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PECULIARITIES OF SUCCINAMIDE TOXICOKINETICS AS AN ANTIDIABETIC AGENT UPON SINGLE INHALAION EXPOSURE

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Abstract. The toxicokinetics of such antidiabetic agents, as β-phenylethylamide of 2-oxysuccinanilic acid (β-PhEA-OSA) and its metabolites (2-hydroxyphenylsuccinamide (2-HPhSA) and β-phenylethylsuccinamide (β-PhESA)) upon a single inhalation influence of β-PhEA-OSA on the male rodents at the levels Lim_{ac} ma Lim_{ch} has been studied. The quantification of studied substances in the blood plasma of male rats was conducted with the use of high-performance liquid chromatography (HPLC) followed by the spectrophotometric determination. The compounds were identified in blood plasma within 30 mins after the inhalation; the maximum plasma concentration of β-PhESA was estimated to be higher than the concentration of β-PhEA-OSAA and 2-HhHS. The compounds were shown to circulate for a long period in the blood stream; the 2-HPhS was counted to have the longest half-life in average; the biotransformation of β-PhEA-OSAA was calculated to be more intensive in comparison with its excretion.

Kew words: *antidiabetic agent, toxicokinetics, chromatography.*

Introduction. Chemical-pharmaceutical manufacture is considered to be a resource-intensive branch, being referred to rather ecologically dangerous enterprises according to international classification [1,2].

Overall, the percentage of disturbances, caused by chemical manufacture conditions among the other pathological states, constitutes 23%. What is more, professional intoxication ranks fourth within general and professional morbidity [3].

Nowadays the concerns about health legislation improvement, particularly, approach of hygienic standards of productive environment quality to the international norms, have been supposed as the one of high importance due to Ukraine entry to the European Union and European economic community .

Modern approaches to the hygienic regulation of drugs and the assessment of their safety primarily require comprehensive toxicological studies, accompanied by toxicity parameters determination as well as exploring the toxicokinetics data of various drugs upon various conditions and duration of exposure; studying the peculiarities of specific biological effects of the compounds and further compound hygienic regulation within the biological field also require special attention [4].

Thorough research of toxicokinetics of a particular xenobiotic promotes a detailed analysis of the speed as well as intensity of compound absorbtion, distribution within the organs and tissues; the research also allows studying the thrust and quantitate of biotransformation processes, elimination speed of the exogenous substance during a period of time. Undoubtedly, such data makes rather important contribution to the prophylactic toxicology.

The aim of our study was to determine the main toxicokinetic profiles of the antidiabetic agent upon a single inhalation influence of a male rodent organism.

Materials and methods of research

The experiments are conducted on nonlinear male rats. Representative sample was formed via random selection of animals from the general population; the grouping was based on randomization method [5]. All experimental studies were conducted in accordance with the Ethical Principles of Experiments on Animals [6].

Toxicokinetic studies included the determination of concentrations of antidiabetic agent and its metabolites - β-PhESA and 2-HPhSA - in blood plasma of rodents within discrete time intervals: 0.5, 0.45, 1, 1.5, 2, 4, 6, 12, 24, 48 h. upon single inhalation exposure of the antidiabetic agent at the threshold of acute inhalation infuence Lim_{ac} (27.9 mg/m^3), followed by the calculation of the main toxicokinetic parameters. The simulation of the inhalation flow of the antidiabetic agent - a succinic acid derivative (ADA-SAD) was carried out by intranasal installations at the threshold of acute inhalation effect of the compound - Lim_{ac} (27.9 mg/m^3), that corresponds to an intranasal dose of 6.7 mg/ml. The conversion was carried out according to the MR 1.1.5-121-2005 "Justification of the MPC of drugs in the air of the working zone and the atmosphere of the inhabited areas" of the Ministry of Health of Ukraine [7].

Results & Discussion

The analysis of the dynamics of the concentration of ADA-SAD and its metabolites upon a single inhalation exposure to the substance showed that the studied compounds were present in plasma 30 minutes after the exposure. Concentration of ADA-SAD in blood plasma was shown to gradually increase and reach a maximum value of 31.6 ± 2.3 ng/μl in 2 hours. Concentrations of metabolites β-PhESA and 2-HPhSA increase rapidly within the first hour of observation and reach levels of 8.3 ± 0.64 ng/μl and 30.6 ± 3.4 ng/μl respectively. The studied compounds were found to circulate in systemic blood flow for a long time and were still identified in blood plasma 48 hours after the exposure.

The increased rate of metabolites formation and consequent earlier identification of their maximum plasma concentrations in comparison with the parent compound itself is supposed to be related to the cytochrome P-450 enzyme functioning, particularly, to such isoforms of the enzyme as CYR1A1, CYR2B1 and CYR4B1; mentioned isoforms were detected in the mucous epithelium of

the upper respiratory tract, actively involved into intense metabolism as well as partial absorbtion of ADA-SAD into the blood plasma in the form of metabolites as well as the parent compound.

Slight fluctuation of 2-HPhSA plasma concentration was observed within the experiment; such values were found to be significantly lower in comparison with the other metabolite. The pharmacokinetic curve reflecting the quantitative content of 2-HPhSA in blood plasma demonstrates no clear peaks and thus appears to have a form of a plateau. The dynamics of the investigated compounds concentrations is presented in Fig. 1

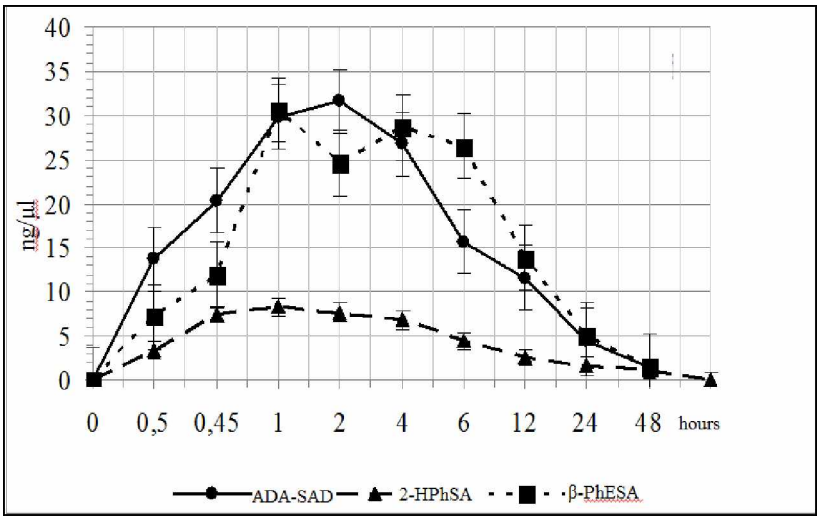

Fig. 1. Averaged toxicokinetic profiles of the antidiabetic agent and its metabolites (β-PhESA and 2-HPhSA) in the blood plasma upon a single inhalation exposure (n=5; ±SD).

In contrast, the other metabolite - β-PhESA - showed significant kinetic features: β-PhESA concentrations had almost equal values in comparison with concentrations of the parent compound; the quantitative levels of β-PHESA were calculated to be 4 times greater than the value of the other metabolite - 2-HPhSA.

Furthermore, we calculated the basic toxicometric parameters of ADA-SAD as well as its metabolites (β-PhESA and 2-HPhSA).

The analysis of the main toxicokinetic parameters of the studied compounds demonstrated that metabolites had higher values of $T_{1/2}$ than the parent compound (almost 2 times greater than the value of ADA-SAD itself); while such metabolite as 2-HPhSA showed more pronounced value changes. The high values of $T_{1/2}$, in turn, are closely related to the clearance level, which characterizes the rate of elimination of the substance from the systemic blood flow via excretion, in particular, 2-HPhSA was recorded to have the smallest value of elimination rate compared with the other metabolite and ADA-SAD. The obtained values of the half-life $T_{1/2}$ strongly indicate a significant delay of parent compound output and its metabolites from the systemic circulation.

Since renal filtration is limited to drugs that are not bound to blood plasma proteins, the studied substances could be assumed to be strongly bound to plasma proteins; what is more, the elimination of the substances from plasma is largely due to extrarenal clearance [8]. According to the literature, the lipophilic compounds, in particular, a large number of drugs, nonionized molecules of water-soluble compounds as well as substances of low molecular weight, are known to be reabsorbed by renal tubules. Lipophilic drugs are considered to be reabsorbed much easier from the primary urine, and then back to the systemic circulation than the hydrophilic ones. Prolonged tubulo-glomerular recirculation of lipophilic xenobiotics leads to a significant decrease in the elimination rate; thus, the action of the compounds is significantly prolonged [9].

It is worth noting, that the initial compound ADA-SAD upon a single inhalation exposure had rather high values of the elimination rate constant and relatively low clearance, indicating a slowdown of the withdrawal rate of the compound from the circulation by renal excretion and acceleration of compounds biotransformation; such facts were confirmed by relatively low values of $T_{1/2}$ in comparison with analogous values of compound metabolites.

The analysis of the mean values of the area under the toxicokinetic curve AUC0-48 showed that the β-PhESA metabolite had the highest value (possibly due to low clearance level), low elimination rate constant and relatively high half-life of the compound; such data, in turn, leads to prolonged compound circulation in blood plasma.

In order to describe the dynamics of the compound concentration within serum/plasma, mathematical models of pharmaco/toxicokinetics were used. As the units of the system-organism, the relative indicators were used: the units were considered as chambers (compartments), being the parts of the system within which the xenobiotic is evenly distributed. Xenobiotic concentration gradually reduced via the following mechanisms: biotransformation (metabolic transformation) and excretion. Both processes were combined and described with such a parameter as k_{el} (elimination rate constant), which characterizes the rate of xenobiotic excretion from an organism through either biotransformation or excretion [10].

It was established, that the ADA-SAD had the greatest value of k_{el}; thus, the parent compound was shown to have the highest intensity and speed of the metabolic transformation, while the metabolite β-PhESA was observed to have the smallest value of k_{el}.

Therefore, the obtained results strongly indicate that upon a single inhalation exposure the parent compound biotransformation predominates over its excretion; such data could be confirmed by the increase of elimination rate constant of ADA-SAD. The changes of k_{el}, mentioned above, are supposed to happen due to relatively low clearance as well as the increase in time taken to achieve the average maximum metabolites concentration in comparison with ADA-SAD. The latter is probably realized by the metabolic systems of the liver. Upon a single inhalation exposure, the parent

compound is metabolized in the upper respiratory tract surface; thus, prolonged metabolite circulation is provided.

Conclusions

The antidiabetic drug has been proved to remain for a rather long period of time within the systemic blood flow in the form of initial compound upon single inhalation exposure. The elimination of the antidiabetic agent upon single-use influences is conducted via biotransformation that somewhat prevails over the excretion of the unchanged compound, as it was shown by high elimination rate of ADA-SAD, while systemic clearance values are relatively low and average maximum metabolites concentrations are shifted in time relatively to the initial compound.

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ОСОБЛИВОСТІ ТОКСИКОКІНЕТИКИ СУКЦИНАМІДА З АНТИДІАБЕТИЧНОЮ АКТИВНІСТЮ ПРИ ОДНОРАЗОВОМУ ІНГАЛЯЦІЙНОМУ ВПЛИВІ

Лалименко О., Кудря М., Завгородній І.

Анотація. Вивчена токсикокінетика сукцинатвмісного антидіабетичного засобу β-фенілетиламіду 2-оксисукцинанілової кислоти (β-ФЕА-ОСАК)/його метаболітів 2-гідроксифенілсукцинаміду (2-ГФСА) та β-фенілетилсукцинаміду (β-ФЕСА) в умовах одноразової інгаляційної дії субстанції β-ФЕА-ОСАК на рівні Lim_{ac} та Lim_{ch} на організм щурів-самців. Кількісне визначення досліджуваних сполук у плазмі крові щурів-самців проведено з використанням розробленого нами метода високоефективної рідинної хроматографії (ВЕРХ) зі спектрофотометричним детектуванням. Встановлено, що сполуки ідентифікуються в плазмі крові вже через 30 хвилин, середня максимальна концентрація β-ФЕСА вище в порівнянні з β-ФЕА-ОСАК та 2-ГФСА, сполуки тривало циркулюють у системному кровотоці, найбільш тривалий період напіввиведення має 2-ГФСА, процеси біотрансформації β-ФЕА-ОСАК дещо переважають над його екскрецією.

Ключові слова: *антидіабетичний засіб, токсикокінетика, хроматографічний аналіз.*

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