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ORIGINAL ARTICLE

Assessment of therapeutic effect of human choriogonadotropin in a chemical cystitis model

Serhat Tanik [a,*], Kürsad Zengin [b], Sebahattin Albayrak [a], Abdullah Gurel [a], Muhittin Atar [a], Sevinc Sahin [c], Nevin Tuzcu [d], Mehmet Tuzcu [e], Muhammet Abdurrahim Imamoglu [a], Mesut Gurdal [a]

[a] *Department of Urology, Bozok University, School of Medicine, Yozgat, Turkey*
[b] *Department of Urology, Afyon Kocatepe University, School of Medicine, Afyon, Turkey*
[c] *Department of Pathology, Bozok University, School of Medicine, Yozgat, Turkey*
[d] *Department of Pharmaceutical Microbiology, Cumhuriyet University, Faculty of Pharmacy, Sivas, Turkey*
[e] *Department of Pathology, Cumhuriyet University, Faculty of Veterinary Medicine, Sivas, Turkey*

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Rat

Abstract In this study, female rats induced with chemical cystitis were administered the hormone human choriogonadotropin (HCG), and it was aimed to reveal the usefulness of HCG in the treatment of interstitial cystitis/bladder pain syndrome. The materials for this study were 32 Wistar albino female rats. The study groups were formed as follows: the cystitis group (Group 1), the cystitis + HCG protection group (Group 2), the cystitis + HCG treatment group (Group 3), and the control group (Group 4), with eight rats in each group. In this study, blood and urine samples were taken from the rats, they were euthanized, and their bladders were removed for glutathione, malondialdehyde, tumor necrosis factor alpha, and interferon gamma measurements. It was observed that tissue damage in Group 2 was lower than that in the other two groups. Glutathione levels in Groups 2 and 4 were significantly higher than in Groups 1 and 3 ($p = 0.01$). Malondialdehyde levels of Groups 2 and 4 were significantly lower than the values in Groups 1 and 3 ($p < 0.001$). When the cystitis groups were compared in terms of their interferon gamma and tumor necrosis factor alpha levels, the lowest interferon gamma and tumor necrosis factor alpha levels were detected in Group 3. It was found that HCG has positive effects on experimental cystitis in rats. This study revealed that HCG should be researched as a therapeutic agent and formed a step for studies to be carried out on this subject.

Conflicts of interest: All authors declare no conflicts of interest.

* Corresponding author. Department of Urology, Bozok University, School of Medicine, Seyh osman Mah. Adnan menderes bulvarı, No: 66 Yozgat, 66000, Turkey.
E-mail address: tanikserhat@gmail.com (S. Tanik).

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Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a sterile chronic inflammation characterized by the thinning of the bladder epithelium or ulcerated lesions, in which pelvic pain, ulcers, and the symptoms of pollakiuria are prominent. While the etiology of IC/BPS cannot be fully explained, autoimmunity, presence of toxic substances in the urine, and psychiatric pathologies are the suspected mechanisms [1]. It is indicated that disorders in sexual functions may occur, in addition to deterioration of the quality of life in about one-third of IC/BPS patients [2]. In a study carried out in the USA, it was forecast that the prevalence of IC/BPS among women may rise to 12% [3,4]. The objective of the treatment is to eliminate the symptoms, but no curative treatment is known as yet [2]. There are studies indicating that the bladder epithelium damage detected in IC/BPS patients results from inflammation [5]. While limited inflammation is observed in most of the patients, the occurrence of symptoms may be quite variable, and the reason for this is that the inflammatory process is affected by different cytokines [1,6].

Human choriogonadotropin hormone (HCG) is a hormone of glycoprotein structure involved in the physiological adaptation during pregnancy. The presence of HCG receptors in many tissues, including the urinary system, was shown in humans and other species [7,8]. HCG has effects such as proliferation, angiogenesis, immune tolerance, and apoptosis in target tissues [9–12]. Studies have explained that the decrease of complaints of IC/BPS patients during pregnancy is due to the effect of HCG on bladder epithelium [13].

As no experimental study assessing the efficiency of HCG in the chemical cystitis model has been encountered, this study aimed to investigate the therapeutic effect of HCG in the rat model with induced chemical cystitis by comparing the histopathological changes, and the inflammation and oxidative stress markers.

Materials and methods

All animals were provided by the Animal Laboratory of Cumhuriyet University. Animals were housed in a light-controlled (12-hour light/dark cycle) and temperature-controlled (22–24°C) room, and were given rodent chow and tap water *ad libitum*. This study was carried out by the approval granted by the Cumhuriyet University Animal Experiments Local Ethics Board (resolution number B.30.2.CUM.01 00 00 50/4).

Thirty-two Wistar albino female rats weighing 230–250 g, with an average age of 3 months, were used in this study. The chemical cystitis group [14] was induced by injecting 75 mg/kg of intraperitoneal cyclophosphamide (Endoxan; Eczacibasi-Baxter, Istanbul, Turkey) four times in total at 3-day intervals, in accordance with the protocol reported by Vizzard and Boyle [14]. Cyclophosphamide-induced chemical cystitis in previous studies was used as an experimental IC rat model [15]. Rats were randomly allocated to one of four groups ($n = 8$ per group). The study groups and all the administrations to the groups are as follows:

Group 1 (the cystitis group): 75 mg/kg of intraperitoneal cyclophosphamide (Endoxan; Eczacibasi-Baxter) was injected four times in total at 3-day intervals.
Group 2 (the cystitis + HCG protection group): 75 mg/kg of intraperitoneal cyclophosphamide was injected four times in total at 3-day intervals, and two doses of subcutaneous 10 iu/mL of HCG were injected on days before first and second doses of the injection of cyclophosphamide.
Group 3 (the cystitis + HCG treatment group): 75 mg/kg of intraperitoneal cyclophosphamide was injected four times in total at 3-day intervals, and two doses of subcutaneous 10 iu/mL of HCG were injected on days after first and second doses the injection of cyclophosphamide.
Group 4 (the control group): Four doses of 2 mL 0.9% NaCl were injected intraperitoneally once every 3 days.

At the end of the study, general anesthesia (1.25 g/kg) was given intraperitoneally in the rats of all the groups. Blood samples were taken after the anesthesia; 4–5 cc of blood was taken from the rats under anesthesia and they were euthanized. Their bladders were removed during necropsy. One-fourth of the bladders taken were fixed with formalin for histopathological studies. The remaining portions were used for the measurements of glutathione (GSH) and malondialdehyde (MDA). As a result of the deaths seen in all working groups during the study, the data of six rats in all groups were subjected to statistical evaluation.

Histopathological examination

The tissues were blocked in paraffin using the standard methods. Serial sections at a thickness of 5 μm taken from the paraffin blocks were stained using hematoxylin and eosin, and examined under a light microscope. The scores in Table 1, similar to the lesion scoring used by Gokakin et al. [16], were used in the assessment of the pathologic lesions.

Oxidative stress marker measurement

Tissue samples were taken from the bladder in order to colorimetrically analyze the reduced GSH and malondialdehyde (MDA) levels using the spectrophotometric method. After the tissues were immediately fixed at 4°C using 0.15M KCl, they were homogenized using a refrigerated homogenizer for a period of 3 minutes. After the

Table 1 Scores used in the assessment of the pathologic lesions (maximum score 10).

Hyperemia	1
Degeneration of epithelium	1
Necrosis and loss in epithelium (mild)	2
or	
Necrosis and loss in epithelium (severe)	3
Mononuclear cell infiltration	2
Hemorrhage (light)	2
or	
Hemorrhage (severe)	3