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THE ACUTE TOXICITY STUDY OF NEW AMINO ACID-CONTAINING DERIVATIVES OF 1,4-NAPHTHOQUINONE

Introduction

One of the essential steps in the investigation of new chemical compounds are, of course, the study of their toxicological properties. This fact makes it possible, first of all, to obtain the information about the class of the toxicity of these substances and to calculate the parameters of their toxicity (LD_{50}, LD_{16} and LD_{84}), which will help to determin with the dosages for the further subsequent screening studies about the estimated properties of the compounds [3].

Our attention was drawn with the eleven original derivatives of 1,4-naphthoquinone (NH), modified by including the amino acid residue in its structure - leucine, arginine, α and ß-alanine, histidine, glycine, aspartate, glutamate, methionine and tyrosine. All materials were synthesized at the National University "Lviv Polytechnic" led by professor V.P. Novikov.

Interest to the naphthoquinone derivatives resulting from the presence of their donor-acceptor properties, which explains their efficacy in preventing early disorders of the respiratory chain in hypoxia [7]. Also the derivatives of naphthoquinone (vitamin K) have some anabolic properties, which leads to the biomass improvement, the oxidative phosphorylation increase, ATP synthesis and creatine phosphate in muscles [8]. That’s why we can see clearly the fact of the presence of the antihypoxic and antiischaemic activities in the aminobenzokinones [4] and also antihypoxic, antiischaemic and cerebroprotective properties of the new amino acid-containing derivatives of 1,4-naphthoquinone [9, 10].

The presence of the chemical structure of the investigated derivatives of amino acid residues that can bring new interesting features, increases the interest in them. It is well known that, for example, the amino acid glycine, despite its inhibitory processes in the central nervous system, has the acting-protective effect [8, 11] and glycine-containing derivatives of 1,4-NH shows the expressed neuro-and cardioprotective effects [10]. Other amino acid residues also can be characterized by the useful properties. It is known that the experimental combined use of the azafen and alanine, aspartate or glutamate leads to the likely increase of the nootropics acting- protective effect during the exhausting physical exercise [1].

In connection with the above mentioned ideas we have found out the interest to investigate the toxicological properties of the amino acid-containing derivatives of 1,4-naphthoquinone and define the parameters of their acute toxicity, which has become the aim of our research.

Materials and Methods

The research of the acute toxicity of the new amino acid-containing derivatives of 1,4-naphthoquinone with the laboratory codes (I-XI) has been held at 185 nonlinear rats of the both sexes, weighing 160-190 g according to the guidelines of the State Pharmacological Center the Ministry of health care Ukraine [3]. The studying substances have been injected once intraperitoneally (w / v), and the compound the laboratory codes VI - also intragastrically, subcutaneously, intramuscularly.

Each dose has been tested on a group of the rats which consisted of 5 individuals. Observation has been conducted for 14 days. The Index of the LD_{50}, its confidence interval and also the LD_{16} and LD_{84} have been calculated using the graphical method of Litchfield-Wilcoxona [5].

The obtained data are presented in the Table 1 and 2.

Results and Discussion

Characterizing the data of the acute toxicity of the studying amino acid derivatives of 1,4-naphthoquinone (Table. 1.), it should be mentioned that all compounds of this series, according to the classification of Sidorov K.K. (1973) [3], the largest index of LD_{50} can be classified as Class IV (low-toxic substances) because their LD_{50} indexes are in the range of 290-785 mg/kg for the enteral dosing in the rats' body.

Table 1

The acute toxicity of the derivatives of 1,4-naphthoquinone for the rats with intraperitoneal administration and the major features of the acute poisoning

The labora-tory com-pounds codes	The chemical structure and the names of the compounds	LD_{50} and its confidence interval, mg / kg	The main features of the acute poisoning
I	Potassium salt of 2-leucine-3-chloro-1,4-naphthoquinone	290 (266÷328)	The excitation of the central nervous system in the form of periodic spasms
II	Potassium salt of 2-arginine-3-chloro-1,4-naphthoquinone	785 (758÷828)	The excitation of the central nervous system in the form of periodic spasms

III	Potassium salt of 2-α-alanine-3-chloro-1,4-naphthoquinone	502 (462÷548)	In small doses – depression of the CNS (lack of exercise and lateral position and then transition to the excitement - periodic spasms). In large doses – The excitation of the central nervous system in the form of periodic spasms
IV	Potassium salt of 2-β-alanine-3-chloro-1,4-naphthoquinone	480 (438÷532)	CNS depression – side position with the transition to the excitation (spasms)
V	Potassium salt of 2- gistidine -3-chloro-1,4-naphthoquinone	520 (486÷556)	The excitation of the central nervous system in the form of spasms
VI	Potassium salt of glycine-2-3-chloro-1,4-naphthoquinone	552 (525÷589)	Depression of the central nervous system: hypodynamia, lateral position
VII	Dipotassium salt of 2-aspartate-3-chloro-1,4-naphthoquinone	690 (638÷740)	The excitation of the central nervous system in the form of spasms
VIII	Dipotassium salt glutamate-2-3-chloro-1,4-naphthoquinone	700 (674÷736)	The excitation of the central nervous system in the form of periodic spasms

IX	Potassium salt of 2-methionine-3-chloro-1,4-naphthoquinone	440 (408÷482)	The excitation of the central nervous system in the form of periodic spasms
X	Potassium salt of 2-tyrosine-3-chloro-1,4-naphthoquinone	730 (694÷775)	In small doses – depression of the CNS (hypodynamia and lateral position). In large doses – the excitation of the central nervous system in the form of periodic spasms
XI	Citrate arginine-2-3-chloro-1,4-naphthoquinone	740 (638÷792)	The excitation of the central nervous system in the form of periodic spasms

Thus, analyzing the dependence of the LD_{50} index upon the nature of the amino acid residue derivatives of 1,4-naphthoquinone, it should be mentioned that the presence in the studying compounds structures of the arginine residues (II, XI), aspartate (VII), glutamate (VIII) or tyrosine (X) is favorable to reduction of the acute toxicity of these substances, indicatied by the growth in the value of the LD_{50} index. In contrast, the presence of the leucine (I) or methionine (IX) in the structure of derivatives of 1,4-naphthoquinone is favorable to increase of the acute toxicity of the substances indicatied by the decrease of the LD_{50} index.

In the study of the acute toxicity of amino acid derivatives of 1,4 - naphthoquinone has been found that the pattern of the acute poisoning in the rats in the vast majority of these substances (I-III, V, VII-XI) has been characterized by the features of CNS excitation, the provements were the periodic spasms and increased pain sensitivity in response to a mechanical stimulation of the tail. Only under the influence of the compounds with laboratory code IV (containing residue β-alanine) and VI (containing glycine residue) have resulted in the CNS depression that has

been shown in hypodynamia and lateral position of the animal. In all cases, death came against the background of the oppressed breathing.

It should also be mentioned that all studying amino acid derivatives of 1,4 - naphthoquinone have been caused in urine in red color of varying intensity, which may be a sign of their urinary excretion in the same condition. This coincides with the observations of the researchers [10] on the effects of other amino acid derivatives of 1,4-naphthoquinone.

Since the compound with laboratory code VI (potassium salt of glycine-2-3-chloro-1,4-naphthoquinone) with the further researches of its acting-protective properties has appeared to be the most promising for the in-depth study of its pharmacological properties, there has been an interest of studying its acute toxicity in various routes of the administration in bodies of the rats. The obtained data are presented in Table. 2.

Table 2

Parameters of the acute toxicity of potassium salts of glycine-2-3-chloro-1, 4 - naphthoquinone for the rats at different ways of the administration, mg / kg

The ways of the administration	LD_{16}, mg/kg	LD_{50}, mg/kg	LD_{84}, mg/kg
intragastrically	724	840 (816÷872)	946
subcutaneously	638	726 (702÷756)	794
intramuscularly	544	614 (592÷644)	702
intraperitoneally	486	552 (525÷589)	636

From the data table 2 it is clearly shown that the compound VI has the lowest toxicity at the intragastric administration in the body: LD_{50}index is 840 (816 ÷ 872) mg / kg. Acute toxicity of this compound in terms of LD_{50} increases in the following order: intragastric <subcutaneous <intramuscularly <vnutrishnoocherevenne

administrations. However, according to the classification by K.K. Sidorov (1973) [3] the compound VI according to the index LD_{50} in the parenteral and enteral routes of the administration may be assigned to the Class IV of low toxic substances because its LD_{50} index stays within 552-726 mg / kg for intraperitoneal, intramuscular and subcutaneous administrations, while for intragastric administration is 840 mg / kg.

The clinical picture of acute poisoning with the compound VI in a single intramuscular administration in the bodies of the rats at doses that were lower than LD_{50}, has been characterized by hypodynamia, which occured within 5-10 minutes after the administration of the substance. In some animals in 20-30 minutes there have been the lateral positions, while there has been a suppression of the pain reflex to a mechanical stimulation of the tail. Death came in the side position against the background of the respiratory depression in 6-18 hours after the administration of the compound. The animals that have survived at 2-3 days after the administration of the substance have practically no difference from the intact rats.

Similar signs of poisoning of the compound VI have been observed in intramuscular, subcutaneous and intragastric administrations in the body and differed only by the time delay of their occurrence.

Thus, the pattern of the acute poisoning of the rats by the compound VI has been characterized by the influence on the CNS. The cause of the death of the rats is probably the inhibition of the bulbar vital centers of the brains. The degree and time of the poisoning by the potassium salt of 2 - glycine-3-chloro-1 ,4-naphthoquinone (compound VI) has been caused by its dose and way of administrations into the body.

In conclusion it should be mentioned that the most effective substance for the further studies of its acting-protective effects (compound VI) in the terms of LD_{50} (552 mg / kg, i / o) is close to the standard actoprotector bemetile (581.5 mg / kg, w / v) [2], but it has turned out to be more active than the reference drug, as manifesting actoprotective effect in 4.5 times lower doses [6], which may be a sign of a greater usage of its therapeutic effect.

Thus, summarizing the results of the study, we should mentione that **:** amino

acid-containing derivatives of 1,4-naphthoquinone is low toxic substances and has the interest of the further research of their antihypoxic, antiischaemic and actoprotective properties. In our opinion, quite promising substance is the potassium salt of glycine-2-3-chloro-1 ,4-naphthoquinone (the compound VI) in this aspect.

Conclusions

1. The studying amino acid-containing derivatives of 1,4-naphthoquinone due to their the largest index LD_{50} can be assigned to the Class IV of the low toxic substances according to the classification by Sidorov K.K. (1973).

2. The presence in the structure of 1,4-naphthoquinone derivatives of the arginine residues (II, XI), aspartate (VII), glutamate (VIII) or tyrosine (X) has decreased the acute toxicity of these substances.

3. The presence in the structure of derivatives of 1,4-naphthoquinone residue of the leucine (I) or methionine (IX) is favourable to the increase of the compounds acute toxicity .

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