

Pharmacological Research of Loravit Suppositories

Iryna V. Herasymova¹, Oleksandr I. Tykhonov², Oleg S. Shpychak³,
Nataliya V. Khokhlenkova¹, Maryna V. Buryak¹, Vasil N. Koval⁴

¹Department Technology of Drugs, National University of Pharmacy, Kharkiv, Ukraine, ²Department of Technology of Perfumes and Cosmetics, National University of Pharmacy, Kharkiv, Ukraine, ³Drug Technology Department named after D.P. Salo, National University of Pharmacy, Kharkiv, Ukraine, ⁴Department of Pharmacy of the National Pirogov Memorial Medical University, Vinnytsya, Ukraine

Abstract

Introduction: The work represents the data about the worldwide spread age of allergic diseases both among adult and childish population. The given information is released according to the literary data analysis, including the WHO data. By studying these literary data, we prove the actuality of new antiallergic medicines creation, particularly, in pediatrics. **Materials and Methods:** The experimental part represents the study results of antihistamine activity, acute toxicity, and safety of new drug for the children's allergic disease medication. The research has been using pharmacological, physiological, and instrumental methods alongside the mathematical statistics method. **Results and Discussion:** As a result for investigations, we defined, that Loravit, a new antihistamine drug, prevents the conglomeration of homocytotropic antibodies, suppresses the mastocytes degranulation, which speaks of its manifested antiallergic activity. While investigating the drug's acute toxicity, the Loravit test sample in 5000 mg/kg dose was defined not to cause the animals death and influences the mass coefficients of internal organs, which proves the absence of meaningful test sample toxicity in the given dose, which characterizes it as non-toxic. **Summary:** By the investigation of Loravit suppositories pharmacological activity, its test samples were proven to obtain a manifested antiallergic activity. By the results of toxicological investigations, the new loratadine hydrochloride-based drug is considered to be mostly non-toxic (toxicity Class V, LD₅₀ >5000 mg/kg) according to the general toxicity classification. The results of investigations allow to consider the developed drug to be perspective for further production and market implementation.

Key words: Acute toxicity, allergic diseases, antihistamine activity, loratadine hydrochloride, Loravit suppositories

INTRODUCTION

According to the statistics, more than a third part of Earth inhabitants suffers from allergy. The increasing of allergic pathology patients is shocking. The "allergy" term was first proposed by an Austrian pediatrician Clemens Peter Freiherr von Pirquet. This definition indicates a change in the sensitivity of the organism to substances with which the organism previously contacted.^[1]

At first, the allergic reactivity was connected to the development of inflammatory reaction in the point of allergen influence. Later, with the data discovery for histohematic barriers role, the new views on the allergy development mechanism have formed. Allergic reactivity started to get reviewed as the biologically advisable, highly specific and sensitive reaction at the allergen income to the organism, due to the damage of

natural histohematic barriers, both mucous and skin, and the barrier penetrability as the most important factor of allergy predilection.^[2]

Allergic reaction terminology and classifying were reviewed on the beginning of 00 s by the Commission of European Academy of Allergy and Clinical Immunology and were accepted everywhere. According to the new terminology, allergy is a hypersensitivity reaction, caused by the immune mechanisms. Today, allergy is a medicosocial problem

Address for correspondence:

V. Herasymova Iryna, Department Technology of Drugs,
National University of Pharmacy,
Valentynivska Street 4, 61168, Kharkov, Ukraine.
Tel.: +38-093-123-18-41.
E-mail: iryna_herasymova@ukr.net

Received: 26-01-2018

Revised: 21-02-2018

Accepted: 03-03-2018

because of versatile etiologic factors, immunological pathogenesis violations, wide specter of allergens, ecology's growing role, climate changes, population migration, etc., according to the WHO data. Allergy is one of the most spread non-infectious diseases and is spectated among 35% population.^[3]

According to the epidemiological researchers in many countries, 40% children suffer from allergic diseases, where bronchial asthma, allergic rhinitis, and atopic dermatitis are dominating.^[4-6] For instance, in the UK, the allergic diseases are registered among 32.2% teenagers, in Australia and New Zealand, 50% children suffer from allergy. In Turkey, the allergic pathology is spread among 16.5...34.7% children, depending on the atmospheric air pollution, where asthma takes from 0.2 to 4.9%.^[7-9]

Today, allergic rhinitis is in five of the most spread chronic diseases and, according to the ISAAC data, is registered among 10–30% adult and near 42% childish population.^[10-12] The frequency of this illness is increasing in every country and childish AR illness is from 4% to 32%.^[13-16]

According to the given information, we must admit that the creation of new antiallergic drugs in a form of suppositories for children is one of the important pharmaceutical science tasks alongside with today practice. That is why, we designed a technology and composition of new antihistamine suppositories for children with loratadine hydrochloride.

Experimental part

To confirm the drug-specific activity and safety, we held the pharmacological investigation of Loravit test samples.

The investigation of specific pharmacological action to reveal the expected antiallergic activity of Loravit test sample was held in compliance to State UMOH expert center SE.^[17,18] The tests were made according to the National “general ethical principles of animal experiments” (UA, 2001), compliant to the European directive on the protection of animals used for scientific purposes (Strasbourg, 1986).^[19]

The severity of given test sample action was estimated by its ability to prevent from hypersensitivity reaction of instant and slowed type in the test of indirect mastocytes degranulation and conjunctival probe^[20] compared to referent sample, Claritin syrup. The test was made on guinea pigs (550–600 g) and white breedless rats (150–195 g). As the given drug is prescribed for rectal application, it was applied rectally on the animals in the dose of 0.25 mg/kg, calculated (basing on the children daily dose) as conditionally therapeutic using the coefficients of special durability by Yu and Rybolovlev.^[21]

The study of antiallergic Loravit test sample properties in the conjunctival probe was held on the guinea pigs, with a rectally applied test sample in the dose of 0.25 mg/kg and 1 ml/kg

referent sample during 21 days. The animals from a positive control group were everyday given the purified water. On the 21st day, in 40 min after the test samples appliance, every animal was given 1 drop 1% histamine water solution per palpebram. The left eye was taken as a control. The mucous membrane reaction was registered 15 min after allergen appliance and was expressed in points by the following scale:

- 0 point - no reaction;
- 1 point - weak reaction (barely noticeable eyelid edema, short-term eye itching, slight eye channel redness, and slight lacrimation);
- 2 points - moderate reaction (moderate edema of eye lids and conjunctive, pre-corneal sclera and eye channel redness, lacrimation, and short-time itching);
- 3 points - expressed reaction (strong clear and conjunctive edema, their whole redness, immense lacrimation, and highly expressed frequent itching).

To be fully informed about possible antiallergic features of given test sample, we held the *in vitro* allergologic test - a reaction of indirect mastocytes degranulation, which is a result of peritoneal rat mastocytes with a blood serum of sensitized animal and corresponding allergen, and shows an ability of investigated test sample to prevent from homocytotropic antibodies appearance. To set the test, we used the white breedless rats (female and male), sensitized 1% glair solution 3 times every other day 0.2 ml per injection, by the following scheme: The first injection was subcutaneous and two other were intramuscular into the hip area. The animals from test groups were rectally applied with an investigated sample in 0.25 mg/kg dose and the referent sample in 1 ml/kg dose. The animals of negative control group were injected with purified water. On the 21st day, the animals were given a general ether narcosis and a serum for the test. The previous experiments were held using the solution with egg white concentration, causing not higher than 10% non-specific degranulation. The preparations were made on the slides, painted with 0.3% alcohol solution of neutral red. The 0.03 ml mastocytes from the intact animal peritoneal exudate were added with 0.03 ml serum of sensitized or intact animals and 0.03 ml eye white solution. The following controls were accounted:

- 0.03 ml mastocytes suspension, 0.03 ml tested serums, and 0.03 ml saline;
- 0.03 ml mastocytes suspension and 0.06 ml saline;
- 0.03 ml mastocytes suspension, 0.03 ml saline, and 0.03 ml eye white solution.

The preparations were afterward incubated for 15 min at 37°C and investigated under the light microscope. Every camera contained 100 cells, which were not contacting each other. The cells were divided into two categories: Normal and degranulated. The reaction was considered negative, if the degranulated cell quantity was <10%. The experiment results are represented in Table 2.

While investigating the toxicologic features of drugs, the acute toxicity definition is a stage of information gathering

Table 1: Influence of Loravit test sample on the ophthalmic reaction severity

Animal groups	n	Dose	Ophthalmic reaction, points
Positive control	6	-	3 (3–3)
Allergin	6	50 mg/kg	2 (1–2)*
Claritin	6	1 ml/kg	2 (1–3)*

n (animal quantity in the group)=6, *: Index deviations, reliable to the positive control, $P < 0.05$

Table 2: The Loravit test sample influence on mastocyte degranulation

Animal groups	Dose	n	Degranulated cells quantity, %
Intact control	-	6	1.67±0.42
Positive control	-	6	15.17±1.49*
Test sample (Loravit)	0.25 mg/kg	6	6.67±1.17**/**
Referent sample (Claritin)	1 ml/kg	6	6.00±1.10**/**

n (animal quantity in the group)=6, *: Index deviation, reliable to the intact control data, $P < 0.05$; **: Index deviations, reliable to the positive control, $P < 0.05$

about the given drug danger/safety for the health during the short-time usage of high doses. One of the main toxicologic features of the drug is an average lethal dose index (LD_{50}) and its standard deviation ($LD_{50} \pm m$). To recreate the clinic of acute intoxication and to define the LD_{50} , the acute toxicity of Loravit test sample was studied on white breedless rats weighing 240–280 g according to the State Enterprise “State Expert Center of the Ministry of Health of Ukraine” recommendations. The suppositories were applied rectally. The application way was selected corresponding to the prognosed usage of test sample in clinical practice. According to the State Enterprise “State Expert Center of the Ministry of Health of Ukraine” recommendations,^[22] the limiting index at the drug acute toxicity definition is the Maximal IV class toxicity dose, considering the application way (5000 mg/kg per rectum according to Ukrainian State Pharmacopeia). If the Loravit test sample is considered non-toxic, the following tests can be considered pointless. Considering this, the test sample acute toxicity was defined starting from 5000 mg/kg. The animals were spectated during 2 weeks. The test sample toxicity grade was valued according to the change of animals overall state, lethality, test sample influence on animals body mass, and internal organ coefficients [Table 3].

The obtained experimental data were processed by the method of variation statistics (calculated the arithmetical mean and standard error).^[23] For comparison of the normal distribution, one-factor ANOVA and Newman-Calex for multiple comparisons were used, for non-parametric data, the Kruskal–Wallis (ANOVA) and the Mann–Whitney criterion.

Table 3: Loravit acute toxicity investigation

Animal groups	Dose, mg/kg	Spectated effect, dead/general animal quantity in a group	
		Males	Females
«Loravit»	5000	0/6	0/6

n (animal quantity in the group)=6

Table 4: Loravit suppositories influence on the body mass growth among the experimental group animals (white breedless rats)

Animal groups	Animal mass growth, g, $\sqrt{X} \pm S_x$		
	3 day	7 day	14 day
Males			
Intact control	3.33±1.05	10.00±2.24	17.50±2.82
Loravit	10.00±1.29*	7.50±5.74	28.73±8.03
Females			
Intact control	7.50±1.12	12.50±1.12	20.00±1.83
Loravit	7.50±2.14	14.17±2.01	20.83±2.71

n (animal quantity in the group)=6, *: Index deviation, reliable to the intact control data, $P \leq 0.05$

The verification of the normality of the distribution of factual data was performed using Leven’s test.^[24] Differences between groups were considered statistically significant at $P < 0.05$.

When using the Mann–Whitney test, the significance level for multiple comparisons is listed with the Bonferroni correction according to the formula $P = p_0/k$,^[24] where $p_0 = 0.05$, k is the number of pair comparisons, which in this study is equal to 2: “Intact control - negative control,” “intact control - test sample,” and level of significance $P = 0.0250$. Statistical processing of the data was performed using the Statistical 6.0 software package.^[23,24]

RESULTS OF THE RESEARCH AND THEIR DISCUSSION

The conjunctival probe test during the Loravit samples antiallergic features investigation, indicated the manifested conjunctive edema and chemosis, accompanied by lacrimation, conjunctive, and eyelid hyperhemia. The maximal edema was developed 10–15 min after histamine application. The severity of ophthalmic reaction among every positive control group animal was valued with 3 points [Table 1].

The animals, who got the test and referent samples, were spectated to have the ophthalmic reaction lowering, close to the positive, which severity was valued with 1–2 points [Table 1].

Table 5: Mass coefficients of mature white breedless rat internal organs at experimental groups

Animal groups	Investigated organs mass coefficients, $\bar{X} \pm S_x$									
	Liver	Kidneys		Heart	Lungs	Spleen	Adrenals	Thymus	Testicles	
		Right	Left						Right	Left
Males										
Intact control	3.29±0.06	0.30±0.01	0.33±0.02	0.33±0.02	0.62±0.03	0.41±0.04	0.018±0.002	0.126±0.010	0.61±0.02	0.62±0.02
Loravit	3.28±0.01	0.30±0.01	0.30±0.02	0.29±0.02	0.58±0.04	0.36±0.02	0.016±0.002	0.113±0.015	0.57±0.03	0.57±0.03
Females										
Intact control	3.27±0.14	0.32±0.01	0.31±0.01	0.31±0.01	0.64±0.02	0.36±0.02	0.017±0.002	0.126±0.012	0.62±0.01	0.62±0.01
Loravit	3.12±0.13	0.32±0.01	0.32±0.01	0.32±0.02	0.62±0.03	0.37±0.01	0.016±0.002	0.119±0.008	0.60±0.02	0.60±0.02

n (animal quantity in the group)=6

The investigation helped to define the manifested antihistamine action of Loravit test sample abreast the Claritin syrup as the referent sample.

The allergologic test, which results are given in Table 2, defined the Loravit test sample to prevent from homocytotropic antibodies conglomeration in blood, suppresses the mastocyte degranulation, therefore, defines the expressed antiallergic activity.

The *in vitro* test helped to define the manifested antihistamine action of Loravit test sample abreast the Claritin syrup as the referent sample.

The obtained results analysis evidences about the expressed antihistamine and antiallergic features of tested sample on the patterns of conjunctival probe and indirect mastocyte degranulation.

Basing on the given data, we can confirm that the Loravit test sample, during the oral usage in the conditionally therapeutic dose (0.25 mg/kg), possesses the antiallergic features, which experimentally confirms the rationality of theoretical summary about the new Loravit antihistamine suppositories contain.

According to the tests of toxicological features, there were no lethal accidents among the animals during the rectal application of Loravit in 5000 mg/kg dose: The animals were tidy, had good appetite, normally reacted on light/sound irritants, urination and defecation were normal, and there was no violation in breathing and no spasms. There also were not any side effects during the test. At the end of spectating term (the 14th day), the animals were removed from the experiment by the decapitation under the narcosis to get the macroscopic review and definition of internal organs mass coefficients.

The examination of skin, physiological holes, the autopsy, and macroscopic examination of rats' internal organs, who were applied with the tested sample, possessed no manifestations of irritations, inflammations, or any other

pathological processes. The size, color, consistency, and location of internal organs of tested rats did not differ from the intact animals. The test of animal mass and mass coefficient dynamics for internal organs showed the absence of Loravit test sample toxicity in a dose of 5000 mg/kg at the single-time rectal appliance for white breadless mature rats [Tables 3-5].

That is why, the test samples of Loravit suppositories in a dose of 5000 mg/kg were resulted not to cause the animals' death, not to influence the internal organs mass coefficients, which talks about no significant toxicity and can be characterized as almost non-toxic (Toxicity V class, $LD_{50} > 5000$ mg/kg) according to the substances general toxicologic classification.

Following the recommendations of State Enterprise "State Expert Center of the Ministry of Health of Ukraine," the average lethal dose determination for the given pattern is impossible.

Summary

1. We studied the specific pharmacological activity and acute toxicity of Loravit suppositories for different childish allergies medication.
2. The investigations, made on the conjunctival probe and indirect mastocyte degranulation, prove the Loravit suppositories to express an antihistamine activity abreast the referent sample of Claritin syrup.
3. To summarize the investigation of toxicological features, we defined that Loravit suppositories are practically non-toxic and have a V toxicity class, $LD_{50} > 5000$ mg/kg, according to the general toxicological classification of substances.

REFERENCES

1. Igea JM. The history of the idea of allergy. *Allergy* 2013;68:966-73.

2. Gushchin IS. Overcoming allergens of the tissue barrier is the decisive form of predisposition to allergy. *Patologicheskaiia fiziologiiia i eksperimentalnaia terapiia* 2009; 1:8-12.
3. Martynov-Radushinskii AA, Martynov AI, Ilina NI, Luss LV, Fedoskova TG, Nazarova EV. The influence of negative environmental factors on the properties of plant pollen. *Effektivnaia Farmakoterapiia* 2015;20:32-35.
4. Baranov AA, Khaitov RM. *Allergology and Immunology*. Moscow: Union of Pediatricians of Russia; 2010. p. 248.
5. Oie F, Burbela EI, Volianska LA, Niu L. Epidemiological Study of the Spread of Bronchial Asthma among School Children in the city of Ternopil. *Proceedings of Interregional Conference Pediatricians. Problematic Issues of Diagnosis and Treatment of Children with Somatic Pathology*. Kharkiv (Ukraine); 2012. p. 150-1.
6. Kryvopustov SP. Allergic rhinitis in pediatric practice. *Dytiachyi likar* 2010;2:18-23.
7. Beyhun NE, Soyer OU, Kuyucu S, Tuncer A, Sekerel B, Saçkesen C, *et al.* A multi-center survey of childhood asthma in Turkey-I: The cost and its determinants. *Pediatr Allergy Immunol* 2009;20:72-80.
8. Chernysheva OE, Iulish EI. Modern ideas about the pathogenesis of bronchial asthma. *Sovrem Pediatr* 2010;2:67-72.
9. Soyer OU, Beyhun NE, Demir E, Yildirim S, Boz AB, Altinel N, *et al.* A multicenter survey of childhood asthma in Turkey-II: Utilization of asthma drugs, control levels, and their determinants. *Pediatr Allergy Immunol* 2009;20:172-9.
10. Robert A. Nathan management of patients with allergic rhinitis and asthma. *South Med J* 2009;102:935-41.
11. Davila I. Effect of bilastine upon nasal obstruction. *J Invest Allergol Clin Immunol* 2011;21:2-8.
12. Scadding GK, Durham SR. BSACI guidelines for the management of allergic and non-allergic rhinitis. *Clin Exp Allergy* 2008;38:19-42.
13. Meltzer EO, Blaiss MS, Derebery MJ, Mahr TA, Gordon BR, Sheth KK, *et al.* Burden of allergic rhinitis: Results from the pediatric allergies in America survey. *J Allergy Clin Immunol* 2009;124:S43-70.
14. Li J, Sun B, Huang Y, *et al.* China alliance of research on respiratory allergic disease (CARRAD). A multicenter study assessing the prevalence of NS in patients with asthma and/or rhinitis in China. *Allergy* 2009;64:1083-92.
15. Sur DK, Scandale S. Treatment of allergic rhinitis. *Am Fam Phys* 2010;81:1440-6.
16. Shumnaia TE. Epidemiology of allergic diseases in children living in the industrial region. *Pediatr* 2015;94:189-92.
17. Order of the Ministry of Health of Ukraine from December 14 2009, № 944. On Approval of the Procedure for Pre-Clinical Examination of Medicines and Examination of Materials for Preclinical Study of Medicinal Products; 2004.
18. Gatcura VV. *Methods of Primary Pharmacological Research of Biologically Active Substances*. Moscow: Medicine; 1974. p. 143.
19. European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. ETS 123. Strasbourg: Council of Europe; 1986. p. 51.
20. Mironov AN, Bunatian ND. *A Guideline Stopreclinical Drug Research. Part I*. Moscow: Grif I K; 2012. p. 944.
21. Iu R, Rybolovlev RS. Dosage of substances form animals according to constants of biological activity. *Doklady Akademii Nauk SSSR* 1979;247:1513-6.
22. State Enterprise Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. *State Pharmacopoeia of Ukraine*. 2nd ed., Vol. 1. Kharkiv: State Enterprise Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines; 2015. p. 1128.
23. Lapach SN, Chubenko AV, Babich PN. *Statistical Method Sinbiomedical Research using Excel*. 2nd ed. Kharkiv: MORION; 2001. p. 320.
24. Oi UR. *Statistical analysis of medical data. Application of the STATISTICA Software Package*. 3rd ed. Moscow: Media Sfera; 2006. p. 312.

Source of Support: Nil. **Conflict of Interest:** None declared.