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Artificial cystitis model: The response of Sprague-Dawley rats' bladder to surfactant instillation

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Abstract

Aim: Chemical cystitis is a disease that causes mortality and morbidity. Its treatment is really difficult. We aimed to present a low-cost, successful, easy-to-access, and easily applicable treatment option for the treatment of this disease.

Materials and Methods: Sixteen Sprague-Dawley rats were divided into 4 groups. 1 group is a sham group, 2nd is saline, 3rd surfactant before chemical cystitis, and 4th group is surfactant after chemical cystitis. On the 4th day, all groups are sacrificed and their bladders are sent for histochemical examination. The pathological data of the subjects were evaluated according to the levels of hemorrhage, edema, inflammation, and congestion. Data were evaluated by scoring between 0-2. 0 none, 1 Moderate, and 2 highly present.

Results: There was no statistically significant difference found between the groups in terms of all parameters ($p=0.111$). The difference was not found statistically significant in the pairwise comparison of the groups ($p>0.05$).

Conclusion: Surfactant had no preventive or therapeutic effect on the chemical cystitis developed in mice. The simple saline application seems to be more effective.

Introduction

Cystitis is defined as general inflammation of the bladder [1]. The inflammation can be bacterial and non-bacterial and also can be acute and chronic. The severity of the disease ranged from mild to severe such as suprapubic discomfort to significant hemorrhage, which can be life-threatening. Non-bacterial cystitis can occur secondarily from radiotherapy and chemically irritation of the bladder [2]. Despite all the medical knowledge and developments of our age, there are still great difficulties in the treatment of chemical cystitis in the field of urology. Inadequate treatments significantly reduce the quality of life of people suffering from this disease.

Radiation and chemical cystitis can be misdiagnosed because they mimic other types of cystitis. Anamnesis is very important in the diagnosis of the disease. Radiation cystitis is seen in patients who have received radiotherapy. Chemical cystitis is usually seen in patients who have received chemo/immunotherapy intravesically or intravenously. In addition, it can also occur when the bladder is directly exposed to chemical irritants (overly sensitive women use large amounts of soap, etc., for cleaning). Bacterial cystitis must be excluded from the diagnosis [3, 4].

The lack of standardization in treatment and the lack of a successful treatment method leaves physicians in hesitation. In addition, the lack of randomized prospective studies for treatment, lack of experience, suspicion of efficacy in treatment, and fear of complications make doctors uneasy about the treatment of this disease.

There have been many studies conducted with surfactants in recent years. Studies are showing that surfactant forms a protective barrier by reducing surface tension [5]. In the light of this information, we aimed to use surfactants in our experimental study. We can treat chemical cystitis permanently with surfactant. Then it will shed light on future studies.

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Materials and Methods

We performed our study in an experimental medical application and research center animal laboratory after obtaining the approval of the animal ethics board (Health Sciences University Hamidiye Animal Experiments Local Ethics Committee, 2019-09/03- 46418926-605.02).

The source equation method was used to determine the appropriate sample size should be used in order to ensure statistical competence by using a small number of animals when determining the power analysis. (Error degree of freedom = E = Total Animal Number – Number of Groups) $10 < E < 20$ indicates that the number of animals is sufficient (*). E=16-4=12 for our study.
* Jaykaran C, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother. 2013;4(4):303–6.

16 Sprague-Dawley male rats weighing 200-300 g were used. Female rats synthesize different degrees of estrogen during the menstrual cycle. Since the antioxidant property of estrogen is known, the study was planned with male rats. The animals were brought to the environment to acclimatize to the new environment for 7-10 days. They were fed with regular tap water and rat chow as much as they could drink and eat, and they were illuminated under white light for an average of 12 hours during the day and 12 nights. The living spaces are arranged to be a calm and stress-free environment.

Sedation was done by ketamine (85 mg/kg). Anesthesia was done by xylazine (12.5 mg/kg) intraperitoneally. Local anesthesia was done by Catha-gel. Chemical cystitis was induced by intraperitoneal administration of a single dose of 150 mg/kg cyclophosphamide, as described in the literature [4, 7, 8]. 16 Sprague Dawley rats were divided into four groups. Chemical cystitis was formed in the 4th group on the 1st and the others on the second day.

On the 1st day, nothing was done to the 1st group. The 2nd group was instilled with saline, and the 3rd group was instilled with 0.6cc surfactant intravesically. On the 2nd day, chemical cystitis was created in the first 3 groups as planned, while 0.6 cc surfactant instillation was performed in the 4th group. On the 3rd day, nothing was done to the 1st group. Saline was instilled in the 2nd group, and surfactant was instilled in the 3rd and 4th groups. On the 4th day, all animals were sacrificed using the cervical dislocation method, and their bladders were excised for histopathological examination. (Figure 1), (Table 1).

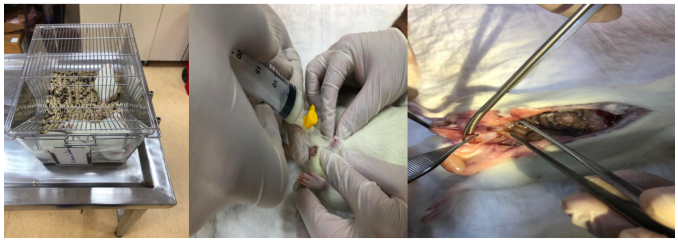

Figure 1. Housing, surfactant instillation, bladder excision.

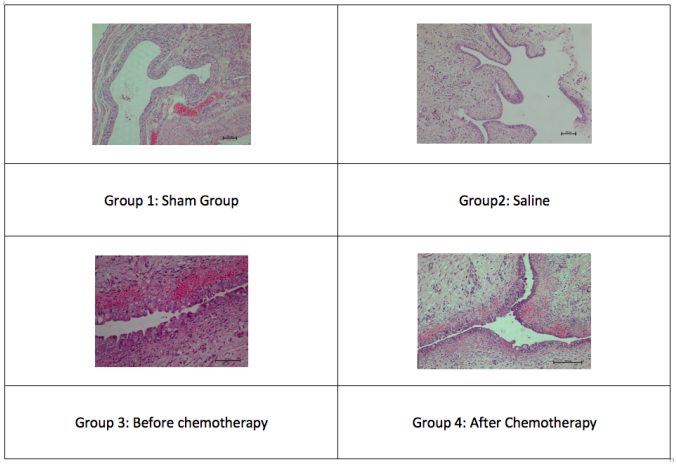

Figure 2. Evaluation of the degree of inflammation by hematoxylin-eosin staining of tissues under the light microscope.

Histopathologic analysis

A single independent pathologist performed histopathologic analysis. She completed the analysis under a light microscope. She does not know the groups. The study was randomized and double-blinded. Until the macroscopic examination, the vesical tissues were fixed in 10% formol. Stained with hematoxylin, eosin, and Masson's trichrome for histochemical analysis. (Figure 2).

Statistical analysis

As a statistical method in the analysis; data analyses were performed using SPSS Statistics 20.0 software (SPSS Inc., Chicago, IL, USA). As a statistical method in the analysis of data in the research; descriptive analyzes were given with frequency distributions, percentage, mean, standard deviation or median values. Chi-Square test was used to measure the difference between groups data.

Qualitative and quantitative data

The pathological data of the subjects were evaluated according to the levels of hemorrhage, edema, inflammation, and congestion. Data' were assessed by scoring between 0-2. 0 none, 1 Moderate, and 2 highly present.

Results

16 rats were included in our study. On the 3rd day of the study, one rat died in the saline group due to an anesthetic complication.

The pathological data' total scores was shown in Table 2.

There was no statistically significant difference between the groups regarding hemorrhage ($p=0.107$). The difference was not found statistically significant in the pairwise comparison of the groups ($p>0.05$) (Table 3).

There was no statistically significant difference between the groups regarding edema ($p=0.180$). The difference was not found statistically significant in the pairwise comparison of the groups ($p>0.05$) (Table 4).

Table 1. Groups and treatment plans.

	1st-day	2nd-day	3rd-day	4th-day
1st group	Do nothing	CC	Do nothing	Sacrification and then histopathological examination
2nd group	IV S	CC + IV S	IV S	Sacrification and then histopathological examination
3rd group	IV Surfactant	CC	IV Surfactant	Sacrification and then histopathological examination
4th group	CC	IV Surfactant	IV Surfactant	Sacrification and then histopathological examination

CC: Chemical cystitis, IV: Intra Vesical, S: Saline.

Table 2. Number of groups and signs of inflammation.

	SNU	Hemorrhagia	Edema	Inflammation	Congestion
BC (Group 3)	1	2	1	2	2
	2	0	0	1	1
	3	0	1	1	1
	4	0	1	0	1
AC (Group 4)	1	2	2	2	2
	2	2	1	2	1
	3	2	1	2	2
	4	1	1	1	1
Sham (Group 1)	1	1	1	1	1
	2	2	2	2	2
	3	2	2	2	2
	4	1	2	2	1
S (Group 2)	1	0	1	0	0
	2	0	0	0	0
	3	1	1	1	1

SNU: Serial number of units, BC: before chemical cystitis, AC: After Chemical cystitis, S: Saline.

Table 3. Distribution and statistical results of hemorrhage as a sign of inflammation according to groups.

		Groups				Total	p
		BC	AC	Sham	S		
Hemorrhagia	0	3 (75%)	0 (0%)	0 (0%)	2 (66.67%)	5	0.107
	1	0 (0%)	1 (25%)	2 (50%)	1 (33.33%)	4	
	2	1 (25%)	3 (75%)	2 (50%)	0 (0%)	6	
Total		4	4	4	3	15	

SNU: Serial number of units, BC: before chemical cystitis, AC: After Chemical cystitis, S: Saline.

Table 4. Distribution of edema as a sign of inflammation according to groups and statistical results.

		Groups				Total	p
		BC	AC	Sham	S		
Edema	0	1 (25%)	0 (0%)	0 (0%)	1 (33.33%)	2	0.180
	1	3 (75%)	3 (75%)	1 (25%)	2 (66.67%)	9	
	2	0 (0%)	1 (25%)	3 (75%)	0 (0%)	4	
Total		4	4	4	3	15	

SNU: Serial number of units, BC: before chemical cystitis, AC: After Chemical cystitis, S: Saline.

There was no statistically significant difference between the groups in terms of inflammation (p=0.198). The difference was not found statistically significant in the pairwise comparison of the groups (p>0.05) (Table 5).

There was no statistically significant difference between the groups in terms of congestion (p=0.111). The difference was not found statistically significant in the pairwise comparison of the groups (p>0.05). (Table 6)

Discussion

Many prophylactic and operational methods are used in chemical cystitis. Hyperhydration and Mesna (2-

Table 5. Distribution and statistical results of leukocyte infiltration as a sign of inflammation according to groups.

		Groups				Total	p
		BC	AC	Sham	S		
Inflammation	0	1 (25%)	0 (0%)	0 (0%)	2 (66.67%)	3	0.198
	1	2 (50%)	1 (25%)	1 (25%)	1 (33.33%)	5	
	2	1 (25%)	3 (75%)	3 (75%)	0 (0%)	7	
Total		4	4	4	3	15	

SNU: Serial number of units, BC: before chemical cystitis, AC: After Chemical cystitis, S: Saline.

Table 6. Distribution and statistical results of lcongestion as a sign of inflammation according to groups.

		Groups				Total	p
		BC	AC	Sham	S		
Congestion	0	0 (0%)	0 (0%)	0 (0%)	2 (66.67%)	2	0.111
	1	3 (75%)	2 (50%)	2 (50%)	1 (33.33%)	8	
	2	1 (25%)	2 (50%)	2 (50%)	0 (0%)	5	
Total		4	4	4	3	15	

SNU: Serial number of units, BC: before chemical cystitis, AC: After Chemical cystitis, S: Saline.

mercaptoethanol sodium sulfonate) are most commonly used for prophylaxis. In addition, Alum (1-2%) solution, oral/intravesical antifibrin applications, and hyperbaric oxygen therapy are also used [2, 9, 10]. The effects and successes of these drugs are limited.

In the literature about treatment of cystitis, there are studies with various surfactants. In the study of Thompson et al., chondroitin sulfate was used to treatment of cystitis. Chondroitin sulfate acts locally by initiating and entertaining a urothelial coating, a biofilm formation, thus repairing the damage of the glycosaminoglycan barrier of the bladder epithelium (**). Sodium hyaluronate was used by Sommariva et al. and symptoms such as dysuria and pain from chemical and radiation cystitis were solved for 97% of patients (***). Sodium pentosanpolysulphate was another treatment agent for cystitis used by Manikandan et al. And Duthie et al. in special groups and they have reported successful results (****, *****).

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*** Sommariva ML, Sandri SD and Ceriani V: Efficacy of sodium hyaluronate in the management of chemical and radiation cystitis. Minerva Urol Nefrol 62: 145-150, 2010.

**** Manikandan R, Kumar S and Dorairajan LN: Hemorrhagic cystitis: A challenge to the urologist. Indian J Urol 26: 159 166, 2010.

***** Duthie G, Whyte L, Chandran H, Lawson S, Velangi M and McCarthy L: Introduction of sodium pentosan poly-sulfate and avoidance of urethral catheterisation: Improved outcomes in children with haemorrhagic cystitis post stem cell transplant/chemotherapy. J Pediatr Surg 47: 375-379, 2012.

Surfactant is known to reduce surface tension in the lung and is essential for the success of respiration, especially in newborns [5]. There are four subtypes of surfactant. Surfactants A and D direct the defense cells, while B and C have been described in the literature as regulating surface tension [5, 11].

In the light of this mechanism, the places where the surfactant is secreted in the body have been investigated, and several studies have demonstrated its role in the urinary system. In the study of Beileke et al., it was shown that surfactant is secreted from Sertoli cells in the testis. It has been demonstrated that surfactant is secreted at a lower rate in patients with testicular tumors [12].

In the study of Bassorgun et al., it was shown that surfactant is secreted from the renal tubules. In addition, it has been observed that the surfactant is secreted less than usual in patients with renal cell tumors [13].

In the study of Hashimoto J et al., it was shown that surfactant reduces the proliferation and adhesion of Escherichia coli, which is the most common urinary tract pathogen bacteria, in the urethelium [14].

In the study of Pechey et al., Although there are such promising studies in the literature, it was found that the effect of surfactant was not different from the sham group in our study. It has been shown that simple serum physiological administration is more beneficial to rats.

Conclusion

On the formation of chemical cystitis or in developed cystitis, no preventive or therapeutic effect of surfactant was detected in our animal model. The simple saline application seems to be more effective.

Ethics approval

We performed our study Health Sciences University Hamidiye Animal Experiments Local Ethics Committee, after obtaining the approval of the animal ethics board (2019-09/03- 46418926-605.02).

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