# THE EFFECT OF SEX STEROIDS ON THE AORTIC ENDOTHELIUM OF RATS WITH THERMAL INJURY

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## ABSTRACT

**Objective:** Thermal injury-induced physiopathological events are related to an acute inflammatory reaction. This study was designed to observe the role of sex steroids on the aortic endothelia of rats with burn injury.

**Methods:** Male Wistar Albino rats were given burn trauma (n=30), and the rats were injected subcutaneously with either 17β estradiol benzoate ($E_2$, 10mg/kg) or an androgen receptor blocker cyproterone acetate (CPA, 25 mg/kg) or vehicle immediately after the burn and at 12 h. At the 24$^{th}$ hour of burn injury, the rats were decapitated. Blood samples to measure serum TNF levels were collected. The aortas of the rats were processed for both light and scanning electron microscopy. Student's t test was used for statistical analysis.

**Results:** Antiandrogen and $E_2$ treatments depressed burn induced elevation in serum TNF levels. Both light and electron microscopy revealed a morphology in accordance with TNF results. The endothelia of aortas were preserved significantly better than in the vehicle group. The adhesion of blood cells on endothelium was reduced in the antiandrogen or $E_2$ - treated groups.

**Conclusion:** In conclusion, we may predict that treatment with estrogens or antiandrogens may have an ameliorating effect on systemic inflammation induced by burn.

**Key Words:** Burn injury, Sex steroids, Aorta, Microscopy

## INTRODUCTION

Burn-induced injury produces local and systemic damage due to the release of several inflammatory mediators. The physiopathological events after thermal injury are related to an acute systemic inflammatory reaction (1). Shortly after thermal injury, a number of cytokines (interleukin-1, TNF) are induced and inflammatory response develops (1). Overproduction of cytokines and the activation of endothelial cells lead to production of inflammatory active substances. TNF is among the most important cytokines

*Marmara Medical Journal 2003;16(2):97-102*

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involved in the acute inflammatory reaction following thermal injury (2,3). It enhances the endothelial adhesiveness of leukocytes and stimulates neutrophils and monocytes promoting their adherence, phagocytosis and degranulation (1). Burn injury leads to vascular thrombosis and occlusion by thermal damage to the vascular network and may cause changes in the endothelium. The resulting response is vasoconstriction due to the release of ET-1 and a reduction in vasodilator PGI2/ NO (4, 5) response.

Animals with thermal injury showed significant loss of systemic vascular resistance and fall in mean arterial pressure (6,7). Meanwhile, alteration in vascular smooth muscle receptor activity has been hypothesized to occur (8). Also maximal rise of the spontaneous intravascular platelet aggregation index was observed in studies on burn injury (9). The frequency of thromboembolic complication in burn patients has been shown to range from 0.4 -7 % (1, 4). Leukocytes and the process of leukocyte adherence have been implicated in the pathogenesis of organ dysfunction after thermal injury (8, 10).

Recent clinical studies in trauma patients have verified that susceptibility to and death from sepsis is higher in men than in women (11-13). Female animals demonstrated normal or enhanced immune response as opposed to markedly decreased immune response in male mice in recent clinical studies (11-13). Besides, the suppressive effect of androgens on immunity have been reported on normal immune functions as well as in autoimmune diseases (14,15). However, it remains unclear whether sex steroids have any effect on vascular integrity challenged with thermal injury. So the aim of this study was to investigate the role of endogeneous testesterone or exogenous estradiol on the aortic endothelia of rats with burn injury.

## MATERIALS AND METHODS

**Animals:** Male Wistar Albino rats (250-300g) were kept in a light and temperature-controlled room on a 12:12-h light-dark cycle, where the temperature (22±0.5 °C) and relative humidity (65-70 %) were kept constant. They were fed a standard diet and water ad libitum. The experiments were approved by the Marmara University, School of Medicine, Animal Care and Use Committee. Surgical procedures and burn trauma were conducted under anesthesia performed by intraperitoneal (ip) injection of a mixture of ketamine (100mg/kg) and chlorpromazine (12.5mg/kg).

**Burn injury.** Under anesthesia, the dorsum of the rat was shaved and exposed to 90 °C water bath for 8 seconds, to induce full-thickness burn involving 30% of the total body surface area. Fluid resuscitation was made (20 ml/kg saline; subcutaneously, sc) and the animals were placed on a heating pad until they recovered from anesthesia.

**Experimental design.** The rats were treated sc twice- immediately after burn and at 12 h- with either 17β estradiol benzoate (10 mg/kg, in olive oil; Sigma) or an androgen receptor blocker cyproterone acetate (CPA, 25 mg/kg; Schering A.G.) or oil alone (vehicle, 1 mg/kg; Komili) before they were decapitated at the 24$^{th}$ hour. Trunk blood obtained by decapitation was taken into tubes, which were kept on crushed ice. Serum samples were kept at -50 °C until TNF levels were assayed.

**Measurement of TNFα levels.** A TNFα enzyme-linked immunabsorbent assay kit (Endogen, Mass, USA) was used with the manufacturer's instructions without any modifications. Optical densities were read on a plate reader set at 450 nm. The concentration of TNFα in the serum samples was calculated from the standard curve, multiplied by the dilution factor and expressed as pg /mL.

**Light microscopy,** (LM). The rat aortas were fixed in 10% buffered formalin and dehydrated in increasing concentrations of ethanol, then cleared in toluene and embedded in paraffin. Sections 5 μm thick were stained with toluidin blue (TB) and observed under an Olympus photomicroscope (Tokyo, Japan).

**Scanning electronmicroscopy,** (SEM). Specimens were fixed, dehydrated and dried with liquid $CO_2$ under pressure with critical point dryer (Bio-Rad E 3000, Hertfordshire, UK), then coated with gold approximately 400 Å in a sputter

coating unit (Bio-Rad SC 502, Hertfordshire, UK). Coated specimens were veiwed in a Jeol JMS SEM (Tokyo, Japan).

**Statistical analysis**. All data are expressed as means ± SEM with eight to ten rats per group. Instat statistical package (GraphPad Software, San Diego, CA, USA) was used. Following the assurance of normal distribution of data, one–way analysis of variance (ANOVA) with the Tukey-Kramer post-hoc test was used for multiple comparison. Values of $p<0.05$ were regarded as significant.

## RESULTS

*Light microscopy:* The control aorta demonstrated regular endothelium with intact intima layer containing elastic fibres (Fig. 1a). In the vehicle-treated animals with burn injury, aorta endothelium was disorganized and muscle cell contraction was present (Fig. 2a). In the estradiol-treated group, almost regular endothelium and elastic fibers were observed (Fig. 3a). As a result of CPA treatment, the muscle bundles among the elastic laminae showed a contracted morphology, but the luminal contour was regular (Fig. 4a).

*Scanning electronmicroscopy*: In the control group, the luminal surface of the aortas was characterized with intact longitudinal relief of elongated and orderly populated endothelial cells with a few blood cells sticking to the luminal surface (Fig. 1b). In the vehicle group, burn injury resulted in a disorganized surface appearance, and numerous blood cells stuck to the lumen were prominent (Fig. 2b). In the estradiol-treated group, the luminal surface

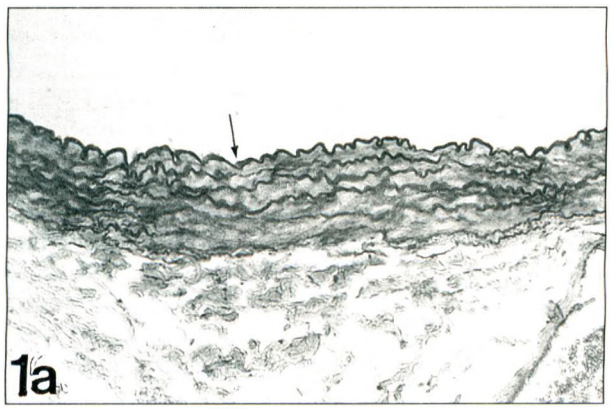
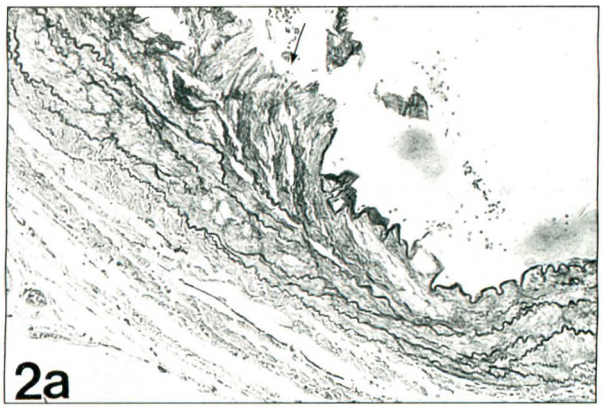




**Fig. 1:** Control aorta a) regular endothelium with intact intima layer (arrow) containing elastic fibres, TB X100, b) luminal surface of aortas was characterized with intact longitudinal relief of elongated and orderly populated endothelial cells (arrows) with a few blood cells sticking to the luminal surface, SEM.



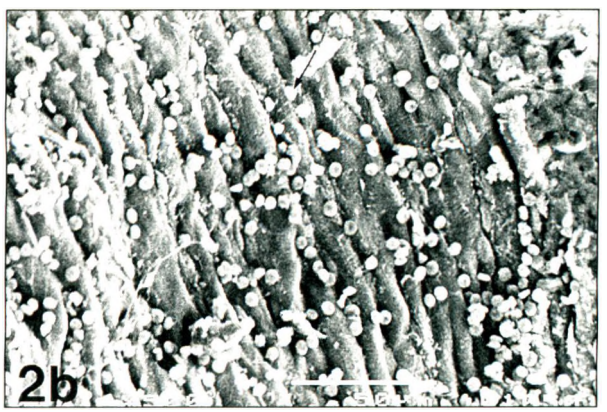
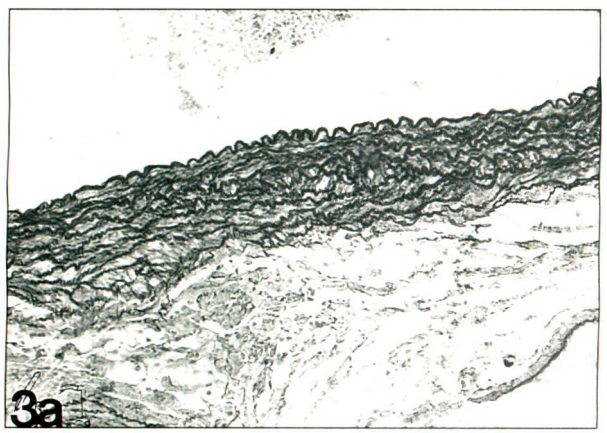




**Fig. 2:** Vehicle-treated group, a) aorta endothelium was disorganized (arrow) and muscle cell contraction was present TB X100, b) a disorganized surface appearance, and numerous blood cells (arrow) stuck to the lumen were prominent, SEM.

exhibited nearly no detectable surface changes as compared to the control group, and decreased blood cell adhesion was observed in this group (Fig. 3b) . In the CPA- treated group, the acanthotic eyhrocytes were partially stuck to the luminal surface, which showed almost characteristic regular cobblestone appearance. (Fig. 4b).

*Serum TNF levels*: In the vehicle-treated burn group (44.03±6.90 pg/ml; $p<0.05$), serum TNF level was significantly increased compared to the control group ( 25.55±1.92 pg/ml; $p<0.05$) (Fig. 5). This burn-induced rise in serum TNF level was abolished by treatment with estradiol (27.54 ±3.45 pg/ml; $p<0.05$) or CPA (29.06±5.72 pg/ml; $p<0.05$).



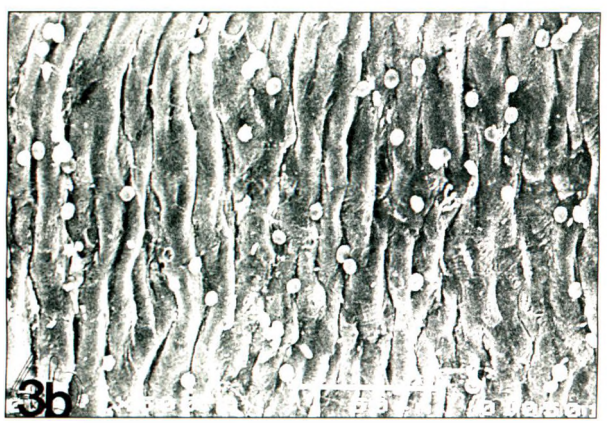




**Fig.3:** Estradiol-treated group, a) almost regular endothelium and elastic fibers were observed TB X100, b) luminal surface exhibited nearly no detectable surface changes as compared to control group. SEM.

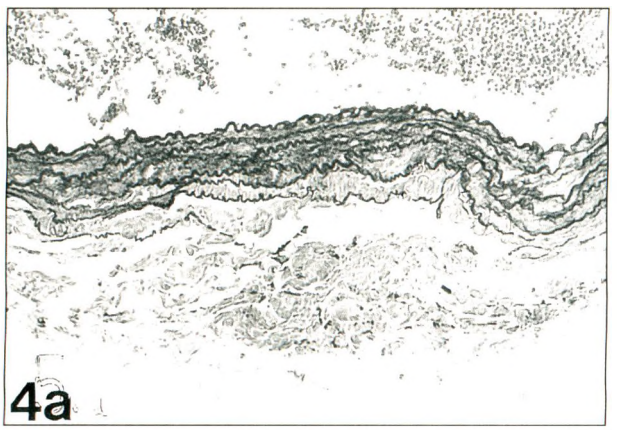




**Fig.4:** CPA treatment, a) the muscle bundles among the elastic laminae showed a contracted morphology, but the luminal contour was regular TB X100, b) the eyhrocytes (arrows) were partially stuck to the luminal surface, which showed almost characteristic regular cobblestone appearance. SEM.

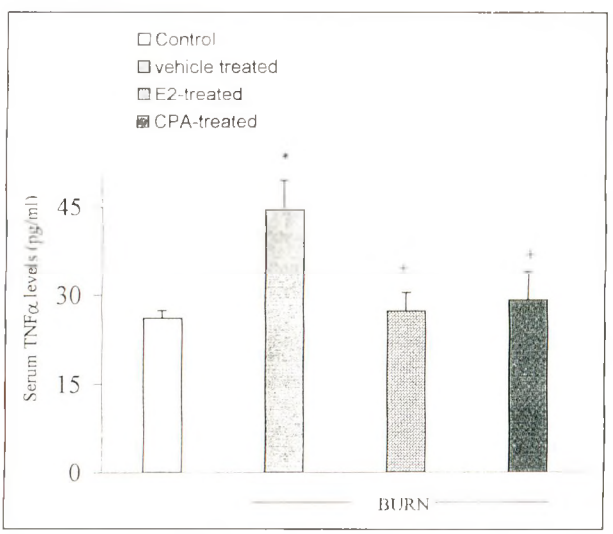



**Fig.5:** Serum TNF levels in the control, vehicle, estrogen and CPA treated rats.
* $p<0.05$, compared to control group,
\+ $p<0.05$, compared to vehicle-treated group.

## DISCUSSION

In the present study, CPA and estradiol treatment did not cause any significant changes in the morphology of the luminal surface of burn-injured aorta endothelium. However, there was a significant decrease in the number of adhering blood cells when compared to vehicle group where the regular topography of the endotelial layer was disrupted. The presence of estradiol prevented the burn-induced morphological changes indicating endothelial damage. In concert with estradiol, CPA also seems to exert an ameliorating effect on luminal morphology of the aorta endothelium.

The model of a 30% total body surface area burn injury results in hypotension and hypoxemia in the burn group (10). Burned animals show a sustained 20-30% fall in mean arterial blood pressure (7). The frequency of thromboembolic complications in burn patients has been estimated to range from 0.4% to 7% (4). Thermal injury leads to activation of strong cytokines. Platelets are inflammatory cells (16); when the endothelium is damaged, platelets adhere to it, thereby becoming activated. Activated platelets release a number of substances such as serotonin, PAF, TNFα, cationic proteins and proteolitic enzymes (collagenase and elastase) that modify tissue integrity (9). Most possibly the antiinflammatory effects of estrogen on burn injury involve mechanisms that reduce both neutrophil infiltration and TNFα which have leading roles in the inflammatory processes. Hence, it is clarified that in the presence of estradiol less platelets adhere to the luminal surface of aorta endothelium.

Prostacyclin and the endothelium derived nitric oxide (NO) are produced by endothelium and have leading roles in managing vascular tone. Studies have shown that NO precursor L-arginine and the NO-donor compound FK 409 which inhibit platelet aggregation and adhesion in the vascular endothelium, lead to an inhibition in balloon injury-induced intimal hyperplasia (17). Moreover, in some studies it is suggested that the ameliorating effect of estrogen may be mediated by the stimulation of prostaglandin synthesis by the vessel wall (17,18).

In the development of cardiovascular disease, the gender difference has been documented in human and animal studies (19,20). Many studies revealed that estrogen might have protective effects on cardiovascular morbitidy (17,18,21). The increased risk of coronary heart disease in young women with bilateral ovariectomy and the beneficial effect of estrogen replacement therapy in postmenaposed women further support a role for estrogen in protecting against the development of coronary artery disease (22). It is suggested that estrogens show their effect through a direct action on blood vessels which contain specific high affinity receptors for estrogen (17).

Many studies predict that androgenic steroids may lead to vasospasm, platelet hyperaggregability and thrombotic cardiovascular diseases (19,23). In experimental studies of arterial thrombosis and platelet aggregability models, gender differences have been observed and also an increase in both arterial thrombus formation and platelet aggregability is observed by testesterone pretreatment (23). It is predicted that testesterone receptor blockade inhibits the vasoconstrictive activity of thromboxane A2 and/or enhances the release of vasodilator prostacyclin in the aortic-coronary circulation, causing a better organ perfusion after trauma (23).

Our study demonstrated that both estrogen and antiandrogen treatments which lead to an increase in plasma estradiol levels (24) lead to a decrease in burn-induced inflammation in the endothelia of aortas. The pronounced effects of these treatments may be, in part, due to the limiting effect of estrogen on tissue injury by depressing neutrophil infiltration and by reducing the release of inflammatory cytokines. In conclusion, we may predict that treatment with estrogens or antiandrogens may ameliorate systemic inflammation induced by burn.

## REFERENCES

1. *Deveci M, Eski M, Şengezer M, Kısa U. Effects of cerium nitrate bathing and promp burn wound excision on IL-6 and TNF-α levels in burned animals. Burns 2000;26:41-45.*

2. Sparkes BG. Immunological responses to thermal injury. Burns 1997;23:106-113.
3. Arturson G. Pathophysiology of the burn wound and pharmacological treatment. The Rudi Hermans lecture.1995. Burns 1996;22: 255-274.
4. Rue LW 3rd, Cioffi WG JR, Rush R, McManus WF, Pruitt BA Jr. Thromboembolic complications in thermally injured patients. World J Surg 1992;16:1151-1154.
5. Murpy JT, Duffy S, Purdue GF, Hunt JL. Thermal injury alters endothelial vasoconstrictor and vasodilator response to endotoxin. J Trauma 1999;47:492-499.
6. Drueck C, Welch GW, Pruitt BA Jr. Hemodynamic analysis of septic shock in thermal injury: treatment with dopamine. Am Surg 1978;44:424-427.
7. Lang C, Dobrescu C. Attenuation of burn-induced changes in hemodynamics and glucose metabolism by the PAF antagonist SRI 63-675. Eur J Pharmacol 1988;156:207.
8. Cioffi WG, DeMeules JE, Gamelli RL. Vascular reactivity following thermal injury. Circ Shock 1988;25:309-317.
9. Lukoianova TI, Kozel'skaia LV. Functional damage of blood vessels and intravascular platelet aggregation in burns. Bull Eksp Biol Med 1983;95:20-22.
10. Mileski W, Gates B, Sigman A, et al. Inhibition of leukocyte adherence in a rabbit model of major thermal injury. J Burn Care Rehabil 1993;14:610-616.
11. McGowan JE, Barnes MW, Finland N, Bacteremia at Boston City Hospital: occurrence and mortality during 12 selected years (1935-1972) with special reference to hospital-acquired cases. J Infec Dis 1975;132:316-335.
12. Nuytinck H, Offerman W,Goris RJ. Whole body inflammation in trauma patients. Arch Surg 1988;123:1519-1524.
13. Bone RC. Toward an epidemiology and natural history of SIRS (systemic inflammatory response sendrome). JAMA 1992;268:3452-3455.
14. Viselli SM, Stanziale S, Shults K, Kovacs WJ, Olsen NJ. Castration alters peripheral immune function in normal male mice. Immunology 1995;84:337-342.
15. Walker SE, Besch-Williford CL, Keisler DH. Accelerated deaths from systemic lupus erythematosus in NZBx NZW F1 mice treated with testesterone-blocking drug flutamide. J Lab Clin Med1994;124:401-407.
16. Şan T, Moini H, Emerk K, Bilsel S. Protective effect of defibrotide on perfusion induced endothelial damage. Thromb Res 2000;99:335-341.
17. Shan J, Resnick LM, Qin-Yue L, Xl-Chen W, Barbagallo M, Pang PK. Vascular effects of 17β estradiol in male Spraque-Dawley rats. Am J Physiol (Heart Circ Physiol 35) 1994;266:H967-H973.
18. Farhat MY, Lavigne MC, Ramwell PW. The vascular protective effect of estrogen. FASEB J 1996; 10(April):615-624.
19. Hayashi T, Fukuto JM, Ignarro LJ, Chadhuri G. Gender differences in atherosclerosis: possible role of nitric oxide. J Cardiovasc Pharmacol 1995;26:792-802.
20. McGill HC Jr, Stern MP. Sex and atherosclerosis. Atherscler Rev 1979;4:157-242.
21. Maddox YT, Falcon JG, Ridinger M,Cunard CM, Ramwell PW. Endothelium-dependent gender differences in the response of the rat aorta. J Pharmacol Exp Ther 1987;240:392-395.
22. Zhang A, Altura BT, Altura BM. Sexual dimorphism of vascular smooth muscle responsiveness is dependent on anions and estrogen. Steroids 1991;56:524-526.
23. Matsuda K, Ruff A, Morinelli TA, Mathur RS, Haluska PV. Testesterone increases thromboxane A2 receptor density and responsiveness in rat aortas and platelets. Am J Physiol (Heart Circ. Physiol,36) 1994; 267: H887- H893.
24. Özveri E, Bozkurt A, Haklar G, et al. Estrogens ameliorate remote organ inflammation induced by burn injury in rats. Inflamm Res 2001;50:585-591.