

## ARTICLE

# Study of the Antitumor Activity of the Drug Dekoglitz on Two Tumors and Some Aspects of Its Mechanism of Action

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### ABSTRACT

**Aim:** Evaluation of the antitumor activity of the new drug Dekoglitz in animals with tumor strains of Sarcoma 45 in comparison with the drug dekocin, from which it was obtained, as well as with 5-fluorouracil and etoposide, and on ovarian tumors (OT) in comparison with the drug dekocin and identification of the effect of Dekoglitz on NA synthesis and internucleosomal DNA degradation. **Methods:** The study of preparations was carried out on 68 outbred rats with transplanted C-45 and OT tumors. The alkylating effect of the drugs was studied on cells tumor of Sarcoma 180. **Results:** The antitumor activity of dekoglitz on Sarcoma 45 was high, about 98/96%, with a remission rate of 80%. Its effect was 28-24% higher than that of dekocin. On OT, the effect of decoglitz with intraperitoneal administration reached 89/76% with a remission rate of 40%, with oral administration 96/86% with a remission rate of 60%. **Conclusion:** The study of the new drug Dekoglitz on animals with a tumor of Sarcoma 45 revealed its higher activity (by 20-27%) in comparison with the original Dekocin, 5-fluorouracil and etoposide with a lower level of side effects. On OT, the effect of Dekoglitz was 35-40% higher, especially after oral administration. Apparently, the great ability to suppress the synthesis of NA and carry out internucleosomal degradation and fragmentation of tumor DNA by the new drugs dekoglitz explains its antitumor efficacy, which is greater than that of Dekocin (K-18) in experiments on tumors.

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## 1. Introduction

The pronounced general toxic effect of a large number of used cytostatics, rapidly developing resistance, and the lack of sensitivity of a number of tumors to existing drugs dictate the need to create new anticancer drugs.

The use of medicinal preparations based on licorice root has been around for several millennia. The main active ingredient in licorice root is the triterpenoid glycyrrhizic acid (GA). GA as a solubilizer of many water-insoluble organic substances is used to create low-dose, low-toxic drugs<sup>[1]</sup>.

For example, practically insoluble in water, gossypol and its derivatives, hydrocortisone, prednisolone, kracil, nistatine and other drugs in combination with the monoammonium salt of glycyrrhizic acid (MASGA) pass into aqueous solutions<sup>[2,3]</sup>. All these positive properties of GA and its derivatives are associated with its ability to form supramolecular complexes, which in aqueous solutions have very low critical micelle concentration values. All researchers note the very low toxicity of preparations with GA, MASGK and their derivatives, created on their basis. In addition to the above properties, GA and its derivatives exhibit a pronounced anti-inflammatory, analgesic effect, anti-edema, hypotensive, virus-neutralizing effect, improves tissue regeneration both in the early manifestations of a viral disease and in ulcerative forms.

However, GA was not used for combination with anticancer drugs. We are developing new promising substances based on tropolone alkaloids, of which dekocin, a derivative of the alkaloid colchicine, revealed activity in animal studies with 10 tumor strains, which was the highest (above 80%) on Sarcoma S 180, RShM-5 (cervical cancer) and AKATOL<sup>[4]</sup>, which allowed this drug to be proposed for clinical trials. The obtained clinical data of the antitumor drug dekocin indicate a high sensitivity of skin cancer to 3-4% dekocin ointment, which was also effective in combination with radiation<sup>[5,6]</sup>. However, dekocin is insoluble in water, which complicates both its parenteral administration and bioavailability. In this regard, we used the method of molecular encapsulation of the drug dekocin with glycyrrhizic acid (HA), which has effective solubilizing properties. A new water-soluble supramolecular complex of Dekocin and HA was obtained, which differs in physicochemical parameters from the original Dekocin, as well as a 2.6-fold decrease in toxicity, which is named Dekoglitz.

The aim of this work was to study the antitumor activity of a new colchicine derivative Dekoglitz in animals with tumor strains of Sarcoma 45 and ovarian tumor (OT) in comparison with the effect of dekocin, 5-fluorouracil

and etoposide, as well as to study the effect of Decoglitz on DNA/RNA synthesis and internucleosomal tumor degradation in comparison with the effect of dekocin (K-18) and etoposide.

## 2. Methods

### 2.1 Tumoral Strains

Transplantable tumors, murine sarcoma 180, and two strains of rat sarcoma 45 and ovarian tumors (OT) were used in the work. Strain Sarcoma 180 was purchased from the Tumor Strains Collection Bank (Institute of Carcinogenesis, N.N. Blokhin Russian Cancer Research Center, Russian Academy of Medical Sciences) Moscow, Russia. The strains of Sarcoma 45 and ovarian tumors (OT) were purchased from the Tumor Strains Collection Bank (Institute of Oncology of Kazakhstan). The tumor strains were passaged to the strain protocol.

### 2.2 Antitumor Drugs

The following drugs were used in the work: etoposide (Etoposide phosphate, Bristol-Myers Squibb); 5-fluorouracil (Getwell Pharm acuticals, India); the K-18 (Dekocin) and its derivative Dekoglitz (tropolone alkaloids, colchicine derivatives) developed by Prof., Z. M. Enikeeva at the Republican Specialized Scientific Practical Medical Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan (RSNPMTSO&R MH RUz).

### 2.3 Animals

In the experiment white outbred, mice weighing 18-20 g (60 individuals) and rats weighing 90-140 g (68 individuals) were used. The animals were kept on a standard diet under natural lighting conditions and had free access to water and food in the vivarium at the RSNPMTSO&R MH RUz.

At the end of the experiment, all rats and mice were euthanized under ether anesthesia, in accordance with the International Rules for the Protection of Vertebrates. All experiments were performed in accordance with the recommendations and requirements of the "World Society for the Protection of Animals (WSPA)" and "European Convention for the Protection of Experimental" (Strasbourg, 1986).

#### 2.3.1 Investigation Antitumor Activity of Drugs

Tumor subinoculation was carried out according to generally accepted methods: tumors of Sarcoma 45 and OT were inoculated subcutaneously with a suspension of tumor cells, 30-60 mg in 0.3-0.5 ml of nutrient medium per rat

<sup>[7]</sup>. Treatment of animals began 4 days after tumor implantation, drugs were injected in all groups 10 times, and all experimental groups were injected with drugs in a volume of 0.3 ml per 100 g rat. The animals were slaughtered on the 19-21st day after tumor implantation, the animals were sacrificed using humane methods of working with laboratory animals. Before the introduction and at the end of the experiment, the body weight of the animals was determined.

During the experiment, in order to study the dynamics of tumor growth, the volumes of tumors through the skin of animals were measured in the treated and control groups of mice (in 3 projections) at the beginning of the experiment, every 5 days after the start of treatment, and before slaughter. At the end of the experiment, the efficacy in sacrificed mice was determined by the volume (V) of the extracted tumor tissue, as well as by the tumor mass in the compared groups. Tumor growth inhibition was calculated using the formulas<sup>[7]</sup>. The tolerability of the treatment was judged by the death of the mice; for an indirect assessment of the possible hematotoxicity in the sacrificed mice, the spleen weight was determined.

### 2.3.2 Alkylating Action

The effect of drugs on the synthesis of DNA and RNA was studied on Sarcoma 180 tumor cells in vitro. A cell suspension from tumor tissue was obtained according to the method<sup>[8]</sup>. Cells with a titer of 10,000 were cultured in medium (RPMI-1640 containing 5% fetal bovine serum (FBS), 2mM L-glutamine, 10U/ml penicillin and 100mcg / ml streptomycin), with the absence and presence of therapeutic (TD) investigational drugs, for 24 hours at 370C in an atmosphere of 5% CO<sub>2</sub>.

### 2.3.3 Isolation of DNA/RNA

DNA/RNA preparations from Sarcoma 180 cells were isolated by two methods, a) phenol-chloroform method<sup>[9]</sup> and according to the protocol of the kit-kit “DNA-sorb-B” (InterLabService), Russia. The DNA/RNA concentration

was determined by adsorption at a wavelength of 260 nm on an SF-26 spectrophotometer (Russia).

For the analysis of internucleosomal DNA degradation, total DNA/RNA preparations were treated with the RNase A enzyme according by method<sup>[9]</sup>. DNA/RNA electrophoresis was analyzed in 1.5% agarose gel for 4 h, 60V according to the method<sup>[9]</sup>.

Statistical processing was performed using Statistica, version 6.0. The level of statistical significance was taken as p <0.05.

## 3. Results

### 3.1 Study of Antitumor Activity, in vivo

The study of the antitumor activity of the drugs on the Sarcoma 45 strain began 4 days after tumor subinoculation the drugs were injected 10 times. The slaughter was carried out on day 21. In the control group, there was a mortality of 25%, in the experimental groups with the use of dekoglits and dekocin, there was no death of animals, in the group with 5-fluorouracil all animals died after 10-fold administration, in the group with etoposide, 30% of the animals died.

In group 2, the drug Dekoglitz showed high antitumor activity in 98/96%, 80% of regressed tumors were observed, while the drug caused a slight decrease in body weight (by 5%) and an increase in the spleen by 20% (Table 1).

In group 3, the antitumor effect of the drug dekocin was less high - 70/72%, the drug caused a slight decrease in body weight (by 6%) and spleen (by 20%).

In group 4, the drug 5-fluorouracil at a dose of 15 mg/kg caused the death of all animals on day 15 after inoculation and its antitumor effect could be assessed on day 12 when measuring the volume of tumors in 2 animals, which was in relation to the control for this day is 76%, however, due to the death of animals, it was impossible to assess the effect of the drug on body weight and spleen.

**Table 1.** Antitumor activity of the drug Dekoglitz in comparison with Dekocin, 5-FU and Etoposide in rats with tumor Sarcoma 45

(Treatment with drugs was carried out on the 4th day after tumor implantation. The slaughter was carried out on the 21st day)

Groups of animals	Number of animals before and after treatment		The mass of animals (gr)		Tumor volume, (cm <sup>3</sup> )		
	before	after	before	after	5th day	12th day	after
Control	8	6	131.0±9.3	121.3±9.0	0.3±0.04	2.1±0.5	2.7±0.8
Decoglitz 20 mg/kg	6	6	102.0±5.8	97.0±6.0	0.2±0.1	0.2±0.1	0.04±0.01*
Dekocin 15 mg/kg	6	6	132.0±17	124.0±14.4	0.1±0.02	0.5±0.13	0.8±0.07*
5-FU 15 mg/kg	6	0	109.0±4.4	-	0.1±0.05	0.48±0.08	-
Etoposide 8 mg/kg	6	4	114.0±9.0	106.0±8.0	0.2±0.1	0.7±0.16	0.6±0.16

**Note:** in the treatment groups n = 6, in the control n = 8; \* differences are statistically significant in comparison with control at P <0.05.

Table 1. Continued.

Groups of animals	Weight tumors (gr)	Weight Spleen (mg)	% inhibition of tumor growth		
			by volume	by mass	% regression
Control	2.5±1.1	0.5±0.03			
Decoglitiz 20 mg/kg	0.04±0.01	0.6±0.04	98	96	80
Dekocin 15 mg/kg	0.7±0.07*	0.4±0.03	70	72	0
5-FU 15 mg/kg	-	-	77	-	0
Etoposide 8 mg/kg	0.6±0.06*	0.3±0.02	78	76	0

Table 2. Antitumor activity of the drug Dekoglitiz in comparison with Dekocin in rats with ovarian tumor

(Treatment with drugs was carried out on the 4th day after tumor implantation. 10 injections of substances. The slaughter was carried out on the 19th day)

Groups of animals	Weight of animals (gr)		Tumor volume (cm <sup>3</sup> )		Weight tumors (gr)	Weight spleen (mg)	% inhibition of tumor growth		
	before treatment	after treatment	for 8 <sup>th</sup> day	for 10 <sup>th</sup> day			by volume	by mass	% regression
Control	160±10.6	162±10.2	1.7±0.5	2.8±0.5	2.5±0.6	0.9±0.07			
Dekocin, 15 g/kg	97±1.3	118±0.02	0.2±0.02	1.3±0.1*	1.4±0.4*	0.8± 0.1	54	44	
Decoglitiz 20 mg/kg, (intra-peritoneal)	119±4.6	135±7.0	0.7±0.1	0.3± 0.02*	0.6±0.1*	0.8±0.07	89	76	40
Decoglitiz 40 mg/kg, (orally)	110±3.2	139±5.7	0.6±0.1	0.1±0.01*	0.1±0.03*	0.8±0.1	96	86	60

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

In the 5th group, the antitumor effect of the drug etoposide was 78/76%, the drug caused a slight decrease in body weight (by 7%) and a more pronounced decrease in the spleen weight (by 40%).

Thus, the new drug Dekoglitiz showed the highest activity, both in comparison with dekocin, from which it was obtained, and known cytostatics, moreover, its effect was higher than the comparison drugs by 20-28%, and there was no such side effect as the effect on the spleen. It should be noted that Dekoglitiz was studied at a dose that in relation to LD<sub>50</sub> was significantly lower than that of dekocin, i.e. for GA and MASGK derivatives, it was noted<sup>[10]</sup> that their activity manifests itself in doses 2-4 times less than the maximum tolerated.

The study of the antitumor activity of the drugs on the Ovarian Tumor (OT) strain began 4 days after tumor transplantation the drugs were injected 10 times. There was no death of animals during the experiment. The slaughter was carried out on the 19th day.

In group 2, the drug dekocin was 54/44% active, while the drug caused a slight decrease in body spleen weight (by 11%) and an increase in body weight by 21% (Table 2).

In group 3, the antitumor effect of the drug Dekoglitiz

at a dose of 20 mg / kg with intraperitoneal injection was less high - 89/76% than when exposed to Sarcoma 45, but caused tumor regression in 40% of animals. The drug caused a slight decrease in the spleen (by 11%), body weight was 13% more than the initial one.

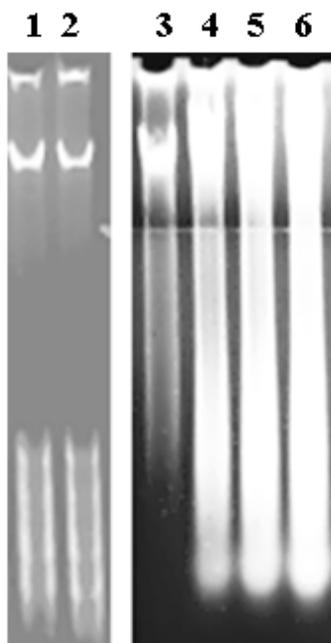
In the 4th group, the drug Dekoglitiz at a dose of 40 mg/kg with oral administration had a higher antitumor effect 96/86%, while it caused tumor regression in 60% of animals, the drug had side effects only in a slight decrease in the spleen (by 11%), body weight was 26% more than the initial one.

### 3.2 Study of the Mechanism of Action, in vitro

The high antitumor activity of the drug Dekoglitiz, as well as its further study as a cytostatic, involves the study of such aspects of its mechanism of action as alkylating, the influence on DNA/RNA synthesis, internucleosomal DNA degradation, and topoisomerase II activity. The effect of K-18 and Decoglitiz on DNA and RNA synthesis was investigated in sarcoma 180 cells in vitro in comparison with etoposide, which is a known inhibitor of topoisomerase I/II.

Figure 1 shows the results of DNA/RNA electrophore-

sis of tumor cells cultured in the absence of preparations (lanes 1,2) and using etoposide, K-18 and Dekoglitz (respectively, lanes 4-6).



**Figure 1.** Influence of preparations on native DNA, nucleosoma degradation of DNA and activity topoisomerase II tumor cells of the Sarcoma 180, in vitro

Lanes 1 and 2 native DNA/ RNA of Sarcoma 180 (not treated with RNase A). Lanes 3-4, aliquots of DNA treated with RNase. Lane 3 control, without the using of cytostatics, Lane 4 Etoposide, Lane 5 K-18 (Dekocin), Lane 6 Dekoglitz. Electrophoresis carries out in to 1.5 % TAE agarose gel, 4h, and 60V and visualized by UV transilluminator after staining with ethidium bromide.

In aliquots of DNA/RNA not treated with the enzyme RNase, the electrophoregram shows a high native of nuclear DNA and RNA molecules (Figure 1, lanes 1, 2). In aliquots of DNA/RNA treated with the enzyme RNase, the electrophoregram shows DNA degradation in the form of a plume (Figure 1, lanes 4-6).

The results of the electrophoresis showed: Etoposide, K-18, and Dekoglitz contributed to internucleosomal DNA degradation by:  $75.7 \pm 3.3$ ,  $86.7 \pm 3.7$ , and  $94.5 \pm 1.7$ , respectively. Also, according to the pattern of DNA fragmentation, electrophoregram, Etoposide, K-18, and Dekoglitz inhibited topoisomerase II activity by  $57.6 \pm 2.7$ ,  $64.6 \pm 2.3$ , and  $79.6 \pm 3.0$ , respectively (Figure 1, Table 3).

Regarding the effect of the three drugs on topoisomerase II (TOPO-II) of Sarcoma 180 tumor cells, the activity of this enzyme was determined visually by the pattern of electrophoresis of fragmented DNA in a gel. Etoposide, K-18, and Dekoglitz inhibited TOPO II activity by  $57.6$

$\pm 2.7$ ,  $64.6 \pm 2.3$ , and  $79.6 \pm 3.0$ , respectively (Figure 1, Table 3).

The results, the effect of the studied drugs on the synthesis of DNA/RNA showed: a) Etoposide, K-18 and Dekoglitz inhibited DNA synthesis by  $64.9 \pm 2.7$ ,  $85.6 \pm 2.3$ ,  $95.7 \pm 3.7$ , respectively; b) Etoposide, K-18, and Dekoglitz inhibited RNA synthesis by  $30.0 \pm 3.0$ ,  $60.5 \pm 1.7$ ,  $65.9 \pm 2.7$ , respectively (Table 3).

**Table 3.** Influence of antineoplastic preparations on synthesis DNA/RNA, TOPO II activity and DNA nucleosoma degradation of cells of the Sarcoma 180 tumor, in vitro

Antitumor preparations (TD)	DNA nucleosoma degradation, in %	Activity TOPO-II in %	Inhibition	
			DNA synthesis in %	RNA synthesis in %
Control	0±0	0±0	0±0	0±0
Etoposide 8 mkg/ml	75.7±3.3	57.6±2.7	64.9±2.7	30.0±3.0
K-18 15 mkg/ml	86.7±3.7	64.6±2.3	85.6±2.3	60.5±1.7
Dekoglitz 20 mkg/ml	94.5±1.7	79.6±3.0	95.7±3.7	65.9±2.7

Thus, the results of this experiment showed a high alkylating activity of Dekoglitz to targets DNA/RNA and its inhibitory effect on topoisomerase II, resulting in DNA fragmentation, and then cell apoptosis.

#### 4. Conclusion

The study of the new drug Dekoglitz on animals with a tumor of Sarcoma 45 revealed a very high activity with 80% tumor regression, which was 20-27% more than the original Dekocin, 5-fluorouracil and etoposide with a lower level of side effects. Dekoglitz also had a high effect on OT tumor when administered intraperitoneal, which was 30-40% higher than the effect of Dekocin (40% of tumors regressed), however, Dekoglitz showed an even higher activity after oral administration, where 60% of tumors regressed.

This Dekoglitz effect is confirmed by a more intense effect on the synthesis of DNA and RNA of tumor cells. Apparently, the great ability to suppress the synthesis of NA and the activity of topoisomerase II and to carry out internucleosomal degradation of tumor DNA by the new drug Dekoglitz explains its antitumor efficacy, which is greater than that of Dekocin (K-18) in experiments on tumors.

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## Conflict of Interest

The authors declare no conflict of interest.

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