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Original Article *Özgün Araştırma*

EFFECTS OF CORTICOSTEROID AND ANTIHISTAMINIC INJECTIONS ON COLD-INDUCED STRESS ON RAT BLADDER TISSUE: AN EXPERIMENTAL STUDY

Kortikosteroid ve Antihistaminik Enjeksiyonların Sıçan Mesane Dokusunda Soğuk Kaynaklı Stres Üzerine Etkileri: Deneysel Bir Çalışma

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ABSTRACT

Objective: Cold is a stress-inducing factor that can cause changes in bladder function in various ways. The present study is intended to investigate the effects of corticosteroid and antihistaminic treatment on acutely and chronically induced cold stress.

Material and Methods: Forty-two female rats were randomly divided into seven groups as follows: Control group; Acute cold-stress (ACS) group; ACS+ corticosteroid (CORT) group; ACS+CORT + Antihistaminic group; Cronic cold-stres (CCS) group; CSS+CORT group; CSS+CORT+Antihistaminic group. On the 15th day after these treatments, bladders of the rats were harvested for histopathological examinations under general anesthesia and fixed with 10% neutral buffered formaline. *Hematoxylin and eosin* and toluidine blue stainings were performed.

Results: The comparison based on mast cell count yielded the highest value in the CCS group in comparison to the control group. The lowest value was harvested in the CCS + CORT group. The comparison between the CCS groups revealed the highest polymorphonuclear leucocyte (PNL) values in the lamina propria in the CCS group, whereas the CORT and CORT + Antihistaminic treatments were found to have significantly decreased the PNL values in the lamina propria. While the PNL counts in the epithelium were high in the ACS and CCS groups, the results in the ACS and CCS groups that were treated with CORT and/or antihistaminic were revealed to be similar with those in the control group. It was discovered that antihistaminic injection in addition to CORT decreased the lymphocyte counts in epithelium in CCS more efficiently than CORT alone did.

Conclusion: The present research revealed that corticosteroid treatment reduced inflammatory cell infiltration and decreased mast cell count. A more evident amelioration was observed particularly in chronic cold stress.

Keywords: *Cold stress, cystitis, rat, corticosteroid, antihistaminic*

ÖZ

Amaç: Soğuk, mesane fonksiyonunda değişik şekillerde hasara neden olabilen stres indükleyici bir faktördür. Bu çalışmada kortikosteroid ve antihistaminik tedavisinin akut ve kronik olarak indüklenmiş soğuk stres üzerine etkilerini araştırmak amaçlanmıştır.

Gereç ve Yöntemler: 42 adet dişi sıçanrastgele yedi gruba ayrılmıştır. Gruplar şu şekildedir;

Kontrol grubu, Akut soğuk stress (ACS) grubu; ACS+ kortikosteroid (CORT) grubu; ACS+CORT+antihistaminik grubu; Kronik soğuk stress (CCS) grubu; CSS CORT grubu; CSS+CORT+antihistaminik grubu. Bu uygulamalardan sonraki 15. günde, genel anestezi altında sıçanlara sistektomi uygulandı ve mesaneleri çıkarıldı. Çıkarılan dokular %10 nötral tamponlu formalin içerisinde fikse edilip takip edildikten sonra hematoksilen eozin ve Toluidin mavisi ile boyandı.

Bulgular: Mast hücre sayısına göre yapılan değerlendirmede, kontrol grubuna kıyasla en yüksek değer CCS grubunda gözlenmiştir. En düşük değer CCS + CORT grubunda elde edilmiştir. CCS grupları arasındaki karşılaştırmaya göre, lamina propriadaki en yüksek polimorfonükleer lökosit (PNL) değerleri CCS grubunda görülürken, CORT ve CORT+Antihistaminik tedavilerinin, lamina propriadaki PNL değerlerini önemli ölçüde azalttığı gözlendi. Epiteldeki PNL sayıları ACS ve CCS gruplarında yüksek iken, CORT ve/veya antihistaminik ile tedavi edilen ACS ve CCS gruplarındaki sonuçların kontrol grubundakilerle benzer olduğu ortaya çıkmıştır. CORT'a ek olarak antihistaminik enjeksiyonun, CCS'deki epitelde bulunan lenfosit sayısını, sadece CORT'dan daha verimli bir şekilde azalttığı görüldü.

Sonuç: Mevcut çalışma, kortikosteroid tedavisinin, enflamatuar hücre infiltrasyonunu ve mast hücre sayısını azalttığını ortaya koymuştur. Özellikle kronik soğuk stresde daha belirgin bir iyileşme olduğu gözlenmektedir.

Anahtar Kelimeler: *Soğuk stress, sistit, sıçan, kortikosteroid, antihistaminik*

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INTRODUCTION

Cold stress of environmental stressors is listed among the most commonly studied models thanks to its physiological and psychological effects (1). Especially people and animals living in cold areas frequently have to face the adverse effects of cold stress on organisms (2). Working and living in cold conditions may increase the risk of cold-induced chronic and degenerative diseases. Cold stress syndrome caused by cold exposure disrupts energy metabolism and immunological functions and leads to endogenous or secondary diseases (3). It has been found out that stress induced by low temperatures in professional settings where employees spend a large portion of the day adversely affects productivity. Moreover, such issues as attention and perception related problems, shivering, fatigue, decreased blood flow to extremities, pupil dilatation, and skin inflammation (4). Construction workers, in particular, are exposed to and feel the adverse effects of cold environment at construction sites more severely than employees in other professions (5). In previous studies, cold stress has been systematically created in rats and post-exposure neuronal loss has been reported in their CA1 and CA3 areas. However, the mechanism pertaining to neuronal loss and cold stress is still unclear. In order to reestablish homeostasis during stress, cortisol is released. Yet, prolonged stress may result in hyperactivation of the HPA axis and prolonged increases in corticosteroid (CORT) level may cause irreversible damages in the hippocampus (3). Cold stress may lead to various functional and structural dysfunctions in gastrointestinal system as well. Cold stress has been reported to have induced gastric and mucosal legions, decrease in phospholipid levels in mucosal barrier and stomach's absorptive capacity, gastric emptying, reduced gastric mucosal thickness and gastric enterochromaffin cell count and reduced serotonin content of enterochromaffin cells (6-12). Additionally, histopathological dysfunctions, such as hemorrhage, hyperemia, and epithelial damage may occur in the small intestine and damage in intestinal mucosa has been observed to deteriorate over the course of a prolonged stress (2,13). Besides, cold stress has been reported to increase mast cell population and IgM, IgA, IgG, and IL-7 levels in the small intestine and has been shown to trigger infiltration in the small intestine (2,14,15). Environmental temperature plays a significant role in the regulation of blood pressure. A study has revealed that offspring of dams exposed to cold suffers from high systolic and diastolic blood pressure and low urine volume and sodium excretion, which are due to increased sympathetic activity (16). Cold is a stress-inducing factor that can cause changes in bladder function in various ways. It can directly affect cold-sensitive afferent fibers in the bladder. The available literature suggests that bladder's response to cold results from a detrusor contraction specifically mediated by menthol-sensitive unmyelinated C-fibers. Furthermore, cold can modify bladder's tissue structure. Exposure to cold has been shown to induce histological changes, such as urothelial damage, glycosaminoglycan (GAG) layer disruption, inflammation and interstitial cystitis (17). These are caused by mast cell infiltration. Cold stress leads to urothelial damage. Besides, neuropeptides, such as substance P, are released from primary afferent fibers during stress and they activate the mast cells in the bladder. These findings account for the role of various stress conditions in the pathophysiology of chronic bladder diseases (18). Demir et al. have found that in vivo exposure to cold causes serious bladder damage. This urothelial damage and lamina propria along with mast cell infiltration and PNL and its inflammation have been found to be more significant in the chronic cold stress group. These histological variations have been associated with reduced bladder contraction (18).

The present study aims to investigate the effects of corticosteroid and antihistaminic treatment on acutely and chronically induced cold stress.

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MATERIALS AND METHODS

This study was approved by Kafkas University Experimental Animal Research Ethics Committee (Date: 29.01.2015; decision number: 2015/02). Animal procedures were performed according to the principles in "Guide for the Care and Use of Laboratory Animals" (19). In this study, 42 female Sprague Dawley rats weighing 220-300 g were used. The rats were supplied by the Kafkas University Experimental Research Center. All the rats were housed in pairs in suitable cages in an animal room maintained at standard humidity (45-50%) and temperature (22±2°C) in 12-hour light and 12-hour darkness. They were fed with standard food and water ad libitum.

Randomization

The 42 female rats were randomly divided into seven groups. Randomization was carried out by giving the rats sequential numbers and randomly assigning them to groups using a random-numbers table.

Experimental procedure

The groups are as follows:

Group 1 (Control (C), n=6): Untreated group

Group 2 (Acute cold-stress (ACS), n=6): Acute cold-stress group treated at +4^{0}C for 8 h

Group 3 (ACS+corticosteroid (CORT), n=6): CORT injected after being treated at +4^{0}C (ACS) for 8 h

Group 4 (ACS+CORT+Antihistaminic, n=6): group CORT- and antihistaminic-injected after being treated at +4^{0}C (ACS) for 8 h

Group 5 (Cronic cold-stress (CCS), n=6): Chronic cold-stress group treated at +4^{0}C for 4 h daily for 21 days

Group 6 (CSS+CORT, n=6): CORT-injected after being treated at +4^{0}C for 4 h daily for 21 days

Group 7 (CSS+CORT+Antihistaminic, n=6): CORT- and antihistaminic-injected after being treated at +4^{0}C for 4 h daily for 21 days

The rats in Group 1 were not treated, while the ones in Group 2 were kept at +4^{0}C for 4 h as described in the related literature to induce ACS (18). The ACS rats in Group 3 were injected with one dose of CORT intraperitoneally (IP), and the ones in Group 4 with a single dose of CORT IP (1 mg/ml, Dekort, Deva Holding A.Ş, İstanbul, Türkiye) and antihistaminic IP (3 mg/kg, Feniramin, Sandoz İlaç Sanayi ve Tic. A.Ş, İstanbul, Türkiye) following the creation of ACS. The rats in Group 5 were kept at +4^{0}C for 4 h over 21 days daily as described in the related literature to induce CCS (18). The CCS rats in Group 6 were injected with a single dose of CORT, while the ones in Group 7 with a single dose of CORT and antihistaminic via intraperitoneally after the creation of CCS.

On the 15th day after these treatments, the rats were anesthetized with ketamine hydrochloride (50 mg/kg, Ketalar®, Pfizer, Turkey) and Xylazine (10 mg/kg, Rompun®, Bayer, Canada). Then a midline lower abdominal incision was made. Bladders of the rats were harvested for histopathological examinations and fixed with 10%neutral buffered formaline.

Histopathological Evaluation

Tissue samples were put in 10%neutral buffered formalin. At the end of the 48-hour fixation, the tissues were subjected to routine tissue processing protocol. The tissues were embedded into liquid paraffin blocks and 4-micron thick sections were cut out from each paraffin block with microtome (Leica RM 2125 RTS). Then they were stained with routine hematoxylin-eosin (H&E) method for histopathologic examination and Toluidine Blue method for mast cell count. The stained preparations were examined under a light microscope (Zeiss AxioScope A1, x400 magnification). The following parameters were examined in each section:

Mast cell count in the lamina propria

Polymorphonuclear leukocyte (PNL) count in the epithelium

PNL count in the lamina propria

Lymphocyte count in the epithelium

Lymphocyte count in the lamina propria

The examination was carried by counting all the cells in all the sections.

Statistical Analysis

The data were analyzed with IBM SPSS Statistics Data Editor Version 21. Independent-samples t-test was employed to determine the difference between the means of two groups and the significance of this difference. The obtained results were tabulated. A 95%confidence interval was adopted for the purpose of the study. Differences of $p<0.05$ were considered significant.

RESULTS

The mean and standard deviations of mast cell count of the groups in different histological locations are presented in Table 1.

In consideration of the mast cell counts in the lamina propria, the comparison of Group C with the other groups yielded a significant difference in the CCS group (p=0.02). Moreover, a decrease was observed in the CCS+CORT group in comparison with Group C and this decrease was found to be statistically significant (p=0.004). The mast cell counts in the lamina propria in Group 2, 3, 4, 5, and 7 were found to be similar to the ones in Group C. The two-group comparison revealed that the mast cell count in the lamina propria was higher in the ACS group than in the CCS group (p=0.004). The comparison between the ACS and CCS+CORT groups yielded a decrease in the CCS+CORT group and this decrease was found to be statistically significant (p=0.000). A significant decrease was obtained in the CCS+CORT group as a result of the comparison between the ACS+CORT+Antihistaminic group and the CCS+CORT group (p=0.04).

The comparison of Group C with the ACS group and the CCS group in terms of the PNL count in the epithelium revealed a significant increase in the ACS and CCS groups (p=0.01 and 0.00, respectively). The results in the other groups were found to be similar with Group C.

The comparison between the control group and the other groups in terms of PNL counts in the lamina propria revealed a significant difference solely in the CCS group (p=0.02). The results of the other groups were similar with Group C. The comparison between the ACS and CCS groups led to higher PNL counts in the lamina propria in the CCS group (p=0.05). Nevertheless, significant decreases in both groups were observed as a result of the comparison of the CCS group with the CCS+CORT and CCS+CORT + antihistaminic groups (p=0.02 and 0.03, respectively).

The comparison between the control group and the other groups in terms of the lymphocyte counts in the epithelium revealed a significant decrease in Group 3 (ACS+CORT) and 6 (CCS+CORT) (p=0.014 and 0.002, respectively). Furthermore, a significant increase was observed in the CCS+CORT group as a result of its comparison with the ACS+CORT group (p=0.00). The comparison between Group 6 (CCS+CORT) and Group 7 (CCS+CORT + antihistaminic) in terms of the lymphocyte count in the epithelium yielded a significant decrease in the CCS+CORT+Antihistaminic group (p=0.013). No significant difference was observed between the groups in terms of the lymphocyte count in the lamina propria. Toluidine blue staining images of experimental groups can be seen in Figure 1.

Table 1: Results of histopathological changes of all groups.

Groups	n	Mean	Std. Deviation

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Mast cell count in the lamina propria	1	5	5,20	1,48
	2	5	5,60	1,67
	3	6	4,33	3,27
	4	6	9,67	7,97
	5	6	2,83	2,14
	6	6	2,00	1,26
	7	6	6,67	9,09
PNL count in the epithelium	1	5	,00	,00
	2	5	1,00	,71
	3	6	,50	,84
	4	6	1,00	1,55
	5	6	1,33	,52
	6	6	,67	1,03
	7	6	,83	,75
PNL count in the lamina propria	1	5	,80	,84
	2	5	3,60	3,91
	3	6	3,00	3,22
	4	6	7,00	9,36
	5	6	17,33	13,08
	6	6	2,33	2,42
	7	6	3,17	3,71
Lymphocyte count in the epithelium	1	5	1,20	,84
	2	5	,80	,84
	3	6	,50	,84
	4	6	2,33	2,94
	5	6	,83	,98
	6	6	4,00	1,90
	7	6	1,33	1,03
Lymphocyte count in the lamina propria	1	5	6,20	3,19
	2	5	3,00	1,58
	3	6	3,17	2,14
	4	6	9,00	10,18
	5	6	6,50	11,54
	6	6	6,67	5,24
	7	6	4,67	2,80

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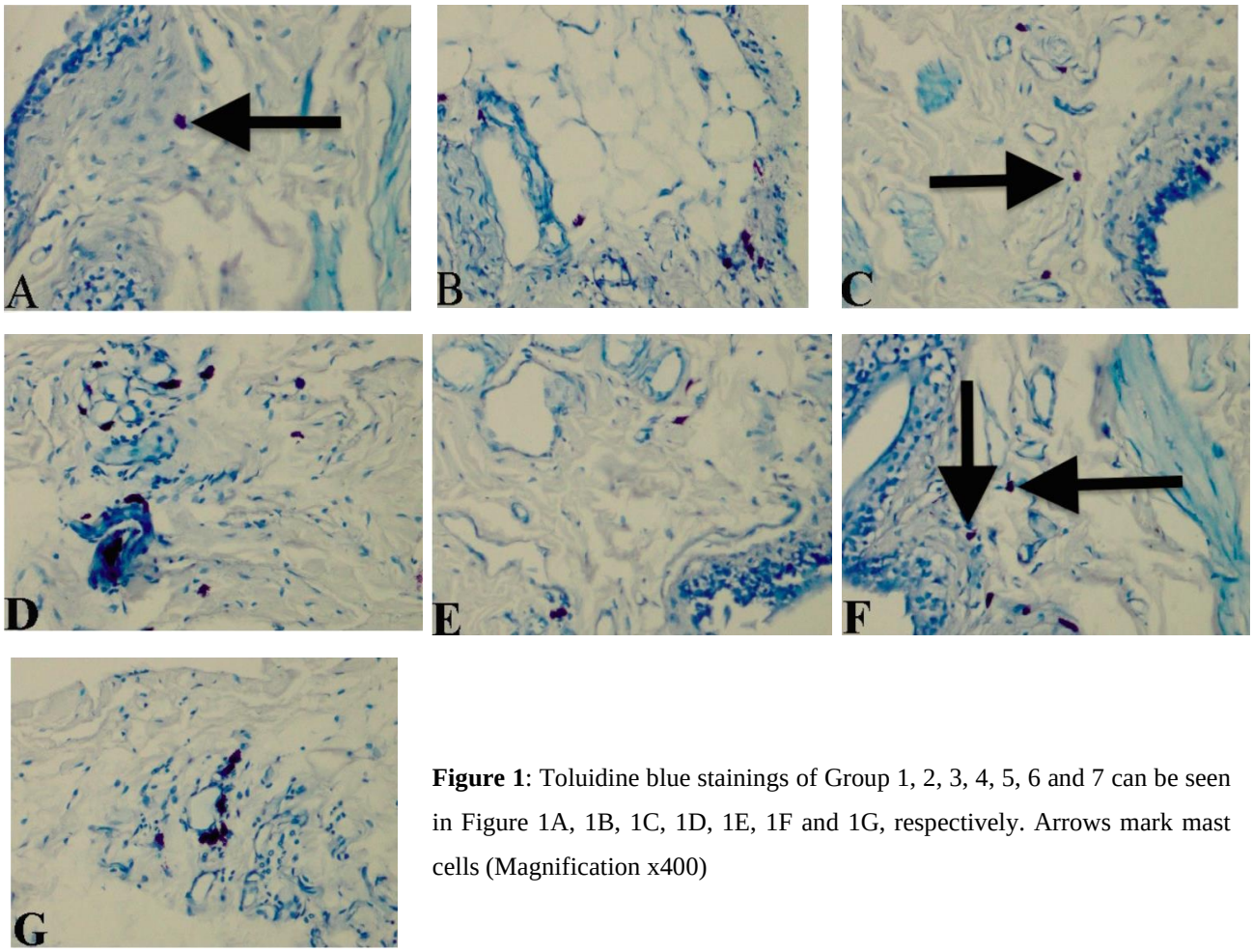

Figure 1: Toluidine blue stainings of Group 1, 2, 3, 4, 5, 6 and 7 can be seen in Figure 1A, 1B, 1C, 1D, 1E, 1F and 1G, respectively. Arrows mark mast cells (Magnification x400)

DISCUSSION

The present study investigated the damage in the bladder wall caused by acute and chronic cold stress and the effects of CORT and antihistaminic injection on this damage. The histopathological evaluations were performed in consideration of mast cell count in lamina propria, PNL count in epithelium, PNL count in lamina propria, lymphocyte count in epithelium, and lymphocyte count in lamina propria. Accordingly, the comparison based on mast cell count yielded the highest value in the CCS group in comparison of the control group. The lowest value was harvested in the CCS+CORT group. The evaluation in consideration of the PNL counts in the lamina propria revealed a significant difference only in the CCS group in comparison with the control group. The other groups were found to have similar values with the control group. Yet higher PNL counts were observed in the lamina propria in the CCS group as a result of the comparison between the ACS and CCS groups. The comparison between the CCS groups revealed the highest PNL values in the lamina propria in the CCS group, whereas the CORT and CORT+Antihistaminic treatments were found to have significantly decreased the PNL values in the lamina propria. But no significant difference was observed during these two treatments. While the PNL counts in the epithelium were high in the ACS and CCS groups, the results in the ACS and CCS groups that were treated with CORT and/or antihistaminic were revealed to be similar with those in the control group. A significant decrease was observed in the ACS+CORT and CCS+CORT groups in terms of the lymphocyte count in the epithelium. It was discovered that antihistaminic injection in addition

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to CORT decreased the lymphocyte counts in epithelium in CCS more efficiently than CORT alone did. Accordingly, the CORT treatment was revealed to decrease inflammatory infiltration in inflammatory damage that occurred in the bladder in ACS and CCS. Antihistaminic administered along with CORT particularly in CCS was observed to have considerably helped decrease the lymphocyte infiltration in the epithelium.

Cold is among the environmental stressors and exhibits various effects on organisms. Cold stress stands out as a risk factor for cardiovascular diseases in a community, especially for women (20). While acute cold stress may pose critical health problems for people living in cold climates, chronic cold stress is a serious issue particularly for people working outdoors. It has been observed that cold-stress stimulation of the soles during pregnancy has been reported to have increased blood pressure especially after the second week, urinary protein excretion and epinephrine and norepinephrine concentration. An increase has been determined in subendothelial fibrinoid deposits in the glomerular capillary (21). Previous studies have shown that cold induces apoptosis and cold-induced apoptosis is mediated by ROS (22). A study on zebrafish manifested that cold stress increases oxidative stress in liver. The α-lipoic acid and a low level of glutathione subsequently administered to zebrafish have been observed to decrease cold-induced oxidative stress and tissue damage. Thus, it has been concluded that decreased oxidative stress promotes tolerance of cold (23). Bladder's response to cold originates from cold receptors located in the walls of lower urinary tract. This operation is supplied by unmyelinated afferent C-fibers (24). Exposure of a certain part of skin to cold leads to urinary urgency, which is referred to as acute cold-induced urgency (ACIU). TRPM8 receptors were found to regulate this mechanism and the inhibition of these receptors to alleviate ACUI (25).

A study has compared the morphologies of bladder tissues of interstitial cystitis (IC) and cold stress groups and degranulated mast cells in the mucosa, leukocyte infiltration in the lamina propria, vacuole formation in the urothelial cells, dilated intercellular spaces and altered proliferative activity were found to have increased in the stress group in comparison with the control group. The similarity of these findings with the histopathological findings of interstitial cystitis shows that cold causes an IC-like condition in bladder (17). The present study attempted to treat the inflammatory process likely to occur in bladder with a single dose of CORT and antihistaminic and revealed that a single dose of CORT could reduce inflammatory cell infiltrations. Especially the administration of a single dose of antihistaminic in addition to CORT in CCS was shown to further alleviate the condition and further decrease the inflammatory cell infiltration in comparison of the sole administration of CORT. The fact that mast cell count was manifested to decrease with the injection of CORT in CCS can be suggested as a critical parameter in controlling the inflammatory process. The better results that concern the lymphocyte count in the epithelium and which were achieved with the administration of CORT+Antihistaminic also remarkably contributed to this process and resulted in a more efficient treatment.

This study showed that administering CORT and antihistaminic produced more significant effects to alleviate inflammation in CCS than in ACS. A 21-day cold exposure in CCS allowed for the introduction of leukocytes effective in chronic inflammation into the process. CORT was understood to prove more efficient in the treatment of chronic inflammation occurring as such than of acute inflammation. CORT is a drug that exerts diverse effects on organisms, several of which are as follows:

Supression of leukocyte flow to the inflammation areas

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Effect on leukocyte, fibroblast, and endothelium function

Supression of the effects of humoral factors influential in inflammatory cases

Reduction in the number of monocytes, T-cells, eosinophils, and basophils in the circulatory system but increase in the number of neutrophils

Reduction in the expression of MHC class II molecules and Fc receptors on the cell surfaces of monocytes and macrophages and suppression in the synthesis of proinflammatory cytokines, such as IL-2, IL-6, and TNF-α from these cells

Inhibiton of delayed-type hypersensitivity reaction

Inhibiton of the formation of IL-2 associated with T-lymphocytes (26)

CORT's higher level of effectiveness in chronic inflammation occurring in CCS than in ACS results from CORT's being more effective in cells present particularly in chronic inflammation. Especially, the ability of a single dose of CORT to lead to this effect allows for an easier and cheaper treatment without resorting to repetitive administrations to produce an anti-inflammatory effect.

The present research investigated the effects of corticosteroid and antihistaminics on the inflammation caused by cold stress on bladder tissue and revealed that corticosteroid treatment reduced inflammatory cell infiltration and decreased mast cell count. A more evident amelioration was observed particularly in chronic cold stress.

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