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Cytogenotoxicity and Lipid Peroxide Induction in Actinomycin D Post-treated Mice

KEYWORDS: Actinomycin D, Cytogenotoxicity, Lipid peroxide induction, Swiss mice

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ABSTRACT *Actinomycin D, a polypeptide antibiotic, is primarily used as an investigative tool in cell biology to inhibit transcription and is also commonly prescribed in the treatment of a variety of cancers. However, its reported carcinogenicity and mutagenicity in different test systems necessitated the detail study on its post-treated cytogenotoxic effects. In the present study, all the three tested doses of actinomycin D (0.4, 0.6 and 0.8 mg kg-1) induced significant (p £ 0.05 or p £ 0.01) percentages of aberrant metaphases and chromosomal aberrations in bone marrow cells of mice. But, micronuclei induction was not increased and the drug was not mitotoxic. Lipid peroxide induction was increased in bone marrow, although not significantly, but decreased significantly (p £ 0.05 or p £ 0.01) in liver and testis tissue. The possible mechanisms of such effects have been discussed. The findings can be utilized in dose escalation and in formulating a suitable treatment schedule of this drug.*

INTRODUCTION

Actinomycins are a class of polypeptide antibiotics isolated from soil bacteria of the genus Streptomyces. Of from, the most significant is actinomycin D (AD). It is presently obtained as a fermentation product of Streptomyces parvulus (Cragg and Newman, 1999). AD is primarily used in cell biology research as an inhibitor of transcription. Popularly known as dactinomycin, AD was introduced into clinic as the first antitumour antibiotic after the approval of US FDA in 1964. Along with other drugs, AD is prescribed for the treatment of rhabdomyosarcoma and Wilm's tumor in children, Ewing and Kaposi sarcomas, carcinomas of breast, bladder and ovary, low risk gestational trophoblastic neoplasia, high risk chronic lymphocytic leukaemia, etc. (Eriksson et al., 2012; Cagayan 2012). AD liposomes also enhance antitumour effects in non-small cell lung cancer (Guo et al., 2012). However, AD is reportedly a positive carcinogen in animals. It was mutagenic in in vitro and in vivo test systems, including human fibroblast, leucocytes and HeLa cells. Therefore, there was an urgent need for the assessment of its detail cytogenotoxicity and lipid peroxide induction potential in mice in vivo.

MATERIALS AND METHODS

Dacmozen (Dactinomycin for injection), manufactured by Korea United Pharm. Inc., Korea and VHB Life Sciences Ltd., Mumbai was used as the test chemical. Taking the human therapeutic dose, relative body weight and surface area of a person to that of a mouse into considerations, AD 0.4, 0.6 and 0.8 mg kg-1 bodyweight of mice were selected for testing. Cyclophosphamide (CY), an alkylating anticancer drug, @ 40 mg kg-1 and 0.9% sodium chloride @ 10 ml kg-1 b. w. were tested as positive and negative controls respectively. All the treatments were intra-peritoneal. Swiss albino mice (Mus musculus), of 8-10 weeks old and 15-20 g b. w. each, were employed in the experiments with the permission of the Institutional Animal Ethics Committee (IAEC), following the guidelines of CPCSEA, Government of India. Ninety healthy mice (45 female + 45 male) were grouped randomly 18 each (9 female + 9 male). 0.9% sodium chloride, CY 40 mg kg-1 and AD 0.4, 0.6 and 0.8 mg kg-1 were treated to five different groups. From each group, 6 mice (3 female + 3 male) were employed in chromosomal aberration (CA) and mitotic index (MI) study from bone marrow cells at 24 h post-treatment, another six for micronucleus test (MNT) at 30 h post-treatment from polychromatic erythrocytes (PCEs) and the remaining 6 for LPO (lipid peroxidation) test from bone marrow, liver and testis tissues at 24 h post-treatment. For mitotic metaphase CA study, MI study and MNT, the procedures of Choudhury et al. (2000) were followed. Percentages of aberrant metaphases, CAs (excluding gaps) per 100 metaphases, percentages of dividing cells and MN per 1000 PCEs in them were calculated. For LPO test from bone marrow, liver and testis tissues, the malondialdehide (MDA) equivalents were estimated spectrophotometrically following the procedure of Devasagayam et al. (2003). MDA equivalents in nano moles mg-1 fresh weight tissue were calculated. The data generated for AD and CY treatments were compared with that of the negative control mice. The significance of differences among them was assessed from the statistical tables of Kastenbaum and Bowman (1970), and two-tail paired t-tests were conducted for LPO test.

RESULTS AND DISCUSSION

CY induced significantly (p £ 0.01) increased percentages of aberrant metaphases, CAs per 100 metaphases and MN per 1000 PCEs (Table-1). This is in complete agreement with the earlier reported clastogenic action of CY, which has been reviewed by Anderson et al. (1995). MI in CY treated mice did not differ significantly from that of the negative control mice. Thus, CY is not mitotoxic, which is in agreement with the earlier report of Sladek (1971). CY induced MDA equivalents in nano moles mg-1 of bone marrow, liver and testis tissues were increased from that of the negative control mice. Such increase only in the liver tissue of male mice is statistically significant (p £ 0.01) (Table-1).

AD 0.4, 0.6 and 0.8 mg kg-1 induced significantly increased (p £ 0.05 or p £ 0.01) percentages of aberrant metaphases and CAs per 100 metaphases (Table 1). Thus, AD is clastogenic in mouse bone marrow cells, which is in agreement with the earlier reports of Cherry and Hsu (1982) and Hashimoto et al. (1995). Peculiarly, AD was found more clastogenic in male mice. AD binds selectively to the GC-rich sequences of DNA and blocks RNA polymerase progression during transcription, intercalates into DNA and interferes in the normal functioning of topoisomerases leading to DNA strand breaks and illegitimate recombinatons (Cherry and Hsu, 1982; Wolf et al., 2009; Bailey et al., 1994). In the present study too, AD induced increased number of chromatid breaks. MI study showed increased percentages of dividing cells in AD treated mice, although not significantly (Table-1). In earlier reports, S and G2 phases were found sensitive to AD to inhibit cell growth. It blocked G1 and slowed down the rate of cell cycle progression through S and G2 /M phase (Wu and Yung, 1994). Thus, AD is neither an inhibitor nor an enhancer of mitosis. However, the increased percentages of dividing cells in the present study might have occurred due to the accumulation of cells by delaying the cell cycle progression. Contrary to the reports of Hashimoto et al. (1995), here all

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the tested doses of AD failed to induce increased number of MN in PCEs at 30 h post-treatment (Table-1). This might have been due to the use of very low doses of AD for testing, or due to its delaying effects in cell cycle progression prior to the entry of cells into mitosis so that 30 h interval could be insufficient for MNT.

LPO induction in bone marrow was increased from that of the negative control mice, although not significantly. However, it was decreased significantly (p £ 0.05 or p £ 0.01) in liver and testis tissue (Table-1). This indicates that AD has the potential of LPO induction. But its decrease in liver and testis might have been due to the detoxifying enzymes of liver and its restricted entry into testis by crossing the blood-testis barrier. Wozniak et al. (2005) also noticed AD-induced changes in antioxidant enzyme activity, concentration of lipid peroxide products and compared the participation of reactive oxygen species in the cytotoxic action of the drug. AD induced lipid peroxide radicals might be responsible for the induction of other free radicals that affect DNA and other vital biomolecules, which consequently lead to cytogenotoxic effects. Taking these results into considerations, dose escalation and treatment schedules of AD can be revised suitably for better clinical effects.

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Table 1 – Actinomycin D and cyclophosphamide induced cytogenotoxic effects on bone marrow cells and their lipid peroxide induction in different tissues of mice

Chemicals	Dose (mg kg^{-1} b.w.)	Number of mice treated & gender	Average percentage of aberrant metaphases ± SD	Average CAs (excluding gaps) per 100 metaphases ± SD	Average percentage of dividing cells ± SD	Average MN per 1000 PCEs ± SD	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
Na Cl (0.9 %)	10 (ml kg^{-1})	3f	2.58 ± 0.66	0.64 ± 0.03	2.21 ± 0.03	1.92 ± 0.77	47.76 ± 16.34	55.63 ± 9.67	--
		3m	3.02 ± 0.47	0.33 ± 0.29	2.99 ± 0.04	3.61 ± 0.83	59.09 ± 14.07	72.17 ± 9.76	68.20 ± 2.22
AD	0.4	3f	12.66 ± 5.03*	16.88 ± 9.34**	4.68 ± 1.26	0.33 ± 0.28	51.68 ± 3.94	43.97 ± 7.50*	--
		3m	12.22 ±3.67*	20.44 ± 4.53**	3.35 ± 0.31	0.50 ± 0.86	71.35 ± 8.01	42.30 ± 11.20**	30.51 ± 1.55**
	0.6	3f	11.77 ± 1.01*	14.66 ± 3.05**	4.48 ± 0.54	2.33 ± 1.25	98.53 ± 32.57	51.79 ± 10.77	--
		3m	18.88 ± 3.35**	24.66 ± 2.90**	5.45 ±0. 21	1.50 ± 1.00	76.53 ± 10.68	45.76 ± 3.41*	29.23 ± 1.15**
	0.8	3f	12.66 ± 3.05*	16.21 ± 4.07**	4.66 ± 1.10	2.50 ± 0.86	93.98 ± 73.21	46.79 ± 2.47	--
		3m	23.55 ± 2.14**	26.44 ± 5.54**	4.86 ± 0.79	1.83 ± 0.76	57.77 ± 24.11	38.07 ± 10.19	36.79 ± 2.44**
CY	40	3f	63.39 ± 0.35**	110.34 ± 4.35**	2.91 ± 0.00	17.80 ± 1.05**	80.54 ± 26.82	61.53 ± 5.34	--
		3m	51.28 ± 0.68**	106.79 ± 3.35**	3.31 ± 0.02	29.17 ± 4.02**	72.34 ± 6.04	104.74 ± 11.74**	72.56 ± 1.23

Na Cl: Sodium Chloride; AD: Actinomycin D; CY: Cyclophosphamide; b.w.: body weight; f: female; m: male; CAs: Chromosomal aberrations; PCEs, Polychromatic erythrocytes; MN, micronuclei; MDA: Malondialdehyde; FW: Fresh weight; n moles: nano moles; SD: standard deviation; * p £ 0.05, ** p £ 0.01.

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