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Research on Anti-Inflammatory Activity of Mezsozifen in Prepubertal Age Rats

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Abstract The antiexudative and antiproliferative activity of Mezsozifen was studied in rats of one-month age in comparison with Ibuprofen. It was established that Mezsozifen, which is a mixture of herbal extracts, exhibits a distinct antiexudative and antiproliferative effect in prepubertal animals. In terms of its anti-inflammatory activity, Mezsozifen is approximately 2.5 times more effective than Ibuprofen. It is believed that Mezsozifen can be recommended as an anti-inflammatory agent in the prepubertal period.

Keywords Inflammation, Exudation, Proliferation, Mezsozifen, Ibuprofen, Prepubertal period

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) occupy one of the most important places in pediatric practice and are constantly in the centre of attention of specialists. This attitude of pediatricians towards NSAIDs is stipulated by the fact that they are used quite often in children's pathologies accompanied by pain, fever and inflammation in infectious diseases, as well as traumatic injuries, connective tissue diseases, neuralgia, headache and toothache [1]. At the same time, the use of NSAIDs, when it is required to achieve a detoxifying and analgesic effect, is especially important for pediatrics, because these symptoms of pathology, and especially a hypothermic state, are observed with children much more often than with adults [2]. In terms of consumption, this group of preparations is one of the most popular preparations in the world. However, despite the undoubted clinical efficacy, even short-term, the use of NSAIDs is fraught with the development of side effects in every fourth case, and in 5% of patients it can pose a serious threat to life [3,4].

In contrast to the adults, whose body are a self-regulating biological system with stable functional activity of organs and systems, the children's body is characterized by continuous changes in physiological processes [5].

Unfortunately, the list of NSAIDs used in pediatric practice is very limited and includes preparations that have been used for many years and have sufficiently compromised themselves due to the high frequency of adverse reactions (development of gastropathy, Reye's syndrome) [5]. In this regard, the development and discovery of new, less toxic and more effective antiphlogogenic preparations is of great interest. The researches in recent years has shown that herbal extracts are a vital source of potentially useful new preparations for the prevention and treatment of diseases in which inflammation occupy central place in the pathogenesis of them.

We have previously shown that the sum of extracts of herbal plants has a high anti-inflammatory activity (AIA) [6-8]. However, the AIA efficacy of this compound in the prepubertal period has not been investigated. The importance of this study is associated with the fact that the child's body is in constant growth and development. The intensity of changes depends on the period of childhood with the differentiation in all organ systems, maturation and the inclusion of the body's enzyme systems asynchronous, age-dependent, changes in the functional active organs of the endocrine child's systems [5]. According to the requirements for the introduction of new medicinal substances, experimental studies are required on laboratory animals of the appropriate age, which can be extrapolated to the age of humans. In this regard, the experimental studies on animals of both sexes one month old corresponds to the age of children 4.5-5 years of age [12].

Aim of research was to study anti-inflammatory activity of mezsozifen in prepubertal age rats.

2. Material and Methods

Experimental studies were carried out on one-month-old rat pups of both sexes weighing 50-70 g. It is known that at this age, there are no significant differences in the physiological aspect between male and female. Before the

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beginning of the experiment, all laboratory animals were carefully examined, weighed, their age and physical activity were taken into account. During the entire period of preparation for the experiment and during its conduct, laboratory animals were kept in a vivarium at a temperature of 20-25℃ with humidity of at least 50% in a well-ventilated room and day/night light mode in standard plastic cages, with 6 individuals in each, with a standard diet, where the daily requirement is compiled in accordance with the age of the animals.

A dry extract (conventionally named Mezsozifen) from herbal plants was obtained as follows: Aerial parts of Hypericum perforatum L., Ziziphora pedicellata Pazij et Vved. and Mediazia macrophylla as well as root and rhizome parts of Glycyrrhiza glabra L. dried separately in a dark place at room temperature for 10-12 days. Considering that the soil contains various bacterial spores, they were treated with special methods, then the dry raw materials were separately crushed to a size of 4-6 mm and mixed in a mass ratio of these components, 1.25: 1.0: 1.25: 1.5. Aqueous extraction was carried out at a temperature of 93-95℃ for 3 hours. After filtration, the obtained extract was evaporated in vacuum to obtain a dense residue, which was then dried in a thermostat at 60℃.

Inflammatory edema of rat pups' paw was modeled by subplantar injection of dextran, which is widely used to assess the anti-inflammatory activity of new potential preparations [9,10]. Experimental models of aseptic arthritis were reproduced by subplantar injection of 6% aqueous dextran solution in a volume of 0.05 ml into the hind paw of rat pups. Animal divided to seven groups and each group consisted 6 rat pups. The animals of 1st group was control and they administered distilled water. The animals of 2-, 3- and 4-groups received the various doses of Mezsozifen 5, 10 and 25 mg/kg, respectively and animals of 5-, 6- and 7-group received Ibuprofen in doses of 10, 25 and 50 mg/kg, respectively. The above preparations were preventively administered intragastrically with a metal canula 1 hour before the introduction of phlogogen. Measurement of the paw volume of animals was carried out using a plethysmometer [11] before and in 1, 2, 3, and 4 hours after the injection of dextran. The value of anti-inflammatory activity (AIA, %) of preparations was calculated using the formula:

$$AIA = Vcon\text{-}Vexp/Vcon \times 100 = in\ \%.$$

Antiproliferative activity of Mezsozifen was investigated on the model of "cotton granuloma" [12,13]. This model was created by implanting a sterile cotton swab (weighing 10 mg) under the back skin of rat pups between the shoulder blades. The operation was performed within aseptic conditions under general anesthesia. Animals of the first - third groups on the day of surgery and for the next seven days once a day were intragastrically administered Mezsozifen - 5, 10 and 25 mg/kg, respectively and the animal of fourth to sixth groups were administered Ibuprofen in doses 10, 25 and 50 mg/kg before feeding. Control animals (seventh group) received drinking water in appropriate volume. 24 hours after the last administration of the preparations (on the eighth day), the animals were euthanized under general anesthesia, and cotton balls with the formed granulation tissue around them were removed, weighed on an electronic scales (SINKO, Japan, 2014) and dried at 60℃ to constant weight. The degree of the proliferative phase was judged by the difference between the mass of the dried granuloma and the initial mass of the ball. The exudative reaction was assessed by the difference in the weight of raw and dried granulomas [14,15]. The permission of the Ethical Committee of Uzbekistan was taken before the beginig of experiments. The experiments were carried out in accordance with the "Rules and Regulations for Carrying Out Animal Research Work", as well as the rules adopted in the European Convention for the Protection of Vertebrate Animals used for Experimental Research or for Other Scientific Purposes (ETS No. 123, Strasbourg, March 18, 1986).

2.1. Statistical Analysis

The results of experimental studies were investigated statistically using the standard StatPlus 2009 software package. Significance of indicators (M±m) and differences between the samples were determined using the Student t-test. The difference was accepted significant at a probability level of 95% or more ($p<0.05$).

3. Results and Discussion

The results of the experimental studies showed that in rat pups, Dextran causes the development of distinct edema, the degree of which exceeds the initial one by 144.0% after one hour, which, as can be seen from the data in the table 1, persists without significant changes in the next four hours.

Dextran is known to increase the release of inflammatory mediators such as histamine and serotonin from mast cells [9,10], which leads to an increase in membrane permeability and the development of edema. Ibuprofen is considered one of the most effective NSAIDs used for children [16]. Referring to this fact, it was chosen as a comparison preparation. Preventive administration of Ibuprofen at a dose of 10 mg/kg caused some (15.3-34.5%) suppression of the exudation process in rat pups. Increasing the dose of the preparation by 2.5 times led to a significant suppression of the exudation process. At the same time, the AIA of the preparation within the indicated periods of the experiment (after 1, 2, 3, and 4 hours) was 30.5-55.2%, respectively. A further increase in the dose of Ibuprofen (50 mg/kg) did not lead to a clear increase in its AIA. Consequently, Ibuprofen in rats of prepubertal age exhibits a distinct antiphlogogenic effect, especially at a dose of 25 mg/kg. A similar effect, but a higher anti-inflammatory effect, was noted by us for the preparation Mezsozifen.

Thus, an hour after the start of dextran injection, the volume of the paws of animals receiving Mezsozifene at a

dose of 5 mg/kg increased by 82.7% compared to the initial and by 73.1%, 63.5% and 50.0%, respectively, after two, three and four hours after the injection of phlogogen. The AIA value was 40.3, 44.1, 47.6 and 55.2%, respectively. From the data in Table 1, it can be seen that a two fold increase in the dose of the preparation led to a more expressed suppression of the exudation process, where AIA was 45.8, 51.5, 58.7 and 63.8%, respectively, at the indicated observation periods. Under the influence of a higher dose of Mezsozifene, the noted effect did not undergo statistically significant changes in comparison with the previous one. Consequently, Mezsozifen has a expressed antiphlogogenic activity and surpasses Ibuprofen in its effectiveness. The effective anti-inflammatory dose of Mezsozifen in prepubertal animals is 10 mg/kg, which is 2.5 times less than the corresponding dose of Ibuprofen.

According to modern concepts, inflammation of various etiologies proceeds with the simultaneous course of three processes: exudation, alteration and proliferation. It has been established that the exudative stage of inflammation with increased permeability of various capillaries ends with the proliferation of mesenchymal cells [17].

Since the investigated preparations have a distinct inhibitory effect on the exudation process, it was of interest to study their effect on the proliferative phase of inflammation. The latter is investigated by implanting cotton swab under the skin of animals [18]. At the same time, the technique allows one to simultaneously assess the effect of the substances under study on the exudative (the difference between raw and dried granulomas) and proliferative reactions (the difference between the mass of the dried granuloma and the initial mass of a cotton ball).

Table 1. Antiexudative activity of the anti-inflammatory activity of Ibuprofen and Mezsozifen in prepubertal rats with dextran inflammation

Groups	Dose mg/kg	Volume of paw, sm^3/AIA				
		initial	1 hour	2 hours	3 hours	4 hours
Control	-	0.50± 0.01	1.22 ± 0.02*	1.18 ± 0.02*	1.13 ± 0.02*	1.08 ± 0.03*
Ibuprofen	10	0.51± 0.02	1.12 ± 0.04* 15.3	1.04 ± 0.04* 22.1	0.97 ± 0.03* 26.9	0.89 ± 0.03* 34.5
Ibuprofen	25	0.54 ± 0.01	1.04 ± 0.06* 30.5	0.96 ± 0.06* 38.2	0.87 ± 0.07* 47.6	0.80 ± 0.06* 55.2
Ibuprofen	50	0.52 ± 0.02	1.06 ± 0.05* 25.2	0.98 ± 0.06* 32.3	0.91 ± 0.07* 38.1	0.83 ± 0.06* 46.6
Mezsozifen	5	0.52 ± 0.03	0.95 ± 0.07* 40.3	0.90 ± 0.07* 44.1	0.85 ± 0.06* 47.6	0.78 ± 0.06* 55.2
Mezsozifen	10	0.57 ± 0.02	0.96 ± 0.08* 45.8	0.90 ± 0.07* 51.5	0.83 ± 0.06* 58.7	0.78 ± 0.06* 63.8
Mezsozifen	25	0.56 ± 0.02	0.97 ± 0.08* 43.1	0.91 ± 0.08* 48.5	0.84 ± 0.07* 55.6	0.80 ± 0.06* 58.6

Note: *****-in comparison with initial index (P<0.05);
AIA - anti-inflammatory activity in %.

Table 2. Antiexudative and antiproliferative activity of various doses of Mezsozifen and Ibuprofen in prepubertal rats

Groups	Dose mg/kg	Wet granuloma mass, mg	Dry granuloma mass, mg
Control	-	207.50 ± 7.26	58.83 ± 4.21
Ibuprofen	10	142.83 ± 9.29*	38.83 ± 3.96*
Ibuprofen	25	117.82 ± 9.67*	25.50 ± 2.65*
Ibuprofen	50	105.33 ± 8.23*	25.51 ± 2.51*
Mezsozifen	5	135.67 ± 10.45*	36.66 ± 3.46*
Mezsozifen	10	124.17 ± 8.59*	31.17 ± 3.27*
Mezsozifen	25	129.50 ± 6.50*	32.67 ± 2.65*

Note: *-in comparison with control index (P<0.05).

The results of the carried out research showed that the compounds under study have an expressed antiexudative effect. From the data in table 2, it can be seen that Ibuprofen inhibits the exudation process by 31.2, 43.2 and 49.2% at doses of 10, 25 50 mg/kg, respectivily and Mezsozifen at doses of 5, 10 and 25 mg/kg by 34.6, 40.2 and 37.6%, respectively. It can be seen that Mezsozifen is practically not inferior to Ibuprofen in its activity, especially at doses of 5 and 10 mg/kg. These data are in agreement with the results of a previous series of experiments, where the antiphlogogenic activity of preparations was investigated in a model of dextran-induced aseptic arthritis.

The calculation of the antiproliferative activity of the studied antiphlogogens showed that the degree of proliferation decreases under the influence of Ibuprofen by 34.0, 51.5 and 56.6% in doses of 10, 25 and 50 mg/kg, respectively. Practically the same effect was obtained at animals receiving Mezsozifene. Thus, the preparation at a dose of 5 mg/kg suppresses the intensity of proliferation by 38.0%, and at a dose of 10 mg/kg by 47.0%. From the data in Table 2 it can be seen that an increase of the dose of Mezsozifene by 2.5 times did not lead to a noticeable change in the results compared to the previous one.

It is noteworthy that this preparation, which is a combination of herbal extracts in 2 times less dose has the same effect as Ibuprofen. This fact indicates a higher antiexudative and antiproliferative activity of Mezsozifen.

In our opinion, the main mechanism of Mesozzifen's antiexudative action is its antioxidant property, due to the flavanoids contained in it [19]. In addition, the preparation inhibits the activity of kinins, an enzyme of hyaluronidase, suppresses the concentration of anti-inflammatory interleukins and increases the content of anti-inflammatory interleukins [6,7].

Thus, the results of this study allow us to recommend Mezsozifen as a preparation for the treatment of inflammatory diseases of children.

4. Conclusions

1. Mezsozifen, which is a mixture of herbal extracts, has a distinct antiexudative and antiproliferative effect in prepubertal animals.
2. An effective anti-inflammatory dose of Mezsozifene is 10 mg/kg in prepubertal rats.
3. In terms of its anti-inflammatory activity, Mezsozifen is approximately 2.5 times more effective than Ibuprofen.
4. Mezsozifene can be recommended as an anti-inflammatory agent in the prepubertal period.

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