

THE INFLUENCE OF SUBLINGUAL TABLETS OF THIOTRIAZOLIN AND DECAMETHOXIN ON ALLERGY MARKERS AND TOXICITY INDICATORS

Kucherenko L. I., Belenichev I. F., Chonka O. O., Moryak Z. B., Gylevskaya O.
Zaporizhzhia State Medical University, Ukraine

At present, various injuries and inflammatory processes of the oral mucous membrane of various etiologies are one of the most common conditions of diseases.

According to the World Health Organization, oral mucosa accounts for 38% of all diseases, and currently this pathology accounts for about 62%. Despite numerous studies in this area, oral mucous membrane diseases are a topical problem in modern fundamental and clinical medicine and pharmacy.

Despite a large arsenal of anti-inflammatory, antibacterial, wound healing, traditional treatment of this pathology does not always give the expected result. In addition, quite often, traditional therapy provides an increase in complications, making it difficult to treat a particular patient. In this regard, the development and creation of drugs that can effectively affect the main links-target inflammatory diseases of the oral mucous membrane is an urgent task of modern pharmacology.

The incidence of oral mucous membrane diseases in adults has reached 98%. OMMDs are among the diseases that have been known since ancient times. With the development and progress of humanity, the incidence of inflammatory diseases is increasing dramatically. If still in the beginning of XX century the given disease met at persons of 40 years and more senior, already in 80-90s years of CVZTP all over the world noticeably "have rejuvenated". This was revealed by epidemiological surveys of the population, based on an analysis that involves the definition of indicators characterizing the state of periodontal tissues. Medication-assisted treatment continues to be essential today. However, the drugs used, each of which acts on a specific link in the pathological process, have a number of side effects and do not always allow to quickly stop the inflammation of periodontal tissues.

For the treatment of these diseases a comprehensive treatment is used, namely, the therapy includes not only antimicrobials, but also necessary drugs to maintain the immune system of the body.

The development of dental services in recent years, especially in market conditions, has brought to the fore the issues of improving the quality of treatment. One of the main directions to ensure the quality of dental care is the search for new drugs to treat pathologies. Therefore, the task for creating new drugs is the most urgent in the pharmaceutical industry.

Having studied the range of medicines in the pharmaceutical market of Ukraine and abroad, it was found that decamethoxin is most commonly used. As decamethoxin has a number of side effects, it is advisable to use it in combination with antioxidant. One of the well-known antioxidants, which has been actively used in the pharmaceutical market for 20 years, is thiotriazolin.

Therefore, together with the BLD "Pharmatron" and the staff of the Medical University at the Department of Pharmaceutical Chemistry, under the guidance of Professor I.A. Mazur, it was proposed to use decamethoxin with thiotriazolin in the ratio (1:100) to create new sublingual tablets.

The widespread use of known, as well as the development of new original drugs necessitates the mandatory preclinical evaluation of both their specific pharmacological activity and toxic properties. At the same time, the risk assessment of toxic manifestations on the basis of experimental studies provides not only for the study of general toxic properties caused by the chemical structure of active substances.

One of the main parameters in the development of new any drug forms is to determine their toxicity.

Therefore, the purpose of our work was the study of allergy markers and acute toxicity indicators of sublingual tablets in a combination of decamethoxin and thiotriazolin

Materials and methods. In the course of our work we used sublingual tablets of series №1 (the average weight of the tablet is 0.8g), which were obtained in the laboratory of "Standardization and technology of pharmaceutical products" at the Department of Pharmaceutical Chemistry of Zaporozhye State Medical University. In the same way, 30 not pedigree white rats were used. Acute toxicity of decamethoxin and thiotriazolin tablets (1:100) was determined using the Kerber method in the modification of A.O. Loit and M.F. Savchenkov using the classification of K.K. Sidorov.

Determination of acute toxicity

In order to establish the average lethal dose (LD50) of the studied drug, it was injected intragastrically in the form of an aqueous solution using a metal probe, and intraperitoneally, once to 5 groups (at each route of administration) of laboratory animals (not pedigree white rats), 6 heads each. Several doses of tablets were administered, including a dose that did not cause death of any animal and a dose that caused death of all animals in the group. After the tablets were injected, the animals remaining alive were monitored for two weeks.

After two weeks of observation, the deceased animals and the living animals were examined by a pathologist.

The allergenic and skin-irritating effect of thiotriazolin tablets with decamethoxin was studied on white outbred rats weighing 180-190 g of female (2 groups - control and experience of 10 animals each), according to the recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and other recommendations. On the lateral surface of the torso of animals, the hair was cut in a 4 × 4 cm area.

0,5 g of tablet mass was applied to this area of the skin, after which the animals were placed for 4 hours in individual cages to prevent drug licking. The application of the tablet mass was carried out by 20 repeated cutaneous applications 5 times a week.

The skin reaction was taken into account daily on the scale of assessment of skin samples. The first test was carried out after 10 applications (in case of allergy detection, further application of the substance was supposed to be stopped). If the result is negative or doubtful, the number of applications must be brought to 20. The results of the allergenic activity of the tablet mass are evaluated by the method of cutaneous applications according to an appropriate scale, which we introduce at table 1.

Table 1 Scale for assessing application skin tests

Reaction	Designation Conventions	Description
negative	-	No skin change
doubtful	+ -	Slight erythema without edema
weakly positive	+	Erythema and edema at the site of application
positive	++	Erythema, edema, papules
sharply positive	+++	Erythema, edema, papules, isolated vesicles
very sharply positive	++++	Erythema, edema, papules, confluent vesicles

The study of allergenic properties in the test of histamine release by blood basophils.

The effect of the studied tablets on the intensity of histamine release was studied on whole blood cells of six donors (6 repetitions in total): three healthy donors and three patients with grade II periodontitis. Donors with inflammatory diseases of the oral cavity did not receive any therapy. The test tablets were taken in several dilutions for each repeat. As 88 controls, various dilutions of anti-IgE and OVA were used, as well as a negative control without the addition of any agents. The heparinized blood obtained from six donors in a volume of 8–9 ml was precipitated by centrifugation under mild conditions (150 g for 8 minutes), the plasma was removed, and the precipitate was dissolved in PIPES buffer. Then centrifugation was repeated under the same conditions for 10 minutes, the supernatant was taken and again restored to PIPES. Agents and blood components dissolved in PIPES buffer were incubated separately for 15 min at 37 ° C, after which 225 µl of blood components dissolved in PIPES buffer were mixed with 100 µl of tablets or control substance in the required dilution. The mixture was incubated for 1 hour at 37 ° C. After that, the samples were centrifuged at 200g for 10 minutes and supernatants were taken. To assess the level of histamine yield, thin-layer chromatography followed by spectrophotometry was used. The sensitivity limit of this method is 10 ng / ml.

The results of the study were calculated using the standard statistical package of the licensed program STATISTICA® for Windows 6.0 (StatSoftInc., No. AXXR712D833214FAN5), as well as SPSS 16.0 and Microsoft Office Excell 2003. Distribution normality was assessed by the Shapiro-Wilk test. Data are presented as mean values. The significance of differences between the mean values was determined by the Student criterion with a normal distribution. In the case of a non-normal distribution or analysis of ordinal variables, the U Mann-Whitney test used. For comparison of independent variables in more than two samples, analysis of variance (ANOVA) with normal distribution or the Kruskal-Wallis criterion for a distribution other than normal were used. For all types of analysis, differences $p < 0.05$ (95%) were considered statistically significant.

The results of toxicological studies presented in Table 2 indicate that a single intragastric administration of thiotriazolin tablets with decamethoxin in a dose of 20,000 mg/kg did not cause any animal death.

With further injection of 30 000 mg/kg, 1 rat died within 36 hours and 5 remained alive.

A dose of 40 000 mg/kg killed 3 animals out of 6 during this time. The administration of thiotriazolin tablets with decamethoxin at a dose of 50 000 mg/kg caused the death of 4 animals at night for 1-2 days of observation. A single intragastric administration of the tablets in a dose of 60 000 caused 100% animal death during the day.

Table 2. Results of experiment in determining the acute toxicity of thiotriazolin decamethoxin tablets in a single intragastric administration to white not pedigree rats after 2 weeks of observation

Dose, mg/kg (group number)	20 000 (1)	30 000 (2)	40 000 (3)	50 000 (4)	60 000 (5)
Survived	6	5	3	2	0
Dead	0	1	3	4	6

Observations of animals receiving intermediate doses of thiotriazolin tablets with decamethoxin allowed us to determine LD50.

The picture of acute poisoning of animals that received a toxic dose of thiotriazolin tablets with decamethoxin was characterized by frequent defecation, urination, increased retardation, reduced approximate research activity, twitching of

the hind legs, then piling on the side while moving, pathological breathing according to Chane-Stokes and the death of animals from respiratory paralysis. Animals that received intermediate doses of thiotriazolin tablets with decamethoxin and survived, within 12 hours after the drug was administered, showed depression of breathing, heart function, approximate research activity, spontaneous twitching of limb muscles. The above symptoms disappeared on 2-3 days.

It should be taken into account that the studied tablets contained 0.2 grams of thiotriazolin, 0.002 grams of decamethoxin and 0.598 grams of pharmacologically inert auxiliary substances. LD50 of thiotriazolin substance during intragastric administration of rats was 10300 mg/kg and decamethoxin was 600 mg/kg. Studies have shown that when applying 20 repeated cutaneous applications of the tablet mass for 4 weeks (5 times a week) during the entire period of observation of animals, no visible reactions were observed.

The appearance of the skin at the place of application of the tablet mass of the control and experimental (application of tablets with thiotriazoline and decamethoxin) did not differ (Table 3).

Table 3 The results of the study of the allergenic activity of thiotriazolin tablets with decamethoxin according to the scale for assessing application skin tests at 4 weeks of observation (20 applications)

Groups n=10	Reaction designation	Conventions	Reaction description
Thiotriazolinum tablets with decamethoxinum	negative	-	No skin change
The control	negative	-	No skin change

Based on the studies, we can conclude that the studied tablets of thiotriazolin and decamethoxin with 4-week use (20 applications) do not cause allergic reactions in the cellular type and do not cause allergic dermatitis.

It is known that the content of histamine is especially high in mast cells, basophils, as well as cells of the gastric mucosa and platelets. Its release during an allergic reaction occurs during the degranulation of 181 cells, mediated by cross-linking the allergen with IgE molecules on the cell membrane. As a result of the reaction, we detect the amount of histamine released, after incubation of blood cells together with the studied drug in a wide range of doses, as well as two controls - ovalbumin and antibodies to IgE. After analysis, we obtained the following primary data (Table 4). From the data obtained, it is seen that the negative control, as expected, did not cause the release of histamine by blood basophils. Positive control (anti-IgE), on the contrary, induced histamine release in all six cases, and for donors with periodontitis the values were higher than for healthy donors. This indicates a higher sensitivity of blood basophils in these donors. Incubation of blood with various dilutions of thiotriazolin and decamethoxin tablets led to a slight release of histamine, and only in cases with donors with oral diseases, however, these values were noticeably lower than the control ones (after stimulation of blood cells with antibodies against IgE). It is also worth noting that the rate of histamine release was not dose-dependent (Table 4). Thus, it was found that the blood basophils of healthy donors do not release histamine in response to the effects of thiotriazoline and decamethoxin tablets. However, more sensitive blood basophils obtained from donors with periodontitis did not dose-dependently release a small amount of histamine. In general, tablets with thiotriazolin and decamethoxin do not have histamine-releasing properties.

Table 4. The effect of thiotriazolin tablets with decamethoxin on histamine release by basophils in the blood, ng / ml.

Dilution of agents	ZD1	ZD2	ZD3	P1	P2	P3
Tablets 1000 mcg / ml	bld	bld	bld	31	bld	bld
Tablets 100 mcg / ml	bld	bld	bld	11	bld	bld
Tablets 10 mcg / ml	bld	bld	bld	42	bld	bld
Tablets 1,0 mcg / ml	bld	bld	bld	9	bld	bld
Tablets 0.1 mcg / ml	bld	bld	bld	bld	bld	bld
Tablets 0.01 mcg / ml	bld	bld	bld	bld	bld	bld
anti-IgE 1:100	211	266	321	1453	1265	1187
anti-IgE 1:300	67	88	74	347	421	644
anti-IgE 1:900	13	21	34	112	98	112
OVA 100 мкг/мл	bld	bld	bld	67	34	54
OVA 10 mcg / ml	bld	bld	bld	32	19	21
OVA 1 mcg / ml	bld	bld	bld	17	bld	bld
Control	bld	bld	bld	bld	bld	bld

Note: HD - healthy donor, P - periodontitis, BLD - below the limit of definition

Conclusions:

1. Thiotriazolin tablets with decamethoxin belong to the VI class of toxicity.
2. Thiotriazolinum tablets with decamethoxinum at 4-week application (20 applications) do not cause allergic reactions on a cellular type and do not cause allergic dermatitis.
3. Tablets with thiotriazolin and decamethoxin do not have histamine-releasing properties
4. The results obtained are experimental justification for further more in-depth studies.

Conflict of interest: none.

References.

1. Danilevskij, N.F., & Borisenko, A.V. (2010). Zabolevanija parodonta. [Periodontal disease]. Kyiv. [in Russian].
2. Mazur, I.P. (2010). Farmakologichni zasoby dlja mistsevoho likuvannia tkanyh parodonta [Pharmacology for the treatment of periodontal tissues]. *Modern dentistry*, 5, 47-52. [in Ukrainian].
3. Borisenko, A.V. (2013). Zabolevanija parodonta [Periodontal Disease]. Kyiv. [in Russian].
4. World Health Organization. Oral health. (2018). Retrieved from <https://www.who.int/news-room/fact-sheets/detail/oral-health>.
5. Modell B. (2012.) *Epidemiology of Oral Clefts 2012: An International Perspective* Cobourne MT (ed): Cleft Lip and Palate. *Epidemiology, Aetiology and Treatment*. Vol 16. Basel: Front Oral Biol. Karger. <https://www.karger.com/Article/Abstract/337464>.
6. Glendor U. (2009) Aetiology and risk factors related to traumatic dental injuries-a review of the literature. *Dent Traumatol*. 25 (1): 19-31. <https://www.ncbi.nlm.nih.gov/pubmed/19208007>.
7. Petersen P E, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C (2015). The global burden of oral diseases and risks to oral health. *Bull World Health Organ*; 83 (9): 661-669.
8. Gozhaja, I.N. (2012). Risk rozvitija zabolevanij parodonta pri nalichii hronicheskikh socialnyh stressov u klinicheskij zdorovyh lic [Parodontologija] 1.21-25. [in Russian].
9. Cepov, L.M. (2010). Hronicheskij generalizovannyj parodontit: ot patogeneza k lecheniju [Dental Jug] 9.32-34.
10. Chekman I. S. Pharmacophore: creation of medications: (review of the literature and own studies) / Chekman I. S. // *Zhurn. AMS of Ukraine*. – 2010. – Vol. 16, № 3. – P. 424-437

Key words: oral mucosa diseases, model mixture, thiotriazoline, decamethoxinum, animals, toxicity, tablets, activity.