Cyclooxygenase Inhibition Aggravates Ischemia–Reperfusion Injury in the Perfused Guinea Pig Heart: Involvement of Isoprostanes

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Objectives. Postischemic contractile dysfunction in the heart may be due, in part, to isoprostanes, thought to accumulate during myocardial reperfusion. This study tested whether cyclooxygenase (COX) inhibitors increase the amount of isoprostanes and, consequently, lead to deterioration of postischemic heart function.

Background. Isoprostanes are bioactive prostaglandin-like compounds that are formed in vivo directly by free radical–catalyzed peroxidation of arachidonic acid. In particular, 8-iso-prostaglandin (PG) F_2 alpha is a potent vasoconstrictor.

Methods. Isolated working guinea pig hearts underwent 30-min low flow ischemia followed by reperfusion, 15 min in a nonworking mode and 20 min performing pressure–volume work. Hearts were perfused with or without 100 μmol/liter acetylsalicylic acid (ASA), 3 or 10 μmol/liter indomethacin or 1 μmol/liter SQ 29548, a thromboxane-A_2 (TxA_2) receptor antagonist able to abolish the vasoconstrictive actions of 8-iso-PGF_2 alpha. External heart work (EHW) and coronary resistance were compared before and after ischemia. Coronary release and tissue content of 8-iso-PGF_2 alpha were also determined.

Results. During reperfusion, 8-iso-PGF_2 alpha release increased tenfold compared with the preischemic value in all groups. However, in ASA- and indomethacin-treated hearts, 8-iso-PGF_2 alpha levels were ~15-fold higher than in control hearts (5.4 vs. 0.35 pg/ml, respectively). Postischemic tissue levels of 8-iso-PGF_2 alpha were also markedly higher: 215 (indomethacin) and 301 (ASA) pg/ml g dry weight versus 43 pg/mg dry weight for control hearts (p < 0.05). Treatment of hearts with COX inhibitor led to a reduction in recovery of EHW (40% vs. 71%, p < 0.05) and seemed to be due to impaired myocardial oxygenation: Coronary venous oxygen was lower (67% of control values), whereas anaerobic metabolism (lactate release vs. pyruvate consumption) was enhanced. Coronary resistance was correspondingly elevated (164% of control values). SQ 29548 caused all variables to revert to control values.

Conclusions. These data demonstrate that in the guinea pig heart, COX-inhibiting drugs exacerbate loss of cardiac function after ischemia. The enhanced production of isoprostanes favors coronary vasoconstriction and leads to myocardial oxygen deprivation.

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of isoprostanes in modulating the extent of acute ischemia–reperfusion injury in the presence of COX inhibitors and identify a pharmacologic interaction of potential therapeutic importance, namely, application of a “thromboxane” receptor antagonist also able to block isoprostane-mediated effects.

**Methods**

**Working heart preparation.** The investigation was performed in conformance with the “Position of the American Heart Association on Research Animal Use” adopted by the Association in November 1984. Isolated hearts from male guinea pigs (230 to 280 g) were prepared as previously described in detail in reports from this laboratory (11). Briefly, animals were stunned by neck dislocation, and the hearts were initially retrogradely perfused through the aorta at a constant pressure of 80 cm H\(_2\)O initially. Coronary flow and differences in P\(_{O2}\) of the perfusate were used (13).

**Physiologic measurements.** The aortic pressure pulse and left ventricular filling pressure were measured directly using pressure transducers (model 1050 BP, ADInstruments, Australia). Mean aortic pressure, heart rate and ejection time of stroke volume were derived from the pressure signal. Coronary flow and aortic flow were determined by means of a two-channel flowmeter (model T 206, Transonic Systems Inc). Partial pressure of oxygen in the coronary venous effluent (P\(_{O2}\)) was measured using a Clark O\(_2\) microelectrode (Bachofer, Reutlingen, Germany). To characterize cardiac function, external heart work (EHW) was defined as the sum of pressure–volume work and acceleration work (12) and cardiac output as the sum of aortic flow and coronary flow. To calculate myocardial oxygen consumption (M\(_{VO2}\)) (per g heart weight), coronary flow and differences in P\(_{O2}\) of the perfusate (P\(_{O2}\) held constant at 630 mm H\(_{2}\)O) and P\(_{O2}\) were used (13).

**Experimental protocol.** The perfusion protocol is schematized in Figure 1 and was chosen to ensure only mild loss of postischemic function under control conditions, facilitating the detection of deleterious effects of COX inhibition.

After an initial 20-min work phase (work phase 1) under strictly standardized conditions (mean left ventricular filling pressure 8.8 mm Hg, mean arterial pressure 63 mm Hg) during which baseline hemodynamic variables were obtained, normothermic low flow ischemia was induced by perfusing hearts for 30 min in the Langendorff mode at a coronary flow of 1 ml/min, equivalent to ~10% of the spontaneous coronary flow during work. The following 15-min phase of reperfusion with the oxygenated KHB was initiated by elevating the coronary flow to a constant 5 ml/min, with the hearts remaining in the nonworking Langendorff mode of perfusion. In the first 5 min of this reperfusion interval, coronary venous effluent was collected to measure the early myocardial pyruvate consumption and the release of lactate and 8-iso-PGF\(_{2\alpha}\). Hearts were then resubjected to pressure–volume work for a second period of 20 min (work phase 2) and all hemodynamic variables (see Physiologic measurements) were again determined. Recovery of a given variable was defined as the posts ischemic value versus the preischemic value after 20 min of work, expressed in percent. Control hearts (n = 6) received no additives to the perfusate. In all experiments involving the COX inhibitors ASA (n = 6) or indomethacin (n = 4 for each concentration), these agents were applied throughout the experiment. Stock solution of indomethacin was added directly to the perfusion medium to achieve final concentrations of 3 or 10 \(\mu\)mol/liter. The fresh stock solutions of ASA and SQ 29548 were infused either into the left atrial cannula (working phase) or the aortic

**Figure 1.** Perfusion protocol. After an initial period (20 min) of pressure–volume work (Work 1), hearts were subjected to 30 min of low flow ischemia (1 ml/min), followed by the reperfusion phase (R) (15 min), during the first 5 min of which coronary venous effluent was collected, and a second period of 20 min of pressure–volume work (Work 2). The following groups were investigated: Control = perfusion with Krebs-Henseleit buffer alone; ASA = 100 \(\mu\)mol/liter ASA applied throughout; INDO = 3 or 10 \(\mu\)mol/liter indomethacin applied throughout; SQ = 1 \(\mu\)mol/liter SQ 29548 applied throughout; ASA + SQ = ASA plus SQ 29548 applied throughout; ASA + SQ(R) = ASA throughout but SQ 29548 applied only from the onset of the reperfusion period; INDO + SQ(R) = 10 \(\mu\)mol/liter indomethacin throughout but SQ 29548 applied only from the onset of the reperfusion period.
feed line (Langendorff phase) at rates calculated to yield the desired end concentration in the cardiac perfusate. The choice of concentrations of ASA and indomethacin was based on reports in which, after 15 min of application, 100 μmol/liter ASA (14) and 10 μmol/liter indomethacin (15) sufficed to abolish irreversibly the synthesis of COX products of endothelial cells.

SQ 29548 was infused either at the onset of work phase 1 (SQ 29548 alone, n = 4; ASA plus SQ 29548, n = 6) and then continued until the end of the study protocol or was given only at the onset of reperfusion (ASA plus SQ 29548[R] and indomethacin plus SQ 29548[R], n = 4 each). The selected concentration of 1 μmol/liter of SQ 29548, by itself, has not been associated with alterations in ventricular performance but has conferred beneficial effects on isolated guinea pig hearts perfused with exogenous isoprostanes (8).

**Determination of lactate release and pyruvate consumption.** Coronary venous effluent was collected through the pulmonary artery at the end of work phase 1 and in the early reperfusion period (first 5 min), and aliquots were stored at −20°C to await analysis. The concentrations of lactate and pyruvate were determined by high performance liquid chromatography (8). The concentration values, multiplied by the coronary flow rate, yielded cardiac release. Subtraction of the pyruvate value from the inflow concentration (0.3 mmol/liter) before multiplication yielded cardiac consumption.

**Determination of 8-iso-PGF₂α.** Measurements of 8-iso-PGF₂α concentrations in the coronary effluent were performed using a commercially available enzyme immunoassay (SPI-BIO, Massey Cedex, France). Effluent was collected at the end of work phase 1 and during the first 5 min of reperfusion and stored at −80°C until analyzed. At the end of the experiment, four hearts each from the control, ASA and indomethacin groups were clamped with aluminum tongs cooled to the temperature of liquid nitrogen and stored at −80°C until analyzed. Frozen ventricular tissue was pulverized under liquid nitrogen. A portion of the powdered tissue was used to determine the dry/wet weight ratio. Extraction of 8-iso-PGF₂α from ~100 mg of frozen powdered ventricular tissue was achieved with 3 ml of 0.5 mol/liter perchloric acid. After neutralization, isoprostane levels were determined by enzyme immunoassay (RD-Systems, Wiesbaden, Germany). All results were expressed in pg/mg dry heart weight. The average recovery of 8-iso-PGF₂α spiked into tissue samples was 106%. All values were corrected by this amount.

**Materials.** SQ 29548 was purchased from SPI-BIO and ASA and indomethacin from Sigma (Deisenhofen, Germany). All other reagents were obtained from Merck (Darmstadt, Germany). SQ 29548 (2.58 mmol/liter) was dissolved in distilled water and ASA (100 mmol/liter) and indomethacin (1 mmol/liter) in KHB solution immediately before application to the hearts; all three solutions were slightly alkalized with NaOH (pH < 8.5).

**Statistical analysis.** Results are expressed as mean value ± SEM, and the number of experiments varied from four to six. Statistical evaluation was carried out by one-way analysis of variance with post hoc comparison by the Student-Newman-Keul test. A p value < 0.05 was considered statistically significant.

**Results**

**Hemodynamic data.** Hemodynamic and functional variables are summarized in Table 1. There were no statistically significant differences between the various groups for any variable measured at the end of work phase 1.

After exposure of control hearts to 30 min low flow ischemia, coronary flow and aortic flow recovered to 75 ± 2% and 72 ± 3%, respectively, at the end of work phase 2, whereas heart rate recovered fully (Table 1). The difference from work phase 1 in cardiac output resulted in a modest but significant reduction in EHW (Table 1, Fig. 2). Coronary vascular resistance, calculated from the mean aortic pressure/coronary flow ratio, rose from the 5th to the 15th minute of reperfusion (Table 2); this taken as a sign of waning reactive hyperemia. Coronary resistance rose further in work phase 2.

**Effect of COX inhibition.** Treatment of hearts with ASA at a concentration fully inhibiting COX did not modify myocardial contractility during the preischemic period (Table 1). However, ASA significantly worsened the recovery of coronary flow and aortic flow (Table 1) and induced a significantly lower recovery of EHW of only 40 ± 6% versus 71 ± 3% for control hearts (Fig. 2). Furthermore, in the presence of ASA, coronary vascular resistance during the early reperfusion period (first 5 min) and in work phase 2 was greatest than that in the untreated preparations (Table 2).

Effects similar to those produced by ASA were obtained with indomethacin, which, with increasing concentrations, caused a reduction in posts ischemic recovery of all variables except heart rate (Table 1). Coronary flow recovered to 59 ± 3%, aortic flow to 52 ± 5% and EHW to 54 ± 5% at an indomethacin concentration of 3 μmol/liter (data not shown) versus 57 ± 4%, 47 ± 7% and 48 ± 6%, respectively, with 10 μmol/liter indomethacin (Table 1, Fig. 2). As in the case of ASA, coronary vascular resistance during restoration of flow after 30 min of ischemia as well as during work phase 2 was marked higher in the indomethacin group than in the control group (Table 2).

**Additional TxA₂ receptor blockade.** The “thromboxane” receptor antagonist SQ 29548 did not significantly alter the pattern of functional recovery observed in the control group, and there was even a modest trend toward a beneficial effect (Table 1, Fig. 2). Thus, functional recovery of all measured variables after ischemia and reperfusion was significantly higher in the SQ 29548 group than in the ASA and indomethacin groups (Tables 1 and 2, Fig. 2). The addition of SQ to ASA throughout the experiment resulted in improved recovery (p < 0.05) of EHW (79 ± 2% vs. 64 ± 3%), coronary flow (75 ± 3% vs. 55 ± 1%) and aortic flow (67 ± 1% vs. 39 ± 7%) (Table 1, Fig. 2). Importantly, SQ 29548 improved posts ischemic myocardial recovery of ASA- and indomethacin-treated hearts to control levels. This improvement was also seen when
the application of SQ 29548 started exclusively with reperfusion (Table 1, Fig. 2). In addition, the ASA- and indomethacin-induced elevation of coronary resistance during the initial reperfusion phase was abolished (Table 2).

**Metabolic data.** **Myocardial oxygenation.** $\text{MVO}_2$ of control hearts and hearts perfused with SQ 29548 decreased during work phase 2 to $\sim80\%$ of that during work phase 1 (Table 1).

This decrease corresponded well with the decreased performance of EHW in these two groups of hearts (Fig. 2). In contrast, in hearts perfused with inhibitors of COX, postischemic $\text{MVO}_2$ amounted to $\sim60\%$ of the preischemic value,

### Table 1. Preischemic and Postischemic Functional and Hemodynamic Variables of Different Groups of Hearts

<table>
<thead>
<tr>
<th>Group and Work Phase</th>
<th>Heart Rate (beats/min)</th>
<th>Coronary Flow (ml/min per g)</th>
<th>Aortic Flow (ml/min per g)</th>
<th>EHW (mJ/min per g)</th>
<th>$\text{MVO}_2$ (mol/min per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>249 ± 5</td>
<td>10.6 ± 0.6</td>
<td>39.9 ± 3.5</td>
<td>425 ± 32</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td>W1</td>
<td>243 ± 6</td>
<td>8.0 ± 0.2*</td>
<td>29.0 ± 3.1*</td>
<td>304 ± 28*</td>
<td>5.6 ± 0.1*</td>
</tr>
<tr>
<td>W2</td>
<td>256 ± 5</td>
<td>10.6 ± 0.8</td>
<td>39.7 ± 2.0</td>
<td>407 ± 21</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>SQ 29548 (n = 4)</td>
<td>238 ± 7</td>
<td>8.5 ± 1.0</td>
<td>30.1 ± 3.2*</td>
<td>312 ± 32*</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>ASA (n = 6)</td>
<td>248 ± 5</td>
<td>10.1 ± 0.7</td>
<td>36.1 ± 3.2</td>
<td>377 ± 29</td>
<td>6.7 ± 0.5</td>
</tr>
<tr>
<td>W1</td>
<td>232 ± 8</td>
<td>5.5 ± 0.5*†‡§§</td>
<td>14.2 ± 3.4*†‡§§</td>
<td>154 ± 31*†‡§§</td>
<td>4.0 ± 0.3*†‡§§</td>
</tr>
<tr>
<td>ASA+SQ 29548 (n = 6)</td>
<td>249 ± 3</td>
<td>10.5 ± 1.0</td>
<td>40.3 ± 5.2</td>
<td>414 ± 41</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>W1</td>
<td>237 ± 6</td>
<td>6.1 ± 1.0*</td>
<td>19.1 ± 5.5*</td>
<td>203 ± 43*#</td>
<td>4.4 ± 0.7*</td>
</tr>
<tr>
<td>ASA+SQ 29548 (R) (n = 4)</td>
<td>240 ± 5</td>
<td>9.9 ± 0.5</td>
<td>34.8 ± 3.6</td>
<td>365 ± 33</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>W1</td>
<td>227 ± 10</td>
<td>10.3 ± 0.6</td>
<td>41.5 ± 4.4</td>
<td>423 ± 39</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>W2</td>
<td>233 ± 12</td>
<td>7.5 ± 0.5*</td>
<td>28.5 ± 3.9</td>
<td>292 ± 35*</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>Indo+SQ 29548 (R) (n = 4)</td>
<td>244 ± 10</td>
<td>11.1 ± 1.0</td>
<td>45.7 ± 2.1</td>
<td>464 ± 24</td>
<td>7.4 ± 0.7</td>
</tr>
<tr>
<td>W1</td>
<td>227 ± 15</td>
<td>7.8 ± 0.3*</td>
<td>31.3 ± 1.4*</td>
<td>317 ± 12*</td>
<td>5.6 ± 0.3*</td>
</tr>
</tbody>
</table>

* $p < 0.05$ versus work phase 1. † $p < 0.05$ versus control and SQ 29548 groups at matched time point. ‡ $p < 0.05$ versus ASA+SQ 29548 group at matched time point. § $p < 0.05$ versus Indo+SQ 29548 (R) at matched time point. Data presented are mean value ± SEM. ASA = acetylsalicylic acid; EHW = external heart work; Indo = indomethacin; $\text{MVO}_2$ = myocardial oxygen consumption; R = SQ 29548 (1 mol/liter) applied from the onset of reperfusion; all other drugs were applied throughout the experiment (ASA: 100 mol/liter; Indo: 10 μmol/liter); W1 = work phase 1; W2 = work phase 2.

### Table 2. Coronary Vascular Resistance Determined at End of Early and Late Phase of Reperfusion and at End of Postischemic Work Phase

<table>
<thead>
<tr>
<th>Group</th>
<th>$R_1$ (mm Hg/ml per min)</th>
<th>$R_2$ (mm Hg/ml per min)</th>
<th>$W_2$ (mm Hg/ml per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>4.6 ± 0.3</td>
<td>6.6 ± 0.6</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>SQ 29548 (n = 4)</td>
<td>4.0 ± 0.2</td>
<td>6.1 ± 0.4</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>ASA (n = 6)</td>
<td>6.5 ± 0.5†‡§§</td>
<td>7.5 ± 0.3</td>
<td>11.8 ± 0.8†‡§</td>
</tr>
<tr>
<td>Indo (n = 4)</td>
<td>6.3 ± 0.3§§</td>
<td>7.4 ± 1.8</td>
<td>10.2 ± 0.9§</td>
</tr>
<tr>
<td>ASA+SQ 29548 (n = 6)</td>
<td>4.4 ± 0.3</td>
<td>6.3 ± 0.5</td>
<td>7.9 ± 0.4</td>
</tr>
<tr>
<td>ASA+SQ 29548 (R) (n = 4)</td>
<td>4.1 ± 0.5</td>
<td>6.5 ± 0.6</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>Indo+SQ 29548 (R) (n = 4)</td>
<td>4.6 ± 0.5</td>
<td>6.9 ± 0.5</td>
<td>7.4 ± 0.3</td>
</tr>
</tbody>
</table>

* Coronary vascular resistance was measured after the first 5 min ($R_1$) and subsequent 10 min of reperfusion ($R_2$) and after the second work phase ($W_2$). † $p < 0.05$ versus control and SQ 29548 groups. ‡ $p < 0.05$ versus ASA+SQ 29548 and ASA+SQ 29548 (R) groups. § $p < 0.05$ versus Indo+SQ 29548 (R) group. Data presented are mean value ± SEM. ASA = acetylsalicylic acid 100 μmol/liter applied throughout; Indo = indomethacin (10 μmol/liter applied throughout); R = SQ 29548 (1 μmol/liter) applied only from the onset of reperfusion.
whereas EHW levels had recovered to only 40% to 50%. The addition of SQ 29548 restored MVO₂ (and EHW) to control levels (Table 1).

To address whether reduced MVO₂ was in accordance with decreased function or was the cause of the functional deterioration of hearts perfused with ASA or indomethacin, we compared coronary flow during work phase 2 with the PV O₂ of the respective hearts (Fig. 3). Figure 3 (top panel) clearly shows that coronary flow recovered to only ~55% of the preischemic value in hearts treated with COX inhibitors. SQ 29548 restored recovery of coronary flow to control levels. PV O₂ (Fig. 3, bottom panel) was significantly lower in hearts perfused with ASA or indomethacin than in the other groups. Because PA O₂ was identical in all cases, those hearts with low recovery of coronary flow were trying to extract more oxygen from the perfusate, a phenomenon again normalized by SQ 29548. These findings indicate that the lowered coronary flow during work phase 2 was not matched to the oxygen demand of the hearts with inhibited COX.

Ratio of lactate release to pyruvate consumption. The molar ratio of lactate release to pyruvate consumption of the hearts immediately before ischemia (work phase 1) was on the order of ~1 (~1 μmol/min for each metabolite) and did not differ between the groups (data not shown). With the beginning of reperfusion (first 5 min), more lactate was released from the hearts into the coronary venous effluent, whereas pyruvate consumption decreased compared with that during work phase 1. This decrease resulted in a general increase in the ratio, as depicted in Figure 4, indicating a shift toward anaerobic metabolism. Comparison of these data with those of Table 2 and Figure 2 reveals a parallel relation to coronary resistance and an inverse relation between recovery of function and the postischemic ratio of lactate release to pyruvate consumption. Thus, hearts with impaired reperfusion and poor postischemic functional recovery (ASA- and indomethacin-treated hearts) actually consumed less pyruvate and produced more lactate during early reperfusion than did hearts of the other groups.

Release of 8-iso-PGF₂α. Figure 5 (top panel) demonstrates myocardial release of 8-iso-PGF₂α during the various experimental conditions. Preischemic control perfusion in the absence of any drugs was accompanied by a baseline 8-iso-PGF₂α release of 0.02 ± 0.01 pg/ml in the coronary effluent, whereas early reperfusion was associated with a substantial, more than tenfold, increase in 8-iso-PGF₂α release (Fig. 5). In hearts perfused with the COX inhibitors ASA or indomethacin (10 μmol/liter), a comparable pattern of 8-iso-PGF₂α release was found, that is, an 8-iso-PGF₂α efflux that was augmented approximately tenfold during the initial part of the reperfusion phase compared with the preischemic value. However, the rate of formation of 8-iso-PGF₂α in hearts perfused with ASA or indomethacin was ~20-fold greater than that in control hearts during the preischemic phase, and release was further enhanced after ischemia. Thus, ASA and indomethacin identically potentiated the release of 8-iso-PGF₂α during early reperfusion to levels >200-fold above the preischemic control rate.

Content of 8-iso-PGF₂α in postischemic heart tissue. Figure 5 (bottom panel) shows the tissue content of 8-iso-PGF₂α immediately after the end of work phase 2. Levels of 8-iso-PGF₂α in ASA-treated hearts were, on average, sevenfold those of
Known effects of COX inhibitors and thromboxane receptor antagonists. In clinical studies, ASA has been shown (20) to be efficacious in patients with ischemic heart disease, probably because of the antiplatelet action of ASA. Although it is an effective antithrombotic agent, a direct cardioprotective effect of ASA on the ischemic myocardium itself has not yet been demonstrated. On the contrary, a lack of effect of ASA on myocardial infarct size in a canine model of coronary occlusion was reported by Bonow et al. (9). Other investigators have demonstrated that ASA even tended to increase infarct size (21), with similar findings for indomethacin (22).

The poor results of COX inhibition by ASA-like drugs have been related to the inhibition of PG formation, chiefly prostacyclin (PGL2), with their vasodilatory and cardioprotective properties (23). Because the effects of indomethacin on collateral blood flow have been found to be inadequate (22), increases in MV02 or direct metabolic or cellular effects of COX inhibition have been suggested (23). Closely related questions arose in exploring the mechanisms underlying the cardioprotective activity of TxA2 receptor antagonists, like SQ 29548, in myocardial ischemia–reperfusion injury (24).

Surprisingly, the beneficial action is not mitigated by aspirin-like drugs (25), which can effectively block production of TxA2 and other thromboxane receptor activators (PGG2, PGH2). However, the receptor antagonists are not specific; SQ 29548 is very effective in blocking coronary vasoconstriction and platelet activation elicited by isoprostanes (8,26).

The isoprostanes. It is reasonable to speculate that inhibition of COX will cause shunting of AA toward production of other biologically active metabolites (e.g., lipoxigenase products or nonenzymatic oxidation products) that could be vasoconstrictors with thromboxane-like activity. One such group of compounds is that of the isoprostanes (6), which are generated by free radical mechanisms. Two representatives, 8-iso-PGF2a, and 8-iso-PGE2, were recently found (8) to be very potent coronary vasoconstrictors, reducing flow by ~45% at submicromolar concentrations. Interestingly, we found that SQ 29548 completely abrogated these vasoconstrictive actions, suggesting that isoprostanes also act in the heart through TxA2 or closely related receptors. Furthermore, Delanty et al. (27) reported urinary excretion of 8-iso-PGF2a to be increased, both in a canine model of coronary thrombolysis immediately after reperfusion and in patients with acute myocardial infarction given lytic therapy. However, causal involvement of isoprostanes in ischemia and reperfusion injury has not yet been demonstrated. In the present study, the consequences of COX inhibition were studied in an isolated heart model of controlled myocardial ischemia and reperfusion. Our results reveal a significant impairment of ventricular recovery in hearts treated with either ASA and indomethacin. The deleterious effects were completely abolished in the presence of SQ 29548, and COX inhibition was associated with a greatly augmented release of 8-iso-PGF2a before ischemia but especially in the early reperfusion period. Moreover, postischemic tissue levels of the isoprostane were significantly higher in hearts treated with ASA or indomethacin.

Discussion

AA can be enzymatically oxidized in the heart principally through the COX pathway (16). Under basal conditions the concentration of nonesterified AA is low (2), but an accumulation has been reported in the ischemic myocardium (17). As a consequence, there is enhanced generation of eicosanoids within the injured tissue, most notably TxA2 and PGL2 (18,19).

Although the role of COX products in the regulation of coronary flow remains unresolved, reperfusion injury may be influenced by the balance between the different metabolites of AA and thus by inhibition of the COX pathway.

Figure 5. Top panel. Cardiac release of 8-iso-PGF2a (in pg/ml) as measured in coronary effluent during the last 5 min of preischemic work (work phase 1 [W1]) and during the first 5 min of reperfusion (R1). Bottom panel. Tissue content of 8-iso-PGF2a (in pg/mg dry weight [dwt]) at the end of work phase 2. Perfusion was with KHB without (control [CON]) or with 100 μmol/liter ASA or 10 μmol/liter indomethacin (INDO), respectively. Data shown are mean value ± SEM of four hearts under each condition. In all groups, reperfusion was associated with a dramatic increase in 8-iso-PGF2a generation. Treatment of hearts with ASA or indomethacin was associated with markedly higher generation of 8-iso-PGF2a, especially during the first 5 min of reperfusion, as well as significantly higher postischemic tissue levels than in control hearts. *p < 0.05 versus work phase 1. †p < 0.05 versus control group.
Mechanism of action. The ASA- and indomethacin-treated hearts, although identical to control hearts before ischemia, were characterized by significantly lower postischemic coronary flow and aortic flow and performance of EHW, indicating that inhibition of COX impaired ischemic tolerance or augmented reperfusion injury, or both. It could be argued that the more pronounced decrease of coronary flow in ASA- and indomethacin-treated hearts during work phase 2 than in control hearts could have been appropriate and was prompted by the depressed postischemic work performance. Coronary flow is normally regulated to maintain a balance between myocardial oxygen supply and demand (28). Indeed, the significantly lower coronary flow in ASA- and indomethacin-treated hearts was associated with a decrease in MVO$_2$. However, there was also a change in oxygen extraction. With ASA and indomethacin, the coronary arterovenous oxygen difference widened, indicating a significant increase in oxygen extraction by the myocardium. This increase implies that the postischemic work performance was limited by coronary oxygen supply: Had coronary flow been adequate, there would have been no need for greater extraction. In support of this conclusion, indomethacin has reportedly reduced myocardial oxygen supply to postischemic rabbit hearts in vitro (29). A causal link to the isoprostanes is seen on analysis of the coronary flow changes and metabolic variables in the presence of SQ 29548. Postischemic coronary flow, MVO$_2$, and coronary PVO$_2$ values in the groups receiving ASA plus SQ 29548 and indomethacin plus SQ 29548 at the onset of reperfusion (i.e., with blocked isoprostane effects) were all in the range observed in control hearts. Because applying SQ 29548 only at the onset of reperfusion was as effective as it being present during ischemia as well, the effect of COX inhibition would seem to relate more to reperfusion injury.

Further evidence for an isoprostane-induced coronary flow limitation comes from the early reperfusion period. Higher levels of coronary resistance occurred in hearts treated with ASA or indomethacin than in the control hearts but not in the presence of SQ 29548. Because SQ 29548 given alone did not notably affect postischemic vascular resistance or coronary flow, this drug does not possess intrinsic vasodilator activity. Moreover, hearts with COX inhibition were characterized by increased release of lactate as opposed to a suppressed consumption of pyruvate. The ratio of lactate release to pyruvate consumption accentuates a shift toward anaerobic metabolism and has been found to correlate well with myocardial metabolic changes associated with hypoxia (30). The demonstrated relation between vascular resistance, release of 8-iso-PGF$_{2\alpha}$ and the lactate/pyruvate ratio during early reperfusion suggests that there is a disturbance of microvascular perfusion in the ASA and indomethacin groups. The higher level of vascular resistance in work phase 2 in those groups also suggests a vasoconstrictor effect persisting throughout reperfusion. Pertinently, an increase in coronary resistance has been reported in response to COX inhibition (31). In patients with coronary artery disease, indomethacin therapy was also accompanied by a fall in coronary flow that was due to a significant increase in coronary resistance (32). In the isolated rat heart, indomethacin suppressed the increase in coronary flow induced by hypoxia, and the simultaneous release of unknown vasoconstricting factors was proposed (3).

It is noteworthy that both COX inhibitors failed to affect preischemic coronary flow, which suggests that the increase in isoprostane formation under basal conditions is insufficient to cause coronary vasoconstriction in the intact heart. However, with the advent of ischemia–reperfusion, generation of AA and oxygen free radicals is greatly exacerbated. Then, shunting AA away from the COX pathway may lead to critical levels of vasoconstricting metabolites. Owing to their lipophilic nature, the isoprostanes measured in the saline effluent are probably only the “tip of the iceberg.” Indeed, we found postischemic levels of 8-iso-PGF$_{2\alpha}$ in the ventricular tissue of the ASA- and indomethacin-treated hearts to be five to seven times higher than in control hearts. The levels of 200 to 300 pg/mg dry weight convert to concentrations of up to $2 \times 10^{-7}$ mol/liter, if based on tissue water (85% of tissue wet weight). Such concentrations have been shown to decrease coronary flow significantly when infused (8).

Conclusions. The data presented here suggest that the potentially deleterious effects of COX inhibitors reside in the shifting of AA metabolism, away from PG formation and toward the isoprostanes. Aggravation of ischemia–reperfusion injury by these potent vasoconstrictor compounds could be clinically relevant, because increasing numbers of patients with coronary heart disease routinely take ASA. Despite obvious reservations concerning extrapolation of the present model to the in vivo situation, especially with respect to questions of drug concentration, COX inhibitors should probably be used with some caution in patients with severe coronary artery disease. The protection observed with the TxA$_2$ receptor antagonist SQ 29548 against isoprostane-induced vasoconstriction, particularly during reperfusion, may be a therapeutically exploitable principle.

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References


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