Acta Veterinaria (Beograd), Vol. 44. No. 5—6, 337—344, 1994.

UDK 619:575.224.232

EFFECT OF CLOPROSTENOL ON THE MITOTIC INDEX AND SCE FREQUENCY IN CULTURES OF HUMAN LYMPHOCYTES

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(Received, 3. May 1994.)

Genotoxic and cytotoxic effects of cloprostenol (a functional analogue of prostaglandin F-2-alpha) in human lymphocyte cultures were investigated by monitoring the frequency of sister chromatid exchange (SCE) and by determining the mitotic index (MI) of cells.

The treatment with cloprostenol led to a significant increase in SCE level after the administration of 1,5 and 10 μg/ml concentrations; whereas, at the 2 μg/ml concentration there was no significant change in SCE frequency per cell in relation to the level of the negative control. Therefore, it might be concluded that cloprostenol is a genotoxic agent. On the other hand, mitotic index values did not depart markedly from those in the negative control. This means that cloprostenol did not produce cytotoxic effects within the administered concentrations.

Key words: cloprostenol, prostaglandin F-2-alpha, genotoxicity, sister chromatid exchange (SCE), mitotic index (MI)

INTRODUCTION

Prostaglandins are synthesized in all tissues of mammals (Alberts et. al., 1989) in response to different stimuli; and it is well known that they modulate a great number of biochemical processes (Oliw et al., 1983; Chiu and Richardson, 1985).

Prostaglandin F-2-alpha and its functional analogue - cloprostenol, produce a series of physiological effects via binding to specific membrane receptors. It has been shown that astrocytes of rats in culture possess PGF-2-alpha receptors bound to phospholipase C (Kitanaka et al., 1991). After binding of PGF-2-alpha to the receptor, Gp protein is activated and it stimulates phosphoinositide specific phospholipase C which breaks down phosphoinositide-4,5-diphosphate into inositol-1, 4,5-triphosphate and diacylglycerol.

Inositol-triphosphate increases the level of intracellular Ca^{2+}, whilst diacylglycerol activates the enzyme protein kinase C (PKC). PKC can phos-

phorylate a great number of proteins, particularly at serine and threonine residues. The activity of PGF-2-alpha does not only imply an increase in intracellular Ca^{2+} induced by inositol-triphosphate, but also direct stimulation of Ca^{2+} release from intracellular resources (Morimoto et al., 1990; Rodway et al., 1991). It has been shown that PGF-2-alpha modulates cAMP response via PKC activation. It is considered that PKC acts upon the catalytic part of the enzyme adenylate cyclase (Wada et al., 1991).

According to the existing data the effect of PGF-2-alpha on the genetic material of eukaryotic cells has not been thoroughly investigated. It has been found (Chegini et al., 1991) that both in small and large luteal cells in the cow PGF-2-alpha causes chromatin decondensation, without a change in the volume of the cell nucleus. PGF-2-alpha stimulates proliferation of clonal osteoblasts MC3T3-E-1 mostly by stimulating phospholipase C (Hakeda et al., 1991) which results in an intensified synthesis of DNA. In addition to this, PGF-2-alpha stimulates proliferation of primary hepatocytes and increases the expression of protooncogene c-myc (Skouteris et al., 1991).

On the basis of experimental data showing that PGF-2-alpha has an impact on eukaryotic cell chromatin, we undertook to investigate some possible genotoxic properties of the preparation Sinhrogal^{R} which contains cloprostenol as an active component. Cloprostenol is a synthetic analogue of PGF-2-alpha with a slower biotransformation time and a more profound effect.

The use of cloprostenol is primarily based on its luteinic effect, namely its functional and morphological regression of the corpus luteum, in which way an effective synchronization of oestrus in domestic animals is achieved.

MATERIALS AND METHODS

Among various modifications (Pfeiffer 1974; Evans and O'Riordan 1975; Buckton and Evans 1975) of basic methods for the cultivation of human peripheral blood leucocytes (Arakaki and Sparkes, 1963) and chromosomal preparations based upon them (Moorhead et al., 1960), we have chosen for this work the slightly modified protocol described by Evans and O'Riordan (1977). Human lymphocyte cultures were prepared from heparinised whole blood samples obtained from a healthy man under 30 years of age. With the aim to provide successive vizualization of SCE, 5-bromo-2'-deoxyuridine (BUdR, Sigma Chemical Co., final concentration 25 μM) was added in each culture.

At 47 h and 30 min. after the beginning of incubation at 37°C, cloprostenol (CAS number 40665-92-7, Sinhrogal^{R}, ICN Galenika) was added to the cultivation vials in amounts to obtain final experimental concentrations of 1,2 5 and 10 μg/ml. Both negative and positive (10^{-6} M MNNG) controls were used.

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) centrifugation and resuspension, the cell suspension was dropped on microscopis slides, air dried and aged for 96 hours before staining.

Differential staining for the inspection of sister chromatid exchange (SCE) rate was performed according to the FPG procedure described by Perry and Wolff (1974).

For each of the experimental concentrations as well as for controls, 30 well spread mitoses (Wulf et al., 1984) were inspected for SCE scoring according to the established criteria (Perry and Thompson 1984), while the mitotic activity was analysed according to the standard procedure. Statistical analysis of experimental values was performed by Student's t-test.

RESULTS AND DISCUSSION

Analysis of the effect of cloprostenol in cultures of PHA activated human peripheral lymphocytes in vitro was based on two parameters, i. e. SCE frequency per cell and MI values.

SCE frequency was observed in order to ascertain some possible genotoxic effects of cloprostenol. The results are shown in Table 1 and Figure 1. The SCE level in the negative control was 5,92± 0,26; whereas the use of the positive control (10^{-6} M MNNG) gave rise to a significant increase of SCE ($p < 0,001$) per cell up to 13,12± 1,11. Within the investigated cloprostenol concentrations it was noticed that a significant ($p < 0,05$) increase in SCE level occurred at the lowest concentration (1 μg/ml). However, at 2 μg/ml there was a slight decrease in SCE value per cell. Thus, SCE level did not show any marked departure from the negative control values. At 5 and 10 μg/ml concentrations there was a significant ($p < 0,001$) increase in SCE values per cell.

Table 1. The effect of cloprostenol on SCE frequency in lymphocyte cultures of human peripheral blood

Concentration of cloprostenol	MI	SCE per cell X min-Xmah	X SCE	SD	SE	Xk = 100%
negative control	5,60	3 – 9	5,92	1,26	0,26	100
positive control	3,83	7 – 28	13,12**	5,41	1,11	221,62
1 μg/ml	5,98	4 – 10	6,84*	1,67	0,34	115,54
2 μg/ml	5,34	4 – 12	6,56	1,94	0,40	110.81
5 μg/ml	6,35	5 – 11	7,71**	1,54	0,36	130,24
10 μg/ml	6,15	6 – 13	8,35**	1,46	0,32	141,05

SD - standard deviation
SE - standard error
MI - mitotic index
* - $p < 0,05$
** - $p < 0,001$

On the other hand, MI values (Table 1 and Figure 2) showed no significant departures in the positive control or at any experimental concentrations in relation to the negative control level. The lowest MI(3,83%) was observed after the use of the positive control (10^{-6} M MNNG) whereas MI values in the negative control and at the experimental concentrations of cloprostenol ranged from 5,34% to 6,35%.

The experimental approach in the analyses of SCE frequency in most cases proved to be one of the most sensitive and reliable methods for the

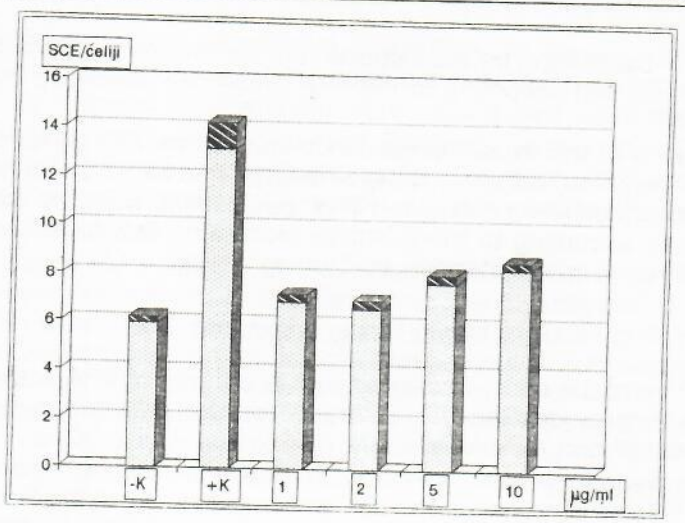

Figure 1. The effect of cloprostenol on the SCE frequency

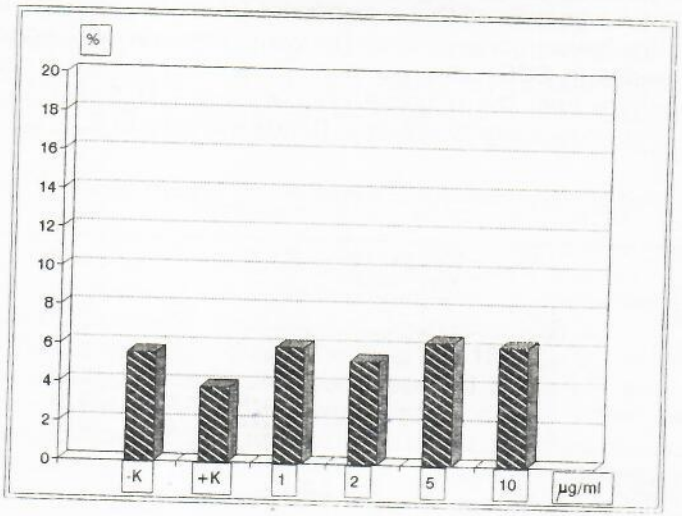

Figure 2. Mitotic index values after treatment with cloprostenol

detection of damage to the DNA molecule caused by various chemical genotoxic agents (Perry and Evans, 1975; Abe and Sasaki 1977; Wolff 1977; Perry 1980). In addition, many authors have pointed at the existence of a strong linear correlation between an induced SCE frequency and the appearance of genetic mutations (Carrano et al., 1978; Siriani and Huang 1980; Nishi et al., 1984; Brooks et al., 1984).

Our results are in full agreement with those that show that PGF-2-alpha increases SCE level per cell in cultures of human peripheral lymphocytes (Das et al., 1989).

Therefore, on the basis of the results obtained, it can be concluded that cloprostenol is a potentially genotoxic agent. However, if administered properly in the breeding and reproduction of domestic animals, the level of human exposure becomes negligibly low. Namely, it is indispensable to take care and not use animals as food for at least 24 hours after the administration of a cloprostenol injection. During that time, cloprostenol is metabolized so that 13 hours after an intramuscular injection the level of cloprostenol in heifers' plasma is almost negligible (Pichová et al., 1983.)

On the other hand, on the basis of the results obtained for MI values it is noticeable that cloprostenol did not produce any cytotoxic effects within the range of the concentrations used.

The molecular mechanism mediating the genotoxic effect of cloprostenol remains a secret for the time being.It is possible to presume that intracellular events after phospholipase C stimulation, bring about a change in the existing cell physiology, which, among other things, leads to an increase in various types of damages to the DNA molecule.

Although the mechanism of the mutagenic effect of cloprostenol is unexplained for the time being, it might be of some interest to note that PGF-2-alpha can modify genetic damage caused by some mutagenic agents (Das et al., 1989). It has also been noticed that some tumor cells contain a larger quantity of PGF-2-alpha (Bennet et al., 1981), whilst many mutagenic and carcinogenic agents stimulate PGF-2-alpha synthesis in cells (Levine 1977). That is why it is thought that one of the mechanisms by which mutagens and/or carcinogens induce DNA molecule damage is this change in the levels of prostaglandin or their precursors.

There are hypotheses that PGF-2-alpha can activate oncogenes and in that way contribute to the appearance of malignant cells (Das et al., 1986). However, precise mechanisms for the appearance of some damage or changes in the DNA molecule are not known, and remain a subject for further investigation in this field.

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UTICAJ KLOPROSTENOLA NA MITOTSKU AKTIVNOST I FREKVENCU SCE U KULTURAMA LIMFOCITA ČOVEKA

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SADRŽAJ

Ispitivani su genotoksični i citotoksični efekti kloprostenola (funkcionalnog analoga prostaglandina F-2-alfa) u kulturama humanih limfocita praćenjem frekvence razmene sestrinskih hromatida (SCE) i određivanjem mitotske aktivnosti (MI) ćelija.

Tretman kloprostenolom doveo je do značajnog porasta u nivou SCE nakon primene koncentracija od 1, 5 i 10 μg/ml, dok pri koncentraciji od 2 μg/ml nije došlo do značajne promene u frekvenci SCE po ćeliji u odnosu na nivo kod negativne kontrole. Prema tome, moglo bi se zaključiti da je kloprostenol genotoksičan agens. S druge strane, vrednosti mitotoskog indeksa ne odstupaju značajno od onih kod negativne kontrole, što znači da kloprostenol nije ispoljio citotoksične efekte u okviru primenjenih koncentracija.