



Chromosomal Aberrations and Micronucleus assay of bone marrow cells following Salinomycin administration

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Abstract: Chromosomal mutation is an important event in carcinogenesis therefore; study of DNA damage at the chromosome level is an essential part in genetic toxicology. The current study was planned to assess the possible chromosomal damage in the bone marrow cells of mice as a result of administration of Salinomycin (SAL) compound. In this study adult male and female balb/c mice were treated with different concentration of Salinomycin (i.p.). The frequencies of micronucleated (MN) polychromatic erythrocytes in mouse bone marrow cells were analyzed after 30 hours exposure of the compound. Induction of chromosomal aberrations in the cells was also serially determined by Giemsa method to evaluate the mutagenic effects after 24 hours exposure. Incidence of dicentrics and centric rings increased almost linearly up to 5 mg/kg. Furthermore, the frequencies of complex aberrations increase as doses of Salinomycin increase.

Keywords: Salinomycin; Chromosomal Aberration; Micronucleus Test; Mouse; Bone marrow; Erythrocytes (polychromatic, normochromatic); Genotoxicity

I. Introduction

Cytogenetic methods, such as conventional chromosome analysis and micronucleus assay have been used as suitable methods for evaluating delayed chromosome aberrations, because they can score the aberration rate at each cell level and measure its distribution among cells under observation (Zhang *et al.*, 2006). The mammalian *in vivo* chromosome aberration test is used for the detection of structural chromosome aberrations induced by test compounds in bone marrow cells of animals, usually rodents. Structural chromosome aberrations are of two types, chromosome or chromatid. An increase in polyploidy indicates that a chemical has the potential to induce numerical aberrations, with the majority of chemical mutagens. Aberrations are of the chromatid-type, but chromosome-type aberrations also occur which can be detected during mitosis and are best scored during metaphase (Savage, 1975). Also, Micronucleus assays have emerged as one of the preferred methods for assessing chromosome damage because they enable both chromosome loss and chromosome breakage to be measured reliably also because micronuclei can only be expressed in cells that complete nuclear division a special method was developed that identifies such cells by their binucleate appearance when blocked from performing cytokinesis. Exposure to mutagens and carcinogens may lead to genomic instability therefore micronucleus (MN) test is part of a battery of tests that many new products must go through prior to bringing them to market. The use of certain chemical agents may either arrest or reduce the mutagenic and/or carcinogenic potential of some toxicants. Further, Free radicals are responsible for the induction of damage to the cellular DNA that leads to the formation of chromosome aberrations. Antioxidants are known to scavenge free radicals, thereby decreasing the degree of such effects. Salinomycin had been reported as an inducer of free radicals therefore this study was planned to assess and evaluate the clastogenicity effect of Salinomycin (if any) after short time exposure in mice bone marrow cells. Also to check for the chronological changes of chromosome related with the doses and observe whether chromosome instability was induced *in vivo*.

II. Materials and methods

Chemicals

Salinomycin (SAL) and Colchicine were purchased from Sigma, US. Giemsa's stain and DMSO (dimethyl sulfoxide) was procured from Glaxo India Ltd., Mumbai, India.

Animals and Grouping

Male and female inbred balb/c mice weighing about 20g were obtained from the Laboratory Animal Division, following the approval of Institute animal and ethic committee of Central drug Research institute, Lucknow India (109/10/Toxicol/IAEC). The animals were maintained under standard conditions of humidity (50 ± 5 %),

temperature ($25 \pm 2^{\circ}\text{C}$) and dark and light (12 h light/ 12h dark cycles) with free access to food and water. The experiments were conducted in accordance to the guidelines by the animal facility of the institute.

Grouping

The balb/c mice were divided into following four groups each containing six animals (three male and three female) and treated as follows:

Group I: vehicle treated controls

Group II: SAL, 1mg/kg/day

Group III: SAL, 3mg/kg/day

Group IV: SAL, 5mg/kg/day

Dose preparation

1, 3 and 5mg/kg Salinomycin prepared in Dimethyl sulphoxide DMSO were intraperitoneally administered to balb/c mice.

Chromosome aberration assay

Clastogenicity activities of Salinomycin in mice was examined by chromosomal aberration assay. Male and female mice were given two doses of 1, 3 and 5mg/kg of Salinomycin within 24hrs via intraperitoneal route (i.p.). Colchicine (4 mg/kg) was administered 2hours prior to sacrifice of the experimental animals. At autopsy, the bone marrow cells were collected from both the femora by flushing in warm sodium citrate (37°C). The cells were incubated in KCl (0.75%) at 37°C in a water bath for 20 min. Following incubation the material was centrifuged at 1000 rpm for 5 min. The supernatant was decanted and the cell pellet was fixed with cold -methanol (acetic acid/methanol, 1:3 (v/v)) overnight. The fixed cells was centrifuged and re-suspended in a small volume of the fixative, which was dropped on a clean, grease-free slide previously chilled in 50% ethanol, ignited on a smoke less flame (spirit lamp), air-dried and stored in a dust-free condition until staining. The slides were stained in the following day in 5% buffered Giemsa (pH 7.0) for 10 min, air-dried, and mounted in DPX.

Scoring of Chromosome Aberrations

At least 100 good metaphase spreads were examined per animal. Chromosome aberrations observed in bone marrow metaphases were scored as follows: (1) fragments(chromosome and chromatid types), identified as un-rejoined acentric fragments derived from a chromosome or chromatid severance, including terminal deletions and interstitial deletions;(2) chromatid (or isochromatid) gaps, identified as an achromatic site along the length of achromatid that was less than the width of the chromatid; (3) dicentrics. All bone marrow metaphases were scored in the same fashion to allow consistent comparisons.

Mitotic index

The number of dividing cells, including late prophase and metaphases (3000 cells/animal) were counted. The mitotic index was calculated as the number of dividing cells /1000 cell/ animal.

Bone marrow micronucleus assays

In bone marrow MN test, adult animals were sacrificed by cervical dislocation 30hr after Salinomycin exposure. Slides of bone marrow were prepared according to Schmid (1976) with some modification (Goncharova *et al.*, 1988). Cell suspensions and slides were prepared following Cole *et al.* (1971; 1981) and Stoyel and Clark (1980). The frequencies of MNPCEs and the ratio of polychromatic erythrocytes to total erythrocytes were estimated on the basis of observation of no less than 1000 PCEs and 500 erythrocytes per animal. The results comparing MNPCE frequencies was done by using Student's t-test for the PCE/NCE ratio comparison.

III. Results

Induction effects on chromosomal aberration in mice bone marrow cells

Structural chromosomal aberrations observed in the present study were in the form of chromatid breakage and rings (Figures 1 ii & iii). Significant increase ($p < 0.05$) was observed in the number of chromatid breakages. Also, the total number of structural chromosomal aberrations increased significantly ($p < 0.005$) in the 3 and 5mg/kg treated groups after 24 h of treatment. However, the increase in the number of total structural aberrations reached a highly significance in the 5mg/kg treated mice ($p < 0.05$). Significant changes in the mitotic indices could be detected between the control groups and the different groups treated with Salinomycin for the different periods of treatment used in the study. The bar graph representing clastogenicity effect Salinomycin is also shown (Figure 1b).

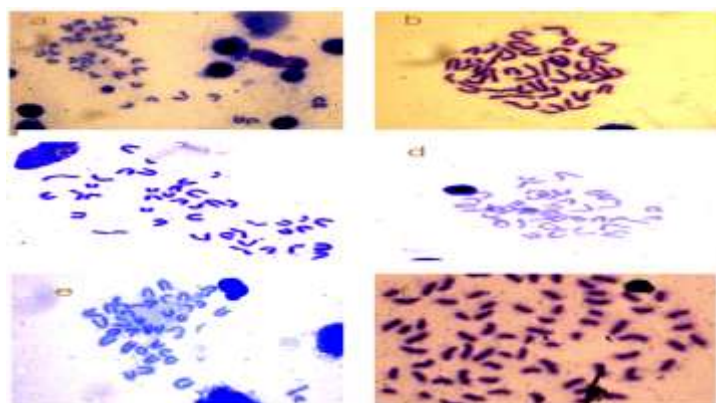


Figure 1 (a). Photomicrographs of chromosomes at metaphases from bone marrow of mice. (i) Normal chromosome (ii) Ring chromosomes (iii) Chromatid break.

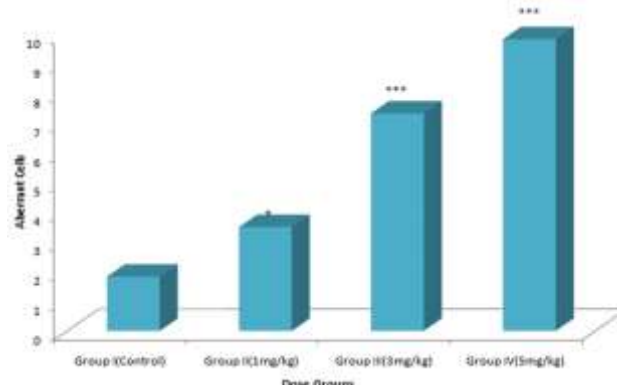


Figure 1(b). Aberrant cells in mice bone marrow following treatment with Salinomycin. * $p < 0.05$ and * $p < 0.001$ were considered statistically significant.**

The production of micronuclei from chromosomal aberrations by Salinomycin

The dynamics of Salinomycin-clastogenesis was considered in balb/c mice following intrapritoneal administration of Salinomycin at different doses (1, 3 & 5 mg/kg), the yield of Salinomycin -induced MNPCEs increased with increasing doses being found 30 h after injection (Figure 21). An analysis of the PCE/NCE ratio indicates depression of the bone marrow erythropoetic function beginning from 30 h after exposure to a higher Salinomycin dose (5mg/kg). The percentage of PCEs in a pool of bone marrow erythrocytes was the lowest at 1mg/kg. The effect of Salinomycin treatment on micronucleus test was presented in bar graph (Figure 21b).

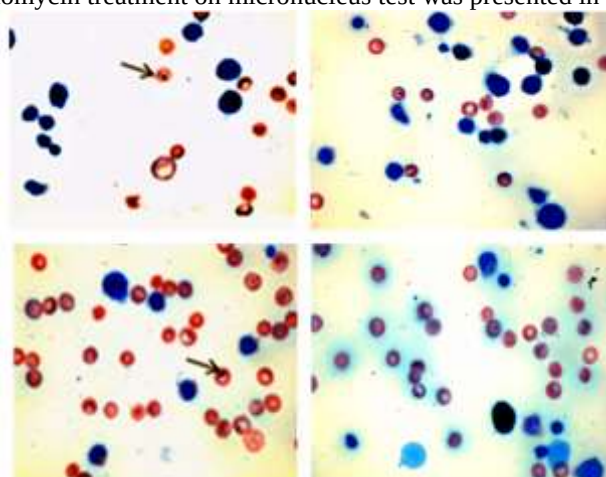


Figure 2. (a) Photomicrographs of bone marrow cells showing Micronucleated Polychromatic Erythrocytes in different mice treated with different doses of Salinomycin (200X).

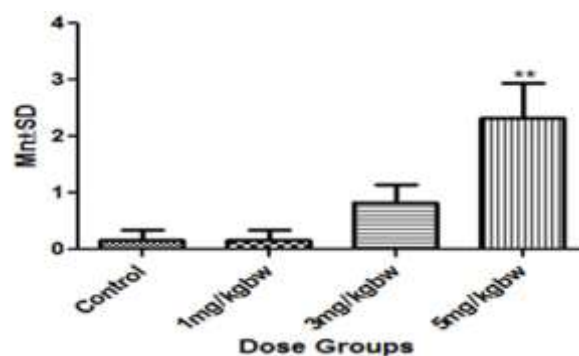


Figure 2(b). Effect on Micronucleated Erythrocytes in mice following Salinomycin.

IV. Discussion

An increase in the structural chromosomal aberrations was observed in the mice following treatment with different double doses of Salinomycin (1, 3 and 5mg/kg). The significant chromosomal aberrations observed were mainly in the form of chromatid break (deletion, gap, break and fragments). Similar observations were made earlier following exposure of Piroxicam (Mohamed *et al.*, 2011). Therefore, it may be concluded that Salinomycin exerts its clastogenic effects after the G1 stage of the cell cycle. Also, micronucleus test, a known cytogenetic screening technique shows potential aneugenicity effect of Salinomycin. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the otherwise anucleated cytoplasm. An increase in the frequency of micronucleated polychromatic erythrocytes in Salinomycin treated animals support clastogenic and aneugenic effect of Salinomycin.

Conflict of interest: the authors have declared no conflicts of interest.

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