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IN VITRO AND IN VIVO CYTOGENETIC ANALYSIS OF THE EFFECTS OF CLOPROSTENOL ON MAMMALIAN CELLS

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The aim of this study was to evaluate possible genotoxic effects of cloprostenol(Sinhrogal$^{R)}$, a synthetic analogue of prostaglandin F-2 alpha, which is used for synchronization of estrus in domestic animals. The mutagenic potential of the test substance was investigated on human peripheral blood lymphocytes (in vitro) and bone marrow cells of intramuscularly treated BALB/c AnNCR male mice (in vivo), by monitoring numerical and structural chromosome aberrations, as well as determination of the mitotic index. There were no indications for a mutagenic potential of cloprostenol within the experimental concentrations and doses applied in this investigation.

Key words: cloprostenol, prostaglandin F-2 alpha, genotoxicity, chromosome aberrations, mitotic index

INTRODUCTION

Epidemiological evidence and data from animal experiments strongly implicate the importance of hormones in development and/or maintenance of malignant cells (Henderson et al., 1982). Having in mind that most cancers originate as a consequence of genetic changes, possible mutagenic effects of hormones -especially steroids, have been studied in various test-systems. However, the influence of nonsteroid hormones and mediators requires deeper genotoxicological characterization.

According to the available data, mediator PGF-2 alpha has genotoxic action (Das et al., 1989), may stimulate proliferation of cultured cells (Hakeda et al., 1991), cause chromatin decondensation of small and large luteal cells (Chegini et al., 1991) whereas metabolities of PGF-2 alpha can bind to DNA (Karmali et al., 1976). It is interesting that many mutagens and carcinogens stimulate PGF-2 alpha synthesis in cells (Levine, 1977). Moreover, some tumor cells contain an excess of PGF-2 alpha (Bennet et al., 1981) and it is thought that changes in prostaglandin levels can be the mechanism by which mutagens and carcinogens induce DNA damage.

The objective of our study was to investigate possible genotoxic properties of cloprostenol (Sinhrogal^R) which is a synthetic analogue of PGF-2 alpha with a slower biotransformation time and a more profound effect. Cloprostenol is used in veterinary medicine since its luteinic effect synchronizes estrus and ovulation in domestic animals therefore allowing successful artificial insemination.

MATERIALS AND METHODS

Test substance. Cloprostenol (CAS No 40665-92-7) is a functional analogue of prostaglandin F-2 alpha (PGF-2 alpha), chemically (16-m-chlorphenoxy)-17, 18, 19, 20-tetranol PGF-2 alpha; molecular weight 424,92. Cloprostenol is used in veterinary medicine mainly for synchronization of estrus. The investigations were performed with Sinhrogal^R (ICN Galenika) which contains cloprostenol as an active component.

Controls. The negative control was prepared in ICN Galenika as a placebo preparation containing all compounds present in Sinhrogal^R except the active one. The positive control N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was dissolved in acetone and added to cultures to obtain a final concentration of 10^{-6} M, whereas animals were treated intramuscularly with a dose of 50 mgkg^{-1}.

In vitro test. Human peripheral blood lymphocyte cultures were set up according to the slightly modified protocol described by Evans and O'Riordan (1975). Heparinised whole blood samples (0.8 ml) obtained from healthy men under 30 years of age were added to vials with 9.2 ml of Parker 199 medium containing 30% of inactivated calf serum (SERVA) and 0.04 mg/ml of hytohaemagglutinin (Wellcome). Cultures were incubated for 72 hours at 37^{0}C. Exactly 47 h and 30 min after the beginning of incubation, cloprostenol (Sinhrogal^R, ICN Galenika) was added to the cultivation vials in such amounts as to obtain final experimental concentrations of 1, 2, 5 and 10 μg/ml. Two hours before harvesting, colcemide (CIBA) was added to the cultures to achieve a final concentration of 0.5 μg/ml. After hypotonic treatment (0.075 M KCl) followed by three repetitive cycles of fixation in methanol-acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped on chilled, grease free microscopic slides, air dried and stiained in 20% Giemsa (Kemika) for 15 minutes. For each of the experimental concentrations, as well as for controls, 150 mitotic plates were examined on coded slides.

In vivo test. The BALB/c AnNCR inbred strain of mouse was used for *in vivo* assay. Animals were kept under standard conditions: (21 ± 1)^{0}C, humidity 55-60%, food and water *ad libitum.* During the course of the experiment their body weight was 20-25 g. For each of the experimental concentrations (1, 3 and 5 mg/kg), as well as for controls, 10 male animals were treated intramuscularly with Sinhrogal^R and control preparation respectively. At 24 hours after treatment the animals were injected intraperitoneally with colchicine (4 mg/kg). Chromosome preparations were made according to the method of Hsu and Patton (1969). Cytogenetic analysis implied observation of heteroploid and polyploid cells, structural chromosomal aberrations and mitotic activity.

Statistical analysis of the obtained results was performed with Student's t-test.

RESULTS AND DISCUSSION

Genotoxicological analysis of the effects of cloprostenol included assessment of numerical (aneuploid and polyploid cells) and structural aberrations (chromosome and chromatid gaps and breaks) in PHA-activated human peripheral blood lymphocytes and bone marrow of BALB/c AnNCR male mice. In order to evaluate possible cytostatic or cytotoxic effects of cloprostenol, the mitotic index (the percentage of cells during mitosis) was established for each examined concentration, negative and positive controls.

The results of the *in vitro* cytogenetic test are shown in Table 1. The level of structural aberrations for experimental concentrations of cloprostenol ranged from 1.97% to 3.47% and those changes were not statistically significant in comparison to the value of the negative control (2.00%). Metaphase chromosomes isolated from cultures treated with 10 μg/ml of cloprostenol are shown in Figure 1. Only treatment with the positive control (10^{-6} M MNNG) caused

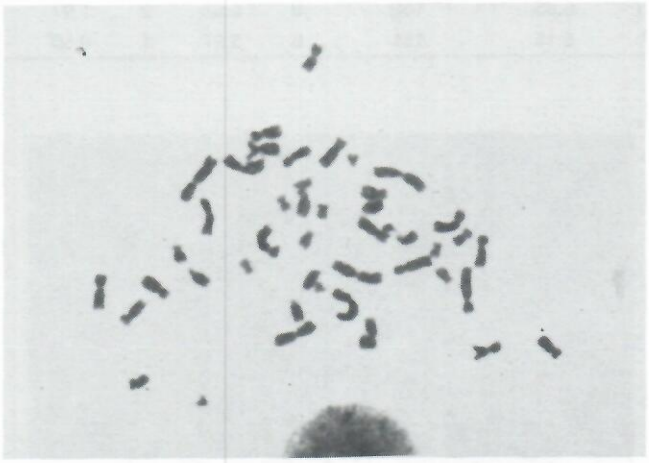

Figure 1. Giemsa stained human metaphase chromosomes isolated from a culture treated with 10 μg/ml of cloprostenol

a significant ($p<0.01$) increase in the percentage of mitoses with gaps and breaks. As for numerical aberrations, the number of polyploid and aneuploid cells was not significantly changed either after administration of cloprostenol, or after treatment with positive control.

In order to ascertain wherther cloprostenol can exhibit genotoxic effects in vivo, chromosome preparations from the bone marrow of BALB/c inbred strain

were analyzed after single administration of cloprostenol (Table 2). The level of structural as well as numerical aberrations was similar to the values observed in cultured human peripheral blood lymphocytes obtained in this experiment. Only the positive control (MNNG) gave rise to a significant increase in aneuploid cells ($p<0.01$) and cells with structural aberrations ($p<0.001$) (an example is shown in Figure 2), whereas the level of polyploid cell did not depart markedly from the negative control. On the other hand, there were no significant changes in the levels of numerical and structural aberrations for all cloprostenol concentrations applied. A metaphase spread from an animal exposed to the highest dose of cloprostenol is presented in Figure 3.

Table 1. The effects of cloprostenol in lymphocyte cultures of human peripheral blood

Conc.of cloprostenol	Mitotic index (%)	Number of cells observed	Aneuploid cells		Polyploid cells		Cells with gaps and breaks	
			No	%	No	%	No	%
negative control	5.60	150	7	4.67	2	1.33	3	2.00
positive control (MNNG)	3.83	150	11	7.33	5	3.33	22	14.67**
1 μg/ml	5.98	144	6	4.17	1	0.69	5	3.47
2 μg/ml	5.34	153	4	2.61	2	1.31	3	1.96
5 μg/ml	6.35	152	9	5.92	2	1.97	4	2.63
10 μg/ml	6.15	151	6	3.97	1	0.66	3	1.97

** $p<0.01$

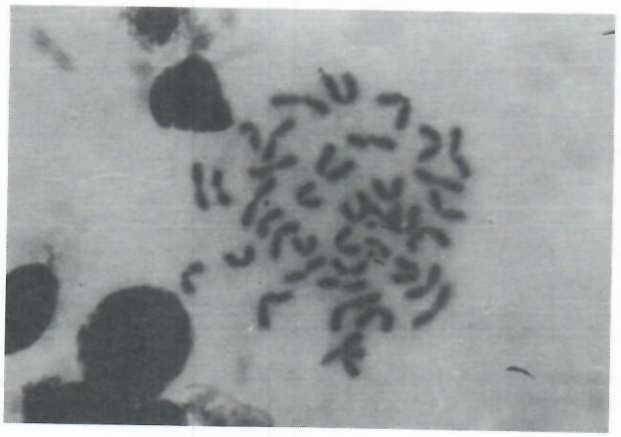

Figure 2. Acentiric fragments (indicated by arrows) as a consequence of the mutagenic action of MNNG in mouse cell

In this experiment mainly structural chromosomal aberrations like gap and break damage of chromosomes, which indicate clastogenic properties could be

detected. For the scoring of gaps and breaks we used criteria described by Brogger (1982).

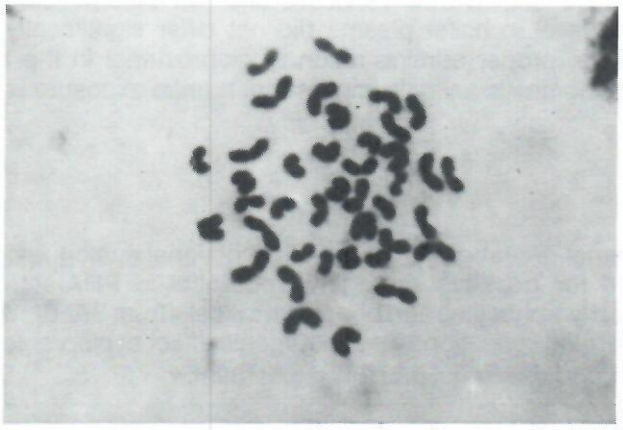

Figure 3. Metaphase spread from mouse bone marrow after treatment with 5 mg/kg of cloprostenol

The values of mitotic index (MI) are shown in Tables 1 and 2. Obviously, variations in the values of MI are not significant either for all experimental concentrations administered *in vitro*, or for all doses of cloprostenol injected into mice.

Table 2. The effects of cloprostenol on bone marrow cells of BALB/cAnNCR male mice

Dose of cloprostenol	Number of treated animals	Mitotic index (%)	Number of cells observed	Aneuploid cells		Polyploid cells		Cells with gaps and breaks	
				No	%	No	%	No	%
negative control	10	2.05	500	21	4.20	5	1.00	13	2.60
positive control (MNNG)	10	1.65	500	44	8.80**	7	1.40	77	15.40***
1 mg/kg	10	1.95	500	20	4.00	6	1.20	10	2.00
3 mg/kg	10	2.60	500	31	6.20	8	1.60	11	2.20
5 mg/kg	10	2.55	500	29	5.80	6	1.20	9	1.80

** $p<0.01$ *** $p<0.001$

Therefore, on the basis of the results obtained by the cytogenetic analyses described in this paper it can be concluded that cloprostenol has no expressed genotoxic properties. It should be mentioned, however, that the same agent was characterized as potentially genotoxic in SCE (sister chromatid exchange) test in vitro (Djelić et al., 1994). This discrepancy can be accounted for by the higher

sensitivity of the SCE test in comparison to the analysis of structural and numerical chromosomal aberrations (Perry, 1980). These findings indicate that cloprostenol might be designated as a relatively weak mutagen. In addition, it should be pointed out that cloprostenol is rapidly metabolized in treated animals. Pichova et al. (1983) have shown that 13 hours after an intramuscular injection of cloprostenol its level in heifer plasma did not differ significantly from control values. Therefore, proper administration of cloprostenol in the breeding and reproduction of domestic animals implies that human exposure is almost negligible.

CONCLUSION

Cloprostenol (Sinhrogal^{R}), a synthetic functional analogue of PGF2 alpha, was evaluated for possible mutagenic potential in PHA-activated human peripheral blood lymphocytes and bone marrow cells from BALB/c AnNCR inbred mice. There were no indications for a mutagenic effect of cloprostenol within the concentrations and doses used in this experiment.

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IN VITRO I IN VIVO CITOGENETIČKA ANALIZA EFEKATA KLOPROSTENOLA NA ĆELIJAMA SISARA

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SADRŽAJ

Cilj ovih istraživanja bio je da se izvrši evaluacija moguših genotoksičnih efekata kloprostenola (Sinhrogal‰) – sintetičkog analoga prostaglandina R2 alfa koji se upotrebljava za sinhrnizaciju estrusa domaćih životinja. Mutageni potencijal test substance ispitivan je na humanim limfocitima periferne krvi (in vitro) i kostnoj srži intramuskularno tretiranih mužjaka miševa soja BALB/cAnNCR (in vivo). Praćene su numeričke i strukturne hromazomske aberacije i određivan je mitotski indeks(procenat ćelija u mitozi). Kloprostenol nije ispoljio mutagene efekte u okviru eksperimentalnih koncentracija i doza upotrebljenih u ovom eksperimentu.