Thrombin inhibition with melagatran does not attenuate renal ischaemia-reperfusion injury in rats

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Abstract

Background. Renal ischaemia-reperfusion (IR) is associated with activation of the coagulation system and inflammation within the kidney. The aim of the present study was to examine the effects of selective thrombin inhibition with melagatran on kidney morphology and function in rats subjected to renal IR.

Methods. Sprague–Dawley rats underwent renal IR (35 min of bilateral renal arterial clamping), or sham surgery. Treatment groups were: (i) IR–Saline, (ii) IR–Melagatran, (iii) Sham–Saline, and (iv) Sham–Melagatran. Twenty minutes prior to renal IR, the rats were administered a bolus dose of saline vehicle or melagatran [0.5 μmol/kg, subcutaneously (s.c.)] followed by a continuous infusion throughout (0.08 μmol/kg/h, s.c.). Forty-eight hours after IR, renal function was assessed in anaesthetized animals and kidney histology was analysed semi-quantitatively.

Results. Rats in group IR–Saline showed an approximate 85% reduction in glomerular filtration rate, 5-fold increases in fractional urinary excretion rates of sodium, potassium and water, and marked renal histological abnormalities, compared with sham (P < 0.05). Renal histopathological changes in the cortex and outer medulla were characterized by tubular necrosis and atrophy, tubular cast formation and interstitial inflammation. In addition, there was significant vascular congestion in the inner stripe of the outer medullary zone. Melagatran treatment had no significant effects on any of the abnormalities in kidney morphology or function in rats subjected to renal IR. Plasma melagatran concentrations were within a range known to exert significant antithrombotic effects, throughout the study period.

Conclusions. Thrombin inhibition with melagatran did not ameliorate abnormalities in kidney morphology or function 48 h after renal IR. These results indicate that melagatran is not renoprotective in rats subjected to renal IR.

Keywords: acute renal failure; coagulation; ischaemia-reperfusion; melagatran; thrombin; thrombin inhibitor

Introduction

Ischaemia is a common cause of acute renal failure (ARF) in critically ill patients [1]. In the intensive care unit, ARF affects 5–20% of patients and contributes independently to co-existing diseases, and severity of illness scores, as well as to the high mortality of 70–80% in this patient group [1].

Activation of the coagulation system may have important roles in the pathogenesis of ischaemic ARF [1]. In support of this notion, increased thrombin generation has been demonstrated as fibrin deposition in the kidney microvasculature and tubuli in both experimental and clinical ischaemic ARF [2–4]. Fibrin deposition and platelet aggregation in glomerular and peritubular capillaries may cause microthrombosis leading to decreased glomerular filtration rate (GFR) and impaired renal perfusion [1–3]. The formation of fibrin-containing tubular casts could lead to tubular obstruction, increased tubular pressure, back-leak of filtrate, and decreased GFR [3,4]. Moreover, thrombin has been shown to elicit pro-inflammatory responses after renal ischaemia-reperfusion (IR) and these effects seem to be mediated by activation of protease-activated receptor (PAR)-1 [5,6]. Thus, thrombin could promote renal ischaemic injury both by reducing renal perfusion and oxygenation, and by stimulating the subsequent inflammatory response. The suggested
mechanism initiating thrombin production in renal IR is the exposure of blood to tissue factor in the subendothelium, or on activated leucocytes, in the kidney microvasculature. This occurs when the renal microvascular endothelium is injured [7], and tissue factor expression increased [8], after renal IR. Accordingly, administration of tissue factor antisense oligonucleotides has been shown to attenuate renal IR-injury [8].

Melagatran is a selective and powerful inhibitor of thrombin activity and the conversion of fibrinogen to fibrin [9]. Melagatran can also inhibit thrombin’s activation of PAR-1 and PAR-4 [10]. In addition, melagatran diminishes platelet activation [9,10]. Consequently, we hypothesized that melagatran might exert protective effects in renal IR. Hence, the aim of the present study was to examine the effects of melagatran on kidney morphology and function in a model of renal IR-injury in rats. Although melagatran, the first direct thrombin inhibitor extensively investigated for prevention of thromboembolic events, was withdrawn from further clinical development in February 2006 due to concerns over liver safety, the results obtained with melagatran could be applicable for other thrombin inhibitors with similar molecular properties.

Subjects and methods

General procedures

Male Sprague–Dawley rats (Harlan, Horst, The Netherlands) weighing approximately 250 g were used. Bilateral renal IR was carried out on the animals anaesthetized with xylazine [10 mg/kg, intraperitoneally (i.p.)] and ketamine [75 mg/kg, i.p.]. Through flank incisions, renal arteries were clamped for 35 min by non-traumatic microvascular clips. During sham surgery, renal arteries were dissected and manipulated, but no clips were applied. Rectal temperature was kept at 37–38°C. After surgery, fluid losses were replaced by administration of 5 ml of warm (37°C) isotonic saline i.p., and the rats were returned to their cages. The rats had free access to normal rat chow and tap water throughout. All experiments were approved by the regional ethics committee in Göteborg. Chemicals were from Sigma (St. Louis, MO, USA) if not stated otherwise. Melagatran (AstraZeneca, Mölndal, Sweden) was stored and prepared as described [11].

Protocol

Rats were divided into four study groups: (i) IR–Saline (n = 10), (ii) IR–Melagatran (n = 11), (iii) Sham–Saline (n = 9) and (iv) Sham–Melagatran (n = 7), and were subjected to renal IR or sham surgery. Twenty minutes prior to renal arterial clamping or sham manipulation, isotonic saline or melagatran, was administered in a subcutaneous (s.c.) bolus dose of 0.5 μmol/kg in group IR–Melagatran, and 0.7 μmol/kg in group Sham–Melagatran, respectively. After the bolus dose, a s.c. infusion of melagatran (0.08 μmol/kg/h in group IR–Melagatran and 0.4 μmol/kg/h in group Sham–Melagatran) was initiated and maintained for 48 h (Alzet pump 1003D, B&K Universal AB, Sollentuna, Sweden).

As melagatran is eliminated principally by the kidneys [12], lower doses were administered to the rats subjected to renal IR. Based on previous studies [11], and pilot experiments, the doses were expected to produce plasma melagatran concentrations of approximately 0.5 μmol/l. This concentration was targeted as it has been shown to markedly prolong thrombin time (TT) and activated partial thromboplastin time (APTT), to reduce platelet activation and to exert potent antithrombotic effects in models of arterial and venous thrombosis in vivo [9,11,13–18].

Renal clearance experiments

Forty-eight hours after renal IR, the rats were anaesthetized with thiobutabarbitral (Inactin, 100 mg/kg i.p.), placed on a heating table, and prewarmed for renal clearance experiments, as described [20]. An arterial line was connected to a pressure transducer (Smiths Medical, Kirchseeon, Germany) for monitoring of mean arterial pressure (MAP) and heart rate (HR) using a data acquisition program (Biopac MP 150, Biopac Systems, Santa Barbara, CA, USA). The urinary bladder was catheterized for urine collection into pre-weighed vials. Rectal temperature was kept at 37°C. Throughout the experiment, the rats received isotonic saline in a total volume of 10 ml/kg/h. After completion of the surgical preparation, a 40 min equilibration period was allowed before renal clearance measurements during three consecutive 20 min periods. GFR was determined by measuring renal 51Cr-EDTA clearance (51Cr-ethylene-diaminetetraacetic acid, Amersham, Buckinghamshire, UK), as described [20]. Arterial blood samples (0.3 ml) were replaced by equivalent volumes of 4% bovine serum albumin in isotonic saline. Following renal clearance measurements, arterial blood gases were taken (ABL 510 blood-gas analyser, Radiometer, Copenhagen, Denmark) and blood was collected for analyses of plasma concentrations of melagatran. Urine and plasma samples were analysed for sodium, potassium and radioactivity, as described [20]. Fractional urinary excretion rates of sodium (FE Na%,), potassium (FE K%,), and water (FE H2O,%), were estimated as the ratio of their respective clearances to that of 51Cr-EDTA, × 100. Renal clearance data are presented as the average of the three clearance periods.

Plasma melagatran concentrations

Plasma melagatran concentrations were analysed in groups Sham–Melagatran (n = 7) and IR–Melagatran (n = 11) at 48 h after reperfusion, immediately following clearance measurements (vide supra). In addition, plasma melagatran levels were determined in separate groups of animals subjected to renal IR following
Thrombin inhibition in renal ischaemia-reperfusion

**Table 1. Systemic haemodynamics and renal excretory function 48 h after renal ischaemia-reperfusion**

<table>
<thead>
<tr>
<th></th>
<th>BW (g)</th>
<th>MAP (mmHg)</th>
<th>HR (bpm)</th>
<th>UV (μl/min/g KW)</th>
<th>UNaV (μmol/min/g KW)</th>
<th>UKV (μmol/min/g KW)</th>
<th>P-Na (mmol/l)</th>
<th>P-K (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham–Saline (n = 9)</td>
<td>262 ± 20</td>
<td>124 ± 4</td>
<td>388 ± 12</td>
<td>13.3 ± 2.2</td>
<td>0.63 ± 0.21</td>
<td>0.88 ± 0.12</td>
<td>146 ± 0</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Sham–Melagatran (n = 7)</td>
<td>265 ± 20</td>
<td>117 ± 5</td>
<td>396 ± 13</td>
<td>8.5 ± 2.2</td>
<td>0.42 ± 0.16</td>
<td>0.64 ± 0.07</td>
<td>145 ± 0</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>IR–Saline (n = 10)</td>
<td>207 ± 7</td>
<td>117 ± 4</td>
<td>398 ± 8</td>
<td>8.3 ± 1.2</td>
<td>0.29 ± 0.06</td>
<td>0.30 ± 0.02</td>
<td>145 ± 0</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>IR–Melagatran (n = 11)</td>
<td>212 ± 5</td>
<td>116 ± 4</td>
<td>390 ± 7</td>
<td>8.0 ± 1.0</td>
<td>0.29 ± 0.09</td>
<td>0.24 ± 0.02</td>
<td>145 ± 1</td>
<td>4.4 ± 0.2</td>
</tr>
</tbody>
</table>

Renal clearance data in thiobutabarbital anaesthetized rats 48 h after renal ischaemia-reperfusion. BW, body weight; MAP, mean arterial pressure; HR, heart rate; UV, urine flow rate; KW, kidney weight; UNaV, urinary sodium excretion; UKV, urinary potassium excretion; P-Na, plasma sodium concentration; P-K, plasma potassium concentration; IR, ischaemia-reperfusion; and NS, non-significant. Values are means ± SEM. Statistical analyses were performed using two-way analysis of variance (ANOVA). ‘IR effect’ denotes effect of factor surgical intervention; ‘Melagatran effect’ denotes effect of factor treatment; and ‘IR × Melagatran’ denotes interaction between the two aforementioned factors.

**Results**

**Body weights and systemic haemodynamics**

Rats subjected to renal IR showed an approximate 10% reduction in body weight compared with sham 48 h after the ischaemic insult (P < 0.001, Table 1). There were no significant differences between groups in MAP or HR (Table 1). No significant haemorrhages, and no deaths, occurred throughout the study.

**Kidney function**

Renal IR produced an approximate 85% decline in GFR (P < 0.05, Figure 1) and about 5-fold increase in FENa, FEK and FEH2O compared with sham (P < 0.05, Figure 2). In addition, urinary potassium excretion in absolute values (UKV) was reduced in group IR–Saline compared with sham (P < 0.05, Table 1). There were no significant differences between IR- and sham-groups in urine flow rate, urinary sodium excretion, or in plasma sodium and potassium concentrations (Table 1). Melagatran treatment decreased UKV (P < 0.05), but had no statistically significant effects on GFR, FENa, FEK, FEH2O and plasma potassium concentration in rats with renal IR-injury (Table 1, Figures 1 and 2).

**Plasma melagatran concentrations**

Plasma melagatran concentrations in group IR–Melagatran were 0.72 ± 0.11, 0.57 ± 0.06 and 0.18 ± 0.03 μmol/l, at 0 h, 24 h and 48 h of reperfusion, respectively. The Sham–Melagatran group had plasma melagatran concentrations of 0.51 ± 0.08 μmol/l at 48 h of reperfusion.

**Blood gases**

Rats subjected to renal IR showed decreased blood pH and increased arterial pO2 and oxygen saturation compared with sham (P < 0.05), with no differences

Kidney histology

Following renal clearance measurements, kidneys were excised, decapsulated, weighed and immersion-fixed in 4% formaldehyde in phosphate buffer (pH 7). Sections of kidneys were stained with haematoxylin-eosin and processed for semi-quantitative histological assessments by light microscopy as previously described [20]. The following variables were quantified separately in the renal cortex, outer (OSOMZ) and inner (ISOMZ) stripe of the outer medullary zone, and in the inner medulla (IM): tubular necrosis, tubular atrophy, tubular dilatation, tubular cast formation, interstitial inflammation with mononuclear leucocytes, interstitial polymorphonuclear (PMN) neutrophil infiltration, interstitial fibrosis, fibrin deposition, microthrombosis and vascular congestion. Analyses were made by an investigator blinded for treatment group using an arbitrary scale where 0 = no changes, 1 = mild focal changes, 2 = modest diffuse changes and 3 = severe diffuse changes, as described [20].

Statistics

Values are means ± SEM, except for semi-quantitative data, which are presented as the median with 25th and 75th percentiles. Differences between groups were analysed by one-way and two-way analyses of variance (ANOVA) followed by Bonferroni’s post hoc test. Histological data were analysed by non-parametric Kruskal–Wallis’ and Mann–Whitney’s tests. A value of P < 0.05 was considered statistically significant. The statistical software program SPSS 11.5.1 was used (SPSS Inc., Chicago, IL, USA).
Both groups subjected to renal IR showed significant tubular necrosis, tubular atrophy and dilatation, tubular cast formation and interstitial inflammation, in the cortex and outer medulla (P < 0.05 vs sham, Tables 3 and 4, Figure 3). In addition, vascular congestion with erythrocytes, and interstitial PMN neutrophil infiltration, was evident specifically in the ISOMZ of kidneys with IR-injury (P < 0.05, Table 4, Figure 3). In the IM, histological changes were sparse and there was no vascular congestion (data not shown). Renal fibrin depositions or microthrombi were not detected in any of the study groups (data not shown). Glomeruli appeared normal in all groups.

There were no statistically significant differences between groups IR–Saline and IR–Melagatran in any of the investigated histological variables (Tables 3 and 4, Figure 3). Melagatran treatment tended to decrease tubular necrosis in the cortex of kidneys with IR-injury (P = 0.058, Table 3). No abnormalities in kidney histology were observed in group Sham–Melagatran (data not shown).

**Discussion**

In the present study, bilateral renal IR caused a pronounced decline in GFR, alterations in tubular function and severe renal histopathological changes. However, thrombin inhibition with melagatran did not attenuate abnormalities in renal morphology or function when assessed 48 h after the ischaemic insult.

In the present study, plasma melagatran concentrations at the time of renal arterial declamping were 0.72 μmol/l, and at 24 h after reperfusion 0.57 μmol/l, in group IR–Melagatran. Melagatran in these concentrations has previously been shown to cause almost complete thrombin inhibition and to markedly inhibit platelet activation and thrombosis formation in vivo [9,11,13–18]. For instance, plasma melagatran concentrations of approximately 0.6 μmol/l completely prevented the development of thrombosis formation in a model of vena cava thrombosis in the rat [14]. In a rat model of arterial thrombosis it was found that plasma concentrations of 0.15 μmol/l produced a 50% anti-thrombotic effect [15]. Furthermore, plasma concentrations in the range of those achieved in the present study (0.6–0.8 μmol/l) have been shown to markedly reduce tissue fibrin depositions, measured by a quantitative assay, in endotoxemic rats with disseminated intravascular coagulation [17]. Notably, in that study it was also observed that increased plasma melagatran concentrations (from 0.8 to 8 μmol/l) did not achieve any further reduction in tissue deposition of fibrin. In the current study, plasma melagatran concentrations were below 1.0 μmol/l throughout, thus ruling out the possibility of melagatran-induced inhibition of endogenous fibrinolysis [9].

At 48 h after IR, plasma melagatran concentrations had declined to approximately 0.2 μmol/l, a level at which melagatran still effectively inhibits thrombin activity and thrombosis formation [13,15,16].

### Kidney histology

Macroscopically, kidneys subjected to IR were clearly enlarged, pale and appeared oedematous. Both IR-groups had significantly increased kidney weights compared with sham (P < 0.05, Table 2). There were no significant differences in pCO₂, base excess or plasma bicarbonate levels between study groups (Table 2).

Between saline- and melagatran-treated groups (Table 2), melagatran treatment significantly decreased arterial haemoglobin concentrations compared with saline infusion (P < 0.05, Table 2). There were no significant differences in pCO₂, base excess or plasma bicarbonate levels between study groups (Table 2).

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Thrombin inhibition in renal ischaemia-reperfusion

Table 2. Arterial blood gases and haemoglobin concentrations 48 h after renal ischaemia-reperfusion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham–Saline (n = 9)</th>
<th>IR–Saline (n = 10)</th>
<th>IR–Melagatran (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/l)</td>
<td>146 ± 3</td>
<td>132 ± 7</td>
<td>143 ± 2</td>
</tr>
<tr>
<td>pO2 (kPa)</td>
<td>9.4 ± 0.2</td>
<td>9.7 ± 0.3</td>
<td>10.5 ± 0.2</td>
</tr>
<tr>
<td>pCO2 (kPa)</td>
<td>6.4 ± 0.2</td>
<td>6.1 ± 0.2</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>86.5 ± 0.7</td>
<td>87.5 ± 1.2</td>
<td>89.9 ± 0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.03</td>
<td>7.40 ± 0.00</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>HCO3 (mmol/l)</td>
<td>27.8 ± 0.4</td>
<td>27.5 ± 0.6</td>
<td>27.7 ± 0.6</td>
</tr>
<tr>
<td>BE (mmol/l)</td>
<td>2.5 ± 0.4</td>
<td>2.6 ± 0.6</td>
<td>2.0 ± 0.7</td>
</tr>
</tbody>
</table>

Arterial blood gases in thiobutabarbitral anaesthetized rats 48 h after renal ischaemia-reperfusion. Hb, haemoglobin; pO2, partial pressure of oxygen; pCO2, partial pressure of carbon dioxide; SaO2, oxygen saturation; HCO3-, bicarbonate; BE, base excess; IR, ischaemia-reperfusion; and NS, non-significant. Values are means ± SEM. Statistical analyses were performed using two-way analysis of variance (ANOVA). ‘IR effect’ denotes effect of factor surgical intervention; ‘Melagatran effect’ denotes effect of factor treatment; and ‘IR × Melagatran’ denotes interaction between the two aforementioned factors.

Table 3. Histopathological scores in the kidney cortex 48 h after renal ischaemia-reperfusion

<table>
<thead>
<tr>
<th>Histopathological Scores</th>
<th>Sham–Saline (n = 9)</th>
<th>IR–Saline (n = 10)</th>
<th>IR–Melagatran (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular necrosis</td>
<td>0 (0–0)</td>
<td>2 (1.75–2.25)*</td>
<td>1 (1–2)*</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0 (0–0)</td>
<td>1 (0–1)*</td>
<td>1 (0–1)*</td>
</tr>
<tr>
<td>Tubular dilatation</td>
<td>0 (0–0)</td>
<td>1 (1–1.25)*</td>
<td>2 (1–2)*</td>
</tr>
<tr>
<td>Tubular casts</td>
<td>0 (0–0)</td>
<td>1 (1–2)*</td>
<td>1 (1–2)*</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0 (0–0)</td>
<td>0 (0–0.25)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Interstitial mononuclear inflammation</td>
<td>0 (0–1)</td>
<td>1 (1–1)*</td>
<td>1 (1–1)*</td>
</tr>
</tbody>
</table>

Histopathological scores in the kidney cortex 48 h after renal ischaemia-reperfusion (IR) in rats treated with melagatran (IR–Melagatran) or isotonic saline (IR–Saline). Histological abnormalities were scored using an arbitrary scale from 0–3 (Methods). Scores for group Sham–Melagatran were 0 (0–0) for all investigated variables and were excluded from table. Data are presented as median and 25th and 75th percentiles. Kruskal–Wallis’ and Mann–Whitney’s tests were used for statistical analyses. *P < 0.05 vs sham.

Table 4. Histopathological scores in the kidney outer medulla 48 h after renal ischaemia-reperfusion

<table>
<thead>
<tr>
<th>Histopathological Scores</th>
<th>Sham–Saline (n = 9)</th>
<th>IR–Saline (n = 10)</th>
<th>IR–Melagatran (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSOMZ</td>
<td>Tubular necrosis</td>
<td>0 (0–0)</td>
<td>2 (1.75–2.25)*</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0 (0–0)</td>
<td>0.5 (0–1)*</td>
<td>0 (0–1)*</td>
</tr>
<tr>
<td>Tubular dilatation</td>
<td>0 (0–0)</td>
<td>1 (1–1.25)*</td>
<td>2 (1–2)*</td>
</tr>
<tr>
<td>Tubular casts</td>
<td>0 (0–0)</td>
<td>2 (1–2)*</td>
<td>2 (1–2)*</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>0 (0–0)</td>
<td>0 (0–0.25)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Interstitial mononuclear inflammation</td>
<td>0 (0–0)</td>
<td>1 (1–1)*</td>
<td>1 (1–1)*</td>
</tr>
</tbody>
</table>

Histopathological scores in the kidney outer medulla 48 h after renal ischaemia-reperfusion (IR) in rats treated with melagatran (IR–Melagatran) or isotonic saline (IR–Saline). Histological abnormalities in the outer (OSOMZ) and inner (ISOMZ) stripe of the outer medullary zone were scored using an arbitrary scale from 0–3 (Methods). Scores for group Sham–Melagatran were 0 (0–0) for all investigated variables and were excluded from the table. PMN denotes polymorphonuclear neutrophils. Data are presented as median and 25th and 75th percentiles. Kruskal–Wallis’ and Mann–Whitney’s tests were used for statistical analyses. *P < 0.05 vs sham.

The lower plasma concentration at 48 h could be explained by the fact that rats with renal IR-injury recover kidney function during the second day after the ischaemic insult [21], as melagatran is eliminated from plasma principally by glomerular filtration [12]. In addition, a relatively low maintenance dose of melagatran was chosen as pilot studies revealed that doses any higher caused drug accumulation and fatal bleeding complications in rats with renal IR-injury. Taken together, plasma melagatran concentrations in the present study were clearly within the range of those well-known to cause pronounced thrombin inhibition, in particular during the first 24 h after the ischaemic insult when kidney injury is initiated [1] and the coagulation system activated [22].

One might speculate that activation of the coagulation cascade could contribute to tubular obstruction and outer medullary vascular congestion, in kidneys with IR-injury [3,4]. Our results clearly indicate that thrombin does not importantly contribute to outer medullary vascular congestion after IR and this is in accord with previous studies using thrombin inhibitors heparin and antithrombin [23,24]. It has previously been shown that heparin reduces tubular obstruction 1 h after IR [4]. In the present study, melagatran did not decrease tubular obstruction when examined at 48 h after IR. Our finding is in agreement with the results of Mizutani et al. [24] indicating that thrombin inhibition with antithrombin does not diminish tubular dilatation at 24 h after IR. Taken together, these data suggest that thrombin may contribute to tubular obstruction early after IR, but that this effect is not sustained until later time points.
Notably, we were unable to detect intratubular and intravascular fibrin in saline-treated rats with renal IR-injury in the present study. Most likely this could at least partly be explained by our histological method, i.e. light microscopy of sections stained with haematoxylin-eosin, not being sensitive enough to detect modest increases in fibrin. In support of this notion, Enestrom et al. [3] were able to demonstrate increased renal fibrin after IR utilizing sensitive immunohistochemical methods, although fibrin was not detectable on plain light microscopy. In addition, it should be noted that histological analyses were only performed at 48 h after reperfusion in the present study. Thus, our findings at this time point does not rule out that significant differences between groups could have been detected earlier during the study course. Consequently, and in accord with previous studies demonstrating renal fibrin deposition at 1 h and 24 h after IR [3,22], it is reasonable to assume that there was an early increase in kidney fibrin generation after renal IR also in the present study.

Thrombin’s activation of PAR-1 and -4 can elicit pro-inflammatory responses, and PAR-1 deficient mice have been shown to develop less renal inflammation, and decreased plasma creatinine levels 24 h after renal IR [6]. Our results demonstrate that melagatran did not reduce renal interstitial inflammation or PMN neutrophil infiltration after IR. This finding suggests that melagatran, at least in the concentrations of the present study, did not effectively inhibit PAR activity and downstream inflammatory pathways. Furthermore, one might speculate that anti-inflammatory effects of thrombin inhibitors are a prerequisite for renoprotection in renal IR injury. In support of this notion, thrombin inhibition with hirudin and heparin in doses that decreased inflammation also attenuated the increase in plasma creatinine after IR [6,25]. However, the discrepancy between those results, and the data in the present study, could also be explained by the fact that renal IR injury was more severe in the current study. Accordingly, thrombin inhibition with heparin and antithrombin has been shown not to be renoprotective in models of more severe renal IR [24,26].

Finally, recent studies suggest that thrombin could actually have beneficial effects after renal ischaemia [27,30]. Thrombin activates protein C, and may thereby, have positive effects on renal function after IR [28]. Indeed, plasma melagatran concentrations equivalent to those in the present study have been shown to decrease protein C activation [29]. Moreover, thrombin may stimulate tubular regeneration and recovery after renal IR by stimulating proximal tubular cell proliferation and by activation of the anti-apoptotic phosphatidylinositol 3-kinase/Akt pathway [27,30]. Thus, one might speculate that the lack of renoprotective consequences of melagatran in the present study could at least partially be explained by the inhibition of thrombin’s potentially positive effects on tubular integrity. However, it should be pointed out that we were unable to detect any significant negative effects of melagatran on kidney morphology or function. Moreover, it may be argued that bleeding and decreased haemoglobin concentrations in melagatran-treated rats could have counteracted renoprotective effects by reducing renal oxygen delivery. However, although renal blood flow was not measured, there were no significant reductions in MAP, arterial pO2 or oxygen saturation in animals receiving melagatran. In addition, tissue oxygenation is also determined by oxygen extraction, which has been shown to increase in the rat kidney during haemorrhage [31]. It is, therefore, unlikely that the modest reduction in haemoglobin levels (129 g/l in IR–Melagatran vs 143 g/l in IR–Saline) caused a significant decrease in renal oxygenation that could have contributed to hypoxic tissue injury or impaired tubular recovery.

In conclusion, selective thrombin inhibition with melagatran did not improve kidney function or attenuate renal histopathological abnormalities in rats with renal IR-injury. Thrombin does not seem to be an important factor in the development of renal IR-injury.

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