

Investigation of Mechanism Activity of Antitumor and Radiosensitizing Activity of Preparations K-26 and K-26w

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Abstract: Introduction. Tropolone alkaloid – colchicine, there is very interesting object for synthesis of its derivatives, with such properties as, alkylation, a low toxicity, high antineoplastic activity and especially overcoming of multidrug resistance (MDR). We had been developed the antineoplastic preparation K-26 derivative of colchicine. K-26 has shown high cytotoxic activity on 60 lines of tumoral cells of the human *in vitro*, at National Institute of the Cancer of the USA (NCI). Further on the basis of K-26 its water-soluble form named term K-26w has been received. The work purpose. Studying of the mechanism of action of preparations K-26 and K-26w on: alkylating ability, mitotic activity, topoisomerase II, MDR2, p53 and colony-forming cells spleen (CFCs). Materials and methods. All researches have been carrying out by a standard technique. Studying mitotic activity of preparations was carrying out on duodenum and tumor CaPa after preparation influence. On models of a tumor of the Sarcoma 180 action of preparations has been investigated: the alkylating - on synthesis DNA/RNA, nucleosoma DNA degradation, activity topoisomerase II; on an expression MDR2 and p53 genes. Studying CFCs carry out by a standard technique on outbred mice. Results. K-26, K-26w and etoposide inhibited in cells of the Sarcoma 180: synthesis DNA/RNA on 84/65%, 95/85% and 55/35%, accordingly, in relation to the control; activity topoisomerase II on 80%, 90% and 60% accordingly. By method RT-PCR it is shown, K-26, K-26w and etoposide: inhibited an expression of MDR2 gene on 83%, 91% and 62%; increase expression p53 gene to 74%, 88% and 55%, accordingly, under the relation of the control of referential gene GARDH (100%). High ability K-26 and K-26w in an induction apoptosis tumoral cells and CFCs to 12 units is shown. Conclusion. Revealed ability K-26 and K-26w to suppress synthesis DNA/RNA activity topoisomerases, to stimulate p53, and also to suppress an expression of a multidrug resistance MDR2 gene, it explains their high antineoplastic activity which is connected with mitotic activity leading to cell fission synchronization, and radiosensitization activity. Special interest represents found at K-26w and K-26 suppression MDR2 as they are aimed for treatment of such resistant tumor as a kidney cancer. Stimulation CFCs which provides formation of haemopoietic and immune cells can protect an organism from their intensive cytotoxic action.

Keywords: K-26, K-26w, Sarcoma 180, Topoisomerase II, MDR2, p53, CFCs

1. Introduction

Under the grant (№ PZ201709069) by us it is developed as

an antineoplastic preparation derivative colchicine K-26 [1], shown high cytotoxic activity *in vitro* at National Institute of the Cancer of the USA (NCI), and, in particular, on such

tumors as a cancer of a kidney and melanoma. On the basis of K-26 water-soluble analogue K-26w has been received, for both preparations their antineoplastic activity *in vivo* on 6-8 transplantable tumors of animals [2-4] has been studied.

Also by us ability of these substances to strengthen irradiation action on intact mice with entered K-26 and K-26w to an irradiation in a dose 1/3 from LD₁₆, is shown, that has caused the big destruction of animals, than in the control, and value FCD (the factor changing doses) equal 0.65-0.7 has been found [5, 6]. It causes them radiosensitization action, thereupon we suppose to study new antineoplastic preparations K-26 and K-26w on animals with tumors as a radio sensitizer with antineoplastic action for parenteral (K-26w) and external application (K-26).

High antineoplastic activity of preparations, and also their further studying as radio sensitizers, assumes studying of such parties of the mechanism of action, as alkylating abilities (influence on synthesis of DNA and RNA, on nucleosoma degradation and DNA fragmentation), influence on activity topoisomerase II and drug resistance.

It has been established, that derivatives trapolone alkaloids promote induction colony-forming cells of spleen (CFCs) [5], thereupon studying of this feature of new preparations represented the big interest.

Purpose of the present research was studying mitotic and alkylating activity, influence on topoisomerase II, on drug resistance and p53, and also on CFCs of new preparations.

2. Materials and Methods

2.1. Tumoral Strains

In work mouse transplantable tumors the Sarcoma 180 and CaPa have been used. Tumoral strains have been got from bank of collection tumoral strains (Institute Carcinogenesis the Russian Oncological Centre of Science of N. N. Blohina of the Russian Academy of Medical Science) Moscow, Russia.

2.2. Antitumor Drugs

Commercial drug Etoposide has been received from companies EBEWE (Switzerland).

Trapolone alkaloids, colchicine derivatives K-26 and K-26w, are developed, Dr., professor Enikeeva Z. M., in the Republican Specialized Scientific - Practical Medical Center of Oncology and Radiology MH RUz (SSPMCOR MH RUz).

2.3. Animals

In experiment white outbred mice with weight in limits 18-20g have been used. Animals contained on a standard diet till 6-12 individuals at a natural mode of illumination and had an easy approach to water and food in vivarium at (SSPMCOR MH RUz). All mice lulled under a radio narcosis, according to the International rules on protection of vertebrate animals. All experiments have been executed in conformity with recommendations and requirements "The World society of protection of animals (WSPA)" and

"The European convention on protection experimental" (Strasbourg, 1986).

2.4. Mitotic Activity

Studying mitotic activity (mitotic an index) was spent at intraperitoneal introduction of preparations to mice in the dose making 1/2 (LD₁₆), in 30, 60 minutes and each hour within days. After decapitation animals, took away 1sm of a duodenal gut for histological research and fixed in mix Bowen. Mitotic index (MI) and mitotic activity (MA) defined a standard histology method [7].

2.5. Alkylating Action

Influence of preparations on synthesis of DNA and RNA has been studied on cells of a tumor the Sarcoma 180. Approximately cells on 10.000 cultivated, in the medium (RPMI-1640 supplemented with 5% fetal bovine serum, 2mM L-glutamine, 100 IU/mL penicillin, and streptomycin 100mg/mL), without and with therapeutic doses (TD) of drugs for 24 hrs. at 37°C in an atmosphere of 5% CO₂.

2.6. Dna/Rna Isolation

For total DNA/RNA isolation from tumor cell of the Sarcoma 180, the DNA-SORB B kit (Iterlabservice, Moscow, Russia) was used according to the manufacturer's protocol. Concentration DNA/RNA defined on adsorption at wave length of 260 nm on the device spectrophotometer (SF-26, Russia). Electrophoresis of DNA/RNA analyzed in 1.5% gel agarose, during 4 hrs. 60V by method [8].

2.7. Rt-pcr

For total RNA isolation from tumor cell of Sarcoma 180 and spleen of model of mice, the (EZ1-RNA Tissue Mini Kit) was used according to the manufacturer's protocol. For synthesis of cDNA on template RNA, the REVERTAL-L kit (Iterlabservice, Moscow, Russia) was used according to the manufacturer's protocol). The analysis of an expression of genes was used RT-PCR as described in articles, for Mdr2 and GARDH [9], for p53 [10].

2.8. Colony-Forming Cells of Spleen (CFCs)

Studying of influence of preparations on number endogenous CFCs to carry out on outbred mice on apparatus "THERATRON" with aggregate capacity 112 Gray/min, source Co⁶⁰ in the sublethal dose equal 6 Gray. Then preparations unitary entered in various therapeutic doses and 1mg/kg. For 9 days after an irradiation, animals were decapitated under ether narcosis, and data analyzed change of weight of a body and a spleen and thymus, and also number of the former colonies in a spleen by a method [11].

3. Results and Discussion

Antimitotic action of colchicine is known, preservation of

this property is necessary for its derivative as potential antineoplastic preparations. Mitosis blockade by colchicine it is result to increase in number of cells in mitosis.

At studying, mitotic index (MI) epithelial crypt on bowels [7], in various terms after introduction K-26 in comparison with initial colchicine it is shown, that the maximum delay mitotic divisions observed at introduction colchicine ($35.73 \pm 0.26\%$) after 6 hours, and for K-26 MI were more low- about 23%, however in comparison with the control on 19% above.

At the same time as at studying mitotic activity of these substances on cellular culture of line CaPa (cancer of a pancreas of the person) influence K-26 (which it was defined by calculation of quantity of sharing cells on 2000 counted and was expressed in per mille (%)), has been found [12], that in tumoral culture K-26 possesses big antimitotic action than colchicine. The MI of cultures of a tumoral tissue after influence K-26 was 120%, while for colchicine it is equal 80% were equal. Calculation MI intact cells has made 52%.

Thus, K-26 possesses big MI on tumor CaPa than colchicine. Also difference of influence K-26 on normal cells, where K-26 MI was lower than at colchicine is shown. Results of this experiment have shown that in tumoral cells preparation K-26 has rendered the big ability to stop their division than colchicine.

Influence of K-26 and K-26w on synthesis of DNA and RNA has been investigated on sarcoma 180 cells in comparison with etoposide which is known inhibitor of topoisomerases I/II.

Figure 1 shows an electrophoregram for assessing DNA native, nucleosoma DNA degradation, and the topoisomerase activity which was assessed by the degree of DNA fragmentation. Results electrophoresis have shown: etoposide, K-26 and K-26w promoted nucleosoma DNA degradation on (70.1 ± 4.7), (85.6 ± 3.0) and (91.3 ± 3.3) accordingly, figure 1. Also, on a picture of a fragmentation of DNA electrophoregram of etoposide, K-26 and K-26w inhibited activity of topoisomerase II on (55.5 ± 4.7), (63.2 ± 3.7) and (69.5 ± 1.7) accordingly, (Figure 1, Table 1).

Further, on cells of a tumor of the Sarcoma 180, influence

of investigated preparations on synthesis DNA/RNA has been investigated: etoposide, K-26 and K-26w inhibited DNA synthesis on 54.1 ± 4.7 , 84.0 ± 3.7 and 95.6 ± 3.0 , accordingly; etoposide, K-26 and K-26w inhibited synthesis RNA on 35.2 ± 5.7 , 65.2 ± 2.0 and 65.5 ± 2.3 , accordingly (Table 1).

Concerning influence of three preparations on topoisomerase II (TOPO-II) cells of the Sarcoma 180 tumor, activity of this enzyme, has been defined visually on a picture electrophoresis of DNA fragmentation in agarose gel. Etoposide, K-26 and K-26w inhibited activity TOPO-II on (55.5 ± 4.7), (63.2 ± 3.7) and (69.5 ± 1.7) accordingly (Figure 1, Table 1).

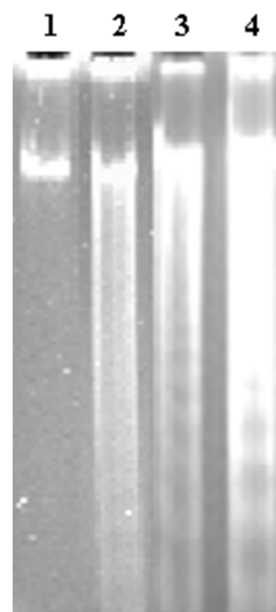


Figure 1. Influence of preparations on native DNA, nucleosoma degradation of DNA and activity topoisomerase II tumoral cells of the Sarcoma 180, in vitro.

Lane 1 control, native DNA lambda bacteriophage (DNA- λ), Lane 2 etoposide, Lane 3 K-26, Lane 4 K-26w. Electrophoresis carries out in to 1.5% TAE agarose gel, 4h, 60V and visualized by UV transilluminator after staining with ethidium bromide.

Table 1. 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 | DNA nucleosoma degradation, in% | Inhibition | | |
|------------------------|---------------------------------|-----------------------|--------------------|--------------------|
| | | Activity TOPO-II, in% | DNA synthesis, in% | RNA synthesis, in% |
| Control | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 | 0 \pm 0 |
| Etoposide | 70.1 \pm 4.7 | 55.5 \pm 4.7 | 54.1 \pm 4.7 | 35.2 \pm 5.7 |
| K-26 | 85.6 \pm 3.0 | 63.2 \pm 3.7 | 84.0 \pm 3.7 | 65.2 \pm 2.0 |
| K-26w | 91.3 \pm 3.3 | 69.5 \pm 1.7 | 95.6 \pm 3.0 | 65.5 \pm 2.3 |

Thus, results of this experiment have shown: K-26w inhibited synthesis DNA/RNA on 95/65%, K-26 on 84/65%, and etoposide within 55/35%. K-26 and K-26w inhibited topoisomerase II within 63-69%, and etoposide on 55%. On electrophoregram, investigated preparations, in all variants the fragmentation of DNA within 70-90%, as a result of activity suppression topoisomerase II, figure 1 is observed.

As is known, inhibitors topoisomerases (etoposide and doxorubicin) intercalation between the nucleic bases of

DNA double-stranded also form a threefold complex from DNA-topoisomerase (12, 13). Formation of a threefold complex "DNA-preparation-topoisomerase" leads to loss ligation to activity topoisomerase and, as consequence, to occurrence of ruptures in DNA chain. As a result of this endocellular process, there is no restoration of the broken off thread of DNA (14, 16). The cells are late in a G2-phase and then perish (13, 16).

On the basis of it, well-known, that the basic mechanism

of action of ionizing radiation on cells, this damage native DNA. Similar action is characteristic and for inhibitors topoisomerases. Therefore their use, both at chemotherapy and in a combination with an irradiation causes the big interest [17].

Further, at animals with a tumor the sarcoma 180, treatment preparations K-26 and K-26w and, in parallel experience with etoposide, has been investigated an expression of genes of multidrug resistance MDR2 and tumoral suppressor p53. As the control referential gene GARDH, have been used, Figures 2, 3.

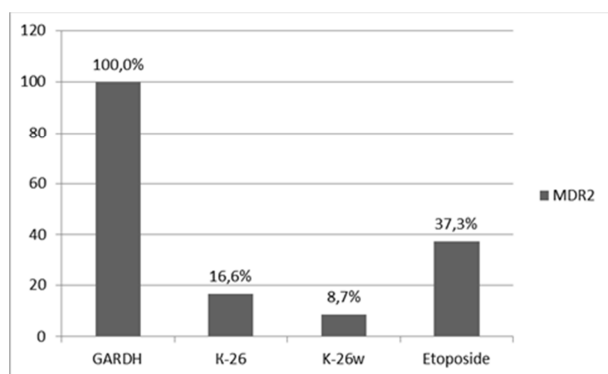


Figure 2. RT-PCR. Influence of antineoplastic preparations K-26, K-26w and etoposide on expression *mdr2* gene of Sarcoma 180 cells, in vivo.

K-26, K26w and etoposide inhibited an expression of MDR2 gene on 83.4%, 91.3% and 62.7%, accordingly (Figure 2), in relation to control referential gene GARDH. It is obvious, that K-26 and K-26w less promotes development of multidrug resistance in comparison with etoposide.

K-26, K26w and etoposide increase expression p53 gene on 74.5%, 88.4% and 55.2%, accordingly (Figure 3).

Thus, by a method RT-PCR it is shown, K26w has shown high activity in inhibition of expression MDR2 and in

expression increase p53. It is established, that in tumor cells low level of MDR2 gene expression under the influence of preparations K-26w and K-26 in comparison with influence etoposide is observed.

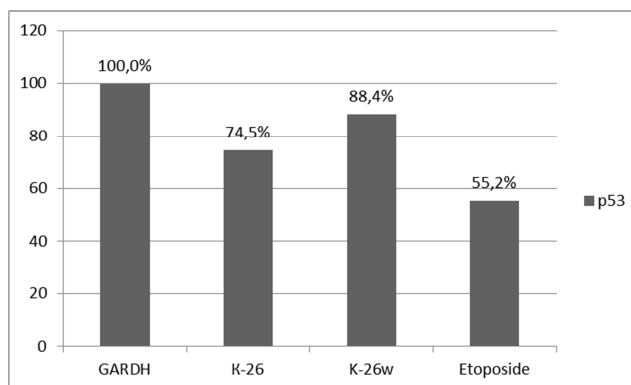


Figure 3. RT-PCR. Influence of antineoplastic preparations K-26, K-26w and etoposide on expression *p53* gene of Sarcoma 180 cells, in vivo.

And the gene expression p53 considerably increases to 74-88%, (at etoposide to 55%), that defines big ability K-26w and K-26 to induce apoptosis tumors. In it specifies also ability of preparation K-26 to expressed DNA nucleosoma degradation (Figure 1).

Studying of influence of preparations K-26 and K-26w in comparison with the irradiated control on CFCs are presented in Table 2. At intraperitoneal introduction of preparations in a therapeutic dose and 1 mg/kg, in 2 hours after an irradiation has shown that after irradiation influence, for intact animal's colony-forming cell ability raises. In a spleen it is formed from 3 to 5 (an average – 4.0) colonies while at the mice which have been not subjected to an irradiation, was 1-3 (2.0) colonies, (Table 2).

Table 2. Influence preparation K-26 and K-26w on CFCs, a spleen and thymus intact and the irradiated animals (not purebred mice).

| Groups animals | Change of weight of animals body to 9 days, (g) | Spleen weight, (mg) | %, weights of spleen in relation to the control 1 | Absolute number CFCs |
|----------------------------|---|---------------------|---|----------------------|
| 1. Intact* | +5.5 | 78.0±3.7 | 183.9 | 2.0±0.4 |
| 2. Irradiation (control 1) | +1.2 | 42.4±1.8 | 100 | 4.0±0.57 |
| 3. K-26 (1 mg/kg) | +6.7 | 94.0±2.9 | 221.7 | 11.2±1.4 |
| 4. K-26 (22 mg/kg) | +9 | 96.4±2.7 | 227.4 | 8.0±0.6 |
| 5. K-26w (1 mg/kg) | +9.6 | 97.2±0.8 | 229.2 | 14.0±0.8 |
| 6. K-26w (32 mg/kg) | +9.2 | 95.2±2.4 | 224.5 | 10.0±0.9 |

Table 2. Continued.

| Groups animals | %, CFCs in relation to intact group | %, CFCs in relation to the control 1 | Weight thymus (mg) | %, weights thymus in relation to the control 1 |
|----------------------------|-------------------------------------|--------------------------------------|--------------------|--|
| 1. Intact* | 0 | -50 | 40.0±2.1 | |
| 2. Irradiation (control 1) | 100 | 0 | 19.8±1.5 | |
| 3. K-26 (1 mg/kg) | 460 | 180 | 32.0±3.0 | 61.6 |
| 4. K-26 (22 mg/kg) | 300 | 100 | 34.5±3.0 | 74.2 |
| 5. K-26w (1 mg/kg) | 600 | 250 | 37.8±1.9 | 90.9 |
| 6. K-26w (32 mg/kg) | 400 | 150 | 36.6±2.8 | 84.8 |

*P < 0.01 - in relation to intact group.

Influence preparation K-26 and K-26w on CFCs, a spleen and thymus intact and the irradiated animals (not purebred

mice).

Preparation K-26 in a dose of 1 mg/kg promoted induction

CFCs to 11.2 units, and in a dose of 22mg/kg to 8 units, that has made accordingly 460% and 180% in comparison with intact and with group of the control with an irradiation. Spleens and weight thymus were in both cases more than at the irradiated control. Weight of a spleen and thymus were in both cases more than at the irradiated control.

K-26-w in a dose of 1 mg/kg promoted induction CFCs on 14.0 ± 0.8 units, that has made 600% in comparison with intact and 250% in comparison with group of the control with an irradiation. The weight of a spleen in this group was on 129% more than in intact to group, and on 229.2% more than in irradiation group. The weight thymus was on 5.5% less, than in intact to group and on 90.9% more than in control group.

Colchicine and colchamine in the literature [18] are carried to radiomimetic, to the substances similar on action with an irradiation, their ability to induction CFCs therefore is clear. It is necessary to notice, that K-26 and its salt K-26w owing to the structural features because of introduction oxy- and amines fragments in a molecule colchicine which transforms it into substance of freely radical nature [5], also is radiomimetic and stimulations CFCs promote.

Probably, introduction of such substance is a signal stromal environment of a bone brain to protection of an organism against its cytotoxic influence. The induction of emission CFCs which occurs at an irradiation, when an organism, being protected, throws out some colonies (4-5 units) from a bone brain, amplifies in a case with a new preparation, and stimulation more quantities CFCs when there are future haemopoetic and immune cells, protects an organism from their cytotoxic action [5].

4. The Conclusion

The carried out researches have shown, that preparations K-26 and K-26w possess a number of properties for damage tumor: mitotic activity, in high degree as the alkylating, promote nucleosoma degradation and DNA fragmentation, to inhibit activity topoisomerases I and II, as causes its high antineoplastic effect, and, theoretically as radiosensitizing.

The more activity topoisomerases suppress with new preparations, and is stimulated p53 – inductor apoptosis, the above overcoming of drug resistance which concerning these substances represents special interest as they are aimed for treatment of such resistant tumor as a kidney cancer. Thus ability to emission CFCs protects an organism from consequences of their cytotoxic action.

Conflict of Interest

The authors declare that they have no competing interests.

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