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Study of Toxicology of the Antitumor Drug Colchiprit-Neo (K-20)

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ABSTRACT

Objective: *Colchiprit-neo was administered orally to rats at dosages of 30.0, 61.0, and 120 mg/kg for 30 days in order to assess its chronic toxicity.* ***Methods:*** *The effects on behavior, body weight, peripheral blood composition, kidney function, liver enzyme levels, and internal organ pathology were evaluated after rats were given Colchiprit-neo at the recommended dosages. To assess the reversibility of any possible harmful effects, a one-month recovery time was incorporated.* ***Results:*** *According to the study, rats' behavior, body weight, and blood composition were unaffected by repeated oral treatment of Colchiprit-neo. During therapy, the liver's alanine aminotransferase (ALT) level rose, but during the recovery phase, all tested values went back to normal. The 30 mg/kg dose did not cause any toxicity in the parenchymal organs, according to pathomorphological analysis.* ***Novelty:*** *The findings indicate that Colchiprit-neo is safe for long-term usage because it does not show substantial chronic toxicity at therapeutic levels. This work offers significant preclinical evidence in favor of its possible therapeutic use without endangering the major organ systems.*

INTRODUCTION

Currently, dozens of effective antitumor drugs created from natural compounds are known [1]. We also changed the structure of colchicine and colchamine in order to obtain less toxic and more active compounds on their basis [2]. The new drug Colchiprit-neo is obtained on the basis of colchicine, which, when administered intraperitoneally, has a toxicity of 54.6 mg/kg, and was developed for the treatment of the bladder. However, when administered orally, its toxicity is reduced by 35.7 times, which allows the use of higher doses. We have shown on a number of animal tumors that the antitumor activity of the drug Colchiprit-neo when administered orally higher than at intraperitoneal, and it is intended for the treatment of tumors in oral form.

The aim of the chronic toxicity studies of the drug Colchiprit-neo is to establish the nature and severity of its damaging effect on the body of experimental animals.

RESEARCH METHOD

The general toxic effect of the drug Kolkhiprit-neo was studied on 4 groups of rats (6 animals in each) of both sexes. The first group of rats served as a control, which were administered distilled water in the volume of the drug and in the same way as the experimental rats, the 2nd, 3rd, 4th groups were experimental rats, to which the drug was administered daily, intragastrically through a tube in doses of 30 (1/40 of LD_{50}), 61, (1/20) and 122 (1/10) of LD_{50} mg/kg, respectively, for 30 days.

All animals were kept in standard vivarium conditions and were on a complete food and water ration.

To identify the effect of the drug on the functional state of organs, generally accepted research methods were used: daily observation of the condition and behavior of animals, determination of integral indicators, morphological composition of peripheral blood (hemoglobin level, g / l; number of erythrocytes, $x10^{12}$; percentage of reticulocytes, 1%, number of platelets, $x10^{9}$; leukocytes $x10^{9}$ and leukogram). The number of platelets and reticulocytes was counted using Peters' diluting solution.

Renal function was assessed by diuresis over 4 hours with a water load (5% of body weight), urine acidity (pH), creatinine, the presence of sugar and proteins (qualitative reactions), and microscopy of urinary sediment.

The functional state of the liver was determined using test kits produced by the company Pharmacodiagnostika, Tashkent. The content of glucose, total protein, alanine, aminotrispherase (ALAT, AST) in serum was determined.

Blood for the study of the morphological composition of peripheral blood was taken from rats by cutting off the tip of the tail, and for biochemical analyses, blood was taken during decapitation of rats with preliminary application of anesthesia (sodium ethaminal) at 10, 20 and 30 days, as well as 1 month after the end of the course of drug administration.

The obtained results are presented in the corresponding tables. t- criterion.

At the end of the course of drug administration and after 1 month of the recovery period , internal organs were examined microscopically, the mass of the heart, liver, kidneys, spleen, thymus, and sex glands was determined. Pieces of organs were placed in a 12% formalin solution for histological examination.

RESULTS AND DISCUSSION

LD_{50} of the drug for rats when administered orally was 1217 (1067.5-1917.3) mg/kg. LD_{16} = 1338.7 mg/kg, LD_{84} = 1825.5 mg/kg, therapeutic dose = 30 mg/kg.

A chronic toxicity study conducted on rats showed that in the first 10 days of daily intragastric administration of the drug at doses of 30, 61, and 122 mg/kg did not cause significant differences from the control group of animals. The animals were neat, although their fur became dull, there was a decrease in activity and weight loss depending on the dose used. After 20 days of daily administration of the drug in the studied doses, an even greater difference in the appearance of the animals of the experimental groups was revealed: the fur was tousled, dull, with a grayish tint, activity was reduced, the animals lost weight compared to the control. After 30 days, these phenomena became even more noticeable: the animals "hunched over", the fur was tousled, dull, the animals became even less active. Weight loss in animals was observed at all observation periods, while in the control there was an increase in weight, there were no deaths of animals in the experimental groups of animals at all observation periods.

After 1 month of the recovery period, the experimental animals were practically no different from the control group in appearance, behavior, food consumption and water drinking. However, it should be noted that in the control group, the rats gained 20% in weight compared to the initial weight, while in the experimental groups it only reached the initial values.

Analysis of the composition of the peripheral blood of rats, carried out every 10 days of the first month showed that in the first 10 days the level of hemoglobin, the number of erythrocytes decreased insignificantly in all experimental groups of rats, and an increase in the dose of the drug led to a more pronounced decrease in these indicators compared to the control (Table 1). The percentage of reticulocytes (which can serve as an indicator of the weakening of the regenerative capabilities of the bone marrow) and the number of leukocytes also decreased. Here, a dose dependence is also observed, i.e. an increase in the dose of the drug leads to a more pronounced decrease in these indicators. No pathological changes were observed in the leukocyte formula. The number of thrombocytes remained at the level of control values (Table 1).

After 20 days of daily intragastric administration of Colchiprit, the hemoglobin level significantly ($p > 0.05$) decreased in all experimental groups of rats by an average of 12.5% compared to the control. The number of erythrocytes decreased by an average of 31.5% ($p > 0.01$). The percentage of reticulocytes decreased by 20.8% (1 on average), and the number of leukocytes by 22.8% ($p > 0.05$) (Table 1).

Table 1. Changes in the composition of peripheral blood of rats under the influence of the drug K-20 with its daily intragastric administration for 10 and 20 days (M+ m ; n=6, p*<0.01)

Tests	In 10 days				Through 20 days			
	Groups				Groups			
	1gr. counter diss.	II gr 0.83 mg/kg	Shgr 1.7 mg/kg	IV gr 3.4 mg/kg	I group of counter diss.	II gr 0.83 mg/kg	Shgr 1.7 mg/kg	IV gr 3.4 mg/kg
Hemoglobin, g\l	147.0 +5.5	135.0+5.0	133.0+5.0	130.0+5.0	145.0+6.0	127.0+3.0*	125.0+2.5*	123.0+2.0*
Erythrocytes, $x10^{12}$ /l	6.0 ± 0.6	5.7. ± 0.6	5.3 ± 0.5	5.1 ± 0.5	5.8 ± 0.6	4.3 ± 0.4	4.0 ± 0.4	4.0+0.4
Reticulitis, %	5.4±0.5	5.1+0.5	4.7+0.5	4.5+0.5	5.3+0.4	4.2+0.5	4.1+0.4	4.1+0.4
Platelets, x 10\9\l	645.0+21.0	635.0+23.0	600.0+17.0	605.0+13.0	700.0+15.0	608.0+13.0	615.0+12.5	610.0+20.0
Leukocytes, x 10\9\l	12.5+0.9	11.1+0.8	10.7+0.9	10.0+0.9	12.3+1.1	9.8+0.2*	9.5+0.3	9.3+0.5
NEUTROPHILS:								
Band cells, %	3.0+0.3	4.0+0.4	3.7+0.4	3.5+0.4	2.5+0.2	2.7+0.3	2.8+0.3	2.7+0.3
Segmented, %	23.0+3.0	24+2,5	25.0+2.5	22.0+3.0	25.0+2.5	26.0+3.0	25.0+2.5	24.0+2.5
Eosinophils,%	3.0+0.3	2.5+0.2	2.3+0.3	3.0+0.3	3.0+0.3	4.0+0.4	3.3+0.4	3.5+0.4
Monocytes, %	2.0+0.2	2.0+0.2	2.0+0.2	2.0+0.2	2.0+0.2	2.5+0.2	2.0+0.2	2.3+0.3

Lymphocytes, %	70.0+3.5	67.5+3.0	66.6+3.5	69.5+6.0	67.5+3.5	64.8+3.2	66.9+3.1	67.5+3.5

P <0.01 compared to control.

Daily use of the drug Colchiprit for 30 days resulted in an even more pronounced decrease in the hemoglobin level by 17.1%, the number of erythrocytes by 33.4%, the percentage of reticulocytes by 37.1% (p > 0.01) in relation to the control. The number of leukocytes decreased on average by 22.4% compared to the control (Table 2).

Table 2. Changes in the composition of peripheral blood of rats under the influence of the drug K-20 for 30 days and after 1 month recovery period (M+ m ; n=6, p*< 0.05)

Tests	In 30 days				After 1 month of recovery period			
	Groups				Groups			
	1gr. counter diss.	**II gr 0.83 mg/kg**	**Shgr 1.7 mg/kg**	**IV gr 3.4 mg/kg**	**I group of counter diss.**	**II gr 0.83 mg/kg**	**Shgr 1.7 mg/kg**	**IV gr 3.4 mg/kg**
Hemoglobin, g\l	146.0+4.5	125.0+3.5*	120.0+3.5*	119.0+3.0 *	147.0+5.5*	141.0+4.5 *	140.0+4.5*	139.0+4.0*
Erythrocytes, x10 ^{12} /l	6.2+0.6	4.5+0.5*	4.0+0.4*	3.9+0.4 *	6.5+0.6	5.4+0.6	4.9+0.5 *	4.7+0.5 *
Reticulitis, %	5.6+0.5	4.0+0.4 *	3.4+0.4 *	3.2+0.4 *	5.8+0.6	4.8+0.5	4.3+0.4	4.1+0.4
Platelets, x 10\9\l	650.0+20.0	615.0+17.0	655.0+21.0	675.0+25.0	675.0+25.0	700.0+15.0	625.0+20.0	605.0+25.0
Leukocytes, x 10\9\l	12.5+0.5	9.7+0.3 *	9.5+0.5 *	9.2+0.8 *	12.7+1.3	10.8+1.2	10.0+0.1	10.2+0.8
NEUTROPHILS:								
Band cells, %	2.0+0.2	3.0+0.3	2.5+0.3	2.3+0.2	3.0+0.3	2.5+0.3	2.7+0.3	3.0+0.3
Segmented, %	25.0+0.3	23.0+2.0	22.0+24.0	21.0+0.2	26.0+0.3	25.0+0.5	23.0+0.3	25.0+0.3
Eosinophils,%	4.0+0.4	3.0+0.3	3.5+0.4	3.7+0.4	3.0+0.3	3.5+0.4	3.7+0.4	4.0+0.4
Monocytes, %	2.0+0.2	2.0+0.2	2.0+0.2	2.0+0.2	2.0+0.2	2.5+0.2	2.0+0.2	2.0+0.2
Lymphocytes, %	67.0+3.0	69.0+1.0	70.0+3.0	71.0+1.5	66.0+4.0	67.5+2.5	68.6+2.4	66.0+4.0

P<0 , 0 5 in relation to control

After 1 month of the recovery period, the hemoglobin level, the number of erythrocytes in the experimental groups increased, approached the control, but still did not reach the data of the control group of rats. Thus, the hemoglobin level in the experimental groups remained reduced by 4.1%, the number of erythrocytes by 11.5%, the percentage of reticulocytes by 20.3%, the number of leukocytes by 20% (p > 1.05)

Thus, the obtained results indicate that the drug Colchiprit, applied daily intragastrically in doses of 30, 61 and 122 mg/kg, has a toxic effect on the peripheral blood of rats. Its more pronounced toxic effect is manifested on days 20-30 in doses of 61 and 122 mg/kg.

Discontinuation of the drug during the recovery period did not lead to complete restoration of the studied parameters (hemoglobin, erythrocytes, leukocytes, reticulocytes), although their values approached the control parameters.

Liver function in rat experiments was studied using the most sensitive tests 10, 20 and 30 days after the start of drug administration and a 1-month recovery period. The protein level in all experimental and control groups did not change during the entire study period. The exception was group 3, where the total protein increased from 78 6.0 to 96 10.0 g/l or by 23% after 10 administrations. At all other times, the total protein in this group did not differ from the control group (Table 3). As can be seen from Table 4, AST in the experimental groups did not differ from the control during the entire study period).

Table 3. The effect of the drug Colchiprit on some indices of liver function in rats (M ± m ; n =5;)

Doses mg/kg	Research time, days							
	In 10 days				In 20 days			
	Total protein, g/l	ALT mol/l	AST mol/l	Glucose mol/l	Total protein, g/l	ALT mol/l	AST mol/l	Glucose mol/l
1-contact	78 ± 6.0	0.3 ± 0.02	0.2 ± 0.02	4.7 ± 0.3	78 ± 6.0	0.3 ± 0.03	0.2 ± 0.03	4.7 ± 0.3
II-30	80 ± 6.6	0.3 ± 0.03	0.2 ± 0.02	4.4 ± 0.3	80 ± 7.0	0.4 ± 0.04	0.2 ± 0.02	4.7 ± 0.3
III-61	96 ± 10	0.2 ± 0.02	0.2 ± 0.02	4.4 ± 0.3	84 ± 7.0	0.3 ± 0.02	0.2 ± 0.02	4.7 ± 0.3
IY-122	80 ± 6.0	0.3 ± 0.02	0.2 ± 0.02	4.4 ± 0.3	78 ± 6.0	0.3 ± 0.02	0.2 ± 0.02	4.7 ± 0.3
After 30 days After 1 month of recovery period								
1-contact	76 ± 6.0	0.6 ± 0.05	0.4 ± 0.03	5.8 ± 0.5	81 ± 7.0	0.7 ± 0.05	0.45 ± 0.04	5.4 ± 0.4
II-30	72 ± 5.6	0.5 ± 0.04	0.35 ± 0.03	5.9 ± 0.6	80 ± 7.0	0.6 ± 0.04	0.4 ± 0.04	6 , 0 ± 0, 8
III-61	76 ± 6.0	0.3 ± 0.03	0.2 ± 0.02	5.9 ± 0.6	80 ± 7.0	0.5 ± 0.04	0.38 ± 0.04	6 , 8 ± 0, 6
IY-122	82 ± 7.0	0.4 ± 0.03	0.27 ± 0.03	6.4 ± 0.6	78 ± 5.6	0.4 ± 0.05	0.27 ± 0.03	5 ,7 ± 0, 4

P *< 0.05 relative to control.

A study of the glucose content in the blood serum showed that after 10 administrations of Colchiprit in all the studied doses, this indicator did not change significantly (Table 3). After 20 days, a dose-dependent increase in the glucose content in the blood serum was observed in groups 2; 3 and 4, respectively, from 4.6 ± 0.4 to 5.0 ± 0.4; 6.5 ± 0.5 and 7.7 ± 0.6 mmol/l by 9.41 and 67%.

After 30 days, the glucose content in the blood serum of both the experimental and control groups did not change significantly. After 1 month of the recovery period, the glucose content was increased at a dose of 30 mg/kg from 5.4 ± 0.4 to 6.9 ± 0.8 mmol/l or 28%, at a dose of 60 mg/kg – to 6.8 ± 0.6 mol/l or by 26% and at a dose of 122 mg/kg – to 5.7 ± 0.4 mmol/l or 6%.

Thus, repeated administration of the drug Colchiprit affects the liver of experimental rats, which is expressed in an increase in the glucose content in the blood serum, and even after 1 month of the recovery period this effect was preserved, but was expressed less strongly. At the same time, the content of ALT in the blood serum of experimental animals remained within the normal range, and a short-term increase in total protein at a dose of 61 mg / kg after 10 days of administration is most likely associated with the specific activity of the drug.

Data on some indices of the functional state of the kidneys in rats that were administered daily intragastrically, through a tube, the drug Colchiprit in doses of 30, 61 and 122 mg/kg during periods of 10; 20; 30 days and 1 month of the recovery period are presented in Table 4, from which it is evident that diuresis in rats for 4 hours with a water load (5% of body weight) did not change significantly regardless of the dose of the drug and the time of its administration compared to the control group of rats.

Table 4. The effect of the drug Colchiprit on the functional state of the kidneys of rats with its chronic daily use for 30 days and after 1 month of the recovery period (M + m ; n = 6;) ($p < 0.05$)

N	Animal groups, doses	In 10 days		In 20 days		In 30 days		1 mc east period	
		Diuresis in 4 hours, %	Creatine mol/l	Diuresis in 4 hours, %	Creatine mol/l	Diuresis in 4 hours, %	Creatine mol/l	Diuresis in 4 hours, %	Creatine mol/l
1.	1g. Cont. dis.water	90.0 + 10.0	0.225	93.5 + 10.0	0.193	90.0 + 1.0	0.175	94.3% + 5.7	0.228
2.	2g. Colchiprit 30 mg/kg	86.4 + 8.5	0.193	87.0 + 9.0	0.158	85.0 + 9.5	0.145	93.5 + 2.5	0.350
3.	3gr. Colchiprit 61 mg/kg	82.7 + 7.3	0,210	88.0 + 8.0	0.175	83.5 + 6.5	0.085	84.5 + 6.5	0.290
4.	4g. Colchiprit 122 mg/kg	83.7 + 7.3	0.175	81.0 + 10.0	0.175	86.0 + 9.0	0.315	89.5 + 7.0	0.360

The excreted urine was completely transparent, light straw-colored, urine acidity (pH) fluctuated within 7-8. Protein and sugar in the urine (quantitative reactions) were absent at all times of the study. Microscopy of urinary sediment without pathological changes. The experiments conducted in increasing creatinine in the blood serum on the 30th day of the experiment at a dose of 122 mg / kg (group 4 rats in the first 10 and 20 days of administration) the indicator did not significantly differ from the control (1 gr.) After 30 days in group 4, where the drug was used at a dose of 122 mg / kg, the creatinine level increased by 80%, and in gr. 3 it decreased by 45%. During the recovery period, creatinine remained significantly elevated.

Thus, the drug Colchiprit has an effect on renal diuresis during the studied period of time, but exhibits its toxic effect in the long term in all experimental groups, as evidenced by an increase in the creatinine content in the blood serum.

At the end of a month-long experiment on rats, which were daily administered the drug Colchiprit intragastrically through a tube in doses of 30, 61 and 122 mg/kg, and also after a 1-month recovery period, the animals were killed by decapitation and pieces of internal organs (brain, lungs, thymus, heart, liver, spleen, adrenal glands, kidney, stomach, intestines and gonads) were placed in a 12% formalin solution for histological examination.

Macroscopic examination of the rats' internal organs during visual inspection did not reveal any changes in comparison with the control group of rats, except for the color of the liver, which became yellowish-grayish depending on the dose of the drug used. After 1 month of the recovery period, the liver of the experimental rats did not differ in color from the control group.

Examination of the stomach and intestines revealed no changes associated with the administration of the drug compared to the control. The use of the drug Colchiprit did not have a significant effect on the mass of the organs under study either after 30 days of daily use or after 1 month of the recovery period.

As a result of the pathomorphological study of the internal organs of 4 groups of rats (brain, lungs, thymus, heart, liver, spleen, adrenal glands, kidney, stomach, intestines and gonads**).** After using colziprit for 30 days in a chronic experiment, it was shown : - a dose of 30 mg/kg with long-term oral administration is well tolerated by animals of both sexes and does not have a pronounced toxic effect on the functions of vital organs and systems.

The use of 61 and 121 mg/kg of the drug causes a number of negative effects: more toxic effect on the kidneys and liver. The effect on other organs is less toxic, compared to the control (first) group.

CONCLUSION

Fundamental Finding: With a reduction in hemoglobin, erythrocyte, reticulocyte, and leukocyte counts, the study showed that Colchiprit-neo exhibited dose-dependent hemotoxicity when given at therapeutic levels over a 30-day period. However, following a month-long recuperation period, these effects were reversible. The medication also impaired liver function by raising blood glucose, while enzyme levels stayed within normal ranges, and caused minor renal damage, as evidenced by elevated creatinine levels. 30 mg/kg was a well-tolerated dose that had no appreciable negative effects on critical organ systems. **Implication:** With the possibility of oral administration in tablet or capsule form, these results imply that Colchiprit-neo may be reasonably safe for use as a cytostatic drug at therapeutic levels. More research should be done on its reversible toxicity profile before using it in clinical settings, especially for cancer treatments. **Limitation:** Although recovery was seen in the rats used in the study, more research is needed to determine the long-term implications, particularly in people. It's also necessary to investigate the effects of greater dosages and longer exposure times. **Future Research:** In order to evaluate Colchiprit-neo's safety and effectiveness in people, investigate its molecular mechanisms of action, and establish the ideal therapeutic dosages, future research should concentrate on clinical trials. Additional preclinical research analyzing its long-term toxicity in various animal models and determining any possible drug interactions would also be beneficial.

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