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IN VIVO GENOTOXICITY STUDIES OF PRULIFLOXACIN

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ABSTRACT

In vivo genotoxicity of Prulifloxacin, a difluorinated antibacterial drug, was evaluated by employing mouse *In vivo* chromosomal aberration test in bone marrow cells. Mice (6-8 weeks) were segregated in to 10 groups (n=6) for time dependent study (12 and 24 hrs). Two groups of animals were injected distilled ,water intraperitoneally for use as vehicle control Another two groups were administered Cyclophosphamide (10mg/kg) and the remaining six groups of animals were administered Prulifloxacin (33.3, 66.6 and 133.3 mg/kg), two groups for each dose through the same route. Animals were sacrificed 12 and 24 h after treatment by cervical dislocation. Ninety minutes before sacrifice animals were injected 0.2 ml of Colchicine (4 mg/kg). Bone marrow was flushed in 0.056 % KCL, centrifuged for 10 min and fixed in cold fixative; flame dried and stained in 10% buffered Giemsa stain. Statistically significant increase in chromosomal aberrations, reduction in mitotic index and increased percentage of abnormal metaphase were observed at the highest dose (133.3 mg\kg) of the drug. This increase in percentage of damage was lesser than the positive control group while more than the control group. This result seems to indicate that Prulifloxacin is a weak c1astogen in the bone marrow cells.

Keywords:- Genotoxixity, Prulifloxacin, Chromosomal aberrations and Mitotic.

INTRODUCTION

Prulifloxacin is fluoroquinolone and widely used in medicine. The principle of its therapeutic activity, as for all fluoroquinolones, is the inhibition of gyrase, a specific Prykaryguc enzyme. Gyrase catalyses the conversion of relaxed DNA in to negatively super coiled DNA. A combination of gyrase and topoisomerase -I is required to correct DNA topology during replication and transcription. The inhibition of gyrase by quinolones triggers replication arrest and cell death. Prulifloxacin is given to treat infections of the lower respiratory system, urinary tract, sinuses, the skin, bones and joints, and prostate. Prulifloxacin is also used for inhalational anthrax, STDs, severe bronchial infections, infectious diarrhea, typhoid fever, and pneumonia [1].

It has been reported that fluoroquinolones also show cross-reactivity with other enzymes involved in the process of DNA replication. These compounds thus induce a variety of genotoxic effects due to their ability to inhibit mammalian topoisomeraseII activities including induction of transient DNA strand breaks during replication, chromosomes condensation and disjunction during meiosis. As a consequence of these effects fluoroquinolones have shown genotoxic effects in pro-and eukaryotic test systems. However it has to be taken in to account that several orders of magnitude higher concentrations as for gyrase are normally needed for topoisomerase-Il inhibition. There are several reports on the photogenotoxicity of many fluoroquinolones. Recent reports on the photogenotoxicity indicated that fluoroquinolones have potent genotoxicity under UV or visible irradiation [2-4].

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Based on their specific mode of action and, enzyme inhibition, and in contrast to chemical mutagens which directly interacts with DNA, no effect levels can be defined for fluoroquinolones, which act by enzyme inhibition. If no enzyme inhibition takes place no genotoxic effect can be induced. Nevertheless, the safety of fluoroquinolones in antimicrobial therapy requires careful evaluation on case by case basis. Therefore, *In vivo* genotoxicity data's of several quinolones are presented and discussed in several published papers [5-7]. But in our knowledge, there have been no published studies investigating chromosomal aberration frequencies in mouse bone marrow model. The aim of present study was to investigate any possible effects of Prulifloxacin on bone marrow chromosomal aberrations (CA) and mitotic index (MI) in the bone marrow of mice.

MATERIAL AND METHODS

Chemicals

Prulifloxacin procured as a gift sample from SAGA Laboratories Pvt. Ltd., Ahmedabad, Cyclophosphamide obtained from Cadila healthcare Ltd., Ahmedabad, Colchicines from himedia Laboratories, Mumbai and Giemsa stain was procured from Sd fine Chemicals Pvt. Ltd., India.

Animals

Swiss albino mice with an average body weight of 25 ▫ 5 g were used in the present study. The mice were obtained from central animal facility of S K Patel College of Pharmaceutical Education and Research, Mehsana. They were housed in polypropylene cages and given food and water *ad libitum* and maintained under good laboratory practice (GLP) condition at a temperature of 22 ± 2^0 *C* and] 2/ 12-h light/dark cycle and at a relative humidity of 50 ± 15%.

Experimental design

Swiss albino Mice (6-8 weeks old) of either sex were segregated into 10 groups (n=6). Two groups of animals were injected distilled water intraperitoneally for use as vehicle control. Another two groups were administered Cyclophosphamide (10 mg/kg) and the remaining six groups of animals were administered Prulifloxacin (33.3, 66.6 and 133.3 mg/ kg), two groups for each dose through the same route. Animals were sacrificed 12 and 24 h after treatment by cervical dislocation.

Chromosomal aberration assay

After the treatment with Prulifloxacin (12-24 h before scarify), the animals were injected 0.2 m1 (4 mg/kg) of colchicine through intraperitoneal injection and were sacrificed by cervical dislocation after 90 min. Bone marrow from both the femurs was collected in hypotonic solution (0.056%KCL) and incubated at 37°C for 20 min and fixed in cold fixative solution (Methanol: Acetic Acid; 3: 1). The permanent slides were prepared by the dropping the cell suspension on pre chilled blank slides and these slides were gently flame dried. The prepared slides were stained with Giemsa stain and observed at 100 x oil immersion [8-10].

Screening of aberration

One-hundred well spread metaphases per animal were examined to a total of 600 metaphases for each treatment/control group. Different types of aberrations were studies like, Chromatid/chromosome gap arid break, ring, stickiness and fragmentation. The gaps were not included in the calculation for the analysis. Stages of mitotic index among 500 cells were calculated per animal [11-13].

Statistics

Results were expressed as Mean ± S.E.M. Statistical significance between two groups was tested using unpaired two-tailed student's t-test as appropriate using computer based fitting program (Prism, Graph pad.). Differences were considered to be statistically significant if $p < 0.05$.

RESULT AND DISCUSSION

The data of bone marrow chromosomal aberration assay are depicted in table 1. The results of current study revealed that the mice of negative control group showed 8.15±O.13 and 4.01 ±O.63, 10.02+0.79 and 4.11+0.60 % of total aberrations and % aberrations (excluding gaps) in 12 and 24 hrs studies, respectively (Table 1). In the positive control group the 12 and 24 hrs study mice showed 20.23±1.08 % aberrant metaphases with 7.7+0.35 % aberrations (excluding gaps) per hundred metaphases, and 25.82+0.96 % aberrant metaphases with 11.58±0.75 aberrations per hundred metaphases, respectively. The increased percentages of aberrant metaphases and percent of CAs (excluding gap) in both studies of the positive control group are statistically highly significant (p<O.OO 1) when compared to that of the respective negative control groups of mice (Table 1). Prulifloxacin 33.3, 66.6 and 133.3 mg/kg induced average percentages of aberrant metaphases in the mice were 8.31 +0.20 and 3.0±0.25, 11.72±0.84and5.3±0.28, 14.48+0.81 and 6.13+0.31, 12hrs after exposure and 11.6±1.24and4.93±O.36,12.7±O.98 and6.35±0.65,15.22+0.85 and6.03±0,55, respectively after 24 hrs of exposure of Prulif1oxacin. The percentages of aberrant metaphases and aberrations per hundred metaphases induced by all the three tested doses of Prulifloxacin in mice are significantly higher than their respective vehicle control groups of mice (Table 1).

Mice of the negative control group showed 5.75±0.59 and 6.20±O.87 average percentage of mitotic index for 12 and 24 hrs studies, respectively. Their counter parts in the positive control group showed 3.09+0.64.ancl 2.12+0.38 average percentage of mitotic index, respectively, with significant difference from that of the negative control mice (Table 1). In the groups of mice that received Prulifloxacin 33.3, 66.6 and 133.3 mg/kg, the average percentages of dividing cells was 5.75+0.60, 5.45+0.70 and 5.51±O.30 respectively 12 hrs after

administration of Prulifloxacin. While after 24 hrs it was 6.14±O.58, 5.90±0.56 and 5.94±0, 84, respectively which is significant compare to negative control (Table 1).

The genotoxic nature of several fluoroquinolone antibacterial drugs is unequivocal. These drugs inhibit DNA gyrase in bacterial cells which plays a crucial role in DNA replication in these organisms. In eukaryotic cells the DNA associated protein which is most preferred target for fluoroquinolones is topoisomerase II because it has structural and functional similarity with bacterial gyrase. The drug stabilize DNA-gyrase complex and stimulate topoisomerase II cleavage of DNA. Fluoroquinolones also show cross-reactivity with other enzymes involved in the process of DNA replication. These compounds thus induce a variety of genotoxic effects due to their ability to inhibit mammalian topoisomerase II activities including induction of transient DNA strand breaks during replication, chromosomes condensation and disjunction during meiosis [12-14].

The mouse *In vivo* chromosomal aberration assay is one of the most frequently used tests for the quantification of genetic damage induced by chemicals in somatic cells *In vivo*. The test has been recommended for routine analysis and data obtained are considered highly relevant in human context. In the present investigation the positive control, Cyclophosphamide has shown significant chromosomal aberrations compare to negative control. Therefore, it is proven as mutagenic agent and jus6fied it as positive group in our study. The exact mechanism is underlying the mutagenic activity of Cyclophosphamide is not known. It summarized that Cyclophosphamide generates aldophosphamide and phosphor amide mustard, probably exert a direct mutagenic effect on chromosomes [15].

Prulif1oxacin, when administered at three different doses, viz., 33.3, 66.6 & 133.3 mg/kg body weight of Prulifloxacin have shown many changes in the chromosome including gaps, breaks, fragmentation, stickiness and ring, compared to control at 12 and 24 hrs. The percentage of aberration that observed for different dose of Prulifloxacin after 12 and 24 hrs treatments, with/without gap were increased significantly as compared to control group. The percentage of aberration was higher in groups treated with Prulifloxacin as compared to control, but the same frequencies were looks lesser when compared with the positive control group. When these observations were compared with the different doses of Prulit1oxacin, we got that percentage of chromosomal aberration increases significantly as dose of Prulifloxacin increase.

The Mitotic Index is the indicator of cellular proliferation rate. The Mitotic Index assay is used to characterize proliferating cells and to identify compounds that inhibit or induce mitotic progression. It is the biomarkers of the cell division. Nearly none effect was seen in mitotic index in the groups treated with different doses of Prulifloxacin for different durations. But in groups, treated with the higher doses of Prulifloxacin shows slight reduction in mitotic index, shows its weak clastogenicity.

Prulifloxacin appears to exert its genotoxic action through binding to the gyrase-DNA complex, stabilizing it and preventing enzyme turnover. This complex is tem1ed as cleavable complex. The formation of these complex results in a double strand break in the helix with both free ends of the helix attached to the enzyme by way of phosphotyrosine linkages. It is likely that cleavable complex stabilization plays a key role in Prulifloxacin genotoxic activity and this may occur at multiple stages in the cell cycle, including mitosis, but there may be other mechanisms of quinolone involvement in topoisomerase II-mediated DNA damage [14,16].

We think that our results, together with other published data, indicating that Prulifloxacin is mutagenic up to some extent but is less mutagenic compared to Cyclophosphamide. These genotoxic effects being compatible with the role of inhibition of topoisomerase in the DNA metabolism. So on the basis of above study; we can say that Prulifloxacin have weak c1astogenic potential. But it needs further detailed investigations on other in vitro and *In vivo* test systems before reaching to definitive conclusions.

Fig 1. Showing different chromosomal abnormalities in Prulit10xacin treated animals. (a) Normal chromosomes (b) Fragmentation (c) Chromatid break and gap (d) Chromosomal, Chromatid breaks and ring (e) Chromatid gap (f) Stickiness

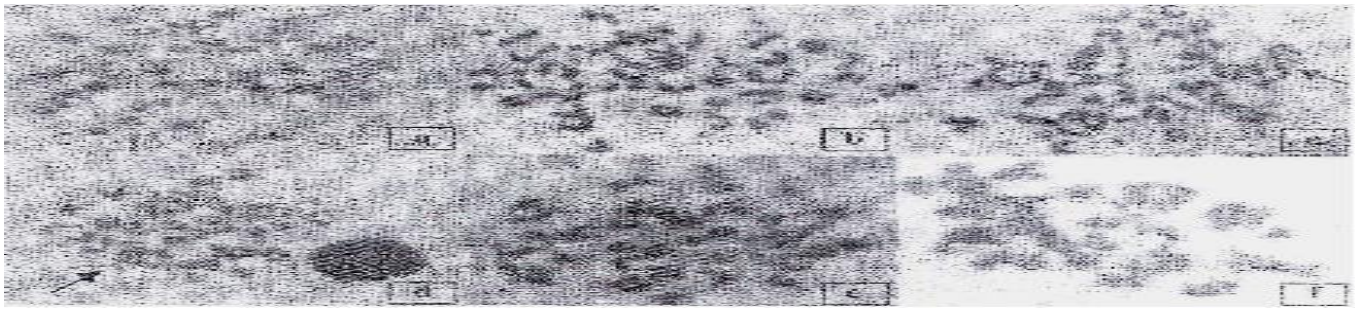

Table 1. Percentage frequency of chromosomal aberrations and mitotic index induced by Prulifloxacin at different dose levels and time intervals, (single close study).

Group	Duration	Chromosome		Chromatid		F	S	R	% Aberrations with gap Meant S.E.M.	% Aberrations without gap Mean+S.E.M.	Mitotic Index Mean+S.E.M.
			B	G	D						
Control	12hrs.	6	9	5	7	2	7	9	8.15+0.13	4.01+0.63	5.75+0.59
CP(2U mg/kg)	12hrs.	23	28	3	D	4	15	24	20.23±1.08***	7.7+0.35***	3.09+0.64*
GF-(66.6miT/ku)	12hrs.	9	8	7	-		7	S	8.31±0.20””	3.0+0.25*””	5.75+0.60S
GF-(66.6miT/ku)	12hrs.	“	7	5	9	2	10	15	11.72+0.84”###	5.3+0.28###	5.45+0.70#
GH(H3.3gA.jp	12hrs.	13	15	6	8	7	11	17	14.48±0.81***##	6.13±0.31*##	5.51±0.30##
Control	24hrs.	7	8	7	9	2	7	“	10.02±0.79	4.11+0.60	6.20+0.87
CP(20 mg/kg)	24hrs.	30	32	5	9	7	17	31	25.82±0.96***	11.58±0.75***	2.12*0.38**
OP(33.3niR/kii)	24hrs.	13	10	5	3	2	9	13	11.6+1.24###	4.93±0.36###	6.14±0.58###
CF(66.6 mg/kg)	24hrs.	10	8	7	9	3	10	17	12.7±0.98###	6.35+0.65*###	5.90+0.56###
OF(133.3g/kg)	24hrs.	15	12	7	9	3	12	19	15.22±0.85**###	6.03±0.55*###	5.94+0.84##

G-gap, B-break, F- fragmentation, S-stickiness, R- ring Each value represents the Mean SEM for each group (n=6) *p<O.Ol, **p<O.Ol, *'"*p<O, OO1 Vs. Control, # p<O.05, ## p<O.Ol, ###p<O.OOl Vs. CP

CONCLUSION

The finding of mouse bone marrow chromosomal aberration study suggests that Prulifloxacin have weak mutagenic effect in the chromosome structures and mitodepression. As per our conclusion Prulifloxacin has a weak c1astogenic effect in the somatic cells of mouse *In vivo*.

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