STUDY OF THE DISTRIBUTION OF AMLODIPINE IN ORGANS OF POISONED ANIMALS

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The aim of the investigation is the study of the distribution of amlodipine in organs of poisoned animals, using developed highly sensitive and selective techniques for the analysis of amlodipine in biological objects.
Materials and methods. To study the distribution of amlodipine in organs of poisoned animals, rats weighing 200–250 g were used. Amlodipine besylate solution was administered to rats using a probe in the stomach from the calculation of 70,0 mg/kg, after 3 hours rats were decapitated. For the study blood, urine, heart, liver, kidneys, lungs, stomach and intestine with contents and spleen were taken. Control experiments were delivered with the appropriate organs in parallel. Extraction of the substance was carried out with a mixture of methanol and acetonitrile (2: 3), purification was carried out by extraction of impurities with diethyl ether at pH 2,0–3,0 and TLC-method. Amlodipine was identified in biogenic extracts by the TLC-method in three systems of mobile solvents. The substance content was determined by spectrophotometry methods in the UV region of the spectrum.
Results and discussion. It is established that the highest amount of amlodipine was found in the stomach and intestine with the content, which is typical for acute poisoning. Less amount of amlodipine is found in the liver, kidneys and urine – organs and liquids that provide active detoxification of the body. According to the results of the research, it was found that in case of lethal poisoning, amlodipine for forensic toxicological studies should be carried out in urine, stomach with contents, intestine with contents, liver, spleen and kidneys.
Conclusions. The distribution of amlodipine in organs of poisoned animals with oral administration was studied. It is established that in case of lethal poisoning with amlodipine, for forensic-toxicological research it is necessary to be conducted in urine, stomach with contents, intestine with contents, spleen and kidneys. The developed methods can be proposed for introduction into the practice of the Bureau of Forensic Medical Examination, toxicological centers.
Keywords: amlodipine besylate, distribution in organs and biological fluids of poisoned rats, forensic toxicological examination.

1. Introduction

Amlodipine besylate belongs to a group of blockers of calcium channels, derivatives of 1,4-dihydropyridine, is used to treatment of arterial hypertension and vasospastic forms of angina pectoris [1, 2]. Amlodipine reduces peripheral and coronary resistance, improves coronary blood flow, reduces intracellular overload with calcium, suppresses platelet aggregation. Amlodipine in modern hypertension therapy is widely used in conjunction with the preparations of the main groups of antihypertensive drugs: perindopril [3], ramipril and lisinopril [4], indapamide [5]. When amlodipine is used, there are possible side effects: arterial hypotension, tachycardia, headache, nausea, dizziness, visual impairment, and depression. The main manifestations of amlodipine overdose are hyperglycemia, metabolic acidosis, electrolyte imbalance, sinus bradycardia, collapse [1]. According to the literature sources, amlodipine in case of overdose can provoke the development of breast cancer [6], cause ischemia of the optic nerve [7]. Deadly poisoning with amlodipine may accompany drug overdoses or suicidal cases. Fatal doses for children and adults range from 0,9 to 4,1 mg/kg [8, 9].

One of the important stages in the forensic toxicological examination is the choice of corpse organs to investigate them for the presence of substances that could be the cause of poisoning. The right choice of organs of a deceased person makes it possible not to lose toxic substances, to carry out screening, identification and quantitative determination of them. The algorithm of investigation of biological objects for the presence of a toxic substance involves several stages: the development and validation of identification and quantification methods for the test substance on model solutions, the application of developed techniques for studying the model mixes of toxic substances and biological material, approbation of techniques on organs of poisoned animals and introduction of the algorithm research on human body material. The last stage is performed in the forensic-toxicological department of the bureau of forensic medical examination of the city or region by forensic experts. In literature there are limited data on the distribution of amlodipine in the poisoned animal bodies that do not have a systematic character [10].

The aim of the investigation is the study of the distribution of amlodipine in organs of poisoned animals, using developed highly sensitive and selective techniques for analysis of amlodipine in biological objects.

2. Materials and methods

For the study, model mixtures of 10,0 g of chopped liver of animal origin and 1,0 ml of aqueous solution of amlodipine besylate containing 1000,0 μg of the drug were thoroughly mixed and left for a day. Amlodipine was extracted from biological fluids using 5,0 ml of blood and 10,0 ml of urine with addition of 1,0 ml of aqueous solution of amlodipine besylate containing 500,0 μg of the drug, stirred and left for a day. Control experiments were put in parallel.

Extraction of amlodipine from a biological material using methanol and acetonitrile (2:3) was carried out according to the developed method [10]: 10 % solution of chloride acid to pH 2,0–3,0 and a mixture of methanol and acetonitrile (2:3) in a volume of 10,0 ml, was thoroughly mixed. The vials with a mixture were shaken on an automatic shaker for 10 minutes, centrifuged for 5 minutes at 2500 rpm. The liquid over sediment was separated and the extraction was repeated by two portions of eluent of 10,0 ml.

Extraction purification of the extract from the liver tissue was performed according to the procedure: the combined extracts were filtered through a paper filter in a separating funnel containing 50,0 ml of 2,5 % aqueous sodium sulfate solution, were acidified with 10 % acid chloride solution to pH 2,0–3,0 (according to the universal indicator) and the biogenic impurities were extracted twice with portions of diethyl ether of 10,0 ml. The ether layers were separated and not further investigated. The water-methanol-acetonitrile solution was alkalized with 20 % solution of sodium hydroxide to pH 7,0–7,5 and amlodipine-base was extracted three times with portions of chloroform of 10,0 ml. The chloroform extracts were combined and centrifuged for 10 minutes at 5000 rpm for the destruction of stable emulsions. Chloroform extracts were filtered through a paper filter ("red tape") with 1,0 g sodium sulfate anhydrous, were evaporated to a dry residue which was dissolved in methanol, and then quantitatively was transferred to 5,0 ml volumetric flask. The volume was brought to the mark with methanol and examined by thin layer chromatography-method (TLC).

TLC-purification and identification of amlodipine in the extracts were carried out using the following procedure: 1,0 ml of amlodipine methanol solution, which corresponded to 2,0 g of biological material, was evaporated to 0,3–0,5 ml and was applied in the form of a strip of length 2 cm on the starting line of the chromatographic plate for high-performance thin-layer chromatography (production of Estonia, silicagel KSKG, fraction 5–20 microns, thickness of the layer 130± ±25 microns, plate size 20×20 cm). At a distance of 2 cm from the strip, a standard methanolic solution (20,0 μg/ml) of amlodipine was applied to the spot. At a distance of 2 cm from the point that corresponded to the witness, the extracted from the control sample, as obtained above, was applied. Chromatography was carried out in a chamber of a volume of 500 cm^3, in which 50,0 ml of a system of organic solvents – chloroform – methanol (9:1) was introduced, followed by saturation of the chamber in solvent pairs for at least 30 minutes. The length of the run of the front of the moving phase is 7 cm. The chromatographic plate was dried at room temperature, after which its part, where the witness and the extract from the control sample were located, were treated with the most sensitive reagent of Dragendorff for Munie (sensitivity of the reagent – 3,0–5,0 μg of the substance in the sample); $Rf_{amlodipine}$=0,51–0,53, the impurities were located on the line of start or on the finish line.

In order to confirm the results of the identification of amlodipine by TLC-method, were used chromatographic plates for high-performance thin-layer chromatography, a system of mobile solvents – chloroform-acetone-isopropanol – 25 % solution of ammonium hydroxide (7:7:7:2), $Rf_{amlodipine}$=0,55–0,57 and chloroform – acetone – 25 % solution of ammonium hydroxide (15:15:1), $Rf_{amlodipine}$=0,49-0,51.

At the level of finding the spot of a standard solution of the amlodipine from part of the chromatographic plate, which was not treated by the reagent of Dragendorff for Munie, was removed the layer of sorbent with an area of 4–5 cm^2. The layer of sorbent was transferred to the filter. The amlodipine-base was eluted three times with portions of methanol of 5,0 ml. The resulting solution was filtered through a filter ("red tape") and was evaporated to a dry residue. The dry residue was dissolved in chloroform, and then quantitatively was transferred to a volumetric flask of 10,0 ml, was brought to the mark with a solvent. Chloroform solution was used for quantitative determination by spectrophotometry in the UV region of the spectrum using the techniques developed.

The quantitative determination of amlodipine by spectrophotometry in the UV region of the spectrum was carried out under the conditions [11]: the optical density of the chloroform solution obtained after purification was determined on a spectrophotometer SF-46, cell thickness 10 mm; λmax=365±2 nm. The solution, which was obtained from a control experiment, was used as a solution for comparison. The concentration of amlodipine in a solution (C, μg/ml) was calculated from the calibration graph or by the linear equation of the optical density and its concentration: A=0,0329+0,0225 C, where A is the optical density of the chloroform solution of amlodipine; C is the concentration of amlodipine solution, μg/ml. The interval of the linearity of the calibration graph is 5,0–35,0 μg/ml, the lower limit of determination is 5,0 μg/ml, the correlation coefficient is 0,9997. The relative uncertainty of the mean result in the analysis of amlodipine in model solutions was ε=±1,64 %. It is established that following the methods of extraction of amlodipine with a mixture of methanol and acetonitrile (2:3), purification by diethyl ether extraction and TLC-method, TLC-method identification and quantitative determination of amlodipine content by spectrophotometry in the UV region of the spectrum 76,21–84,07 % amlodipine (ε=±4,91 %) can be determined in the liver tissue.

Extraction of amlodipine from blood was carried out according to the developed method: to the mixture was added 5,0 ml of purified water and 10,0 ml of 10 % solution of acid chloride, left for 2 hours, periodically stirred. The mixture was centrifuged for 5 minutes (5000 rpm), the centrifuge was separated. Extraction of the substance from the precipitate was repeated using the above experimental techniques.

For extraction purification from impurities, the centrifuges (pH 2,0–3,0) were combined, transferred to a separating funnel and impurities were extracted twice with portions of diethyl ether of 5,0 ml. The ether layers were separated and not further investigated. The acidic water layer was alkalized by a 20 % solution of sodium hydroxide to pH 7,0–7,5. The amlodipine-base was extracted three times with portions of chloroform of 5,0 ml. Purification and identification by TLC-method, quantitative determination of the content of amlodipine by spectrophotometry in the UV region of the spectrum was carried out using the above methods.

For extraction of amlodipine from urine the mixture was acidified with 10 % solution of acid chloride to pH 2,0–3,0. The following stages of extraction purification, identification and quantitative determination of the content of amlodipine were performed using methods of analysis of amlodipine in the blood. It is established that following the methods of extraction of amlodipine from biological fluids it is possible to determine in the blood – 48,66–53,07 % amlodipine (ε=±4,32 %), in urine – 75,37–80,38 % amlodipine (ε=±3,21 %).

3. Results

For the study of the distribution of amlodipine in organs of poisoned animals, rats weighing 200–250 g that did not receive food during the day were used. Amlodipine besylate solution was administered to rats using a probe in the stomach from the calculation of 70,0 mg/kg (LD_{50} was 40–100 mg/kg), after 3 hours rats were decapitated. For the study blood, urine, heart, liver, kidneys, lungs, stomach and intestine with contents and spleen were taken. Control experiments were delivered with the appropriate organs in parallel. The stages of extraction, purification, identification and quantitative determination of the content of amlodipine were performed using above methods of analysis of amlodipine. Depending on the weight of the organs or fluids under investigation, the volumes of the extractants was changed. It is established that the highest amount of amlodipine was found in the stomach and intestine with the content, which is typical for acute poisoning. Less amount of amlodipine is found in the liver, kidneys and urine – organs and liquids that provide active detoxification of the body. According to the results of the research, it was found that in case of lethal poisoning, amlodipine for forensic toxicological studies should be carried out in urine, stomach with contents, intestine with contents, liver, spleen and kidneys. The developed methods can be proposed for introduction into the practice of the Bureau of Forensic Medical Examination, toxicological centers.

4. Discussion

The results of research are important for forensic toxicological analysis, since in literature there are limited data on the distribution of amlodipine in the poisoned animal bodies that do not have a systematic character [10]. According to the data of Shormanov V. K. and Kvachakhiya L. L. for the analysis of

amlodipine in the organs of rats were used extractions with acetone, purification of extracts in a column with silica gel 160 μm with eluent-ethanol-hexane (7:3). These methods are characterized by significant losses of matter during isolation and duration of analysis. The result of using these techniques is the selection of a very limited number of organs and fluids recommended for forensic toxicology analysis. Despite the use of highly sensitive methods for identification and quantification (TLC, GC/MS and UV spectrophotometry), only the stomach, blood and kidneys can be used in the study. This choice of organs does not give an objective picture of poisoning and can cause loss of substance during analysis.

As a result of our studies it was found that in the case of lethal poisoning with amlodipine, forensic toxicology analysis should be carried out in the urine, stomach with contents, in the intestine with contents, liver, spleen and kidneys. The choice of important organs and fluids is due to the processes of localization of amlodipine and its detoxification. Advantages of the obtained results are approbation of the developed methods for the analysis of amlodipine in biological objects and the use of results in the selection of organs and fluids for forensic toxicological analysis. The choice of organs and liquids with the maximum content of amlodipine allows to identify and to determine the substance in native form in the presence of biogenic impurities. Disadvantages – the results of the study were obtained on animals, therefore it is important to test the analysis methods on the organs and fluids of people who died in the fatal poisoning of amlodipine.

The results of the study can be recommended for the use in the practice of forensic toxicologists in the study of cadaveric material. It is planned to develop tandem methods of high-performance liquid chromatography and gas chromatography with mass spectrometry for the analysis of amlodipine and its metabolites in animal organs and cadaveric material.

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