The Bioscan 5 (2) : 317 - 320, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

CYTOGENETICS EFFECTS OF ADRIAMYCIN IN BONE MARROW CELLS OF SWISS ALBINO MICE

C. KUSUM LATA AND K. RUDRAMA DEVI*
Humam Genetics Laboratory, Department of Zoology,
Osmania Univercity Hyderabad - 500 072
E-mail: rudramadevi_k@yahoo.com

KEY WORDS
Somatic cells
Adriamycin, Mice
Chromosomal aberration.

Received on :
19.01.2010
Accepted on :
28.04.2010

***Corresponding author**

ABSTRACT
In present investigation the cytogenetic effect of Adriamycin were studied in invivo animal model by using analysis of chromosomal aberration in somatic cells of mice. Three concentration were selected and used to analysisthe frequency of chromosomal aberration .As the concentration increased a significant increase in the frequency of chromosomal aberration in somatic cells of Adriamycin treated mice was observed thus the results indicate the clastogenetic nature of adriamycin in mice.

INTRODUCTION

A number of antineoplastic drugs are used to combat with different types of cancer which have also shown to be mutagenic in various test systems. Various antineoplastic drugs such as cisplatin, cyclophosphamide, Tamoxifrn Genecitabine and Paclitaxel etc have shown to be clastogenic effects in various test systems (Garrone *et al.,* 1993; Takeda *et al.,* 2001; Padmalatha Rai and Vijaylakhmi, 2001; Boffetta *et al.,* 2007; Padmanabham *et al.,* 2008).

Adriamycin, one of most commonly used anthracyclin is effective in malignant lymphomas, the drug is particularly beneficial in a wide range of pediatric and adult sarcomas. It has been show that chemotherapist agents including anthracyclins cause gene mutation, chromosomal aberrations rearrangents 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 and shuker, 1994; Ben Yehuda *et al.,* 1996). Further a significant increase was reported in patients involved in cytostatic treatment (chamber *et al.,* 1984). Because of the extensive and increasing use of adriamycin in successfully therapy regimes, an understanding of the mutagenic properties are important. Hence an attempt was made to study the potential mutagenic effect of adriemycin in mice system.

MATERIALS AND METHODS

Eight week old healthy laboratory bred Swiss albino mice (*Mus musculus*) weighing 25 ± 3g of were maintained under standard laboratory conditions at temperature 22ºC relative humidity 50 ± 10% and 12 h photo period commercial pellet diet (Hindustan Lever India) and deionised water were provided by labium.

In the present study the various split doses of Adriamycin (4, 8, 16mg/kg bw) was injected intraperitoneally to the animals for four consecutive days and the animals were killed after 72hr of administration of the test chemical. The treatment for 48 hr was kept to allow bone marrow cells to complete the two cell cycles. The control and treated group of animals were scarified after 6 hr of the last treatment by cervical dislocation. The bone marrow was flushed out into clean glass petri dishes with hypertonic solution (0.56% KCl) to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37ºC for 45 minutes. Four slides were prepared from control and three groups of experimental animals. The staining was done within 24 hr of preparation according to the method of Preston *et al.,* (1987). The slides were screened for 250 well spread metaphases per animal to examine the presence of various types of chromosomal aberrations like gaps breaks, fragment, chromatid separations and polyploids in control and treated group of animals. The data was analysed using Chi-Square test.

RESULTS

The data on the genotoxic effects of adriamycine evaluated from bone marrow cells of mice after 24, 48, 72 hrs of administration of the drug was furnished in (Table 1 and 2) .These include changes in different type of chromosomal aberrations.

The frequency of chromosomal aberration in mice treated with various doses of adriamycin 4, 8 and 16 mg/kg body weight were 6.4, 10.4 and 16.4 % at 24 hrs of administration

Table 1: Frequency of chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of adriamycin for 24, 48 and 72 hrs interval

Dose (mg/kg) and duration oftreatment (hrs)	24 hrs Normal Metaphases Scored (%)	24 hrs Abnormal Metaphases Scored (%)	48 hrs Normal Metaphases Scored (%)	48 hrs Abnormal Metaphases Scored (%)	72 hrs Normal Metaphases Scored (%)	72 hrs Abnormal Metaphases Scored (%)
Control	244 (97.60)	6 (2.40)	242 (96.80)	8 (3.20)	242 (96.80)	8 (3.20)
4 mg/kg	234 (93.60)	16 (6.40)	229 (91.60)	21 (8.40)	219 (87.60)	31 (12.40)
8 mg/kg	224 (89.60)	26 (10.40)	214 (85.60)	36 (14.40)	209 (83.60)	41 (16.40)
16mg/kg	209 (83.60)	41 (16.40)	207 (82.80)	43 (17.20)	202 (80.80)	48 (19.20)

*$p < 0.05$ The values in parentheses are percentages.

Table 2: Classification of various types of chromosomal aberrations in somatic cells of mice analysed after 24, 48 and 72 hrs treatment with various doses of adriamycin

Dose (mg/kg) Duration of Treatment (hrs)	Structural aberrations Gaps	Breaks	Fragments	Exchanges	Numerical aberrations Polyploidy	Chromatid separations	Total no. of Aberrations(%)
24 hrs							
Control I	4(8.00)	1(2.00)	0(0.00)	0(0.00)	0(0.00)	1(2.00)	2(4.00)
4.0	5(10.00)	2(4.00)	1(2.00)	0(0.00)	0(0.00)	1(2.00)	4(8.00)
8.0	7(14.00)	1(2.00)	3(6.00)	0(0.00)	0(0.00)	2(4.00)	6(12.00)
16.0	9(18.00)	1(2.00)	4(8.00)	3(6.00)	0(0.00)	1(2.00)	9(18.00)
48 hrs							
Control II	4(8.00)	1(2.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(2.00)
4.0	6(12.00)	1(2.00)	3(6.00)	0(0.00)	0(0.00)	1(2.00)	5(10.00)
8.0	8(16.00)	2(4.00)	4(8.00)	1(2.00)	0(0.00)	1(2.00)	8(16.00)
16.0	10(20.00)	4(8.00)	3(6.00)	2(4.00)	0(0.00)	2(4.00)	11(22.00)
72 hrs							
Control III	4(8.00)	1(2.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(2.00)
4.0	9(18.00)	2(4.00)	3(6.00)	1(2.00)	0(0.00)	1(2.00)	7(14.00)
8.0	11(22.00)	5(10.00)	4(8.00)	1(2.00)	0(0.00)	1(2.00)	11(22.00)
16.0	17(34.00)	7(14.00)	4(8.00)	3(6.00)	0(0.00)	1(2.00)	15(30.00)

Gaps and polyploids are not included in total aberrations. The values in the parenthesis are percentages.

respectively when compared to controls (2.4%) (Table 1). The aberrations consisted mostly gaps, breaks, fragments, exchanges and chromatid separation. Gaps were significantly high at 24 hrs. Fragments in the treated mice were more compared to controls. Exchanges were observed at only 16 mg/kg weight. No polyploidy was observed (Table 2). The data was analyzed statistically using χ^2. The results were found to be significant ($p < 0.05$).

At 48 hrs of administration for the various doses of Adriamycin with 4, 8 and 16 mg/kg body weight the frequency of chromosomal aberration in the treated group of mice were 8, 14 and 20 % respectively when compared to control animals 3.2% (Table 1). At 48 hrs of the drug administration the frequencies of the gaps, breaks, fragments, chromatid separation and exchanges were increased in the mice when compared to that of the controls (Table 2). There were no Polyploids. The data was analysed statistically using χ^2. The results were found to be significant ($p < 0.05$).

At 72hrs of administratation of drug the total frequencies (%) of chromosomal aberration in the treated mice were increased to 12, 16 and 18 % respectively when compared to controls 3.2 %.Gaps have increased in the treated mice over controls. Breaks were observed but fragments (photo) were increased in treated mice. Exchanges in the Adriamycin treated mice increased when compared to control values of 0.00%. No polyploidys were found but chromatid separation in the mice increased over controls. Differences in the frequency of chromosomal aberration between control and treated mice in somatic cells of mice were analysed using χ^2 test and the values were found to be significant ($p < 0.05$).

DISCUSSION

At 72hrs of administratation of drug the total frequencies (%) of chromosomal aberration in the treated mice were increased to 12.4, 16 and 18 % respectively when compared to controls 3.2 %.

The doxorubicin induced a significant increase ($p < 0.01$) the frequency of chromosome abnormalities, these results being consistent with those reported (Anderson *et al.*, 1998).

The present results are accordance that Larramendy *et al.,* (1980), the frequency of chromatid-type aberrations exhibited a direct-correlation with the dose in mice treated for 6h but not for 12 h. On the other hand, chromosome-type aberrations detected 12 hrs after injection were directly correlated with the dose of adriamycin, the genotoxic effects of the metacentric-like chromosomes induced by adriamycin arise either from translocations involving entire chromosomes arms or from

aberrations of the exchange type between 2 short arms of acrocentric chromosomes.

Similar results were reported by Au and Hsu (1980) the genotoxic effects of adriamycin on somatic cells were studied 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 bone-marrow cells the frequency of chromosome breakages peaked at 5 h or 1 day for the bone marrow Univalent formation was increased overall.

The results of present studies are comparable to Venkatesh *et al.,* (2007) the effect of various concentrations of doxorubicin (DOX)-induced genotoxic effects in mice bone marrow was studied. Treatment of mice with different concentrations of DOX resulted in a dose-dependent elevation in the frequency of micronucleated polychromatic (MPCE) as well as normochromatic (MNCE) erythrocytes in mouse bone marrow

The similar results were reported by aydemir and Bilallug, (2004) to evaluate the effects of chromosomal aberrations induced by Doxorubicin (DXR) in bone marrow cells of Wistar rats.

The present results are accordance that Mehmet Dogan Gülkac (2004) induction of chromosomal aberrations (CA) in rat bone marrow cells by injecting DXR (90 mg/kg body wt). Animals treated with single dose of DXR presented a statistically significant increase in total number of CA. The present results are coincided with Amany A. Tohamy *et al.,* (2003) , the induction of chromosomal aberrations in the bone marrow cells of mice treated with with cyclophosphamide (CP) (2.5 mg/kg bw, i.p.) adriamycin (ADR) (12 mg/kg bw, i.p.) and *cis*-diamminedichloroplatinum-II (cisplatin) (5 mg/kg bw, i.p.) investigated.their was increased number of cells with structural chromosomal aberrations scored after the treatment in bone marrow cells.

The present results are accordance to Prahalathan *et al.,* (2006), investigated the ADR-induced clastogenicity and apoptosis in the bone marrow of rats. The animals were randomly divided into eight groups consisting of six rats each. Five groups were administered ADR (20 mg/kg body weight, i.v.) to induce genotoxicity; The effects of adriamycin were monitored by DNA strand breaks, chromosomal aberrations, micronucleus assay and apoptotic studies in the bone marrow cells of rats after 24 h following single dose of ADR treatment. ADR treatment caused significant clastogenicity and apoptosis in rat bone marrow cells.

Wistar rat cells treated with DXR *in vivo*. The animals were treated by gavage for micronucleus assay (MN) and chromosome preparations. Control groups received a single dose of DXR, rat bone marrow cells developed significantly fewer MN and chromosomal aberrations than those treated with DXR alone. In contradictory Marvin Meistrich *et al.,* (1990) failed to observed increases in chromosomal aberration in the ADR-treated mice at the 6 mg or 8 mg/kg doses. The genotoxic effect of the anticancer drugs such as 5-fluouracil, cyclophasphamide, cisplatin in animal model has published else where Rao (2006). A significant increase in the frequency of chromosomal aberration in somatic cells of mice were reported by antihelminthic drugs (Rudrama Devi and Reddy, 1995), antiasthamatic drugs (Kameswari *et al.,* 1991), heavy metal such as lead (Rudrama devi and Reddy, 1988), chromium (Kiran *et al.,* 1999), cadmium chloride (Rajitha and Rudrama devi, 1999) and Cyclophasphamide and fluorouracil (Rao *et al.,* 2006, Shobha Rani *et al.,* 2006).

REFERENCES

Anderson, D., Basaran, N., Blowers, S. D. and Edwards, A. J. 1998. The effect of antioxidant on bleomycin treatment in in vitro and in vivo genotoxicity assays. *Mutata Res.* **329(1):** 37 - 47.

Au, W. W. and Hsu, T. C. 1980. The genotoxic effects of adriamycin in somatic and germinal cells of the mouse**.** *Mutat. Res.* **79:** 351–361.

Aydemir, N. and Bilallug, R. 2004. Genotoxicity of two anticancer drugs, gemcitabine and topotecan, in mouse bone marrow in vivo: Mutation Research / Genetic *Toxicology and Environmental Mutagenesis.* **537(1):** 43–51.

Ben-Yehuda, D., Krichevsky, S., Caspi, O. 1996. Microsatellite instability and p53 mutations in therapy related leukemia suggest mutator phenotype. *Blood,* **88**, 4296–4303.

Boffetta P., O. van der Hel, et al., 2007. Chromosomal Aberrations and Cancer Risk : Results of a Cohort Study from Central Europe. *Am. J. Epidemiol.* **165(1):** 36-43.

Chambers, S. H., Bleehan, N. M. and Watson, J. V. 1984. Effect of cell density on intracellular adriamycin concentration and cytotoxicity in exponential and plateau phase EMT6 cells. *Br. J. Cancer.* **49:** 301-306.

Garrone O., Del Mastro, L., Mariani G. L., Ardizzoni, A., Venturini, M. and Rosso, R. 1993. Feasibility of a cyclophosphamide, methotrexate and 5-flurouracil regime intravenously administered with and without granulocyte–colony stimulating factor in surgically treated breast carcinomas. *Minerva Med.* **84(9):** 467-72.

Gulkac, M. D., Akpinar, G., Ustun, H. and Ozon Kanli, A. 2004. Effects of vitamin A on doxorubicin-induced chromosomal aberrations in bone marrow cells of rats. *Mutagenesis.* **19:** 231-6.

Kiran, B., Irene, D. and Devi, R. K. 1999. Mutagenicity of chromium in bone marrow cells of mice. *Trends in Life Science.***14(2):** 93-96.

Kameshwari, M., Kalyana swami, B. and Devi, R. K. 1991. Evaluation of theophyllin mutagenicity in swiss albino mice. *J. Agri. Biol. Res.* **7(2):** 108-11

Larramendy M. L., Dulout, F. N., Bianchi, N. O. and Olivero, O. A. 1980. In vivo dose response relationship in bone marrow cells of mice treated with adriamycin. *Mutat Res. Oct.* **79(2):** 133–40.

Marvin L. Meistrich, Maria, E. A. B. van Beek, Jan C. Liang, Susan L. Johnson and Lu, J. 1990. Low Levels of Chromosomal Mutations in Germ Cells Derived from Doxorubicin-treated Stem Spermatogonia in the Mouse. *Cancer Research.* **50:** 370-374.

Padmalatha Rai, S. and Vijaylakshmi, K. K. 2001. Tamoxifen citrate induced sperm shape abnormalities in the invivo mouse. *Mut. Res.* **492:** 1-6.

Padmanabhan S., Tripathi, D. N. Vikram, A. Ramarao, P. and Jena, G. B. 2008. Cytotoxic and genotoxic effects of methotrexate in germ cells of male Swiss mice Mutation Research/Genetic *Toxicology and Environmental Mutagenesis.* **655(1-2):** 59-67.

Povirk, L. and Shuker, D. 1994. DNA damage and mutagenesis induced by nitrogen mustards. *Mutat. Res.* **318**: 205–226.

Preston, R. J., Dean, B. J., Galloway, S., Holdem, H., Mcfeeand, M. F. and Shelby, M. 1987. Mammaliam invivo cytogenetic assay. Analysis of crromosomal aberration in bone marrow cells. *Mt. Res.***189:**157-165.

Prahalathan, C., Selvakumar, E. Varalakshmi, P., Kumarasam, P. and Saravanan, R. 2006. Salubrious effects of lipoic acid against adriamycin-induced clastogenesis and apoptosis in Wistar rat bone marrow cells.

Toxicology. **222(3):** 225-232.

References and further reading may be available for this article. To view references and further reading you must purchase this article.

Rao, K. 2006. Genotoxic effects of cyclophosphamide *in vivo* and *in vitro* test systems. Ph. D submitted thesis to Osmania University.

Rajitha, A. and Rudrama Devi, R. K. 1999. genotoxicity of cadmium in somatic and germ cells of mice. *Agri. Bio. Res.* **15(1and2):** 1-8.

Rudrama Devi, K. and Reddy, G. M. 1995. Mutagenecity of antihelmintic drug in mammal4.Effedct of tetramizole on somatic cells of swiss male mice. *Cell and Chromosome Research.***12(2and3):** 21-123.

Rudrama Devi, K. and Reddy, P. P.1988. Genotoxic effect of heavy metals in mice 1: effwct of lead acetate on bone marrow erythrocytes of mice. *Cell and Chromosome Research.* **1(1):** 36-37

Shobha Rani M., Devi, R. K. and Chakravorthy, A. 2006. Induction of chromosomal aberration in bone marrow cells of mice by chromium.*Trends in Life Science.* **21(10):** 11-15.

Sandoval, C., Pui, C. H. and Bowman, L. C. 1993. Secondary acute myeloid leukemia in children previously treated with alkylating agents, intercalating topoisomerase II inhibitors, and irradiation. *J. Clin. Oncol.* **11**: 1039–1045.

Tohamy, A. A. 2003. El-Ghor AA, El-Nahas SM and Noshy MM. Beta-glucan inhibits the genotoxicity of cyclophosphamide, adriamycin and cisplatin. *Mutat. Res.* **541:** 45-53.

Takeda, Y., Yoshizaki, I., Nonaka, Y., Yanagie, H., Matsuzawa, A. and Eriguchi, M. 2001. Docetaxel alone or orally combined with 5–fluorouracil and its derivatives: effects on mouse mammary tumor cell line MM2 in vitro and in vivo. *Anticancer Drugs.* **12(8):** 691–8.

Venkatesh, P., Shantala, B. Jagetia, G. C., Rao, K. K. and Baliga, M. S. 2007. Modulation of Doxorubicin-Induced Genotoxicity by Aegle marmelos in Mouse Bone Marrow: *A Micronucleus Study Integrative Cancer Therapies.* **6(1):** 42-53.