Oestrogen protects against ischaemic acute renal failure in rats by suppressing renal endothelin-1 overproduction

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ABSTRACT

We investigated whether the treatment with 17β-oestradiol has renal protective effects in male rats with ischaemic acute renal failure (ARF). We also examined if the effect of 17β-oestradiol is accompanied by suppression of enhanced endothelin-1 production in postischaemic kidneys. Ischaemic ARF was induced by clamping the left renal artery and vein for 45 min followed by reperfusion, 2 weeks after contralateral nephrectomy. Renal function parameters such as blood urea nitrogen, plasma creatinine and creatinine clearance were measured to test the effectiveness of the steroid hormone. Renal function in ARF rats markedly decreased 24 h after reperfusion. The ischaemia/reperfusion-induced renal dysfunction was dose-dependently improved by pretreatment with 17β-oestradiol (20 or 100 μg/kg, intravenously). Histopathological examination of the kidney of untreated ARF rats revealed severe lesions, such as tubular necrosis, proteinaceous casts in tubuli and medullary congestion, all of which were markedly improved by the higher dose of 17β-oestradiol. In addition, endothelin-1 content in the kidney after the ischaemia/reperfusion increased significantly by approx. 2-fold over sham-operated rats, and this elevation was dose-dependently suppressed by the 17β-oestradiol treatment. These results suggest that oestrogen exhibits protective effects against renal dysfunction and tissue injury induced by ischaemia/reperfusion, possibly through the suppression of endothelin-1 overproduction in postischaemic kidneys.

INTRODUCTION

It is well known that the incidence of cardiovascular disease is lower in females prior to menopause compared with males and postmenopausal females. Oestrogen replacement therapy attenuates the incidence of cardiovascular events in postmenopausal women [1]. The identification of the specific mechanisms involved in oestrogen-related cardiovascular defence has been the objective of numerous experimental studies [2,3].

Key words: acute renal failure, endothelin-1, ischaemia, oestrogen, reperfusion.
Abbreviations: ARF, acute renal failure; ET, endothelin.
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Furthermore, we examined the effect of oestrogen on the renal content of ET-1, which is a deleterious mediator in the pathogenesis of ischaemic ARF [6–9].

MATERIALS AND METHODS

Animals and experimental design
Male Sprague–Dawley rats were used. At 8 weeks of age, the right kidney was removed through a small flank incision under pentobarbital anaesthesia (50 mg/kg, intraperitoneally) 2 weeks before the study. After a 2-week recovery period, these rats were separated into four groups: (i) sham-operated control, (ii) untreated ischaemic ARF, (iii) ischaemic ARF pretreated with 17β-oestradiol (20 μg/kg, intravenously) and (iv) ischaemic ARF pretreated with 17β-oestradiol (100 μg/kg, intravenously). To induce ischaemic ARF, the rats were anaesthetized, and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded with a non-traumatic clamp for 45 min, and then the clamp was released to allow reperfusion. 17β-Oestradiol or vehicle (a mixture of 2.5% ethanol, 30% polyethylene glycol 400 and 67.5% saline) (1 ml/kg) was injected 15 min before reperfusion. In sham-operated control rats, the kidney was treated identically, except for the clamping. Animals exposed to 45-min ischaemia were housed in metabolic cages at 24 h after reperfusion. After urine was collected for 5 h, blood samples were taken, and then left kidneys were excised under pentobarbital anaesthesia.

Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences (Osaka, Japan).

Blood and urine measurements
Blood urea nitrogen and creatinine levels in plasma or urine were determined as described previously [7].

Histological studies
The kidneys were preserved in phosphate-buffered 10% formalin, embedded in paraffin wax, cut at 4 μm, and stained with haematoxylin and eosin. The evaluations were made in a blind manner.

Renal ET-1 assay
ET-1 was extracted from the kidney, according to the method described in [10]. The radioimmunoassay for ET-1 was performed as described previously [11].

Statistical analysis
Values were expressed as means±S.E.M. Data were analysed for significant differences between the sham-operated and untreated ARF groups using the Student’s unpaired t test. Statistical analysis for renal functional studies was performed using one-way analysis of variance followed by a Dunnett-type multiple comparison test. Histological data were analysed using the Kruskal–Wallis non-parametric test combined with a Steel-type multiple comparison tests. For all comparisons, differences were considered significant at P < 0.05.

RESULTS
As shown in Table 1, renal function of rats subjected to a 45-min ischaemia showed a marked deterioration when measured 24 h after the reperfusion. Untreated ARF rats showed significant increases in blood urea nitrogen, plasma creatinine, urine flow, and a significant decrease in creatinine clearance, compared with sham-operated rats. The administration of 17β-oestradiol (20 or 100 μg/kg) to ischaemic ARF rats dose-dependently attenuated the ARF-induced renal dysfunction.

Histopathological examination revealed severe lesions in the kidneys of untreated ARF rats (1 day after the 45-min ischaemia). These changes were characterized by tubular necrosis in outer zone outer stripe of medulla, proteinaceous casts in tubuli in inner zone of medulla and medullary congestion and haemorrhage in outer zone inner stripe of medulla. Treatment with 17β-oestradiol at 100 μg/kg clearly prevented the development of all these lesions.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effect of 17β-oestradiol on renal function and ET-1 content 1 day after the ischaemia/reperfusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Values are means±S.E.M. (n = 7). * P &lt; 0.01, compared with sham rats; † P &lt; 0.05, ‡ P &lt; 0.01, compared with untreated ARF rats. BUN, blood urea nitrogen; Pcr, plasma creatinine; Ccr, creatinine clearance; UF, urine flow.</td>
</tr>
<tr>
<td>Sham</td>
<td>26.9±1.7                                      0.68±0.03                                      4.09±0.28                                      29.4±1.8                                      0.67±0.02</td>
</tr>
<tr>
<td>Untreated ARF</td>
<td>142±14 *                                   3.67±0.45*                                    0.82±0.23*                                    78.2±10 *                                    1.39±0.24 *</td>
</tr>
<tr>
<td>ARF + 17β-oestradiol (20 μg/kg)</td>
<td>90.7±11 †                                    2.34±0.30†                                    1.33±0.26                                    68.4±6.9                                      0.86±0.13†</td>
</tr>
<tr>
<td>ARF + 17β-oestradiol (100 μg/kg)</td>
<td>45.9±4.1 †                                   1.12±0.12 †                                   3.07±0.31 †                                   50.8±5.3 †                                    0.60±0.08 †</td>
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To evaluate whether the preventative effects of 17β-oestradiol on ischaemia/reperfusion-induced renal injury are accompanied by a suppression of enhanced ET-1 production in the kidney of ischaemic ARF rats, renal ET-1 content was determined 24 h after reperfusion. As shown in Table 1, renal ET-1 contents were significantly increased in rats exposed to the 45-min ischaemia, being about twofold over sham-operated rats. The increased renal ET-1 content was abolished by the higher dose of 17β-oestradiol.

**DISCUSSION**

The current study showed that 17β-oestradiol was capable of preventing the renal dysfunction and tissue injury induced by ischaemia/reperfusion. We also found that the effects of 17β-oestradiol were accompanied by a decrease in renal content of ET-1, a deleterious mediator in the pathogenesis of ischaemic ARF. Thus, 17β-oestradiol appears to suppress the enhanced ET-1 production in renal tissues and the consequent renal damage in this model of ARF.

There is accumulating evidence indicating that ET-1 plays an important role in the pathogenesis of ischaemic ARF. This view is based on findings that administration of a selective ETA receptor antagonist or non-selective ET_α/ET_β receptor antagonist to ischaemic ARF rats attenuates the ischaemia/reperfusion-induced impairment of renal function [7,12–14]. In addition, it has been shown that ET-1 mRNA expression and ET-1 content are elevated in the postischaemic kidney [6–9]. Wilhelm et al. [9] observed a marked increase of ET-1 in the peritubular capillary network, suggesting that ET-1-induced vasoconstriction may play a pathophysiological role in ischaemia/reperfusion-induced tubular necrosis. Taken together, it seems that enhanced local production of ET-1 and its action occur in the kidney after ischaemia/reperfusion. In the present study, we also observed that renal ET-1 content in untreated ARF rats increased significantly 24 h after reperfusion, and the increases in ET-1 content were reduced to the sham level by treatment with the higher dose of 17β-oestradiol. Thus, it is conceivable that the suppressive effect of 17β-oestradiol on the renal ET-1 overproduction would attenuate ET-1 actions on the kidney in ischaemic ARF rats and, eventually, ameliorate the renal dysfunction and tissue injury.

Some studies using cultured endothelial cells reveal that 17β-oestradiol attenuates ET-1 production via oestrogen receptors [4] and inhibits angiotensin II-induced mitogen-activated protein kinase signalling to ET-1 transcription [5]. These findings have been supported by Dubey et al. [15], who also provided evidence that oestradiol metabolites 2-hydroxyoestradiol and 2-methoxyoestradiol potently inhibit ET-1 synthesis by means of an oestrogen receptor–independent mechanism, and showed that these effects of oestradiol metabolites may be mediated by inhibition of mitogen-activated protein kinase activity [15]. A more interesting observation is that overexpression of oestrogen receptor-α in endothelial cells results in a dramatic decrease in ET-1 secretion [16]. On the other hand, it is not clear as to the mechanism for suppression of enhanced renal ET-1 production by 17β-oestradiol in ischaemic ARF. There is no circumstantial evidence regarding whether oestrogen receptor antagonists block the suppressive effect of 17β-oestradiol on ET-1 production and renal injury in postschaemic kidneys. Further experiments are required to clarify the molecular mechanisms by which 17β-oestradiol has renoprotective effects in ischaemic ARF.

In conclusion, the present results demonstrate that pretreatment with 17β-oestradiol attenuates the development of ischaemia/reperfusion-induced ARF. The mechanisms by which 17β-oestradiol has protective effects on ischaemic ARF would include suppression of renal ET-1 overproduction after ischaemia/reperfusion. This suppression would eventually reduce ET-1 actions on renal vasculature and tubules in ischaemic ARF rats.

**REFERENCES**