Organic Chemistry: An Indian Journal

Research | Vol 13 Iss 1

Investigation of Mechanism Action of Some Preparations Obtained Based on Tropolone Alkaloids

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Received: February 27, 2017; **Accepted:** March 6, 2017; **Published:** March 15, 2017

Abstract

Conducted investigation have shown that the studied of new preparations possess full line nearby damaged tumour properties: mitotic activity, at high degree-alkylation, promote between nucleosome degradation and fragmentation DNA, inhibits topoisomerases I and II and possess ability of overcoming drug stability, and above, than etoposide and doxorubicin, possess the expressed cytotoxic action, as causes their high anticancer effect. Thus ability to emission CFUs protects an organism from consequences of their cytotoxic action. Ability to induction CFUs could be explained by their structural characteristics that causes their expressed properties radiomimetic. High of induction CFUs, side by side with suppression of growth of tumour, promotes at influence of some substances to increase of immunity and haemopoietic indicators, at average induction CFUs at number of substances immunity and haemopoietic indicators after the spent treatment, as not so considerably decrease at application of commercial anti-tumour preparations.

Keywords: ***Tropolone alkaloid; Antitumor; Colchicine; Colchamine; Mechanism action***

Introduction

Expressed total toxic effect, quickly increasing resistance of an organism to using preparations, and also variety of forms of oncological diseases dictates necessity of expansion of an arsenal available of preparations. At the National scientific centre of oncology Ministry of Health of RUz the large number group of new substances has working out based on tropolone alkaloids Colchicine and Colchamine with the activity equal or exceeding activity of known applied antitumor preparations (vincristine, taxole, ethopozide, cyclophosphane, 5-ftoruratsil and xelods) [1].

For all of preparations of this line, and in particular, for K-19, Decovine and K-20 have been conducted investigation of their toxic properties and antitumor activity, as in NCI (USA) *in vitro*, and also *in vivo* on 6-8 strains of tumours [2-4].

Citation: Enykeeva ZM, Ibrogimov AA, Abdirova ACH, et al. Investigation of Mechanism Action of Some Preparations Obtained Based on Tropolone Alkaloids. Org Chem Ind J. 2016;13(1):109.

Was presented for publication the data about studying influence of K-19, Decovine and K-20 on such tumoral targets as DNA, RNA and thopoizomerases where high activity of these preparations in inhibition of synthesis of DNA and RNA, exceeding effect ethoposide and doxorubicin, used as control is revealed, preparations inhibited activity thopoizomerases I and II and promote between nucleosoma to degradation of DNA of tumor of AKATOL about 90% [5]. For more full studying of mechanism of action of these preparation also it was studied them mitotic activity, influence on drug stability (DS), immunity and CFUs. The mechanism action of new preparations from set of obtained results. The aim this work is studying of mitotic activity, impact on DS, on mutagenicity, CFUs, immunity.

Materials and Methods

Mitotic activity-on crypt bowels intact of mice which entered intra-abdominal studied of preparation at dose 1/2 LD_{16}. After 30, 60 minutes and each hour within days to animals drive in decapitation and for the histologic investigation has taken 1 sm 12-tiperstnoy entrails, fixed in mix of Bouena, filled in paraffin and then histologic preparations painted hematoxylin+eosins, by quantity of cells in cript and quantity of cell in division calculated mitotic an index (MI) and mitotic activity (MA).

Influence of preparation on DS was conducted on cells *S. cerevisiae* (cultivated by method of Kojin's with addition of therapeutic doses of preparations, and also on model of resistant cells of yeast to cytostatics). Investigation of influence on CFUs was conducted on mice by instrument "THERATRON" with capacity 112 Gr/min., source of Co^{60} in the sub lethal dose equal of 6 Gr for 9 days after an irradiation [6,7]. For studying of index of immune status in system *in vivo* conducted studying of an expression of receptors immunocompetent cell in spleen to day of face. Statistical processing was conducted by using of the program Statistica, version 6.0. For level of the statistical importance was accepted $p<0.05$.

Results and Discussion

You can see from the FIG. 1, all studied substances cause increase mitotic an index in 1 hour after injection (basically in 2 times in comparison with control and in 1, 1-15 times-with Colchicines). Then the quantity of mitoses were grow during 3 hours and reached almost for all substances of the maximum values later 6 hours and remained that within even 6 hours, then by 18 hours decreased to control level.

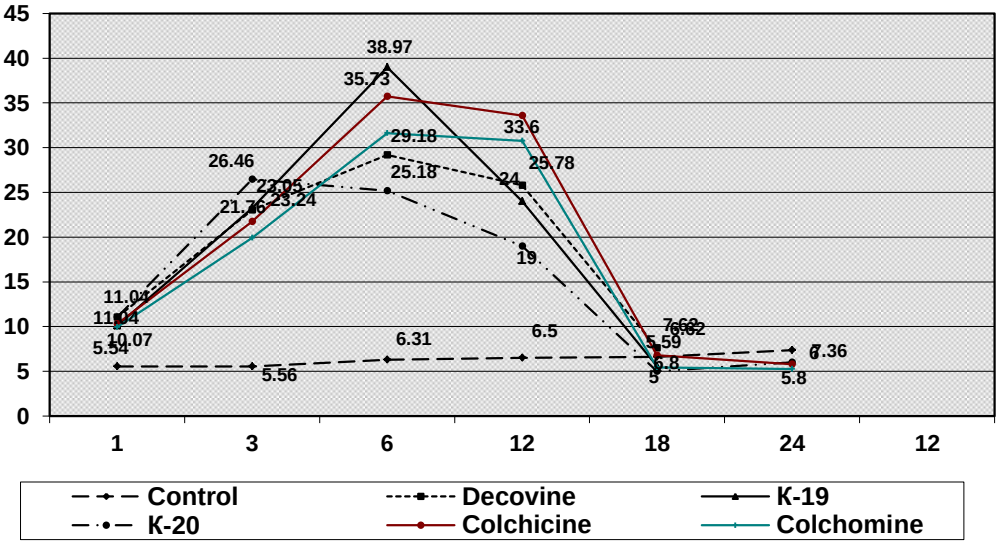

FIG. 1. **Mitotic index new derivatives at comparison with initial alkaloids.**

Values of MI epithelium crypt intestines in various terms after introduction of preparations at K-19 there was the highest MI-38.97%+0.33%, above, than at Colchicines and Colkhamine. Values of MI of Decovine (29.68%+0.33%) and K-20 (25.18%+0.87%) lower, than at Colchicines, however on 19% to 20% high in comparison with control. For the comparison mitotic activity of new preparations on crypts intestines from them mitotic the index in tumour involves the data obtained about studying cytotoxic and mitotic properties of some new connections on tumoral cellular culture of line of CaPa.

In work [8] it has been found that all amine derivatives of Colchicine which are and substances studied, in tumoral culture possess mitotic an index within 93% to 120% while at Colchicines it is equal 80% (mitotic an index intact cell of 52%). Thus, amine derivatives Colchicines possess in comparison with Colchicine, as rule, smaller MI on normal cells (cript intestines) and more expressed mitotic influence on tumour. Influence of preparations Decovine, K-19, K-20 in therapeutic doses on DS was studied during 10 days, estimated level proliferation intact and resistant cell of yeast *S. cerevisiae* strain XII. A number known tubulin interactive preparation was comparison preparations: Colchicine, have known for resistance and the initial substance for obtain of new derivatives, and also taxol, vincristine and ethoposide, was besides compared action to other known antitumor preparations doxorubicin and methotrexate.

It has shown in TABLE 1, that after a day after influence derivatives Colchicine do not suppress growth of cell, and commercial preparations suppress growth intact cell of yeast on 27% to 39%. After 3 days incubation action of commercial preparations decreases, except taxol at which most active of all applied substances suppression of growth of investigated cell is observed, and action of Colchicines derivatives grows-from 32% till 54%. During this period Colchicines causes growth of cells till 24%.

TABLE 1. **Influence of therapeutic doses of investigating agents on growth suppression intact cell of yeast *Saccharomyces cerevisiae* strain XII$_7$.**

S. No.	Preparation	Therapeutic doses investigating preparation mg/кg	Day	Three day	10 day
			% growth cell		
1	Control		100.0	100.0	100.0
2	Decovine	50	100.0	60.0	30.0
3	K-19	40	93.0	46.0	50.0
4	K-20	4	100.0	67.5	13.0
5	Taxol	15	72.5	42.4	50.00
6	Vincristine	0.4	69.7	81.6	-
7	Doxorubicine	1.8	65.6	89.8	-
8	Methotrexate	0.6	68.7	82.4	-
9	Etoposide	15	60.4	85.7	--
10	Colchicine	0.2	10.0	124.0	42.00

For 10 days in comparison with the commercial preparations which action doesn`t cause suppression of growth of cell, behind an exception taxol (50% of suppression of growth), preparations Decocine till 70%, K-19 till 50% and K-20 till 87%

are suppressed by growth intact cells. Colchicine also till 58% suppresses growth of cell during this period. At the TABLE 2 has shown the data about incubation investigated preparations with resistant to using agents cell of yeast *S. cerevisiae* strain XII_7.

If commercial preparations after day caused growth of cell derivatives Colchicine K-20 suppressed growth about 34.5%. After 3 days from all commercial preparations only taxol suppressed resistant cells (on 43.2%) while Colchicines and its derivatives reduced growth of cell from 23.5% (Decovine) till 51.6% (K-20).

You can see distinctive feature of derivatives Colchicines is both their delayed action, and their influence on the resistant cells, shown through 3 days in what there is a similarity with one of commercial preparations taxol (except K-20 which in day suppresses growth of cell). It is necessary to note and absence of resistance Colchicines in 10 days on intact models of cell of yeast and through three that resistant.

TABLE 2. **Influences of investigated agents on level of growth of cells of yeast *Saccharomyces cerevisiae* strain XII_7, resistant to corresponding agents.**

S. No.	Preparation	Therapeutic doses investigating preparation mg/кg	Day	Three day
			% growth cell	
1	Control		100.0	100.0
2	Decovine	50	103.7	76.5
3	K-19	40	95.7	62.5
4	K-20	4	65.4	48.40
5	Taxol	15	103.7	56.8
6	Vincristine	0.4	114.6	105.6
7	Doxorubicin	1.8	114.0	125.60
8	Methotrexate	0.6	100.05	100.0
9	Etoposide	15	126.3	122.17
10	Colchicine	0.2	110.0	75.4

At molecular level it is possible to notice that K-19, Decovine and K-20 don`t promote activation transmembrane transport fiber Pdr5p responsible for medicinal stability (function Pdr5p is connected with emission of medicines from cells).
Thus, it is possible to do conclusion about overcoming of DS of derivatives Colchicines.

At studying of influence of preparations on immune system of mice with the intertwined tumour the Sarcoma 180 preparations Decovine, Colchiprit (K-20) and Colkhaminol (K-19) entered 10-times in therapeutic doses (TD), studying of indicators of the immune status conducted on spleens of mice at day cat, according to methodic recommendations (Zaljalieva M. V, and etc., 1999, 2004) on influence of preparations on differentiation antigens lymphocytes (TABLE 3).

TABLE 3. **Average indexes of some parameters of immunity at mice control and in experiment.**

	CD3	CD4	CD8	CD16	CD19	CD95
Control	40.7 ± 2.3	48.7 ± 3.3	42.3 ± 3.9	49.3 ± 1.45	37.3 ± 1.2	39.3 ± 6.0
К-20	44.75 ± 5.5	56.25 ± 6.2	55.25 ± 5.5	59.5 ± 0.5	58.25 ± 5.7	57.5 ± 6.7
К-19	46.3 ± 2.6	47.7 ± 3.9	41.3 ± 3.1	43.8 ± 3.3	37.8 ± 2.8	35.0 ± 2.3
Decovine	42 ± 1.34	29 ± 1.15	21.8 ± 0.7	28 ± 0.57	27 ± 1.34	44.0 ± 4.0
The note: $^{*}p<0.05$; **-p<0.001-reliable difference relatively control						

Investigation of superficial receptors lymphocyte has been conducted to an inductive phase of the immune reply after injection of preparations. It has been established that preparation of К-20 caused stimulation of all studied superficial receptors from 1.1 till 1.56 times.

Preparation К-19 causes inhibition receptors CD16 (natural to killing cells) and CD95 (receptors apoptosis) on 11% on comparison with control ($p<0.05$). Receptors $CD3^{+}$-lymphocyte also have been little raised relatively control. It is noted authentic distinctions in an expression of receptors CD4, CD8, CD19 (helpers, cytotoxic T-lymphocyte, V-lymphocyte).

However, as whole influence on the immunity, shown in some decrease in receptors CD16 and CD95 preparation К-19, doesn`t assume the expressed failure of immunity at preparation application in clinic [9]. Decovine didn`t suppress receptors of CD3 and CD95 and caused suppression of all other receptors, but promoted preservation of activity of cages of immune system that was shown both in reactions with mitogens the ConA, and with ArM [10]. Influence on CFUs preparations Decovine, К-19 and К-20 were studied in comparison with the irradiated control. All preparations were entered unitary in dose of 1 mg/kg and in therapeutic dose intra-abdominal in 2 hours after an irradiation (TABLE 4).

All substances, including initial alkaloids, possess stimulating ability on an exit CFUs, however in different measure: Colchicine in TD also considerably stimulates CFUs to 17 times, Colchamine in 1mg/kg dose-in 15 times [11], Decovine in 2-3 times, К-19 in 3-9,6 times, К-20 in 9,7-13 times in respect to intact with an animal. The greatest possesses ability substance К-20, and in wide range of concentration. Thus, this property inherent initial alkaloids is available for the substances based on its, but in different measure.

TABLE 4. **Influence of preparations on CFUs, spleen and thymus intact and the irradiated animals (non-pedigreed mice).**

No	Group	Doses preparations mg/kg	Changing mass of body of animals at 9 day g.	Mass of spleen (mg)	Absolute number of CFUs	Mass of thymus (mg)

1	Intact		+5.5	78 ± 5.22	2 ± 0.40	40 ± 3.6
2	Irradiation (Control 1)		+1.2	47.2 ± 7.0	4 ± 0.44	20 ± 2.7
3	К-19	1	+10	111.3 ± 12.3	19.25 ± 4.2	31.25 ± 7.2
4	К-19	30	+13.6	113.8 ± 5.3	6 ± 2.4	44.0 ± 4.8
5	К-20	1	+10.5	98.8 ± 7.5	26 ± 4.4	43.75 ± 9.4
6	К-20	4	+10.50	107.5 ± 5.9	19.5 ± 1.8	42.5 ± 3.2
7	Decovine	1	+4	68.0 ± 3.0	8.2 ± 1.4	26.4 ± 5.0
8	Decovine	50	+6	45.0 ± 2.8*	5.8 ± 3.1	22.8 ± 3.6

Studied substances at TD stimulated from 6 till 19.5 units of CFUs, and at injection dose of 1 mg/kg stimulation CFUs under the influence of preparations was above (from 8 till 26 unit). It was reflected in their immune status, К-20 did not reduce immunity after the spent treatment at 10-times injection in TD, consequence of treatment by preparation К-19 doesn`t assume the expressed failure of immunity at application of preparation. Decovine did not suppress receptors CD3 and CD95, caused suppression of all other receptors, but promoted preservation of activity of cell of immune system (TABLE 5).

As stimulation CFUs is one of factors of formation new haemopoietic and immunocompetent cell, as thereby promote restoration of indicators of peripheral blood. At studying haematological the indicators obtained in experiment at treatment of animals with different strains of tumours, find out the haemostimulating action of preparations which had positive influence on haematological indicators at animals-cancer delivery, normalizing the general analysis of blood at experimental animals.

TABLE 5. **Antitumor activity and hematological indicators of experimental animals with subinoculation strains АКАТОL after treatment by preparations К-19, К-20 and Decovine.**

№	**Group of animals**	**Dose (mg/kg) and number of introduction**	**% IGT Volume/ mass**	**% remission of tumor**	**Mass of spleen, g**	**Erythrocyte 10^{12} /l**	**Leukocyte 10^9 /l**
1	Control				0.36 ± 0.04*	1.9 ± 0.2	3.0 ± 0.1
2	К-19	(35)-8	100/100	100	0.28 ± 0.16	1.7 ± 0.2	1.36 ± 0.21
3	К-20	(5)-8	93/87	50	0.68 ± 0.21	3.7 ± 0.2	2.88 ± 0.35
4	Decovine	(50)-5	100/100	100	0.23 ± 0.1	1.3 ± 0.2	0.893 ± 0.23
The note: in treatment groups n=8, at control n=10; *distinctions are statistically authentic in comparison with control at P <0.05. GIT-growth inhibition of tumors							

Restriction for cytostatic, as rule, is their active influence on processes hematopoiesis that causes level of incidental effects. Investigation of potential antitumor preparations on model CFUs detected spontaneous their influence on reparation processes in system hemopoiesis. All new preparations possess mitotic activity, in different measure-alkylation activity,

promote internucleosoma to degradation and fragmentation of DNA by means of inhibition tophoizomerazes I and II [5] and possess ability of overcoming DS, and above, than etoposide and doxorubicin, possess the expressed cytotoxic action, as causes their high antitumor effect.

However along with considerable suppression of growth of the tumour, new substances not so considerably reduce immunity and haemopoetic indicators, and some substances raise both that and another after the conducted treatment. Colchicines in the literature [12] it is refer to radiomimetic, to the substances similar on action with an irradiation. It is necessary to note, as K-20 and some other owing to the structural features because of introduction alkylation fragments are stronger radiomimetic, than initial alkaloid, and in major to measure strengthen emission CFUs. Besides, the originality of all new substances consists in their paramagnetic properties. Proceeding from theoretical reasons, calculations of spectra EPR, it is possible to assume that the location not coupled electron is tropolone ring in it oxy-iminne to the form that informs the connection which is in this form an ion-radical character [13].

It increases their ability to easier π-π-or n-to-interaction with organic molecules-targets what fibers of type tubuline or nucleotides are that explains big alkylation activity of new derivatives colchicine, both at influence on nucleonic acids, and on tubuline through which it is carried out them mitotic action, and certainly, as substances of the is free-radical nature, they strengthen radiomimetic influence on a marrow.

Probably, their introduction is a signal stromal marrow environment to protection of an organism against their cytotoxic influence (as with an irradiation when an organism, being protected, throws out some colonies from marrow) that induces emission CFUs and by that protects an organism from consequences of their cytotoxic action. It, possibly, causes also properties unusual to antitumor preparations to raise immunity, and absence at number of preparations of negative influence on hemopoiesis. In the literature, there is no such class of the anti tumour substances capable stimulate CFUs, on level with suppression of growth of tumour [14,15]. As a rule, antitumor preparations with the different mechanism of action possess a large quantity of by-effects: low toxicity, suppress immunity, possess of DS.

Conclusion

From the obtained results, it can be conclusion follows that the new created substances capable to considerable stimulation CFUs (more 15-20 units), don`t possess the expressed collateral influence on immunity and hematopoiesis as do not suppress stem cell of marrow, and do not lead to occurrence of the expressed resistance. The substances of this number not capable to considerable stimulation CFUs (4-8 unit) as Decovine, possess collateral influence on immunity and hemapoiesis, however their collateral action will be nevertheless lower, than at known cytostatic.

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