A study on toxicity, local irritative effect of and allergic response to a novel intranasal medication containing N-phenylacetyl-L-prolylglycine ethyl ester

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Key words: N-phenylacetyl-L-prolylglycine ethyl ester, medication for intranasal delivery, pharmacology, acute toxicity, allergic reaction.

Development of novel medications for delivery of active ingredients with systemic absorption and high bioavailability is an actual challenge for modern pharmaceutical and medical science. Nowadays, the number of diseases of the central nervous system is continuously growing. These conditions lead to impairment of mnemonic and intellectual brain functions, to a decrease in mental alertness and memory in particular, which results in deterioration in the quality of life, sometimes in disability and patients' partial or complete dependence on other people. The existing variety of nootropics does not fully respond to modern criteria of clinical science and practice due to insufficient effectiveness and neuroavailability. Recently, scientists have drawn attention to the potential of intranasal administration for delivery of active ingredients with systemic effect to human blood flow. Intranasal administration for delivery of active ingredients will enhance neuroavailability and, thus, a therapeutic effect of drugs. At the Departments of Medicines Technology, Pharmacology and Medical Formulation of ZSMU, a composition of the novel medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery was developed as the result of complex physical and chemical, microbiological and biopharmaceutical experiments. The medication contains 1 % of N-phenylacetyl-L-prolylglycine ethyl ester, 5 % of Glycerin and Poltava Bischoffe (standardized solution prepared at the Department of Medicines Technology of ZSMU), Sodium carboxymethyl cellulose solution and Tween 80 (1 %). Given the above, the urgent task is to study some safety parameters of the developed dosage form.

The aim of the research is to study some toxicological parameters, local irritative effect of and allergic response to an effective dose of created medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery.

Materials and methods. The created medications for intranasal delivery were used as materials for each test. These medications contained N-phenylacetyl-L-prolylglycine ethyl ester (Noopept), Glycerin and standardized Poltava Bischoffe (5 % each), Sodium carboxymethyl cellulose solution, and Tween 80 (1 %). N-phenylacetyl-L-prolylglycine ethyl ester (CAS №157115-85-0), obtained from Shijiazhuang Prosperity Import and Export Co., Ltd., China. Purity: ≥98 %. Poltava Bischoffe (standardized solution prepared at the Department of Medicines Technology of ZSMU), Polysorbate 80 (obtained from Limited liability company “Symbias”, Kyiv), Sodium carboxymethyl cellulose (obtained from Limited liability company “Symbias”, Kyiv), Glycerin (obtained from Limited liability company “Symbias”, Kyiv), Benzalkonium chloride (obtained from Limited liability company “Istok-Plus”, Zaporizhzhia). A study of acute toxicity, allergic response and irritating effect on skin, and cutaneous anaphylaxis reaction was conducted on white rats. Local irritative effect (Conjunctival allergen provocation test, CAPT) of created medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery was determined on guinea pigs in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and other recommendations. Results were statistically processed by means of the standard statistical package of the licensed program Statistica for Windows 13 (StatSoft Inc., Nr: JPZB041382130ARC110-J). For all analysis types the P-value < 0.05 (95 %) was considered statistically significant.

Results. One-time intranasal delivery of the maximum allowable volume of the medication under research (0.4 ml) to the rats weighing 100 g in a dose of 40 mg/kg did not result in death of any of 6 animals of the experimental group overnight. In the course of studying potential local irritative effect of the intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester, two experimental animals 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 other eight experimental animals. Daily application of the studied medication for intranasal delivery (0.5 g) to a shaved area of the lateral surface of the animals' bodies (4 × 4 cm) during 5 days, and consequent one-time application of the intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester (0.3 g), did not result in anaphylactic shock development. No visible reactions were detected in the experimental animals after 20 more skin applications of the intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester during 4 weeks (5 times per week). The skin area exposed to application in animals of the control and experimental groups looked the same.

Conclusions. Complex studies of some toxicological parameters (such as mortality, dynamics of body weight change, local irritative effect and allergic response to the effective dose of a novel medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery) have been performed. Summarizing obtained results, 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. Thus, further research of the novel medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal administration has a potential perspective.
інтрааназальних досліджень. Перспективним є продовження досліджень місцевоподразнювальної та алергізувальної дії ефективної дози нової інтрааназальної форми з етиловим ефіром N-фенілацетил-L-пролілгліцину.

Мета роботи – виявлення окремих параметрів токсичності, місцевоподразнювальної та алергізувальної дії ефективної дози створеної інтрааназальної форми з етиловим ефіром N-фенілацетил-L-пролілгліцину.

Матеріали та методи. Для кожного дослідження як матеріали використовували розроблені інтрааназальні форми, що містили 1 % етилового ефіру N-фенілацетил-L-пролілгліцину (ноопепт), по 5 % гідрогелю та бішофіту повільного (стандартизовано), розчин натрію карбоксиметилцелюлози та 1 % тянь-80. Етиловий ефір N-фенілацетил-L-пролілгліцину (CAS №157115-85-0, отримані від Shijiazhuang Prosperity Import & Export Co., Ltd., China. Purity: ≥98 %), бішофіт повільний (стандартизований розчин на кафедрі технології ліків ЗДМУ), полісорбат-80 (ТОВ Синбіас, м. Київ), натрій КМЦ (ТОВ Синбіас, м. Київ), гідрогелю (ТОВ Синбіас, м. Київ), бензаконю хлорид (ТОВ «ІСТОК-ПЛЮС», м. Запоріжжя).

Гостра токсичність, алергізувальну та цифроводушні рідкість активність, активну шкірну анафілаксію вивчали на білих щурах, а дослідження місцевоподразнювальної дії (концентраційна проба) інтрааназальної форми з етиловим ефіром N-фенілацетил-L-пролілгліцину виконали на мурзаках відповідно до рекомендацій ДФЦ МО З України та згідно з іншими рекомендаціями. Статистично результати оброблювали, застосовуючи ліцензійний пакет програми Statistica for Windows 13 (StatSoft Inc., № JPS204382130ARC/10-J). Для всіх видів аналізу статистично значущими вважали різницю p < 0,05 (95 %).

Результати. Одноразове інтрааназальне введення максимально припустимого об’єму 0,4 мл препарату шаром масою 100 г у дозі 40 мг/л не викликало загибелі жодного з 6 тварин групи дослідження протягом доби. Вивчаючи можливу місцевоподразнювальну дію інтрааназального гелю з етиловим ефіром N-фенілацетил-L-пролілгліцину, у 2 з 10 тварин зареєстрували слабке почервоніння кон’юнктиви відразу після введення препарату. В інших 8 тварин не було змін слизової оболонки очей. Щоденне нанесення на вистрижену ділянку бокової поверхні тулуба тварин (4 × 4 см) 0,5 г інтрааназальної форми, яку вивчали, протягом 5 дб, а потім однократного нанесення 0,3 г інтрааназального гелю з етиловим ефіром N-фенілацетил-L-пролілгліцину не спричинило розвиток анафілактичного шоку. Після нанесення 20 повторних нашкірних аплікацій інтрааназального гелю з етиловим ефіром N-фенілацетил-L-пролілгліцину протягом 4 тижнів (5 разів на тиждень) виявили слабке почервоніння кон’юнктиви відразу після введення препарату. В інших 8 тварин не було змін слизової оболонки очей.

Висновки. Здійснили комплексні дослідження окремих параметрів токсичності (летальність, динаміка зміни маси тіла, місцевоподразнювальної та алергізувальної дії) ефективної дози нової інтрааназальної форми з етиловим ефіром N-фенілацетил-L-пролілгліцину. За результатами, що отримали, цей препарат не викликає місцевоподразнювальну й алергізувальну реакцію, не проявляє загальну токсичність дію в умовах інтрааназального введення. Перспективним є продовження досліджень нової інтрааназальної форми з етиловим ефіром N-фенілацетил-L-пролілгліцину.

Ключові слова: N-фенілацетил-L-пролілгліцин, назальна лекарственна форма, фармакологічні існування, остра токсичність, алергізувальна дія.
Development of novel medications for delivery of active ingredients with systemic absorption and high bioavailability is an actual challenge for modern pharmaceutical and medical science.

Nowadays, the number of the central nervous system diseases is continuously growing. These conditions lead to impairment of mnemonic and intellectual brain functions, to decrease in mental alertness and memory in particular, which results in deterioration in the quality of life, sometimes in disability and patients’ partial or complete dependence on other people [1–4].

The existing variety of nootropics does not fully respond to modern criteria of clinical science and practice due to insufficient effectiveness and neuroavailability.

Recently, scientists have drawn attention to the potential of intranasal administration for delivery of active ingredients with systemic effect to human blood flow. Intranasal administration for delivery of active ingredients will enhance neuroavailability and, thus, a therapeutic effect of drugs [5,6].

Various compounds are used as nootropic agents, such as: cerebral vasodilators (Vinpocetine, Nicergoline), antioxidants and membrane protectors (Mexidol, Pyritinol), pyrrolidone derivatives (Piracetam and its derivatives), GABAergic drugs (γ-aminobutyric acid, Picamilonum, Phenibut), calcium channel blockers (Nimodipine, Cinnarizine), drugs increasing cholinergic transmission (Ipidacrinum, Donepezilum, Galantamine), herbal preparations (Ginkgo Biloba), neuropeptides and their analogues (Cerebrolysin, Semax, Noopept) [7,8].

At the Departments of Medicines Technology, Pharmacology and Medical Formulation of ZSMU a composition of the novel medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery was developed as a result of complex physical and chemical, microbiological and biopharmaceutical experiments. The medication contains 5 % of N-phenylacetyl-L-prolylglycine ethyl ester, 5 % of Glycerin and Poltava Bischoffite (standardized solution prepared on the Department of Medicines Technology of ZSMU), Sodium carboxymethyl cellulose solution and Tween 80 (1 %).

Aim

The aim of the research is to study some toxicological parameters, local irritative effect of and allergic response to effective dose of created medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery.

Materials and methods

The created medications for intranasal delivery were used as materials for each test. These medications contained N-phenylacetyl-L-prolylglycine ethyl ester (1 %), Glycerin and standardized Poltava Bischoffite (5 % each), Sodium carboxymethyl cellulose solution and Tween 80 (1 %). The created medications for intranasal delivery were used as materials for each test. These medications contained N-phenylacetyl-L-prolylglycine ethyl ester (1 %), Glycerin and standardized Poltava Bischoffite (5 % each), Sodium carboxymethyl cellulose solution and Tween 80 (1 %). N-phenylacetyl-L-prolylglycine ethyl ester (CAS №157115-85-0, obtained from Shijiazhuang Prosperity Import and Export Co., Ltd., China. Purity: ≥98 %), Poltava Bischoffite (standardized solution prepared on the Department of Medicines Technology of ZSMU), Polysorbate 80 (obtained from Limited liability company “Synbias”, Kyiv), Sodium carboxymethyl cellulose (obtained from Limited liability company “Synbias”, Kyiv), Benzalkonium chloride (obtained from Limited liability company “Istok-Plus”, Zaporizhzhia).

Possible toxic effect of the medication containing N-phenylacetyl-L-prolylglycine ethyl ester was studied on outbred albino rats weighing 98–110 g in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine. Each group consisted of six animals. The intranasal gel under research, containing N-phenylacetyl-L-prolylglycine ethyl ester, was administered intranasally with syringe dispenser in the maximum allowable amount of 0.4 ml. Possible pathological changes in behavior and appearance, as well as death of animals were recorded within 14 days. Autopsy and morphological studies were performed only in dead animals [9].

Allergic response and irritating effect on skin of the intranasal medication was studied by means of skin application 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. On the lateral surface of the body of animals, an area of a 4 × 4 cm was shaved. This area was exposed to application of 0.5 g of a dosage form, then the animals were located in individual cells to prevent licking off the drug. The application of the intranasal 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.

The research on local irritative effect (Conjunctival allergen provocation test (CAPT)) of created medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery was carried out on guinea pigs weighing 480–520 g (2 groups – control and experimental with ten 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 animals from the control group received distilled water administration into conjunctival sac. The observation lasted for 3 days. Allergic reaction was assessed 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 medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery was conducted on outbred albino female rats weighing 180–190 g (two groups – control and experimental with ten animals in each) in accordance with recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine.

An area of a 4 × 4 cm was shaved on the lateral surface of the animals’ bodies. It was exposed to application of 0.5 g of a dosage form, then the animals were located in individual cells to prevent licking off the drug. Sensitization of animals was estimated in 5 days after the last drug application. For this, 0.3 g of the intranasal gel was applied once on the skin of the ear. The analysis of the anaphylactic shock intensity 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.

Results were statistically processed by means of the standard statistical package of the licensed program Statistica for Windows 13 (StatSoft Inc., N JP-Z8041382130ARCN10-J). 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. For all analysis types the P-value < 0.05 (95 %) was considered statistically significant [8–12].

The experiments involving the animals were carried out in accordance with the International Standards for the Care and Use of Laboratory Animals and with Council Directive 86/609/EEC regarding the protection of vertebrate animals used for experimental and other scientific purposes [13,14].

**Results**

In researches by R. U. Ostrovskaya et al. in 2002, it has been found that N-phenylacetyl-L-prolylglycine ethyl ester belongs to Toxicity Class IV (practically nontoxic), LD50 of which is 5078 mg/kg when administered intraperitoneally [7]. Considering the fact that in our research other method of drug administration was used (intranasal delivery), it is necessary to provide a study on some toxicological parameters (mortality and dynamics of body weight change) of the effective dose (40 mg/kg) of N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery. Thus, it has been found that one-time intranasal delivery of the maximum allowable volume of the medication under research (0.4 ml) to the rats weighing 100 g in a dose of 40 mg/kg did not result in death of any of 6 animals of the experimental group overnight (Table 1).

After one-time administration of the medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery, the animals were under observation. No visible pathological changes, as well as changes in behavior of the animals occurred on day 1, 7, and 14 of the experiment. It has been found that dynamics of body weight change was within the physiological norm (Table 2).

In the course of studying potential local irritative effect of the intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester, two of ten experimental animals developed a slight reddening of the conjunctiva immediately after the administration. No changes in mucous membrane of the eyes were observed in the other eight experimental 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.

Then, the study of cutaneous anaphylaxis reaction to medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery was conducted. As the result, it has been found that a daily application of the studied intranasal medication (0.5 g) on a shaved area of the lateral surface of the body of animals (4 × 4 cm) during 5 days, and then one-time application of the intranasal gel (0.3 g), did not result in development of anaphylactic shock. No symptoms of anaphylactic shock due to intranasal gel application (0.3 g) after 6, 12 and 24 hours. Thereby, the studied intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester does not induce allergic reactions of anaphylactic type when administered to animals during 5 days.

The conducted research of allergenic effect of the intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester has revealed the following: no visible reactions were detected in the experimental animals after 20 more skin applications of the intranasal gel containing N-phenylacetyl-L-prolylglycine ethyl ester during 4 weeks (5 times
Further studies of the new nasal form with ethyl ester of N-phenylacetyl-L-prolylglycine should be conducted. They are studies on rheological parameters, chemical and microbiological stability.

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### Conflicts of interest

Authors have no conflict of interest to declare.

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### Conclusions

A series of complex studies of some toxicological parameters (such as mortality, dynamics of body weight change, local irritative effect and allergic reaction to the effective dose of a novel medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal delivery) have been performed. Summarizing obtained results, 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. Thus, further research of the novel medication containing N-phenylacetyl-L-prolylglycine ethyl ester for intranasal administration has a potential perspective.

### Prospects for further research

Further studies of the new nasal form with ethyl ester of N-phenylacetyl-L-prolylglycine should be conducted. They are studies on rheological parameters, chemical and microbiological stability.

### References


