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

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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 (1), these patients develop 67 percent of testicular atrophy and subfertility (2, 3), which is probably due to ischemia-reperfusion injury (4). 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 (5, 6). 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-μm-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 than one-fourth of a seminiferous tubular section, even in continuum or patchy), or extended (if germ cell necrosis was more than 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 into account. Previously described HE criteria for apoptosis were applied to detect apoptotic cells in seminiferous tubules.

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 germinal 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.
Figure 1

Table 1: HBO effect on germ cell maturation and germinal cells

<table>
<thead>
<tr>
<th>Germ cell changes</th>
<th>Without HBO</th>
<th>With HBO</th>
<th>Apoptosis</th>
<th>Results</th>
<th>Number of seminiferous tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (μm)</td>
<td>20.1±2.1</td>
<td>22.0±2.3</td>
<td>0.01</td>
<td>0.001</td>
<td>200</td>
</tr>
<tr>
<td>Number of spermatogonia</td>
<td>500*</td>
<td>600*</td>
<td>0.05</td>
<td>0.01</td>
<td>200</td>
</tr>
<tr>
<td>Maturation index</td>
<td>1.2±0.1</td>
<td>1.4±0.2</td>
<td>0.01</td>
<td>0.001</td>
<td>200</td>
</tr>
</tbody>
</table>

DISCUSSION

Even when testes are detorsionned in the first 4 hours, some testicular injury develops (1, 2, 3). 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 (4). A previous study shows the positive effect of treating the subject with HBO during reperfusion (4). 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 this study, Kolski evaluated the effect of HBO on rat testis following detorsion (5). In our study, Kolski evaluated the effect of HBO on rat testis following detorsion by measuring the thicknesses of germinal epithelium (6). A thicker epithelium was the sign of an effective treatment. We have observed that, even in the same tests, 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 testes 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 (6). 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.

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