NSAIDs on scar thickness and collagen build-up in the infarcted area (Bulkley and Roberts, 1974; Kloner *et al*., 1978; Brown *et al*., 1983; Hammerman *et al*., 1983a,b; Mannisi *et al*., 1987; Vivaldi *et al*., 1987). Moreover, prostaglandins have been implicated in the regulation of fibroblast proliferation (Otto *et al*., 1982). Therefore, it seems rational to link the effects of ASA therapy to its anti-inflammatory action. However, in the present study, ASA treatment did not affect prostaglandin levels during the inflammatory phase after infarction. Secondly, fibrosis in the spared myocardium, remote from the infarct, rather than in the infarcted area itself was affected; scar thinning and scar collagen content were not significantly altered. On the other hand, inflammation may not

Table 2 Functional parameters during Langendorff perfusion

		Sham	MI	MI + ASA
n	Day 8	9	9	8
	Day 21	11	10	8
Systolic pressure (mmHg)	Day 8	68 ± 4	57 ± 6	64 ± 8
	Day 21	75 ± 5	51 ± 4*	49 ± 4*
$+(dP/dt)_{max}$ (mmHg/s)	Day 8	5237 ± 846	2972 ± 548*	3744 ± 743
	Day 21	6967 ± 806	3162 ± 332*	3165 ± 557*
$-(dP/dt)_{max}$ (mmHg/s)	Day 8	1338 ± 95	991 ± 98*	1136 ± 133
	Day 21	1282 ± 96	885 ± 62*	831 ± 75*
Coronary flow (ml/min)	Day 8	10.0 ± 1.3	8.6 ± 0.6	8.8 ± 0.9
	Day 21	11.3 ± 0.7	10.5 ± 0.6	11.0 ± 1.4
CF/wet weight (ml/min/g)	Day 8	13.0 ± 1.7	9.6 ± 0.9	8.6 ± 0.8*
	Day 21	11.6 ± 0.6	11.1 ± 0.8	11.8 ± 1.4

MI, myocardial infarction; MI + ASA, ASA-treated myocardial infarction; systolic pressure, left ventricular systolic pressure; $+(dP/dt)_{max}$ and $-(dP/dt)_{max}$: maximum velocity of pressure rise and decline, respectively. CF/wet weight: coronary flow per g of wet cardiac tissue.
*: Significantly different from sham values.

be limited to the infarcted area itself (Sulpice *et al*., 1994). The observation that at 8 days after infarction, when cardiac edema is known to be over its maximum (Fishbein *et al*., 1978), increased tissue water content was more pronounced with ASA therapy, suggests still some interference with local inflammatory response to infarction. Other possibilities include a role for thromboxane, direct or indirect in the prevention of release of platelet-derived growth factor (Vissinger *et al*., 1993; Lanas *et al*., 1994). Finally, effects of medication unrelated to inactivation of cyclo-oxygenase, for example by metabolites of ASA (Haynes *et al*., 1993), cannot be excluded.

Another important aspect of the effect of ASA is that the prevention of collagen build-up in non-infarcted tissue after infarction did not result in a further increase of left ventricular cavity diameter in a relaxed state. Diastolic pressure–volume curves in infarcted hearts were not altered by ASA treatment (data not shown), indicating that also during distension by pressures that are physiological during diastole, left ventricular dilation was not aggravated by ASA treatment. Therefore, it is likely that ASA treatment did not aggravate the initial process of breakdown of pre-existing collagen fibres correlating with post-infarct dilation (Whittaker *et al*., 1991). Likewise, the slightly lower collagen content of the infarcted tissue did not result in further thinning of this area, which has been described with steroids and NSAIDs (Brown *et al*., 1983; Hammerman *et al*., 1983a,b; Mannisi *et al*., 1987). An effect of ASA treatment on interstitial fibrosis of spared myocardium, but not on replacement fibrosis of infarcted tissue, suggests that these are indeed two different processes governed by different control mechanisms. Corday *et al.* (1975) pointed out that in dogs, in an experimental model of myocardial infarction, replacement fibrosis was not limited to the infarcted area, but was also present in areas remote from the infarct, as a result of focal necrosis. Because we have not observed focal necrosis in the sections of spared myocardium analysed for fibrosis in the rat coronary artery ligation model, this indicates that we indeed quantified interstitial fibrosis in non-infarcted areas, and that replacement fibrosis could be considered limited to the infarcted area itself.

In the present study, only total collagen content was measured. Collagen type, cross-linking, fiber organization and fiber thickness could also alter the mechanical properties of the myocardium. We cannot exclude that not only the quantity, but also the characteristics of the collagen were affected by ASA treatment. For example, ASA treated hearts could have a higher proportion of thin collagen filaments, as is also suggested by the photomicrographs shown in Figure 1.

Since large myocardial infarction did not decrease heart dry weight to body weight ratios, hypertrophy of spared myocardium is indicated. Although we have not actually measured myocyte dimensions, comparable heart dry weights make it unlikely that ASA treatment would reduce cardiomyocyte hypertrophy in addition to collagen content, as is the case with ACE-inhibitor and angiotensin II blocker therapy (van Krimpen *et al*., 1991; Smits *et al*., 1992). There was no significant effect of ASA therapy on *in vitro* left ventricular performance. If anything, there may be a tendency to delay the onset of left ventricular dysfunction. After ASA treatment, the significant depression of peak

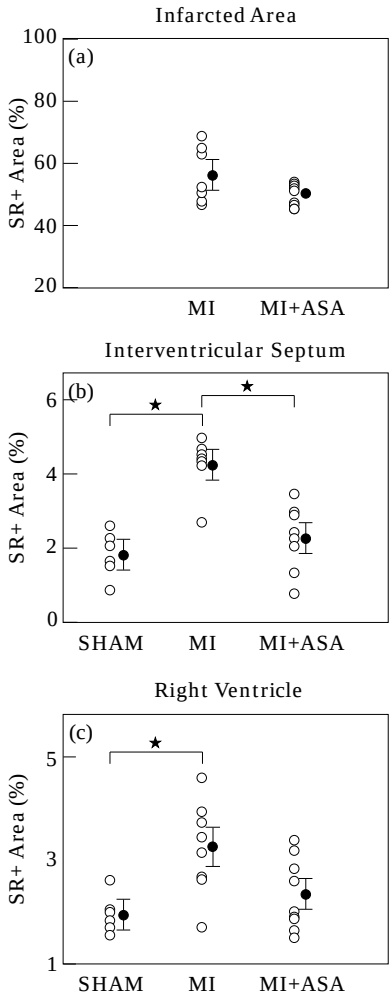

Figure 4 Collagen content, as measured by picrosirius red stained (SR+) tissue area and expressed as percentage of total tissue area, in infarct centre (a), interventricular septum (b) and right ventricle (c). MI: myocardial infarction; MI+ASA: ASA-treated myocardial infarction. Open symbols: values in individual hearts; Closed symbols: group means ± S.E.M. *:$P<0.05$.

velocity of contractility and relaxation in infarcted hearts, was limited to the latest time point (21 days after infarction). In pressure overloaded hearts, interstitial fibrosis rather than ventricular hypertrophy has been associated with decreased ventricular compliance (Brilla *et al*., 1991). Reduced stiffness of spared myocardium could be one mechanism by which a reduced interstitial collagen might attenuate the incidence and severity of post-infarct heart failure in the long-term. Although determination of interstitial collagen content of spared myocardium and the measurements of left ventricular function were not done at the same time point, nearly complete prevention of interstitial fibrosis at 14 days makes it unlikely that the treatment effect would be absent at the other time points during the healing period. Moreover, retardation of the healing period by ASA, implying a temporary nature of the observed treatment effects, is unlikely because of the lack of effect on collagen deposition in the infarcted area. However, the effect on interstitial fibrosis of remodeled, spared hypertrophic myocardium by ASA treatment, did not result in an altered diastolic pressure–volume relationship of the whole left ventricle (data not shown). Thus, at present, the consequence of pharmacological interference with collagen synthesis during remodeling after infarction, for cardiac function, remain unclear.

In conclusion, the results from the present study show that 25 mg/kg/day ASA, which causes a more marked inhibition of thromboxane than of prostaglandin synthesis in rats, seems pharmacologically equivalent to the use of chronic low-dose aspirin treatment in patients. This dose affected interstitial and perivascular fibrosis in the spared myocardium, while leaving wound healing, measured as scarring and thinning of the infarcted area, and compensatory hypertrophy relatively unaffected. Besides a possible delay in the development, aspirin treatment had no effect on final *in vitro* left ventricular dysfunction.

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