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

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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 ([2, 4]), which is probably due to ischemia-reperfusion injury ([5]). 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 ([6, 7]). 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 720o 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 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

Rat No	Germ cell maturation					
	Complete *			Incomplete *		
	C	HO1	HO7	C	HO1	HO7
1	16	71	0	171	429	295
2	0	118	118	317	372	372
3	8	269	9	303	211	460
4	0	288	164	150	212	336
5	0	203	0	289	297	398
6	0	220	148	83	280	352
7	56	236	94	322	264	376
8	35	23	182	333	477	297
9	0	0	180	231	0	320
10	17	118	88	297	372	402
Sum	132	1546	983	2496	2914	3608
Frequency	0,026	0,309	0,196	0,499	0,582	0,721

Germ cell changes														
Germinal epithelial necrosis						Multinuclear Bizarre cells **			Apoptosis **			Nuclear vacuolization **		
Extensive *			Partial *											
C	HO1	HO7	C	HO1	HO7	C	HO1	HO7	C	HO1	HO7	C	HO1	H7
202	0	115	111	0	90	171	39	68	217	205	313	91	137	68
137	0	0	46	0	0	158	78	245	247	117	127	158	156	166
98	0	0	91	20	31	178	205	128	237	254	353	217	68	138
266	0	0	84	0	0	138	68	9	138	137	310	128	98	196
149	0	25	62	0	77	284	59	117	225	78	211	205	245	39
380	0	0	37	0	0	117	68	176	39	68	215	88	284	147
94	0	0	28	0	30	107	9	235	372	147	30	172	205	274
70	0	0	62	0	21	205	284	177	294	411	22	176	91	128
154	500*	0	115	0	0	176	0	78	147	0	352	117	0	216
110	0	0	76	0	10	127	49	139	254	284	303	166	235	117
1660	0	140	712	20	259	1661	859	1372	2170	1701	2820	1518	1519	1489
0,332	0	0,028	0,142	0,004	0,051	0,332	0,171	0,274	0,434	0,340	0,564	0,303	0,303	0,297

*: Number of seminiferous tubuli
HO1: One session Hyperbaric Oxygen therapy group
**: Number in 500 seminiferous tubuli
HO7: Seven sessions Hyperbaric Oxygen therapy group
• Total tubule necrosis due to the accidental main testicular artery severance (excluded from the statistics)

DISCUSSION

Even when testes are detorsionned 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.

CORRESPONDENCE TO

Temuçin SENKUL, MD. GATA Haydarpasa Training Hospital Department of Urology 81327 Üsküdar / Istanbul TURKEY Phone number: +90 532 664 31 89 Fax number: +90 216 348 78 80 e-mail: senkul@ixir.com

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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 Baloglu
Associated Professor, Department of Pathology, GATA Haydarpasa Training Hospital

Ercüment Kiliç
Department of Urology, GATA Haydarpasa Training Hospital