

aggravated conditions for simulating acute inhalation poisoning, the maximum possible concentration of the hexyl ester of 5-aminolevulinic acid disintegration water aerosol was reached, equal to 72,2 mg / m³. Based on its physical properties, hexyl ester of 5-aminolevulinic acid is able to pollute the air environment only in the form of a disintegration aerosol. In accordance with the guidelines [1], the achieved level of exposure to the drug cannot be used as a qualifying hygienic criterion for establishing the hazard class of hexyl ester of 5-aminolevulinic acid in case of inhalation.

BIBLIOGRAPHY

1. Гигиенические нормативы 1.1.9-23-2002. Гигиенические критерии для обоснования необходимости разработки ПДК и ОБУВ (ОДУ) вредных веществ в воздухе рабочей зоны, атмосферном воздухе населенных мест, воде водных объектов: утв. постановлением гл. гос. сан. врача Респ. Беларусь 31.12.2002, № 149.

TOXICOLOGICAL EVALUATION OF LOCAL IRRITATING EFFECT AND APPLICATION TO MUCOUS MEMBRANES OF THE EYES OF HEXYL ESTER OF 5-AMINOLEVULINIC ACID AND ITS REGULATION

M. Atroshko¹, E. Vlasenko²

¹*Belarusian State University, ISEI BSU,
Minsk, Republic of Belarus
atroshkomikhail@gmail.com*

²*Scientific Practical Centre of Hygiene,
Minsk, Republic of Belarus
evgenii_vlasenko@mail.ru*

The hexyl ester of 5-aminolevulinic acid is a very perspective growth-control tool for plants. In that case it is very important to investigate it for some unpleasant effects and to regulate its usage, due to the risk of accidental contact during exploitation with delicate and dangerous parts of the body.

Keywords: hexyl ester of 5-aminolevulinic acid, irritation, rats, rabbits.

Materials and methods

The study of local irritating properties was carried out on 7 white outbred male rats, the severity of erythema, the value of skin edema and its intensity were evaluated according to [1].

A study of the characteristics of the irritating effect of hexyl ester of 5-aminolevulinic acid on the mucous membranes of the eyes was performed on male rabbits. hexyl ester of 5-aminolevulinic acid in the amount of 50 µl of a 50 % aqueous solution was introduced into the lower conjunctival arch of the rabbit's right eye, the left eye (50 µl of distilled water) served as a control. Visual monitoring of the condition of the mucous membranes of the eyes of rabbits was carried out for 14 days. The manifestation of signs of irritation of the mucous membranes of the eyes was recorded - conjunctival and corneal hyperemia, eyelid edema, discharge from the eye [1].

The research results were processed by conventional methods of variation statistics. A critical level of significance when testing statistical hypotheses was accepted $p \leq 0,05$.

Results and discussion

In the process of studying the local irritating effect of hexyl ester of 5-aminolevulinic acid, no signs of hyperemia and visually significant changes in the status of the skin of experimental animals were detected. Under the epicutaneous action of hexyl ester of 5-aminolevulinic acid, erythematous manifestations were not visually observed on the skin of experimental and control animals (0 points when assessing the severity of erythema), and there was no increase in the instrumentally measured thickness of the skin fold of animals compared to the background (intensity gradation - lack of reaction, assessment of edema in points - 0 points). The surface of the skin at the sites of applications was similar to that of the control, the skin was not densified, peeling or with foreign formations. Thus, the total quantitative assessment of the degree of induction of erythema and edema for control and experience under the influence of hexyl ester of 5-aminolevulinic acid is 0 points. Therefore, under the conditions adopted for evaluating the skin-irritant effect when tested in laboratory animals [1], hexyl ester of 5-aminolevulinic acid is not capable of inducing pronounced local irritant properties.

The instillation of hexyl ester of 5-aminolevulinic acid into the lower conjunctival arch of the right eye for 1 hour in rabbits leads to profuse lacrimation, moderate redness of the conjunctival vessels and swelling of the eyelids (4 points). As a result, manifestations of blepharospasm were observed (the eye is completely closed). Symptoms of damage to the mucous membranes of the eye persisted for the next 9 days of observation (1 point). The

amount of excretion moisturizes the eyelids and surrounding tissues on the 1st day after exposure, discharge from the eye is recorded during 5 days of observation (3 points)

The final classification assessment of the damaging effect of hexyl ester of 5-aminolevulinic acid by summing the intensity points of each of the symptoms of irritating effects on the mucous membranes of the eyes (8 points in total) allows us to classify, according to [1], this compound as class 3 — chemical compounds with pronounced irritative properties. The local inflammatory process caused by a single instillation of hexyl ester of 5-aminolevulinic acid is pathophysiologically characterized as serous blepharoconjunctivitis.

BIBLIOGRAPHY

1. Требования к постановке экспериментальных исследований для первичной токсикологической оценки и гигиенической регламентации веществ: инструкция: утв. М-вом здравоохранения Респ. Беларусь 14.12.04. — Минск, 2004. — 43 с.

ANALYSIS OF HUMAN CHORIONIC GONADOTROPIN USING HIGH-RESOLUTION TANDEM MASS-SPECTROMETRY

D. Babaryko^{1,2}, I. Huliya^{1,2}, Y. Bakakina¹, V. Syakhovich^{1,2}

¹*National Anti-Doping Laboratory,
Lesnoy, Republic of Belarus*

²*Belarusian State University, ISEI BSU,
Minsk, Republic of Belarus
dv.babaryko@antidoping.by
dashababariko@mail.ru*

Human chorionic gonadotropin (hCG) is tropic protein hormone secreted by the adenohypophysis. Hormone is included in the prohibited list in all sports. In this study, methodological approach of human chorionic gonadotropin specific peptides obtaining using «bottom-up» proteomics and its detection using liquid chromatography - high-resolution tandem mass spectrometry was developed.

Keywords: human chorionic gonadotropin, high performance liquid chromatography, tandem mass-spectrometry.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone with a molecular weight of about 36 kDa, consisting of two different alpha and beta subunits. Beta-subunit is specific for hCG, while alpha subunit is common for all gonadotropic hormones. The carbohydrate part, which is characterized by significant heterogeneity, accounts for about 30 % of the molecular weight of the protein. There are N- and O-linked carbohydrate chains.

Human chorionic gonadotropin is used by male athletes with the aim of enhancing the secretion of endogenous steroid hormones, while maintaining the testosterone/epitestosterone ratio have been described. hCG is included in prohibited list in all sports on competition and non-competition period (class S2 – peptide hormones, growth factors, related substances, and mimetics).

Due to the existence of several hCG isoforms, the heterogeneity revealed in the composition largely depends on the features of the analysis method used in the study. In the case of determining hCG in the urine, the situation is difficult, since the spectrum of isoforms is more complicated than in the case of blood serum.

In this study the methodological approach to obtain specific peptides of human chorionic gonadotropin using «bottom-up» proteomic approach and their analysis using liquid chromatography – high-resolution tandem mass spectrometry in human urine was developed.

Urine samples consisting hCG at a known concentrations were purified and concentrated using ultrafiltration. Hydrolysis of hCG was carried out using trypsin Proteomics Grade with preliminary protein alkylation. The peptides were separated by HPLC method on reversed-phase column and analyzed using high-resolution tandem mass-spectrometer LTQ Orbitrap Discovery. Mass-spectrometric detection was carried out using Full Scan, Auto MS/MS and Target MS/MS.

More than 90 % of the alpha and beta subunit peptides with varying degrees of protonation were identified. Protein detection limit was 1 ng/ml. List of characteristic peptides precursor and product ions, which will be used as indicators of hCG using as doping has been compiled. Figure 1 shows the mass spectrum of fragment ions of one of the specific hCG beta subunit peptides.