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ORIGINAL PAPER

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The effect of sustained and local administration of epidermal growth factor on improving bilateral testicular tissue after torsion

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Abstract Epidermal growth factor (EGF) modulates Leydig cell proliferation, steroidogenesis, spermiogenesis, and Sertoli cell activity. It plays an important role in repairing ischemia-reperfusion injury in different tissues. The aim of this study was to evaluate the effects of sustained and local administration of EGF on improving bilateral testicular tissue after torsion. A total of 57 Wistar albino rats were used. For the EGF transport system, 1×2 cm gelatin films containing 2 μg EGF were used. Torsion was created by rotating the right testis 720° in a clockwise direction for 4 h in all groups except the control group. Then, in the torsion group, bilateral orchiectomy was performed. After returning the torsioned ipsilateral testes to their normal state, the bilateral testes were wrapped by 1×2 cm unloaded gelatin films in the gelatin (G7 and G21) groups and, by 2 μg EGF loaded gelatin films in the EGF 7 and EGF 21 groups. The testes were removed on the seventh and 21st days, respectively, for biochemical and histological examination. Histologically, Johnsen's spermatogenesis criteria and mean seminiferous tubule diameter (MSTD) measurements were used. The EGF7 group did not show significant loss of Sertoli cells, while in the G7 group the number of these cells decreased. The ipsilateral ischemic testis of the EGF21 group showed Leydig cell hyperplasia, and the contralateral non-ischemic testes in this group were similar to the control group. In the G21 group, the bilateral testes showed Sertoli cell only syndrome in some sections, and most of the cells were undergoing apoptosis. The mean spermatogenesis scores and MSTD in the EGF7 and EGF21 groups were higher than in the G7 and G21 groups ($P < 0.05$). Malondialdehyde levels were significantly lower in the EGF groups than in the G groups ($P < 0.05$). Glutathione peroxidase (GSH-Px) levels in the G21 group were significantly higher than in the EGF21 group. Our study shows that local and sustained EGF release after testicular torsion improves bilateral testicular injury. EGF administration may be a new treatment choice for bilaterally injured testis after detorsion without removing the twisted testis.

Keywords Epidermal growth factor · Testis · Torsion · Ischemia · Reperfusion · Treatment

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Introduction

Testicular torsion is a common surgical emergency among boys and young men. In spite of prompt diagnosis and either orchiectomy or preservation of the affected testis, infertility remains a significant sequel. Abnormal semen analyses have been noted in 40–60% of patients after ipsilateral testicular torsion [1]. In terms of diminished fertility after ipsilateral testicular torsion, adverse effects of this phenomenon on the contralateral blood flow and histopathological alterations have been well evaluated in a number of both clinical and experimental studies [1, 2, 3, 4, 5, 6]. Among the several proposed causes for contralateral damage, a reflex decrease in contralateral testicular blood flow is probably the most likely [2]. Testicular injury resulting from torsion and detorsion resembles the phenomenon of ischemia-reperfusion (I/R) injury observed in other tissues. During the detorsion process, a huge amount of molecular oxygen is

supplied to the tissues and abundant amounts of free oxygen radicals, which are responsible for reperfusion injury, are produced [7]. These potentially reactive oxygen species (ROS) in turn promote cell destruction through peroxidation of the membrane lipids [8]. Testicular torsion/detorsion causes germ cell-specific apoptosis that is induced by reactive oxygen species arising from reperfusing leukocytes, and leads to an increase of thiobarbituric acid reactive substances in the testes [9]. The preservation of twisted testes through a detorsion procedure could cause further deterioration by way of reperfusion injury, indicating the importance of the removal of the damaged testis to minimize long-term histopathological alterations in the contralateral testis [3, 5].

Epidermal growth factor (EGF) is a polypeptide of 53 amino acids. Endogenously, it is secreted in exocrine fashion from the submandibular glands and the Brunner glands of the duodenum. EGF is involved in the complex regulation of cell proliferation in hormone sensitive tissues [10]. The binding of EGF to its receptor initiates a cascade of events culminating in biological effects such as the stimulation of cell proliferation [11]. EGF is a potent mitogen for cells of mesodermal and ectodermal origin. During its fetal and adult development, the testis is under the control of hormones, cytokines and growth factors [12]. EGF has been reported to modulate Leydig cell proliferation [13], steroidogenesis [14], spermatogenesis [12, 15, 16] and Sertoli cell activity [17, 18]. The elimination of circulating plasma EGF by sialoadenectomy of adult mice caused a marked decrease in male fertility without a concomitant decrease in gonadotrophin and androgen concentrations [19]. Daily administration of EGF significantly increased the number of spermatozoa in streptozotocin-induced diabetic mice [20]. EGF has trophic effects on both enterocytes and myocytes in the small bowel, causing cellular proliferation [21]. Heparin-binding EGF decreased reactive oxygen species production in the intestinal epithelial cells [22]. Similarly, EGF significantly protects against intestinal I/R injury [23]. EGF exerts a beneficial influence on the course of I/R induced pancreatitis and this effect seems to be related to a reduction in the activation of the pro-inflammatory interleukin cascade, the improvement of pancreatic blood flow, and an increase in pancreatic cell growth [24]. The expression of EGF and EGF receptor in renal tubules may play an important role in repair following ischemic renal damage [25]. It is therefore postulated that EGF may decrease bilateral testicular injury in ipsilateral testicular torsion. An experimental study was therefore designed to evaluate the effects of EGF on improving testicular injury.

Materials and methods

Preparation of gelatin films

Gelatin films were prepared by molding aqueous gelatin solution (5% w/v) into siliconized Petri dishes. After 24 h of drying, glutaraldahyde solution (1%, 20 ml) was added in each mold in order to obtain stable cross-linked structures. The cross-linking process was carried out for 30 min at room temperature; the films were then washed with distilled water and dried in oven at 37°C. For the preparation of EGF containing films, EGF (Sigma Aldrich, St Louis, Mo.) was added initially into the aqueous gelatin solution and the same procedure was followed. The loaded amount of EGF was 2 μg for each gelatin film. All gelatin films were sterilized for 60 min immediately before use by UV.

In vivo applications

A total of 57 male Wistar albino rats weighing 240–280 g were divided into six groups. All surgical procedures were performed while the rats were under a single intramuscular injection of 60 mg/kg ketamine, and 9 mg/kg xylazine (both from Eczacıbası, Turkey). In the control group, bilateral orchiectomy was performed. In the other groups, after shaving the scrotal region, the right testes, excluding the epididymis, were rotated 720° in the clockwise direction and left for for 4 h under sterile conditions. The twisted testes were fixed medially and laterally to the scrotum by a transmesoorchial suture to eliminate extra trauma of the testicular parenchyma. Groups were defined as follows: control group (C; $n=8$), torsion group (T; $n=9$): the right testes were torsioned, and after 4 h the bilateral testes were removed. Gelatin 7 and 21 (G7, $n=10$ and G21, $n=10$) groups: the testes were torsioned, and after 4 h detorsioned and 1×2 cm unloaded gelatin films were wrapped around the bilateral testes and epididymis. The testes were fixed to the tunica albuginea with a 5/0 atraumatic silk suture. On the seventh and 21st days, the testes were removed from G7 and G21, respectively. Each testis was divided into three pieces for biochemical and histopathological examination. EGF7 ($n=10$) and EGF21 ($n=10$) groups: the testes were torsioned, and after 4 h were detorsioned and 2 μg EGF containing gelatin films were wrapped around the bilateral testes and epididymis. The testes were fixed to the scrotum with 5/0 atraumatic silk suture. On the seventh and 21st days, the testes were removed. Each testis was divided into three pieces for biochemical and histopathological examinations.

Histology

Light and electron microscopy

One piece of testis was immediately fixed in Bouin's solution for histological examination. The specimens were embedded in paraffin, and 5 μm thick sections were cut and stained using the haematoxylin and eosin method (H-E).

Another piece of testis was fixed by immersion in 2.5% gluteraldehyde followed by post fixation in 1% osmium tetroxide in phosphate buffer. After dehydrating in ethanol gradients at room temperature, the tissue samples were embedded in epoxy resin. Semithin (1 μm) sections were cut and stained using the toluidine blue-azure II method. These sections were examined by light microscopy (Olympus BH-2) in a blinded fashion. The thin sections (70 nm) were cut and stained with uranyl acetate and lead citrate. These sections were examined in a Zeiss 9S2 electron microscope (EM).

The mean seminiferous tubular diameter (MSTD) was calculated for each testis by measuring 20 separate tubular diameters using an ocular micrometer under low power, selecting the smallest, roundest seminiferous tubules within the field. If the MSTD of the testis was ≥270 μm, it was considered normal for an adult rat [27].

Spermatogenesis was assessed histopathologically using Johnsen's mean score count on 2.5% gluteraldehyde fixed tissue sections. All tubular sections in one section of testis tissue were evaluated systematically and each was given a score of from 1 to 10 according to the following criteria: score 10 indicates complete spermatogenesis with many spermatozoa (spermatozoa are here defined as cells having achieved the small head form of the spermatozoon). Germinal epithelium organized with a regular thickness leaving an open lumen. Score 9: many spermatozoa present but germinal epithelium disorganized with marked sloughing or obliteration of lumen. Score 8: only few spermatozoa (< 5–10) present in the section, score 7: no spermatozoa but many spermatids present, score 6: no spermatozoa and only few spermatids (< 5–10) present, score 5: no spermatozoa, no spermatids but several or many spermatocytes present, score 4: only few spermatocytes (< 5) and no spermatids or spermatozoa present, score 3: spermatogonia are the only germ cells present, score 2: No germ cells but Sertoli cells are present, and score 1: no cells present in the tubular section [28].

Normal Sertoli cell and myoid cell numbers per tubule are ten and eight, respectively, in adults [29, 30]. Eight or fewer Sertoli cells, and six or fewer myoid cells were regarded as decreased.

Biochemical studies

Glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) levels were measured.

A piece of testis was washed twice with cold saline solution, placed into polypropylene tubes, labeled, and stored in a −40°C freezer until processing.

MDA assay

MDA levels were measured to evaluate lipid peroxidation in the tissue homogenate. After the testis was cut into small pieces, tissues were homogenized (Tempest Virtishear, Model 278069; Virtis, Gardiner, N.Y., USA) for 3 min. All procedures were performed at +4°C. Testis tissue was homogenized in 1 ml 50 $mmol^{-1}$ Tris HCl buffer containing 180 $mmol^{-1}$ cold potassium chloride and 10 $mmol^{-1}$ EDTA, at a final pH of 7.4. The mixture was shaken and kept cold until centrifuged. MDA in testis tissues was determined by the method of Uchiyama and Mihara [31]. A 3 ml aliquot of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid solution were added to 0.5 ml of 10% tissue homogenate pipetted into a tube. The mixture was heated in boiling water for 45 min. After cooling, the colored part of the solution was extracted into 4 ml of n-butanol by vortexing. The absorbance was measured in spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom, UK) at 535 and 525 nm ($\dot{\varepsilon} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$). MDA values were expressed as nmol/mg of protein tissue.

GSH-Px assay

GSH-Px activity estimation was based on the following principle: GSH-Px catalyses the decomposition of hydrogen peroxide to water and singlet oxygen that converts the reduced form of glutathione to oxidized form. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured. GSH-Px activity was expressed as U/mg protein [32].

Statistical analysis

Data were expressed as means ± SD. Differences between groups of continuous data were compared by the Kruskal-Wallis test and Mann-WhitneyU-test using SPSS software (SPSS 9.05; SPSS Chicago, Ill.). A value of $P < 0.05$ was considered significant.

Results

Histological evaluation

Control group

Bilateral testes showed no histological abnormalities (Fig. 1).

Torsion group

Diffuse interstitial hemorrhage was determined in the ipsilateral ischemic (right) testes (Fig. 2). Myoid cells and Sertoli cells were decreased in number. Basal membrane was normal but focal thinning was noticed. Mild edema was observed around the seminiferous

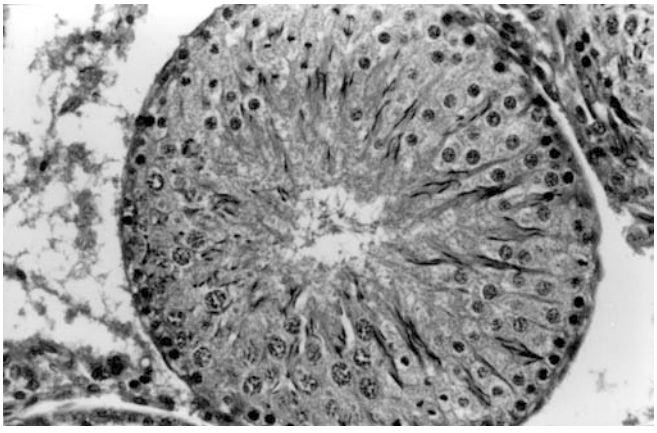

Fig. 1 No pathological changes were found in the control group (H-E×100)

tubules. The nuclei of germ cells were normal and, a few vacuoles were present in the cytoplasma on EM. Although contralateral non-ischemic testes showed only interstitial edema on light microscopy, EM examination revealed findings similar to the ipsilateral testes.

G7 group

Bilateral testes showed vasodilatation. Myoid cells had disappeared, the basal membrane was irregular and undulating. There was obvious edema within the seminiferous tubules. There were degenerated cell residues in the lumen of the seminiferous tubules. Focal infarct and peritubular fibroblast activation were observed. Sertoli cells were decreased in number. Tight junctions between the Sertoli cells were observed to be thinner on EM.

G 21 group

Affected testes had obvious interstitial edema, for some sections infarct and only Sertoli cell syndrome were found. These Sertoli cells were degenerate and showed a

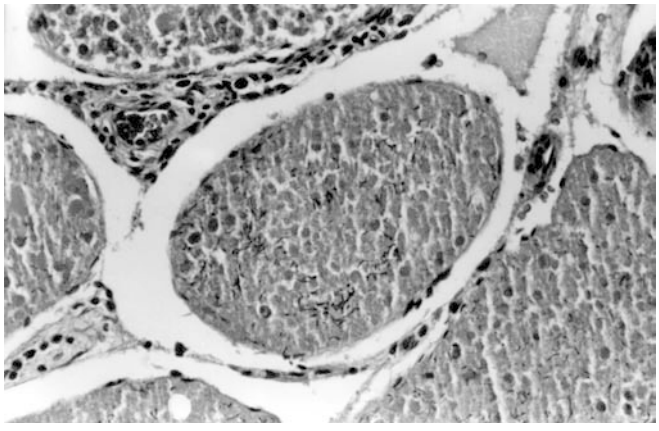

Fig. 3 Total infarct in the G21 group (H-E×100)

decrease in numbers (Figs. 3, 4). Myoid cells had disappeared. The basal membrane was irregular and undulating. Chromatin condensation just under the nuclear membrane and nuclear fragmentation were seen in spermatogenetic cells. These changes indicate that these cells were undergoing apoptosis. Apparent degeneration and loss of intercellular junctions were noticed on EM. In the contralateral testes, histopathological findings were similar to those for the ipsilateral testes. There were lipid droplets among the necrotic cells within the seminiferous tubules.

EGF7 treatment group

The bilateral testes showed interstitial edema; the basal membrane, myoid cells and Sertoli cells were normal. Cell borders were evident and healthy, cells in the lumen were immature. The junctions between Sertoli cells were observed to be separated on EM.

EGF21 treatment group

The numbers of myoid cells and Sertoli cells were decreased, but the basal membrane was normal in the

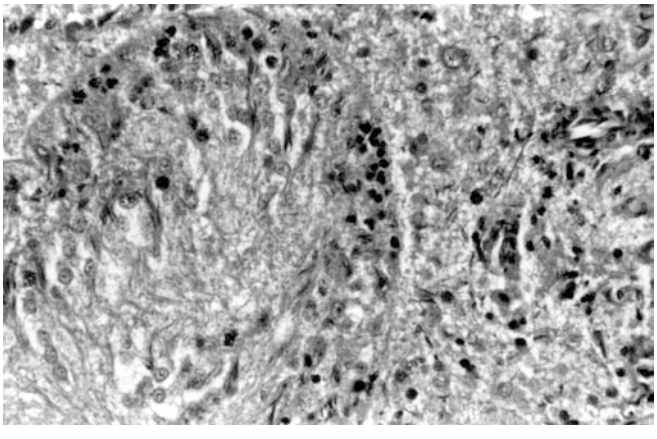

Fig. 2 Interstitial hemorrhage in a contralateral testis of the torsion group (H-E×100)

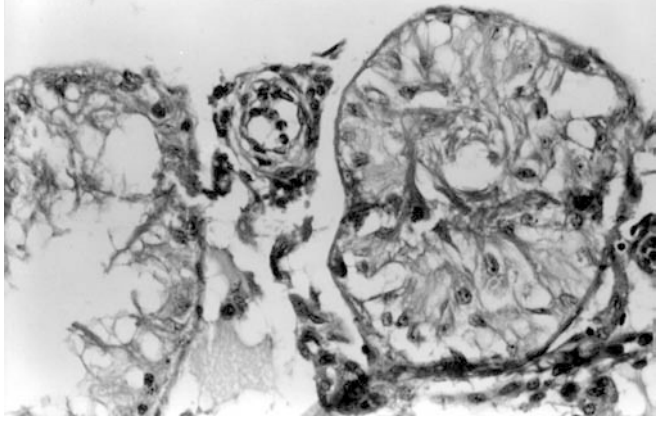

Fig. 4 Sertoli cells only and decreased diameter of the seminiferous tubules in the G21 group (H-E×100)

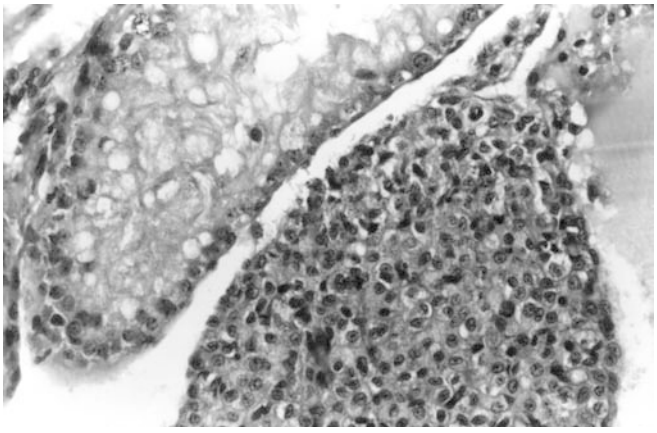

Fig. 5 Leydig cell proliferation in an ipsilateral testis of the EGF21 group (H-E×100)

ipsilateral testes. Leydig cell proliferation was also detected (Fig. 5). Figure 6 shows immature spermatogenesis in the ipsilateral testes. In the contralateral testes, spermatogenetic cells showed chromatin condensation beneath the nuclear membrane in the upper parts of the seminiferous tubules. There was mild edema within the seminiferous tubules. Overall, tissue appearance was normal. The junctions between the Sertoli cells were damaged on EM.

The mean Johnsen's spermatogenesis score for bilateral testes in each group is shown in Table 1. Mean spermatogenesis scores of the EGF treatment groups were significantly higher than in the G7 and G21 groups ($P < 0.05$).

The MSTD values of the bilateral testes in all groups are shown in Table 2. MSTD values of the G7 group were significantly lower than in EGF7 group ($P < 0.05$). MSTD values of the G21 group were significantly lower than in the EGF21 group ($P < 0.05$). MSTD values of the contralateral testes in the EGF21 group and control group did not differed significantly ($P > 0.05$). MSTD values for the ipsilateral and contralateral testes differed significantly in the EGF21 group ($P < 0.05$).

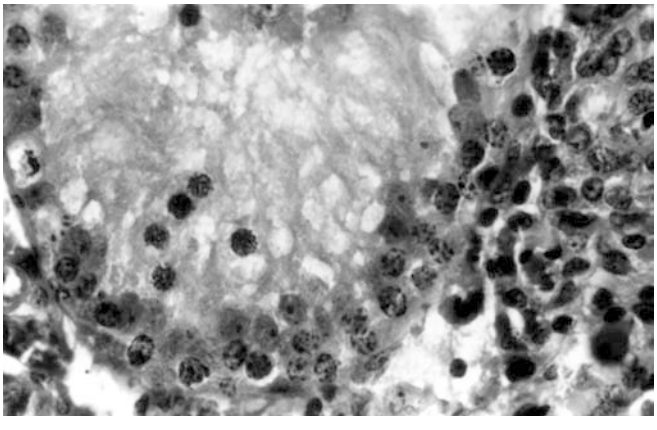

Fig. 6 Immature spermatogenic cells in an ipsilateral testis of the EGF 21 group (H-E×200)

Table 1 The mean Johnsen's spermatogenesis scores of bilateral testes in each group. *P < 0.05, significantly lower than in EGF groups

Groups	Mean Johnsen's scores	
	Ipsilateral	Contralateral
Control	10.0 ± 0.0	10.0 ± 0.0
Torsion	9 ± 0.0	9 ± 0.0
EGF7	6.9 ± 0.7	7.8 ± 0.4
G7	5.1 ± 0.7*	5.9 ± 0.6*
EGF21	6.1 ± 0.9	8.7 ± 0.5
G21	1.8 ± 1.4*	2.6 ± 1.2*

In the control group, MDA and GSH-Px levels were 82 ± 22 nmol/mg and 333 ± 25 U/mg, respectively. These values were the mean values for both of the unprocessed testes. The mean MDA and GSH-Px levels for the ipsilateral and contralateral testes are shown in Figs. 7 and 8, respectively. In all groups, the MDA levels in the bilateral testes were significantly higher than those in the control group ($P < 0.05$), except for the contralateral testes in the EGF21 group ($P > 0.05$).

The mean levels of MDA in the EGF7 group were significantly lower than in the G7 group ($P < 0.05$). MDA levels were also significantly lower in the EGF21 group than in the G21 group ($P < 0.05$). No significant difference was detected between the EGF7 and EGF21 groups ($P > 0.05$). In the torsion group, MDA levels were significantly higher than in the other groups ($P < 0.05$).

GSH-Px levels in the bilateral testes showed significant differences between the control and other groups ($P < 0.05$), with the exception of contralateral testes in the EGF21 group ($P > 0.05$). The GSH-Px levels in the bilateral testes did not differ significantly in the EGF7 and G7 groups. GSH-Px levels in EGF21 were significantly lower than in G21 ($P < 0.05$).

Discussion

Gelatin has great potential in pharmaceutical and biomedical applications because of its very high biocompatibility and biodegradability properties, in addition to being nontoxic and economical. In the pharmaceutical

Table 2 The mean seminiferous tubular diameter (MSTD) values of bilateral testes in all groups. *P < 0.05 significantly lower than in EGF groups

Groups	MSTD	
	Ipsilateral	Contralateral
Control	272 ± 2.2 μm	272 ± 2.2 μm
Torsion	271 ± 1 μm	273 ± 0.8 μm
EGF7	260 ± 2 μm	264 ± 0.9 μm
G7	241 ± 1.5 μm*	246 ± 1.7 μm*
EGF21	243 ± 3.9 μm	267 ± 3.8 μm
G21	215 ± 2.6 μm*	220 ± 1.8 μm*

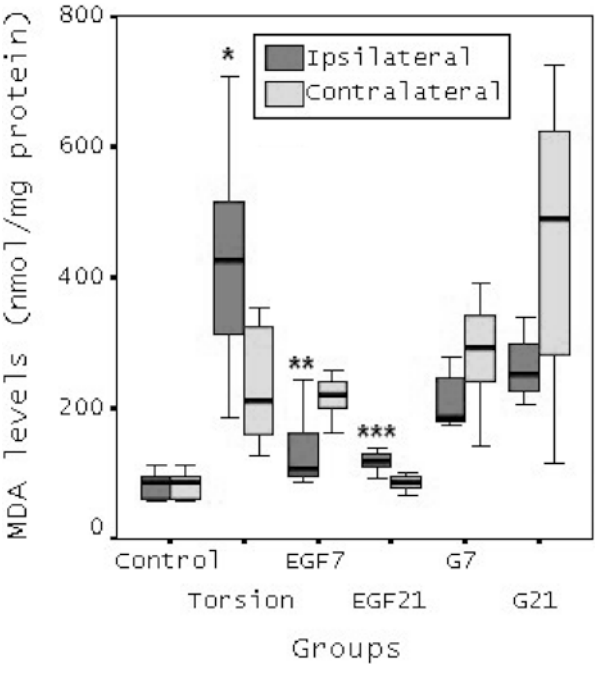

Fig. 7 Malondialdehyde concentrations in the various groups. $*P<0.05$ when compared with other groups, ** $P<0.05$ when compared with G7, and *** $P<0.05$ when compared to G21

industry, microspheres, microcapsules or gel forms of gelatin are widely used as carriers for bioactive agents such as drugs, hormones or growth factors. In wound healing applications, gelatin structures have achieved a very effective delivery of growth factors without causing any mononuclear cell infiltration or foreign body reactions in the wound area [33, 34]. Although gelatin is highly soluble in aqueous media, its solubility can be controlled by using cross-linkers such as aldehydes and carbodiimides. In previous in-vitro studies, fluorescein labelled bovine serum albumin [34] or colchicine [26] carrying gelatin sponges and microspheres prepared with no cross-linkers were degraded completely and released their contents in the first day. The addition of a gluteraldehyde cross-linker, depending on the amount added, extended this degradation period from days to weeks [35]. In the present study, in order to prevent the immediate dissolution of membranes in in vivo conditions, EGF containing gelatin membranes were lightly cross-linked by immersing them into cross-linker solution. Since the films were quite thin and lightly cross-linked, it was expected that EGF would demonstrate its effect very quickly, activating cell proliferation and accelerating wound healing. The results support these expectations. EGF treatment groups showed obvious biochemical and histological improvement compared to the gelatin groups.

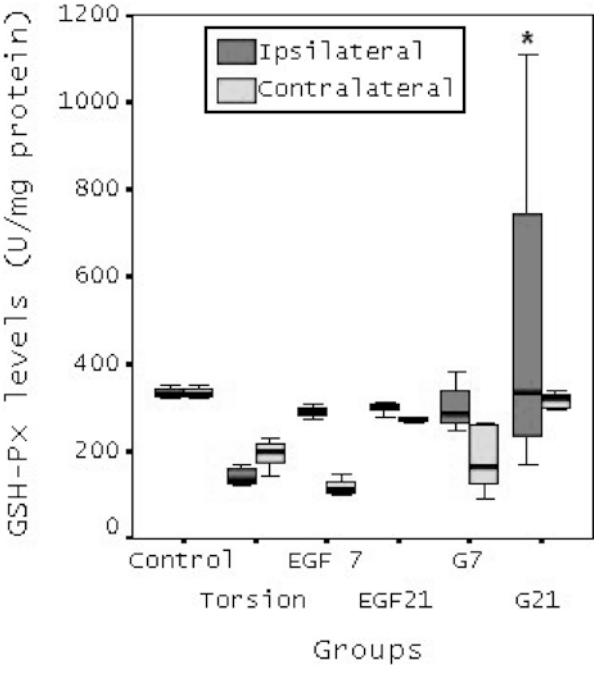

Fig. 8 GSH-Px concentrations in the various groups. * $P<0.05$ when compared to other groups

The distribution half-life of EGF in plasma is a few minutes and the elimination half-life about an hour. Most of the injected EGF is taken up by the liver and degraded; less than 1% is excreted in the bile. Subcutaneously administrated EGF had been detected in serum up to 12 h after administration [36]. Buckley et al. reported that sustained release of EGF from subcutaneous pellets accelerated the process of wound repair in rats, whereas daily injections of EGF were much less effective [37]. The efficiency of sustained release of EGF has also been shown in successful studies of the skin and cornea [34, 38]. Local and sustained EGF administration is more effective and highly economical compared to systematic administration.

EGF leads to the proliferation and differentiation of many cell types. Epidermal growth factor receptor (EGFR) has been shown in Leydig, Sertoli and peritubular cells [39]. It is also present in the epididymes, since this is a site of maturation of spermatozoa [40]. EGF significantly protects against intestinal I/R injury [23]. In previous studies, EGF was shown to decrease reactive oxygen species production in the intestine, pancreas and renal tissue [22, 24, 25].

Reactive oxygen species (ROS) are regularly formed during the process of normal cell respiration. However, their development is kept at physiologically low levels by intracellular free radical scavengers [41]. Overproduction of ROS as well as a decreased antioxidant capacity can result in tissue injury. Testicular torsion and detorsion, affecting mostly germ cells, suggests that lipid peroxides, which are highly toxic, may be formed in these cells rather than in peritubular, Leydig or Sertoli cells [42]. ROS has been shown to play a role in infertility caused by defective sperm function [43]. Anti-oxidation enzymes form an essential part of the cellular defense against ROS. GSH-Px in the testes may protect sperm from oxidant or pro-oxidant damage, particularly during periods of rapid proliferation and gametogenesis

[44]. It has been shown in some studies that natural scavengers are consumed during ischemia [45]. Similarly, in our study after 4 h of torsion, GSH-Px levels were significantly decreased compared to the control group. While on the seventh day of torsion GSH-Px levels among the groups did not differ and were the same as the control, by the 21st day the GSH-Px levels in the G groups were found to be higher than in the EGF treatment groups. GSH-Px is an inducible enzyme. Increased GSH-Px levels in the G21 group compared to EGF21 can be explained by increased GSH-Px synthesis to fight ROS. In group G7, GSH-Px levels did not increase, probably due to insufficient time for GSH-Px synthesis. In the EGF groups, GSH-Px levels did not increase, we think that EGF itself has removed the ROS from the tissue so that further release of GSH-Px was not necessary. MDA levels in the G21 group were also higher than in the EGF21 group supporting this opinion. MDA levels did not differ among the EGF7 and EGF21 groups. This shows that the effect of EGF began early during reperfusion injury and reduced the amount of damage or led to improvement of the tissue. These findings were supported by the histopathological results. Spermatogenic cells were preserved with 1 week of EGF treatment. On the other hand, in the ipsilateral testes no spermatozoa was detected and spermatozoa were reduced in the contralateral testes. At very early stages of reperfusion, spermatozoa could be damaged due to the presence of ROS in the tissue before the EGF levels reached a high enough value. After 21 days, the contralateral testes showed good recovery in the EGF treatment group. MDA levels for the contralateral testis in the EGF21 group were not different from the control group. They were also similar histopathologically. However, in the G21 group, tissue damage was observed in the bilateral testis but was more severe in the ipsilateral testis. In the G21 group, the apoptosis of spermatogenic cells and Sertoli cell only syndrome were observed. MSTD values and mean spermatogenesis scores in the EGF21 group were better than in the G21 group, suggesting that EGF plays an important role in the recovery of the bilateral testis tissue after I/R injury.

In the untreated groups (G7, G21), we observed severe injury in testis tissue and spermatogenic cells of the bilateral testis, which was proportional to the recovery period. Tissue damage in the G21 group was more severe than in the G7 group. Cosentino et al. performed 720 degrees of ipsilateral spermatic cord torsion for various periods of time (0, 1, 3, 5, 9, or 12 h). They showed that in all rats the long-term (after 6-weeks of recovery) changes were consistently more pathologically significant than the short-term changes. Our results also support these findings. Cosentino et al. were somewhat surprised to find that many parameters showed significant pathological damage after exposure to only 1 h of spermatic cord torsion. Indeed, no long-term regeneration was observed histologically. They were therefore particularly concerned with these results, as the currently accepted treatment of spermatic cord torsion in peripubertal boys is to untwist the cord in the hope that the damaged testicular tissue will regenerate and contribute to subsequent fertility. They have shown that the histological changes observed here correlate well with the fertility of the animals and suggest that attempts to salvage a testis after spermatic cord torsion may be futile, and should be critically reviewed [46].

The critical time for preventing contralateral testicular damage was reported to be 3 h [47]. In our study, a 4-h duration of torsion was selected to induce contralateral testicular damage without leading to ipsilateral necrosis. Therefore, we could observe the effects of EGF on the bilateral testes. Akgür et al. showed that testicular blood flow does not return to normal after 720 degrees of testicular torsion lasting more than 3 h, but in our study after 4 h torsion, we did not detect any necrosis [4]. A 4 h torsion period was sufficient for a contralateral influence. Although after 4 h only mild injury was observed in the contralateral testes, after 21 days of reperfusion we observed severe testicular damage. Therefore, we believe that injury to the contralateral testis is caused by reperfusion after detorsion and not by ischemia. Damage to the testicular tissue, especially to the germ cells, is mostly caused by reperfusion rather than ischemia [3, 4, 5, 9]. Niguyen et al. reported that the twisted testis showed diffuse interstitial hemorrhage, infarction, and lymphocyte infiltration on routine microscopy. The contralateral testis was normal on routine microscopy, whereas ultrastructurally, occasional myelin bodies were noted after torsion for 8 h followed by orchiectomy. Marked histopathological abnormalities occurred in the contralateral testis in the testicular salvage group [5]. The degree of contralateral testicular degeneration differs according to the age of the animal and the duration of ipsilateral spermatic cord torsion [47, 48].

The mechanism of contralateral testicular damage after ipsilateral torsion is unclear. Several theories have been propounded, including autoimmunization, subclinical episodes of contralateral testicular torsion, release of acrosomal enzymes and paired neuroendocrine or vasomotor responses during torsion. Animal studies have demonstrated that ipsilateral testicular torsion causes a decrease in the contralateral testicular blood flow and an increase in blood flow after detorsion [2, 4, 5, 6].

To prevent contralateral testicular damage, various treatments have been proposed including the early removal of the twisted testis [3], NO administration after torsion [49], and chemical sympathectomy [50]. There is a clear need for a chemical agent which ameliorates contralateral testicular damage without removal of the twisted testis. In the present study, we used local and sustained EGF for bilateral testicular recovery after ipsilateral testicular torsion, and we suggest that EGF aids the recovery of bilateral testicular tissue after I/R injury.

In conclusion, sustained and local EGF administration may be a new treatment for bilaterally injured testis

after detorsion, without removing the twisted testis. Germ cells can be protected with this treatment. However, further studies are needed to determine the adequate dose of EGF and the duration of treatment.

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