Comparison of Intravesical Application of Chondroitin Sulphate and Colchicine in Rat Protamine/Lipopolysaccharide Induced Cystitis Model

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Received December 2012
Accepted June 2013

Purpose: To investigate beneficial effect of the readily available colchicine through its intravesical application on protamine/lipopolysaccharide induced interstitial cystitis model in rat and to compare its efficacy to the chondroitin sulphate available for clinical use.

Materials and Methods: Twenty-four Wistar female rats were assigned to control (C), interstitial cystitis (IC), chondroitin sulphate (CS) and colchicine (Col) groups. IC, CS and Col groups received protamine sulphate and lipopolysaccharide (PS/LPS) instillation. Testing agents CS and Col were administered a day after PS/LPS inoculation into the bladders. Rats in Group C received saline solution. CS and Col groups received 1 mL CS (0.2%) and 1 mL Col (0.05 mg/mL). The treatment agents were left in bladders for one hour's duration. Animals were sacrificed 5 days after the inoculation and the bladder tissues were examined histologically to evaluate the amount of extravasated leucocytes, mast cell concentration (by counting total number of cells per 10 high power field (hpf; 1 hpf = ×400 magnification) as well as interstitial tissue edema for each bladder.

Results: Intravesical application of CS reduced significantly the leucocyte and mast cell infiltration as well as interstitial edema compared to group C . The level of reduction in leucocyte and mast cell infiltration in Col group was comparable to that of CS, although the interstitial edema was not resolved.

Conclusion: Intravesical administration of Col decreased leucocyte and mast cell infiltration to the same extent of CS in PS/LPS induced bladder inflammation in rat. Col may be an alternative to other treatment modalities for painful bladder conditions such as IC.

Keywords: cystitis; disease models; animal; rats; chondroitin sulfates; therapeutic use.

INTRODUCTION

Interstitial cystitis (IC) is a chronic debilitating disease of the urinary bladder affecting the individuals, 90% of those being in female gender.[1] It is characterized by painful bladder symptoms, presented with urinary frequency, urgency, and nocturia. Most of the patients have recurrences and requires additional therapies. Beside oral medicines such as pentosan polysulphate, intravesical instillation of hyaluronic acid and chondroitin sulphate (CS) are currently used in the IC treatment. Alteration of urothelial glycosaminoglycan (GAG) layer leading to cause increased bladder permeability is the supposed pathogenetic mechanism, and the symptom relief in IC patients has been reported from intravesically GAG solutions, including CS.[2] The proposed mechanism of this beneficial GAG therapy is restoration of the GAG layer.[3] However, the pathogenetic mechanism suggesting the disruption of GAG barrier is controversial as increasing number of studies suggest the presence of both common inflammatory state with abundance of activated mast cells in IC.[4,5] Some analgesic and anti-inflammatory agents against IC has been used intravesically in parallel to these reports, and the search of effective and definitive therapy still continues.[6,7] To investigate IC pathophysiology different models of bladder inflammation have been studied in experimental animals whereby IC was induced by intravesical administration of an irritant or immune stimulant, systemic and environmentally induced inflammation.[8] The appearance of bladder inflammation, including edema, inflammatory cell infiltration, epithelial damage, fibrosis, venous congestion, and hemorrhage are common to all these previous IC models. In a model, bladder damage was induced in rats by injecting hydrochloric acid in saline solution through the cannula into the bladder, whereas several other models used only protamine sulfate (PS) to initiate bladder inflammation.[9,10] Furthermore, the combined intravesical instillation of PS and lipopolysaccharide (LPS) has been reported to induce IC rat model.[11] Colchicine (Col) is a tricyclic alkaloid. It has analgesic and anti-inflammatory effects through inhibition of granulocyte migration into the inflamed area inhibiting mitotic activity and affecting cells with high turnover. Thus, it inhibits various leukocyte functions and depresses their action at the site of the inflammation.[12] Its application in the inflammatory bladder condition has not been yet reported. In this study, we compared the intravesical Col with the well known CS against PS/LPS induced IC rat model to investigate its potential effect.

MATERIALS AND METHODS

The Maltepe University Institutional Animal Care and Experimental Use Committee approved the protocol of this study. Twenty-four Wistar female rats weighing 175-200 gr were assigned into four groups of 6 animals each [control (C), interstitial cystitis (IC), chondroitin sulphate (CS) and colchicine (Col) groups]. All groups received intramuscular ketamine (50 mg/kg) and xylasine 4 mg/kg for anesthesia. A sterile 24 gauge angiocath was inserted into the bladder through the urethral opening and all treatment agents were administered with 2 mL syringe. Saline solution of 1 mL was instilled into the urinary bladder of group C waiting 75 minutes. IC, CS and Col groups received PS (Sigma-Aldrich, Chemie Gmbh, Munich, Germany) 10 mg/mL in the urinary bladder waiting 30 minutes followed by 2 mg/mL LPS (Sigma-Aldrich, Chemie Gmbh, Munich, Germany) instillation waiting additional 45 minutes. For treatment purpose, a day after the PS/LPS inoculation, rats in C and IC groups s received 1 mL saline solution, and rats in CS and Col groups received 1 mL CS (Gepan Instill Farmatek, Istanbul, Turkey) and 1 mL 0.05 mg/mL Col (Sigma-Aldrich, 0.5 mg colchicine powder solved in 10 mL sterile H_2O solution) through 24 gauge angiocath intravesically, respectively. Treatment agents were left in the bladder in 1 hr of duration. Animals were sacrificed with high dose anesthesia 5 days later and their bladders were removed and stored in 10% formalin solution. The samples were blindly reviewed by a pathologist. The specimens were cut by a longitudinal section and both two pieces of a bladder specimen were processed for routine histopathological examination. Formalin fixed specimens were embedded in paraffin and 3 micrometer thick sections from each paraffin block were stained with Hematoxylin and Eosin (H&E) and in order to the detect mast cells with Toluidine Blue (TB; Bio-Optica, Milan, Italy) respectively.

Severity of inflammation was examined by using optical microscope (Olympus BX51 , Tokyo, Japan) in each section according to 4 criteria including; leukocyte infiltration (by

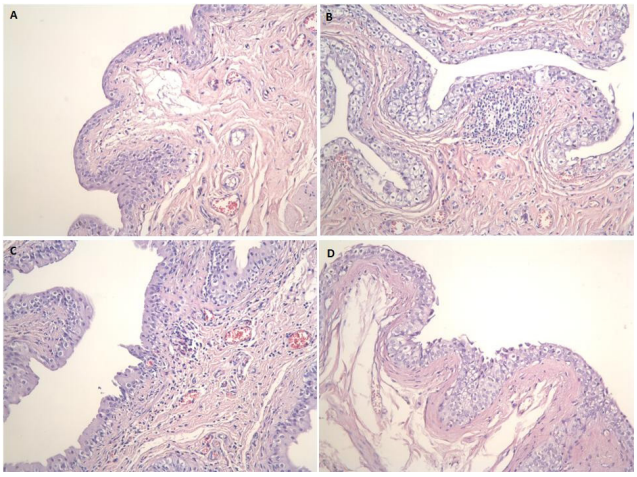

Figure 1. Normal urothelial mucosa in control group (A). Inflammatory reaction composed of mainly lymphocytic aggregate in protamin and lipopolisacharide induced interstitial cystitis (B) which was reduced following intravesical application of chondroitin sulphate (C) and colchicine (D). H & E × 200 magnification; 1cm = 63.2 micrometer.

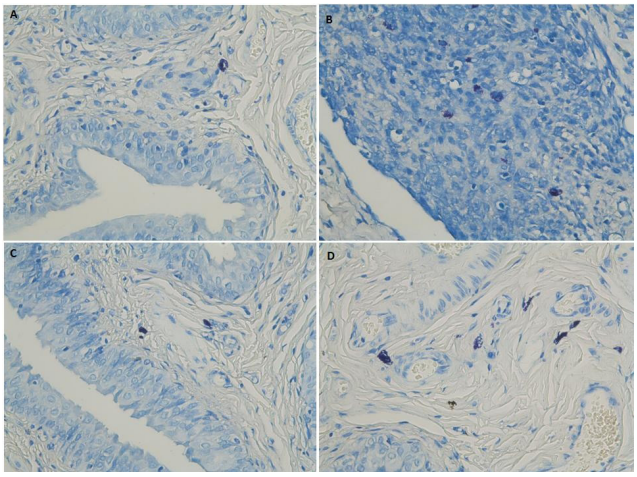

Figure 2. Normal urothelial mucosa in control group with limited number of mast cells (A). Inflammatory reaction composed of mainly lymphocytes accompanied with numerous mast cells in protamin and lipopolisacharide induced interstitial cystitis (B) which was reduced following intravesical application of chondroitin sulphate (C) and colchicine (D). Toluidine Blue × 400 magnification; 1 cm = 31.6 micrometer.

counting extravasated leukocyte number per 10 high power field (hpf; 1 hpf = × 400 magnification]), edema (0 = no edema, 1 = mild edema; an increase of less than twice the width of submucosa, 2 = severe edema; an increase of more than twice the width of submucosa), mast cell infiltration (by counting total number of mast cells per 10 hpf (Table and Figures 1 and 2).

All values are expressed as the mean ± SD and statistical significance was determined using Kruskal Wallis and Mann Whitney *U* tests. $P < .05$ was considered as statistically significance.

RESULTS

Mean leucocyte count per 10 hpf in C, IC, CS and Col groups were 3.67 ± 3.14, 59.17 ± 37.29, 14.0 ± 18.6 and 17.3±21.74, respectively (Table). Intravesical administration of CS and Col reduced significantly leucocyte count ($P = .007$) (Figures 1 and 2). Mean mast cell count per 10 hpf in C, IC, CS and Col groups were 21.67 ± 5.01, 40.17 ± 6.43, 24.50 ± 9.5 and 22.83 ± 13.36, respectively (Table). Intravesical application of CS and Col reduced significantly mast cell infiltration ($P = .027$) (Figures 1 and 2). The bladder tissue edema was negative in C and CS groups whereas positive in IC and Col groups. The level of reduction in leucocyte and mast cell infiltration in Col group was to the same extent of CS group ($P = .335$ and $P = .517$ respectively), although the interstitial edema was not resolved.

DISCUSSION

IC in generally is a painful disease with its devastating symptoms. Its exact causes and pathogenesis remain to be identified. Several animal models have been developed to investigate IC. Most of the models were based on the hypothesis that an initial insult to the bladder is due to a permeability change in its GAG lining, leading noxious urinary solutes to penetrate the underlying epithelium and smooth muscle, in turn resulting in inflammatory reactions.[13] Although, among the current therapies intravesical CS and hyaluronic acid have been suggested to restore the GAG layer, the protective role of GAG layer against noxious urinary solutes and whether instillation of such agents into the bladder restores the damaged mucosal barrier is controversial.[14] Using chamber experiments showed that GAG contributes little or nothing to mammalian urothelial barrier function.[15] Besides, many other treatment alternatives are tried due to unsatisfying results with GAG replenishing agents, among these antihistamines act on mast cell involvement and intravesical dimethyl sulphoxide was proven to reduce bladder inflammation and

stabilize mast cells.[16] In an in-vitro bladder IC model, a reduction of tumor necrosis factor alpha (TNF-α) induced interleukin 6 release after treatment with hyaluronic acid and CS was observed which indicated that their anti-inflammatory action played the predominant role in the treatment of IC.[17] This opinion led us to evaluate the supposed beneficial effect of Col, an old and cheap molecule which is underestimated among a wide variety of available anti-inflammatory drugs, and to compare its effect on bladder inflammation to that of CS. Since it inhibits various leukocyte functions and depresses the action of the leukocytes and of the fibroblasts at the site of the inflammation, it is commonly used in chronic diseases such as familial Mediterranean fever, primary biliary cirrhosis, alkaline esophagitis, psoriasis, Behçet's disease, aphthous stomatitis, chronic urticaria unresponsive to antihistamine. Its anti-inflammatory effect has been linked to its disruption of microtubules in neutrophils thereby inhibiting their migration with chemotactic factors. Furthermore, Col was also shown to alter the distribution of adhesion molecules on the surface of both neutrophils and endothelial cells, leading to the inhibition of interaction between endothelial cells and leucocytes interfering with their transmigration.[18] The evidence suggested that the anti-inflammatory effect of Col was through various pathways.[19] The previous studies reported that the suppression of enzymes such as caspase-1, endothelial nitric oxide synthase 3 or other mediators of chemotaxis led to inflammatory restraint following Col administration.[18] The data presented in our study showed that intravesical Col instillation reduced both leucocyte and mast cell counts in a rat model of bladder inflammation in a comparable level to that of CS. However, the bladder tissue edema in IC did not disappear in Col group in contrast to CS group which might be due to Col's inability to restore bladder GAG layer unlike CS. In parallel to previous reports suggesting that the agents used for painful bladder conditions reduced either inflammation or mast cell activity, our findings can be helpful in addressing the problems associated with chronic inflammation in the human bladder.

There are limitations of our study; first, the present model is not identical to chronic human IC as it induces a short-term acute inflammation in the bladder and, the confirmation of IC in rat bladder remains within histological evaluation without comparison of urinary frequency in treatment groups due to lack of metabolic cages; second, although ketamine was reported to induce cystitis itself in some cases, it is known that this was encountered especially among ketamine abusers. Additionally, the bladder tissues in Group C did not show evidence of cystitis in the present study.[20] Lastly, different concentrations of Col may be further investigated as no previous data is available in the literature for its intravesical instillation.

CONCLUSION

In conclusion, with the exclusion of edema, intravesical administration of Col decreased leucocyte and mast cell infiltration to the same extent of CS in PS/LPS induced IC. This potential action may be useful on bladder inflammation and should be further investigated.

CONFLICT OF INTEREST

None declared.

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