Hyperbaric oxygen treatment time for cyclophosphamide induced cystitis in rats.

E. OZTAS¹, A. KORKMAZ², S. OTER², T.TOPAL²

¹Department of Medical Histology and Embryology, ²Department of Physiology
Gulhane Military Medical Academy, 06018 Etilik/ANKARA/TURKEY

Oztas E, Korkmaz A, Oter S, Topal T. Hyperbaric oxygen treatment time for cyclophosphamide induced cystitis in rats. Undersea Hyperb Med; 31(2):211-216. The aim of this study was to evaluate the prophylactic potential of hyperbaric oxygenation treatment and the timing of hyperbaric oxygen (HBO₂) therapy for cyclophosphamide-(CYP) induced cystitis in rats. Forty male Sprague-Dawley rats were divided into 5 groups. Four groups received a single dose of CYP (100 mg/kg) intraperitoneally (i.p.) at the same time (group 1 served as the control). Group 2 received CYP only; group 3 received HBO₂ treatment (2.8 atmospheres absolute, 90 minutes, twice daily) before and the day after CYP. Group 4 received HBO₂ before and on the day of CYP administration. Group 5 received HBO₂ on the day of and the day after CYP. CYP injection resulted in severe cystitis. Prophylactic HBO₂ treatment did not prevent the severe cystitis. After CYP injection, however, HBO₂ treatment attenuated CYP-induced hemorrhagic cystitis in rats. Hyperbaric oxygen has a beneficial effect on repairing and healing bladder damage, though it does not function to prevent CYP-induced hemorrhagic cystitis.

INTRODUCTION

Cyclophosphamide (CYP) is widely used to treat a number of neoplastic diseases (1). Hemorrhagic cystitis has been recognized as a common and dose-limiting side effect after using CYP and ifosfamide, a synthetic analog of CYP. The incidence of this side effect varies from 2 to 40% in patients on long-term, low-dose treatment with CYP, whereas it can be as high as 75% in patients receiving high intravenous doses (2). Prevention is the best way to reduce the side effects of CYP. Despite the use of preventive measures such as mesna (2-mercaptoethane sulfonate) administration, high fluid intake, diuretics, forced diuresis, and urine alkalinization, bladder protection is not always achieved (3).

It has been proposed that the urotoxicity of CYP is mainly attributable to the renal excretion of acrolein, an urotoxic metabolite of CYP (4). Urothelial damage occurs following direct contact with acrolein, which causes edema, hemorrhage, ulceration, leukocyte infiltration and necrosis (1).

HBO₂ therapy is used for several urological diseases (5). HBO₂ has a beneficial effect in radiation-induced cystitis as indicated in prospective trials (6,7). Less frequent, but equally encouraging, are reports of the value of HBO₂ in CYP-induced hemorrhagic cystitis (8,9).

HBO₂ induces the healing of tissue damage, and decreases edema, necrosis and leukocyte infiltration (10,11). Several studies have also shown the beneficial effects of HBO₂ on preserving cellular homeostasis with respect to ATP levels and in maintaining normal cellular osmolarity (12). Despite these effects, HBO₂ alone does not provide complete protection against CYP-induced cystitis (9).

Recently, it has been shown that endogenous inflammatory mediators such as platelet activated factor (PAF), tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) are involved
in cystitis by increasing nitric oxide (NO) production (13-16). Increased cytokine expression after CYP-induced cystitis, alone or in combination with other inflammatory mediators or growth factors, may contribute to alter the lower urinary tract after cystitis (17). Many studies have shown that HBO$_2$ is not only a therapeutic agent that increases dissolved oxygen in plasma, but that it also affects inflammatory processes. HBO$_2$ significantly decreases the adhesion and rolling of PMNL possibly through downregulating intercellular adhesion molecule-1 (ICAM-1) expression (18-20).

Although HBO$_2$ treatment is generally preferred clinically when cystitis resists other treatment modalities such as mesna, high fluid intake, diuretics, and forced diuresis, consensus does not exist on the appropriate time to start HBO$_2$ therapy against CYP-induced cystitis (5). Only one reference was found that mentions controlled in vivo studies, investigating the prophylactic potential of hyperbaric oxygenation treatment for CYP induced cystitis in rats (21). In our study, we examined the optimum timing of HBO$_2$ treatment on hemorrhagic cystitis and tried to ascertain whether the beneficial effects of HBO$_2$ depend on preventing cystitis or on repairing bladder damage.

**METHODS**

**Animals**

Forty male Sprague-Dawley rats weighing 180-230 g. were divided into 5 groups, as shown in Table 1. They had free access to food and water. The Gulhane Military Medical Academy Animal Care and Use Committee approved the experimental protocol.

<table>
<thead>
<tr>
<th>Groups (n:8)</th>
<th>Day-1</th>
<th>Day-2</th>
<th>Day-3</th>
<th>Day-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(AM)</td>
<td>(PM)</td>
<td>(AM)</td>
<td>(PM)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>Saline</td>
<td>-</td>
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<tr>
<td>CYP</td>
<td>-</td>
<td>-</td>
<td>CYP</td>
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<td>HBO$_2$</td>
<td>HBO$_2$</td>
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<td>HBO$_2$</td>
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<td>HBO$_2$/CYP</td>
<td>HBO$_2$</td>
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<td>CYP+ HBO$_2$</td>
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<tr>
<td>CYP/ HBO$_2$</td>
<td>-</td>
<td>-</td>
<td>CYP+ HBO$_2$</td>
<td>HBO$_2$</td>
</tr>
</tbody>
</table>

CYP; Cyclophosphamide, HBO$_2$; Hyperbaric oxygen treatment.

**CYP and HBO$_2$ administration**

To induce cystitis, rats were given a single dose of CYP (100 mg/kg.) intraperitoneally (i.p.). Animals were treated with HBO$_2$ following the CYP administration. A steel animal hyperbaric oxygen chamber was flushed with 100% oxygen at first and chamber pressure was increased to 2.8 atmospheres absolute (atm abs.) over 10 minutes. Decompression to normobaric air at the end of the session was completed gradually over 5 minutes. A temperature of 22-26 °C and oxygen flow of 15 l per minute was maintained in the medium for 90 minutes (8, 9).

**Tissue preparation**

After 48 hours of cystitis induction, the rats were anesthetized using a combination of ketamine HCl (85 mg/kg) and xylazine HCl (12.5 mg/kg) i.p. injection. The bladders were
removed intact, residual urine evacuated, and then they were weighed for edema and fixed for 24 hours in 10% buffered formalin. The tissues were embedded in paraffin and stained with hematoxylin-eosin. A pathologist blinded to the study group rated the mean histological damage, including edema, hemorrhage and inflammation on a scale of 1 (normal) to 4 (severe changes). Mucosal ulceration was scored as 1 (normal), 2 (epithelial denuding), 3 (focal ulceration), and 4 (widespread epithelial ulceration).

**Statistics**

The data were expressed as means ± SEM and p<0.05 was considered statistically significant. All of the numeric data were analyzed first using the Kruskal-Wallis test to determine whether there were differences between the groups. The Mann-Whitney U test was used to analyze two groups consecutively.

**RESULTS**

All histologic parameters and bladder weight/body weight (bl.w/b.w) ratios have been summarized in Table 2. Control animals had histologically normal bladders (Fig.1) with assigned scores of 1 for all parameters and no hematuria. CYP showed severe histological changes (Fig.2) and macroscopic hematuria continued at the end of the study. CYP caused approximately a 3-fold increase in bl.w/b.w ratios. The prophylactic HBO₂ (group 4) did not show any protective effect (Fig.4) regarding cystitis (p=0.62 for edema, p=0.71 for hemorrhage and inflammation and p=1.00 for ulceration compared with CYP) but group 5 (HBO₂ exposure after CYP administration) showed beneficial effects such as bladder damage repair (p=0.004 for edema, p=1.00 for hemorrhage, p=0.001 for inflammation and ulceration when compared with CYP). Between groups 3 and 5 there was no significant difference in any of the histological parameters (p=0.25 for edema, p=1.00 for hemorrhage and p=0.71 for inflammation and ulceration, Figs.3,5).

### Table 2. Comparison for histologic damage in rat bladder (mean ± S.E.M)

<table>
<thead>
<tr>
<th>Groups (n:8)</th>
<th>Edema</th>
<th>Hemorrhage</th>
<th>Inflammation</th>
<th>Ulceration</th>
<th>Bl.w/Bw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>0.84±0.03</td>
</tr>
<tr>
<td>CYP</td>
<td>3.71±0.28</td>
<td>4.00±0.21</td>
<td>4.14±0.26</td>
<td>4.71±0.18</td>
<td>2.69±0.11</td>
</tr>
<tr>
<td>HBO₂/CYP/ HBO₂</td>
<td>2.71±0.42</td>
<td>4.00±0.30</td>
<td>2.14±0.34</td>
<td>1.28±0.18</td>
<td>1.47±0.05</td>
</tr>
<tr>
<td>HBO₂/CYP</td>
<td>3.42±0.36</td>
<td>4.14±0.26</td>
<td>4.28±0.28</td>
<td>4.71±0.18</td>
<td>2.15±0.06</td>
</tr>
<tr>
<td>CYP/HBO₂</td>
<td>2.00±0.30</td>
<td>4.00±0.21</td>
<td>2.14±0.34</td>
<td>1.42±0.20</td>
<td>1.42±0.06</td>
</tr>
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</table>

*a* p<0.05 compared with CYP group,

*b* p<0.05 compared with HBO₂/CYP/HBO₂ group,

*c* p<0.05 compared with HBO₂/CYP group.
**Fig. 1.** Normal rat bladder histology (Saline) with a score of 1 for edema, hemorrhage, inflammation and ulceration. Intact urothelium, blood vessels, no edema or infiltrate present, smooth muscle layers without alterations. (H&E, 25x).

**Fig. 2.** Severe cyclophosphamide-induced cystitis with a mean histologic score of 3 for edema, 4 for hemorrhage, inflammation, and 5 for ulceration. Marked edema, extensive urothelial damage, urothelium was absent from many regions of the bladder, marked inflammatory infiltrate with abundant lymphocytes and polymorphonuclear blood cells. (H&E, 50x).

**Fig. 3.** Group 3 bladder histology with mean histologic scores 2 for edema and inflammation and 4 for hemorrhage; no ulceration seen. Urothelium present, mild to moderate vascular congestion, mild edema and few white blood cell infiltrates. HBO₂ treatment considerably improved the inflammatory changes induced by CYP (H&E, 200x).

**Fig 4.** Group 4 (prophylactic HBO₂ only) bladder histology with mean histologic scores of 3 for edema, 4 for hemorrhage and inflammation, and 5 for ulceration. Prophylactic HBO₂ treatment has not improved the inflammatory changes induced by CYP (H&E, 50x).

**Fig. 5.** HBO₂ treatment after CYP resulted in similar findings as those of group 3. Scores 2 for edema and inflammation, 4 for hemorrhage, and 1 for ulceration. HBO₂ treatment considerably improved the histological changes induced by CYP (H&E, 50x).
DISCUSSION

Koss first reported on the histological changes seen in rats whose bladders were exposed to CYP metabolites (22). He described evidence of epithelial loss, ulceration, edema, capillary dilatation, submucosal hemorrhage, fibrosis and smooth muscle necrosis. In our study, the CYP administrated groups’ microscopic sections revealed the histological changes as described before. This existence of the histologic changes was accepted as cystitis.

Recent studies have indicated that CYP induced cystitis is not only due to the direct contact of acrolein with bladder mucosa but also involves inflammatory mediators (13-16). The beneficial effects of HBO2 have been demonstrated clinically following bladder damage (23).

Many studies indicate that HBO2 has anti-inflammatory properties. These include, attenuating cytokine induction such as TNF-α, IL-1, and IL-6 (11, 24, 25). It is well known that iNOS synthesis is strongly induced by IL-1 and TNF-α and that inhibition of these inflammatory mediators decreases iNOS expression (12). HBO2 also downregulates ICAM-1 expression leading to decreased PMNL rolling on microvasculature (19). Giving HBO2 may have a beneficial effect on inflammation in CYP-induced cystitis by optimizing the anti-inflammatory milieu. The establishment of elevated tissue oxygen pressure by HBO2 might allow a marked improvement in all of the histological changes induced by CYP. In our study, the anti-inflammatory effects of HBO2 have been observed in groups 3 and 5 after which cystitis had occurred. While HBO2 probably could not have prevented CYP-induced cystitis, it may have accelerated tissue repair. This result correlates with previous studies (8,9).

In irradiated tissue, HBO2 stimulates the formation of new vessels, and with it improve the healing process of injured tissue. This stimulation of neoangiogenesis by HBO2 has been documented in animals (24) as well as in some clinical studies (25-27).

In the present study, our results were similar to those working on HBO2 prophylaxis of hemorrhagic radiation cystitis. But, in contrast with Haders’ result, we propose that HBO2 might not be useful for prophylaxis of CYP-induced hemorrhagic cystitis. The present data added to the existing experimental data, timing of HBO2 application, indicate that HBO2 is not effective as prophylactic agent for the cyclophosphamide-induced hemorrhagic cystitis.

In our experiment, HBO2 displayed significant beneficial effects on healing bladder damage. The timing of the HBO2 treatment appeared to be a critical factor, with less injury occurring if the HBO2 treatment preceded CYP. Consequently, it would be considered that HBO2 is “likely to be beneficial” and more effective in repairing tissue damage in CYP-induced cystitis. Thus, it appears to be reasonable that there is no need to use HBO2 for the prophylaxis of CYP-induced cystitis.

ACKNOWLEDGMENTS

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REFERENCES