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INVESTIGATION OF THE INFLUENCE OF FLUCTUATING CURRENTS AND APROTININ ON THE INFLAMMATORY REACTION OF TISSUE BASOPHILES IN EXPERIMENTAL CONDITIONS

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Since floating currents at the initial stages of inflammation are capable of restricting and suppressing inflammatory processes, it would be possible to strengthen their therapeutic effect by fluctuophoresis of drugs that have anti-inflammatory effects, in particular, aprotinin. Brain development of the inflammatory process is associated with the effect on the reaction of tissue basophils that emit inflammation mediators through degranulation. The aim of the research was to study the mechanism of influence of fluctuarization and fluctuophoresis with aprotinin on the process of degranulation of tissue basophils in the experimental process of aseptic inflammation. Animals of one experimental group (EG1) conducted fluctuarization with the device of low-frequency electrotherapy "Radius-01" (Belarus), and the second (EG2) - fluctuophoresis with a preparation on the basis of aprotinin. Degree of degranulation was determined by microscopic counting of four types of tissue basophils in film preparations. The results of the study showed that in animals that had fluctuophoresis with aprotinin (EG2), tissue basophils with dense and diffuse placements of granules in the cytoplasm were detected (the lowest degree of degranulation was from 47.0% to 37.5%, which means 20.5 % less than after fluctuarization and by 27% than in the control group. The obtained data indicate a pronounced anti-inflammatory effect of fluctuophoresis with aprotinin due to inhibition of functional activity of tissue basophils.

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Introduction

Rational application of physiotherapeutic procedures in various pathological processes provides sedative, analgesic, anti-inflammatory, antiseptic, desensitizing, haemostatic and anti-oedema effects, changing microcirculation and rheology, peripheral and central hemodynamics, tissue trophic and metabolism, immunobiological and compensatory adaptive processes, and reactivity of the organism.

In today's dental practice the methods of physiotherapeutic influence are widely implemented, which, in combination with other therapeutic and prophylactic measures, can inhibit the development of inflammatory processes in the maxillofacial area.

Fluctuation for the therapeutic purposes uses sinusoidal AC low power and low voltage or partially rectified, or rectified, with chaotically altered the amplitude and frequency from 100 Hz to 2000 [1]. Fluctuating currents cause pronounced local reactions, increase reparative regeneration of tissue structures [2, 3]. In our opinion, the use of drugs that have anti-inflammatory action, through fluctuophoresis, which increase their therapeutic effect, is quite promising.

According to professional literature, in the early stages of inflammation fluctuating currents are able to limit and suppress inflammation, and this may be due to the influence of the reaction of tissue basophiles (TB), which are one of the effector cells that secrete inflammatory mediators (histamine, serotonin, prostaglandins, etc.) by degranulation [4].

Tissue basophiles (TB, mast cells, mastocytes, and labrocytes) are highly specialized immune cells of the connective tissue of vertebrate, analogues of basophiles of blood. They play an important role in inflammatory reactions.

It is known that the main function of mast cells is the accumulation of chemical mediators of inflammatory reaction. Labrocytes contain a large number of cytoplasmic granules, the content of which during activation (for example, during an allergic reaction) is released into the surrounding tissue (degranulation). Other components of the granules of smooth cells are histamine, which increases vascular permeability, neutral protease, eosinophilic chemotoxic factor of anaphylaxis (ECF-A). Degranulation of mast cells also occurs as a result of complementary molecules that are involved in immune responses. Histamine causes the contraction of smooth muscle tissue (mainly in bronchioles), dilates blood vessels and increases their permeability (mainly postcapillary venules). After the excretion of histamine, it is immediately inactivated [5].

Aprotinin is an inhibitor of proteolysis and kininogenesis; it has anti-inflammatory, anti-fibrinolytic and anti-shock effects. The ability of aprotinin to reduce the production of pro-inflammatory interleukins and, simultaneously, to stimulate the formation of anti-inflammatory interleukins, allows it to be used to reduce the effects of surgical trauma and postoperative pain syndrome [6].

The purpose of our study was to investigate the effect of fluctuorization and fluctuophoresis with aprotinin on the process of degranulation of tissue basophils in the experimental process of aseptic inflammation.

Materials and methods

For the purpose of studying the anti-inflammatory effect of fluctuating currents and

aprotinin, 42 Wistar female rats weighting 180-200 g were divided into 3 experimental groups, each of which was divided into 2 subgroups, depending on the time the animals were removed from the experiment. For 48 hours before the experiment, animals were subjected to depilation of sites in place of physiotherapeutic effect. All animals were subjected to modelling an acute aseptic inflammation of the intestinal mesentery using a single retroperitoneal injection of 0.1 ml of 1% solution of carragenin [7]. After 30 minutes following carrageenan solution injection, the animals of the first experimental group (EG1- 14 rats) were exposed to fluctuorization using low electrotherapy device "Radius - 01" (Belarus) and the second experimental group of animals (EG2 - 14 rats) was exposed to fluctuophoresis with the drug on the basis of aprotinin ("Contriven", Biopharma, JSC (Kyiv)). The control group was composed of animals without corrective effect on the inflammatory process (CG - 14 rats).

30 minutes after the physiotherapeutic effect on 7 animals from, the first subgroups of EG1 and EG2 under general anesthesia was taken from the experiment and carried out an intestinal mesentery sampling for determination of the degree of degranulation of the labrocytes, after 60 minutes the animals from the second subgroup of EG1 and EG2. The rats from the control group, 7 animals from each subgroup in terms of 60 and 90 minutes from the beginning of the experiment were taken form the experiment. The choice of such terms of study was due to the fact that in the development of carrageenan inflammation in the first 30 – 90 minutes, the most involved are histamine and serotonin [8].

To obtain free peritoneal labrocytes, a piece of mesentery was consolidated on a slide, fixed in a 10% neutral formalin, specimen were dyed with toluidine blue, and examined under a microscope with an optical zoom of 1000 [9]. Tissue basophiles (TB) were identified and counted according to the types: the first type - with low content of granules in the cytoplasm located near the membrane; The second type - with an average content of granules, located diffusely; The third type is large cells with dense and diffuse placement of granules in the cytoplasm; The zero type is degranulated cells with signs that the integrity of the cytoplasm membrane is impaired [10]. The degree of degranulation was evaluated as the ratio of the number of zero-type cells to the total number of detected cells, expressed in a percentage.

All studies were carried out according to the Law of Ukraine "On Protection of Animals from being Abused" No. 3447-IV, the European Convention for the Protection of Vertebrate Animals used for Research or Other Scientific Purposes of 18.03.1986, the Order of the Ministry of Education, Science and Youth And sport of Ukraine "Procedure for conducting scientific establishments of experiments, experiments on animals".

The statistical processing of the results of the study was carried out by calculating the arithmetic mean, mean square deviation, and compared with the Student criterion. The probability of the obtained results was at the level of significance not less than 95%, $p \leq 0.05$.

Results and discussion

Results of determining the content of tissue basophiles in the inflammatory site are presented in Table 1.

Table 1
The content of tissue basophiles in aseptic carrageenan inflammation and its correction by fluctuating currents and aprotinin.

Animal groups	Types of tissue basophiles	Amount of tissue basophiles			
		The first subgroup (n=7)		The second subgroup (n=7)	
		Abs.	%	Abs.	%
Control Group (CG) (n=14)	1	140,6±6, 37	27,1±0,74	150,4±6,95	29,0±0,75
	2	91,1±6,94	17,6±0,63	91,0±6,38	17,5±0,63
	3	10,1±1,07	1,9±0,23	11,1±1,57	2,1±0,24
	0	277,0±16,79	53,4±0,83	267,2±15,07	51,4±0,83
Experimental group (EG1) (n=14)	1	152,1±11,52**	31,8±0,8**	158,3±8,73**	33,1±0,81**
	2	80,0±8,21	16,7±0,65	82,4±8,34	17,2±0,65
	3	13,1±1,35*	2,8±0,28*	12,0±1,29	2,5±0,27
	0	233,0±8,6**	48,7±0,86**	225,4±7,52**	47,2±0,86**
Experimental group (EG2) (n=14)	1	158,1±7,99**##	35,7±0,86**, ##	138,0±10,36**,##	38,1±0,96**,##
	2	64,0±4,8**, #	14,5±0,63**, #	75,4±6, 8**,##	20,8±0,81**, ##
	3	12,1±1,46*	2,8±0,29*	13,0±0,82** ,#	3,6±0,37** ,#
	0	208,0±14,55**	47,0±0,9**	135,6±11,13**,##	37,5±0,96**,##

*Note: * - Reliable difference with control group with probability> 95% ** - Reliable difference with control group with probability> 99%, # - Reliable difference from EG1 with probability> 95%, # # - Reliable difference with EG1 with probability> 99%.*

In animals that had fluctuophoresis with aprotinin (EG2), after 30 minutes of physiotherapy (first subgroup), 35.7 % of the first type of TB, 14.5 % of the second type of TB, 2.8% of the third type of TB were obtained from the abdominal cavity , The rest - TB of zero type. Similar rates at the same time in the animals of the first experimental group were 31.8 %, 16.7 %, 2.8 % and 48.7%, respectively, and in the control group it was 27,1 %, 17,6 %, 1,9% and 53,4 % (Fig. 1).

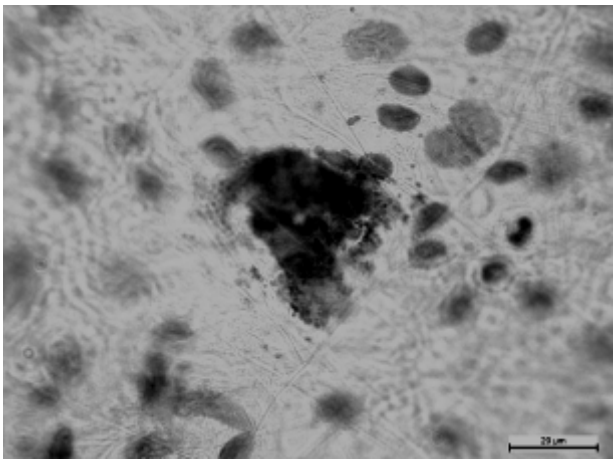

Figure 1. Degranulated tissue basophil with violation of the integrity of the cytoplasmic membrane (type 0) in the rat of the control group (CG). Toluidine blue.

It was established that the degree of degranulation of TB in EG1 was 48.7 %, which is 8.8 % lower than the control group (53.4 %) ($p < 0.01$), and in EG2 – 47 % and 12 % respectively ($P < 0.01$)

60 minutes after fluctuorization, the animals of the first experimental group (EG1) received TB from the first, second, third and zero type respectively 33.1 %, 17.2 %, 2.5% and 47.2 %. The degree of degranulation was 47.2 %. It is 8.2 % less than in the control group. In animals of the second experimental group, after fluctuophoresis with aprotinin, 38.1 % of the first type of TB were received, 20.8 % were of the second type, 3.6% of the third, and the rest - of zero. The degree of degranulation was 37.5 %, which is 27 % less than in the control group.

On the background of fluctuarization (EG1) TB of first type were mainly manifested (the most degranulation degree - of 31.8 % (first subgroup) to 33.1 % (second subgroup), the second type cells were present in small amounts (up to 17.2 %) during the whole period of observation. The third type of TB did not exceed 3%.

During the studying of the intensity of degranulation of TB with carragine peritonitis on the background of using the fluctuophoresis with aprotinin compared with the usual course of inflammation, TB percent of the first type (the largest degree of degranulation) was the largest in subgroup - 38,1%, which is significantly higher than the content of cells in the animal control group – 29 % ($p < 0.01$). Number of second type TB in these animals tended to gradually increase - from 14.5% in the first subgroup to 20.8 % - in the second (Figure 2).

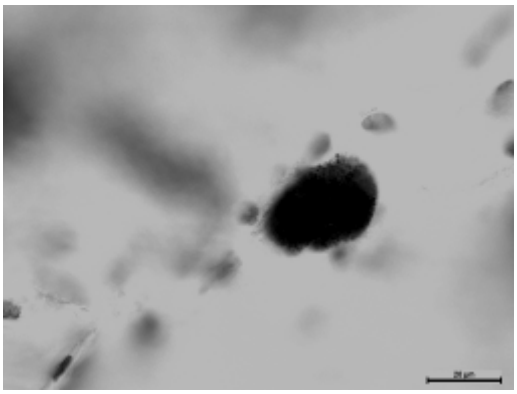

Fig. 2. Tissue basophil with medium content of granules located diffusely (type 2) in rat of the second group (EG2). Toluidine blue.

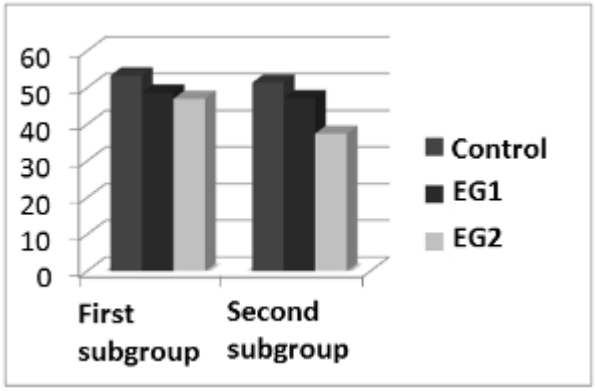

Fig. 3. The degree of degranulation of tissue basophils in aseptic carragine inflammation and its correction by fluctuating currents and aprotinin.

Fig. 3. The degree of degranulation of tissue basophiles in aseptic carrageenan inflammation and its correction by fluctuating currents and aprotinin.

It should be noted that during the entire period of observation in rats of the second experimental group, which were conducted fluctuophoresis with aprotinin, tissue basophiles with dense and diffuse placements of granules in the cytoplasm were detected (the lowest degranulation rate ranged from 47.0 % in the first subgroup to 37.5 % - in the second). The obtained data indicate that the use of fluctuophoresis with aprotinin inhibits the functional activity of tissue basophils and this is confirmed by statistical confidence in the second experimental group of animals (Fig. 3).

Thus, in the mechanism of anti-inflammatory action of aprotinin, introduced into the site of inflammation with the help of a fluctuating current, the inhibition of functional activity of mast cells, which is the source of initial mediators of inflammation, plays a significant role.

Conclusion

The obtained results of experimental studies morphologically substantiate the ability of fluctuophoresis with aprotinin to produce actively influence on the pathogenesis of acute aseptic inflammatory process. One of its mechanisms is the inhibition of the degranulation process of tissue basophils in the area of the affection. The results of the performed studies showed that after fluctuophoresis with aprotinin, the degree of degranulation of tissue basophiles was by 20.5 % less than after fluctuarization, and by 27 % less than in the control group.

Results of the study in an experiment with an anti-inflammatory effect of fluctuophoresis with aprotinin require further thorough clinical study.

References

1. Пономаренко Г.Н. Основы доказательной физиотерапии / Г.Н. Пономаренко. - СПб: В.МедА, 2003. - 224 с.
2. Фізіотерапія / [Я. М. Федорів, М. С. Регеда, І. Г. Гайдучок та ін.] – Львів: Магнолія, 2011. – 558 с.
3. Клінічна фізіотерапія. / Под ред. Оржешковський В.В. - Київ, 2001 - 86с.
4. Шутова Н.А. Роль еозинофілів та їх взаємодія із тучними клітинами у патогенезі запалення: автореф. дис... канд. мед. наук: 14.03.04 - «Патологічна фізіологія» / Н.А. Шутова ; Харк. нац. мед. ун-т. — Х., 2008. — 19 с.
5. Bagley E.O. Mast cells - key participants in the pathogenesis of immune-inflammatory diseases / E.O. Bagley, A.I. Dubikov. // Scientific-clinical rheumatology. - 2015 - # 2. - P. 182-189.
6. Дементьева И.И. Апротинин: безопасность применения в хирургической практике / И.И. Дементьева, М.А. Чарная, Ю.А. Морозов. // Анестезиология и Реаниматология. - 2007. - №2. - С. 69-71.
7. Клименко Н. А. Роль опиоидных пептидов в реакции тучных клеток очага воспаления / Н. А. Клименко, В. А. Перин // Експериментальна і клінічна медицина. - 2010. - № 4. - С. 10-13
8. Di Rosa M. Stadiums are the mediator of acute inflammatory response induced in rats in different sites without carrageenan and turpentine / Di Rosa M, Giroud JP, Willoughby DA. // Journal of Pathology. - 1971 - № 104. - P. 15-29.
9. Klimenko N.A. To the method of morphological study and calculation of mast cells erosion of serous cavities / N.A. Klimenko // Physiol. Journ Academy of Sciences of the USSR. - 1977. - Vol. 23, No. 5. - P. 705-707.
10. Клименко Н. А. Морфологические критерии интенсивности дегрануляции свободных и фиксированных тканевых базофилов в зависимости от их типа / Н. А. Клименко, С.В. Татарко // Морфология. - 1997. – Т. 110, № 1. - С.100-103.

Реферат

ДОСЛІДЖЕННЯ ВПЛИВУ ФЛЮКТУРУЮЧИХ СТРУМІВ ТА АПРОТИНІНУ НА ЗАПАЛЬНУ РЕАКЦІЮ ТКАНИННИХ БАЗОФІЛІВ В УМОВАХ ЕКСПЕРИМЕНТУ

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Ключові слова: флюктуоризація, флюктуофорез, апротинін, запальний процес, тканинні базофіли

Оскільки флюктуруючі струми на початкових стадіях запалення здатні обмежувати та пригнічувати запальні процеси, то підсилити їх лікувальний ефект можна було б шляхом флюктуофорезу лікарських препаратів, що володіють протизапальною дією, зокрема, апротиніну. Гальмування розвитку запального процесу пов'язане з впливом на реакцію тканинних базофілів, які виділяють медіатори запалення шляхом дегрануляції. Метою дослідження було вивчення механізму впливу флюктуоризації та флюктуофорезу з апротиніном на процес дегрануляції тканинних базофілів при експериментальному процесі асептичного запалення. Тваринам однієї дослідної групи (ДГ1) проводили флюктуоризацію приладом низькочастотної електротерапії "Радиус – 01" (Беларусь), а другої (ДГ2) - флюктуофорез з препаратом на основі апротиніну. Ступінь дегрануляції визначали шляхом мікроскопічного підрахунку чотирьох типів тканинних базофілів у плівкових препаратах. Результатами дослідження встановлено, що у тварин, яким проводили флюктуофорез з апротиніном (ДГ2), було виявлено тканинні базофіли зі щільними та дифузними розташуваннями гранул у цитоплазмі (найнижчий ступінь дегрануляції - від 47,0 % до 37,5 %, тобто на 20,5 % менший, ніж після флюктуоризації і на 27 %, ніж у контрольній групі). Отримані дані свідчать про виражений протизапальний ефект флюктуофорезу з апротиніном за рахунок пригнічення функціональної активності тканинних базофілів.

Реферат

ИССЛЕДОВАНИЕ ВЛИЯНИЯ ФЛЮКТУРИРУЮЩЕГО ТОКА И АПРОТИНИНА НА ВОСПАЛИТЕЛЬНУЮ РЕАКЦИЮ ТУЧНЫХ КЛЕТОК В УСЛОВИЯХ ЭКСПЕРИМЕНТА

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Ключевые слова: флюктуоризация, флюктуофорез, апротинин, воспалительный процесс, тучные клетки.

Так как флюктурирующие токи на начальных стадиях воспаления способны ограничивать и угнетать воспалительные процессы, усилить их лечебный эффект можно было бы путем флюктуофореза лечебных препаратов, владеющих противовоспалительным действием, в частности, апротинина. Торможение развития воспалительного процесса может быть связано с влиянием на реакцию тучных клеток, которые выделяют медиаторы воспаления (гистамин, серотонин, простагландини и др.) путем дегрануляции. Целью исследования было изучение механизма влияния флюктуоризации и флюктуофореза с апротинином на процесс дегрануляции тучных клеток при экспериментальном процессе асептического воспаления. Крысам одной опытной группы (ОГ1) проводили флюктуоризацию аппаратом низкочастотной электротерапии "Радиус – 01" (Беларусь), а другой (ОГ2) - флюктуофорез с препаратом на основе апротинина. Степень дегрануляции определяли путем микроскопического подсчета четырех типов тучных клеток в пленочных препаратах. Результатами исследования установлено, что у крыс, которым проводили флюктуофорез с апротинином (ОГ2), определялись тучные клетки с плотными и диффузными гранулами в цитоплазме со степенью дегрануляции от 47,0 % до 37,5 %, то есть на 20,5 % меньше, чем после флюктуоризации и на 27 % меньше по сравнению с контрольной группой. Полученные данные свидетельствуют об выраженном противовоспалительном эффекте флюктуофореза с апротинином за счет угнетения функциональной активности тучных клеток.