Evaluation of the EHF- therapeutic influence on mice's prostate state with experimental chronic prostatitis

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ABSTRACT
Prostatitis is a common disease in men, and its pathogenesis remains to be clarified. The extremely high frequency (EHF) - therapy of the prostatitis is used as one of the methods of treating. The current study aims to evaluate the preventive effect of EHF-therapy during the treatment of the hemodynamic model of experimental chronic prostatitis. The study was carried out on 15 inbred male mice. The animals were divided into 3 groups. Results revealed that the subpopulation of cytotoxic lymphocytes increased insignificantly in the inflammatory prostate in comparison with that in the untouched animals. After the treatment the number of CD8+ cells decreased more than 2 times.

Keywords: Chronic prostatitis, experimental, EHF therapy, Immune response.

Background

Chronic prostatitis takes one of the first places among the diseases of the male reproductive system[1, 2]. In addition, it is one of the psycho traumatic pathological conditions in men[3]. Prostatitis is a common disease in men, and its pathogenesis remains to be clarified. The interaction of inflammation, inflammatory cells and cytokines leads to the development and progression of chronic prostatitis[4]. Until now, the problem of treating this disease remains relevant. According to the literature, the alternative therapy, such as phytotherapy, acupuncture, and pelvic floor physical therapy are in wide use in treating chronic prostatitis[5, 6]. The extremely high frequency (EHF) - therapy of the prostatitis is used as one of the methods of treating[7]. The expediency of using it in chronic prostatitis is caused by the influence on the immune system, activation of regenerative and decreasing of exudative inflammation in the prostate tissue, the normalization of microvasculature disorders[8, 9]. However, in the literature the results of clinical researches without a parallel study of the morphological changes and cellular composition of the prostate predominate.

Aim

To evaluate the preventive effect of EHF-therapy during the treatment of the hemodynamic model of experimental chronic prostatitis.

Objectives

On planning the research the following tasks were set:
1. To determine the cellular composition of prostate tissue in mice treated by the EHF-therapy in experimental chronic prostatitis.
2. To assess the effect of the EHF-therapy on the inflammatory process (using the counting blood leukocytes) in experimental chronic mice prostatitis.
3. To determine the immune response and functional activity of corpulent cells in intact mice, in mice with experimental chronic prostatitis, and in mice with experimental chronic prostatitis under the influence of the EHF-therapy.

Methods

The study was carried out on 15 inbred male mice. The animals were divided into 3 groups. The 1st (№ 5) – untouched mice. The 2nd (№ 5) - mice with the experimental model of chronic prostatitis. Chronic prostatitis was designed by using the hemodynamic model proposed by Knyazkin I.V. (2001). It was modeled by the rectal irritant containing from the mixture of 10% water solution of Dimexidum and turpentine in a volume ratio of 4:1 in the amount of 0.2-0.25 ml, the irritant is injected in the upper section of the rectum to a depth of 10-12 mm. The model is used in 28 days after the injection of this mixture[10]. The 3rd (№ 5) – mice with an experimental model of chronic prostatitis treated by the EHF-therapy in 17 days after injection; there were 10 sessions of EHF- therapy on the reflex zone of the perineum for a period of 30 minutes daily in the morning.
Before and during the course of EHF-therapy other physical treatments were not applied. The apparatus of the EHF-therapy "Jav - 1" (Moscow region, Fryazino production) was used for irradiation. The prostate was removed in 7 days after the injection of irritant and creating the chronicle prostatitis. 0.5 ml of blood for leukocyte counting was taken by the decapitation of each animal. The experiment was conducted in autumn; the animals were kept in vivarium and had an equal food rations equivalent. All experiments were started after ethics committee approval and according with Guide for the Care and Use of Laboratory Animals and Laboratory Animal Welfare Regulations[11]. The pieces of the prostate were fixed in buffered formalin solution and embedded in paraffin by standard methods. We studied 5 microns thick prostate sections using the following methods.
1. The Hematoxylin-eosin dye was used to assess the nature of the influence on the studied organs.
2. The technique of Romanovsky-Giemsa stain was used for the counting leukocytes in blood films.

3. The immunohistochemical methods using suitable monoclonal antibodies were used to identify subpopulations of CD4+, CD8+, CD68+ cells in the prostate.
4. The toluidine blue dye by Unna technique was used for controlling the tissue mucopolysaccharides and heparin in corpulent cells of the prostate.

The morphological assessment of the prostate sections (with the calculation of CD4 +, CD8 + and CD68 + cells per 1 mm^2 in each slice using Sigma Scan Pro 5) was conducted during the research. The parameters used in the article are: M – an average arithmetic size; m – an average mistake of average arithmetic size. The statistical reliability was defined by Student's criterion (t), $p \leq 0,05$.

Results.

A characteristic picture of this organ was observed in the untouched mice: branched adenomere was filled with the secretion. The predominance of proliferation, metaplasia of macrophages into fibroblasts and fibrocytes, degeneration of unstriated muscle fibers were revealed in studying the sections of the mice prostate with the experimental chronic prostatitis. The prostate is characterized by an isolated lesion of the epithelium of ducts and a considerable amount of gland cells. The perigland infiltration by lymphocytes, monocytes, plasma cells was registered more often. The microvascular diameter was narrowed. The hyaline thrombuses were identified in the blood vessels. The alteration and infiltration of the stroma around the veins with leukocytes, as well as stroma loosening, were observed due to the exudation.After the EHF-therapy treating mice of the 3rd Group the alveolar ducts of tubular glands were lined with stratified prismatic epithelium. The lesion of the duct epithelium was found only in 20% of cases. The metaplasia of macrophages into fibroblasts and degeneration of unstriated muscle fibers was found in 15% of the sections. After the toluidine blue dye by the Unna technique the total number of the must cells did not change in the mice with the chronic prostatitis. However, in the mice with the chronic prostatitis the number of cells in the phase of ejection – removing the active substances was increased; in this group the percentage of degranulation cells was 80%.

The number of leukocytes of the untouched mice was as follows: lymphocytes – 88.5 in one field of view (o.f.v.), segmented neutrophils – 8 in o.f.v., monocytes – 2.5 in o.f.v. The marked lymphocytosis (98 o.f.v.) and monotcytopeniya (1 o.f.v.) were revealed in the mice with chronic prostatitis. The significant ($p < 0,05$)% increase of phagocytes were revealed after the EHF - therapy treating mice: segmented neutrophils and monocytes increased by 8.5 and 2.5 times respectively by comparison with the number of leukocytes in mice with chronic prostatitis, what indicate the stimulation of nonspecific immunity[12].

The immunohistolochemical methods reveal that the number of T-helper cells was 6,48 ± 1,94 (1 mm^2) in untouched mice (table 1). It was noted, the number of T-helper cells in the mice with chronic experimental prostatitis was increased in comparison with that in the untouched animals. While the number of T-helper cells decreased in 2.95 times after the EHF-therapy treating mice. The subpopulation of cytotoxic lymphocytes increased insignificantly in the inflammatory prostate in comparison with that in the untouched animals. After the treatment the number of CD8+ cells decreased more than 2 times. The number of macrophages in the mice with chronic prostatitis veraciously decreased in 1.9. After the course of EHF-therapy the number of CD68+ cells approximated to that in the untouched group, indicating the increase of nonspecific system resistance[5, 8].

Conclusions:

1. In the chronic experimental mice prostatitis the decrease in CD8+ and CD68 + cells and a tendency to increase in CD4+ sections of the prostate was observed.
2. The 30-minute EHF-therapy for 10 sessions has a preventive effect: the morphological picture and the number of immunocompetent cells of the prostate tissue in mice and number of phagocytes in the peripheral mice blood approximated to that in the untouched group.

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Table 1: ubpopulation structure CD 4+, CD8+, CD68+ lymphocytes of a prostate of experimental groups of animals (M±m).

	CD4	CD8	CD68
1st group (untouched mice)	6,48±1,94	3,46±1,81	5,03±1,27
The 2nd group (mice with the experimental model of chronic prostatitis)	6,79±2,32	3,73±1,53	2,62±1,07*
The 3rd group mice with an experimental model of chronic prostatitis treated by the EHF-therapy	2,20±0,96*	2,45±1,04*	3,84±1,49*

The note. * $p<0,05$ in comparison with indicators in intact group.