

The role of apoptosis and the effect of epidermal growth factor on proapoptotic BNIP 3 in an experimental rat priapism model

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Background/aim: This study aimed to investigate the effects of apoptosis-inducing Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP 3) and antiapoptotic epidermal growth factor (EGF) on the pathophysiology of experimental low-flow priapism.

Materials and methods: Twenty-four adult Sprague-Dawley rats were divided into four equal groups. Group I was the control group. Ischemic priapism was induced for 4 h in Group II rats. In Group III, intraperitoneal EGF at 10 µg/kg was given for 7 days before induction of ischemic priapism for 4 h. In Group IV, intraperitoneal EGF at 20 µg/kg was given for 7 days before induction of ischemic priapism for 4 h. The western blot method was used to determine BNIP 3 expression levels and the TUNEL method was used to determine the apoptotic cells in the cavernosal tissue samples.

Results: Although BNIP 3 expression levels were significantly higher in all three study groups compared to the controls, BNIP 3 was significantly higher in EGF-administered groups when compared to Group II ($P < 0.05$). The TUNEL score of group II was significantly higher than those of the other groups.

Conclusion: Decreased apoptosis in cavernosal tissues obtained by antagonizing the apoptotic effect of BNIP 3 with EGF may facilitate the development of new conservative treatment methods via those pathways.

Key words: Apoptosis, BNIP 3, epidermal growth factor, priapism, rat

1. Introduction

Priapism is defined as a complete or partial erection that continues more than 4 h in the absence of any sexual stimulus. Priapism is classified into three categories: ischemic (veno-occlusive, low-flow), nonischemic (arterial, high-flow), and stuttering priapism (1). Ischemic priapism is the most common form and constitutes more than 95% of all cases. In this pathology, cavernous smooth muscle alterations occur after 4 h due to hypoxia, acidosis, and glycopenia and ultrastructural alterations become significant at the 12th hour. Those changes may result in corporal fibrosis, cavernous smooth muscle dysfunction, and erectile dysfunction. Since treatments after 36–48 h of onset may not be effective to restore erectile functions, emergent treatment and establishment of blood flow to cavernous structures should be provided (2). Deterioration of the metabolic parameters may impair oxidative phosphorylation in the cavernous tissues; intracellular adenosine triphosphate falls much below the levels required for cell survival and apoptotic processes

begin. The rate of erectile dysfunction has been reported as 90% when ischemic priapism exceeds 24 h, occurring due to necrosis and fibrosis of the erectile tissues (3).

Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP 3) is an important proapoptotic mediator that is known to play a critical role in the start of the mitochondrial pathway of apoptosis induced by hypoxia (4,5). BNIP 3 induces mitochondrial permeabilization and impairs mitochondrial membrane potential, which may result in damaged mitochondrial function and subsequent cellular apoptosis (6). Several studies reported that BNIP 3 levels were found to be elevated in hypoxic and acidotic conditions and increased BNIP 3 expression compromised cell survival in many organ systems (7–10).

A number of mediators play roles to prevent apoptosis, among which growth factors have been found to be particularly important (11–13). Epidermal growth factor (EGF)-mediated protection against apoptosis includes increased expression of antiapoptotic Bc-XL protein and prevention of mitochondrial dysfunction, plasma

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membrane permeability, and cytoplasmic vacuolization induced by BNIP 3 (14,15).

Although BNIP 3 was known to increase in hypoxic conditions and EGF has a well-established antiapoptotic activity, no study was conducted to investigate the level of BNIP 3 and the effect of EGF on BNIP 3 levels in cavernous tissues during priapism. Therefore, the aim of the present study was to investigate the levels of BNIP 3 in normal and priapic cavernosal structures and the possible beneficial effect of EGF on apoptosis.

2. Materials and methods

2.1. Animals and the study design

The local ethics committee approved the study (24.03.2011/73). Twenty-four adult male Sprague-Dawley rats (8–10 weeks old) with a mean weight of 245–300 g were used. All animals were housed under constant environmental conditions (in a constant temperature-controlled room on a 12/12-h light/dark cycle) and fed with standard laboratory rat chow and distilled water. They were divided into four equal groups. Xylazine HCl (Rompun 2%, Bayer, Turkey) was applied as a sedative and muscle relaxant at the dose of 10 mg/kg intraperitoneally. Ketamine HCl (Alfamine 10%, Ege Vet, Turkey) was used for dissociative anesthesia at the dose of 50–60 mg/kg intraperitoneally.

The groups were as follows: Group I served as a control group; Group II was the priapism group; Group III was a priapism group administered EGF (E 9664, Sigma-Aldrich, Turkey) at 10 µg/kg intraperitoneally; Group IV was a priapism group administered EGF at 20 µg/kg intraperitoneally.

Before administration, EGF was dissolved in saline. Erection was constituted by vacuum constriction method in Group I rats, with spontaneous termination in 10 min. In Group II, experimental priapism was induced in the manner described by Sanli et al. (16). A rubber band was placed at the base of the erect penis and erection was maintained for 4 h. The rubber band at the base of the penis was removed 4 h later and the penis was removed. According to previous studies, 4 h of priapism was induced (17,18). No drugs or chemicals were given to Group II rats. In Group III, 10 µg/kg EGF was administered intraperitoneally for 7 days and priapism was induced as in Group II on the 7th day; the penises of the animals were removed after 4 h. In Group IV, 20 µg/kg EGF was given intraperitoneally for 7 days and at the end of 7th day, priapism was induced; the penises of the animals were removed after 4 h. We excised the penises immediately after removing the rubber clamp. Penile cavernous tissues were dissected and divided into two parts. The tissues were homogenized in a cold environment with the help of mechanical homogenizer (Ultraturrax, IKA, Germany)

with homogenization buffer solution at a ratio of 1:10 (w/v). The homogenization solution contained 10 mM Tris-HCl (pH 7.4), 0.1 mM NaCl, 0.1 mM phenyl methyl sulfonyl fluoride, and 5 µM soybean as a trypsin inhibitor. The homogenates were centrifuged in a refrigerated centrifuge (Hettich, Germany) at 60,000 × g for 60 min at 4 °C. The obtained supernatants were taken into microcentrifuge tubes and stored at –70 °C to estimate BNIP 3 activity by western blot technique. The second part was fixed in formaldehyde for histopathological TUNEL examination. The sections taken from paraffin blocks at a thickness of 5 µm were taken into polysilicon membranes. Apoptotic cells were identified using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Cat No: S7101, USA) in accordance with the manufacturer's instructions. Preparations were evaluated and photographed with a research microscope (Olympus BH-2). The apoptosis index of smooth muscle and the intensity of staining was scored by the same experienced investigator semiquantitatively on a 4-point scale as follows: 0, absent; 1, very weak; 2, weak; 3, moderate; and 4, intense.

2.2. Statistical analysis

The data were analyzed using the general linear model procedure of SAS software. Data were presented as mean and standard deviation. Significant differences at 5% among treatment means were determined using Fisher's post hoc test for all groups. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Western blot changes

Western blot BNIP 3 expression levels are presented in the Figure. We found significantly higher BNIP 3 expression levels in the corpora cavernosa of Group II rats ($P < 0.05$).

A dose-dependent linear decrease was seen in BNIP levels in the treatment groups (10.7% and 14.7%, respectively) (Table 1).

3.2. Histopathological results

TUNEL scores of group II were significantly higher than those of the other groups. Numerical evaluation of apoptosis according to the TUNEL scores of groups is given in Table 2.

4. Discussion

The incidence of ischemic priapism was reported as 1.5 cases per 100,000 person years. Ischemic priapism is accepted as a compartment syndrome and cessation of blood flow to the corpora cavernosa may result in time-dependent changes such as hypoxia, hypercarbia, and acidosis. Prolonged periods of severe anoxia significantly impair corporal smooth muscle contractility and cause significant apoptosis of smooth muscle cells, which

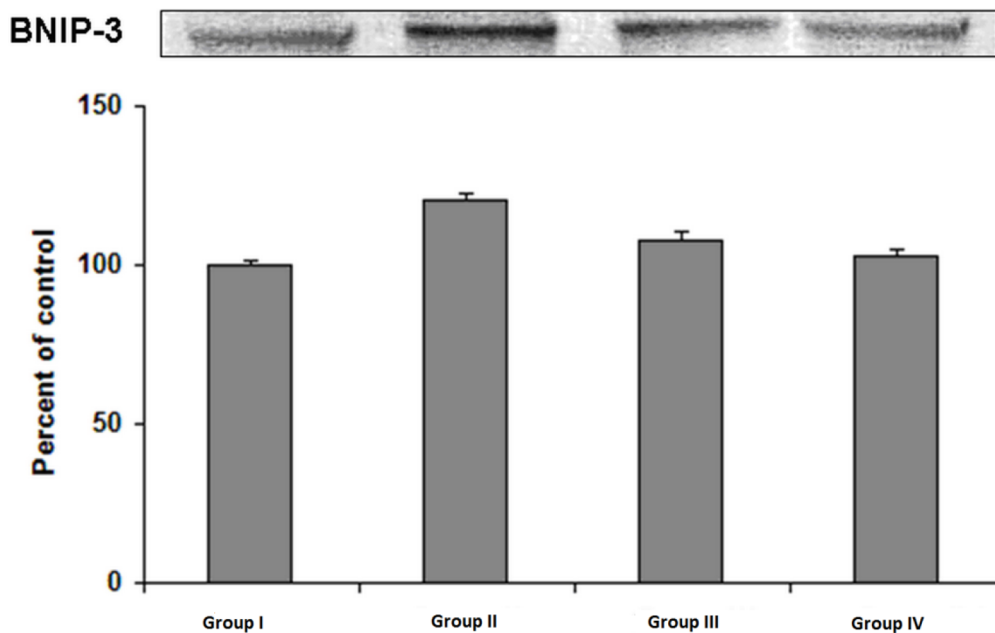


Figure. The effect of EGF on BNIP 3 expression in rats induced with experimental priapism. BNIP 3 expression levels were determined using the western blot method as a percentage of the control.

Table 1. The effect of EGF on BNIP 3 expression.

	Group I	Group II	Group III	Group IV
BNIP 3*	100.0 ± 1.22	120.7 ± 1.68	107.7 ± 3.13	102.9 ± 1.56

The data were analyzed at a significance level of $P < 0.05$ with Fisher's test. The data are presented as mean ± standard deviation.

*: BNIP 3 expression levels were determined using the western blot method as a percentage of the control.

Table 2. TUNEL staining index values according to groups.

Variables	Groups			
	Group I	Group II	Group III	Group IV
Apoptosis index	+1	+3	+2	+1

ultimately results in fibrosis of the corpora cavernosa (19). It is known that hypoxia activates various molecular pathways leading to apoptosis. Caspase activation and mitochondrial membrane perturbation affected by members of the Bcl-2 family are important mediators for developing apoptosis. BNIP 3 is a member of the proapoptotic Bcl-2 family that is overexpressed in hypoxic microenvironments (20). In a previous study, Kubasiak et al. showed that BNIP 3 induced apoptosis in cardiac myocytes during hypoxia (21). Furthermore, Ishihara

et al. demonstrated elevated BNIP 3 expression in renal tubular cells in acute renal failure (22). In addition, a time-dependent increase in BNIP 3 levels in rat hippocampus after extensive brain ischemia was reported by Kastner et al. (23). In accordance with the literature results, our study demonstrated that BNIP 3 levels in the cavernosal smooth muscle cells were found to be higher in the priapism group. Therefore, we believe that manipulation to decrease the level of BNIP 3 expression may be a novel target for further experimental priapism studies.

EGF has been suggested to play a key role to prevent apoptosis (24). EGF exerts its antiapoptotic activity by increasing the expression of antiapoptotic protein Bcl-XL and preventing mitochondrial dysfunction induced by apoptotic members of the Bcl-2 family. Kothari et al. reported that treatment of breast cancer cell line MCF-7 with EGF or IGF protected these cells effectively from BNIP 3-induced cell death (15). They suggested that blocking growth factor signaling in hypoxic regions of tumors might be sufficient for BNIP 3 to induce cell death in these cells. Fabregat et al. reported that EGF suppressed apoptosis in primary hepatocytes (25). Ilio et al. found that treatment of cells with EGF resulted in an inhibition of cell proliferation that could be recovered with increasing concentrations of EGF (26). Thus, the presence of EGF and its receptors can contribute to resistance of cells against apoptosis (27).

We investigated the effect of EGF on BNIP 3, a protein that plays a role in apoptotic processes independently of caspase in priapism, where oxidative damage increases in a time-dependent manner. We found that BNIP 3 expression levels decreased in a dose-dependent and linear manner in cavernosal tissues isolated from animals administered EGF before induction of priapism and, similarly, there was significantly less apoptosis in the cavernosal smooth muscle cells of these animals. The results we found in our study are consistent with the literature. Preventing apoptosis with EGF seems promising for maintaining erectile function.

The first limitation of our study was the absence of a sham group. However, a previous study revealed

comparable findings in sham surgery and control groups (28). In order to reduce animal usage in this experimental study, a sham surgery group was not included. The second limitation was the timing of treatment, as we aimed to investigate the protective efficacy of EGF in the pathophysiology of ischemic priapism. Therefore, we administered EGF before inducing priapism. Although we have demonstrated the protective effect of EGF before inducing priapism, we need new experimental models to show that EGF is effective after inducing ischemic priapism.

The data obtained from the present study are novel, since no other study investigating the BNIP 3 level and effect of EGF on BNIP 3 in cavernosal structures exists in the literature. To our knowledge, this is the first study investigating the BNIP 3 expression in cavernosal tissue and the protective effect of EGF in priapism. Prolonged ischemia results in permanent damage in erectile functions by disturbing cavernosal structures. The apoptotic process is one of the contributing factors to this damage and prevention of apoptosis may give erectile tissues the ability to resist prolonged ischaemia. The present study may provide new insights into the understanding of pathophysiologic processes during priapism and offer new treatment targets, which should be further investigated with subsequent experimental studies including the effect of EGF on oxidative stress and erectile functions.

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