**The article is published in Ukrainian in the journal.
The English text is given in the author's version.**

UDC 612.084:616-092.9:615.099.092

A comparative study of «Capicor» safety in acute experiment

Zhulaу T.S., Shebeko S.K., Shevchenko O.I.\*

National University of Pharmacy, Kharkiv

\* Kharkov Medical Academy of Post-graduate Education

**Key words:** endothelial dysfunction, Meldonium, γ-butyrobetaine, Capikor, acute toxicity.

*One of the main causes in the pathogenesis of chronic ischemia is endothelial dysfunction. The combined use of precursors and analogues of carnitine (Meldonium and γ-butyrobetaine (GBB)) is an interesting decision in terms of optimization of modern anti-ischemic therapy. The concept presented formed the basis for development of the original regulator of the vascular endothelial function with the trade name “Capikor” combining the advantages of Meldonium as a metabolic cytoprotector and GBB as an endothelial corrector. The results of the experimental research concerning the comparative study of “Capikor” acute toxicity are presented in the article. “Capikor” is similar to the reference drug “Mildronate” by its toxicological characteristics. The results of the preclinical study indicate that “Capikor” is a practically non-toxic drug and allow referring it to the V-th toxicity class according to the K.K.Sidorov classification. This is the basis for further study of “Capikor” for the purpose of its introduction into clinical practice as a drug with the metabolic action for treating diseases, in which the endothelial dysfunction occupies a leading position in the pathogenesis.*

**Introduction.** The main cause in the general structure of world mortality is cardiovascular disease (CVDs): each year so many people do not die for no other reason, how many people die from CVDs. According to WHO, the mortality from CVDs amounts 31% of all deaths in the world, which amounted 17.5 millions in 2012, and predictably the mortality from CVDs will increase to 25 millions in 2030[15].

According to statistics, Ukraine occupies a leading position in Europe on indicator of premature mortality from cardio-vascular pathologies. In 2014, premature deaths from cardiovascular pathogenesis accounted for 66% from all deaths, with the standard mortality [coefficient](http://www.lingvo.ua/ru/Search/Translate/GlossaryItemExtraInfo?text=%d0%ba%d0%be%d1%8d%d1%84%d1%84%d0%b8%d1%86%d0%b8%d0%b5%d0%bd%d1%82&translation=coefficient&srcLang=ru&destLang=en) was 961 cases per 100 000 population [11] that, on average, 3 times higher than in most EU countries [12].

### One of the main causes in the pathogenesis of chronic ischemia is endothelial dysfunction. On one side, endothelial dysfunction may be an independent cause of circulatory disorder in the organ, as often provokes vasoconstriction or vascular thrombosis. On the other side, the disorder of regional circulation (venous stasis, ischemia) can also lead to endothelial dysfunction [1]. The most «typical» manifestations of this condition in clinical practice are ischemic heart disease and [cerebrovascular disorders](https://www.google.com.ua/url?sa=t&rct=j&q=&esrc=s&source=web&cd=13&cad=rja&uact=8&ved=0ahUKEwjNkqDIv8LJAhVEqHIKHaX6AacQFghiMAw&url=https%3A%2F%2Fwww.dartmouth.edu%2F~dons%2Fpart_3%2Fchapter_27.html&usg=AFQjCNEoKi2JM2iYFxCIx3xlN27x2iwpEA). However, the a close correlation of angiogenic cochleovestibular disorders and endothelial dysfunction was proved in literature which in clinical practice manifests eustachian tube dysfunction, exudative processes in the middle and inner ear and sensorineural hearing loss.

### Metabolic concept, which implies a normalization of oxidative processes (reduction of fatty acid oxidation and increase of glucose oxidation), and as a result, more efficient use of oxygen, is well-known in modern anti-ischemic therapy. However, recent studies have demonstrated the importance of hemodynamic conception, which primarily consists in endothelial dysfunction correcting and therefore compensation of the impaired circulation. Finally, both approaches increase adaptation of tissues to the functioning in reduced oxygen delivery conditions and, consequently, contribute to the preservation of their structure, integrity and functional activity.

Given the multi-vector pathogenesis of ischemic conditions, medications with polytropic action which provide cytoprotection in unfavorable pathophysiological conditions, have attracted the attention of specialists in recent years. Combined use of carnitine precursors and carnitine analogs (Meldonium and γ-butyrobetaine (GBB)) is an interesting decision in terms of the modern anti-ischemic therapy optimization by combining of metabolic and hemodynamic concepts. The pharmacodynamic preferences of combined use of these substances are characterized by the synergistic phenomenon manifestation in the form of mutual potentiation of their effects. Presented concept has become to the basis for the development of the original drug «Capicor» with binary mechanism of action which combines the advantages of Meldonium as metabolic cytoprotector and GBB as endothelial corrector.

### All the above has led to expediency of carrying out a series of experimental researches by in-depth comparative study of Capicor toxicity and Capicor specific activity for specification of its cytoprotective mechanism.

### Materials and methods. Comparative experimental study of the Capicor toxicological properties in single-dose was conducted as part of scientific research «Pre-clinical study of pharmacological properties of the drug «Capicor» in outbred rats of both sexes. All studies were conducted according to Directive 86/609 EU European Parliament and of the Council of the of 24 November 1986 on compliance with laws, regulations and administrative provisions of the EU on the animal protection used for experimental and other scientific purposes [6, 7, 9, 11, 13, 14].

### Drug «Capicor» [2] production of JSC «Olainfarm» (Latvia) has been used as the basic sample in this study. The drug has been produced in capsules and has the following composition (per 1 capsule):

### Meldonium dehydrate 180 mg;

### Gamma-butyrobetaine dehydrate 60 mg.

### As a reference object Drug «Mildronate» 500 mg [3] production of JSC «Grindeks» (Latvia) as the original meldonium drug has been used as the reference sample.

### The study of acute toxicity of «Capicor» in compared with «Mildronate» has been conducted by the method of the least-squares for probit-analysis of mortality curves by V.B. Prozorovsky. 60 white nonlinear rats of both sexes weighing 150-180 g were used in the experiment. Rats were divided on 10 experimental groups (1-10) for 6 animals in each group. Capicor was introduced of the 1-5 animal groups, Mildronate was introduced of the 6-10 animal groups one time intragastric (i/g) at doses of 1000, 2000, 3000, 4000, 5000 mg / kg respectively for each group. Class toxicity of «Capicor» and «Mildronate» was determined according to the generally accepted classification by K.K. Sidorov [10].

The observation of the animals has been held for two weeks after a single administration of the test and reference samples. Laboratory observation of the survival of the experimental animals, the use of food and water, as well as the manifestations of intoxication symptoms (if they occur): general status, body position changes, conditions of the skin, mucous membrane color, body temperature and the individual symptoms (miosis, watery eyes, diarrhea, changes in the color of urine and faeces, drowsiness, convulsions, etc.) has been conducted throughout the study [4].

All animals in case of death as well as after the end of the study were exposed to autopsy. Macroscopic analysis of the abdominal organs was carried out in order to establish that the death did not occur as a result of manipulation errors as well as to determine of the possible cause of death. Furthermore, weighting of the internal organs (brain, heart, kidneys, liver, and spleen) was conducted and their mass ratios were determined.

The percentage of mortality in each group was determined after 14 days to calculate the median lethal dose (LD50). The LD50 value was determined by means of tables and calculations in according to the method of probit-analysis of mortality curves by V.B. Prozorovsky [8].

**Results and Discussion.** During the study of acute toxicity of Capicor at i/g introduction in rats the following picture of intoxication has been fixed: the visible signs of the toxic effects of the drug on the functional condition of the animals was not registered with the introduction of the drug in doses of 1000-3000 mg/kg. Rats quietly tolerated the introduction of the study drug, and their appearance, behavior and general condition remained unchanged. Further observations during the day were not found in their general condition and behavior abnormalities.

In the early hours of observations some weakness, languor, decreased motor activity, decreased appetite was observed in rats at Capicor introduction in doses 4000 and 5000 mg / kg. Some rats were in an inactive state, response to external stimuli was reduced, and breathing was quickened. For the next 3-5 hours general condition and behavior of animals returned to the physiological norm, and all the manifestations of intoxication disappeared. The next day the animals were free to move around the cage, took water and food, and their general functional state was completely in line with the physiological norm.

During the study in the experimental groups any case of mortality of animals was not registered (table 1).

*Table 1*

**Mortality indicators of rats in the acute toxicity study of**

**Capicor and Mildronate at i/g introduction (n=60)\***

|  |  |  |  |
| --- | --- | --- | --- |
| Group number | Dose,mg/kg | The number of rats per group | The number of dead rats |
| 1-st day | 4-th day | 7-th day | 10-th day  | 14-th day |
| *Capicor* |
| 1 | 1000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 2 | 2000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 3 | 3000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 4 | 4000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 5 | 5000 | 6 | 0 | 0 | 0 | 0 | 0 |
|  | *Mildronate* |
| 6 | 1000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 7 | 2000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 8 | 3000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 9 | 4000 | 6 | 0 | 0 | 0 | 0 | 0 |
| 10 | 5000 | 6 | 0 | 0 | 0 | 0 | 0 |

A similar pattern was observed in the study of the Mildronate toxicological properties in a single i/g introduction. As in the previous case, some signs of intoxication were observed only during the first three hours of observation the drug at introduction in high doses (4000 and 5000 mg/kg). In the future, data manifestations disappeared, and the results of laboratory observations, the animals came back to a normal physiological state, which was within the normal range. Also, as in the study ofCapicor acute toxicity, any case of mortality due to Mildronate single use was not registered (table 1).

Monitor on the dynamics of rat body weight was also conducted during the study of these drugs. Weight gain was determined by an average of 8.0% over a time interval of 14 days, but this was not significant character in compared to the original data. Thus, the negative impact on rat body weight gain at this stage of the research were not observed.

In the study of Capicor toxicological characteristics, pathological examination of the abdominal organs and the brain was carried out after the rat euthanasia on day 14 of the experiment. During the mаcroscopic study of internal organs, all animals were carefully examined for visible pathophysiological signs. In the analysis of significant abnormalities were not observed. Visible mucous membranes were in pink color with no abnormalities, lymph nodes were not enlarged, the organ location was normal, adhesions were not found. All mаcroscopic examination organs (brain, heart, kidneys, liver, spleen) had normal size, color and tissue density. In calculating and analysis of mass coefficient indicators of animal internal organs of probable dynamic changes were not observed. All indicators were within of the physiological norm (table 2).

A similar pattern was observed in the microscopic study of the animal abdominal organs after a single administration of Mildronate. All mass coefficient indicators of internal organs also were within of the physiological norm (Table. 2).

*Table 2*

**Mass coefficients of the rat internal organs in the acute toxicity study of**

**Capicor and Mildronate at i/g introduction (n=60)\***

|  |  |
| --- | --- |
| The mass coefficient, % | *Dose, mg/kg* |
| 1000 | 2000 | 3000 | 4000 | 5000 |
| *Group number* |
| Capicor |
| *1* | *2* | *3* | *4* | *5* |
| brain | 1,04±0,03 | 0,99±0,02 | 1,03±0,04 | 1,07±0,05 | 1,04±0,03 |
| heart | 0,44±0,01 | 0,41±0,01 | 0,44±0,02 | 0,42±0,01 | 0,46±0,02 |
| kidney (right) | 0,30±0,01 | 0,32±0,01 | 0,33±0,02 | 0,32±0,01 | 0,33±0,02 |
| spleen | 0,41±0,01 | 0,38±0,03 | 0,40±0,01 | 0,39±0,03 | 0,42±0,02 |
| liver | 3,44±0,09 | 3,48±0,08 | 3,42±0,09 | 3,43±0,07 | 3,47±0,09 |
|  | Mildronate |
| *6* | *7* | *8* | *9* | *10* |
| brain | 1,00±0,04 | 1,03±0,03 | 0,98±0,05 | 1,03±0,03 | 0,97±0,03 |
| heart | 0,40±0,01 | 0,43±0,02 | 0,41±0,01 | 0,44±0,01 | 0,42±0,03 |
| kidney (right) | 0,32±0,01 | 0,29±0,02 | 0,31±0,02 | 0,33±0,01 | 0,32±0,01 |
| spleen | 0,38±0,01 | 0,41±0,02 | 0,39±0,01 | 0,40±0,03 | 0,39±0,02 |
| liver | 3,45±0,09 | 3,40±0,07 | 3,46±0,08 | 3,45±0,10 | 3,43±0,09 |

The absence of mortality of laboratory animals at i/g introduction of the test and reference products does not allow to calculate the average lethal dose values by probit analysis. This leads to the conclusion that the LD50 value for each of the studied products exceeds the maximum dose, which was used in the experiment. Thus we can say that at i/g introduction of Capicor and Mildronate in rats LD50 is more than 5000 mg/kg. This LD50 value allows referring both studied drugs in the studied route of administration to the V class of toxicity by K.K. Sidorov standard classification. There are practically non-toxic substances [10].

**Conclusions.** Thus, as a result of of the researches on the comparative study of acute toxicity, we can make the following conclusions:

1. Capicor in i/g single administration in rats at doses range 1000-5000 mg/kg does not have toxic effects on the general condition and behavior of animals, and does not cause their death.
2. At this route of administration Capicor LD50 is more than 5000 mg/kg.
3. For toxicological characteristics Capicor is identical to the reference product Mildronate. Namely, Capicor practically does not have toxic effect on the general condition, behavior, food intake and body weight of the animal. Capicor does not have affect on the absolute and relative weight of internal organs. Capicor does not cause any visible changes to the rat internal organs and does not cause their death.
4. Capicor is practically non-toxic agent to the human body and allow to take it to the V class of toxicity by K.K. Sidorov standard classification. There are practically non-toxic substances.
5. All of the above can serve as a basis for further reserch of «Capicor» in order to implement in clinical practice as a cytoprotective drug for the treatment of diseases in the pathogenesis of which endothelial dysfunction takes a leading place.

**References**

1. Долженко М.Н. К вопросу о целесообразности применения метаболической кардиопротекции в эпоху доказательной медицины / М.Н. Долженко // Мистецтво лікування. – 2012. – № 2-3. – С. 3-6.
2. Інструкція для медичного застосування препарату «Капікор» [Електронний ресурс]. – Наказ МОЗ України № 210 від 15.03.2013. – Режим доступу : <http://mozdocs.kiev.ua/likiview.php?id=30553>.
3. Інструкція для медичного застосування препарату «Мілдронат» [Електронний ресурс]. – Наказ МОЗ України № 1095 від 13.12.2010. – Режим доступу : <http://mozdocs.kiev.ua/likiview.php?id=26765>.
4. Коваленко В.М. Експериментальне вивчення токсичної дії потенційних лікарських засобів / В.М. Коваленко, О.В. Стефанов, Ю.М. Максимов,
І.М. Трахтенберг. – В кн.: Доклінічні дослідження лікарських засобів (методичні рекомендації) / За ред. чл.-кор. О.В.Стефанова. – К.: Авіцена, 2001. – С. 74-85.
5. Наказ МОЗ України № 944 від 14.12.2009 р. «Про затвердження Порядку проведення доклінічного вивчення лікарських засобів та експертизи матеріалів доклінічного вивчення лікарських засобів».
6. Настанова СТ-Н МОЗУ 42-6.0:2008. Лікарські засоби. Належна лабораторна практика (видання офіційне) / О. Стефанов, Т. Бухтіарова, В. Коваленко та ін. – К.: Моріон, 2009. – С. 37-68.
7. Настанова СТ-Н МОЗУ 42-6.0:2014. Лікарські засоби. Доклінічні дослідження безпеки як підґрунтя клінічних випробувань за участю людини та реєстрації лікарських засобів (ICH M3(R2)) / О. Нагорна, Т. Бухтіарова, Т. Талаєва та ін. – К. : МОЗ України, 2014. – 45 с.
8. Прозоровский В.Б. Практическое пособие по ускоренному определению средних эффективных доз и концентрации биологически активных веществ. – Санкт-Петербург, 1992. – 42 с.
9. Руководство по проведению доклинических исследований лекарственных средств. Часть первая. – М. : Гриф и К, 2012. – 944 с.
10. Сидоров К.К. О классификации токсичности ядов при парентеральных способах введения. – В кн.: Токсикология новых промышленных химических веществ. – Москва, 1973. – Вып. 13. – С. 47-57.
11. European Detailed Mortality Database [Electronic Resource]. – WHO, Regional Office for Europe, 2015. – Mode of access : http://data.euro.who.int/dmdb.
12. Eurostat regional yearbook 2012. – Luxembourg : Office of the European Union, 2012 – 213 p.
13. Good Laboratory Practice / OECD principles and guidance for compliance monitoring – OECD, 2005.
14. Guide for the care and use of laboratory animals. – 8th edition. – Washington : The National Academies Press, 2011. – 246 p.
15. World Health Statistics 2012. – Geneva : WHO, 2012. – 176 p.