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RESEARCH ARTICLE

CYTOGENETIC CONSEQUENCES OF MITOXANTRONE TREATMENT IN MOUSE BONE MARROW CELLS AND ITS LIPID PEROXIDE INDUCTION POTENTIAL

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ABSTRACT

Mitoxantrone, a synthetic anthraquinone, is highly effective in the treatment of different cancers. But, the recorded correlation between its use and the development of secondary leukemia necessitated the study on its post-treated cytogenetic consequences. In the present study, all the three tested doses of mitoxantrone-induced chromosomal aberrations and micronuclei were significantly increased in mouse bone marrow cells, but the drug was not mitotoxic. It also enhanced the induction of lipid peroxide radicals. Its action on DNA leading to these cytogenotoxic consequences in noncancerous cells during chemotherapy is responsible for the induction of secondary leukemia in cancer survivors. Therefore, mitoxantrone is essentially be made target-specific.

INTRODUCTION

Mitoxantrone (MX), a popular anthraquinone anticancer drug, is the anthracenedione derivative and a synthetic analogue developed in the 1970s in an effort to find a less cardiotoxic doxorubicin derivative (Alderton *et al.*1992; Halterman *et al*., 2010). MX has proven to be highly effective in the treatment of acute nonlymphocytic leukemia, acute myeloid leukemia, relapsed acute lymphoblastic leukemia, solid tumors, recurrent glioblastoma, relapsed mantle cell lymphoma, indolent lymphoma, castration resistant prostate cancer, multiple sclerosis, breast cancer, carcinoma of liver, etc. (Hagemeister *et al*., 2005; Boiardi *et al*., 2008; Garbo *et al*., 2009; Marriott *et al*., 2010; Parker *et al*., 2010; Halterman *et al*., 2010; Kornek *et al*., 2011). Besides, MX-treated apoptotic B16 – F1 cells could be used as a sort of cell vaccine to initiate effective anti-tumor immune-response in mice (Cao *et al*., 2009). It reportedly inhibited the infection of cowpox virus and monkeypox virus (Altmann *et al*., 2012). Although MX is an efficient anticancer agent, its cardiotoxicity has become a matter of concern. Therefore, attempts have been made for the production of its analogues with 3 to 7 carbon atom side chains starting from beta alanino, cyclopropyl, cyclopentyl, cyclohexyl and benzyl groups that showed promising results (Hareesh Kumar *et al*., 2011). MX is also a prospective photo-sensitizer for photodynamic therapy of breast cancer (Montazerabadi *et al*., 2012).

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On the other hand, correlation has been recorded between the rising use of MX for breast cancer treatment and the development of secondary leukemia (Mistry *et al*., 2005). MX combined with cyclophosphamide (CY) and fluorouracil, and radiation therapy also induced therapy related acute myeloid leukemia (Linassier *et al*., 2000). Cancer survivors pre-treated with MX show the recurrence of secondary cancers, mostly therapy related leukemia. Therefore, there was the imperative need of intensive study on the cytogenetic consequences of MX treatment and on the possible mechanism(s) involved in the recurrence of secondary cancers.

MATERIALS AND METHODS

Oncotron, a mitoxantrone injection for i.v. infusion, manufactured by Sun Pharmaceutical India Ltd., Mumbai, India, was used as the test drug. Oncotron is a synthetic anticancer drug belonging to the class anthracenediones. Chemically, mitoxantrone is 1, 4-dihydroxy-5, 8-bis [{2-(2-hydroxymethyl) – amino}-ethylamino] 9, 10-anthracenedione dihydroxide. It is a hydroxyquinone bound to aliphatic side groups with amino functionalities and is structurally related to the anthracycline drug-doxorubicin but lacks aminosugar moiety. Ledoxan, a cyclophosphamide (CY) injection, manufactured by Getwell Pharmaceuticals, Gurgaon, India, was used as the positive control chemical. Each vial of ledoxan contains CY mixed with sodium chloride. Chemically, CY is 2-[bis (β-chloroethyl) amino]-1-oxa-3-aza-2- phosphocy clohexane-2-oxide monohydrate. CY is one of the

most widely prescribed antineoplastic drugs for the treatment of various types of cancers and is also extensively tested as a positive control chemical in cytogenotoxicity tests. Sodium chloride (0.9%) was used as the negative control.

Swiss albino mice (*Mus musculus*) of 8-10 weeks old and 15-20g body weight each were procured from M/S Ghosh Enterprise, Kolkata and acclimatized to the hygienic conditions of the animal house of the department at least for 4 weeks prior to their use in the experiments. The animal house was maintained at an optimal temperature of 23±2^{o}C with relative humidity of approximately 50% and a 12h light/dark cycle. The mice were provided with balanced diet and drinking water *ad libitum*. Healthy male and female mice were selected from the acclimatized stock and were employed in the experiment with the permission of the Institutional Animal Ethics Committee, affiliated to CPCSEA, Government of India.

Dose of MX was selected in accordance with its human therapeutic dose, relative body weight and body surface area of mice to that of an adult patient into considerations. In advanced breast cancer and non-Hodgkin's lymphoma the recommended dose of MX single agent is 14 mg/m^2 body surface area and repeated at 21 days interval. In acute non-lymphocytic leukemia the recommended dose of MX as single agent are 12 mg/m^2 daily for 5 consecutive days, totaling to 60 mg/m^2. Accordingly, in the present study, three different doses of MX 0.5, 1.0 and 1.5 mg kg^{-1} body weight were selected for testing. CY 40 mg kg^{-1} and 0.9% sodium chloride 10 ml kg^{-1} body weight were used as the positive and negative controls, respectively. Dilution of the drugs was made in such a way that the volume of each treatment was maintained to 1 ml 100 g^{-1} body weight of mice. All the treatments were intraperitoneal and assessments were made after a single dose exposure for one cell cycle duration. The end points studied were mitotic metaphase chromosome aberration (CA) study and mitotic index (MI) study from bone marrow cells at 24 h post-treatment, micronucleus test (MNT) from polychromatic erythrocytes (PCEs) at 30 h post-treatment, and lipid peroxidation (LPO) test from bone marrow, liver and testis (wherever possible) tissues at 24 h post-treatment.

Ninety healthy mice (45 females, 45 males), each about 18-20g body weight, were selected from among the acclimatized stock and divided randomly into five groups with 18 (9 females and 9 males) in each group. One group, the negative control, was treated with 0.9% sodium chloride at the rate of 1 ml 100 g^{-1} body weight. Another group, the positive control, was treated with CY at the rate of 40 mg kg^{-1} body weight. The other three groups were treated with MX 0.5, 1.0 and 1.5 mg kg^{-1} body weight of mice. From each group, six mice (3 females and 3 males) were employed for CA and MI study, another six for MNT and the remaining six of each group for LPO test.

For mitotic metaphase CA study from bone marrow cells, the slides were prepared following the colchicine- sodium citrate hypotonic– methanol, glacial acetic acid- flame drying–Giemsa technique, as detailed earlier (Choudhury *et al*., 2000). About 150 well-spread metaphases from each animal were scanned and different chromosomal aberrations like chromatid and chromosome gaps and breaks, fragments, minutes, etc. in them were recorded. The percentage of aberrant metaphases and chromosomal aberrations (excluding gaps) per hundred metaphases were calculated. From the same slides, about 2000 cells per animal were considered for MI study. The dividing cells in them were recorded and the percentage of dividing cells was calculated. For MNT from polychromatic erythrocytes (PCEs), the slides were prepared following the simple technique of Choudhury *et al*. (2000). About 2000 blue-tinged PCEs were scanned randomly from each animal. The MNs in them were recorded and MN per 1000 PCEs was calculated. For LPO test from bone marrow, liver and testis tissues, the concentration of thiobarbituric acid reactive substance (TBA-RS) was estimated from spectrophotometer for measuring the malondialdehyde (MDA) equivalents as a marker of lipid peroxides following the procedures of Jena and Patnaik (1995) and Devasagayam *et al*. (2003). MDA equivalents in nano moles mg^{-1} tissue (fresh weight) were calculated.

The data generated after calculating the averages with standard deviation (SD) at different endpoints from the positive control and MX treated groups of female and male mice were compared with the respective data of negative control mice. Statistical tables of Kastenbaum and Bowman (1970), prepared specifically for mutation studies, were used to determine the levels of significance of the differences among them. However, in LPO test, as the calculated values for different tissue samples showed much variations within each groups, significance of their differences from that of the negative control mice were assessed by performing two-tail paired t-tests.

RESULTS AND DISCUSSION

The female and male mice of the positive control group induced high percentages of aberrant metaphases and average CAs per 100 metaphases, which are statistically highly significant ($p \leq 0.01$) when compared to that of the respective mice of the negative control group (Table 1). MX 0.5, 1.0 and 1.5 mg kg^{-1} induced average percentages of aberrant metaphases in the female and male mice were found much increased from that of the respective mice of the negative control group and such increases are statistically highly significant ($p \leq 0.01$) (Table-1). The induced average CAs (excluding gaps) per 100 metaphases in them were also increased significantly ($p \leq 0.01$) from that of the negative control mice (Table 1). The chromosomal aberrations were mostly chromatid breaks, fragments and a few minutes. The average percentages of dividing cells in the female and male mice of the positive control group were slightly increased. MX 0.5, 1.0 and 1.5 mg kg^{-1} induced average percentages of dividing cells in the female and male mice were also increased but not significantly, when compared to that of the negative control group of mice (Table 1). Average MN per 1000 PCEs in the female and male mice of the positive control group were increased and such increases are statistically highly significant ($p \leq 0.01$) compared with that of the negative control group of mice. Whereas, MX 0.5, 1.0 and 1.5 mg kg^{-1} induced average MN per 1000 PCEs in the female and male mice were increased from that of the negative control mice. But such increases are statistically not significant (Table 1).

Table 1. Mitoxantrone and cyclophosphamide induced cytogenotoxic effects on mouse bone marrow cells

Chemicals	Dose (mg kg^{-1} b.w.)	Number of mice treated & gender	Number of metaphases examined	Number of aberrant metaphases	Total CAs (Excl. gaps)	Average percentage of aberrant metaphases ± SD	Average CAs (excluding gaps) per 100 metaphases ± SD	Number of cells examined for MI study	Average percentage of dividing cells ± SD	Number of PCEs scanned for MN test	Total number of MN observed	Average MN per 1000 PCEs ± SD
NaCl	10	3f	465	12	3	2.58 ± 0.66	0.64 ± 0.03	3427	2.21 ± 0.03	6228	12	1.92 ± 0.77
(0.9 %)	(ml kg^{-1})	3m	596	18	2	3.02 ± 0.47	0.33 ± 0.29	3603	2.99 ± 0.04	6365	23	3.61 ± 0.83
MX	0.5	3f	450	61	92	13.55 ± 5.18 **	20.44 ± 5.41**	6000	7.60 ± 0.21	6000	22	3.66 ± 0.76
		3m	450	96	137	21.33 ± 8.18**	30.44 ± 14.08**	6000	8.51 ± 1.20	6000	19	3.16 ± 1.04
	1.0	3f	450	152	299	33.33 ± 1.76 **	66.44 ± 5.54**	6000	4.25 ± 1.53	6000	49	8.16 ± 1.89
		3m	450	175	497	38.88 ± 5.67**	110.88 ± 19.27**	6352	4.64 ± 2.16	6000	53	8.83 ± 0.57
	1.5	3f	450	136	214	30.21 ± 0.76 **	47.55 ± 2.69**	6000	5.55 ± 0.48	6000	44	7.33 ± 1.75
		3m	450	95	151	21.10 ± 5.67**	33.55 ± 7.18**	6000	6.60 ± 0.85	6000	51	8.50 ± 2.64
CY	40	3f	377	239	416	63.39 ± 0.35**	110.34 ± 4.35**	3363	2.91 ± 0.00	10841	193	17.80 ± 1.05**
		3m	427	219	456	51.28 ± 0.68**	106.79 ± 3.35**	3560	3.31 ± 0.02	6066	177	29.17 ± 4.02**

NaCl, Sodium Chloride; MX, Mitoxantrone; CY, Cyclophosphamide; b.w., body weight; f, female; m, male; CAs, Chromosomal aberrations; SD, standard deviation; PCEs, Polychromatic erythrocytes; MN, micronuclei; ****** $p \leq 0.01$.

Table 2. Mitoxantrone and cyclophosphamide induced LPO in different tissues of mice at 24h post-treatment

Chemicals	Dose (mg kg^{-1} b.w.)	Number of mice treated & gender	Bone marrow MDA in n moles mg^{-1} FW ± SD	Liver MDA in n moles mg^{-1} FW ± SD	Testis MDA in n moles mg^{-1} FW ± SD
NaCl	10	3f	47.76 ± 16.34	55.63 ± 9.67	--
(0.9 %)	(ml kg^{-1})	3m	59.09 ± 14.07	72.17 ± 9.76	68.20 ± 2.22
MX	0.5	3f	59.12 ± 10.24	51.98 ± 2.37	--
		3m	60.34 ± 15.44	56.28 ± 5.22	51.53 ± 3.35*
	1.0	3f	176.22 ± 25.44*	111.15 ± 28.10	--
		3m	95.30 ± 10.13*	528.85 ± 34.99**	159.02 ± 17.86*
	1.5	3f	147.23 ± 30.91	63.84 ± 2.14	--
		3m	58.47 ± 26.85	233.71 ± 1.17**	261.66 ± 28.18**
CY	40	3f	80.54 ± 26.82	61.53 ± 5.34	--
		3m	72.34 ± 6.04	104.74 ± 11.74**	72.56 ± 1.23

LPO, Lipid peroxide; NaCl, Sodium Chloride; MX, Mitoxantrone; CY, Cyclophosphamide; b.w., body weight; f, female; m, male; MDA, Malondialdehyde; FW, Fresh weight; SD, standard deviation; n moles, nano moles; ***** $p \leq 0.05$; ****** $p \leq 0.01$.

The positive control group of female and male mice induced MDA equivalents in n moles mg^{-1} tissues of bone marrow, liver and testis were found increased from that of the negative control mice. Of them, only in the liver tissue of female mice such increase is statistically highly significant ($p \leq 0.01$) (Table 2). MX 0.5, 1.0 and 1.5 mg kg^{-1} induced MDA in n moles mg^{-1} tissue of bone marrow, liver and testis in female and male mice were either increased or decreased from that of the respective negative control mice. Some of such variations are statistically significant ($p \leq 0.05$ or $p \leq 0.01$) (Table 2).

CY, the positive control chemical, is a covalent DNA binding agent (Jackson *et al*., 1996). It reportedly induced CAs, sister chromatid exchanges, MN, heritable translocations, specific locus mutations, dominant lethal mutations and other cytogenotoxic effects in mammalian and non-mammalian tests, which have been updated for a review (Anderson *et al*., 1995). It has been recommended to be used as a positive control in genetic toxicity tests (Krishna *et al*., 1995). In the present study too, CY 40 mg kg^{-1} induced significantly ($p \leq 0.01$) high percentages of aberrant metaphases, CAs (excluding gaps) per 100 metaphases and MN per 1000 PCEs (Table 1). These are in complete agreement with its earlier reported clastogenic action in mouse bone marrow (Mohn and Ellenberger 1976; Rohrborn and Basler 1977; Machemer and Lorke 1978; Mavournin *et al*., 1990; Anderson *et al*., 1995; Krishna *et al*., 1995). CY 40 mg kg^{-1} induced percentage of dividing cells in the present study, did not differ significantly from that of the negative control mice (Table 1), which also is in agreement with an earlier report (Sladek 1971). Thus, CY is not mitotoxic. In LPO test, CY-induced MDA equivalents in n moles mg^{-1} of bone marrow, liver and testis tissues are found increased from that of the negative control mice. Such increase in the liver tissue in male mice is statistically highly significant ($p \leq 0.01$) (Table 1). MX reportedly binds to DNA by intercalation (Durr *et al*., 1983) and by electrostatic interaction (LeMaistre and Herzig 1990), induces breaks in DNA strands associated or not with proteins (Lown *et al*., 1984) and cleaves DNA via interaction with topoisomerase II (Topo II) (Tewey *et al*., 1984). Koceva-Chyla *et al*. (2005) examined the MX-induced molecular events and morphological features associated with apoptosis in immortalized cell lines (NIH 3T3 and B14) leading to necrotic mode of cell death and it proved to be highly cytotoxic to both the cell lines. Mazerski *et al*. (1998) studied the intercalative binding of MX to the dodecamer duplex d(CGCGAGCTCGCG)2.

MX is an inhibitor of the enzyme topo II, which is capable of altering the topological state of DNA and is an essential enzyme for cell duplication and viability (Zhao *et al*., 2012). Stabilization of the covalent complex of topo II and DNA seems to be an early event, leading to cell death by interfering with vital functions, such as DNA duplication. The mechanism of action of topo II inhibitors involves stabilization of otherwise transient (cleavable) complexes between topo II and DNA. Collisions of DNA replication forks such as stabilized complexes lead to formation of DNA double strand breaks (Zhao *et al*., 2012). In addition, clinical studies suggested that topo II initiated chromosomal translocations that led to specific types of leukemia (Mistry *et al*., 2005). Thus, inhibition of topo II can lead to the generation of clastogenic DNA double-strand breaks that can in turn manifest mutagenesis (Smart and Lynch 2011). Additional mechanisms such as involvement of transcription and/or generation of oxidative stress may contribute to DNA double-strand breaks induced by MX (Zhao *et al*., 2012). Peroxidative conversion of MX is accompanied by formation of free radial species. Initial peroxidative attack occurs at the aromatic nitrogens of MX. This generation of free radical species might be responsible partly or fully in bringing about the cytogenotoxic effects of MX (Kolodziejczyk *et al*., 1988).

There is strong evidence that interaction of MX with DNA contributes to the cytotoxic action of this drug. Faulds *et al*. (1991) reported that its action was not on a specific phase of the cell cycle. However, Suzuki and Nakane (1994) reported that the cells were sensitive to topo II inhibitors at G_1–S boundary phase, thereby promoting the stabilization of cleavable complexes leading to DNA double-strand breaks and chromosomal aberrations. Frei *et al*. (1992) studied the genotoxicity of MX in somatic and germ cells of *Drosophila*, where MX failed to induce sex-linked recessive lethal mutations. But it was genotoxic in somatic mutation and recombination tests of the wing. They related the genotoxicity of MX to impaired DNA synthesis in cycling cells owing to its ability to inhibit topo II by intercalation into DNA. Besides, MX reportedly induced CAs in Chinese hamster ovary cells (Stetina and Vesela 1991) and human lymphocytes (Medeiros and Takahashi 1994), DNA strand breaks in leukaemic cells (Ho *et al*., 1987), and cytogenotoxic in bone marrow cells of Wistar rat and BALB/C mice (Cecchi *et al*.,1996).

In the present study, all the three tested doses of MX (0.5, 1.0 and 1.5 mg kg^{-1} b.w. of mice)-induced percentages of aberrant metaphases and CAs (excluding gaps) are statistically highly significant ($p \leq 0.01$) compared to those of the negative control mice. The intermediate dose (MX 1.0 mg kg^{-1}) induced comparatively higher percentages of aberrant metaphases and CAs (Table 1). Although the tested doses of MX are much less than that of the earlier studies in different test systems, the results of the present study are in complete agreement with the earlier reports on its clastogenicity. MX-induced CAs frequencies were reportedly more in female mice than their male counterparts, except for the highest dose in Wishar rats (Cecchi *et al*., 1996). But, contrarily, all the tested doses here induced higher percentages of aberrant metaphases and CAs in male mice than their female counterparts except for the highest tested dose (1.5 mg kg^{-1}). Thus, in Swiss albino mice the males are more sensitive than the females. Besides, MX-idncued MN were found increased substantially, particularly with the higher tested doses (Table 1). However, such increases are statistically not significant, but are in the borderline of significance. The increase in MI in all the tested doses of MX from that of the control mice (Table 1), although not significantly, is in complete agreement with that of Cecchi *et al*. (1991). Thus, MX is not mitotoxic to mouse bone marrow cells. Rather, it has enhanced the percentages of dividing cells. MX-induced lipid peroxides, expressed as MDA equivalents in n moles mg^{-1} tissue of bone marrow, liver and testis are found increased, in most of the cases, when compared with that of the negative control mice. Some of such increases also are statistically significant ($p \leq 0.05$ or $p \leq 0.01$) (Table 2). Peculiarly, it

decreased significantly ($p \leq 0.05$) in testis tissue of mice treated with the lowest dose of MX (0.5 mg kg^{-1}). Thus, MX is capable of inducing lipid peroxide radicals and may be other free radicals also. These induced free radicals might be partly responsible in bringing about DNA strand breaks.

Thus, MX intercalates into DNA, stabilizes the DNA-topo II complexes, generates lipid peroxide radicals that lead to DNA strand breaks and consequently to chromosomal aberrations and other cytogenotoxic consequences. MX is capable of killing the cancerous cells, which is the primary objective of cancer chemotherapy. But as the drug is not target specific, during its systemic treatment numerous non-cancerous cells of the patients are also equally affected. Some of such cells sustain with tolerable cytogenotoxic effects and accumulation of such affected cells might be responsible for the recurrence of secondary cancers, mostly leukemia, in cancer survivors pre-treated with MX. Therefore, further research efforts are absolutely necessary to make MX target specific.

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REFERENCES

Alderton, P.M., Gross, J., Green, M.D. 1992. Comparative study of Doxorubicin, Mitoxantrone and Epirubicin in combination with ICRF-187 (ADR-529) in a chronic cardiotoxicity animal model. *Cancer. Res*., 52: 194-201.

Altmann, S.E., Smith, A.L., Dyall, J., Johnson, R.F., Dodd, L.E., Jahrling, P.B., Paragas, J., Blaney, J.E. 2012. Inhibition of cowpox virus and mankeypox virus infection by mitoxantrone. Antiviral. Res., 93: 305-308.

Anderson, D., Bishop, J.B., Garner, R.C., Ostrosky-Wegman, P., Selby, P.B. 1995. Cyclophosphamide: review of its mutagenicity for an assessment of potential germ cell risks. Mutat. Res., 330: 115-182.

Boiardi, A., Silvani, A., Eoli, M., Lamperti, E., Salmaggi, A., Gaviani, P., Fiumani, A., Botturi, A., Falcone, C., Solari, A., Filippini, G., Di Meco, F., Broggi, G. 2008. Treatment of recurrent glioblastoma : can local delivery of mitoxantrone improve survival? *J. Neurooncol*., 88 : 105-113.

Cao, C., Han, Y., Ren, Y., Wang, Y. 2009. Mitoxantrone–mediated apoptotic B16-F1 cells induce specific anti-tumor immune response. *Cell. Mol. Immunol*., 6: 469-475.

Cecchi, A.O., Borsatto, B., Takahashi, C.S. 1996. Cytogenetic effects of mitoxantrone on bone marrow cells of rodents. *Braz. J. Genet*., 19: 411-416.

Choudhury, R.C., Jagdale, M.B., Misra, S. 2000. Cytogenetic toxicity of cisplatin in bone marrow cells of Swiss mice. *J Chemother*., 12: 173-182.

Devasagayam, T.P.A., Boloor, K.K., Ramasarma, T. 2003. Methods for estimating lipid peroxidation : An analysis of merits and demerits. *Indian. J. Biochem. Biophys*., 40: 300 – 304.

Durr, F.E., Wallace, R.G., Citarella, R.V. 1983. Molecular and biochemical pharmacology of mitoxantrone. Cancer. Treat. Rev., 10: 3-11.

Faulds, D., Balfour, J.A., Chrisp, P., Langtry, H.D. 1991. Mitoxantrone – a review of its pharmacodynamic and pharmacokinetic properties of therapeutic potential in the chemotherapy of cancer. Drugs., 41: 400-449.

Frei, H., Clements, J., Howe, D., Wurgler, F.E. 1992. The genotoxicity of the anticancer drug mitoxantrone in somatic and germ cells of Drosophila melanogaster. Mutat. Res., 279: 21-33.

Garbo, L.E., Flynn, P.J., MacRae, M.A., Rauch, M.A., Wang, Y., Kolibaba, K.S. 2009. Results of a phase II trial of gemcitabine, mitoxantrone, and rituximab in relapsed or refractory mantle cell lymphoma. Investigational New Drugs., 27: 476-481.

Hagemeister, F., Cabanillas, F., Coleman, M., Gregory, S.A., Zinzani, P.L. 2005. The role of mitoxantrone in the treatment of indolent lymphomas. Oncologist. 10: 150-159.

Halterman, P., Vogelzang, N.J., Farabishahadel, A., Goodman, O.B. 2010. Mitoxantrone. In: Fig WD, Chau CH, Samal EJ (eds) Drug Management of Prostate Cancer, Part – 2, Springer-Verlag: New York, p 125.

Hareesh Kumar, P., Shiva Prakash, S., Krishna Kumar, S., Diwakar, L., Reddy, G.C. 2011. Synthesis of mitoxantrone analogues and their in vitro cytotoxicity. *Int. J. Chem. Tech Res*., 3: 690-694.

Ho AD, Seither, E., Ma DDF, Prentice, H.G. 1987. Mitoxantrone-induced toxicity and DNA strand breaks in leukaemic cells. *Br. J. Haematol*., 65: 51-55.

Jackson, M.A., Stack, H.F., Waters, M.D. 1996. Genetic activity profiles of anticancer drugs. Mutat. Res., 355: 171-208.

Jena, B.S., Patnaik. B.K. 1995. Effect of age on lipid peroxidation in a short-lived species of reptile, Calotes versicolor. Arch. Gerontol. Geriatr., 20: 263-272.

Kastenbaum, M.A., Bowman, K.O. 1970. Tables for determining the statistical significance of mutation frequencies. Mutat. Res., 9: 529-549.

Koceva-Chyla, A., Jedrzejczak, M., Skierski, J., Kania, K., Jozwiak, Z. 2005. Mechanism of induction of apoptosis by anthraquinone anticancer drugs aclarubicin and mitoxantrone in comparison with doxorubicin : relation to drug cytotoxicity and caspase-3 activation. Apoptosis., 10: 1497-1514.

Kolodziejczyk, P., Reszka, K., Lown, J.W. 1988. Enzymatic oxidative activation and transformation of the antitumor agent mitoxantrone. *Free Radical Biol. Med*., 5: 13-25.

Kornek, B., Bernert, G., Rostasy, K., Mlczoch, E., Feucht, M., Prayer, D., Vass, K., Seidl, R. 2011. Long-term follow-up of pediatric patients treated with mitoxantrone for multiple sclerosis. Neuropediatrics., 42: 7-12.

Krishna, G., Petrere, J., Anderson, J., Theiss, J. 1995. The use of cyclophosphamide as a positive control in dominant lethal and mammalian assays. Mutat. Res., 355: 331-337.

LeMaistre, C.F., Herzig, R. 1990. Mitoxantrone: Potential for use in intensive therapy. Semin. Oncol., 17: 43-48.

Linassier, C., Barin, C., Calais, G., Letortorec, S., Bremond, J.L., Delain, M., Petit, A., Georget, M.T., Cartron, G., Raban, N., Benboubker, L., Leloup, R., Binet, C., Lamagnere, J.P., Colombat, P. 2000. Early secondary acute myelogenous leukemia in breast cancer patients after treatment with mitoxantrone, cyclophosphamide, fluorouracil and radiation therapy. Ann. Oncol., 11: 1289-1294.

Lown, J.W., Hanstock, C.C., Bradley, R.D., Scraba, D.G. 1984. Interactions of the antitumor agents mitoxantrone and bisantrene with deoxyribonucleic acids studied by electron microscopy. *Mol. Pharmacol*., 25: 178-184.

Machemer, L., Lorke, D. 1978. Mutagenicity studies with praziquantel, a new anthelmintic drug in mammalian systems. Arch. Toxicol., 39: 187-197.

Marriott, J.J., Miyasaki, J.M., Gronseth, G., O'Connor, P.W. 2010. Evidence Report : The efficacy and safety of mitoxantrone (Novantrone) in the treatment of multiple sclerosis. Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology., 74: 1463-1470.

Mavournin, K.H., Blakey, D.H., Cimino, M.C., Salamone, M.F., Heddle, J.A. 1990. The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res., 239: 29-80.

Mazerski, J., Martelli, S., Borowski, E. 1998. The geometry of intercalation complex of antitumor mitoxantrone and ametantrone with DNA: molecular dynamics simulations. Acta. Biochim. Pol., 45: 1-11.

Medeiros, M.G., Takahashi, C.S. 1994. Effects of treatment with novobiocin, caffeine and ara-c on human lymphocytes in culture. Mutat. Res., 307: 285-292.

Mistry, A.R., Felix, C.A., Whitmarsh, R.J., Mason, A., Reiter, A., Cassinat, B., Parry, A., Walz, C., Wiemels, J.L., Segal, M.R., Ades, L., Blair, I.A., Osheroff, N., Peniket, A.J., Lafage-Pochitaloff, M., Cross, N.C., Chomienne, C., Solomon, E., Fenaux, P., Grimwade, D. 2005. DNA topoisomerase II in therapy–related acute promyelocytic leukemia. N. Engl. J. Med., 352: 1529-1538.

Mohn, G.R., Ellenberger, J. 1976. Genetic effects of cyclophosphamide, ifosfamide and trafosfamide. Mutat. Res., 32: 331-360.

Montazerabadi, A.R., Sazgarnia, A., Bahreyni-Toosi, M.H., Ahmadi, A., Shakeri– zadeh, A., Aledavood, A. 2012. Mitoxantrone as a prospective photosensitizer for photodynamic therapy of breast cancer. Photodiagn. Photodyn., 9: 46-51.

Parker, C., Waters, R., Leighton, C., Hancock, J., Sutton, R., Moorman, A.V., Ancliff, P., Morgan, M., Masurekar, A., Goulden, N., Green, N., Revesz, T., Darbyshire, P., Love, S., Saha, V. 2010. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukemia (ALLR3): an open-label randomised trial. Lancet., 376: 2009-2017.

Rohrborn, G., Basler, A. 1977. Cytogenetic investigations of mammals. Comparison of the genetic activity of cytostatics in mammals. Arch. Toxicol., 38: 35-43.

Sladek, N.E. 1971. Metabolism of cyclophosphamide by rat hepatic microsomes. Cancer. Res. 31: 901-908.

Smart, D.J., Lynch, A.M. 2012. Evaluating the genotoxicity of topoisomerase-targeted antibiotics. Mutagenesis., 27: 359-365.

Stetina, R., Vesela, D. 1991. The influence of DNA topoisomerase II inhibitors novobiocin and fostriecin on the induction and repair of DNA damage in Chinese hamster ovary (CHO) cells treated with mitoxantrone. Neoplasma., 38: 109-117.

Suzuki, H., Nakane, S. 1994. Differential induction of chromosomal aberrations by topoisomerase inhibitors in cultured Chinese hamster cells. Biol. Pharm. Bull., 17: 222-226.

Tewey, K.M., Rowe, T.C., Yang, L., Halligan, B.D., Liu, L.F. 1984. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science., 226: 466-468.

Zhao, H., Rybak, P., Dobrucki, J., Traganos, F., Darzynkiewicz, Z. 2012 Relationship of DNA damage signaling to DNA replication following treatment with DNA topoisomerase inhibitors camptothecin/topotecan, mitoxantrone, or etoposide. Cytometry., 81: 45-51.

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