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CYTOGENETICAL ANALYSIS OF THE EFFECTS OF UROTOVET^R ON THE CHROMOSOMES OF MAMALS "IN VIVO"

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In many previous trials Urotovet^R, a product of the farmaceutical enterprise ZDRAVLJE – Leskovac, showed extraordinary results as an additive - bactericide in feeds for domestic animals in livestock production. In the digestive tract, its active component (hexamethylenetetramine hydrorhodanide) is transformed in to formaldehyde and hydrorhodanide acid. In previous studies it was shown that formaldehyde possessed genotoxical properties. Based on these facts the cytogenetic effects of Urotovet^R given orally in therapeutic doses to CH3 mice were examined.

In this study Urotovet^R did not express any effects on the production of chromosome aberrations of the structural type, nor did it increase the frequency of cells with numerical chromosome aberrations, i. e. Urotovet^R in this test did not express any genotoxical properties.

Key words: genotoxicity, drugs, Urotovet^R, chromosome aberrations, muhagenes.

INTRODUCTION

In addition to the positive results, the application of antibiotics in livestock production led to some negative occurrences such as the appearance of resistang strains of micooorganisms, allergic reactions and the extistence of residues in both the products and meat of animals. That is why new preparations that do not cause undesirable consequences have been developed and tested.

Urotovet^R, a preparation of the pharmaceutical company ZDRAVLJE from Leskovac, has showen extraordinary results in livestock production in many trials. In pig raising, for instance (Ševković et al., 1981), when 1-2 per cent Urotovet^R was ingested with the feed, the feed consumption was smaller and the weight gain was 8 per cent bigger than when the preparation was not used. Similar results were obtained (Ševković et al., 1981a), with other kinds of domestic animals (the testing of which is still under way) in relation to the reduced incidence of diseases and mortality. Urotovet^R did not express any harmful effects in these previous tests.

In addition to the effects on weight gain and feed consumption, it was shown that the preparation had fungicidal and detoxicating action (Ožegović et al., 1984; Ožegović et al., 1986a). It was found that in some cases Urotovet^R expressed immunostimulating activity (Janković et al., 1988).

Urotovet^R contains hexamethylenetetramine hydrorhodanide as an active component and in the digestive tract it is transformed into formaldehyde and hydrorhodanic acid. In previous papers the possibility of a toxic effect of the first substance was studied (Ožegović et al., 1986; 1986a), but it was pointed out that at therapeutic doses, no toxic effects were observed and both substances acted bacteriologically and chemotherapeutically.

Formaldehyde belongs to the group of genetically hazardous materials which may act mutagenically. Therefore, some cytogenetical tests have already been carried out (Vujošević et. al., 1986; Soldatović et al., 1994). These tests were prepared to determine eventual effects of Urotovet on the hereditary structures, the chromosomes of mammals. The US EPA reccommended the testes for investigating eventual genotoxicity (Preston et al., 1981).

In porcine lymphocyte cultures (Soldatović et. al., 1994) Urotovet^R din not bring about structural and numerical changes in the chromosomes at tha concentrations used (0,1; 0,2; 0,4 mg/ml); similar results were obtained after testing doses of 1/3 LD; 1/9 LD and 1/27 LD in the "in vivo" system on the chromosomes of bone marrow cells in mice (Vujošević et al., 1986).

The purpose of this work was to investigate the effects of Urotovet^R "in vivo" on a greater number of experimental animals at doses of the preparation usually added to the feed (1-2 per cent) over a longer time interval (10-20 days).

MATERIAL AND METHODS

Urotovet^R (hexamethylenetetramine hydrorhodanide), a product of the pharmaceutical company ZDRAVLJE from Leskovac, was tested in an "in vivo" system on the CH3 strain of laboratory mice. The test was performed in two experimental groups of 10 animals, and a control group of 10 animals was in;cluded too. The experimental animals were fed for 10 and 20 days with the prepared feed to which 1 per cent Urotovet^R, had been added.

After treatment, the animals were sacrificed, after being injected intraperitoneally with cochicine (1 μg/g). Chromosome preparations were made according to the method of Hsu and Paton (1969).

Mitotic activity, the occurrence of heteroploid and polyploid cells and structural aberrations of the chromosomes were analysed.

RESULTS AND DISCUSSION

The results of testing Urotovet^R are shown in Tables 1 and 2. The mitotic activity of the bone marrow cells of treated animals did not deviate significantly from the controls (Table 1). It was noticed that in the animals which had ingested the preparation with the feed for 10 days, the average value of the mitotic index

(3,45) was slightly enchanced in relation to the average value of controls (2,75), while the average value of the mitotic index of the animals treated for 20 days was 2,80. These values are within the normal variations in individual animals, while only two from the group treated for 10 days had a mitotic activity above 3,50. On the basis of these data, it is difficult to assert that the preparation affects the intensity of mitotic activity in general, but the slightly increased mitotic activity in the above cases could be ascribed to the immunostimulating properties of the preparation (Jovanović et al., 1988).

Table 1. Mitotic activity of the bone marrow cells of mice ingesting 1 per cent UROTOVET[R] with the feed

	Number of experimental mice in the group	Total of examined mitoses	Mitotic index $\bar{x}$	Iv
Control	10	6300	2,75	2,0 –3,5
10 days[+]	10	6700	3,45	3,0 – 4,5
20 days[+]	10	6400	2,80	2,5 – 3,5

+ The mice ingested 1 per cent UROTOVET[R] for 10 and 20 days with the feed

The chromosomal changes monitored were of the gap and/or chromatid break type. The number of such changes in the groups of animals treated for 10 days and 20 days, was within the normal range of occurence of these changes in control animals (Table 2).

Table 2 Results of the cytogenetical "in-vivo" test in mice ingesting 1 per cent UROTOVET[R] with the feed

	Number of experimental animals in the group	Examined mitoses (Σ)	Diploid cells ($\bar{x}$ in %)	Heteroploid cells ($\bar{x}$ in %)	Polyploid cells ($\bar{x}$ in %)	Srtuctural changes ($\bar{x}$ in %)
Control	10	634	92,20	7,17	1,56	2,10
10 days	10	616	92,08	7,13	1,56	1,51
20 days	10	534	94,18	5,98	1,42	1,42

+ The mice ingested 1 per cent UROTOVET[R] for 10 and 20 days with the feed

We used the criteria provided by Brogger (1982), according to which the term chromatid breaks' means chromosome damage and separation where the distance of the parts of chromatids is equal to or greater than the width of the chromatid, or the fragments are dislocated; on the contrary, the gap-type damage was produced.

Lilp and Korogodina (1981) point out that in experimental mice one might expect even up to 2,8% of such changes. In our experiment the highest value was 1,51% and it regularly occurred in the controls. Similar results were obtained in acute and subchronic (up to 24 and 48 hours treatment) doses à 1/3 LD in the "in vivo" test (Vujošević et al., 1986), as well as in the "in vitro" test in the lymphocyte culture of pigs (Soldatović et al., 1994).

A number of heteroploid cells normally occurs in the chromosome preparation. In this experiment, no significant deviation in the groups of treated animals in relation to the controls(Table 2) was determined. Polyploid cells also normally occur in small numbers in the bone marrow, which indicates that the equalized values 1,56, and 1,42 did not deviate from controls (1,56). Thus, Urotovet^R did not affect the occurrence of numerical chromosome aberrations.

In this study, under the conditions of testing, Urotovet^R did not express any effects in the production of chromosomal aberrations of the structural or numerical type, i. e. Urotovet^R did not express any genotoxical properties.

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CITOGENETIČKA ANALIZA EFEKATA UROTOVETA^R U "IN VIVO" TESTU NA HROMOZOMIMA SISARA

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SADRŽAJ

Preparat Urotovet^R, proizvod farmaceutske industrije "Zdravlje" iz Leskovca, pokazao je vrlo dobra svojstva pri korišćenju u terapijske svrhe. Međutim, kako je aktivna komponenta preparata heksametilentetramin hidrorodanid, koji

se u digestivnom traktu razlaže na formaldehid i rodovodoničnu kiselinu, a poznato je da formaldehid spada u grupu genetički rizičnih materija, bilo je neophodno sprovesti citogenetičke testove, kako bi se ispitali eventualni efekti ove supstance, pri korišćenju terapijskih doza u dužim vremenskim intervalima.

Izvršena je citogenetička analiza mitotskih, metafaznih figura hromozoma ćelija koštane srži miševa soja CH3, nakon 10 i 20 dana davanja jednoprocentnog Urotoveta^{R} sa hranom.

Zapaženo je blago povišenje mitotske aktivnosti ćelija kod nekoliko tretiranih životinja u odnosu na kontrole, što bi se možda moglo dovesti u vezu sa već ustanovljenim blago imunostimulirajućim osobinama preparata; ali srednje vrednosti mitotskog indeksa u globalu, nisu otstupale značajno od onih utvrđenih kod kontrola.

Ustanovljeno j da su sve vrednosti za učestalost analiziranih promena – prekida i gapova na hromozomima, kao i za učestalost pojave aneuploidnih i poliploidnih ćelija, bile u nivou kontrola, odnosno u nivou vrednosti koje se uobičajeno zapažaju kod netretiranih životinja.

Prema tome, citogenetičkim testom, nisu utvrđene promene koje bi mogle da ukažu na genotoksična svojstva ispitivanog preparata, pri njegovom korišćenju u terapijskim dozama.