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Research Article

STUDYING THE ACUTE TOXICITY AND ANTI-TUMOR ACTIVITY OF THE NEW DRUG COLHAMETIN

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Yuldashev Javlonabduraimovich
Samarkand State Medical University, Uzbekistan

Ibragimov Shavkat Narzikulovich
Republican Specialized Scientific And Practical Medical Center Of Oncology And Radiology Of The Ministry Of Health Of The Republic Of Uzbekistan

Shakhanova Shakhnoza Shavkatovna
Samarkand State Medical University, Uzbekistan

Esankulova Bustonoy Sobirovna
Samarkand State Medical University, Uzbekistan

ABSTRACT

The aim of the study was to study acute toxicity during intraperitoneal administration of a new antitumor drug "colhametin" (K-2) in mice and rats and its antitumor activity on 2 strains of tumors, including with a smaller number of injections.

Material and research methods. Acute toxicity of the K-2 preparation after a single intraperitoneal injection was carried out according to the Litchfield and Wilcoxon method on 30 white outbred mice weighing 20±2g of both sexes and 30 male and female rats weighing 140±10g, 5 animals in each group. The study of antitumor activity was performed on 12 outbred mice and 16 outbred rats with transplanted tumors S-180 (Sarcoma-180) and WC (Walker's sarcoma) which were administered drugs on the 4th day after tumor inoculation 10- and 5-fold. The results were evaluated according to standard criteria: tumor growth inhibition (TGI), body weight and spleen of animals Differences were considered significant at $p<0.05$.

Results. The following data were obtained with a single intraperitoneal injection to mice: the average lethal dose of LD50 is 890 (-150 + 172) mg/kg, LD50 with a single intraperitoneal dose of K-2 preparation in rats, LD50 is 410 (-56 + 61) mg/kg, the values are also determined LD10, LD16 and LD84.

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The antitumor activity of the drug K-2 on the tumor strain Sarcoma - 180 was high, 99/90%. On WC tumors the effect of K-2 reached 90/91% with 10-fold administration, and 89/89% with 5-fold administration, however, its effect was accompanied by a decrease in hematopoietic parameters.

Conclusions. The study of the acute toxicity of the substance of the preparation K-2 showed that this preparation belongs to the IV class of low-toxic compounds. On 2 tumors, the effect of the new drug was high, which did not decrease with a decrease in the number of injections.

KEYWORDS

New anticancer drug colhametin, acute toxicity, mice, rats, activity, tumors, sarcoma -180, Walker sarcoma.

INTRODUCTION

Currently, antitumor drugs are largely created from natural compounds and there are dozens of such effective drugs known in oncology [1]. However, despite their high activity, a large number of known cytostatics have a large number of side effects, usually due to their high toxicity [2-5].

Based on colchicine and colchamine, we created new alkaloids that are less toxic and more active than the original alkaloids, as well as other mitotic inhibitors and anticancer drugs [6-9]. The new drug colhametin was synthesized from colhamin, which was widely used in the clinic in the treatment of stage I and II skin cancer in the form of a 0.5-1% ointment [6], and the amino acid methionine [10], which gave a noticeable decrease in toxicity. The basis for the study of colhametin in preclinical trials was the previously detected high cytotoxic activity of K-2 on 60 human tumor lines in vitro at the NCI (National Cancer Institute, USA) [6] and the confirmed activity of the drug on 3 tumor strains in vivo, which was higher by 70% [11] and involves extensive studies of the drug on other strains of tumors.

The aim of this study was to study the acute toxicity of the new drug colhametin (K-2) in mice and rats and to study its antitumor effect on 2 tumors in mice and rats.

Material and research methods. The object of the study was the drug K-2, synthesized from colhamine, developed in the laboratory at the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCOiR). All experiments were performed in accordance with the recommendations and requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Experimental Animals (Strasbourg, 1986).

Determination of acute toxicity of the drug K-2 with a single intraperitoneal injection was carried out according to the method of Litchfield and Wilcoxon on 30 white outbred mice weighing 20±2g of both sexes and 30 male and female rats weighing 140±10g, 5 animals in each group. In this experiment, 30 mice and 30 rats were used.

All studies were carried out on healthy mature animals (mice) quarantined for at least 10-14 days. The test preparation was administered intraperitoneally in a

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single dose at doses ranging from 250 to 1400 mg/kg. The animals were observed hourly during the first day of the experiment in the laboratory, while survival during the experiment, general condition, possible convulsions and death were used as indicators of the functional state of the animals. Then, daily, for 2 weeks in a vivarium, in animals of all groups, observations were made of the general condition and activity, behavioral patterns, frequency and depth of respiratory movements, the condition of the hair and skin, the position of the tail, the amount and consistency of fecal masses, the frequency urination, changes in body weight, and other indicators. All experimental animals were kept under the same conditions and on a general diet with free access to water and food. At the end of the experiment, the average lethal dose (LD50) was calculated and the toxicity class was determined [12, 13].

The study of the antitumor activity of the drug was carried out on transplanted mouse strains of sarcoma-180 (outbred mice) and Walker's sarcoma (outbred rats), obtained from the tumor bank of the Russian Cancer Research Center of the Ministry of Health of Russia and passaged on mice and rat's donors, according to the protocol of the strain [12].

Treatment was started 4 days after inoculation. The drug was administered at a dose of MTD10 (maximum tolerated dose) and TD10 (therapeutic dose) 10- and 5-fold. When evaluating antitumor activity, the percentage of tumor growth inhibition was determined according to the formulas [12]; body weight loss was also determined, for an indirect assessment of possible hematotoxicity in dead and killed mice - spleen weight and hematological parameters. The experiment was terminated no earlier than 7 days after the last administration of the drug to the animals. Groups of animals with saline injections served as controls. Observation period: from the beginning of the experiment, i.e. inoculation of the tumor, before the slaughter of animals - 21 days.

Statistical processing was performed using the Statistica program, version 6.0. The level of statistical significance was taken as $p \leq 0.05$.

Results

The general effect and "acute" toxicity of the substance of the preparation K-2 was determined on mice and rats with a single intraperitoneal injection. Each dose of the substance was tested on 6 animals. Observation was carried out for 14 days. The Litchfield and Wilcoxon method was used to determine the "acute" toxicity parameters. When studying the general effect of acute toxicity in mice, the drug was administered intraperitoneally at doses of 600, 800, 1000, 1200 and 1400 mg/kg as a 4% solution.

The conducted studies have shown that with intraperitoneal administration of the K-2 preparation at doses of 600 and 800 mg/kg for 0.5-1.0 hours, we observed an increase in breathing, immobility and clustering. Increasing the dose to 1000-1400 mg/kg caused abdominal retraction within 10-15 minutes.

As can be seen from the data in Table 1, the death of mice occurred within a day at a dose of 800 mg/kg 2 out of 6, at a dose of 1000 mg/kg 3 out of 6, at a dose of 1200 mg/kg 4 out of 6 and at a dose of 1400 mg/kg 6 out of 6.

The mean lethal dose LD50 with a single intraperitoneal dose is 890 (-150+172) mg/kg, LD10= 631(-164+221) mg/kg, LD16=750 (-161+203) mg/kg and LD84=1020 (- 260+350).

When studying acute toxicity in rats, the drug was administered intraperitoneally at doses of 250, 300,

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400, 500 and 600 mg/kg as a 5% solution. The conducted studies have shown that with intraperitoneal administration of the K-2 preparation at doses of 250 and 300 mg/kg for 0.5-1.0 hours, an increase in respiration, immobility and clustering was observed. Increasing the dose to 400-600 mg/kg caused abdominal retraction within 10-15 minutes, possibly related to the pH of the drug.

Table 1

The results of indicators of "acute" toxicity with intraperitoneal and oral administration of the substance of the drug K-2

Animal species, route of administration	Animal gender	Doses mg/kg	Number of animals in the group / number of dead	LD_{10} -m+m mg/kg	LD_{16} -m+m mg/kg	LD_{50} -m+m mg/kg	LD_{84} -m+m mg/kg
Mice, Intraperitoneally	Males	600	6/0	631 (-164 +221)	750 (-161 +203)	890 (-150 +172)	1020 (-260 +350)
		800	6/2				
		1000	6/3				
		1200	6/4				
		1400	6/6				
Rats, Intraperitoneally		250	6/0	316 (-60 +72)	350 (-48 +53)	410 (-56 +61)	470 (-96 +123)
		300	6/2				
		400	6/3				
		500	6/4				
		600	6/6				

As can be seen from the data in table-1, the death of rats occurred within a day at a dose of 300 mg/kg 2 out of 6, at a dose of 400 mg/kg 3 out of 6, at a dose of 500 mg/kg 4 out of 6 and at a dose of 600 mg/kg 6 out of 6.

The mean lethal dose ld50 with a single intraperitoneal dose is 410 (-56+61) mg/kg, LD10=316 (-60+72) mg/kg, LD16=350 (-48+53) mg/kg and LD84=470 (- 96+123) mg/kg.

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Thus, the study of the acute toxicity of the substance of the drug K-2 showed that this drug belongs to the iv class of low-toxic compounds [13].

Table 2

Toxic doses of colchamethine in comparison with colchamine (mg/kg)

	Mice					Rats				
Substance	LD_{16}	LD_{50}	LD_{84}	MTD_1 (single entry)	MTD_{10} (10 fold)	LD_{16}	LD_{50}	LD_{84}	MTD_1 (single entry)	MTD_{10} (10 fold)
Kolhametin	750	890	1020	631	**100-120**	350	410	470	316	**45-50**
Kolhamin	38	56,0	73	30,0	2,0		30,0		20,0	1,4

The LD50 of colchamine, a drug used in oncology, is 15.9 times higher in mice and 13.7 times higher in rats than its counterpart, colhametin. At the same time, the maximum tolerated doses for 10-fold use (MTD10) in colhametin for mice are 50-60 times lower than in colhametin, in rats - 32-36 times, which indicates a significant decrease in the toxicity of the drug colhametin in comparison with colhamine, and allows you to use large doses of the drug, which, of course, will have a more pronounced effect.

When studying antitumor activity on the S-180 sarcoma strain, the drugs were administered 10 times, K-2 caused TGI (tumor growth inhibition) 99/90% (by volume and weight) (table 3), the drug contributed to an increase in animal body weight by 10% and spleen by 37% in comparison with the control. However, when exposed to the drug, there was a decrease in the number of leukocytes by 45%, erythrocytes by 33%. Colhamine caused a slight activity in 16/18%, while body weight was reduced by 12%, spleens were smaller by 14%, but leukocytes (by 74%) and erythrocytes (by 39%) decreased more significantly than with the use of K-2.

Table 3

Antitumor activity of the drug K-2 in comparison with colchamine on the sarcoma strain S-180

(treatment 4-13 days after tumor inoculation)

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Drugs and disposable doses (mg/kg)	The mass of animals in the experiment, g		Weight tumors, g	Tumor volume, (A x B x C) cm	Weight spleen, g	% TGI	Leuko-cytes, $\times 10^9$/l	Erythro-cytes, $\times 10^{12}$/l
	up to	after						
1. Control	19,0± 3,14	16,9± 3,19	1,460±0,041	0,919± 0,24	0,28± 0,03		7.90± 0.50	1,82± 0.25
2. K-2 (120)	14,80± 2,22	16,44± 2,1	0,150±0,05*	0,009± 0,004	0,38± 0,12	99/90	4.31± 0.25	1,21± 0.23
3. Kolhamin (2)	18,8±1,44	16,48± 2,0	1,197 ±0,081*	0,772± 0,041*	0,24± 0,07	16/18	2.00± 0.25	1,11± 0.11

Note: in treatment groups n=6, in control n=10; *differences are statistically significant in comparison with the control at P≤0.05.

On the tumor of rats with Walker carcinosarcoma (WSC) the treatment of which was started 4 days after inoculation, the effect of K-2 at MPD at a dose of 45 mg/kg with a 10-fold application reached 90/91%, at a dose of 40 mg/kg kg K-2 was effective at 83/85% in terms of volume and mass of tumors, Table 4.

Table 4

Antitumor activity of the drug K-2 on a strain of Walker's carcinosarcoma (WCS) (treatment from day 4 of tumor inoculation: 10 injections of the substance)

Animals groups	Number of animals	Weight of animals, (g)		Tumor volume (cm^3)	Weight tumors (g)	Weight spleen (g)	% Inhibition V/m
		Before experie-nce	After experience				
Control	6/1	81,8±6,56	80,0±5,77	15,1±3,6	14,1±3,30	0,5±0,058	-

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K-2 45 mg/kg	4	81,3±10,08	75,0±5	1,5±0,45*	1,3±0,4*	0,4 ±0,04	90/91
K-2 40 mg/kg	4	86,8±5,68	88,3±8,25	2,5±0,33*	2,1±0,19*	0,5±0,04	83/85
K-2 45 mg/kg 5-fold injection	4	82,5+3,2	92,5± 2,5	1,6±0,03*	1,4±0,09*	0,5±0,04	89/90
Kolhamin 1.4 mg/kg	4	87,5±5,95	80,0±6,9	7,8±1,4*	7,5±1,7*	0,4 ±0,02	48/47

Note: * differences are statistically significant in comparison with the control at P≤0.05.

With a 5-fold administration, the activity of the drug at a dose of 45 mg/kg was 89/90%. The effect of K-2 was compared with the activity of colchamine at a dose of 1.4 mg/kg, the activity of which was 48/47%. In three groups, a slight decrease in body weight and spleen was observed (except for K-2 at a dose of 40 mg/kg and K-2 at a dose of 45 mg/kg with a 5-fold injection).

Thus, this experience has shown that K-2 is effective on WCS tumors both at 10-fold and 5-fold injections.

CONCLUSION

The study toxicity acute of the preparation K-2 substance showed that this preparation belongs to the IV class of low-toxic compounds. LD50 K-2 when administered intraperitoneally in mice was 890 (-150+172) mg/kg, in rats - 410 (-56+61) mg/kg, which is 13.7-15.9 times higher than that of colhamine.

As can be seen from the data obtained, colhametin is not a highly toxic substance, in comparison with the original colhamine, as well as with other known chemotherapeutic drugs (etoposide, doxorubicin, taxol, etc.), which were compared [2,5,7-9]. Animals can tolerate much higher doses of K-2, which increases the possibility of its direct effect on tumors. The natural alkaloid colchamine, due to its high toxic effect on the entire body, even in small doses, cannot achieve a high antitumor effect. The effect of the new drug K-2 on 2 tumors with a 10-fold injection was high, but hematopoietic parameters were reduced.

With a 5-fold application, the effect of the drug was close to 10-fold, but without its side effects, such as a decrease in body weight and spleen. The obtained data on the toxicity and antitumor activity of K-2 in comparison with the original colchamine indicates the receipt of a substance based on colchamine both with a reduced toxicity by 14-15 times, and with a more significant activity, which is more than 40% higher for WCS and more than 70% on sarcoma 180.

The high antitumor activity of the K-2 drug is confirmed by a more intense effect of the new drug on mitotic activity and the ability to suppress the synthesis of NA and the activity of topoisomerases, as well as to suppress the expression of the MDR2 drug resistance gene. Stimulation of CFUs, which ensures the

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formation of hematopoietic and immune cells, can to a certain extent protect the body from its intense cytotoxic effect [6].

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