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Effect of pentoxifylline on veno-occlusive priapism-induced corporeal tissule lipid peroxidation in a rat model

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Abstract *Objective*: To investigate whether pentoxifylline could play a role in attenuation of the hazardous effects of ischemia/reperfusion on corporeal tissue in a rat model of veno-occlusive priapism (VOP).
Materials and methods: Placebo and pentoxifylline were given to eight groups of rats prior to priapism being induced by a vacuum constrictive device for durations of 6 and 12 h, respectively. Half of the groups of rats that underwent the same duration of priapism (ischemic) were subjected to 1 h of detumescence after band removal (reperfusion). One group underwent no manipulation and no drug administration and served as a baseline determination (control). Corporeal homogenates were examined for lipid peroxidation (LP) derived malondialdehyde (MDA) accumulation via thiobarbituric acid assay.
Results: MDA concentration differed significantly between VOP rats and controls ($P < 0.001$) but did not differ significantly between ischemic-only groups and reperfused groups ($P > 0.05$). In the pentoxifylline-pretreated groups, although MDA accumulation tended to be slightly lower than in the placebo groups, the difference was not statistically significant ($P > 0.05$) either in the 6- or 12-h duration priapic groups.
Conclusions: LP, an indicator of radical oxygen metabolite (ROM) induced injury, occurs in rat corporeal tissue during and after abolishment of VOP. Single-dose pentoxifylline pretreatment failed to exert a protective effect on corporeal tissue in a rat model of VOP in terms of attenuation of LP.

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Priapism is a condition characterized by prolonged, painful penile erection not associated with sexual desire. Recurrent episodes of veno-occlusive priapism (VOP), the most common form, classically have been described in patients suffering from certain neurological conditions and hematological disorders. An increasing incidence of priapism after use of antihypertensive, psychotropic drugs and intracavernous pharmacotherapy has been noted. Corporeal fibrosis subsequent to prolongation of insufficient arterial inflow results in impotence, the chief complication of priapism [22].

The oxidant injury can potentially occur during ischemia and reperfusion due to an excess production of radical oxygen metabolites (ROMs) and/or a decrease in antioxidant defenses [8]. ROMs, beside mediating LP in postischemic tissues, attract polymorphonuclear neutrophils, which on activation adhere to the microvascular endothelium, extravasate and release cytotoxic oxidants and proteases contributing to further tissue damage. Drugs that scavenge and inhibit the formation of ROMs and prevent attraction of polymorphonuclear neutrophils were shown to be useful in the treatment of ischemia/reperfusion injury [25].

Pentoxifylline, a methylxanthine derivative, in addition to its platelet aggregation inhibiting and erythrocyte deformability enhancing [2] effects, was also shown to decrease neutrophil adherence, aggregation and subsequent superoxide production [21]. On the basis of these effects, pentoxifylline has been subjected to a wide range of experimental and clinical trials in attempts to evaluate its efficiency in the treatment of vascular impotence [13], enhancement of motility of spermatozoa [6], amelioration of the hazards of intestinal ischemia/reperfusion [11], septic shock [23] and acute renal failure [24]; and varying degrees of efficiency have been reported. Using a

rat model of VOP we investigated: (1) the possibility of lipid peroxidation (LP) occurring in corporeal tissue as a consequence of ischemia/reperfusion and (2) the effect of pentoxifylline on the protection of corporeal tissue from the hazards of ischemia/reperfusion with respect to alteration in LP. Tissue MDA, a product of LP, was measured as an indicator of ROM-induced injury.

Materials and methods

Animals and housing

Ninety mature male Wistar rats, weighing 280–420 g, were used in the study. Rats were obtained from the Experimental Medical Research Center, Çukurova University. Experimental procedures were subjected to ethical review and conducted in accordance with the institutional guidelines. During the procedures rats were left undisturbed in their wire mesh cages, ten to a cage. Water and food were provided ad libitum.

Anesthesia

No anesthesia was used during the experiment.

Materials

The distal 1-cm portion of a resected cone-tipped 50-ml disposable irrigation syringe was used to create a negative pressure around the penis. Constrictive bands were prepared by sectioning 1-mm-width transverse portions from an 18-F Foley urethral balloon catheter.

Drug

Pentoxifylline (Trental, Hoechst AG, Istanbul). The dose and timing of drug administration were decided on the basis of previous experience with pentoxifylline [6, 11, 13].

Procedure

Two pilot procedures were undertaken before the experiment was started. First, 0.4 mg papaverine in 0.05 ml volume was injected into the exposed corpus cavernosum of an anesthetized rat through a 26-G needle and erection of the body and dorsal flip of the penis was noted. Following appropriate shaving and retraction of the preputium of another unanesthetized rat, held in the hands of an assistant, the hollow tip of the syringe was placed firmly around the base of the penis. By gently withdrawing the piston, a negative pressure was created. Subsequent to demonstration of erection a constrictive band previously placed around the tip of the syringe was dropped over the radix penis with the aid of forceps. The erection thus formed except for additional glans engorgement was noted to be comparable to that obtained after intracavernous papaverine injection. Erection was preserved unchanged during the 12 h of observation with the band in place and with the animal left undisturbed in its cage. The main part of the experiment was continued on the basis of this trial. Those in which tumescence with flip formation could not be maintained unchanged throughout the 6 or 12 h of observation were excluded from the study and replaced. On preserving full tumescence and flexion, rats were noted to spurt out urine. At the end of the procedures rats were sacrificed by cervical dislocation. The corpora were harvested for further evaluation of LP immediately after cervical dislocation while the heart was still beating. Hence, in this study the term "ischemia" is used synonymously to refer to the priapic state into which the rats were placed and "reperfusion" is used to refer to the 1st h of detumescence following band removal.

Rats were randomly assigned to nine groups, each consisting of ten rats (Table 1): group 1: control (baseline) group; these animals underwent no manipulation and no drug administration. The corpora of the rats were resected and examined. Groups 2 and 4: ischemic placebo group; saline (placebo) was injected intraperitoneally 1 h prior to vacuum constriction and priapism was induced to last 6 h in group 2 and 12 h in group 4. The corpora of the rats were resected at the end of the period while the constrictive band was in place. Groups 3 and 5: ischemic + reperfused placebo group; these animals were subjected to similar treatment as in groups 2 and 4, but the corpora were resected 1 h after removal of the band. Groups 6 and 8: ischemic pentoxifylline group; rats were subjected to similar treatment as in groups 2 and 4, but 35 mg/kg pentoxifylline was injected intraperitoneally instead of placebo. Groups 7 and 9: ischemic + reperfused pentoxifylline group; these animals were subjected to similar applications as in groups 3 and 5, but 35 mg/kg pentoxifylline was injected instead of placebo.

MDA assay

The thiobarbituric acid assay was used to assess MDA content [16]. The corporeal tissue that had been previously frozen in 0.5 *M* potassium chloride was thawed, weighed, minced and homogenized immediately in 0.5 *M* potassium chloride buffer by use of a Potter-Elvehjem smooth glass homogenizer with a motor-driven Teflon pestle, after which 2.5 ml 1.22 *M* trichloroacetic acid (TCA) was added to 0.5 ml of the homogenate for 15 min. After the addition of 1.5 ml TCA the homogenates were placed in a boiling water bath for 30 min and allowed to cool. Four milliliters *n*-butonol was then added to the mixture and shaken for 3 min. The sample was centrifuged for 15 min at 1500 rpm and the optical density of the supernatant was read at 532 nm against a blank using a spectrophotometer. Trabecular protein content was determined by the method of Schaffner and MDA content was expressed as nmol MDA/mg protein [18].

Table 1 Definitions of the experimental groups of rats tested (n = 10 for each group)

Group	Procedure	
1	Control (baseline)	No manipulation, no drug administration
2	Ischemia placebo	6 h priapism
3	Ischemia + reperfusion placebo	6 h priapism + 1 h reperfusion
4	Ischemia placebo	12 h priapism
5	Ischemia + reperfusion placebo	12 h priapism + 1 h reperfusion
6	Ischemia pentoxifylline	6 h priapism
7	Ischemia + reperfusion pentoxifylline	6 h priapism + 1 h reperfusion
8	Ischemia pentoxifylline	12 h priapism
9	Ischemia + reperfusion pentoxifylline	12 h priapism + 1 h reperfusion

Table 2 Effects of pentoxifylline on MDA accumulation in penile corporeal tissue of priapic rats. Data are means ± SEM (n = 10 for each group)

Placebo			Pentoxifylline		
Group	Procedure	MDA (nmol/mg protein)	Group	Procedure	MDA
1	Control (baseline)	0.165 ± 0.019			
2	6 h ischemia	1.231 ± 0.412*	6	6 h ischemia	1.216 ± 0.259†
3	6 h ischemia + reperfusion	1.329 ± 0.319*‡	7	6 h ischemia + reperfusion	1.317 ± 0.250††
4	12 h ischemia	1.944 ± 0.441*§	8	12 h ischemia	1.919 ± 0.244†§
5	12 h ischemia + reperfusion	1.961 ± 0.222*‡§	9	12 h ischemia + reperfusion	1.911 ± 0.300†‡§

*$P < 0.001$ vs control
†$P > 0.05$ vs placebo of equivalent procedure and duration
‡$P > 0.05$ vs ischemia of equivalent duration and drug
§$P < 0.05$ vs 6-h duration of equivalent procedure and drug

Statistical analysis

All statistical comparisons were carried out using the Wilcoxon matched pairs–signed rank test on SPSS/PC+, the statistical package for the IBM PC. All values were expressed as means ± standard error of the mean (SEM). Differences between experimental groups were considered significant at $P < 0.05$.

Results

Data of MDA concentrations in corporeal homogenates of the nine groups are shown in Table 2. The MDA concentration of each placebo-treated priapic group was significantly higher than that of the control group ($P < 0.001$). For the equivalent procedure, in both the placebo- and pentoxifylline-administered groups, the MDA concentration appeared to be significantly higher in the 12-h priapic-induced groups than in the 6-h priapic-induced groups (group 4 vs 2, group 5 vs 3, group 8 vs 6, and group 9 vs 7, $P < 0.05$, respectively), confirming a time-dependent accumulation of MDA. Although the MDA concentration appeared to be slightly higher in the reperfused placebo-treated groups than in the ischemic-only groups, the difference was insignificant (group 3 vs 2, group 5 vs 4, $P > 0.05$, respectively). MDA concentrations in the pentoxifylline-administered groups failed to show a significant difference compared to the placebo groups within the same procedure and duration (group 6 vs 2, group 7 vs 3, group 8 vs 4 and group 9 vs 5, $P > 0.05$). Additionally, for the same duration, MDA concentrations did not differ between reperfused and ischemic-only groups (group 7 vs 6 and group 9 vs 8, $P > 0.05$, respectively) in pentoxifylline-administered rats.

Discussion

Male rats, which offer the advantage of having many similarities with the behavioral and endocrine aspects of the sexual activities of man [9] and manifesting similar erectile responses to intracavernous vasoactive drug injections [3], represent a suitable animal model for investigations of penile erection. In our priapism model induction of tumescence we preferred the use of an external vacuum device over the administration of a vasoactive agent due to various drawbacks. These include homeostatic disequilibrium due to dilution of the cavernous blood pool by the added volume of the injected agents [19]; interference with the measurement of MDA is found with prostaglandins [5] and phosphodiesterase inhibiting effect of papaverine similar to that of pentoxifylline [12]. Further, precise data on the duration of intracellular events which are triggered by vasoactive agent administration in regard to possible interference with ROM generation or LP are lacking. Moreover, avoiding the use of intracavernous injection enabled us to perform the procedure in unanesthetized animals, eliminating possible interactions with the anesthetics. Morphometric analyses showed minor cavernosal endothelial defects occurring in VOP lasting less than 12 h and significant changes in trabecular ultrastructure taking place in VOP lasting 12 to 24 h, with the smooth muscle cells showing the first signs of transformation into non-contractile fibroblast-like cells [22]. Since the generation of ROM contributes to the triggering of biochemical cascade, which leads to cellular injury, we limited the duration of the experimental priapism to 12 h; a period presumed to cover the most intense of the biochemical reactions taking place preceding the appearance of the first signs of the anticipated histological alterations.

Tissue MDA, a product of LP, has been measured as an indication of ROM-mediated injury based on the findings that plasma and mitochondrial membrane lipids were particularly vulnerable to ROM reactions [17]. A significant increase in MDA concentration in all our priapism-induced groups compared to control groups suggests that ROMs are generated in rat corporeal cells subsequent to ischemia. MDA accumulation in groups 3 and 5, which underwent reperfusion, did not differ significantly from that in groups 2 and 4, which underwent only ischemia, respectively. Lack of an additional reperfusion effect may be interpreted as being due to several factors.

First, trabecular endothelial/smooth muscle cell metabolism may be hypothesized to be capable of attenuating the effects of reperfusion, which to some extent is usually challenged during transition from flaccidity to rigidity. In this respect it will be worthwhile further investigating whether such a defense mechanism exists and whether a smooth muscle relaxing mediator such as nitric oxide; which has also been shown to exert neutrophil adherence [15] and relevant superoxide anion production inhibiting effects [4], contribute to it. In addition, even 6 h of ischemia may have been too long for rat trabecular tissue, therefore triggering a non-reflow phenomenon characterized by the exacerbation of ischemia-induced neutrophil adherence and associated injury to the endothelium within minutes of reperfusion, resulting in cessation of blood flow [1]. Also, by limiting the reperfusion period to 1 h, our experiment may have been terminated too early to demonstrate the anticipated cumulative rise in LP following reperfusion. However, it has been previously shown that ischemia resulted in a significant increase in neutrophil adherence to the microvascular endothelium and that within 10 min of reperfusion ischemia-induced neutrophil adherence was exacerbated [7]. In addition, taking into account that neutrophils constitute a rather late and important secondary source of ROMs in reperfusion through secretion of myeloperoxidase, it seems unlikely that onset of reperfusion effect has been exceeded by the 1 h.

Our work was encouraged by previous studies that showed attenuation of ROM-mediated injuries in human sperm [6] and ischemia/reperfusion-mediated injuries in rat intestine [11] and striated muscle [10] through treatment with pentoxifylline. Although a decreasing trend of MDA accumulation was noted, pentoxifylline pretreatment did not significantly appear to prevent ischemia/reperfusion-induced ROM release in our rat model of priapism.This may be related to several factors.

First, dose and timing of pentoxifylline administration might not be appropriate. The dosage, 35 mg/kg pentoxifylline given intraperitoneally 1 h prior to induction of priapism, was designed on the basis of previous studies with pentoxifylline in rat models and on the basis of the accepted dose limits for humans [6, 11, 13, 23, 24]. The neutrophil membrane-stabilizing effect of pentoxifylline that prevents subsequent release of hydrolytic enzymes and ROM release may be speculated to require earlier administration of the drug prior to the ischemic period. In a previous clinical study pentoxifylline was shown to improve retinal capillary leukocyte velocity when administration was started 24 h before the examination [20]. Likewise, in a rat model of crush injury, pentoxifylline was shown to improve microvascular circulation when administration was commenced 4 weeks prior to the surgical procedure [14]. Since prediction of onset of priapism is unlikely except for a few cases including intracavernous pharmacotherapy, the prerequisite for prolonged administration of the drug prior to onset will be a limiting factor for its use for this purpose. On the other hand, inhibition of neutrophil aggregation and adhesion to the trabecular endothelium are effects of pentoxifylline which may be speculated to be dose dependent and are therefore beyond the effective concentration range with respect to the given dose as in our case. Another explanation for the inefficiency of pentoxifylline can be that the anticipated antioxidant effect of a single dose of pentoxifylline might have been exhausted in the early phases of ischemia and/or reperfusion, thus being overwhelmed by the prolongation of these periods. Repetition of the experiment in additional groups of rats limiting the priapic period to 1 or 2 h might provide an answer to this suggestion, but, since priapism acquires therapeutic intervention especially on prolongation over 4 or 6 h, the probable efficiency of pentoxifylline limited to the first 1 or 2 h would be of little use as an indication for the clinical use of the drug. However, further investigations conducted to assess the pharmacokinetic properties of pentoxifylline with respect to its effects on neutrophil adhesion, degranulation and release of superoxides will provide evidence for the establishment of a safe and appropriate dosing regimen in the search for adjunctive efficiency of the drug throughout the priapic and subsequent detumescence period.

Conclusions

VOP induced by the vacuum erection device in rats represents a suitable model in the investigation of the effects of ischemia/reperfusion on corporeal tissue. Our results provide evidence for the role of ROMs in prolonged VOP. Pretreatment with a single dose of pentoxifylline failed to demonstrate attenuation of ROM-mediated corporeal tissue damage. However, since variations in the biochemical events and responses to agents between different species may occur, our results should not discourage further trials being designed to evaluate the possible adjuvant role of pentoxifylline in priapism in humans.

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