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TOXIC EFFECTS OF PIRPROFEN ON CHROMOSOMES AND BLOOD CRITERIA IN MICE

BY

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INTRODUCTION

Antirheumatics are important group of medicine since large number of people are using them, and in some cases for long periods.

The most common antirheumatic drugs are those the non-steroidal anti-inflammatory drugs with analgesic and anti-pyretic properties. From this class of antirheumatics; the propionic acid derivatives, e.g. Ketoprofen, Ibuprofen, Fenoprofen, Flurbiprofen, Benoxaprofen and Pirprofen (Rengasil)(R). The side effects of non-steroidal antirheumatics should not be over looked when treating patients. As estimate of 80 - 90% of human cancers were reported to be due to their side effects (Amlacher and Ziebarth 1979). Antirheumatics have also been found to have teratogenic activity (Wassef 1980, Maria et al 1980 and Karsh et al., 1980).

Some studies showed that most of the available non-steroidal anti-inflammatory antirheumatics influence deoxyribonucleic acid (DNA)- synthesis (Klein et al., 1980 and Hoffer et al. 1984). This may damage the genetic material and lead finally to cancer production. On the other hand klein and wottawa (1976) and Hoffer et al. (1984) investigated no effect of propionic acid

derivatives, antirheumatic drugs (Ketoprofen-Benoxaprofen-Pirprofen)on DNA-synthesis.

The present study deals with the cytogenetic and haematological effects of pirprofen. It is one of the most recent non-steroidal anti-inflammatory antirheumatic drugs used for treatment of pain and inflammation. Pirprofen shows an excellent analgesic, antipyretic and anti-inflammatory potency, which gives it a wide therapeutic margin. The analgesic and antipyretic action of pirprofen were found to be more potent than that of other non-steroidal anti-inflammatory drugs (Maier et al 1981).

Up tell now, some reports were carried out about genotoxicity of those propionic acid derivative drugs. Hoffer and Tuschi (1982) and Hoffer et al., (1984) reported slight increase in sister chromatid exchange (SCE) rates on human lymphocytes after using pirprofen and Benoxaprofen.

On the other hand Hoffer et al., (1984) using Ames test, non-genetic activity for pirprofen and Benozaprofen were detected. Kullich and Klein (1985) investigated negative results for pirpofen to give any genetic effects on human lymphocytes after administration of the drug for two weeks using usual doses (800 mg/day). The same authors (1986) showed that Pirprofen, Ketoprofen, Ibuprofen and Flurbiprofen had no effect for induction of sister chromatid exchange rates in human lymphocytes when used as usual doses for 14 days.

Few published studies, Chalchat (1985) and Khan (1985), mentioned that pirprofen did not produce any side-effect on the blood of patients during clinical trials.

The aim of our study was to evaluate and shed some light on the cytogenic and haematological effects of pirprofen in mice.

MATERIALS AND METHODS

To study the side-effects of pirprofen on mice chromosomes and blood, the following steps were carried out: Pirprofen (Rengasil)(R), CIBA-GEIGY EGYPT, was obtained as a powder (99.5% active ingredient). It has the following structure:

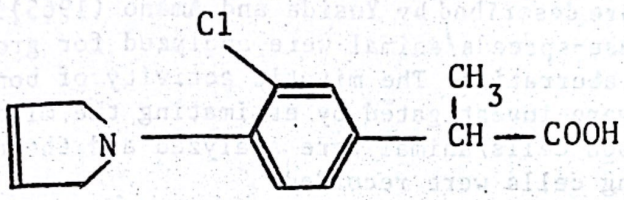

Fig. 1: 2-[3-chloro-4-(3-Pyrrolin-1-YL)-Phenyl]-Propionic acid.

The chemical structure of pirprofen, as shown in Fig. 1 bears certain similarities to that of other anti-inflammatory agents. However, possesses an amino function which gives it amino acid-like properties that account for a certain uniqueness in its chemical, and possibly also in its biological, behaviour. The oral LD_{50} in mice was found to be 900 mg/kg. b.wt (Maier et al.,1981).

Animals:

Swiss mice (22-25 grams) provided by the animal house of the National Research Center were used. Rengasil(R) is disolved in 20% benzyl alcohol and administered orally to female mice every day for 19 days.

Mice were randomly allocated into 4 groups, ten mice each. The first group received the solvent (20% benzyl alcohol) and acted as control. The other three groups

recived three different dose levels of pirprofen (90, 112.5 & 450 mg/kg. b.wt) which correspond to one-tenth, one-eighth and one-half of the LD_{50} respectively.

Procedure for chromosomal aberration:

For carrying out chromosomal analysis, the animals were treated with 0.6 mg/kg. b.wt colchicine two hours before sacrifice. Metaphase spreads of bone marrow cells were prepared according to the standerd cytogenetic procedure described by Yosida and Amano (1965). Fifty metaphase-spreads/animal were analyzed for gross chromosomal aberration. The mitotic activity of bone marrow cells were investigated by estimating the mitotic indices. 1000 cells/animal were analyzed and the number of dividing cells were recorded.

Procedure for haematological investigation:

At the end of treatment, venous blood samples were obtained by means of capillary glass tubings from the retro-orbital plexus under light ether anaesthesia by the procedure described by Schermer (1967). A volume of 1 ml of the blood was collected in small plastic tubes of 1.5 ml capacity containing 0.1 ml of 1.5% potassium salt of ethylenediamine tetracetic acid (EDTA) as anticoagulant. Haematological investigations included determination of haemoglobin concentration, packed cell volume (haematocrit value) and total erythrocytic and leucocytic counts.

The haematological investigationswere carried out by manual Instruction for Coulter, Model M430, Ref. N. : 9242055 present in Auto Analysis Unit, National Research Center, Dokki, Cairo.

The results obtained were analysed using the student's t-test (Fisher and Yates, 1957) to evaluate significant changes between mean values for treated mice and the corresponding mean values of control ones.

RESULTS

Effect on Chromosomes:

The number of cells with chromosomal aberrations after treatment with different doses of pirprofen are tabulated in Tables 1 and 2 .

Animals receiving (90 & 112.5 mg/kg. b.wt) whowed no significant increase in number of cells with aberrations. Those receiving 450 mg/kg. b.wt showed higher percentage of cells with aberration which were mainly breaks, end to end association and centromeric attenuation. The increase in breaks and end to end association were significant ($P < 0.05$). On the other hand the increase in centromeric attenuation, was highly significant ($p < 0.01$).

Effect on Mitotic Activity:

No significant effect on mitotic activity was recorded after treating mice with different doses of the drug (Table 1). Even at high dose of 450 mg/kg. b.wt of pirprofen did not show any significant effect on mitotic activity.

Effect on Blood Picture:

Tables 3 and 4 present the results obtained for the effect of the test-compound on blood picture. Pirprofen did not reveal any significant changes in the hematological data of treated mice from the corresponding control.

DISCUSSION AND CONCLUSION

The present study showed that low doses (90 and 112.5 mg/kg.b.wt) of pirprofen when administered for 19 days did not induce any significant increase in chromosomal aberrations or mitotic activity. These results correlate with those obtained by klein and Wottawa (1976) who reported no effect of ketoprofen (Propionic acid

Table 1: Effect of pirprofen on Chromosomal Aberrations and Mitotic Activity in Mice.

Dose(mg/kg . b.wt)	No. of examined cells in 10 mice.	Total cells with aberrations %		Cells with chromosomal abberations %			Effect on mitotic activity
		No.	X ± S.E.	Break	End to end asso.	Centromeric atten.	X ± S.E.
0.0 Control	500	75	15.00±1.42	4.80±0.32	5.40±0.30	4.80±0.44	57.70±1.16
90	500	86	17.20±0.74	5.60±0.48	5.60±0.48	6.00±0.42	55.40±0.48
112.5	500	90	18.00±0.84	5.60±0.26	6.40±0.64	6.00±0.42	55.20±1.50
450	500	98	19.60±0.66**	6.00±0.42*	6.40±0.26*	7.20±0.32**	55.00±1.90

* Significant at $P < 0.05$

** Significant at $P < 0.01$

Dose(mg/kg.b.wt)	Types of Chromosomal aberrations		
	Break	End to end association	Centromeric attenuation
90	16.66%	3.70 %	25.00 %
112.5	16.66%	18.51 %	25.00 %
450	25.00%	18.51 %	50.00 %

Table 3: Blood Picture of Mice Treated with pirprofen [Mean values ($\bar{X}$) ± S.E.].
N.B.: Values represent means of 10 mice (N = 10).

Dose (mg/kg. b.wt)	Haemoglobin (Hb)(gm/dl)	Haematocrit Hct %	Red blood cells (RBCs) (10^6/ml)	White Blood cells (WBCs) (10^3/ml)
0.0 (Control)	13.85±0.20	46.78±0.40	9.31±0.10	7.43±0.30
90	13.78±0.06	45.89±0.42	9.12±0.07	6.95±0.17
112.5	13.82±0.16	45.94±0.67	9.24±0.12	7.23±0.11
450	13.62±0.04	46.40±0.14	9.25±0.05	7.06±0.25

Table 4: Percentage Decrease of Haematological data from the Corresponding Control Mice.

Dose(mg/kg.b.wt)	Hb (gm/dl)	Hct %	RBCs (10^6/ml)	WBCs (10^3/ml)
90	-0.51%	-1.90%	-2.04%	-6.46%
112.5	-0.22%	-1.80%	-0.75%	-2.69%
450	-1.66%	-0.81%	-0.64%	-4.98%

derivative drug) in DNA-synthesis. Also Hoffer et al (1984) found that Rengasil(R) and Benoxaprofen had no effect on DNA-synthesis. Similar results were obtained by Wottawa et al., (1982), who reported no effect of ketoprofen on sister chromatid exchange rates after three weeks. Slight increase in sister chromatid exchange rates was investigated by Hoffer and Tuschi (1982) after using Rengasil(R) (Pirprofen) and Benoxaprofen on human lymphocyte cultures. Also Benoxaprofen and Rengasil(R) were investigated in three different genetic test systems. In the Ames test, none of the two drugs showed mutagenic activity. In the determination of sister chromatid exchange rates (SCE) with lymphocytes of healthy donors, the two drugs slightly increased (SCE) rates. The two drugs,also,showed no effect on DNA-synthesis (Hoffer et al., 1984). Our results are consisting with the results of Kullich and Klein (1985) who found no effect for pirprofen on the sister chromatid exchange frequencies in human lymphocytes using usual doses of the drug for some weeks. Similar negative results were also obtained by Kullich and Klein (1986), after using pirprofen in a dose of 800 mg/day, ibuprofen, flurbiprofen and ketoprofen. None of the above mentioned propionic acid derivative drugs produced any genetic damage on human lymphocytes after two weeks.

From the present results,it is concluded that pirprofen is not a direct mutagen, when used in low doses.

Using high dose level of the drug (450 mg/kg.b.wt), a significant increase in chromosomal aberrations was recorded. These aberrations were represented by breaks, end to end associations and centromeric attenuations.

In spite of the positive effect of high dose on chromosomes, it did not affect the mitotic activity which indicates that pirprofen has no cytotoxic effect.

The statistical analysis of the haematological data of the three doses did not show any significant changes from the corresponding control mice. These results consist with those of Chalchat (1985) who observed that in France, 3069 patients with rheumatoid arthritis , ankylosing spondylitis or osteoarthritis, were treated with Rengasil(R) (800 - 1200 mg/day) for one year in a multicentre study, did not display serious haematological side effects. Khan (1985) also found that, the routine blood examination before and after treatment with Rengasil(R) (400 mg/day) of 1000 patients suffering from soft tissue rheumatism, did not show any change.

Finally, when talking about the drug, risk and benefit should be considered very carefully, whereas the amount of chromosomal abberations after using high dose of Rengasil(R) (450 mg/kg.b.wt) represents an amount of risk which must be kept in mind during treatment of patients.

SUMMARY

Pirprofen (Rengasil)(R) is one of the recent non-steroidal, anti-inflammatory antirheumatic drugs of propionic acid derivative. This study was carried out on female mice for 19 days using three different doses, to evaluate the cytogenetic and haematological effects of pirprofen on chromosomes and blood picture to get a firm conclusion about its side effects.

At dose levels of 90 & 112.5 mg.kg.b.wt pirprofen did not induce any significant increase in chromosomal aberrations. However after treatment with 450 mg/kg.b.wt a significant increase in chromosomal aberrations was found.

None of the three tested doses had any effect on mitotic activity and blood picture of treated mice. On the other hand, its use in high doses includes amount of risk on cytogenetic activity which must be kept in mind.

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