

# Cytogenetic effects of mitoxantrone on bone marrow cells of rodents

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## ABSTRACT

Mitoxantrone (MTX) is a synthetic compound of the anthracycline class of antibiotics, with antineoplastic properties. MTX interacts with DNA by intercalating into its molecule or by electrostatic interaction. In view of its reduced cardiotoxic effects compared to other anthracyclines, MTX has been used for the treatment of cancer. When tested *in vitro* on human lymphocytes, MTX was found to be clastogenic. In the present study the clastogenic potential of MTX was evaluated *in vivo*, using Wistar rat and BALB/c mouse bone marrow cells. The animals were injected intraperitoneally with different MTX concentrations. MTX induced a significant increase in the total number of chromosome aberrations in all treatment groups. Only fractionated dose treatments showed significant cytotoxicity to bone marrow cells.

## INTRODUCTION

Anthracyclines are a class of antibiotics derived from *Streptomyces*. Chemically, they are pigmented glycosides containing an amino sugar and a tetracycle aglycone, thus corresponding to anthracenediones. Drugs of this class, such as doxorubicin (adriamycin) and daunorubicin (daunomycin), are extensively used for the treatment of human neoplasias. Due to their toxicity, several anthracycline-derived drugs have been isolated or synthesized in order to improve their biological properties (Neidle and Taylor, 1979). Mitoxantrone (MTX), a new synthetic analogue of anthracycline antibiotics, is an antitumor agent that has proven to be highly effective in the treatment of patients with acute leukemia, malignant lymphoma, breast

cancer and, more recently, carcinoma of the liver (Richard *et al.*, 1991).

MTX binds to DNA by intercalation (Durr *et al.*, 1983) and by electrostatic interaction (LeMaistre and Herzog, 1990), induces breaks in DNA strands associated or not with proteins (Lown *et al.*, 1984) and cleaves DNA via interaction with topoisomerase II (Tewey *et al.*, 1984). This mammalian nuclear enzyme alters the topological state of DNA and is also an essential enzyme for cell duplication and viability. There are indications that this enzyme is the major cellular target of a variety of antitumor agents. Stabilization of the covalent complex between topoisomerase II and DNA seems to be an early event, leading to cell death by interfering with vital functions, such as DNA duplication. This interaction appears to play an important role in the cytotoxicity of anthracyclines (Bodley *et al.*, 1989). There is strong evidence that the interaction of MTX with DNA contributes significantly to the cytotoxic action of this drug, which has been reported not to act on a specific phase of the cell cycle (Faulds *et al.*, 1991).

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## MATERIAL AND METHODS

### Chemical agent

MTX (Novantrone, American Cyanamid Company, Lederle Laboratories) is an antitumor agent with the chemical name [1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride] (Figure 1). The drug was supplied by Laboratório Cyanamid Lederle, Divisão de Oncologia do Rio de Janeiro, Brazil.

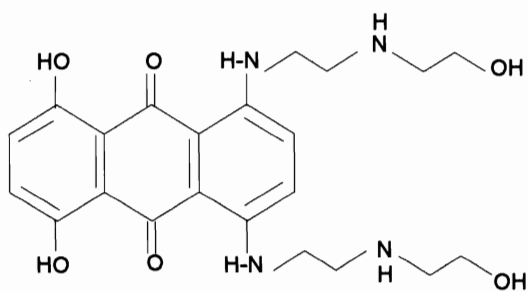


Figure 1 - Chemical formula of mitoxantrone.

### Animal treatment

Wistar rats (*Rattus norvegicus*) and BALB/c mice (*Mus musculus*) weighing approximately 100 g and 30 g, respectively, were obtained from the Biotério Geral, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP.

Rats and mice were divided into six groups of three males and three females each. MTX was administered as a single or as a fractionated dose with a 6-h interval between doses. The negative control group was treated with distilled water, which was also used to dilute MTX.

The drug was injected intraperitoneally (*ip*) and the animals were sacrificed 24 h after administration of the first dose, a time sufficient for one cell division to occur. The animals were sacrificed with ether 90 min after receiving *ip* 0.5 (rats) or 0.3 ml (mice) 0.16% colchicine (Merck).

The following treatment groups were used:

- |                |                             |
|----------------|-----------------------------|
| 1. Control     | distilled water             |
| 2. Treatment 1 | 1.2 mg/kg body weight       |
| 3. Treatment 2 | 2.4 mg/kg body weight       |
| 4. Treatment 3 | 1.2 + 1.2 mg/kg body weight |
| 5. Treatment 4 | 1.2 + 2.4 mg/kg body weight |
| 6. Treatment 5 | 2.4 + 2.4 mg/kg body weight |

Metaphase cells were obtained from rat bone marrow by the method of Ford and Hamerton (1956).

### Slide analysis

Chromosome aberrations (CA) and mitotic index (MI) were the parameters used to evaluate the clastogenic potential of MTX. For CA analysis, the frequency of cells with aberrations, the total number and type of CA and the number of cells with multiple aberrations (cells presenting a CA number of 10 or more) was determined. A total of 100 metaphases/animal were analyzed for CA and the number of metaphase cells was counted in 2000 cells/animal for MI determination.

### Statistical analysis

Data concerning number of cells with CA, total number of CA, and MI were analyzed statistically by the Kruskal-Wallis test using the Statgraphics software (Statistical Graphics System, version 2.7) (1987), with the level of significance set at  $P < 0.05$ .

## RESULTS

### Wistar rat bone marrow system

The total number of CA observed in all groups treated with MTX was significantly higher than the number detected in the control group (Table I). The same result was also obtained for the frequency of cells with chromosome aberrations.

Analysis of the type of chromosome aberrations demonstrated that most of the changes observed were chromatid breaks. Treatment with fractionated MTX doses led to a large number of cells with multiple aberrations (MA) (Table I).

The MI values obtained for the groups treated with MTX doses of 1.2 + 2.4 and 2.4 + 2.4 mg/kg body weight were significantly reduced compared to the control, indicating cytotoxic activity of MTX at the doses used.

Comparison of total CA number from male and female Wistar rats showed a greater sensitivity of females to MTX in all treatments, except for the dose of 2.4 + 2.4 mg/kg body weight (Figure 2). The group that received MTX at the dose of 2.4 mg/kg body weight presented a significantly smaller total number of CA/100 cells than the group that received the same treatment divided into two doses (Table I).

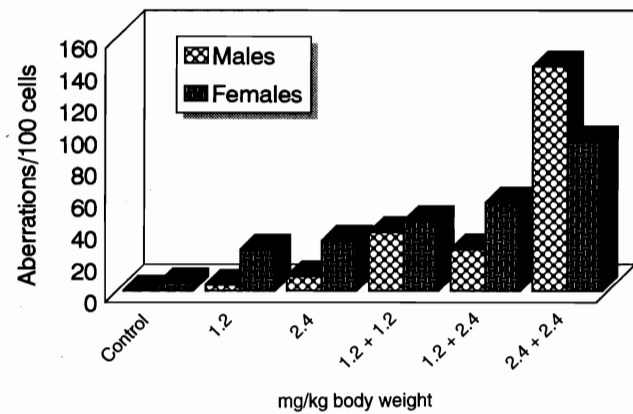
**Table I** - Distribution of the different types of chromosomal aberrations (CA) and the mitotic index (MI) observed in Wistar rat bone marrow cells after treatment with single and fractionated doses (separated by a 6-h interval) of mitoxantrone, and the respective control.

Treatment (mg/kg body weight)	Chromosomal aberrations							CA/100 cells	NCA (%)	MI (%)
	Gaps c	Breaks c ic	ce	tr	qr	ma <sup>+</sup>				
Control	2	3 8	1	0	0	0	2.3	2.2	2.15	
1.2	7	55 26	0	0	0	2	14.7*	10.0*	2.15	
2.4	10	62 45	1	2	0	9	20.3*	14.4*	1.57	
1.2 + 1.2	7	125 55	10	7	2	93	40.6*	20.5*	1.37	
1.2 + 2.4	5	82 41	16	13	0	109	35.7*	18.4*	0.47*	
2.4 + 2.4	4	113 78	17	7	0	173	100.5*	26.2*	0.47*	

c = Chromatid; ic = isochromatid; ce = complex exchange; tr = triradial; qr = quadriradial; ma = multiple aberrations; NCA = number of cells with chromosomal aberrations.

\*Significantly different from control ( $P < 0.05$ ).

<sup>+</sup>Not included in the total number of chromosomal aberrations and cells scored.

**Figure 2** - Comparison of the total number of chromosomal aberrations/100 cells detected in male and female Wistar rats treated with single and fractionated doses of mitoxantrone.**Table II** - Distribution of the different types of chromosomal aberrations (CA) and the mitotic index (MI) observed in BALB/c mouse bone marrow cells after treatment with single and fractionated doses (separated by a 6-h interval) of mitoxantrone, and the respective control.

Treatment (mg/kg body weight)	Chromosomal aberrations								CA/100 cells	NCA (%)	MI (%)
	Gaps c	Breaks c ic	ce	tr	qr	r	ma <sup>+</sup>				
Control	3	22 1	0	0	0	0	0	4.3	3.5	2.14	
1.2	0	152 24	0	1	0	0	3	29.7*	20.9*	2.61	
2.4	1	133 19	0	0	0	0	13	26.0*	17.2*	1.90	
1.2 + 1.2	1	290 53	0	1	0	0	15	59.0*	36.2*	2.93	
1.2 + 2.4	3	480 48	1	1	0	0	106	110.6*	48.3*	1.39	
2.4 + 2.4	2	297 47	2	2	1	1	225	93.9*	41.3*	2.29	

c = Chromatid; ic = isochromatid; ce = complex exchange; tr = triradial; qr = quadriradial; r = centric ring; ma = multiple aberrations; NCA = number of cells with chromosomal aberrations.

\*Significantly different from control ( $P < 0.05$ ).

<sup>+</sup>Not included in the total of chromosomal aberrations and cells scored.

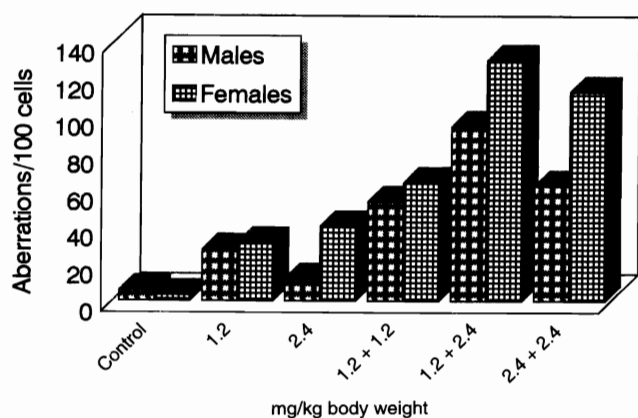
## BALB/c mouse bone marrow system

As also observed for rats, the total number of CA and the frequency of cells with CA were significantly higher compared to the control (Table II). Most of the alterations detected were chromatid breaks. A large number of MA was also observed after treatment with fractionated MTX doses of 1.2 + 2.4 and 2.4 + 2.4 mg/kg body weight.

Comparison of the total number of CA in male and female mice showed a higher sensitivity of females in response to MTX in all treatment groups (Figure 3).

## DISCUSSION

*In vivo* tests are recommended because of the involvement of metabolic activation pathways, and



**Figure 3** - Comparison of the total number of chromosomal aberrations/100 cells detected in male and female BALB/c mice treated with single and fractionated doses of mitoxantrone.

because they permit routes of exposure similar to those for humans (Legator and Ward Jr., 1991). Intra-peritoneal treatment of the animals introduces the compound directly into the peritoneal cavity, where contact with the bloodstream is quite extensive (Klaassen and Rozman, 1991).

MTX was efficient in inducing chromosome aberrations in Wistar rat and BALB/c mouse bone marrow cells (Tables I and II), an effect also observed in *in vitro* test systems such as CHO (Stetina and Veselá, 1991) and human lymphocytes (Medeiros and Takahashi, 1994). The ability of this compound to induce CA is similar to that observed in many other antitumor agents, among them doxorubicin (Capranico *et al.*, 1989), bleomycin (Stubbe and Kozarich, 1987), and cisplatin (De Boer and Glickman, 1989). There is evidence that the chromosome damage induced by antitumor agents is the major cause of cell death.

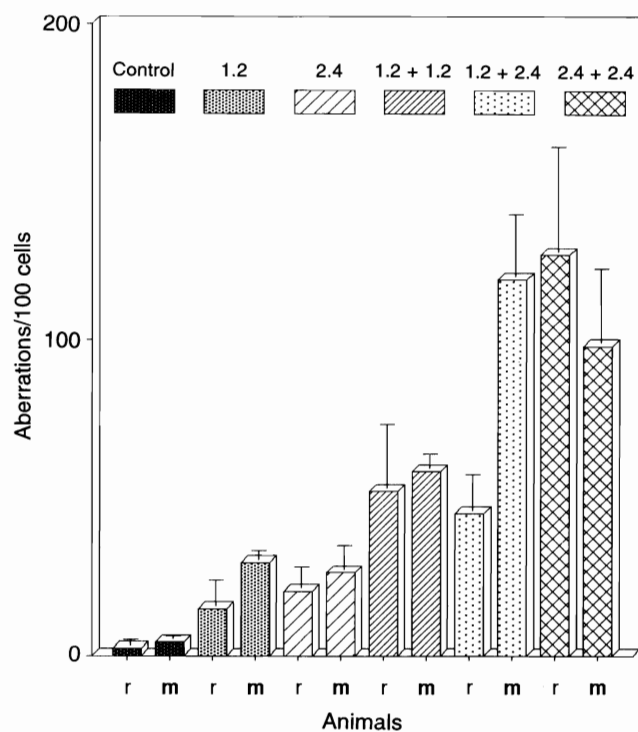
The antitumor drug MTX is known to bind to DNA by intercalation, presenting some differences in its mechanism of action in relation to other intercalating agents: 1) it stabilizes the cleavable DNA topoisomerase II complex, 2) it induces DNA aggregation and compacting via electrostatic cross-binding, and 3) it generates free radicals by oxidative activation processes that induce breaks in the strand of DNA not associated with proteins (Faulds *et al.*, 1991). Mechanisms of intercalation, electrostatic interaction with DNA and nucleic acid condensation or the modulation of DNA topoisomerase I or II activity are believed to be responsible for MTX cytotoxicity (Kapuscinski *et al.*, 1981).

A large number of cells showed MA, especially after the fractionated MTX doses (Tables I and II). These cells presented 10 or more aberrations/cell and since it was not possible to identify all the aberrations which occurred, they were listed separately and were not

included in the CA/100 cells (Preston *et al.*, 1987). Even though all the MTX doses tested induced a high MA rate, these doses were maintained since none of them led to death of the animals. In addition, most of the doses were not cytotoxic. According to Preston *et al.* (1987), the maximum dose of a compound to be used in clastogenicity tests should not induce lethality. The reduction observed in MI indicates that MTX has cytotoxic activity, probably related to the large number of CA produced. Ho *et al.* (1987) correlated the accumulation of breaks in the DNA strands produced by MTX to the cytotoxic activity of the drug on human leukemic cell lineages.

In both rodent species studied here there was a difference in total CA number between males and females. Preston *et al.* (1987) reported that CA induction in bone marrow cells is different between males and females for some agents, with the more sensitive sex varying with the particular agent.

There was a difference between the rodent species studied in relation to total number of CA induced by different MTX doses. At the same drug concentrations, CA number was higher in mice than in rats, except for the highest concentration (2.4 + 2.4 mg/kg body weight), suggesting a difference in sensitivity to the antitumor agent used (Figure 4). Probably, the decreased number of CA observed at the highest MTX dose administered to mice was due to the cytotoxic activity of the drug which masked the result



**Figure 4** - Comparison of the frequencies of chromosomal aberrations in Wistar rats (r) and BALB/c mice (m) treated with single and fractionated doses (mg/kg body weight) of mitoxantrone.

obtained. Nersessian *et al.* (1992) also detected a species difference in sensitivity to certain compounds. These investigators observed that Wistar rat bone marrow cells responded with a 32.2% increase in the frequency of CA induced by cyclophosphamide, whereas mice presented 25%, Chinese hamsters 14.6% and Armenian hamsters 2.8%.

The dose of 2.4 mg/kg body weight produced a greater clastogenic effect (measured as the total number of CA and of cells with CA) in both rats and mice when administered in fractionated doses than as a single dose (Tables I and II). This result may have been due to sensitization induced after administration of the first MTX dose.

The MTX concentrations tested showed an efficient clastogenic activity in single or fractionated doses and some of the higher doses were cytotoxic to Wistar rat and BALB/c mouse bone marrow cells.

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## RESUMO

Mitoxantrona (MTX) é um composto sintético, pertencente à classe dos antibióticos antraciclina, que apresenta propriedades antineoplásicas. Interações com o DNA intercalando-se em sua molécula, ou por interação eletrostática. Por ser um antineoplásico que apresenta efeitos cardiotoxicos menos severos, comparado a outras antraciclina, a sua utilização no tratamento de câncer tem sido muito importante. Ao ser avaliada *in vitro* em linfócitos humanos, a MTX mostrou-se clastogênica. Neste trabalho foi avaliado o potencial clastogênico da MTX *in vivo* em sistema de células de medula óssea de ratos Wistar e camundongos BALB/c. Os animais receberam tratamento intraperitoneal com diferentes concentrações de MTX. Em todos os grupos de tratamento, este composto induziu um aumento estatisticamente significativo no número total de aberrações cromossômicas quando comparado ao grupo controle. A MTX mostrou-se citotóxica apenas quando foi administrada em doses fracionadas.

## REFERENCES

- Bodley, A., Liu, L.F., Israel, M., Seshadri, R., Koseki, Y., Giulian, F.C., Kirschenbaum, S., Sieber, R. and Potmesil, M. (1989). DNA topoisomerase II-mediated interaction of Doxorubicin and Daunorubicin congeners with DNA. *Cancer Res.* 49: 5969-5978.
- Capranico, G., Isabella, P., Penco, S., Tinelli, S. and Zunino, F. (1989). Role of DNA breakage in cytotoxicity of Doxorubicin, 9-Deoxydoxorubicin, and 4-Demethyl-6-Deoxydoxorubicin in murine leukemia P388 cells. *Cancer Res.* 49: 2022-2027.
- De Boer, J.G. and Glickman, B.W. (1989). Sequence specificity of mutation induced by the antitumor drug cisplatin in the CHO *aprt* gene. *Carcinogenesis* 10: 1363-1367.
- Durr, F.E., Wallace, R.G. and Citarella, R.V. (1983). Molecular and biochemical pharmacology of mitoxantrone. *Cancer Treat. Res.* 10: 3-11.
- Faulds, D., Balfour, J.A., Chrisp, P. and Langtry, H.D. (1991). Mitoxantrone - a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the chemotherapy of cancer. *Drugs* 41: 400-449.
- Ford, C.E. and Hamerton, J.L. (1956). A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. *Stain Technol.* 31: 247-251.
- Ho, A.D., Seither, E., Ma, D.D.F. and Prentice, H.G. (1987). Mitoxantrone-induced toxicity and DNA strand breaks in leukaemic cells. *Br. J. Haematol.* 65: 51-55.
- Kapuscinski, J., Darzynkiewicz, Z., Traganos, F. and Melamed, M.R. (1981). Interactions of a new antitumor agent, 1,4-dihydroxy-5,8-bis[[2-(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione with nucleic acids. *Biochem. Pharmacol.* 30: 231-240.
- Klaassen, C.D. and Rozman, K. (1991). Absorption, distribution, and excretion of toxicants. In: *Toxicology - the Basic Science of Poison* (Amdur, M.; Doull, I. and Klaassen, C.D., eds.). 4th edn. Casarett and Doull's Publisher, New York, pp. 50-87.
- Legator, M.S. and Ward Jr., J.B. (1991). Use of *in vivo* genetic toxicity data for risk assessment. *Mutat. Res.* 250: 457-465.
- LeMaistre, C.F. and Herzig, R. (1990). Mitoxantrone: Potential for use in intensive therapy. *Seminars in Oncology* 17: 1 (Suppl.): 43-48.
- Lown, J.W., Hanstock, C.C., Bradley, R.D. and Scraba, D.G. (1984). Interactions of the antitumor agents mitoxantrone and bisantrene with deoxyribonucleic acids studied by electron microscopy. *Mol. Pharmacol.* 25: 178-184.
- Medeiros, M.G. and Takahashi, C.S. (1994). Effects of treatment with mitoxantrone in combination with novobiocin, caffeine and ara-C on human lymphocytes in culture. *Mutat. Res.* 307: 285-292.
- Neidle, S. and Taylor, G.L. (1979). Nucleic acid binding drugs. Some conformational properties of the anti-cancer drug Daunomycin and several of its derivatives. *FEBS Lett* 107: 348-354.
- Nersessian, A.K., Zilfian, V.N. and Koumkoumdjan, N.A. (1992). Comparative investigation of cyclophosphamide action on bone marrow cells of the Armenian hamster and 4 other species of rodents. *Mutat. Res.* 268: 211-215.
- Preston, R.J., Dean, B.J., Galloway, S., Holden, H., McFee, A.F. and Shelby, M. (1987). Mammalian *in vivo*

- cytogenetic assays. Analysis of chromosome aberrations in bone marrow cells. *Mutat. Res.* 189: 157-165.
- Richard, B., Faber, G., Sousa, G.D.E., Faber, I., Rahmani, R. and Cano, J.P.** (1991). Interspecies variability in mitoxantrone metabolism using primary cultures of hepatocytes isolated from rat, rabbit and humans. *Biochem. Pharmacol.* 41: 255-262.
- Stetina, R. and Veselá, 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.
- Stubbe, J. and Kozarich, J.W.** (1987). Mechanisms of bleomycin-induced DNA degradation. *Chem. Rev.* 87: 1107-1136.
- Tewey, K.M., Rowe, T.C., Yang, L., Halligan, B.D. and Liu, L.F.** (1984). Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 226: 466-468.

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