Effects of Intravesical Dexpanthenol Use on Lipid Peroxidation and Bladder Histology in a Chemical Cystitis Animal Model

Omer Bayrak, Ilker Seckiner, Mehmet Solakhan, Metin Karakok, Sakip M. Erturhan, and Faruk Yagci

OBJECTIVE To demonstrate the effects of intravesical dexpanthenol use on bladder histology and lipid peroxidation in a chemical cystitis animal model.

METHODS Thirty-five New Zealand rabbits were divided into 3 groups. Cystitis was conducted with transurethral intravesical hydrochloric acid instillation on the subjects in groups I and II. Then, Group I subjects were transurethrally administered intravesical dexpanthenol therapy twice a week, Group II subjects were given only intravesical isotonic NaCl instillation, and Group III subjects were administered intravesical isotonic NaCl instillation without conducting chemical cystitis to create the same stress. Treatment schemes of all groups were arranged in the same manner. After 6-week therapy, the rabbits were sacrificed and histopathologic investigations were carried out to demonstrate changes in the urinary bladder. Serum and tissue malondialdehyde (MDA) values were examined to investigate the effect of dexpanthenol on lipid peroxidation.

RESULTS We observed that the basal membrane and mucosal integrity were maintained, inflammatory cells were suppressed, and MDA levels decreased in group I, which received dexpanthenol therapy. However, it was also observed that mucosal integrity was spoiled, numerous inflammatory cells were accumulated, and MDA levels were significantly increased in group II, which was administered isotonic NaCl.

CONCLUSION In light of our findings, intravesical dexpanthenol therapy could be a new therapeutic approach in the treatment of interstitial cystitis because of its low cost and acceptable side effects. UROLOGY 79: 1023–1026, 2012. © 2012 Elsevier Inc.

Interstitial cystitis (IC) is a chronic, progressive complex of symptoms characterized by chronic pelvic pain and urgent and frequent urination. The etiopathogenesis of IC cannot be explained and an accepted standard therapy is still nonexistent. 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.[1,2]

Pantothenic acid (vitamin B5) is a molecule that contributes to coenzyme A, which is an essential coenzyme for maintaining life and plays a role in essential fats, cholesterol, and steroid hormones. Pantothenic acid has also been found to decrease myeloperoxidase release from granulocytes, leading to the discovery that inflammatory response formed by the proliferation of free oxygen radicals is also decreased, demonstrating its antiinflammatory effect. Dexpanthenol is the molecule that is the alcohol form of pantothenic acid and accelerates antiinflammatory effects by increasing mitotic division.[3-5]

Malondialdehyde (MDA) is created because of the reaction of products from lipid peroxidation with thiobarbituric acid (TBA). A mutagenic, genotoxic, and carcinogenic compound, MDA is the final product of lipid peroxidation. MDA is used as an indicator of lipid peroxidation by measuring it in tissue, blood, and bodily fluids.[6-9]

In this study, we examined the changes that appear as a result of dexpanthenol administered intravesically in our animal model, which we constructed using the IC model. In addition, we investigated tissue and serum MDA levels to establish the effects of dexpanthenol, the antioxidant effect of which has been proven in previous studies, on lipid peroxidation.

From the Department of Urology, Gaziantep University, Gaziantep, Turkey; Department of Urology, Medical Park Hospital, Gaziantep, Turkey; and Department of Pathology, Gaziantep University, Gaziantep, Turkey

Reprint requests: Omer Bayrak, M.D., University of Gaziantep, School of Medicine, Department of Urology, 27310 Gaziantep, Turkey. E-mail: Dromerbayrak@yahoo.com

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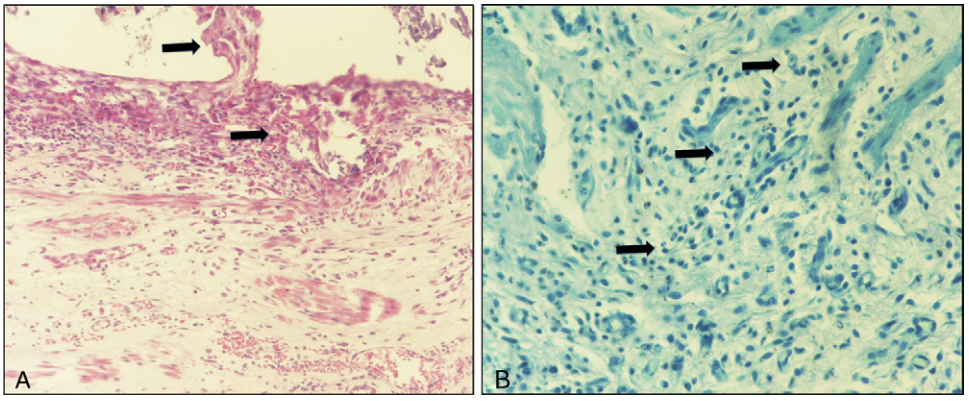

Figure 1. (**A**) Spoiled mucosal integrity caused by the occurring inflammation and numerous leukocyte cells (group II leukocyte cell structures with hematoxylin and eosin) (x200). (**B**) Spoiled mucosal integrity caused by the occurring inflammation, numerous mast cells (group II mast cell structures with toluidine blue) (x200).

Table 1. Comparison of leukocyte and mast cell counts of groups

	Group 1	Group 2	Group 3	P
Mast	3.3 ± 2.7	11 ± 3.3	1.4 ± 0.9	.001
Leukocyte	13 ± 6.3	25 ± 6.6	8.2 ± 2.9	.001

MATERIAL AND METHODS

Animals and the Study Order

Approval from the Local Ethics Committee was obtained for the study, and the study then commenced under decision number 05-2007/18. Forty-seven white, female New Zealand rabbits, with weights ranging between 1200 and 2300 g, were selected as subjects. Urine samples taken from all subjects, included to evaluate the presence of an infection before commencing the study, were examined in a culture environment. Two subjects were excluded from the study because of significant growth in culture 2.

The subjects were divided into 3 groups. There were 15 subjects in each group. Chemical cystitis was formed in subjects in groups I and II through transurethral intravesical hydrochloric acid (HCI) instillation. Chemical cystitis was induced by intravesical instillation of hydrochloric acid (0.2 mL of 0.4 N HCl), as described by Cayan et al[10] and as used in the previous studies of Rivas et al.[11] After anesthesia with an intramuscular administration of 50 mg/kg ketamine and 10 mg/kg xylazine in subjects in groups I and II, one session of hydrochloric acid (0.2 mL, 0.4 N HCl) instillation was conducted. For the application, a sterile 5-Fr feeding tube was implanted transurethrally. After the aspiration of urine in the bladder, HCI was instilled transurethrally and maintained there for 4 minutes.

Group III was established as the control group, and after a similar administration of anesthesia on subjects in this group, 0.9% NaCl intravesical instillation was conducted transurethrally without forming chemical cystitis.

Treatment Protocol

Group I (n = 13). After the formation of chemical cystitis, 10 mL 0.9% NaCl solution containing 500 mg/kg dexpanthenol was instilled through a 5-Fr urethral feeding tube for 6 weeks at 2 sessions per week (on the fourth and seventh days) and maintained for 10 minutes. At the end of the sixth week, the subjects were sacrificed to assess the results, a cystectomy was performed, and histopathologic and biochemical examinations were conducted.

Group II (n = 12). After the formation of chemical cystitis, 10 mL 0.9% NaCl solution was instilled transurethrally through a 5-Fr urethral feeding tube for 6 weeks at 2 sessions per week (on the fourth and seventh days) and maintained for 10 minutes. At the end of the sixth week, the subjects were sacrificed 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-Fr feeding tube for 6 weeks at 2 sessions per week (on the fourth and seventh days) and maintained for 10 minutes. The subjects were sacrificed at the end of the sixth week, a cystectomy was performed, and a histopathologic examination was conducted.

Histopathologic Evaluation

The subjects were sacrificed with 200 mg/kg pentobarbital. After this they were dissected with transabdominal midline incision and a cystectomy was conducted. 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 hematoxylin-eosin; and inflammatory changes, leukocyte counts and mast cells, after staining with toluidine blue, were all evaluated.

An Olympus Bx50 light microscope (Center Valley, PA) 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 were evaluated in each of the 10 subsections by the following scala:

0. No extravascular leukocytes and mast cells
1. Fewer than 20 leukocytes and mast cells
2. 20-45 leukocytes and mast cells
3. >45 leukocytes and mast cells

The scores for all 10 sections were added, divided by 30 (maximum possible score), and multiplied by 100. The leukocytes and the mast cells infiltration scores for an individual bladder

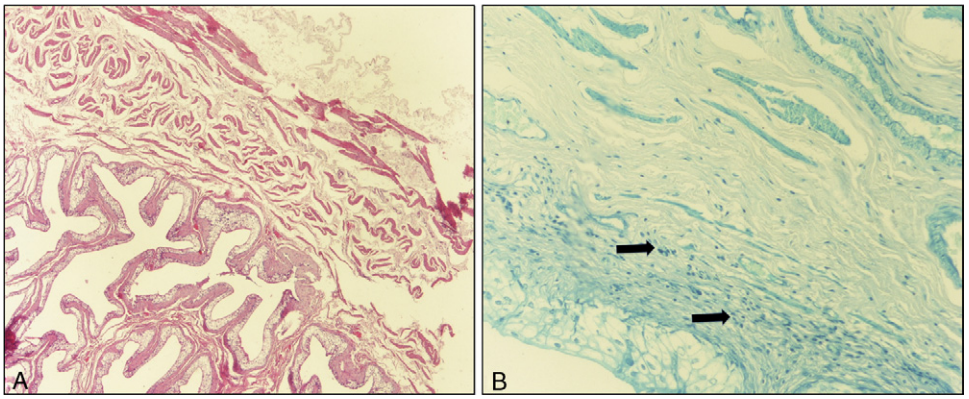

Figure 2. (**A**) Limited inflammatory changes, near normal mucosal integrity, limited leukocyte cells (group I leukocyte cell structures with hematoxylin and eosin) (x200). (**B**) Limited inflammatory changes, near normal mucosal integrity, limited mast cells (group I mast cell structures with toluidine blue) (x200).

Table 2. Comparison of serum and tissue MDA values of groups

	Group 1	Group 2	Group 3
Serum MDA (mmol/L)	3.25 ± 1.51	5.86 ± 2.58*	2.8 ± 1.32
Tissue MDA (μmol/g)	132.3 ± 65.2	398 ± 203.6*	109.1 ± 58.9

* Group II was higher compared with group I, and group III was found to be statistically significant ($P = .001$).

were the average of the 3 cross sections examined. We counted the leukocytes and the mast cells under x200 optical zoom.[12]

Serum and Tissue MDA Evaluation. MDA assessment from peroxidation reaction products by free radicals was performed with the double heating method by Draper and Hadley. The method is based on the principle of producing maximum absorbance in spectrophotometric measurement at 532-nm wavelength by a reaction MDA, which is the final product in fatty acid peroxidation with thiobarbituric acid (TBA).[13] In our study, MDA was investigated in blood samples obtained before the subjects in compliance with said method were killed.

MDA was investigated in tissue sections that were obtained after the rabbits were sacrificed and maintained in aluminum foil at −80°C.[13]

Statistical Analysis

SPSS 11 for Windows (SPSS, Inc., Chicago, IL) statistics software pack was used for statistical estimations. The data were identified as arithmetic mean and standard error. A nonparametric 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 1650 ± 150 g, 1700 ± 150 g, and 1750 ± 150 g, respectively. A statistically significant difference was not established between groups in terms of weight ($P>.05$).

In bladder samples of group 3 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.

After the formation of chemical cystitis, the bladder samples of Group II subjects that were administered 0.9% NaCl demonstrated a spoiled mucosal integrity (Fig. 1A) and the presence of numerous mast and leukocyte cells in the basal membrane as a response to inflammation (Fig. 1B). These findings showed that 0.9% NaCl intravesical instillation did not suppress inflammatory cells, meaning that it does not have a therapeutic effect in the treatment of chemical cystitis.

Mean mast and leukocyte cell counts in group I, which received dexpanthenol treatment, were observed to have significantly decreased compared with group II, which received 0.9% NaCl (Table 1). In the bladder specimen of group I, it was demonstrated that mucosal integrity was maintained (Fig. 2A) and few mast and leukocyte cells were present in the basal membrane in response to inflammation (Fig. 2B).

Mean plasma MDA values of the groups were measured as group I 3.25 μmol/L, group II 5.86 μmol/L, and group III 2.8 μmol/L. The fact that the values of group II were higher compared with groups I and III was found to be statistically significant ($P = .001$, $P = .001$, respectively) (Table 2).

Mean bladder tissue MDA values of the groups were measured as group I 132.3 μmol/g tissue, group II 398 μmol/g tissue, and group III 109.1 μmol/g tissue protein. The increase in group II compared with group III and the decrease in group I compared with group II were found to be statistically significant ($P = .001$, $P = .001$, respectively) (Table 2).

COMMENT

It was demonstrated that the chemical cystitis formed in numerous animal experiments resembled that of interstitial cystitis in terms of both leukocyte counts and mast cell counts, and thus that it is possible to create an animal model for interstitial cystitis. This model can be established with HCl, protamine sulfate, cyclophosph-

amide, and sucralphate.[14,15] In our study, chemical cystitis was created with HCl, and changes that histopathologically were compliant with chemical cystitis in group II 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.[10,12] Further, in compliance with existing literature, our study also established that 0.9% NaCl therapy did not affect leukocyte and mast cell counts in group II patients.

Today, free radicals are suggested to have a significant role in many diseases. The covalent binding of free radicals to the receptors of membranes changes the unsaturated fatty acid/protein ratio and commences lipid peroxidation. Lipid peroxides are unstable and easily break down to form various products including aldehyde, such as MDA. Thus, the measurement of MDA amount reflects the degree of lipid peroxidation in tissues.[6-9] The fact that there was a decrease in serum and tissue MDA levels after dexpanthenol therapy in our study demonstrates the oxidative stress-relieving antioxidant effect of dexpanthenol. Because of these findings, it was established in the treatment of chemical cystitis that the intravesical instillation of 0.9% NaCl solution containing 500 mg/kg dexpanthenol showed an antioxidant effect and caused a significant decrease in serum and tissue MDA value serum.

Pantothenic acid is known to be necessary for normal epithelial functions because of its role in metabolic ways. Its antiinflammatory and antioxidant effects have been proven in previous studies[5,16,17] and were clearly demonstrated in our study as well.

We used dexpanthenol in our experimental chemical cystitis model because of its antiinflammatory, wound healing, epithelization accelerating properties mentioned in the existing literature. We observed that dexpanthenol helps in the recovery of mucosal damage, and that it provided near normal mucosa, intact epithelium, and a basal membrane layer in the bladders of subjects after the treatment.[18-21] We also found that dexpanthenol decreased the MDA levels of serum and tissue, which are the products of lipid peroxidation, to a statistically significant extent because of its oxidative stress-relieving antioxidant effect.

Any studies that were conducted with dexpanthenol therapy alone in IC could not be found in the existing literature. Our study is qualified in this respect, and our findings show that dexpanthenol therapy decreases the said inflammatory cells in comparison with the control group. Although the results of this experimental study are encouraging, it is clear that these results are short-term and the long-term results are questionable.

Because of its low cost, fewer side effects, and ease of use, dexpanthenol is among the candidates for the treatment approaches preferred for IC. In addition to comprehensive studies that evaluate other medical approaches used in the treatment of interstitial cystitis with intravesical dexpanthenol combination therapy, further studies that evaluate the effects of dexpanthenol therapy are also necessary.

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