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STUDY ON ACUTE TOXICITY AND ANTIDOTE ACTIVITY OF LYOPHOBIC DRUG "CALCIUM FOLINATE"

Raikhona A. Khusainova
Doctor of Medical Sciences, Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan.
Email: xusainova79@inbox.ru, info@pharmi.uz

Nilufar K. Abdullaeva
Associate Professor, Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan. Email: author.uzb@mail.ru

Nilufar M. Rizaeva
Master Degree, Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan. Email: author.uzb@mail.ru

Rahmonjon R. Shokodirov
Associate Professor, Tashkent Pharmaceutical Institute, Tashkent, Republic of Uzbekistan. Email: author.uzb@mail.ru

ABSTRACT

To combat the toxic effects of methotrexate on bone marrow cells and GI (gastrointestinal tract) tract causing a disorder of nucleic acid synthesis and the growth of proliferating tissues, a lyophobic drug "Calcium folinate" was used as an antidote for the development of Necrotizing ulcerative stomatitis, enteropathy, and etcetera. It is intended for oral and parenteral administration with the bioavailability approximately 97% for a 25 mg dose, 75% for a 50 mg dose, and 37% for a 100 mg dose. In combination with fluorouracil, it can be used in the palliative treatment on patients with colorectal cancer as well. Calcium folinate solution is intended for intravenous (i / v) administration with the maximum rate of 160 mg/min and intramuscular (i/m), while intrathecal (endolumbar) administration of the solution is strictly prohibited. In form of infusion solutions diluted with 0.9% sodium chloride solution or 5% glucose solution, calcium folinate is used for the treatment of megaloblastic anemia and the therapy and prevention of folate deficiency in cases when oral administration of folic acid is impossible or for some reason ineffective, for example, in patients with parenteral nutrition or severe malabsorption syndrome.

The study of acute toxicity was carried out according to conventional methods on 72 white mice (both sexes) within 14 days. As a result, it was found that under the drug administration, there is a significant decrease in death compared with the control 6.00 to 1.83 (0.43÷3.23). The study of the antidote activity of the drug showed that the drug has a reliable antidote effect.

Key words: Calcium folinate, Methotrexatum, acute toxicity, antidote activity, JV «Jurabek Laboratories».

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About the authors Raikhona A. Khusainova

Corresponding author- Email: xusainova79@inbox.ru, info@pharmi.uz

抽象的

为了对抗甲氨蝶呤对骨髓细胞和胃肠道（胃肠道）的毒性作用，导致核酸合成障碍和增殖组织的生长，使用疏液药物“亚叶酸钙”**作**为坏死性溃疡形成的解毒剂。口腔炎、肠病等。它用于口服和肠胃外给药，25 mg 剂量的生物利用度约为 97%，50 mg 剂量约为 75%，100 mg 剂量约为 37%。**与氟尿**嘧啶合用，也可用于大肠癌患者的姑息治疗。亚叶酸钙溶液用于静脉（i / v）给药，最大速率为160 mg / min**和肌肉内**（i / m），**而**严格禁止鞘内（endolumbar）给药。以 0.9% 氯化钠溶液或 5% **葡萄糖溶液稀**释的输液形式，亚叶酸钙用于治疗巨幼红细胞性贫血和治疗和预防叶酸缺乏的情况下，如果口服叶酸是不可能的或由于某种原因无效，例如对肠外营养或严重吸收不良综合征的患者。

急性毒性研究按常规方法对72**只白鼠**（**雌雄同体**）**在**14**天内**进行。结果发现，在给药下，与对照6.00**相比**，**死亡**显着降低至1.83（0.43÷3.23）。对该药解毒活性的研究表明，该药具有可靠的解毒作用。

关键词：亚叶酸钙，甲氨蝶呤，急性毒性，解毒活性，JV «Jurabek Laboratories»。

INTRODUCTION

The antitumor effect of methotrexate is due to inhibition of the activity of the enzyme dihydrofolate reductase (recovers dihydrofolate into its active form - tetrahydrofolate), as a result, there is a disorder of nucleic acids synthesis, and the growth of proliferating tissues stops. Simultaneously with the antitumor effect under the influence of methotrexate, hematopoiesis is oppressed. In contrast with folic acid, calcium folinate does not require recovery by dihydrofolate reductase for conversion into tetrahydrofolate. Therefore, the blockade of this enzyme by folic acid antagonists does not affect the action of calcium folinate. Calcium folinate, administered at the appropriate time, prevents the toxic effect of methotrexate on bone marrow cells (ensures the preservation of hematopoiesis) and GI tract (gastrointestinal tract).

In oncological practice, calcium folinate is applied as an antidote for the development of toxic effects (necrotizing ulcerative stomatitis, enteropathy, etc.,) in using usual doses of methotrexate, to prevent the possible toxic effect of increased, high and ultra-high doses of methotrexate, as well as in the palliative treatment on patients with colorectal cancer (in combination with fluorouracil).

After oral administration, it is rapidly absorbed from the GI tract. Bioavailability is approximately 97% for a 25 mg dose, 75% for a 50 mg dose, and 37% for a 100 mg dose. When taken orally at a dose of 15 mg, Cmax in the blood is (268 ± 18) mg / ml, Tmax - (1.72 ± 0.8) h, with parenteral administration it is of the same dose - (241 ± 17) mg / ml and (0.71 ± 0.09) h, respectively. It passes through the BBB in moderation and mostly accumulates in the liver. It is metabolized in the liver and intestinal

mucosa, mainly into an active metabolite (5-methyltetrahydrofolate). After oral administration in 30 minutes, it is biotransformed more than 90%, with parenteral administration, metabolism is slower and to a lesser extent (about 66% with i/v and 72% with i/m ad.). $T_{1/2}$ from blood is 6.2 h, regardless of the route of administration. It is excreted by the kidneys (80–90%) and with feces (5–8%).

Intoxication by folic acid antagonists (methotrexate, trimethoprim, pyrimethamine); megaloblastic anemia as a folic acid deficiency with the ineffectiveness of oral therapy with folic acid drugs (including with malabsorption syndrome, malnutrition, congenital deficiency of dihydrofolate reductase); colon cancer, including the rectum (as adjuvant therapy).

Calcium folinate solution is intended for intramuscular (i / m) and intravenous (i / v) administration. Intrathecal (endolumbar) administration of the solution is strictly prohibited. The maximum rate of intravenous administration is 160 mg / min. Infusion solutions are prepared by diluting Calcium folinate with 0.9% sodium chloride solution or 5% glucose solution. The drug is used for the treatment of megaloblastic anemia associated with a deficiency of folic acid, as well as for the therapy and prevention of folate deficiency in cases when oral administration of folic acid is impossible or for some reason ineffective, for example, in patients with parenteral nutrition or severe malabsorption syndrome. All doses are quoted as folinic acid.

Research purpose: preclinical study of the drug "Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan.

MATERIALS AND METHODS

All studies were applied on healthy animals quarantined for at least 10-14 days [1, 2].

The study of acute toxicity was carried out according to conventional methods on white mice (both sexes), body weight 18-22 g, 6 animals per group, 30 mice were used in total.
The drug was applied on experimental animals slowly, intravenously (into the tail vein) in the form of a 4% solution, at doses: 200 mg / kg (0.1 ml / 20 g), 400 mg / kg (0.2 ml / 20 g), 600 mg / kg (0.3 ml / 20 g), 800 mg / kg (0.4 ml / 20 g) and 1000 mg / kg (0.5 ml / 20 g).

Then the animals were placed in separate cages in groups, and were continuously monitored during the first day, and once a day in the next 13 days of the experiment (total observation period was 14 days). At the same time, the clinical picture of intoxication and the lethality of the animals were recorded. The calculation of the average lethal dose (LD50) was carried out according to the Litchfield and Wilcoxon scheme by the Probit analysis method [3].

During the experiment, all animals were kept in standard vivarium with complete food and water diet.

RESULTS AND DISCUSSIONS

After the administration of the drug, a number of symptoms of intoxication, changes in the general condition and other effects characterizing the toxic effect were observed (Table 1).

Table 1

The results of the toxic effect of the drug "Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan

Dose	Result
200 mg/kg	Immediately after the administration of the drug, the animals showed a decrease in motor activity within 2-2.5 hours. After the condition of the animals returned to normal, and during the entire period of the experiment no death of animals was observed.
400 mg/kg	Immediately after administration of the drug, the animals showed a decrease in motor activity and convulsions within 6-8 hours. On the first day, one mouse died.
600 mg/kg	Immediately after administration of the drug, the animals showed a decrease in motor activity, convulsions and deep breathing within a day. After 3 minutes from drug administration, 2 mice died. After 10 minutes, one more mouse died and the death of another one was observed on the day 2.
800 mg/kg	Immediately after the drug administration, animals showed decrease in motor activity, convulsions and deep breathing during 2 days. Immediately after drug administration, 3 mice died. After 3 minutes one more died. After 5 minutes, the death of another one was observed.
1000 mg/kg	Immediately after the drug administration, at the same time with decrease in motor activity, convulsions and deep breathing within 5 minutes, all died.

On the basis of the results of the death of experimental animals, we calculated LD50 of the drug. (Table 2).

Table 2

The result of the study of the indexes of the acute toxicity of the drug

(p=0,05)

"Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan	
Doses	The number of died animals/total
200 mg/kg	0/6
400 mg/kg	1/6
600 mg/kg	4/6
800 mg/kg	5/6
1000 mg/kg	6/6
LD_{50}= 540 (400÷729) mg/kg	

Table 3

Study period: 10.08.2020-28.08.2020

The results of measuring the mass of animals (in grams) taken for the study of acute toxicity

№	"Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan				
	200 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg	1000 mg/kg
1.	20	22	22	20	22
2.	20	21	18	20	18
3.	21	19	19	21	19
4.	19	18	21	19	21
5.	18	22	22	18	22
6.	21	21	20	22	20

Research on antidote activity of the drug

The study on antidote activity was carried out by the method based on the ability of the drug to reduce the toxicity of methotrexate in experimental animals [2]. The experiments were carried out on 72 white mice (both sexes) weighing 20-22 g, in a group of 6x6 animals.

For this purpose, the animals of the experimental groups were injected daily, once, slowly, intravenously (into the tail vein) in the form of a 0.55% solution during four days. Then on the second day of the experiment, one hour after the drug administration to animals of all groups, methotrexate was once orally administered in the form of a 0.6% suspension, at a dose of 150 mg / kg (0.5 ml / 20 g):

control group (control) - animals with test modeling, but without drug administration;

1. test group - animals received the drug "Calcium folinate" lyophilisate for preparation of a solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan, at a dose of 55 mg / kg, in a volume of 0.2 ml / 20 g.

After application methotrexate at a lethal dose, the animals of all groups were observed within 14 days and the death was registered.

The criteria for evaluating the pharmacological activity had a decrease in the death of animals compared to the control.

The results were processed by the method of variation statistics according to the Student's t-test at p = 0.05 [1,2]. The tables show the arithmetic mean values (M), the corresponding standard errors of the mean (m), Student's t-test (t), the number of samples (n), and confidence limits (lower confidence limit ÷ upper confidence limit).

As a result of the study of the antidote activity of the drug, it was found that under the drug administration, there is a significant decrease in death compared with the control, which indicates the antidote activity of the drug (table 4).

Table 4

The results of the study of the antidote activity of the drugs (M±tm; n=6; p=0,05)

Group	The number of died animals
Control	6,00
"Calcium folinate" lyophilisate for the preparation of solution for injection 300	1,83 (0,43÷3,23)

mg, JSC Ltd "Jurabek Laboratories" Uzbekistan	

Table 5

Study period: 24.08.2020-17.09.2020

The results of the study of the antidote activity of the drug (M±tm; n=6; p=0,05)

№	The number of died animals	Average
Control		
1.	6	6,00 (6,00÷6,00)
2.	6	
3.	6	
4.	6	
5.	6	
6.	6	
"Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan		
1.	4	1,83 (0,43÷3,23)
2.	0	
3.	2	
4.	2	
5.	1	
6.	2	

Table 6

LD50 calculation according to the Litchfield and Wilcoxon scheme by Probit analysis of the drug "Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan

Doses	Observed effect	% of observed effect	% of expected effect	The difference between the observed and expected % of the effect	The term for X^2
1	2	3	4	5	6
200 mg/kg	0/6	0	0,45	0,45	0
400 mg/kg	1/6	16,7	21,0	4,3	0,013
600 mg/kg	4/6	66,7	62,0	4,7	0,01
800 mg/kg	5/6	83,3	86,0	2,7	0,006
1000 mg/kg	6/6	98,2	94,5	3,7	0,026

				Sum	0,075

X^2= 0,075x6=0,45

0,45<7,82 at p=0,05 therefore the straight line corresponds to the plotted points

f=5-2=3

LD_{50}= 540 mg/kg

LD_{16}= 370 mg/kg

LD_{84}= 780 mg/kg

$$S = \frac{\frac{LD_{84}}{LD_{50}} + \frac{LD_{50}}{LD_{16}}}{2} = \frac{\frac{780}{540} + \frac{540}{370}}{2} = 1{,}45$$

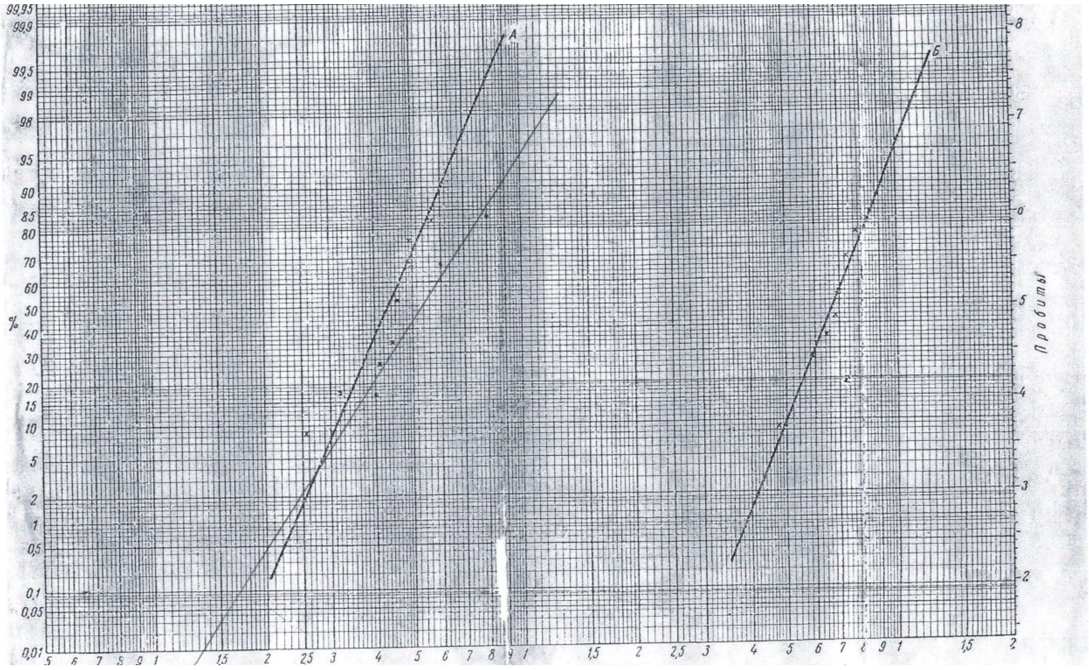
N= 2x6=12

f_{LD50}=1,35

540:1,35 = 400 mg/kg

540x1,35 = 729 mg/kg

LD_{50}= 540 (400÷729) mg/kg



CONCLUSION

The preclinical study of the drug "Calcium folinate" lyophilisate for the preparation of solution for injection 300 mg, JSC Ltd "Jurabek Laboratories" Uzbekistan, was carried out according to the indexes of the acute toxicity and antidote activity. According to the results of the study of acute toxicity, the average lethal dose was identified as LD50 = 540 (400 ÷ 729) mg/kg. The study of the antidote activity of the drug showed that the drug has a reliable antidote effect.

CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of obvious and potential conflicts of interest related to the

publication of this article and report on the contribution of each author.

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