EFFECTS OF DEFIBROTIDE ON STRESS-INDUCED BLADDER MORPHOLOGY

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ABSTRACT

Objective: This study investigates the morphological effect of defibrotide on cold-immobilization stress-induced rat urinary bladder.

Material and Methods: Wistar albino rats were used in this study. After the cold, immobilization stress following the 48 hours of fasting, defibrotide was applied either intraperitonally (16 mg/ml) or intravesically (1200µg/ml). Urinary bladders were removed for light and electron microscopical investigations.

Results: The stress group showed leukocyte infiltration and edema, further vacuoles in the cytoplasm of urothelial cells and loose tight junctions. Additionally, urothelial protrusions towards the lumen were observed. The defibrotide-applied groups displayed regular morphology both under light and electron microscopy level.

Conclusion: These results showed that defibrotide reverse the stress induced morphological alterations. Although the biochemical effects of defibrotide is unknown, a possible explanation could be via modulation of calcium entry into the cells, and increase the permeability and regulation of inflammatory response and/or may have a role in the protection of urothelium by increasing the synthesis of prostaglandins and have a cytoprotective role in stress-induced bladder damage.

Key Words: Defibrotide, cytoprotection, cold-immobilization stress, urinary bladder, ultrastructure

INTRODUCTION

Stress factors effect the body via activating the nervous, immune and endocrine systems (1). Stress factors play a role in many diseases such as ulcerative gastritis, psoriasis, and migraine headaches (2-5). Recent studies on certain bladder disease known as interstitial cystitis (IC) with an unknown etiology have shown that stress factors are major causes of the former disease (6-10). There is no agreement as to the cause of interstitial cystitis. A number of theories have been introduced including infections, vascular or lymphatic obstruction, autoimmunity, genetic, neurogenic and hormonal factors, defaults in the bladder cytoprotection, the presence of toxic substances in the urine, defect on the urothelium and/or the glycosaminoglycan layer and psychiatric causes (11). Experimental studies have shown that stress factors activate bladder mast cells (8,10).

Defibrotide is a sodium salt of single-stranded DNA isolated from bovine lungs by controlled depolymerisation. Electrophoretic and chromatographic analysis of the drug demonstrated that it is composed of a mixture of DNA fragments of various lengths (12). Defibrotide has antithrombotic, profibrinolytic and anti-ischaemic effects without anticoagulant activity. The antithrombotic and fibrinolytic activities of defibrotide are due to its stimulation of plasminogen activator (tPA) and prostacyclin (PGI2) release from the endothelium while decreasing plasminogen activator inhibitor (PAI) release (13,14). Its anti-ischaemic activity is attributed to the reduction of leukotriene B4 (LTB4) levels and the stimulation of prostanoid synthesis (15). Stimulation of prostaglandin L2 and its release have beneficial actions by causing both vasodilatation and inhibition of platelet aggregation, while reduction of leukotriene B4 levels plays an important role in post-ischaemic damage (16,17). Cytoprotective effects of the drug are additionally reported (18).

Cytoprotective effects of defibrotide has been shown concerning on the endothelial cels (18). We think that similar cytoprotective effects may exist on the urothelium, as well. Clinical effects of DF on the

epithelium were evaluated in various studies, but morphological effects have not been evaluated.

The aim of this study is to show whether Defibrotide has a cytoprotective effect on the stress-induced damage of the urinary bladder of the rat.

MATERIALS AND METHODS

Animals

Adult female Wistar Albino rats weighing 180-200 gr were used in this study. They were housed individually in a light and temperature controlled room on a 12/12 light and dark cycle, and fed on a standard pellet lab chow and water ad libitum. 5 animals were used in each group.

Experimental Groups

Following 48 hours of fasting, animals were put into restraint cages for 3 hours at 4°C. After the stress protocol, defibrotide or phosphate buffered solution (vehicle group) was applied either intraperitonally (n=5, 16mg/ml) (stress+df ip group) or intravesically (n=5, 1200µg/ml) (stress+df iv group). Intravesical defibrotide was instilled via PE50 catheter twice a day. The bladder samples from each group were removed at 12 hours, following the second injections under ether anesthesia for light and electron microscopic (both transmission and scanning electron microscope) investigation. Same procedure was applied for the control animals (control group).

Microscopic Preparation

The specimens were fixed in 10% buttered formalin for 48 hours, and prepared for light microscopical investigation. Approximately 7µm thick sections were stained with Hematoxylin-Eosin (H-E) for general morphology.

For transmission electron microscopic (TEM) investigations the specimens were fixed in 4% phosphate buffered gluteraldehyde (pH 7,2 and 0,1 M), postfixed in 1% OsO_4, dehydrated with alcohol series and embedded in EPON 812. Thin sections were stained with uranyl acetate and lead citrate and observed under the Jeol 1200 SX (Japan) transmission electron microscope.

For scanning electron microscopic (SEM) investigations samples were fixed and dehydrated as TEM preparation. Than samples were dried with critical point dryer (Bio-Rad E 3000, Austria) and coated with gold by sputter coater instrument (Bio-Rad SC 502, Austria). Further the surface morphology of the urothelium were observed under Jeol JSM (Japan) scanning electron microscope.

RESULTS

The control group displayed regular bladder mucosa (Fig 1) under light microscope. TEM observations showed regular urothelial cells with fusiform vesicles, normal intracellular junctions (Fig 2) and regular luminal urothelial cells with microridges under SEM (Fig 3).

The stress group showed epithelial disruption, polymorph leukocytes and edema in the lamina propria under light microscope (Fig 4). Cytoplasmic vacuolisation because of the dilated endoplasmic reticulum cisternea, dilatation of the perinuclear spaces and loose intracellular junctions were observed in the urothelial cell layer under TEM (Fig 5). Degeneration of the luminal surface and protrusions of some urothelial cells were observed under SEM (Fig 6).

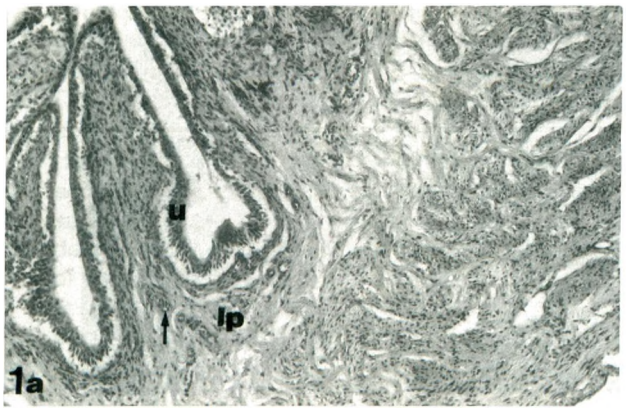
The stress+df ip group light microscopic investigations showed regular urothelium and mucosa (Fig 7), TEM observations showed some vacuolisation in the cytoplasm of the urothelial cells and some loose dilatation of the intracellular space (Fig 8). SEM observations showed normal luminal urothelial cells with microridges (Fig 9).

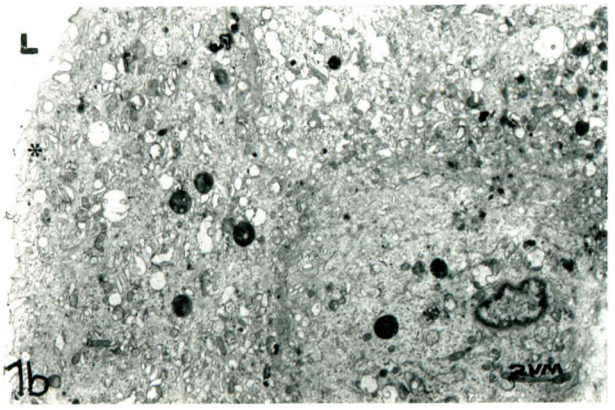
The stress + df iv group light microscopic investigations showed regular morphology of the mucosa (Fig 10). TEM observations showed regular urothelial cells with fusiform vesicles covered with asymmetric unit membranes and normal intracellular junctions (Fig 11). SEM observations showed normal urothelial cells with microridges (Fig 12).

DISCUSSION

Stress conditions effect many systems and organs in the body (19) and are responsible for many diseases such as; ulcerative cystitis, psoriasis, and migraine headache (2-5). IC is one of the disease with unknown etiology. Recent studies have shown that IC is effected by the stress factors as well (6-10). Koziol et al. indicated that pain increased in 60% of patients with IC under stress conditions (6). Meanwhile the results of our previous study and a study by Spanos et al. have shown that stress conditions effect the bladder mast cells (8,10) as well.

The morphological alterations of DF infused rabbit endothelium have been examined at light and electron microscopy level. Although the effecting mechanism was not clear, the author postulated that cytoprotective effect of DF on the endothelium could be due to an increase in prostaglandin synthesis (18).





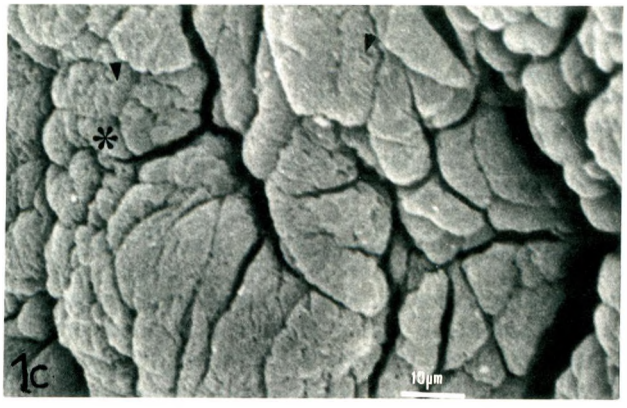
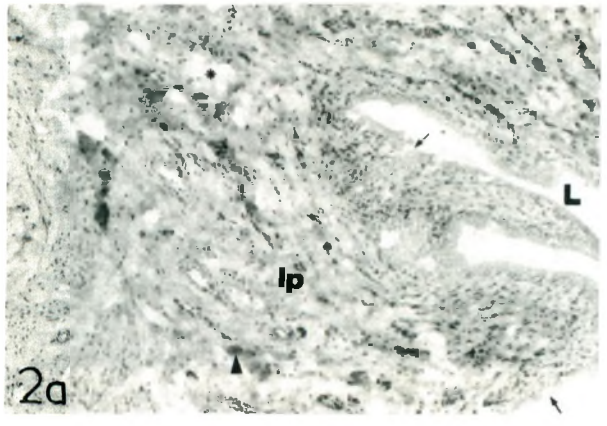


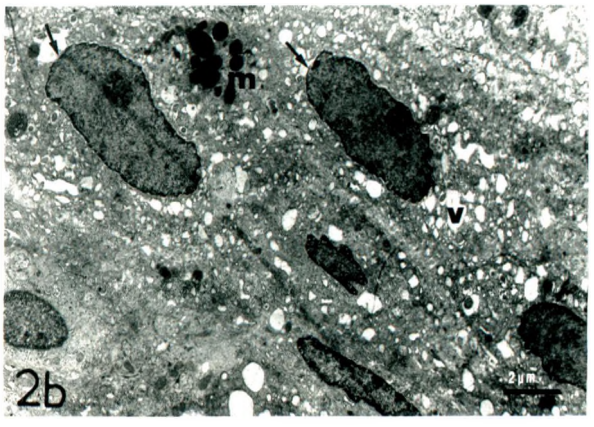
Fig.1.: *Control group:*

1a: Regular urothelium (u), blood vessels (→) in the lamina propria (lp), Hematoxylin-Eosin staining, x33.

1b: Urothelial cells with a fusiform vesicles (★), lumen (L), TEM micrograph.

1c: Urothelial cells (★) with microridges (➤) at the luminal surface, SEM micrograph.





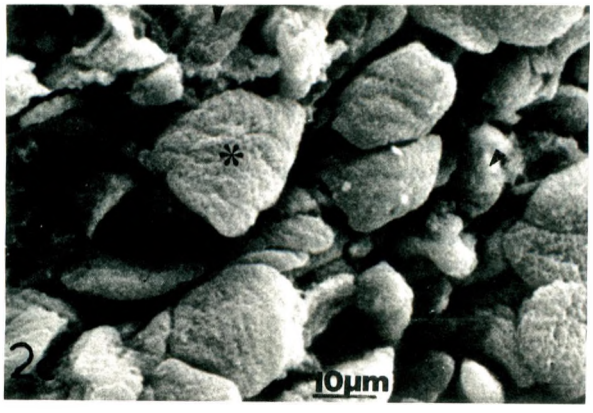
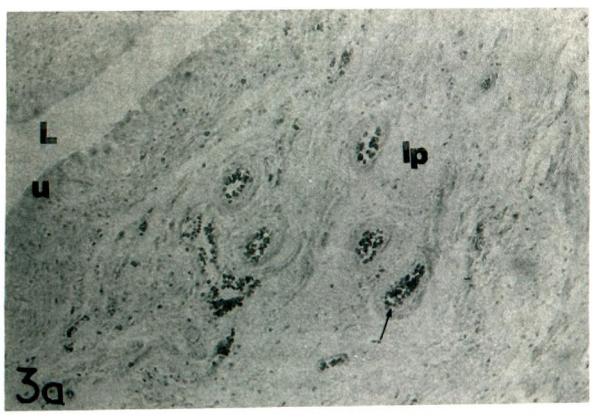


Fig.2.: *Stress group:*

2a: Disruption of the urothelium (→), polymorph leukocyte infiltration (➤) and edema (★) in the lamina propria (lp), lumen (L), Hematoxylin-Eosin staining, x33.

2b: Dilated perinuclear space (→) and endoplasmic reticulum membranes in the cytoplasm (v), mast cell (m) between the urothelial cells, TEM micrograph.

2c: Protrusion of urothelial cells towards the lumen (★) and degenerated urothelial cells (➤), SEM micrograph.



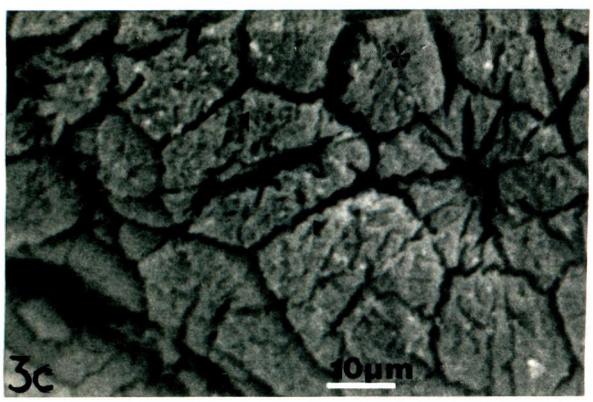
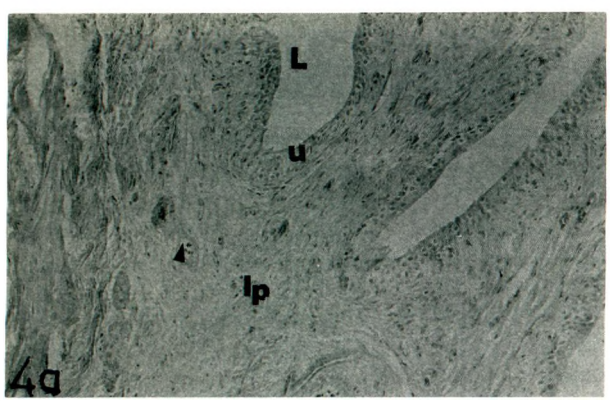


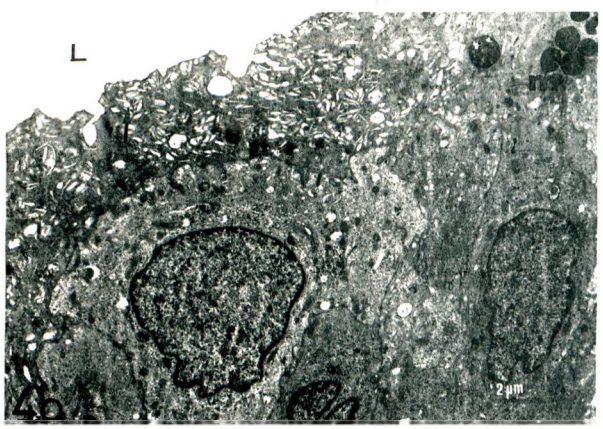
Fig.3.: ***Stress+df ip group:***

3a: Regular urothelium (u), blood vessels (→) in the lamina propria (lp), lumen (L), Hematoxylin-Eosin staining, x33.

3b: Some of the endoplasmic reticulum membrane dilatations in the cytoplasm (v), lumen (L), nucleus (n), TEM micrograph.

3c: Regular luminal urothelial cells (★) with a microridges (➤), SEM micrograph.





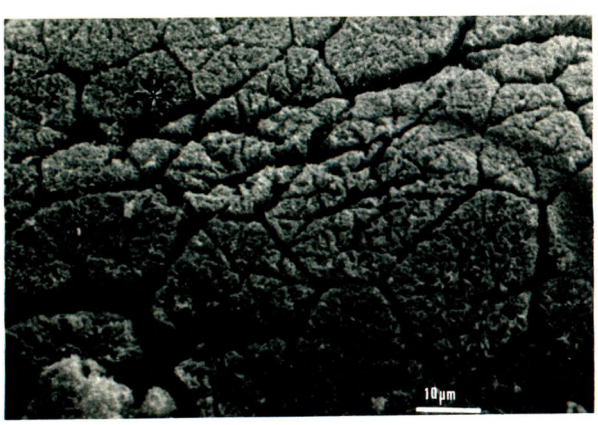


Fig.4.: ***Stress+df iv group:***

4a: Regular urothelial morphology (u), blood vessels (➤) in the lamina propria (lp), Hemetoxylin-Eosin staining, x33.

4b: Regular urothelial cells with fusiform vesicles (→), mast cell (m) between the urothelial cells, lumen (L), TEM micrograph.

4c: Regular urothelial cells (★) with a microridges (➤), at apical surface, SEM micrograph.

In stress induced group we observed edema in the lamina propria, dilated perinuclear space and dilated endoplasmic reticulum membranes. Some of these morphological alterations were also seen in ip. injected DF group. These results showed us stress conditions change the urothelium morphology may be via the destroying of cell membranes and increase the activation of some stress substances such as substance P. Also stress factors activate the mast cell degranulation in urinary bladder of rat (8,10). Applying of defibrotide reverse the effects of stress. But these reversals were not occurring immediately, so we observed some dilatations in defibrotide groups.

The general cytoprotective effect of the DF in vitro, the enzyme releasing via neutrophile activation and the formation of the free radicals are related with the entering of calcium into the cells, this entry being modulated with DF which inhibits the entering in a dose dependent. This effect of DF on calcium translocation also supports similar results about calcium channel blockers such as nifedipine and flunarisine (20). Calcium channel blockers are being used in the symptomatic treatment of IC. Effecting the calcium transfer on the cell membranes may change the functional activities in different cell types. In experimental conditions calcium antagonists decrease the detrusor contractility more than anticholinergics. It is thought that systemic immune answer is related to calcium, which is transferred into lymphocytes (21,22). It is further thought that calcium channel blockers can effect the IC symptoms from several potential mechanisms such as; detrusor relaxation, immune suppresion and relaxation of the smooth muscle of the blood vessels (21).

In this study, we think that the effect of DF on stress induced bladder damage can reverse the modulation of the calcium transfer into the cells and decrease the leukocyte activation and can result with the cytoprotective effect. In the IC patients increase permeatibilty of the epithelial cells, may result in excess calcium entering in the cell. DF application at this stage may be responsible for the modulation of calcium entering into the cell. Therefore, in the IC patints DF can modulate the inflammatory response, the relaxation of the smooth muscle of the blood vessels and detrusor.

The GAG layer and also prostaglandin synthesis in the urothelium play a role in the bladder cytoprotection and thus effects the urothelial permeability. Human bladder mucosa synthesizes different prostaglandings (23). Physiological studies indicated that bladder damage of guinea pigs is due to a decrease of the cytoprotective effect of prostaglandin E2 (24).

We think that instillation of the bladder wall with the DF can increase the prostaglandin synthesis of the urothelium, thus fasten recovery. In this study we found apparent morphological differences between DF treated groups and vehicle groups. The recovery in the DF treated group can possibly be related to an increase of the prostaglandins synthesis due to defibrotide.

The molecular effecting mechanism of the DF is still unclear, however our morphological results showed that defibrotide displayed a reversal mechanism on stress-induced bladder damage.

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