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STUDY OF MUTAGENIC AND ANTIMUTAGENIC ACTION OF ENTEROSORBENTS BY THE EXAMPLE OF MEDICAL PREPARATION ENTEROSGEL (PASTE FOR ORAL ADMINISTRATION)

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РЕЗЮМЕ. Результати проведеного дослідження свідчать про те, що ентеросорбент Ентеросгель (паста для перорального вживання) виробництва ЗАТ ЕОФ "КРЕОМА□ФАРМ" володіє антимутагенними властивостями, що, можливо, відкриває нові підходи до використання препарату для профілактики уражень слизової оболонки ШКТ, викликаних, наприклад, радіонуклідним навантаженням, а також деякими іншими виробничими чинниками, що мають мутагенний ефект.
Ключові слова: ентнросгель, мутагенна дія, антимутагенні властивості.

РЕЗЮМЕ. Результаты проведенного исследования свидетельствуют о том, что энтеросорбент Энтеросгель (паста для перорального применения) производства ЗАО ЭОФ "КРЕОМА□ФАРМ" обладает антимутагенными свойствами, что, возможно, открывает новые подходы к использованию препарата для профилактики поражений слизистой ЖКТ, вызванных, например, радионуклидной нагрузкой, а также некоторыми другими производственными факторами, обладающими мутагенным эффектом.
Ключевые слова: энтеросгель, мутагенное действие, антимутагенные свойства.

SUMMARY. *The results of the conducted research testify that Enterosorbent Enterosgel' (paste for peroral'nogo application) of production of joint_stock*
COMPANY ZAO EOF "KREOMA_FARM" possesses antimutagenicity properties, that, possibly, opens the new going near the use of preparation for the prophylaxis of defeats of mucous membrane of ZHKT, caused, for example, radionuklidnoy loading, and also some other by a production factors, possessing mutagens an effect.
Key words: enterosgel', mutagenicity, antimutagenicity

The medical preparation Enterosgel (paste for oral administration) is a hydrogel of methylated cells and a chemically synthesized enterosorbent. It is known that chemical substances can induce gene, chromosome and genomic mutations and, at the same time, give high specificity for certain types of mutations. In this regard, when assessing the mutagenic potential of a substance, a set of methods is used to record all types of genetic changes.

The standard testing of medical preparations [1] includes four steps: monitoring of gene mutations on microorganisms (Ames test) or on fruit fly; monitoring of pre-dominant lethal mutations in the mice embryoblast; monitoring of chromosomal aberrations in mice bone marrow cells and chromosomal aberrations or sister chromatid exchanges in the human lymphocyte culture.

The common thing for these test systems is that the methods used in them allow solving two different issues gradually: identification of potential mutagens and assessment of their genetic activity.

The purpose of the performed experiments is to study mutagenic and antimutagenic action of Enterosgel medical preparation (paste for oral administration).

According to the objectives, the mutagenic activity of Enterosgel (paste for oral administration) was studied in three tests: induction of reverse gene mutations in Salmonella typhimurium (Ames test without and with metabolic activation); induction of chromosome aberrations in human peripheral blood lymphocyte culture in vitro without and with metabolic activation; induction of chromosome aberrations in mice bone marrow cells in vivo.

The antimutagenic activity of Enterosgel was studied in the test for inducing chromosome aberrations in human peripheral blood lymphocyte culture in vitro without and with metabolic activation.

Test for inducing chromosome aberrations in mice bone marrow cells in vivo

The mutagenic activity of the medical preparation Enterosgel (paste for oral administration) in the test for induction of chromosome aberrations in mice bone marrow cells in vivo was studied in white non-linear mice weighing 18-20 g, obtained from the mouse bank of the laboratory animal resource center of the Private Enterprise "Biomodelservice" (Kiev, E. Potier Street, 14). Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Experimental and control groups included 6 animals.

During the experiment, animals were kept in vivarium in standard plastic cells, in groups of 6 animals, at ambient temperature of 20-22 °C, air humidity of 50-60%, and a standard day-night light regime. Chromosome preparations of bone marrow cells were prepared by the method of H.J. Evans [2]. Statistical processing of the obtained data was carried out using Student's t-test [3].

No animal mortality or clinical symptoms of intoxication were observed upon administration of Enterosgel medical preparation (paste for oral administration) in all tested doses. The behavior of experimental mice did not deviate from the control ones. Enterosgel medical preparation (paste for oral administration) in doses of 5.0 to 0.5 g / kg of the animal body weight did not induce statistically significant excess of the spontaneous frequency of chromosome aberrations. At the same time, statistically significant ($P < 0.001$) excess of the spontaneous frequency of aberrations was noted in the group of animals treated with Cyclophosphamide, which proves the adequacy of the use of this test system for evaluating mutagenic properties of chemical agents.

In the Ames test and in the test for inducing chromosome aberrations in human peripheral blood lymphocyte culture in vitro, Enterosgel medical preparation (paste for oral administration) was used in the following concentrations:

- 10.0 mg / ml - maximum concentration for the entire course for a person;
- 5.0 mg / ml - usual course of treatment for a person;
- 2.50 mg / ml - dose exceeding the daily by 4-fold;
- 1.25 mg / ml - double daily dose;
- 0.625 mg / ml - daily dose for a person

The experiment in both tests was performed in two parallel versions - without metabolic activation and with activation of the microsomal activating mixture - S-9 mix. In versions without metabolic activation, the action of direct mutagens was recorded - the compounds inducing mutations due to the activity of the primary structure of the examined substance. In experiment versions with metabolic

activation the action of promutagenes was recorded - the compounds whose effect is due to the formation of mutagenic metabolites.

Ames test

The presence of a mutagenic effect in the Ames test was taken into account by the induction of reverse mutations from auxotrophy to prototrophy in histidine. In order to identify different types of mutations, two indicator strains of S. typhimurium were used in the experiment: TA - 98 (his D 3052, rfa, Δ uvr B, + R: pkM 101), which registers mutations by the reading frame shift type and TA - 100 (his G 46, rfa, Δ uvr B, + R: pkM 101), which records mutations by substitution type of base pairs obtained from the Bruce Ames Laboratory, Department of Molecular and Cell Biology, University of California at Berkeley, 401 Barker Holl, 94720 0001) in 1993.

In the study of the mutagenic activity of Enterosgel medical preparation (paste for oral administration) by the standard cup test, proposed by D.M. Maron and B.N. Ames [4], a weak mutagenic effect was found in the maximum concentration for the entire course - 10.0 mg / ml in experiments on two strains TA - 98 and TA - 100 without and with metabolic activation, and in the second concentration - 5.0 Mcg / ml (usual course of treatment). However, taking into account the strength of preparation in medium and bacterium (pseudo mutagenic effect that was obtained in a standard cup test), in this case a preincubation test is considered more appropriate, as it lacks a sorbent contact factor with a solid nutrient medium. Its essence lies in the fact that the bacterial suspension, the solution of the examined compound and the activating mixture (if the experiment contains metabolic activation) are incubated together at + 37°C for 0.5 - 3 hours, then a bacterium is withdrawn and added into 2 ml of a molten semi-liquid agar at + 45 °C and layers are formed on selective agar. Further incubation occurs on a cup in agar medium, as in the usual Ames test. The results of this test are presented in Table 1.

The table shows a weak mutagenic effect in the maximum concentration for the entire course - 10.0 mg / ml in the experiment on TA - 98 strain with metabolic activation.

Thus, Enterosgel medical preparation (paste for oral administration), tested in the inverse gene mutation in Salmonella typhimurium (Ames test) with preincubation, shows a weak mutagenic effect only at the maximum concentration for the entire course (10.0 mg / Ml) in a version of the experiment with metabolic activation on the indicator strain TA - 98, which is not limiting when assessing the safety of the medical preparation Enterosgel (paste for oral administration).

Test for the induction of chromosome aberrations in human peripheral blood lymphocyte culture in vitro

The basis for the peripheral blood lymphocytes cultivation and compounding of chromosome preparations was a standard semi-micro-method [5], though with modifications accepted in the mutagenesis laboratory [6]. The selection of metaphase plates for cytogenetic analysis, classification and account of chromosome aberrations were generally accepted. The statistical processing of the obtained data was performed using the Student's criteria [3].

The frequency of aberrations of chromosomes, the number of aneuploid cells (possible carcinogenic risk), and the number of multi-aberrant cells (possible disorders of the DNA repair system) were taken into account.

Thus, in the test for induction of chromosome aberrations in human peripheral blood lymphocyte culture in vitro in experiment versions with and without metabolic activation at concentrations from the maximum for the entire course (10.0 mg / ml) to a daily dose (0.625 mg / Ml) the mutation of the gene activity of Enterosgel (peroral paste) has not been revealed.

Table 1

The multiplicity of the excess of the number of S. typhimurium revertant colonies by different strains in studies with preincubation

Strains		Mutagene	Concentration of medical preparation Enterosgel (paste for peroral use) (mg/ml)				
			10.0	5.0	2.5	1.25	0.625
TA-98	without m/a	++	-	-	-	-	-
	with m/a	+++	+	-	-	-	-
TA-100	without m/a	+	-	-	-	-	-
	with m/a	+	-	-	-	-	-

No effect "?" For TA 98 - to 2-fold, for TA 100 - to 1.8-fold,
Weak mutagenic effect ("+"),
Average mutagenic effect ("++"),
Strong mutagenic effect ("+++").

Studies of the antimutagen activity of the medical preparation were performed in a test for inducing chromosome aberrations in human peripheral blood lymphocyte culture in vitro at the same concentrations as in the study of mutagenic activity.

In the first version, Enterosgel was added to each vial 3 hours after the addition of mitomycin C (the concentration of the mutagen was 10 μg / ml, as in the positive control). The results are shown in Table 2.

According to the table, there was no sufficient decrease in chromosome aberrations when Enterosgel was added three hours after Mitomycin C, although at high doses the tendency to this is clearly visible.

This table also demonstrates that at two higher concentrations - the maximum and usual dose of treatment for the entire course, a statistically significant decrease in aneuploid cells was observed compared with induction by mitomycin C. In other concentrations there was no significant reduction of aneuploid cells.

To exclude the direct interaction of mutagen and sorbent, the same experiment was performed with preincubation. The scheme of "preincubation" presupposed treatment of the human blood cells with mitomycin C, and three hours after the "washing out" of mutagen, a standard cultivation scheme was reproduced (Table 3).

It can be seen from the table that action time of the sorbent is not essential to the manifestation of its antimutagenic properties. At the same time, a significant improvement in all cytogenetic indicators was observed, namely: the frequency of metaphase with aberrations, aneuploid and multi-aberrant cells with two maximum Enstrosgel concentrations.

Table 2

The main cytogenetic parameters of Enterosgel (paste for oral administration) in the culture of human peripheral blood lymphocytes three hours after the addition of mitomycin C.

Examined substance	Average frequency (%±m)		
	Metaphases		Multi-aberrant cells
	With aberrations	Aneuploid	
Negative control	1.0±0.9	11.50±2.30	
Mitomycin-C (control)	14.00±3.4	22.00±4.10	51.0±4.9
Enterosgel (oral paste) mg / ml, added to the culture 3 hours after the addition of mitomycin C			
10.0	7.2±2.7	6.00±2.4*	33.3±4.7*
5.0	8.0±2.7	7.00±2.5*	45.0±4.9
2.5	14.0±3.4	12.00±3.2	50.0±4.9
1.25	14.5±3.4	15.00±3.5	50.0±4.9
0.625	14.5±3.4	16.00±3.6	50.0±4.9

* P <0.001 compared with the control of mitomycin C

Table 3

The main cytogenetic indices of Enterosgel (paste for oral administration) in the culture of human peripheral blood lymphocytes with pre-incubation of mitomycin C

Examined substance	Average frequency (%±m)		
	Metaphases		Multi-aberrant cells
	With aberrations	Aneuploid	
Negative control	1.0±0.9	11.50±2.30	
Mitomycin-C (control)	14.00±3.4*	22.00±4.10*	51.0±4.9
Mitomycin C with preincubation (10 μg / ml) + Enterosgel (oral paste) mg / ml added to the culture 48 hours before the end of cultivation			
10.0	4.0±2.3*	6.00±2.4*	0*
5.0	5.0±2.3*	7.00±2.6*	0*
2.5	8.0±2.7*	8.00±2.7*	14.0±3.5*
Mitomycin C with preincubation (10 μg / ml) + Enterosgel (oral paste) mg / ml added to the culture 24 hours before the end of cultivation			
10.0	4.0±2.3*	5.30±2.3*	0*
5.0	6.0±2.4*	6.00±2.4*	0*
2.5	8.0±2.7	8.00±2.7*	15.0±3.6*

* P <0.001 compared with the control of mitomycin C

Therefore, enterosorbent Enterosgel (paste for oral administration) produced by CSC Environmental protection firm "KREOMA-PHARM" has anti mutagenic properties that possibly open new approaches to the use of the preparation for the prevention of gastrointestinal mucosal lesions caused, for example, by radionuclide load, as well as some other production factors with mutagenic effect.

As a result, it can be concluded that only a complex study of preparations can provide an accurate answer to the question about the presence of mutagenic properties in the examined substance. Furthermore, during the examination of preparations, along with studies of mutagenic activity, it is expedient to investigate antimutagenic activity of pharmaceutical preparations to refine and expand the scope of their application. It should also be noted that an individual approach is important in the study of

each medical substance in order to avoid pseudo-positive mutagenic effects, as in the case of Enterosgel study in the standard Ames cup test.

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