

Chemical cystitis developed in experimental animals model: Topical effect of intravesical ozone application to bladder

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Abstract

Aims: To demonstrate the effects of intravesical ozone treatment on inflammation and epithelial cell damage in chemical cystitis animal model.

Materials and Methods: A total of 30 New Zealand rabbits were divided into six groups. Cystitis was conducted with transurethral intravesical hydrochloric acid instillation on the subjects in Groups IA, IB, IIA, and IIB. Then, Group IA-IB subjects were transurethrally administered intravesical ozone therapy twice a week, while Group IIA-IIB subjects were only given intravesical isotonic NaCl instillation. Group IIIA-IIIB subjects were administered intravesical isotonic NaCl instillation without conducting chemical cystitis in order to create the same stress. Treatment schemes of all groups were arranged in the same manner. Following a 3-week (early period) and 6-week (late period) therapy, the rabbits were sacrificed and histopathologic investigations were carried out in order to demonstrate changes in the urinary bladder.

Results: In our study, we observed that the basal membrane and mucosal integrity were maintained, inflammatory cells were suppressed in Group IA-IB (Early and late period), which received ozone therapy. However, it was also observed that mucosal integrity was spoiled, numerous inflammatory cells were accumulated in Group IIA-IIB, which was administered isotonic NaCl.

Conclusion: Due to its low cost and minimal side effects; ozone therapy could be a new therapeutic approach in the treatment of interstitial cystitis.

Key Words: Inflammation, interstitial cystitis, ozone

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INTRODUCTION

Interstitial Cystitis (IC) is a chronic, progressive complex of symptoms characterized by chronic pelvic pain, and urgent and frequent urination. The aetiopathogenesis of IC cannot be explained and an accepted standard therapy is still non-existent. The clinical course of the disease is remittent,

and symptoms may repeat in most of the patients showing clinical improvement after a while. Therefore, new approaches are constantly appearing in the treatment of IC.^[1-3]

Ozone is an agent employed for disinfection and sterilization, and is used for most illnesses for which medical treatment remains ineffective as an immunomodulator and results are quite satisfactory.^[4] Immunomodulator of ozone can be observed as providing an increase of energy production in lymphocyte and monocyte in the case of acute infection and inducing of H₂O₂ production in leukocyte for chronic infections. Ozone increases interleukin production in the first instance, as well as cytokine. Therefore, cytokine normalizes cellular and humoral immunity. Cell-based immunity efficiency is provided by increasing the amount of T cells

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and also regulating B lymphocyte activity responsible for immunoglobulin synthesis.^[5,6]

Almost all of the studies of the urinary system, that were carried out in order to demonstrate the effects of ozone have been performed on the kidneys, while there is only a limited number of studies investigating the effects on the bladder. In this study, we examined the changes in early and late period that appear due to intravesical ozone treatment administered intravesically on our animal model, which we constructed using the IC model.

MATERIALS AND METHODS

Animals and the study order

Approval from the Local Ethics Committee was obtained for the study in 2007. A total of 32 white, female New Zealand rabbits, with weights ranging between 1500 g and 2000 g were selected as subjects. Urine samples taken from all subjects, included in order to evaluate the presence of an infection before commencing the study, were examined in a culture environment. Two subjects were excluded from the study due to significant growth in culture.

Experimental animals were divided into 6 groups. There were 5 subjects ($n = 5$) in each groups. Chemical cystitis was formed in subjects in Groups IA, IB, IIA and IIB through transurethral intravesical hydrochloric acid (HCl) instillation. Chemical cystitis was induced by intravesical instillation of HCl (0.2 ml of 0.4 N HCl), as described by Cayan et al., and as used in previous studies by Rivas et al.^[7,8]

Following anesthesia with an intramuscular administration of 50 mg/kg ketamine and 10 mg/kg xylazine in subjects in Groups IA, IB, IIA and IIB, one session of HCl (0.2 mL, 0.4 N HCl) instillation was conducted. A sterile 5 French feeding tube was implanted transurethraly. Following the aspiration of urine in bladder, HCl was instilled transurethraly and maintained there for 4 min.

Group IIIA and IIIB were established as the control group, and following a similar administration of anesthesia on subjects in this group, 0.9% NaCl intravesical instillation was conducted transurethraly without forming chemical cystitis [Table I].

Treatment protocol

Group I ($n = 10$): Following the formation of chemical cystitis, 10 ml 0.9% NaCl solution containing 20-25 mcg of ozone per mL of NaCl solution^[4] was instilled through a 5 f urethral feeding tube for 3 weeks in Group IA ($n = 5$), and 6 weeks in Group IB ($n = 5$) at 2 sessions per week (on the 4th and 7th days), and maintained for 10 min (Medozosons-BM was used as an ozone generator). At the end of the 3rd week in Group IA and at

Table 1: Study groups

Group IA	10 ml 0.9% NaCl+20-25 mcg/mL ozone (Following the formation of chemical cystitis) (early period)
Group IB	10 ml 0.9% NaCl+20-25 mcg/mL ozone (Following the formation of chemical cystitis) (late period)
Group IIA	10 ml 0.9% NaCl (Following the formation of chemical cystitis) (early period)
Group IIB	10 ml 0.9% NaCl (Following the formation of chemical cystitis) (late period)
Group IIIA	10 ml 0.9% NaCl (Without forming chemical cystitis) (early period)
Group IIIB	10 ml 0.9% NaCl (Without forming chemical cystitis) (late period)

the end of the 6th week in Group IB; the subjects were sacrificed in order to assess the results, a cystectomy was performed, and histopathologic and biochemical examinations were conducted.

Group II ($n = 10$): Following the formation of chemical cystitis, 10 mL 0.9% NaCl solution as transurethraly instilled through a 5 f urethral feeding tube for 3 weeks in Group IIA ($n = 5$), and 6 weeks in Group IIB ($n = 5$) at 2 sessions per week (on the 4th and 7th days), and maintained for 10 min. At the end of the 3rd week in Group IIA and at the end of the 6th week in Group IIB; the subjects were sacrificed in order to assess the results, a cystectomy was performed, and histopathologic and biochemical examinations were conducted.

Group III ($n = 10$): Without forming chemical cystitis on experimental animals, 10 mL 0.9% NaCl solution was intravesically instilled through a 5 f feeding tube for 3 weeks in Group IIIA ($n = 5$), and 6 weeks in Group IIIB ($n = 5$) at 2 sessions per week (on the 4th and 7th days), and maintained for 10 min. At the end of the 3rd week in Group IIIA and at the end of the 6th week in Group IIIB; the subjects were sacrificed in order to assess the results, a cystectomy was performed, and a histopathologic examination was conducted.

Histopathological evaluation

The subjects were sacrificed with 200 mg/kg pentobarbital. Following this, they were dissected with transabdominal midline incision and a cystectomy was performed. Bladder tissue samples were taken and embedded in paraffin; sections of 4 μm were separated with the help of a microtome. Samples were then stained with H and E, and inflammatory changes, leukocyte counts and mast cells, after staining with toluidine blue, were all evaluated.

An Olympus Bx 50 light microscope and an Olympus PM10SP camera system were used for slide imaging. Each cross-section was divided into 10 subsections, and leukocytes and the mast cell infiltration was evaluated in each of the 10 subsections by the following scalar;

- 0: No extravascular leukocytes and mast cells
- I: Less than 20 leukocytes and mast cells

- 2: 20-45 leukocytes and mast cells
- 3: Greater than 45 leukocytes and the mast cells.

The scores for all 10 stions were added, divided by 30 (maximum possible score) and multiplied by 100. The leukocytes and the mast cells infiltration scores for an individual bladder were the average of the 3 cross-sections examined. We counted the leukocytes and the mast cells under $\times 200$ optic zoom.^[9,10]

Statistical analysis

“SPSS 11 for Windows” statistics software pack was used for statistical estimations. The data were identified as arithmetic mean and standard error. A non-parametric Kruskal-Wallis test was used for group comparisons.

RESULTS

Average weights of the subjects were measured for Group I, Group II, and Group III as 1750 ± 150 g, 1700 ± 100 g, and 1750 ± 100 g, respectively. A statistically significant difference was not established between groups in terms of weight ($P > 0.05$).

In bladder samples of Group IIIA and IIIB subjects that were only administered 0.9% NaCl without chemical cystitis, mucosal integrity was found as normal and inflammation did not occur. Any mast and leukocyte cells that would lead to a pathologic view in the basal membrane were not observed.

Following the formation of chemical cystitis, the bladder samples of Group IIA and Group IIB subjects that were administered 0.9% NaCl demonstrated a spoilt mucosal integrity [Figure 1a] and the presence of numerous mast and leukocyte cells in the basal membrane as a response to inflammation [Figure 1b]. These findings showed that 0.9% NaCl intravesical instillation did not suppress inflammatory cells, meaning that it did not have a therapeutic effect in the treatment of chemical cystitis.

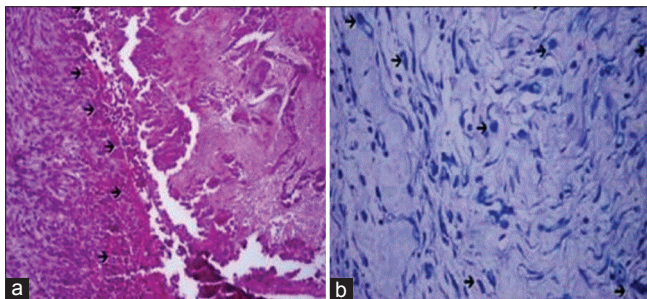


Figure 1: (a) Spoilt mucosal integrity due to the occurring inflammation, numerous leukocyte cells (Group IIb leukocyte cell structures with H and E) ($\times 200$) (b) Spoilt mucosal integrity due to the occurring inflammation, numerous mast cells (Group IIb mast cell structures with toluidine blue) ($\times 200$)

Mean mast and leukocyte cell counts in Group IA and IB, which received ozone treatment, were observed to have significantly decreased compared to Group IIA and IIB, which received 0.9% NaCl ($P = 0.008$, $P = 0.008$) [Tables 2 and 3]. In the bladder specimen of Group IA and IB, it was demonstrated that mucosal integrity was maintained [Figure 2a] and few mast and leukocyte cells were present in the basal membrane in response to inflammation [Figure 2b].

In addition; when comparing Group IA (early period) and IB (late period), it was observed that mast and leukocyte cell counts were decreased in Group IB ($P = 0.69$).

DISCUSSION

It was demonstrated that the chemical cystitis formed in numerous animal experiments resembled that of IC in terms of both leukocyte counts and mast cell counts, and thus that it is possible to create an animal model for IC. This model can be established with HCl, protamine sulphate, cyclophosphamide, and sucralphate.^[11-13] In our study, chemical cystitis was created with HCl and changes that histopathologically were compliant with chemical cystitis in Group IIA-IIB subjects were found.

When the data of existing literature were checked, 0.9% NaCl was demonstrated as ineffective in the treatment of IC in subjects in which chemical cystitis was created.^[7,9] Furthermore, in compliance with existing literature, our study also established that 0.9% NaCl therapy did not affect leukocyte and mast cell counts in Group IIA-IIB subjects.

Ozone treatment shows its effects by immunomodulation, vasodilation, disinfection, right shifting of the HbO_2 dissociation curve, releasing growth factors (e.g. Platelet derived growth factor, Transforming growth factor- $\beta 1$) and increasing antioxidant defence capacity with general and local anti-inflammatory effects.^[4-6,14]

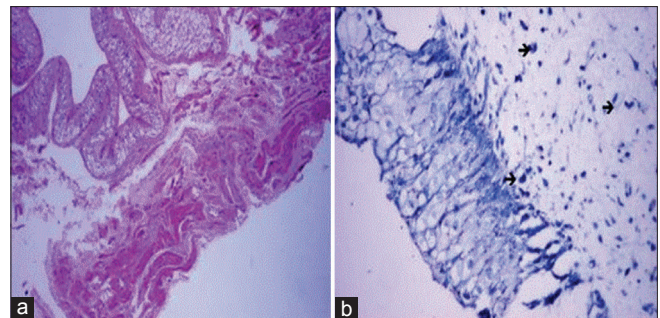


Figure 2: (a) Limited inflammatory changes, near normal mucosal integrity, limited leukocyte cells (Group Ib leukocyte cell structures with H and E) ($\times 200$) (b) Limited inflammatory changes, near normal mucosal integrity, limited mast cells (Group Ib mast cell structures with toluidine blue) ($\times 200$)

Table 2: Comparison of leucocyte and mast cells counts of groups in early period

Type of cell	Group IA	Group IIA	Group IIIA
Mast	4.62±1.80	15.24±1.86	1.98±1.80
Leucocyt	12.54±2.76	43.88±7.56	9.24±2.76

*Group IIA were higher compared to group IA, and group IIIA was found to be statistically significant ($P=0.008$)

Table 3: Comparison of leucocyte and mast cells counts of groups in late period

Type of cell	Group IB	Group IIB	Group IIIB
Mast	3.96±1.47	16.64 2.47	2.64±1.76
Leucocyt	11.88±1.80	46.24±9.38	9.90±2.33

*Group IIB were higher compared to group IB, and group IIIB was found to be statistically significant ($P=0.008$)

Almost all of the studies of the urinary system, that were carried out in order to demonstrate the effects of ozone have been performed on the kidneys while there is only a limited number of studies investigating the effects on the bladder. Gonzalez *et al.* stated that the test subjects treated by rectal ozone insufflation for cisplatin nephrotoxicity had lower serum creatinine levels with inhibited superoxide distimulase, catalase and glutathione peroxidase. It was reported that the renal tissue cleared from the toxic effect more rapidly in test subjects that received ozone and it was also suggested that ozone might prevent the kidney from oxidative damage thanks to this effect. The reason for the rapid healing of the tissue was attributed to the antioxidant enzyme activity stimulated by ozone in the kidneys.^[15] In another experimental study by Calunga *et al.*, the authors performed rectal ozone insufflations on rats with subtotal nephrectomy and observed that the test subjects received ozone treatment had a more active renal compensation mechanism than the ones received no such treatment. The results of the study suggested that ozone treatment prevented vascular damage in the test subjects by increasing angiogenesis and reserving the oxidative damage.^[16] In a study by Caliskan *et al.*, they were conducted to evaluate the effect of ozone therapy, as an immunomodulator and antioxidant in an experimental model of acute pyelonephritis (APN). It was reported that, ozone therapy might be considered as an adjuvant therapy to classical anti-biotherapy to prevent renal inflammation and fibrosis in APN.^[17]

Randomized human model studies on chemical cystitis and ozone treatment for experimental animal models built via methodology has been very rarely performed. Daniluk *et al.* performed a mixture of oxygen and ozone to chronic bladder inflammation and underlined that reproduction in cultures might be prevented by the anti-inflammatory effect and it could be used effectively.^[18] In another study, Null G observed a decrease on drug resistance of bacteria by using intravesical ozonised saline for urinary tract infections.^[19] There were significant decreases of fertility concentration of

microorganisms especially such as *E. coli* in a culture medium observed in other study promoting that approach.^[20] In a case study, Clavo *et al.* performed radiotherapy for a patient having local relapse after radical prostatectomy. It was stated that oxidative damage could be reversed through performing an ozonized bi-distilled water. It was reported that the macroscopic hematuria was eliminated within the 1st week while there was no hematuria in the urine tests within the 3rd week. Furthermore, the tissue damage was healed as seen through cystoscopic analysis at the end of the 8th week.^[21]

The results of our study revealed that near-normal mucosa, sound epithelia and basal membrane layers were existent in the bladders of the in early and late ozone treated groups in parallel with other studies carried out on bladder. It is suggested that the mentioned effects of ozone were brought about through increasing capillary angiogenesis, fibroblast proliferation and collagen formation, and through reducing edema.

CONCLUSIONS

There is no study during which only ozone treatment is applied for IC in literature. This study is unique in this respect. According to the result of this study, ozone treatment decreases the number of leukocyte and mast cells by providing anti-inflammatory effects on experimental cystitis models. Due to its low cost, minimal side effects, and easy usage, ozone treatment is a potentially preferential treatment candidate for IC.

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