A 14-DAY SUBCHRONIC GENOTOXICITY STUDY OF NIMESULIDE IN MOUSE BONE MARROW CELLS IN VIVO

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Summary

A 14-day subchronic genotoxicity of Nimesulide was evaluated by employing mouse bone marrow chromosomal aberration test. The Nimesulide administered orally for 2 weeks at the rate of 1.5, 2.5, 5 mg/kg body weight in swiss albino mice. The results show decreased mitotic activity in all groups of Nimesulide treatments. Similar results concerning the chromosomal aberrations revealed in all treated animals. However statistically significant genotoxicity was seen only with the higher dose of Nimesulide. The obtained results indicate that 2 weeks continuous treatment of Nimesulide is moderately genotoxic in the bone marrow cells of swiss albino mice.

Keywords: Nimesulide, Genotoxicity, Chromosomal aberration, Mitotic index.

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Introduction

It is known that many substances with an anti-inflammatory action influence DNA metabolism[1-2] and can thus give rise to later damage in the genetic material. Various short term studies have shown that they either have only a weak or no genotoxic effect at all [3-6]. It is reported that various non steroidal anti-inflammatory drugs (NSAIDs), including diclofenac, flurbiprofen, ibuprofen, indomethacin, isoxicam, ketoprofen, piroxicam, pirprofen, and tiaprofenic acid, failed to increase sister chromatid exchange (SCE) after 2 weeks of treatment[3]. Since nonsteroidal antiinflammatory drugs are usually administered over long periods, appraisal of the mutagenic risk of these drugs would appear to be especially important.

Nimesulide is a NSAID with marked anti-inflammatory, antipyretic and analgesic properties and is indicated for osteoarthritis, rheumatoid arthritis, reduction of fever, primary dysmenorrhea and for relief of mild to moderate pain. The recommended adult dosage of Nimesulide is 100 mg administered orally as tablets twice-daily. Although there is no doubt about the therapeutic usefulness and necessity of NSAIDs yet some recent findings indicate that Nimesulide has a higher risk of hepatic toxicity, when compared to other marketed NSAIDs[7-8].

Studies on genotoxic potential of Nimesulide are limited. Nimesulide is reported for slightly, but statistically non significant, increases in average SCE suggested that the short-term therapeutic application of Nimesulide produce no genotoxic effects on the chromosomes of peripheral blood lymphocytes[9]. It is also reported that Nimesulide completely suppresses the increase of 8-oxodGuo, a mutagenic oxidative DNA damage, observed during early dextran-sodium-sulphate–induced inflammation[10]. Further, Nimesulide is found to induce a significant increase in the incidence of chromosomal aberrations in vivo in murine bone marrow cells[11]. However, 12 and 24 hrs prior administration of Nimesulide in short-term assay, reported that neither Nimesulide, nor its biotransformed product, is genotoxic in the bone marrow cells of mice[12]. In this study, the genotoxic effect of Nimesulide was evaluated after 2 weeks continues treatment, by using the mouse bone marrow cells in vivo, employing chromosomal aberrations (CA) test in mice.

Material and methods

Drugs and chemicals

Cyclophosphamide (Endoxan-N) was purchased from Cadila Health Care Ltd., Goa, India, Nimusulide was received as a gift sample from ACME Pharmaceuticals Ltd., Kherva, Mehsana, Gujarat, India. Colchicine was purchased from Hi Media Laboratories Pvt Ltd., Mumbai, India. All other chemicals used for the study were of reagent grade and purchased from commercial sources.

Animals

Swiss albino mice (6-8weeks) were procured from institutional animal house of Shri Sarvajanik Pharmacy College, Mehsana. They were acclimatized for 7 days under standard husbandry conditions, i.e.; room temperature of 25 ± 10 C; relative humidity 45-55% and a 12:12h light/

dark photoperiod, with *ad libitum* access to food (commercial mouse pellets) and water throughout the experiments. For the animal experimentation, approval from Institutional Animal Ethical Committee (IAEC) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), OECD guidelines and ICH guidelines[13-14].

Experimental design

Animals were segregated into five separate groups of 5 animals each and oral treatment of 1.5, 2.5 and 5 mg/kg Nimesulide were given for two weeks. The dose selection for Nimesulide was based on the recommended therapeutic doses for humans. Since the recommended oral dose of Nimesulide for adults is 100 mg twice-daily and the selected doses were the lower, middle and higher doses of the human prophylactic doses. Suspensions of Nimesulide were prepared in 0.5% carboxy methyl cellulose (CMC). Negative and positive control groups received 0.5% CMC while positive control animals received Cyclophosphamide (40 mg/kg) intraperitoneally 24 hrs prior to termination.

In vivo chromosomal aberration assay

Animals were given 0.4 ml of 0.05% colchicine intraperitoneally 90 min before sacrify to each mouse in order to stop the mitotic process in metaphase. At the time of death both femurs were dissected out, bone marrow extracted in 0.075M KCl and the cell suspension incubated for 20 min at 37 °C. Cells were collected by centrifugation at 1000 rpm for 10 min and fixed three times with methanol/acetic acid (3:1). Chromosome slides were prepared by dropping the cell suspension onto cleaned slides, which were flame dried and all slides were coded and stained in dilute Giemsa solution. The microscopic observations, performed with a magnification of 100× oil immersion. Hundred well spread metaphase were scored per animal (500 metaphase per treatment group) at random. Mitotic Index (MI) was calculated from 1000 cells/animal and expressed in percentage[15-16].

Scoring of aberrations

Hundred well spread metaphase were scored per animal (500 metaphase per treatment group) at random. All aberrations like chromatid gaps, chromosomal gaps, chromatid breaks, chromosomal breaks, deletion, ring, dicentric ring, exchange, stickiness, acentrics and fragmentation were considered equal regardless of the number of breakages involved. Chromosomal aberrations cells (CA/cell) were calculated including and excluding gaps[17].

Statistical analysis

The calculated average data generated at different end points of the treated groups of mice were compared with the respective data of vehicle/negative control and positive control group. For statistical analysis the one-way ANOVA was applied followed by Tukey's test for multiple pair-wise comparisons using Prism software (PRISM, 1997) as "a posteriori" test were used in all the experiments. The significance of differences was examined at the p-value of 0.05.

Results

Table 1 presents the data of MI recorded in bone marrow cells. A statistically highly significant ($p < 0.001$) reduction in MI was recorded in the bone marrow cells of positive control animals. At the same time with the different doses of Nimesulide, a slight depression in mitotic activity, as indicated by a reduction in MI values, compared to negative control, was noticed. However, this apparent reduction in MIs was not statistically significant at both lower doses of Nimesulide (1.5 and 2.5 mg/kg) however, significantly higher ($p < 0.001$) MI was recorded at the higher doses (5 mg/kg), compared to the negative control.

Table 2 shows data on the induction of CA in dividing bone marrow cells of mice following in vivo exposure to different doses of NM. A statistically significant ($p < 0.001$) increase in the incidence of CAs/cell over the negative control was observed in the positive control mice. These data confirm the sensitivity of the experimental protocol followed in the detection of genotoxic effects. In Nimesulide treated groups, dose related increase in abnormal metaphases and CAs/cell was recorded however significant ($p < 0.001$) increases in CAs/cell was observed only at the higher dose (5 mg/kg). The metaphase analysis of the bone marrow cells revealed the presence of slightly higher frequencies of various types of aberrations, such as Chromosome gap, breaks, ring and deletion in varying frequencies in Nimesulide-treated animals, as compared to negative control.

Table 1: The mitotic index in the bone marrow cells of swiss albino mice at 14-day after Nimesulide treatment.

Groups	Dose (mg/kg)	Number of animals	Number of cells analyzed	Number of dividing cells	% Mitotic index (mean±SD)
NC (CMC)	--	5	5000	410	8.200±0.495
PC (CP)	40	5	5000	52	1.040±0.279[a]
NM-I	1.5	5	5000	404	8.080±0.396[b]
NM-II	2.5	5	5000	369	7.380±0.335[b]
NM-III	5.0	5	5000	299	5.980±0.870[a,b,c,d]

Abbreviations: NC, Negative control; CMC, Carboxyl methyl cellulose; CP, Cyclophosphamide; NM, Nimesulide.
a$p < 0.001$; significant when compared with the negative control (NC).
b$p < 0.001$; significant when compared to positive control group (CP).
c$p < 0.001$; significant when compared to NM-I.
d$p < 0.01$; significant when compared to NM-I.

Table 2: Absolute frequency of cells with number of chromosomal aberrations cells of swiss albino mice at 14-day after Nimesulide treatment.

Groups	Dose	Total AM	Gap*	Break		R	DR	D	Ex	F	S	AF	CA/cell	
				CtB	ChB								Including cells with gap	Excluding cells with gap
NC	--	17	4	4	3	2	-	3	-	-	-	2	0.036 ±0.011	0.028 ±0.013
PC	40	454	78	66	34	101	62	64	26	19	10	34	0.988 ± 0.060[a]	0.832 ±0.129[a]
NM-I	1.5	20	3	3	4	3	2	4	-	-	-	3	0.044 ±0.017[c]	0.038 ±0.013[c]
NM-II	2.5	45	5	13	6	8	2	5	2	-	-	10	0.102 ±0.019[c]	0.092± 0.024[c]
NM-III	5.0	114	22	21	11	13	12	19	4	-	-	22	0.252 ±0.070[a,c,d,f]	0.208 ±0.074[a,b,c,e]

*Includes both chromatid and isochromatid gap.

Data are expressed as mean±SD (n=5). Abbreviations: NC, Negative control; CMC, Carboxyl methyl cellulose; CP, Cyclophosphamide; NM, Nimesulide ; AM, Aberrant metaphases; CtB, Chromatid break; ChB, Chromosome break; R, Ring; DR, Dicentric; D, Deletion; Ex, Exchange; F, Fragmentation; S, Stickiness; AF, Acentric fragments.

a$p < 0.001$; b$p < 0.01$; significant when compared with the NC.
c$p < 0.001$; significant when compared to PC.
d$p < 0.001$; e$p < 0.01$; significant when compared to NM-I.
f$p < 0.001$; significant when compared to NM-II.

Discussion

The mouse in vivo chromosomal aberration assay is one of the most frequently used and sensitive test for the detection of genotoxic profile of chemicals. The test has been recommended for routine analysis and data obtained are considered highly relevant in human context[18-19]. In the present study, a 14-day subchronic genotoxicity of NSAID, namely Nimesulide was evaluated by employing mouse in vivo chromosomal aberration assay. Although the genotoxic potential of other NSAIDs have been evaluated in various studies, [9, 11, 12] according to our knowledge, this is the first report of subchronic genotoxicity studies for Nimesulide with the use of an in vivo chromosomal aberration assay. In the present study, we found that two weeks continues treatment of Nimesulide induced a slight depression in mitotic activity and caused a slight increase the frequencies of chromosomal aberrations in the bone marrow cells of mice. But these effects were not found statistically significant except at the higher dose. This suggests that Nimesulide at higher dose level, possess moderated genotoxicity.

Nimesulide is widely recommended and frequently used in many countries, even without prescription. A study demonstrate that mice with a genetic abnormality in mitochondrial Sod2 expression, resulting increased oxidant stress are sensitized to the prooxidant activity of Nimesulide administered in vivo over a prolonged period of time at doses comparable to human therapeutic doses. It is reported that repeated administration of NM can superimpose an oxidant stress, potentiate mitochondrial damage, and activate proapoptotic factors in mice[19]. At the molecular level, reductive bio activation of the aromatic nitro group of NM might cause oxido reductive stress and induce covalent binding of reactive intermediates to proteins. Although the exact mechanisms of Nimesulide induced genotoxicity is not fully understood but might be because of its nitroaromatic moiety, having potential for multistep nitroreductive bioactivation (6-electron transfer) that produces the potentially hazardous nitro anion radical, nitroso intermediate, and N-hydroxy derivative. These intermediates have been associated with increased oxidant stress and targeting of nucleophilic residues on proteins and nucleic acids[20-22].

In conclusion, as revealed by the CA test used, Nimesulide is capable of inducing DNA damage in mice. The genotoxic effect of Nimesulide is seems to be dependent on the dose of exposure. Further, above finding provide new information that may be more important for the therapeutic use of Nimesulide.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of the manuscript entitled, “A 14-day subchronic genotoxicity study of Nimesulide in mouse bone marrow cells in vivo”.

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