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# A STUDY ON ACUTE TOXICITY, LOCAL IRRITATIVE EFFECT OF AND ALLERGIC RESPONSE TO A NOVEL INTRANASAL MEDICATION CONTAINING THE INTERLEUKIN-1B RECEPTOR ANTAGONIST (IL-1RA)

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For a long time, nasal dosage forms have been used for delivery of active ingredients of various drug classes intended for topical administration, such as vasoconstrictor, anti-allergic and wound healing. Later, scientists investigated the possibility of using nasal administration for delivery of active ingredients with systemic effect into the human body. The transmission rate of active ingredients into the bloodstream using nasal delivery is often similar to parenteral administration. This is probably due to the branched system of capillaries in the nasal cavity and some features of transport [1, 2, 3, 4].

A development of novel medications includes such investigations as: choosing an active ingredient or formulations of active ingredients, conducting physical and chemical, analytical, technological, rheological and biopharmaceutical researches on the choice of auxiliary substances. Then, the drug stability must be studied and the necessity of preservatives and stabilizers inclusion into the composition must be justified [5, 6, 7].

Our research studies, the composition of a novel intranasal medication has been developed. It contains IL-1Ra and auxiliary substances necessary for formation of the dosage form. Considering a necessity of conducting researches on safety of the novel medication [8, 9, 10], it is relevant to study some toxicological parameters, local irritative effect of and allergic response to the novel intranasal medication.

The aim of the research is to study some toxicological parameters, local irritative effect of and allergic response to effective dose of a novel intranasal drug containing IL-1ra.

**Materials and methods.** The studies were conducted on outbred albino rats weighing 98-110 g in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and other recommendations. Each group consisted of 6 animals. The gel under research was administered intranasally with syringe dispenser in the maximum allowable amount – 0,4 ml. Changes in cardiovascular, respiratory, central nervous systems, motor activity and death of the animals were recorded within 14 days.

The study of allergic response to and irritating effect on skin of the nasal gel was conducted on outbred albino rats of both sexes weighing 120-150 g (2 groups – control and experimental with 10 animals in each) in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and other recommendations. On the lateral surface of the body of animals an area of a 4x4 cm was shaved. This area was exposed to application of 0,5 g of the gel, then the animals were kept in individual cells for 4 hours to prevent licking off the drug. The application of the gel was repeated 20 more times by means of cutaneous applications 5 times per week. Skin allergy reactions were analyzed daily according to the scale of evaluation of skin samples. The first test was conducted after 10 applications (in the event of detecting allergic reaction, further application of the medication was intended to stop). In case of negative or doubtful effect, the number of skin applications was to be increased to 20. An evaluation of the analysis results was conducted according to the corresponding scale (table 1).

**Table №1**

**The evaluation scale of the skin application tests**

| Designation of reactions | Symbols | Description of reactions |
|---|---|---|
| negative | - | No changes in skin surface were observed |
| doubtful | ± | Slightly manifested erythema without swelling |
| weakly positive | + | Erythema and swelling of the application area |
| positive | ++ | Erythema, swelling, papules |
| moderately positive | +++ | Erythema, swelling, papules, vesicles |
| strongly positive | ++++ | Erythema, swelling, papules, bullae |

The research on local irritative effect was carried out on guinea pigs weighing 480-520 g (2 groups – control and experimental with 10 animals in each), in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and other recommendations. On the conjunctiva of both eyes of animals from the experimental group 0,01 ml of gel was applied with the dispenser. The rats from the control group received distilled water administration into conjunctival sac. The observation lasted for 3 days. The assessment of the allergic reaction was performed according to a scale: 0 points – no changes in the conjunctiva; 1 point – slight reddening of the conjunctiva; 2 points - reddening of the conjunctiva and swelling.

The study of cutaneous anaphylaxis reaction to the gel was conducted on outbred albino female rats weighing 180-190 g (2 groups – control and experimental with 10 animals in each) in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine. On the lateral surface of the body of animals an area of a 4x4 cm was shaved. This area was exposed to application of 0,5 g of the gel, then the animals were kept in individual cells for 4 hours to prevent licking off the drug. Sensitization of animals was estimated in 5 days after the last drug application. With this purpose, 0,3 g of the gel was applied once on the skin of the ear. The analysis of the intensity of the anaphylactic shock was performed after 6, 12 and 24 hours using the anaphylactic shock index of Weigle et al.: ++++ – a shock with lethal outcome; +++ – a shock with severe symptoms (general convulsions, asphyxia, the animal loses the ability to stand on its paws, falls to the side, no lethal outcome); ++ – a mild shock (slight convulsions, pronounced symptoms of bronchospasm); + – a slight shock (some anxiety, rapid breathing, itchy muzzle, involuntary urination, defecation, disheveled hair); 0 – a shock reaction did not occur, no symptoms were detected.

Statistical processing of the results was conducted by means of the standard statistical package of the licensed program «STATISTICA® for Windows 6.0» (StatSoftInc., №AXXR712D833214FAN5) and «SPSS 16.0», «Microsoft Office Excel 2016». An assessment of the normality of data was estimated according to the Shapiro-Wilk test. The data is described in the form of an average value. The significance of the difference between the average values was determined by the Student's t test in the normal distribution. In case of distribution other than normal or for ordinal variable analysis the Mann–Whitney U test was used. For independent variables comparison in more than two samples analysis of variance (ANOVA) in normal distribution or the Kruskal–Wallis test in distribution other than normal were used [6]. For all analysis types the p-value <0,05 (95%) was considered statistically significant.

**Results**. Determination of acute toxicity parameters of the nasal medication containing IL-1ra has been carried out in accordance with recommendations of the State Expert Center of the Ministry of Health of Ukraine. It has been found that one-time intranasal delivery of the maximum allowable volume of the gel with IL-1ra (0,4 ml, 0,5%) to the rats weighing 100 g in a dose of 20 mg/kg did not result in death of any of 6 animals of the group overnight (table 2).

**Table 2**

**A study on acute toxicity of the gel containing IL-1ra on the rats using intranasal administration**

| The maximum allowable volume, ml | Dosage, mg / kg | The number of rats | | | Mortality, % |
|---|---|---|---|---|---|
| | | *Overall* | *Dead* | *Survived* | |
| 0,4 | 20 | 6 | 0 | 6 | 0 |

No visible pathological changes, as well as changes in behavior of the experimental animals occurred on the 1, 7 and 14 day after one-time intranasal administration of the gel containing IL-1ra. It has been found that dynamics of body weight change was within the physiological norm (table 3).

**Table 3**

**The dynamics of body weight change in rats after one-time administration of the intranasal gel containing IL-1ra, M ± m**

| The groups of animals | Initial state, (g) | the 7th day, (g) | the 14th day, (g) |
|---|---|---|---|
| The nasal gel containing IL-1ra | 100,2 ± 2,7 | 123,2 ± 3,1 | 146,2 ± 2,2 |
| The control group | 107,1 ± 9,5 | 133,1 ± 8,6 | 154,3 ± 4,1 |

In the course of studying potential local irritative effect of the gel containing IL-1ra (0,5%), 1 experimental animal out of 10 developed a slight reddening of the conjunctiva immediately after the administration. No changes in mucous membrane of the eyes were observed in the rest 9 animals. On the second and third day after the administration, none of the experimental animals developed a positive reaction of mucous membrane of the eye. This indicates the absence of irritant action in this dosage form. Thus, it has been found that the studied gel containing IL-1ra (0,5%) does not induce a local irritative effect.

As a result of studies on cutaneous anaphylaxis reaction to the gel containing IL-1ra, it has been found that a daily application of the gel containing IL-1ra (0,5 g) on a shaved area of the lateral surface of the body of animals (4x4 cm) during 5 days, and then one-time application of the gel (0,3 g), did not result in development of anaphylactic shock. No symptoms of anaphylactic shock occurred as a result of the gel application (0,3 g) after 6, 12 and 24 hours. Thereby, the gel containing IL-1ra (0,5% ) does not induce allergic reactions of anaphylactic type when administered to the animals during 5 days.

Then researches of allergenic effect of the gel containing IL-1ra have been conducted by means of skin applications. The researches have revealed the following: no visible reactions were detected in the animals after 20 more skin applications of the gel containing IL-1ra (0,5% ) during 4 weeks (5 times per week). The appearance of the skin area exposed to application in animals of the control and experimental groups was the same (table 4).

**Table 4**

**The results of the study on allergenic effect of the gel containing IL-1ra (0,5%) according to the scale of evaluation of skin samples on the 4th week of observation (20 applications)**

| The groups of animals n=10 | Designation of reactions | Symbols | Description of reactions |
|---|---|---|---|
| The gel containing IL-1ra (0,5%) | Negative | - | No changes in skin surface were observed. |
| The control group | Negative | - | No changes in skin surface were observed. |

Considering the obtained research data, it can be concluded that the gel under study containing IL-1ra (0,5%) neither induces allergic reactions on cellular level, nor provokes allergic dermatitis after 20 applications during 4 weeks.

Conclusions. The complex studies of some toxicological parameters (such as mortality, dynamics of body weight change, local irritative effect of and allergic response to the effective dose of the medication containing IL-1ra for nasal delivery) have been performed. Summarizing obtained result data, it can be confirmed that the medication under study does not cause any local irritative effect and allergic response and does not demonstrate general toxic effects in case of its intranasal delivery.

Conflict of interest: All authors declare that no conflict of interest exists.

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