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INVESTIGATION OF THE GENOTOXIC EFFECTS OF IVERMECTIN ON HUMAN LYMPHOCYTES

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The genotoxic effects of ivermectin, a widely used antiparasitic drug, were investigated. It was tested in various concentrations in PHA-activated human lymphocyte cultures, for the ability to induce sister chromatid exchange (SCE) and micronuclei. In doses of 0.30 mg/ml and higher it significantly increased the SCE frequency. No evidence about genotoxicity of ivermectin could be obtained in the micronucleus test.

Key words: ivermectin, genotoxicity, in vitro, SCE, micronuclei.

INTRODUCTION

Ivermectin is a very potent antiparasitic drug efficient against nematode and arthropod parasites. The exact mechanism of its action is not certain, but it is estimated that paralysis of the parasites is the consequence of increased releasing and/or binding of GABA (Goodman and Gilman, 1990; Martindale, 1989).

There are numerous data about side effects of ivermectin in animals and humans (Addiss et al. 1991; Albiez et al. 1988; Guderian et al. 1991; Huijun et al. 1991. Lankas et al., 1989; Sanford et al. 1988) However, information about its genotoxicity is scanty and consists of the results of Gadzhiev (1984), who investigated the mutagenic effects of ivermectin by the dominant lethal test. Ivermectin was administered subcutaneously to male hybrid mice. In a dose of 2000 mg/kg (tenfold therapeutic dose) ivermectin induced dominant lethal mutations in the stage of late spermatocytes and in a dose of 600 mg/kg (threefold therapeutic dose) in the stages of late and early spermatids. In the therapeutic dose no changes in spermatogenesis were detected.

The aim of the present work was to determine the influence of ivermectin on SCE and micronucleus frequency in PHA-stimulated human lymphocyte cultures.

MATERIAL AND METHODS

Ivermectin (CAS-70161-11-4 (Bla); 70209-81-3 (B1b); 70288-86-7 (mixture) is a semisynthetic derivative of an ivermectin. It is a mixture of about 80% component Bla and 20% B1b (Goodman and Gilman, 1990; Martindale, 1989).

The drug was diluted in dimethylsulfoxide (DMSO, Serva) and added to cultures at the time of setting them up, in concentrations of 0.15, 0.30, 0.60, 1.20 and 2.40 mg/ml. The genotoxic effects of ivermectin were investigated by the SCE and micronucleus test. Whole blood was cultivated according to a somewhat modified method of Evans and O'Riordan (1975) in Parker 199 medium (Institute for immunology and virology, Belgrade). Lymphocyte stimulation was achieved by adding 0.025 ml reconstituted phytohaemagglutinin (Difco Laboratories) per milliliter.

In the SCE test 5-bromo-deoxyuridine (Serva) was added in a concentration of 10 mg/ml and 90 minutes before the preparation colcemid (Serva) was added (0.02 ml/ml) The slides were stained in giemsa solution (Alves and Jonasson, 1978. In the micronucleus test cytochalasine B was added 44 h after setting up, (3 mg/ml). Lymphocytes were prepared by standard procedures and the slides were stained with giemsa (Fenech and Morley, 1985). Functioning of the SCE test was confirmed by adding mitomycin C (ICN-Flow) at a concentration of 30 mg/ml of medium. The positive control in the micronucleus test was a culture of X-irradiated blood (2 Gy). DMSO was the negative control in both tests.

The results were processed by standard statistical methods (Žižić et al. 1993).

In the SCE test well spread mitoses of the second division were observed. The SCE number was determined onn 30 metaphase figures for all concentrations of the drug tested and both controls.

RESULTS AND DISCUSSION

The mean value of SCE per cell in the negative control was 6.93 (Table 1.) At the lowest ivermectin concentration the SCE frequency was 6.67 on average and did not significantly differ from the control. Ivermectin at 0.30 mg/ml enhanced the mean SCE value per cell by 7.79% in comparison to the control, which was statistically significant ($p<0.001$) The mean SCE number was 7.47 At a concentration of 0.60 mg/ml the drug enhanced the arithmetic mean of the number of SCE per cell to 8.03, i. e. to a level 15.87% higher than in the negative control. In comparison with DMSO the difference was statistically significant.

Similar effects on the SCE number occurred at high concentrations (1.20 and 2.40 mg/ml of ivermectin. Namely, at 1.20 mg/ml the antiparasitic provoked 9.07 SCE per cell. This increase of 30,88% in comparison to the cotrol was statistically very significant. At the highest concentration explored the mean SCE frequency reached the value of 9.50. This increase was statistically very significant.

Table 1. Influence of ivermectin on SCE frequency in cultured human lymphocytes.

Trearment	Number of cells scored	Sce/cell (x)	Sce rate (%)
DMSO	30	6.93	100.00
IVERMECTIN (μg/ml)			
0.15	30	6.67	96.25
0.30	30	7.47	107.79*
0.60	30	8.03	115.87*
1.20	30	9.07	130.88*
2.40	30	9.50	137.09*
MITOMYCIN C	30	13.18	115.87*

* statistically significant difference (compared to the negative control) ($P<0.01$)

The significance of differences in the effects of individual doses of ivermectin was as follows (Table 2). The highest dose investigated provoked statistically very significantly more SCE than doses of 0.15, 0.30 and 0.60 mg/ml; the inncrease was not significant in relation to the effect of the 1.20 mg/ml dose. Ivermectin in this dose (1.20 mg/ml) exhibited a statistically very significant effect in comparison with the effects of doses of 0.15, 0.30 ad 0.60 mg/ml. Ivermectin in a dose 0.60 mg/ml of medium induced a significantly different SCE number compared with each of other doses explored. Similarly, the dose of 0.30 mg/ml caused a significantly different number of exchanges in relation to all others that were tested. In the smallest concentration, ivernectin provoked only a slightly higher frequency of sister chromatid exchanges than the diluent alone i. e. significantly less in comparison with all other doses applied.

Table 2. Statistical significance of the difference in the arithmetic mean of SCE number in various ivermestin concentrations

TREATMENT	0.15*	0.30*	0.60*	1.20*	2.40*	DMSO
Mitomycin C	S	S	S	S	S	S
DMSO	I	S	S	S	S	
2.40*	S	S	S	I		
1.20*	S	S	S			
0.60*	S	S				
0.30*	S					

* ivermetin concentration (m/ml)
S statistically* significant difference (:$P< 0.01$)
I significant difference non

As expected, the number of SCE in the positive control was significantly higher than with each concentration of ivermectin applied and the negative control.

In the micronucleus assay, 1000 binucleated lymphocytes were examinend for each ivermectin dose and the controls (Table 3). In the negative control 8 micronucleated cells were discovered. The same number was found for ivermec-

tin concentrqations of 0.15, 0.30 and 1.2 mg/ml. The 0.6 mg/ml dose provoked 9, and the highest dose 10 micronucleated cells per 1000 binucleated ones. In all micronucleated cells there was only one micronucleus per cell.

Table 3. Ifluence ofivermectin on micronucleus frequency in cultured human lymphocytes.

Trearment	Number of cells scored	Micronucleated cells	Micronucleated cells rate (%)
DMSO	1000	8	100.00
IVERMECTIN (μg/ml)			
0.15	1000	8	100.00
0.30	1000	8	100.00
0.60	1000	9	112.50
1.20	1000	8	100.00
2.40	1000	10	125.00
X-IRRADIATION	1000	192	2400.00*

* statistically significant increase (compared to the negative control) ($P<0.01$)

Statistical analysis revealed that there was no significant difference in microucleus frequency induced by ivermectin. However, a significant micronucleus rise was provoked in the irradiated blood culture (positive control).

Ivermectin is an antiparasitic drug whose genotoxic effects are very poorly known. Its mutagenic effects were investigated by Gadzhiev (1984), using a dominant lethal test. With the therapeutic dose no differences in spermatogenesis were revealed. However, a threefold therapeutic dose induced dominant lethal mutations in the stage of late and early spermatids, and a tenfold dose affected male germinative cells in the stage of late spermatocytes.

We have investigated the ability of ivermectin to induce sister chromatid exchanges and micronuclei in PHA-activated human lymphocytes in doses of 2.4, 1.2, 0.6, 0.3 and 0.15 mg/ml of medium. The smallest concentration tested had no significant effect on SCE, but in higher concentrations the effect was statistically significant. Ivermectin did not induce micronuclei in human lymphocytes in vitro.

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ISPITIVANJE GENOTOKSIČNIH EFEKATA IVERMEKTINA NA HUMANIM LIMFOCITIMA

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SADRŽAJ

Ispitivana je genotoksičnost ivermektina, antiparazitika koji se primenjuje u veterinarskoj i humanoj medicini. Primenjeni su test razmena sestrinskih hromatida (SCE) i mikronukleus test, oba u kulturi humanih limfocita.

Medikament je testiran u koncentracijama 0.15, 0.30, 0.60, 1.20 i 2.40 mg/ml podloge. Za pozitivnu kontrolu poslužio je mitomycin C (SCE test), odnosno kultura ozračene krvi (mikronukleus test), a za negativnu rastvarač, DMSO, u oba testa.

Ivermektin je izazvao signifikantno povećanje učestalosti SCE u koncentraciji 0.30 mg/ml podloge i većim. U mikronukleus testu nisu ustanovljeni genotoksični efekti ivermektina.