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RESEARCH ARTICLE CYTOLOGY

ADRIAMYCIN INDUCED CHROMOSOMAL ABERRATIONS IN GERM CELLS OF MICE

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ABSTRACT

An antineoplastic drug, adriamycin was tested for its mutagenic potential in invivo mouse system using analysis of chromosomal aberrations in germ cells of mice. A significant increase in the frequency of chromosomal aberrations in germ cells of mice was observed at all dose levels. The present results suggest that adriamycin is capable of inducing clastogenic activity in mouse system

KEY WORDS

Adriamycin, Chromosomal aberrations, germs cells

INTRODUCTION

A number of antineoplastic drugs are used to combat with different types of cancer which have shown to be mutagenic in various test systems. Various antineoplastic drugs such as cisplatin, cyclophosphamide, Tamoxifin Gemcitabine and Paclitaxel etc have shown clastogenic effects in various test systems [Garrone **et al , 1993,** Takeda **et al, 2001,** Padma Latha Rai et al, 2001, Padmanabhan et al, 2008]. Significant increase in the frequency of chromosomal aberrations in somatic cells has been reported [Kusum Latha and Rudrama Devi, 2010,2011].

Adriamycin is one of the most commonly used drug for treating many cancers Anthracyclin is effective in malignant lymphomas and is particularly beneficial in a wide range of pediatric and adult sarcomas. It has been shown that chemotherapy agents including anthracyclins cause gene mutation, chromosomal aberrations and aneuploids in somatic cells as well as an increased frequency of secondary treatment related tumor in human cancer survivors[Sandoval et al, 1993, Povirk et al, 1994, Ben-Yehuda et al, 1996]. Further a significant increase was reported in patients involved in cytostatic treatment [Chambers et al, 1984] Because of the extensive and increasing use of adrianycin in successful therapy regimes, an understanding of the mutagenic properties are important. Hence an attempt was made to study the potential mutagenic effect of adriamycin in mice system.

MATERIALS AND METHOD

The animals were fed with 4.0, 6.0 and 8 mg/kg of adriamycin orally for four consecutive days. Control group of animals were maintained simultaneously which received 0.1 ml saline and 0.1 ml mytomycin as positive control.

The animals were sacrificed on 60^{th} day after last treatment and meiotic preparations were made. The dissected out testis were teased in 1.2% trisodium citrate solution and incubated at $37^{o}C$ for 40 min and centrifuged for 10 min. The slides were stained with 2% giemsa and screened according to the method of Evan et al [Evans et al, 1964]. A total of 100 well spread metaphases were screened for the presence of structural and numerical derivations in control and treated groups. The data was analyzed stastically using Chi-Square test.

RESULTS AND DISCUSSION

Cytogenetic methods for clastogenic activity of environmental pollutants are an essential part of routine testing programs. The invivo cytogenetic analysis of chromosomal aberration is one of the best methods to evalutate the clastogenic activity of chemicals, drugs and environmental pollutants. Analysis of diakinesis of first metaphase stage of meiosis is a suitable stage for detecting Chromosomal aberration induced in spermatogonia of treated animals.

Various doses of the drug, Adriamycin of 4, 8 and 16 mg/kg body weight have been administered and the results were illustrated (Table) .The frequencies (%) of the chromosomal aberrations in controls were 8.80% when compared to the treated mice were 11.60, 17.20 and 21.60% respectively for 4, 8 and 16 mg/kg body weight of Adriamycin.(table-22). They have shown an increase in the abnormalities with the increase in the dosage. Among the chromosomal number autosomal univalents in controls recorded were 3.60% with that of treated mice were 3.20, 5.20 and 6.80% respectively. Sex chromosomal univalents have also shown an increase from 3.60% to 3.60, 6.40 and 10.40% respectively and among the ploids; ploryloidy in controls were 1.60% when that of Adriamycin treated mice were 2.40, 3.20 and 2.80% respectively. No aneuploidy records were found in controls, when that of treated mice were 0.80, 0 and 1.60% respectively. No translocations were observed in controls when compared with that of Adriamycin treated mice were 1.60, 2.40 and 0% respectively for 4, 8 and 16mg/kg body weight of Adriamycin. Dose effect relationship was observed. The differences in the frequencies of the chromosomal aberrations between the controls and treated mice analysed using X2 test and the results were found to be significant and illustrated ($P<0.01$).Table:1

Albanese [Albenese et al, 1987] used direct and indirect methods for the detection of chemically induced chromosomal damage in male germ cell. The direct methods assess chromosomal damage in the dosed animals but analysis is restricted to the dividing spermatogonia and spermatocytes but in Indirect methods ,chromosomal damage is assessed in the F1 progeny of the dosed male and analysis covers all germ cell stages both methods can provide evidence of germ cell exposure.

Our result with various doses of drug, Adriamycin 4, 8 and 16 mg/kg body weight has been administered and the results were illustrated in Table1-2. The frequencies of chromosomal abnormalities in control were 8.80% when compared to the treated mice were recorded 11.6%, 17.20%, 21.60% and respectively for 4, 8, 16 mg/kg body weight. They have showed an increase in the abnormalities with the increase in doses.

The present results are in accordance to Marvin Meistrich et al [Marvin et al, 1990] The mutagenic effects of doxorubicin (Adriamycin, ADR) on mouse spermatogonial stem cells were examined by analysis of spermatocyte chromosomes and of dominant lethality transmitted through the spermatozoa. Chromosomal translocations were observed in 0.6% of the spermatocytes of mice treated with ADR (2–6 mg/kg).

The results of present studies are comparab to Palo et al [2005]. The clastogenic potential of three different doses of etoposide (10, 15 and 20 mg kg(-1)) in the male germline of mice was assessed from the dividing spermatogonia after a single exposure for one cell cycle duration at 24 h post-treatment. Transmission of such effects was assessed from the frequency of aberrant primary spermatocytes at week 4 post-treatment. All three doses of etoposide were found to be clastogenic to the dividing spermatogonia of mice, and mostly chromatid breaks were induced. The effects also were transmitted through the male germline of mice, which was evident from the prevalence of statistically significant increased percentages of aberrant primary spermatocytes at week 4 posttreatment. Thus, there is every chance that the cytogenotoxic effects of etoposide are transmitted to the next generation through the male germline of post-chemotherapeutic cancer survivors. Therefore it is essential to make etoposide target-specific or modulate its effects.

Au and Hsu [1980] studied the genotoxic effects of adriamycin on germinal cells in mice

treated with single injections of 3, 12 or 24 mg/kg of the drug. From 1 to 5 days post-injection, chromosome aberrations were observed in diakineses-metaphase I cells from the testes. The frequency of chromosome breakages peaked at 5 h or 1 day for the bone marrow at 3 and 5 days for the testis. Univalent formation was increased overall but did not have a dose- and time-dependent relationship. In long-term follow-up studies, adriamycin was found to induce killing of germ cells which resulted in a reduction in the numbers of spermatocytes and sperms, treated with the higher doses. There was complete absence of gametogenetic elements and, eventually, testicular atrophy occurred. In mice treated with 3 mg/kg, there was gradual recovery of spermatogenesis from 50 days onward. Chromosome breaks and translocations were again observed in the recovering spermatocytes. It was concluded that some of the chromosome aberrations must have been induced in the spermatogonial cells which had survived.

Similar results were reported by Marchetti et al [2006]who used a mouse model to investigate the effects of clinical doses of etoposide on the induction of chromosomal abnormalities in spermatocytes and their transmission to zygotes by using a combination of chromosome painting and 4',6-diamidino-2-phenylindole staining. High frequencies of chromosomal aberrations were detected in spermatocytes within 64 h after treatment when over 30% of the metaphases analyzed had structural aberrations. Significant increases in the percentages of zygotic metaphases with structural aberrations were found only for matings that sampled treated pachytene and preleptotene spermatocytes. Chromosomal exchanges were rare. Etoposide treatment of pachytene cells induced aneuploidy in both spermatocytes and zygotes. The studies found that therapeutic doses of etoposide affect primarily meiotic germ cells, producing unstable structural aberrations and aneuploidy, effects that are transmitted to the progeny.

Table 1

Results on the chromosomal aberrations (meiotic cells) germ cells of mice after treatment with various doses of Adriamycin

Time Dose	Total Number of Metaphases Scored	Total Number Of Normal Metaphases Scored (%)	Total No. of abnormal Metaphases Scored (%)
Control	250	228 (91.20)	22 (8.80)
4 mg/kg	250	221 (88.40)	29 (11.60)
8 mg/kg	250	207 (82.80)	43* (17.20)
16 mg/kg	250	196 (78.40)	54* (21.60)

Values in parenthesis are percentages.*P<0.01

Table 2
Classification of abnormal metaphases germ cells of mice analysed after treatment with Adriamycin.

S.No.	Types of aberration	Control	4 mg/kg	8 mg/kg	16 mg/kg
I	Changes in chromosome number				
a.	Autosomal univalents	9(3.60)	8(3.20)	13 *(5.20)	17 *(6.80)
b.	Sex-chromosomal univalents	9(3.60)	9(3.60)	16(6.40)	26(10.40)
c.	Polyploidy	4(1.60)	6(2.40)	8(3.20)	7(2.80)
d.	Aneuploidy	0(0.00)	2(0.80)	0(0.00)	4(1.60)
II	Structural changes in chromosome				
e.	Translocations	0(0.00)	4(1.60)	6(2.40)	0(0.00)

Values in parenthesis are percentages.*P<0.01

Results are in accordance with Marchetti et al [2001]. The study was to characterize the long-term effects of ET on male germ cells using sperm fluorescence in situ hybridization (FISH) analyses. Chromosomal aberrations (partial duplications and deletions) and whole chromosomal aneuploidies were detected in sperm of mice treated with a clinical dose of ET. Semen samples were collected at 25 and 49 days after dosing to investigate the effects of ET on meiotic pachytene cells and spermatogonial stem-cells, respectively. ET treatment resulted in major increases in the chromosomal aberrations in meiotic pachytene (27- to 578-fold) spermatogonial stem-cells (8- to 16-fold). Earlier we reported a higher incidence of sperm head abnormalities in adrimicine treated swiss albino mice [Kusum Latha and Rudrama Devi, 2011]

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