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Research Article

STUDY OF ACUTE TOXICITY AND ANTITUMOR ACTIVITY OF NEW COLCHAMETIN PREPARATION COLCHAMETIN

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ABSTRACT

The aim of the study was to study acute toxicity in different methods of administration in mice and rats of a new antitumor preparation "colchametin" (K-2) and its antitumor activity on 2 strains of tumors

Material and research methods. Acute toxicity of the preparation K-2 with a single intraperitoneal administration was carried out according to the Litchfield and Wilcoxon method in 60 white infertile mice weighing 20□2g. both sexes and 60 male and female rats weighing 140□10g 5 animals in each group, a total of 120 mice and rats were used.

The study of antithumor activity was performed on 12 non-fertile and 16 linear mice with transfused tumors S-180 and ACATON, which was injected with preparations on the 4th day after tumour was administered on day 4 after tumour transfusion 10 times. Evaluation of the results was carried out according to standard criteria: inhibition of tumor growth (SRW), body weight and spleen of animals. Differences at $p < 0.05$ were considered reliable.

Results. Such data at single vnutribryushinny introduction are obtained to mice: the average lethal dose of LD50 makes 890 (-150+172) mg/kg, At oral introduction of medicine K-2 to mice of LD50 is equal to 3250 (-630+650) mg/kg, LD50 at single vnutribryushinny to rats of medicine K-2 LD50 makes 410 (-56+61) mg/kg, LD50 at single oral introduction to rats makes 3250 (-630+650) mg/kg, are defined also at all ways of introduction of LD10, LD16 and LD84 value

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The antitumor activity of the drug K-2 on the tumor strain of Sarcoma 180 was high - about 99/90%. On the ACATON tumor, the K-2 effect during intraperitoneal administration was lower and reached 76/75%, however, its effect on both tumors was accompanied by a decrease in hematopoietic indicators.

Conclusions. The study of the acute toxicity of the substance of the preparation K-2 showed that this preparation belongs to class IV of low-toxic compounds. On 2 tumors, the effect of the new drug was high, but hematopoietic indicators were reduced.

KEYWORDS

New antitumor drug colchametin, acute toxicity, mice, rats, activity, sarcoma tumors 180, ACATON.

INTRODUCTION

Currently, antitumor drugs are largely created from natural compounds and there are dozens of such effective drugs known in oncology [4]. However, despite their high activity, a large number of known cytostatics have a large number of side effects, usually due to their high toxicity. Based on colchicine and colchamine, we created new alkaloids that are less toxic and more active than the original ones, as well as other mitotic inhibitors and anticancer drugs [1]. 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 [1], and the amino acid methionine [6], which gave a sharp 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 (US National Cancer Institute) [1] and the confirmed activity of the drug on 3 tumor strains in vivo, which was higher than 70% [2] and suggests extensive studies of the drug on other tumor strains.

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 of the Republican Cancer Research Center of the Ministry of Health of the Republic of Uzbekistan. 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, a total of 60 mice and 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 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

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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 [3,5].

The study of the antitumor activity of the drug was carried out on transplanted mouse strains of sarcoma S 180 (outbred mice) and KSU (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 rats donors, according to the protocol of the strain [3].

Treatment was started 4 days after inoculation. The drug was administered at a dose of MPD10 and TD10 10- and 5-fold. When evaluating antitumor activity, the percentage of tumor growth inhibition was determined according to the formulas [3], body weight loss was also determined, for an indirect assessment of possible hematotoxicity in dead and killed mice - spleen weight, 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 $p<0.05$.

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. kg 6 out of 6.

The mean lethal dose LD50 for 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 250,300,400, 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

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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 drug K-2

Animal species route of administration	Gender	Dose mg/kg	Number of animals in the group / number of dead	LD10 -m+m mg/kg	LD16 -m+m mg/kg	LD50 -m+m mg/kg	LD84 -m+m mg/kg
Mice intraperitoneal tire	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 intraperitoneal tire		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. kg 6 out of 6.

The mean lethal dose LD50 for 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.

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 [5].

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Table 2

Toxic doses of colhametin versus colhamine (mg/kg)

	Mice					Rats				
Substance	ЛД16	ЛД50	ЛД84	МПД1 (одно-крат)	МПД10 (10-кратн)	ЛД16	ЛД50	ЛД84	МПД1 (одно-кратн)	МПД10 (10-кратн)
colhametin	750	890	1020	631	**100-120**	350	410	470	316	**45-50**
colhamine	38	56,0	73	30,0	2,0		30,0		20,0	1,4

The LD50 of colhamine, 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 (MPD10) 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 colhametin, 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 TPO (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%. Colhamin 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 colhamine on the strain of sarcoma S 180

(treatment 4-13 days after tumor inoculation)

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Drugs and disposable doses (mg/kg)	Weight animal-up to experience, G	Mass of animals after experience, G	Weight tumors, G	tumor volume, (a x b x c)cm	Weight spleen, g	% TPO	Leukocytes, $\times 10^9$/l	red blood cells, $\times 10^{12}$/l
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. colhamine (2)	18,8±1,44	16,48± 2,0	1,197 ±0,081*	0,772± 0,041*	0,24± 0,07	6/18	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) (Table 4), 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

Antitumor activity of the drug K-2 on a strain of Walker's carcinosarcoma

(treatment from day 4 of tumor inoculation: 10 injections of the substance)

Groups animals	Qty animal	Weight of animals (g		Volume tumors (cm3)	Weight tumors (G)	Weight spleen (G)	% brake-nia V/m
		before experience	After experience				
1. 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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2. 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
3. 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
4. K-2 45 mg/kg 5-times	4	82,5+3,2	92,5± 2,5	1,6±0,03*	1,4±0,09*	0,5±0,04	89/90
5.Colhamine 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 3 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 5-fold administration)

Thus, this experience has shown that the drug is effective on KSU tumors both at 10-fold and at a smaller - 5-fold number of injections.

CONCLUSION

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, LD50 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, from which it was obtained. 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 that were compared [7,8] . Animals can tolerate much higher doses of this compound, which enhances 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 KSU 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 formation of hematopoietic and immune cells, to a

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certain extent can protect the body from its intense cytotoxic effect [1] .

REFERENCES

1. Enikeeva Z.M., Ibragimov A.A. A new class of cytostatics with stimulation of colony-forming units on the spleen (CFUs). Tashkent, 2016, from "Fan va texnologiya", 173p.
2. Enikeeva Z.M., Fuzailova T.M., Ibragimov Sh.N., Umarov M., Kholturaeva N.R., Karpysheva I.V. "Study of the antitumor activity of the drug K-2 in the early periods after inoculation of mouse tumors (communication 1)" Pharm. magazine, 2017, No. 3.p.103-107
3. Guidelines for preclinical study of the general toxic effect of drugs. / Guidelines for conducting preclinical studies of drugs. Part one. P.13-23 //Ed. Mironova A.N. - M.-2012.-p.944.
4. Guidelines for chemotherapy of tumor diseases. Ed. N.I. Translator // Ed. "Practical Medicine". Moscow 2013.
5. Cidorov K.K. Toxicology of new industrial chemicals. - M .: Medicine, 1973, issue 3, -47s
6. Enikeeva Z.M. Sulfur-containing Aminoacid Derivatives of Colchicine and Colchamine and Derivatives of Izothiouronium and Mercaptoethilamine. Chemistry of Natural Compounds, 1998, V.35, No. 5, p. 556-563.