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17β-oestradiol reduces cardiac leukocyte accumulation in myocardial ischaemia reperfusion injury in rat

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Abstract

We investigated whether oestrogens modulate the phenomenon of leukocyte accumulation during ischaemia and reperfusion of the myocardium. Anaesthetized rats were subjected to total occlusion (1 h) of the left main coronary artery followed by 1 h reperfusion. Sham myocardial ischaemia–reperfusion rats (Sham) were used as controls. Myocardial necrosis, myocardial myeloperoxidase activity, serum creatinine phosphokinase activity, serum and macrophages tumour necrosis factor (TNF-α) and the myocardial staining of intercellular adhesion molecule-1 (ICAM-1) were evaluated. Myocardial ischaemia plus reperfusion in untreated rats produced marked myocardial necrosis, increased serum creatinine phosphokinase activity (348 ± 38 U/ml) and cardiac myeloperoxidase activity, a marker of polymorphonuclear leukocyte accumulation, both in the area at risk and in the necrotic area (MPO 9 ± 1.1 mU/g tissue and 8.2 ± 1 mU/g tissue, respectively), and induced a marked increase in the macrophage (156 ± 14 U/ml at the end of reperfusion) and serum (344 ± 12 U/ml, at the end of reperfusion) levels of TNF-α. Finally, myocardial ischaemia–reperfusion injury increased ICAM-1 staining in the myocardium. Administration of 17β-oestradiol (5, 10 and 20 μg/kg, i.m. 5 min after induction of myocardial ischaemia–reperfusion injury), lowered myocardial necrosis and myeloperoxidase activity in the area at risk and in the necrotic area, reduced serum and macrophages TNF-α (20 ± 3 U/ml and 9 ± 3 U/ml, respectively) and decreased serum creatinine phosphokinase activity (67 ± 3 U/ml). Oestrogen treatment also blunted the increased staining of ICAM-1 in the injured myocardium. Finally, 17β-oestradiol added in vitro to peritoneal macrophages collected from untreated rats subjected to myocardial ischaemia–reperfusion injury, significantly reduced TNF-α production. Our results suggest that 17β-oestradiol, by inhibiting TNF-α production, limits the deleterious ICAM-1-mediated binding of leukocytes to injured mycardium and protects against myocardial ischaemia–reperfusion injury.

Keywords: 17β-oestradiol; Myocardial ischaemia–reperfusion injury; ICAM-1 (intercellular adhesion molecule-1); TNF-α (tumor necrosis factor-α)

1. Introduction

Several lines of evidence suggest that oestrogens are cardioprotective (Barrett-Connor and Bush, 1991). Although the cardioprotective benefits of oestrogens appear well established, the mechanism of this effect remains unclear and is the subject of intense investigation (Grady et al., 1992).

Alterations in plasma concentrations of lipoproteins, hemostatic factors, glucose and insulin and reductions in arterial blood pressure (Wahl et al., 1983; Stampfer and Colditz, 1991; Mcade and Berra, 1992) have been proposed as possible explanations for the oestrogen-induced cardioprotection. However, these factors alone cannot explain the positive effects of oestrogens on the cardiovascular system.

The involvement of an inflammatory response in the pathophysiology of myocardial ischaemia has also been suggested (Lucchesi, 1990). Leukocyte accumulation in the myocardium may amplify tissue damage by producing cell activation of the myocytes and by releasing deleterious substances such as leukotrienes (Feuerstein, 1984), thromboxane A_2 (Coker and Parrat, 1985), oxygen free radicals

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(McCord, 1985) and platelet activating factor (Braquet et al., 1987).

Adhesion molecules are considered to play a pivotal role in the localization and development of an inflammatory reaction. Intercellular adhesion molecule-1 (ICAM-1) is an adhesion molecule normally expressed at a low basal level on endothelial cells, but its expression can be enhanced by several inflammatory mediators such as Interleukin-1 and tumour necrosis factor (TNF-α) (Wertheimer et al., 1992).

Structurally, ICAM-1 is a member of the immunoglobulin (Ig) supergene family with five Ig-like domains, a single transmembrane region and a short cytoplasmatic tail (Simmons et al., 1988). It is a ligand for at least two members of the CD18 family of leukocyte adhesion molecules: LFA-1 (CD11a/CS18) and Mac-1 (CD11b/CD18) (Marlin and Springer, 1987; Diamond et al., 1990).

Previous findings have suggested that cardiac myocytes express ICAM-1 in response to cytokine stimulation and that ICAM-1 serves as an adhesive molecule for neutrophils on this cell type (Smith et al., 1991). Furthermore, passive immunization with specific antibodies raised against ICAM-1 reduces infarct size and myocardial leukocyte accumulation in an experimental model of myocardial ischaemia in the rat (Ioculano et al., 1994).

In light of these findings we studied whether the cardioprotective effects of oestrogens may be mediated in vivo by a reduction in ICAM-1-mediated leukocyte accumulation in the ischaemic myocardium and therefore we investigated the effects of 17β-oestradiol on the pathological sequelae associated with occlusion and reperfusion of the myocardium in the rat.

2. Materials and methods

2.1. Animal preparation

Female ovariectomized Sprague–Dawley rats weighing 225–250 g were permitted access to food and water ad libitum. Experiments were approved by the Ethical Committee of the University of Messina. Rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed on a heated operating table. Polyethylene catheters (PE 50) were inserted into the common carotid artery for the measurement of blood pressure and heart rate, as reported previously (Caputi et al., 1980). After tracheotomy, the animals were ventilated with room air with a respirator for small rodents (model 7025 Ugo Basile, Varese, Italy) with a stroke volume of 15 ml/kg and a rate of 54 strokes/min to maintain normal pO_2, pCO_2 and pH parameters. An incision was made on the left side of the chest and the fourth intercostal space was exposed. Sutures were placed through the overlapping skin and muscles to permit rapid closure of the chest wall after the surgical procedures. The chest was then opened and the ribs were gently spread. The heart was quickly expressed out of the thoracic cavity, inverted and a 4.0 silk ligature was placed under the visualized left main coronary artery. The ligature was then tied. The heart was returned quickly to the thoracic cavity, the tips of the suture used to produce the coronary ligation were exteriorized through the chest wall and, after the removal of air in the chest with a syringe, the incision was closed by tying the previously placed sutures (Smith et al., 1989). The tips of the sutures were removed after 1 h and the heart was taken out after 60 min of reperfusion (MI/R rats). Sham-operated animals underwent all the previously described surgical procedures apart from the fact that the suture passing around the left coronary artery was not tied (Sham MI/R rats). The animals were treated with 17β-oestradiol (5, 10 and 20 μg/kg, i.m.) or vehicle (1 ml/kg, i.m.) 5 min after the coronary occlusion.

2.2. Quantification of myocardial damage

Infarcted and perfused areas were evaluated with the triphenyl tetrazolium chloride-Evan's blue technique (Klein et al., 1981). At the end of the reperfusion period, the ligature around the left main coronary artery was retightened; 2 ml of Evan's blue dye (2 mg/ml solution) was injected into the jugular vein to stain the area of the myocardium perfused by the patent coronary arteries. The area at risk was, therefore, determined by negative staining. The atria, right ventricle and the major blood vessels were subsequently removed from the heart. The left ventricle was then sliced into sections 3 mm thick parallel to the atrioventricular groove. The unstained portion of the myocardium (i.e., the area at risk) was separated from the stained portion (i.e., the area not at risk). The unstained portion was again sliced into 1-mm-thick sections and incubated in a 1% solution of the triphenyl tetrazolium chloride stain in 20 mM phosphate buffer, pH 7.4 at 37°C for 20 min to detect the presence of coenzyme and dehydrogenase. The necrotic portion of the myocardium, which did not stain, was separated from the stained portion (i.e., the non-necrotic area at risk). Samples from all three portions of left ventricular cardiac tissue (i.e., non-ischaemic, ischaemic non-necrotic and ischaemic necrotic) were weighed and stored at -70°C for subsequent assay of myeloperoxidase activity.

2.3. Biological assay for tumour necrosis factor-α activity

Killing of L929 mouse tumour cells was used to measure TNF-α levels in serum and in peritoneal macrophage supernatants on the basis of a standard micro-elisa assay (Ruff and Gifford, 1980). L929 cells in RPMI 1640 medium containing 5% fetal calf serum were seeded at 3×10^4 cells per well in 96-well microdilution plates and incubated overnight at 37°C in an atmosphere of 5% CO_2 in

air. Serial 1:2 dilution of serum (drawn at the end of reperfusion) and supernatants of peritoneal macrophages, collected at the same time, as previously described (Altavilla et al., 1989), were made in the above-described medium containing 1.0 μg of actinomycin D per ml and 100 μl volumes of each dilution were added to the wells. For the in vitro studies macrophages collected from untreated MI/R rats and sham-operated rats were incubated for 3 h either with RPMI 1640 medium or several doses of 17β-oestradiol (250, 500 and 1000 pg/ml). One TNF-α unit was defined as the amount giving 50% cell cytotoxicity. The TNF-α content in the sample was calculated by comparison with a calibration curve obtained with recombinant murine TNF-α (Nuclear Laser Medicine, Italy).

2.4. Serum creatinine phosphokinase activity

Samples of arterial blood were drawn from the carotid catheter immediately after the end of reperfusion and collected in polyethylene tubes. The blood was kept at 4°C until it was centrifuged at $2400 \times g$ and 4°C for 15 min. The serum was decanted off and aliquots were used for the determination of creatinine phosphokinase activity (CPK) using a commercial kit (CK-NAC activated, Boehringer-Mannheim).

2.5. Myeloperoxidase activity

Polymorphonuclear neutrophil accumulation was investigated using myeloperoxidase activity as a measure (Mullane et al., 1985). MPO activity was determined from cardiac tissue samples (obtained as described above after the end of the reperfusion period), thereby permitting the simultaneous assessment of PMN infiltration and myocardial injury. The samples were first homogenized in a solution containing 20 mM of potassium phosphate buffer (pH 7.4), 0.01 M EDTA, 50 U/ml of a protease inhibitor (aprotinin) in proportions of 1:10 (w:v) and then centrifuged for 30 min at $20\,000 \times g$ at 4°C. The supernatant of each sample was then discarded and the pellet was immediately frozen on dry ice. The samples were kept at a temperature of 0°C for 14 h before sonication. After thawing, the resulting pellet was added to a buffer solution consisting of 0.5% hexacyltrimethylammonium bromide (Sigma, St. Louis, MO, USA) dissolved in 50 mM potassium phosphate buffer (pH 6) containing 30 U/ml of protease inhibitor. Each sample was then sonicated for 1 min at intensity 2 and at a temperature of 4°C. After sonication the samples were chilled on ice for approximately 30 min, and were then centrifuged for 30 min at $40\,000 \times g$ at 4°C. An aliquot of the supernatant was then allowed to react with 0.167 mg/ml *o*-dianisidine dihydrochloride (Sigma) and 0.0010% H_2O_2 and the rate of change in absorbance was measured at 405 nm in a microtitre plate reader. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide/min at 25°C and was expressed in milliunits per g weight (mU/g tissue).

2.6. Immunohistochemistry

ICAM-1 staining was studied in the area at risk at the end of the reperfusion period. For immunohistochemical evaluation 5-μm-thick cryostat sections were stained according to the avidin–biotin–peroxidase complex procedure (Hsu et al., 1981). An average of seven sections per immunohistochemical stain was cut from each sample, air-dried for 30 min and then fixed in cold acetone for 10 min. Endogenous peroxidases were blocked with horse serum for 15 min at room temperature prior to incubation with primary antibodies. Monoclonal antibodies consisted of mouse monoclonal antibodies raised against rat ICAM-1 (clone: IA 29, subclass IgG_1) and were obtained from British Bio-technology Products (Abingdon). A monoclonal mouse IgG_1 antibody was used for the controls. Biotinylated, species-specific second layer reagents were then applied, followed by avidin–biotin–horse radish peroxidase complex as a chromogenic substrate, as previously reported (Hsu et al., 1981). The microscopy image was sent to a computer-assisted image analyser that analysed the changes in staining. Densitometric analysis of the captured image was performed on a PC using image analysis software.

2.7. Cardiovascular measurements

Pressure rate index (PRI) (Gobel et al., 1978) was calculated as the product of mean arterial blood pressure and heart rate reported as mmHg/beats per min $\times 10^{-3}$. Changes in the electrical activity of the myocardium were detected from the ECG in lead II (Biocard, model BC-1, Florence).

2.8. Drugs

17β-oestradiol was obtained from Sigma.

2.9. Statistical analysis

Data are expressed as means ± S.D. and were analyzed by analysis of variance for multiple comparison of results; Duncan's multiple range test was used to compare group means. In all cases, an error probability of less than 0.05 was selected as criterion for statistical significance.

3. Results

3.1. Myocardial infarct size

The area at risk, determined by negative staining following perfusion with Evan's blue stain, showed no signif-

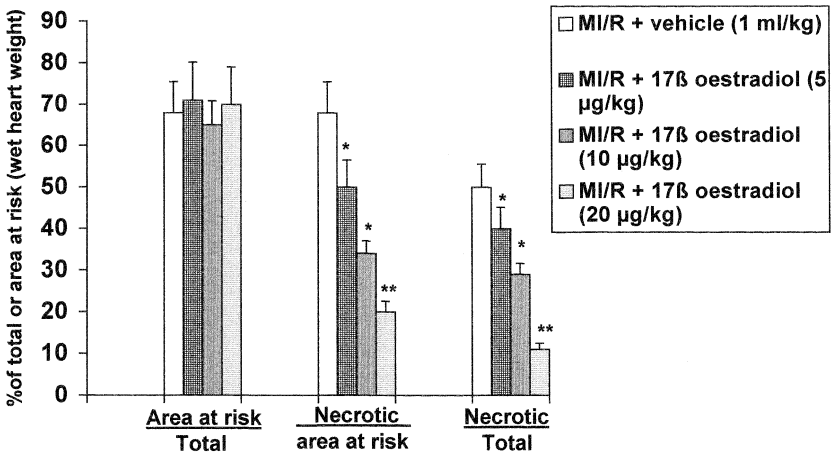

Fig. 1. Effects of vehicle (1 ml/kg i.m., 5 min after the surgical procedures) or 17β-oestradiol (5, 10 and 20 μg/kg i.m. 5 min after the surgical procedures) on the area at risk, indexed to total ventricle (area at risk/total left ventricle $\times$ 100) and necrotic area indexed to area at risk (necrotic area/area at risk $\times$ 100) and to total left ventricle (necrotic area/total left ventricle $\times$ 100) as a percentage of wet weight. Bar heights represent means $\pm$ S.D. of six experiments. * $P < 0.01$ vs MI/R + vehicle. * * $P < 0.005$ vs MI/R + vehicle.

icant differences between experimental groups (Fig. 1), indicating that a similar amount of tissue was jeopardized by occlusion of the main left coronary artery in each group. In contrast, the necrotic area, which was measured by negative staining with triphenyl tetrazolium chloride, indicated that a relatively large amount of the myocardial tissue at risk became necrotic in the MI/R vehicle-treated rats (67 $\pm$ 9%).

Administration of 17β-oestradiol (5, 10 and 20 μg/kg i.m., 5 min after coronary occlusion) reduced in a dose-dependent manner the myocardial necrosis extension (Fig. 1). This significant reduction in necrosis was observed whether the necrotic area was expressed as a percentage of the area at risk or as a percentage of the total left ventricle (Fig. 1). Thus, 17β-oestradiol afforded high cardioprotection and the dose of 20 μg/kg was the most effective.

3.2. Myeloperoxidase activity

Elevated myeloperoxidase activities were found in the area at risk (9 $\pm$ 1.1 mU/g tissue) and in the necrotic area (8.2 $\pm$ 1 mU/g tissue) of the untreated myocardial ischaemia reperfusion injured rats (Fig. 1). In contrast, very low myeloperoxidase activity was measured in sham myocardial ischaemia–reperfusion injury (1.5 $\pm$ 0.3 mU/g tissue in the sham MI/R rats + vehicle; 1.7 $\pm$ 0.2 mU/g

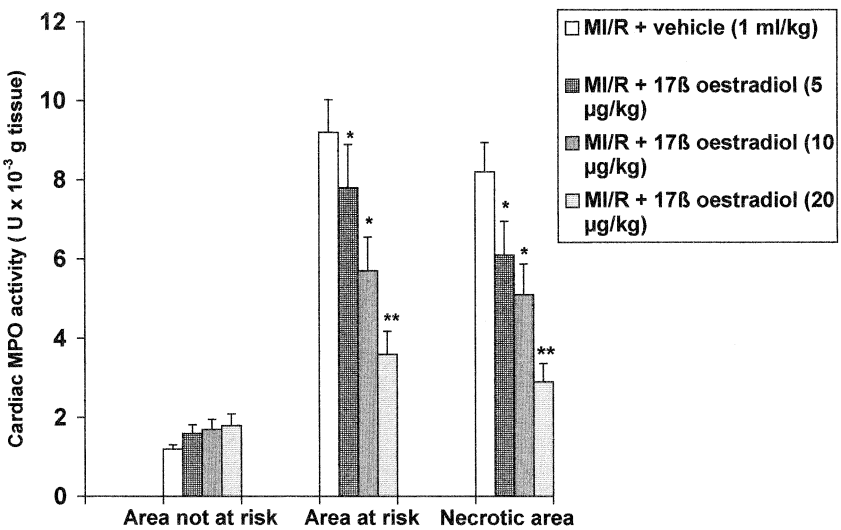

Fig. 2. Effects of vehicle (1 ml/kg i.m. 5 min after the surgical procedure) or 17β-oestradiol (5, 10 and 20 μg/kg i.m. 5 min after the surgical procedures) on myeloperoxidase (MPO) activity in the area not at risk, area at risk and area of necrosis, from rats subjected to myocardial ischaemia–reperfusion (MI/R) injury. Bar heights represent the means $\pm$ S.D. from six experiments. * $P < 0.02$ vs SAO + vehicle. * * $P < 0.001$ vs SAO + vehicle.

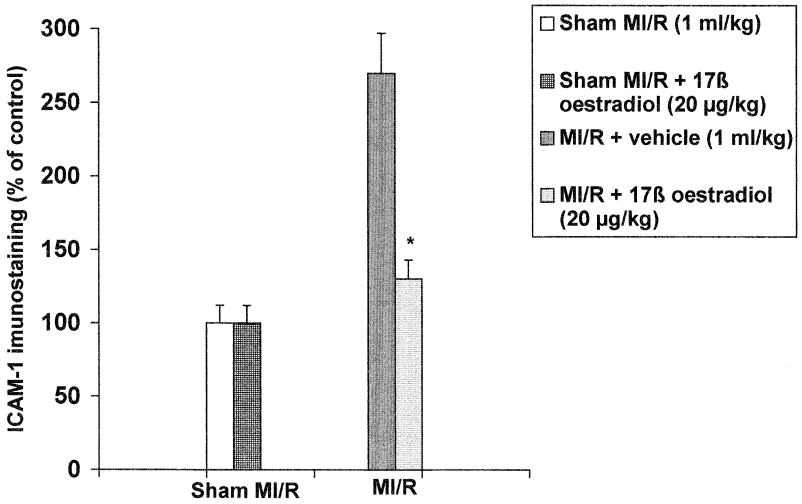

Fig. 3. Effects of vehicle (1 ml/kg i.m., 5 min after the surgical procedure) or 17β-oestradiol (20 μg/kg i.m., 5 min after the surgical procedures) on ICAM-1 immunostaining in the myocardium at risk, from rats subjected to myocardial ischaemia–reperfusion (MI/R) injury. Bar heights represent the means $\pm$ S.D. from six experiments. * $P < 0.05$ vs SAO + vehicle.

tissue in the sham MI/R rats + 17β-oestradiol). Administration of 17β-oestradiol (5, 10 and 20 μg/kg i.m., 5 min after occlusion of the coronary artery) significantly blunted the rise in myocardial myeloperoxidase activity both in the area at risk and in the necrotic area (Fig. 2). Thus, 17β-oestradiol limits the amount of neutrophil infiltration into the ischaemic/reperfused myocardium.

3.3. Serum creatinine phosphokinase activity

Sham myocardial ischaemia–reperfusion-injured rats, given vehicle or the highest dose of 17β-oestradiol, exhibited no significant differences in creatinine phosphokinase levels (30 ± 7 and 35 ± 8 U/ml, respectively). A significant increase of this enzyme was found in the serum of rats subjected to myocardial ischaemia–reperfusion injury and given vehicle (348 ± 38 U/ml). Administration of 17β-oestradiol (5, 10 and 20 μg/kg, i.m.) resulted in blunting of the creatinine phosphokinase activity depletion (180 ± 10, 110 ± 9 and 67 ± 3 U/ml, respectively). These data further support a cardioprotective effect of oestrogen in acute myocardial infarction in rats.

3.4. ICAM-1 staining in myocardium at risk

ICAM-1 staining was studied in myocardium at risk. Immunohistochemical evaluation indicated that a very low constitutive staining of ICAM-1 was present in the myocardium of sham-operated animals (Fig. 3) and in non-ischaemic myocardium of infarcted rats (results not shown). In contrast, samples of the area at risk had an increase in ICAM-1 staining. 17β-oestradiol reduced the increased staining of ICAM-1 (Fig. 3).

3.5. Serum and macrophage TNF-α

Serum and macrophage levels of TNF-α were undetectable in sham-operated rats treated with either vehicle or 17β-oestradiol. TNF-α was significantly increased in both serum and macrophages collected from infarcted rats at the end of the reperfusion period (Table 1). The administration

Table 1
Effects of 17β-oestradiol on serum and macrophage tumour necrosis factor-α (TNF-α) in myocardial ischaemia–reperfusion injury (MI/R)

Treatment	Serum TNF-α (U/ml)	Macrophage TNF-α (U/ml)
Sham MI/R + vehicle	N.D.	N.D.
Sham MI/R + 17β-oestradiol	N.D.	N.D.
MI/R + vehicle	344 ± 12	156 ± 14
MI/R + 17β-oestradiol (5 μg/kg)	230 ± 8 [a]	99 ± 8 [a]
MI/R + 17β-oestradiol (10 μg/kg)	192 ± 11 [a]	73 ± 5 [a]
MI/R + 17β-oestradiol (20 μg/kg)	20 ± 3 [b]	9 ± 3 [b]

Serum and macrophages were collected at the end of reperfusion. Each point represents the mean $\pm$ S.D. from six experiments. Animals received 17β-oestradiol or vehicle (1 ml/kg) 5 min after the surgical procedures.
[a] $P < 0.05$ vs MI/R + vehicle; [b] $P < 0.001$ vs MI/R + vehicle. N.D. = not detectable.

Table 2
In vitro effects of 17β-oestradiol on production of tumour necrosis factor (TNF-α) by peritoneal macrophages collected from rats subjected to myocardial ischaemia–reperfusion injury (MI/R) or sham ischaemia (Sham MI/R)

	Macrophage TNF-α (U/ml)	
	MI/R	Sham MI/R
RPMI (1 ml)	319 ± 13	N.D.
17β-oestradiol (250 pg)	110 ± 10 [a]	N.D.
17β-oestradiol (500 pg)	46 ± 14 [b]	N.D.
17β-oestradiol (1000 pg)	43 ± 15 [b]	N.D.

Peritoneal macrophages were collected from untreated rats subjected to myocardial ischaemia–reperfusion injury at the end of reperfusion. Macrophages were then incubated for 3 h with several doses of 17β-oestradiol or with the vehicle in which oestrogens were dissolved. Each point represents the mean ± S.D. of seven experiments.
[a] $P < 0.05$ vs RPMI; [b] $P < 0.001$ vs RPMI.

of 17β-oestradiol significantly blunted the macrophage and serum levels of this cytokine. Furthermore, 17β-oestradiol added in vitro to macrophages collected from untreated rats subjected to myocardial ischaemia–reperfusion injury significantly reduced TNF-α (Table 2).

3.6. Cardiovascular measurements

The pressure rate index (PRI) was evaluated as index of myocardial oxygen demand. The pressure rate index was comparable for the four groups before coronary artery ligation and decreased to a similar extent following thoracotomy (results not shown). At the end of reperfusion, the pressure rate index was 83.5 ± 7.2 mmHg/beats per min $\times 10^{-3}$ in sham myocardial ischaemia–reperfusion injury. Infarcted rats given vehicle had a marked reduction in pressure rate index throughout the experiment (52.3 ± 5.1 mmHg/beats per min $\times 10^{-3}$). 17β-oestradiol injection (5, 10 and 20 μg/kg, i.m.) markedly improved the pressure rate index (67 ± 4, 75 ± 5 and 81.4 ± 3.3 mmHg/beats per min $\times 10^{-3}$, respectively).

4. Discussion

One mechanism proposed for the vasoprotective effect of oestrogen is favourable modulation of vasoreactivity. This is suggested by reports of the ability of oestrogen to block endothelin-1 and calcium-mediated vasoconstriction in isolated coronary arteries (Jiang et al., 1991; Jiang et al., 1992), to stimulate prostacyclin production (Chang et al., 1980; Makila et al., 1982) and modulate the production of nitric oxide (Garg and Hassid, 1989). However these effects do not fully account for the degree of clinical benefit attributable to oestrogen therapy.

A major factor involved in leukocyte recruitment into inflammatory tissues is thought to be the expression on activated endothelial cells of cytokine inducible adhesion molecules for leukocytes (Harlan, 1987; Osborn, 1990). Leukocyte accumulation in the myocardium has been shown to represent an important aspect of myocardial ischaemia. Indeed neutrophil depletion via the administration of an antiserum, injection of hydroxyurea or extracorporeal filtration, as well as inhibition of neutrophil function with a drug such as ibuprofen reduces the degree of myocardial necrosis following an ischaemic insult (Entman et al., 1991; Mullane et al., 1984). In addition to leukocytes, endothelial adhesion molecules may be implicated in the pathogenesis of experimental and human myocardial ischaemia (Squadrito et al., 1996).

A hypothesis that has not been sufficiently tested is that oestrogen-induced cardioprotection is mediated by inhibition of adhesion molecule-mediated leukocyte accumulation in the ischaemic myocardium.

To test this hypothesis we investigated the effects of 17β-oestradiol in a model of myocardial ischaemia-reperfusion injury in rats. Previous data have shown that tumour necrosis factor (TNF-α) and the adhesion molecules, E-selectin and ICAM-1, are likely to be involved in the pathogenesis of this type of experimental myocardial ischaemia–reperfusion injury (Ioculano et al., 1994; Altavilla et al., 1994; Squadrito et al., 1993).

Our data suggest that rats subjected to occlusion and reperfusion of the main left coronary artery have marked myocardial damage and a marked leukocyte infiltration (as shown by the increase in neutrophil accumulation in the area at risk and the necrotic area of the ischaemic heart). This increase in neutrophil adhesion to the ischaemic myocardium was also accompanied by a marked increase in ICAM-1 staining in the area at risk. Indeed our data suggest that ICAM-1 overproduction occurs following 1 h of reperfusion, while previous findings have indicated that ICAM-1 expression is not increased until after 2 h of reperfusion (Weyrich et al., 1995). The reason for this discrepancy could be due the longer period of occlusion (1 h) that would cause a greater inflammatory reaction during the reperfusion period. Our findings, taken together, suggest that the deleterious leukocyte accumulation in ischaemic cardiac tissue in vivo is mediated by the adhesion molecule ICAM-1. This result is in agreement with results of previous in vitro experiments showing that isolated cardiac myocytes express ICAM-1 in response to TNF-α stimulation, and that ICAM-1 functions as an adhesive molecule for neutrophils on this type of cell (Smith et al., 1991). Furthermore, rats subjected to the myocardial ischaemia–reperfusion injury also had increased levels of both serum and macrophage TNF-α. It can, therefore, be argued that TNF-α also induces the expression of this adhesive receptor on cardiac tissue in vivo.

Our results indicate that 17β-oestradiol reduced the enhanced macrophage and serum levels of TNF-α. This effect is a consequence of a direct inhibition of this inflammatory cytokine: in fact 17β-oestradiol added in vitro to macrophages collected from untreated rats sub-

jected to myocardial ischaemia–reperfusion injury caused a marked inhibition in the production of TNF-α. This finding is in agreement with previous findings showing that oestrogens may down-regulate the production of this cytokine, via inhibition of the mRNA for TNF-α (Shanker et al., 1994).

The administration of 17β-oestradiol reduced ICAM-1 staining and decreased myeloperoxidase activity, an index of leukocyte accumulation. Since leukocyte–endothelial interaction (more specifically the ICAM-1-dependent leukocyte adhesion) is primed by TNF-α, it can be proposed that 17β-oestradiol, by inhibiting this inflammatory cytokine, inhibits ICAM-1 immunostaining and limits leukocyte accumulation.

Occlusion and reperfusion of the left main coronary artery produced marked myocardial necrosis, and oestrogen treatment reduced infarct size. In addition 17β-oestradiol decreased the serum levels of creatinine phosphokinase, thus confirming the cardioprotective effects of this treatment.

The positive effect in the ischaemic myocardium occurred with a clear reduction in myocardial oxygen demand. This finding is interesting: the protective effects of oestrogen could also result from a reduction in oxygen consumption. Furthermore, the reduction in oxygen demand might be the consequence of the inhibition of leukocyte accumulation in ischaemic myocardium.

It could, therefore, be argued that 17β-oestradiol by reducing ICAM-1-mediated binding of leukocytes to the myocardium protects against myocardial necrosis. These effects confirm that 17β-oestradiol exerts strong cardioprotection.

In conclusion we have shown that 17β-oestradiol inhibits in vitro and in vivo TNF-α: the 17β-oestradiol-induced inhibition of the production of this inflammatory cytokine, at least in myocardial ischaemia–reperfusion injury in the rat, reduces cardiac ICAM-1 expression and leukocyte infiltration, in turn limiting myocardial necrosis. These findings would suggest that inhibition of myocardial ICAM-1 may contribute to the cardioprotective effects of oestrogens.

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