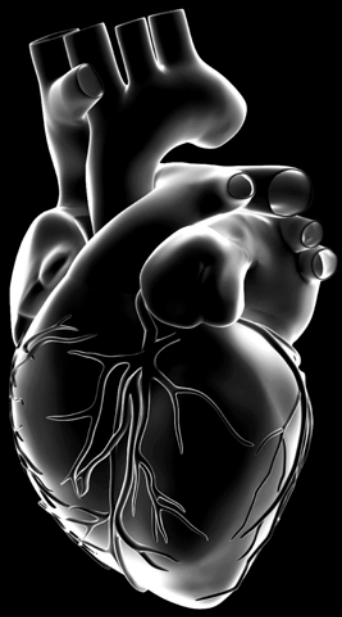
Novel pharmaeutical interventions in experimental atherosclerosis and myocardial infarction

Hoorn, Johanna Wijnanda Anthonia van der



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Negative effects of rofecoxib treatment on cardiac function after ischemia-reperfusion injury in APOE*3Leiden mice are prevented by combined treatment with thromboxane-prostanoid receptor antagonist S18886 (terutroban)

JWA van der Hoorn
JW Jukema
MEA Bekkers
HMG Princen
S Corda
JJ Emeis
P Steendijk

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Abstract

Objective Selective COX-2 inhibition by rofecoxib was associated with increased risk of cardiovascular events. We hypothesized that concomitant treatment with thromboxane prostanoid receptor antagonist S18886 might ameliorate possible negative effects. We evaluated the effects of S18886, rofecoxib, and the interaction of both compounds in a combined treatment on myocardial infarct (MI) size and cardiac function after experimental ischemia/reperfusion injury in hyperlipidemic APOE*3Leiden transgenic mice.

Methods and Results After four weeks of feeding an atherogenic diet, MI was induced by a 30-min ligation of the left anterior descending coronary artery, followed by reperfusion. Oral compound treatment was initiated 90 minutes prior to MI, and continued daily by gavage for seven days. Four treatment groups (n=12, each) were studied: solvent (Control), S18886, rofecoxib, and S18886 plus rofecoxib. One week after MI, the mice were anesthetized and cardiac function was quantified by left ventricular (LV) pressure-volume relationships obtained by miniature pressure-conductance catheters. No significant differences in infarct size were found between groups as measured by morphometry. Compared to Control, treatment with S18886 did not affect heart function whereas the rofecoxib group had significantly lower cardiac output (4.5±0.8 vs. 3.2±1.1 mL/min, $p<0.01$), lower ejection fraction (40±8 vs. 27±11%, $p<0.005$), and increased end-systolic volume (18.6±5.7 vs. 28.6±9.0 μL, $p<0.05$). The group with combined (S18886+rofecoxib) treatment was not different from Control. Statistical analysis showed significant interactive effects between S18886 and rofecoxib indicating that negative effects of rofecoxib on cardiac function were prevented by S18886 treatment.

Conclusion Rofecoxib treatment reduced global and systolic LV function after ischemia-reperfusion injury in APOE*3Leiden mice. These negative effects are prevented by combined treatment with thromboxane prostanoid-receptor antagonist S18886.

Introduction

Cyclooxygenases are the rate-limiting enzymes in prostaglandin (PG) synthesis. They metabolize arachidonic acid to PGH_2, which is followed by cell-specific synthase and isomerase enzymes (**figure 1**). Two cyclooxygenase iso-enzymes are characterized: COX-1 and COX-2. COX-1 is constitutively expressed in most cells to mediate physiological responses and regulate homeostasis. COX-2 is expressed in a few organs including the central nervous system, kidneys and the gonads in a constitutive manner similar to COX-1[1], but shows increased expression in pathological conditions such as inflammation and ischemia and acts as an inflammatory mediator[2]. Based upon these findings, a subclass of nonsteroidal anti-inflammatory drugs, designed to selectively inhibit COX-2 has been developed for the treatment of chronic inflammatory diseases. However, their use has been under debate since an increased risk of cardiovascular events with the COX-2 inhibitor rofecoxib was noted in the Vioxx Gastrointestinal Outcomes Research study (VIGOR)[3] and was also observed in subsequent studies[4,5]. The Adenomatous Polyp Prevention on Vioxx (APPROVe)[6] study also showed a significant increase in the risk of myocardial infarction and atherothrombotic events, leading to the market withdrawal of rofecoxib. Two other large randomized controlled trials assessing the safety of celecoxib [7] and valdecoxib found the same side effects[8]. A recently published meta-analysis based on nine case-control studies and two cohort studies showed an relative risk of 1.35 for serious cardiovascular events with rofecoxib treatment[9]. To explain these observations, the hypothesis has been advanced that COX-2-selective inhibitors shift the balance in the vascular system between the prostanoid thromboxane A_2 (TXA_2) and prostaglandin I_2

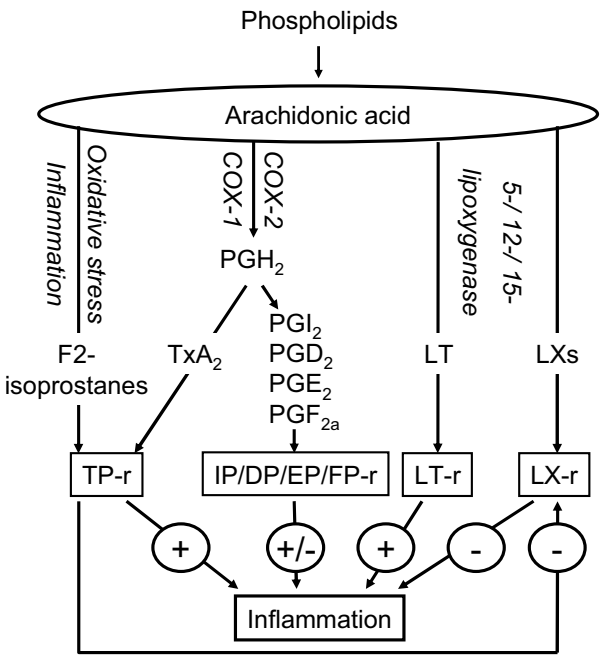

Figure 1. Schematic overview of the production arachidonic acid metabolites. The COX-derived PGs are mediators of inflammation, whereas TXA_2 and the LTs are mainly pro-inflammatory. LXs have anti-inflammatory effects. COX = cyclooxygenase, LT = Leukotriene, LX = lypoxin,PG = prostaglandin, xP receptor = prostanoid receptor, TXA_2 = Thromboxane A_2.

(PGI_2) by suppressing the synthesis of (endothelial) COX-2-derived PGI_2, without changing the (platelet) COX-1-derived TxA_2 synthesis[10]. Increased TXA_2 release has been shown during myocardial ischemia, possibly not only derived from aggregating platelets but also from macrophages and other immunologically reactive cells[11]. Since TXA_2 is responsible for platelet aggregation, vasoconstriction of coronary arteries, and has pro-inflammatory and cytotoxic effects[12], it can be speculated that TXA_2 exerts a deleterious effect during ischemia and reperfusion.

S18886 is a potent and selective antagonist of the thromboxane-prostanoid (TP) endoperoxide receptor[13]. The compound is being developed for the secondary prevention of ischemic events in patients with atherosclerosis. S18886 inhibits platelet aggregation and vasoconstriction induced by the agonists of the TP receptor, without affecting the production of prostacyclin by endothelial cells and other vessel wall cells[14]. We thus hypothesized that concomitant treatment with S18886 might ameliorate negative effects (if any) of COX-2 inhibition on the cardiovascular system, by redressing the functional balance between inflammatory mediator PGI_2 and the pro-inflammatory TXA_2.

The goal of the present study was to further investigate the previously reported negative effects of the COX-2-selective inhibitor rofecoxib on myocardial infarct size and on heart function and to test the hypothesis that concomitant treatment with thromboxane prostanoid receptor antagonist S18886 might ameliorate the anticipated negative effects. We used APOE*3Leiden transgenic mice on a standardized Western type diet - an established mouse model for hyperlipidemia and pharmaceutical interventions[15,16] - and induced myocardial injury by ischemia-reperfusion in order to mimic the human situation with regards to elevated plasma lipid levels and relatively limited infarct size due to early reperfusion by PTCA or thrombolysis.

Methods

Animals

Twelve-week-old male heterozygous APOE*3Leiden transgenic mice backcrossed into a C57BL6/J background and characterized by an ELISA for human apoE[15], were used. The mice were housed in a clean-conventional animal room at the Gaubius Laboratory TNO. Housing conditions were: relative humidity 50-60%, temperature ~21˚C, light cycle 6 am to 6 pm. Mice were housed in macrolon cages. Food and water were supplied *ad libitum*. Animal experiments were approved by the Institutional Animal Care and Use Committee of The Netherlands Organization for Applied Scientific Research TNO, and conformed to the rules and regulations set forth by The Netherlands Animal Experiments Act.

Study design

Fifty APOE*3Leiden mice were fed a standardized Western type diet, containing 15% (w/w) cacao butter, 1.0% (w/w) cholesterol and 0.05% (w/w) cholate[17]. After three weeks, mice were randomized into four groups of 12 or 13 mice each, matched for body weight and plasma cholesterol level. One week later, myocardial ischemia and reperfusion was induced, essentially as described by Michael et al.[18]. One hour prior to the induction of anesthesia (approximately 90 min before the ischemic period), mice were treated orally (100 µL/10 gram bw) with vehicle (1.0% hydroxyethylcellulose) for the control group, 10 mg S18886/kg bw, for the S18886 group, or 10 mg rofecoxib/kg bw for the rofecoxib group. The S18886 plus rofecoxib combination group received 10 mg S18886/kg plus 10 mg rofecoxib/kg (given separately, each in 50 µL/10 gram body weight). Subsequently, drugs or solvent were administered daily by gavage until sacrifice seven days later. The S18886 dose was based on previous studies[13,19,20] showing effective TP receptor antagonism with 5-10 mg/kg/day. Likewise rofecoxib dose was chosen in line with previous studies[21-23] mimicking human use and taking into account approximately 10x higher metabolism in the mouse.

Body weight was determined weekly. Seven days after ischemia-reperfusion, heart function was assessed by LV pressure-volume relationships (as described in detail below), and the hearts were harvested to measure the infarct size. Before excision of the heart, intracardiac blood was drawn and serum was prepared (30 min 37ºC, 30 min 4ºC, 2000 x g for 15 min) and stored at -70ºC.

Induction of myocardial ischemia.

The mice were anesthetized by intraperitoneal injection of a mixture of fentanyl (0.8 mg/kg bw), fluanison (25 µg/kg bw) and midazolam (12.5 µg/kg bw) (FFM). An intratracheal tube was inserted, and the animals were artificially ventilated, using a dedicated mouse ventilator (UNO, Zevenaar, The Netherlands). After left lateral thoracotomy, the left anterior descending coronary artery (LAD) was ligated just distal to the left atrial appendix. After thirty minutes of ischemia (evidenced by blanching in the LV), reperfusion was initiated by removing the ligature. The thorax was closed, and the mouse was allowed to recover on a heating pad[18]. Oxygen support and an analgesic (Nubaine) were given as required.

Hemodynamic measurements

Seven days after myocardial ischemia-reperfusion, the mice were re-anesthetized with FFM, intubated, ventilated, and instrumented to assess LV function by pressure-volume loop analysis. The jugular vein was cannulated for infusion of hypertonic saline to determine parallel conductance. Via the carotid artery a miniaturized pressure-conductance catheter (SPR-839, Millar Instruments, Houston, TX) was positioned into

the LV. The abdomen was opened just below the diaphragm to enable temporary preload reductions by directly compressing the inferior vena cava. The pressure-conductance catheter was connected to a Sigma-SA signal processor (CD Leycom, Zoetermeer, The Netherlands) for on-line display and registration of LV pressure and volume signals. All data were acquired at 2000Hz using Conduct-NT software (CD Leycom) and analyzed off-line by custom-made software (CircLab). LV pressure-volume signals were acquired in steady state to quantify general hemodynamic conditions: heart rate (HR), stroke volume (SV), cardiac output (CO), end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), end-diastolic pressure (EDP), and end-systolic pressure (ESP). Stroke work (SW) was determined as the area of the pressure-volume loop, and the maximal and minimal rate of LV pressure change, dP/dt_{MAX} and dP/dt_{MIN}, were obtained. Effective arterial elastance (E_A) was calculated as ESP/SV. Relaxation time τ was calculated as the time-constant of mono-exponential pressure decay during isovolumic relaxation[24]. To obtain load-independent indices of LV function, we determined pressure-volume relations by recording pressure-volume loops during a gradual preload reduction. The slopes of end-systolic pressure-volume relation (end-systolic elastance, E_{ES}) and end-diastolic pressure-volume relation (end-diastolic stiffness, E_{ED}) quantify systolic and diastolic function, respectively[25]. This approach is illustrated in **figure 2**.

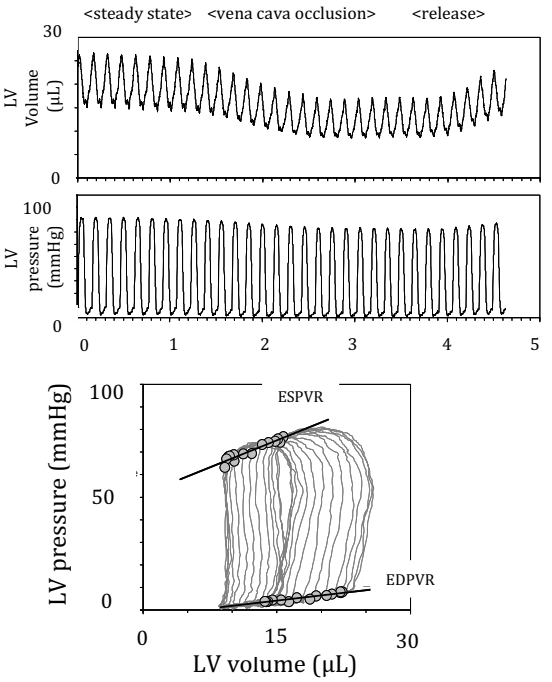

Figure 2. Typical pressure-volume loops to illustrate our methodology. End-systolic and end-diastolic pressure-volume relations (ESPVR, EDPVR) were determined from pressure-volume loops obtained during gradual preload reduction by vena cava occlusion. The slopes of these relations (E_{ES} and E_{ED}) are load-independent parameters of systolic and diastolic LV function. Steady state hemodynamic parameters were derived from pressure-volume loops just prior to vena cava occlusion.

Infarct area, morphometry and immunohistochemistry

After hemodynamic measurements, a cannula was inserted into the aorta, and the heart was stopped in diastole by injection of 0.1M cadmium chloride, followed by perfusion of the heart with sodium nitroprusside (0.1mg/mL saline) for two minutes and perfusion fixation using 4% p-

formaldehyde. The heart was then removed, fixed overnight in p-formaldehyde, and cut into six 1 mm-thick slices, perpendicular to the long axis of the heart. These slices were flat-embedded in paraffin and 3 µm-thick sections were prepared. Sections were stained by the haematoxylin-phloxin-saffran (HPS) method for general histology. To delineate the infarcted area, sections were stained immunohistochemically with a mouse monoclonal antibody against cardiac Troponin T (Lab Vision Co, Fremont, CA; NeoMarkers cat # MS-295-P), and with Sirius red F3B for collagen to delineate the infarcted area. On paraffin sections from the six 1 mm-slices the infarct area and the total LV wall area (including the septum) were measured by morphometry (Leitz Qwin system). The LV area was measured on HPS-stained sections. The infarcted area was measured on Sirius red stained sections. Sirius red staining and Troponin T-immunohistochemical staining were generally fully overlapping, but delineation was easier with Sirius-Red staining and therefore preferred for infarct quantification. The infarct area was expressed as percentage (vol/vol) of the LV wall volume. The volumes were calculated as measured area multiplied by slice thickness. COX-2 immunoreactivity was visualized immunohistochemically[26], using an affinity-purified polyclonal antibody against COX-2 (Cayman Chemical Co, Ann Arbor, MI, cat # 160126).

Plasma assays

At randomization and at sacrifice total cholesterol (Roche Diagnostics, No-1489437) and total triglycerides (Roche Diagnostics, No-1488872) were determined.

Statistical analysis

Differences between groups were analyzed by one-way ANOVA followed by post-hoc Bonferroni correction for multiple comparisons using SPSS for Windows (version 12.0, SPSS Inc, Chicago IL). Differences were considered significant at $p<0.05$. All data are presented as mean±SD (unless otherwise indicated). To specifically test whether the effect of rofecoxib was significantly altered by the absence or presence of S18886 we applied a full factorial univariate linear regression model. If the S18886*rofexocib interaction term included in this model reached significance, this indicated that presence of S18886 significantly influenced the effect of rofecoxib (or *vice versa*).

Results

Body weight and plasma values

Body weights increased during the dietary study phase, and slightly decreased postoperatively, most likely due to the ischemic insult. Plasma cholesterol and triglyceride levels were equal in all groups both at surgery (week 3) and at sacrifice (week 5) (**table 1**).

		Control	S18886	ROF	ROF + S18886
At randomization					
Body weight	(gram)	22.9 ± 1.9	23.0 ± 1.6	24.2 ± 2.5	23.6 ± 2.1
Cholesterol	(mmol/L)	12.4 ± 2.8	13.2 ± 7.1	11.6 ± 4.2	12.7 ± 4.7
Triglycerides	(mmol/L)	2.1 ± 0.6	1.9 ± 0.8	1.8 ± 0.7	2.1 ± 0.9
At sacrifice					
Body weight	(gram)	22.4 ± 1.4	23.0 ± 1.6	24.9 ± 2.2	23.8 ± 1.7
Cholesterol	(mmol/L)	11.9 ± 3.5	14.3 ± 3.0	11.8 ± 6.5	12.1 ± 2.1
Triglycerides	(mmol/L)	1.9 ± 0.6	2.0 ± 0.5	1.8 ± 0.6	2.1 ± 0.5

Table 1. Body weight and plasma lipids. Body weight and plasma cholesterol and triglycerides were measured at randomization and at sacrifice. No significant differences between groups were observed at any time point.

Ventricular volume and infarct size

LV myocardial volume and infarct size did not differ between groups (**figure 3**). In the control group, the 7-day-old infarct constituted 9.0±3.9% of the LV wall volume.

Cardiac function

The results of the cardiac function measurements are shown in **table 2**. ANOVA indicated significant differences between groups for CO, ESV and EF. SW just failed to reach statistical significance (P=0.067). These indices are presented as bar graphs in **figure 4** (left panels). Between-group comparisons showed that the rofecoxib group had significantly lower CO, higher ESV and lower EF compared to the control group, indicating reduced global and systolic LV function. No other significant effects were present, except for a significantly higher CO in the S18886+rofecoxib group compared to the rofecoxib group. The finding that neither

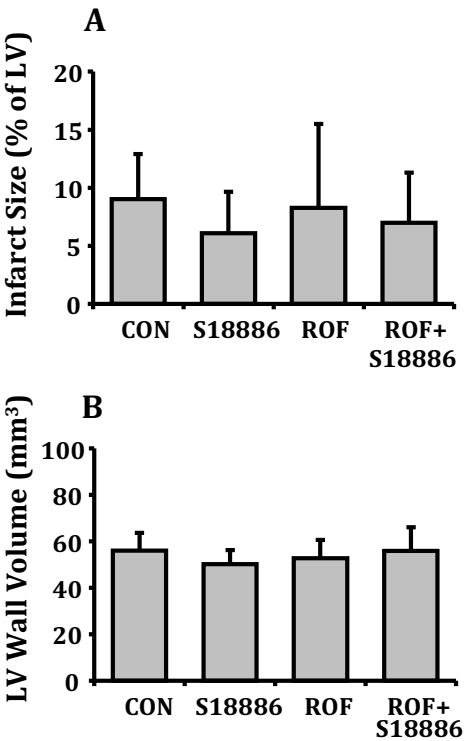

Figure 3. The effect of S18886, rofecoxib (ROF), and their combination on infarct size and LV wall volume. No differences were observed between the groups. Data are presented as mean ± SD.

the S18886 nor the group with combined S18886+rofecoxib treatment showed significant differences with the control group suggests an interactive effect by which the negative effects of rofecoxib are prevented by combined treatment with S18886. To specifically test the rofecoxib-S18886 interactive effects we applied a univariate analysis. The results are presented in **table 2** and for the indices that showed significance also displayed in **figure 4** (right panels). The results show significant interaction for CO, SW and EF, and marginally significant interaction (p=0.074) for ESV. The significant interaction indicates that the effect of rofecoxib is different in the absence and presence of S18886. In all cases the significant negative effect of rofecoxib on cardiac function in absence of S18886 (-S18886) was reversed in presence of S18886 (+S18886).

Cardiac function		Groups					Univariate linear		
		Control	S18886	ROF	ROF + S18886	ANOVA P	S18886 effect	ROF effect	Inter-action
General									
HR	(beats/min)	385 ± 51	369 ± 42	350 ± 58	377 ± 62	0.437	0.735	0.372	0.181
CO	(mL/min)	4.5 ± 0.8	3.9 ± 1.3	3.2 ± 1.1*	4.6 ± 1.3#	**0.017**	0.254	0.393	**0.004**
SW	(mmHg.µL)	800 ± 160	668 ± 260	584 ± 294	819 ± 232	0.067	0.464	0.651	**0.012**
E_A	(mmHg/µL)	6.2 ± 1.3	7.2 ± 3.7	7.8 ± 3.7	5.9 ± 1.9	0.345	0.616	0.860	0.084
Systolic									
ESV	(µL)	18.6 ± 5.7	23.2 ± 10.7	28.6 ± 9.0*	24.4 ± 7.0	**0.044**	0.909	**0.025**	0.074
ESP	(mmHg)	70.3 ± 8.7	64.9 ± 16.4	61.9 ± 13.8	66.5 ± 9.3	0.533	0.900	0.354	0.175
EF	(%)	40.4 ± 8.1	33.4 ± 9.4	27.0 ± 11.0**	35.2 ± 7.5	**0.008**	0.810	**0.032**	**0.006**
dP/dt_{max}	(mmHg/ms)	6.0 ± 1.9	4.7 ± 1.7	4.5 ± 2.2	5.5 ± 2.3	0.263	0.799	0.527	0.063
E_{ES}	(mmHg/µL)	2.1 ± 0.8	1.8 ± 0.6	2.0 ± 1.0	1.7 ± 0.5	0.510	0.158	0.650	0.794
Diastolic									
EDV	(µL)	29.8 ± 6.5	33.3 ± 13.0	37.1 ± 10.1	35.8 ± 8.8	0.293	0.705	0.092	0.396
EDP	(mmHg)	8.4 ± 3.2	9.5 ± 5.3	9.9 ± 5.4	6.7 ± 1.8	0.280	0.400	0.618	0.091
$-dP/dt_{min}$	(mmHg/ms)	4.8 ± 1.1	3.9 ± 9.6	3.8 ± 1.4	4.3 ± 1.3	0.195	0.650	0.442	**0.049**
τ	(ms)	11.5 ± 2.1	12.9 ± 1.4	13.3 ± 3.1	13.3 ± 3.0	0.269	0.353	0.139	0.345
E_{ED}	(mmHg/µL)	0.43 ± 0.20	0.49 ± 0.25	0.47 ± 0.31	0.33 ± 0.15	0.315	0.532	0.376	0.125

Table 2. Cardiac function: general, systolic and diastolic indices. * p<0.05 vs Control group; ** p<0.005 vs Control group; # p<0.05 vs Rofecoxib group (ROF). HR = heart rate; CO = cardiac output; SW = stroke work; E_A = arterial elastance (afterload); ESV = end-systolic-volume; ESP = end-systolic-pressure; dP/dt_{MAX} = maximal rate of pressure increase; E_{ES} = end-systolic elastance; EDV = end-diastolic-volume; EDP = end-diastolic-pressure; $-dP/dt_{MIN}$ = maximal rate of pressure decline; τ = relaxation time constant; E_{ED} = diastolic stiffness.

Immunohistochemistry of COX-2

Immunohistochemical staining for COX-2 showed minor or no reactivity in the myocardium remotely from the infarct area, but was upregulated in cardiomyocytes and vascular smooth muscle cells adjacent to an infarcted area (**figure 5**). Occasionally COX-2 was expressed in scattered cells in the infarcted area (most clearly in conjunction with signs of recent inflammatory activity). No differences were observed between the groups in the present study, as could be expected because infarct size was similar.

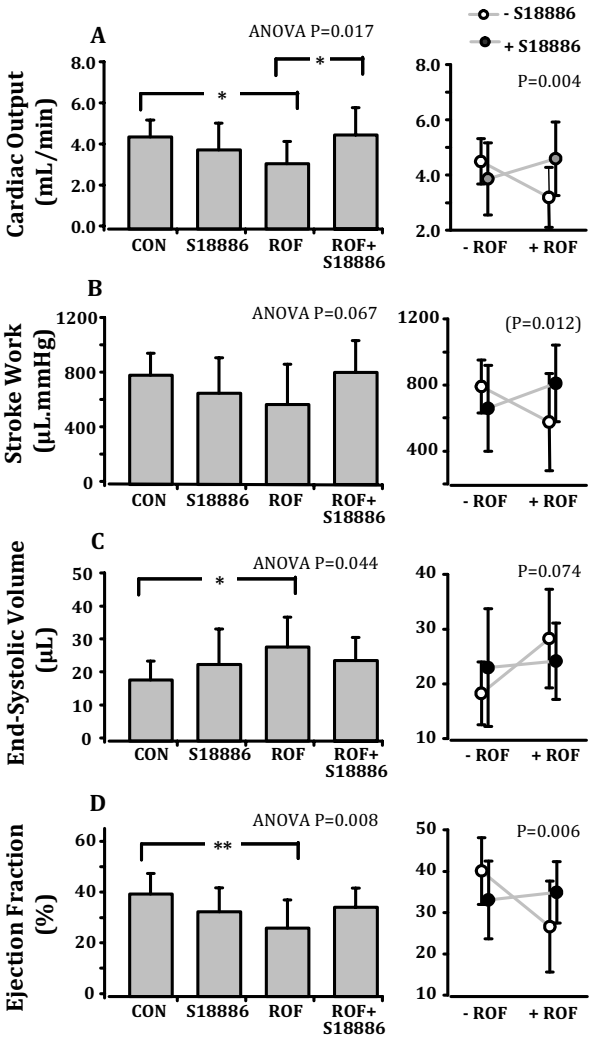

Figure 4. Cardiac output (A), stroke work (B), end-systolic volume (C) and ejection fraction (D) in control (CON) mice and in mice treated with S18886, rofecoxib (ROF), or ROF+S18886. The bar graphs in the left panels show the results for the four groups with ANOVA statistics. The figures in the right panels display the same data from the same four groups but focus on the interactive effects to illustrate the statistical method as described in detail in the text. The P-values refer to the significance of the interactive effect. Full statistics are provided in table 2.

Discussion

In line with previous studies, the present study showed significant negative effects of daily rofecoxib treatment on global and systolic LV function in mice seven days after ischemia reperfusion injury. The main new aspect of our study is the finding that these effects may be prevented by combining the rofecoxib treatment with S18886. The groups did not show differences in infarct size indicating that the hemodynamic effects were not primarily resulting from altered infarct size.

Beneficial effects of TP receptor antagonism have previously been reported in experimental studies of ischemia-reperfusion injury in several species[27,28]. However, the mechanism by which TP receptor antagonists exert their cardioprotective effect is not clear. It does not appear to be the consequence of increased coronary blood flow into ischemic region[29], nor does it appear to be the consequence of a reduced neutrophil accumulation[30]. A hypothesis is that TXA_2 released during reperfusion could result in vasoconstriction and platelet aggregation, contributing to a

no-reflow phenomenon to parts of ischemic myocardial tissue, despite a completely restored blood flow in the large coronary vessels. Other explanations for the benefits of TP-receptor antagonism are an enhanced blood flow during reperfusion, which is related to the increase in tissue viability, an improved utilization of oxygen in myocardial cells[31] or a reduced formation of free radicals[32]. Additionally TP receptor antagonist S18886 has shown anti-inflammatory actions in different murine models of hyperlipidemia and atherosclerosis[19,20,33,34]

In the present study we aimed for 'human like' conditions with regard to plasma lipid levels and ischemia-reperfusion mimicking clinical revascularization by early PTCA or thrombolysis rather than permanent coronary occlusion. Therefore we used animals with slightly elevated plasma lipid levels and obtained a relatively small infarct size. The area-at-risk after ligation of the LAD coronary artery was not measured, because the 7-day interval between the induction of the infarct and sacrifice made such a measurement of little relevance. In previous experiments (unpublished data) we acutely measured an area-at-risk after identical ligation of the LAD of about 38-40 % (vol/vol) of the LV. This would mean that the infarcts typically constituted about 23% of the area-at-risk, suggesting that the ischemic insult was less severe (but more clinically relevant) than in many previous murine myocardial infarction studies, which limits a direct comparison. In the present study, S18886 at 10 mg/kg/day seemed to be associated with a smaller infarct size than control but the difference was not statistically significant (6.1±3.6 vs. 9.0±3.9%, p=NS). Since it has been shown at least in rats[35] that relatively small infarcts (13% of the LV) do not result in reduced global heart function per se, it was not surprising that S18886 did not influence heart function. In fact, if anything, there appeared to be some tendency for depressed cardiac function, which however did not show statistical significance. Rofecoxib did not affect infarct size by itself, but did induce a significant cardiodepression evident from an increased ESV and a

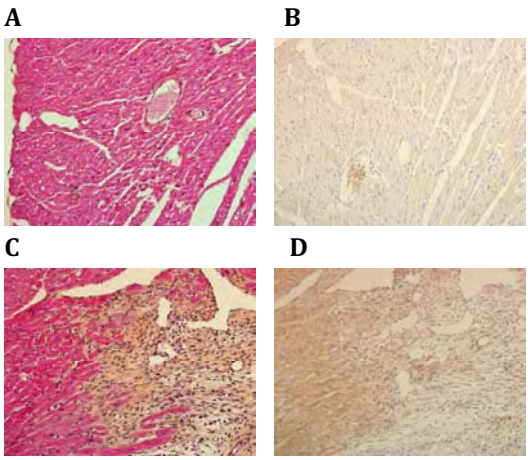

Figure 5. The upper panels show HPS staining (A) and immunohistochemical staining for COX-2 (B) of a section remote from the infarct area. The lower panels show staining for a section adjacent to the infarct. The remote cardiomyocytes do not express COX-2 (B), but the cardiomyocytes adjacent to the infarct area show clear COX-2 reactivity (D). Some COX-2 expression was present in scattered cells in the infarcted area. This example was from the Control group. No between-group differences were observed.

reduced EF and CO and a tendency to reduce SW. When rofecoxib treatment was combined with S18886 these effects were fully prevented. This provides evidence for S18886 to have a significant beneficial interactive effect when added to rofecoxib treatment, which was supported by statistical analysis of the interactive effects.

The use of COX-2 selective inhibitors in animal models of ischemic heart disease has been reported to be both beneficial and deleterious. Recent studies have demonstrated that COX-2 acts as a cardioprotective protein in the late phase of ischemic preconditioning. IP-receptor deficiency led to an increased infarct area[36] and COX-2 expression was associated with a cardioprotective effect after ischemic preconditioning[37]. Recently, impaired systolic function upon COX-2 inhibition was also found in a pig model of MI, with infarct size and EF comparable to our study[38]. Conversely, some studies with murine models of MI[22,23] and chronic heart failure[21] showed improved cardiac function in the group treated with the selective COX-2 inhibitors, without affecting the infarct size. However, in these MI studies the LAD was permanently ligated, which is not comparable to our study. Scheuren *et al*.[37,39] demonstrated, in a rat model of myocardial infarction, that a 4-day treatment with rofecoxib (3 mg/kg/day) resulted in reduced influx of inflammatory and fibroblast-like cells into infarcted tissue, without affecting the infarct size. In line with previous findings[2], we found presence of COX-2 immunoreactivity in heart muscle after ischemia-reperfusion injury suggesting that a change in local PGI_2 production might be involved in the changes in LV function after myocardial infarction. The favorable effects of S18886 may thus be due to restoring the local TXA_2-PGI_2 balance. However, it is also possible that antagonism of TP-receptors inhibits their activation by ligands such as TXA_2 and isoprostanes and subsequently prevents the induction pro-inflammatory pathways. As presented in **figure 1**, the metabolic pathway of arachidonic acid is a balanced and complex network of different cascades. It has been shown that agonizing the TP-receptor may also result in a reduced expression of the lipoxin (LX)-receptor[40], inhibiting the anti-inflammatory effects of the LXs. This might also contribute to the protective effect of S18886.

We conclude that COX-2 inhibition by daily rofecoxib treatment has deleterious effects on cardiac function after ischemia-reperfusion injury in APOE*3Leiden mice. These negative effects on ESV, CO, EF and SW are prevented by concomitant treatment with thromboxane prostanoid-receptor antagonist S18886. This study proves beneficial interaction of S18886 and rofecoxib, and might provide clues to further elucidate the mechanism underlying the increase in cardiovascular risk associated with COX-2 inhibiting treatments.

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Disclosures

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