The Evaluation Of The Role Of Hyperbaric Oxygen Therapy In Preventing The Ischemia-Reperfusion Injury Following Experimental Testicular Torsion

T Senkul, D Erden, C Iseri, K Karademir, S Özkan, H Baloglu, E Kiliç

Citation


Abstract

Testicular torsion is a urologic emergency where the injury is in form of ischemia-reperfusion and methods to lessen the morbidity should be used even after a successful detorsion procedure. In order to test the effect of hyperbaric oxygen therapy (HBO) treatment to reduce the testicular tissue damage and testicular function loss, we used rat testis models and compared the efficacy of a single session HBO treatment with repeated sessions. Young Sprague-Downey rats were used in this study and 4 experimental groups were instituted; 1) Sham: Testicles dissected, 2) Control: 720 degree torsion applied during 4 hours than detorsionned. 3) HBO-1: 4 hour torsion than one session HBO, and 4) HBO-7: 4-hour torsion than 7 sessions HBO. All testicles were removed one week later and germinal changes were evaluated. HBO treatment groups showed significantly high maturation rates when compared to the control group (p<0.05). Germinal epithelial necrosis was seen in all the rats of the control group. The rate of germinal epithelial necrosis was 1/9 in HBO-1 group and 6/9 in HBO-7 group. A decrease in the number of multinuclear bizarre cells and apoptotic cells was found significant only in HBO-1 group. HBO treatment was not found to have affected the number of seminiferous tubules having germinal cells with nuclear vacuolization. Although HBO treatment was found effective in preventing the damage from ischemia-reperfusion injury in rat testis; no significant difference was noted between a single and multiple sessions of HBO treatment.

INTRODUCTION

Testicular torsion is a urologic emergency, which may cause gonadal loss, due to the ischemia-reperfusion injury. The ischemic injury level is proportional to the arterial compression and time spent since the beginning of symptoms. If not treated in 4 to 6 hours, testicular necrosis may ensue. Although the testicular salvage rate following immediate detorsion is reported high up to 90 percent (3), these patients develop 67 percent of testicular atrophy and subfertility (3,4), which is probably due to ischemia-reperfusion injury (3). It was shown that ischemia-reperfusion injury is significantly reduced with treatment of hyperbaric oxygen therapy, on studies done with animal skin flap and skeletal muscle models (3,4). The purpose of this study was to investigate the protective effect of a single and multiple (seven) sessions of HBO following detorsion of rat testicle.

MATERIAL AND METHODS

Young male Sprague-Dawley rats weighing 250 to 300 g were used. All experiments were done in accordance with the regulations of the Animal Ethical Committee of Kowa Co. Ltd. Forty rats were divided in 5 groups as normal morphology (n=5), sham (n=5), control (n=10), HBO-1 (n=10), and HBO-7 (n=10). The animals were anesthetized with single dose 25 mg intramuscular Ketamin hydrochloride. The testicles were exposed by a 2 cm long bilateral vertical incision. In the sham group, 4 hours after testicular exploration, the wound was closed. In the control group, the testicles were rotated 720° and fixed to the scrotum with chromic sutures. Four hours later, the testicles were detorsionned and the wound closed. For both HBO-group rats the testicles were torsionned for 4 hours and detorsionned. Then, one (HBO-1 group) or seven (HBO-7 group) sessions of HBO treatment was applied.

All rats were orchidectomized 7 days after the procedure and
testicles were fixed in Bouin’s solution for 24 hours. Each
testis was dissected along the long axis in 5 mm’s. Two
entire sections form each rat were blocked in paraffin. Two
five- m-thick sections of each block (total 4 sections for each
testis) were stained with Hematoxylen-Eosin (HE) for
microscopic evaluation. Normal testis morphology was set
after evaluating testicles of the normal morphology group
rats. Testicular morphology was evaluated in two groups of
parameters, namely germ cell maturation and germ cell
changes (Table-1). 50 seminiferous tubular sections from
each testis were randomly selected, then examined. The
presence of spermatogonia, spermatocyte, and spermatid
together in any of these 50 seminiferous tubular sections was
taken as the proof of complete maturation. Absence of
maturation up to spermatid level was classified as
incomplete germ cell maturation. In the presence of germinal
epithelium necrosis, the extent of necrotic germ cells within
the seminiferous tubules was set partial (if germ cell necrosis
was less then one-fourth of a seminiferous tubular section,
even in continuum or patchy), or extended (if germ cell
necrosis was more then one-fourth of a seminiferous tubular
section). The numbers of seminiferous tubules containing
any of the multinuclear bizarre germ cell(s) and/or germ
cell(s) showing nuclear vacuole(s) and/or apoptotic cell(s)
were also taken in account. Previously described HE criteria
for apoptosis were applied to detect apoptotic cells in
seminiferous tubules (8).

Presence or absence of set parameters in the study for
evaluating testicular morphology and its extent in presence,
were compared by paired-samples T test to evaluate the
efficacy of short-term and long-term HBO treatment for
testicular torsion model causing ischemia-reperfusion injury
in rats.

RESULTS

Microscopic parameters used in the study for evaluating
testicular morphology were found within normal limits in
both sham and normal group of rats.

Germ cell maturation was found gradually increased in both
hyperbaric oxygen treated groups compared to control group
rats. Complete to incomplete ratio of germ cell maturation
(maturation ratio) was 0.05, 0.53, and 0.27 in the control
group, and HBO-1 and HBO-7 groups respectively. The
HBO treatment groups showed significantly high maturation
rates when compared to the control group (p<0.05).

Germinal epithelial necrosis was seen in all rats of the
control group in different rates (mean 47.4 %). The necrosis
was extended type in 69.9 % and was partial type in 30.1 %
of affected seminiferous tubules.

In the HBO-1 group, only 2 rats revealed necrosis, one with
partial in 20 of the total of 500 tubules, and the other with
total necrosis due to the accidental main testicular artery
severance for which it was excluded from the study. Out of
these 2 rats, germinal epithelial necrosis was not seen in the
HBO-1 group rats.

In the HBO-7 group, 7 of 10 rats were found with germinal
epithelial necrosis. Only 2 of them were associated with
extensive necrosis, and in the remaining rats germinal
epithelial necrosis was only partial. While the frequency of
germinial epithelial necrosis was 1/9 in the HBO-1 group, the
HBO-7 group showed a 6/9-necrosis rate.

Multinuclear bizarre cells and apoptosis were both seen in
all three groups of rats in different ratios. Statistical
differences in decrease in the number of seminiferous
tubules containing multinuclear bizarre cells, and in the
number of seminiferous tubules containing apoptotic cells
were found significant only in the HBO-1 group (p<0.05).
However, the difference between HBO-1 and HBO-7 groups
as to the number of multinuclear bizarre cells and apoptotic
cells in seminiferous tubules, was statistically significant,
denoting the inverse effect of prolonged HBO treatment
(p<0.05).

Hyperbaric oxygen treatment was not found to have affected
the number of seminiferous tubules having germinal cells
with nuclear vacuolization since there was no significance
for each of 3 groups (p>0.05).

All the results were summarized in Table1.
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**DISCUSSION**

Even when testes are detorsioned in the first 4 hours, some testicular injury develops \((1, 2, 4)\). Studies show that the first 60-90 minutes of the reperfusion is critical for reperfusion injury, since the free oxygen radicals originating from neutrophils and parenchymal cells \((10)\).

A previous study shows the positive effect of treating the subject with HBO during reperfusion \((10)\). In our study, following 4-hour-long testicular torsion, spermatogenesis was found significantly disturbed and germinal epithelial necrosis developed in more than half of the seminiferous tubules. But, when the same animals were treated with HBO this epithelial necrosis was only rare. In his study, Kolski evaluated the effect of HBO on rat testis following detorsion, by measuring the thicknesses of germinal epithelium \((9)\). A thicker epithelium was the sign of an effective treatment. We have observed that, even in the same testis, germinal epithelium thicknesses may show variations and concluded that this criterion may not be reliable. That’s why we have preferred to evaluate the germ cell maturation and germinal epithelium necrosis, in order to set objective data on the vitality and functions of the testis.

We have also observed more apoptotic changes on testis treated with 7 sessions of HBO. Probably, apoptosis is an irreversible process, which is impossible to prevent with HBO treatment.

Studies have done on skeletal muscles have shown a beneficial effect of multiple sessions of HBO \((11)\). Although the results we obtained with 7 sessions of HBO treatment seemed better, the difference was not shown to be statistically significant.

Finally, although HBO treatment was found effective in preventing the damage from ischemia-reperfusion injury in rat testis, no significant difference was noted between a single and multiple sessions of HBO treatment.

**REFERENCES**

Author Information

Temucin Senkul
Assistant Professor, Department of Urology, GATA Haydarpasa Training Hospital

Dogan Erden, Professor
Department of Urology, GATA Haydarpasa Training Hospital

Cüneyt Iseri
Associated Professor, Department of Urology, GATA Haydarpasa Training Hospital

Kenan Karademir, Assistant Professor
Department of Urology, GATA Haydarpasa Training Hospital

Sezai Özkan, Assistant Professor
Department of Anesthesiology, GATA Haydarpasa Training Hospital

Hüseyin Baloğlu
Associated Professor, Department of Pathology, GATA Haydarpasa Training Hospital

Ercüment Kılıç
Department of Urology, GATA Haydarpasa Training Hospital