

CYTOGENETIC EFFECTS OF MEBENDAZOLE ON *in vivo* AND *in vitro* MAMMALIAN SYSTEMS

Lusânia M.G. Antunes¹ and Catarina S. Takahashi^{1,2}

ABSTRACT

Mebendazole (MBZ), (methyl-5 benzoyl benzimidazole-2-carbamate), a potent antihelmintic agent, was tested for clastogenicity in Wistar rat bone marrow cells (1300, 1750, 3500 and 7000 mg/kg b.w.) and for both clastogenicity and antimetabolic potential in human peripheral blood lymphocytes in culture (5, 10 and 20 µg/ml culture medium). One-hundred metaphases/treatment were analyzed for induction of chromosome aberrations and 2000 cells/treatment were counted to determine the mitotic index. MBZ did not induce an increase in the frequency of chromosome aberrations, however it was effective in blocking the cell cycle at metaphase.

INTRODUCTION

Benzimidazole derivatives in general have received considerable commercial attention, primarily because of good antifungal, antihelmintic and antineoplastic properties. Unfortunately, some members of this class of compounds also have potential mutagenic, teratogenic or cytotoxic activity (Seiler, 1976; Delatour and Richard, 1976; Higa *et al.*, 1992).

Mebendazole (MBZ), a benzimidazole derivative, is a potent antihelmintic agent. Studies in man (Brugmans *et al.*, 1971) and in various species of domestic animals (Marsboom, 1973) have shown the effectiveness of MBZ against numerous species of nematodes and cestodes. This drug exerts its action by binding to the protein tubulin (Van den Bossche *et al.*, 1982).

MBZ apparently affects cell function at sites associated with the structures and mechanisms responsible for cell division, so that dividing cells would tend to be

damaged by the drug. Indeed, bone marrow toxicity has been reported in clinical trials (Harris, 1979; Murray-Lyon and Reynolds, 1979).

The toxicity of MBZ has already been evaluated in animal systems (mice, rats and dogs) and no liver damage was observed even after long-term administration (Marsboom, 1973). However, Bekhti and Pirotte (1987) described hepatotoxicity as a side effect of MBZ in a patient suffering from multiple hydatid cysts treated with this agent for six months.

Several investigators have suggested that induction of mitotic arrest in *in vitro* systems indicates a teratogenic potential (Delatour *et al.*, 1976). Inhibition of the proliferation of Chang liver cells by MBZ has been detected at a concentration of 0.008 mg/l, a lower concentration than that having a cytotoxic effect (Higa *et al.*, 1992). MBZ also is a potent inhibitor of cell growth and differentiation in micromass cultures. The MBZ concentrations required to inhibit differentiation in micromass cultures are very similar to those observed in the plasma of patients undergoing antihelmintic therapy (Whittaker and Faustman, 1992).

Holden *et al.* (1980) observed mebendazole-induced mitotic accumulation in human lymphocytes that was qualitatively and quantitatively similar to the effects of colcemid (a colchicine derivative). In mouse bone marrow cells, MBZ induced c-mitoses, polyploidy and SCE (Sinsheimer *et al.*, 1990).

¹ Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14049-900 Ribeirão Preto, SP, Brasil.

² Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901 Ribeirão Preto, SP, Brasil. Send correspondence to C.S.T.

Due to the widespread use of this drug in medicine and the increasing concern about mitotic poisons, MBZ was tested for clastogenicity in Wistar rat bone marrow cells and for both clastogenicity and antimutagenic potential in human peripheral blood lymphocytes in culture.

MATERIAL AND METHODS

Mebendazole (CAS Registry No. 31431-39-7) in the form of tablets each containing 100 mg of the active compound was obtained at drugstores as a commercial formulation (Pantelmin, Cilag, Johnson & Johnson). In the *in vivo* treatment the drug was emulsified in distilled water and in the *in vitro* treatment the drug was dissolved in DMSO (Me₂SO, Merck). All test solutions were freshly prepared before each experiment.

Wistar rat bone marrow cells

Four-week old Wistar rats (*Rattus norvegicus*) weighing 100 g were treated by gavage with a single dose of the compound. The MBZ doses tested were 1300, 1750, 3500 and 7000 mg/kg body weight. Six animals per treatment were used (three males and three females). The negative control group received distilled water and the positive control received 30 mg/kg body weight of cyclophosphamide (CP). The animals were sacrificed 24 hours after treatment.

Metaphase cell preparations were obtained from rat bone marrow by the technique of Ford and Hamerton (1956), modified by the Cytogenetics and Mutagenesis Laboratory of FMRP-USP. The animals were injected with 0.16% colchicine (0.5 ml; Merck) one hour and 30 minutes before sacrifice. One-hundred metaphases/rat/treatment were analyzed for the induction of chromosome aberrations, and 2000 cells/animals were counted to determine the mitotic index. Statistical analyses were carried out by the Kruskal-Wallis test.

Human peripheral blood lymphocytes

Human peripheral blood lymphocytes from six normal and healthy 20-30 year old individuals (five for the analysis of chromosome aberrations) were grown in RPMI 1640 medium (Sigma) supplemented with 20% fetal calf serum plus antibiotics. Cells were stimulated with 4% phytohemagglutinin prepared at the Cytogenetics Laboratory of FMRP-USP. The drug concentrations tested were 5, 10 and 20 µg/ml culture medium. Ara-C (2.5 x 10⁻⁹ M) was used as a positive control. MBZ was dissolved in DMSO. The final DMSO concentration was 1% (v/v).

For the analysis of chromosome aberrations (CA), cultures were treated with MBZ 8 h after incubation; the cultures were harvested 48 h after the beginning of

treatment without removing the drug. Colchicine (0.016%, 30 µl/10 ml; Merck) was added one hour and 30 minutes before fixation.

For the analysis of antimutagenic potential, cultures were incubated for 48 h. Cells were exposed to MBZ two or four hours before fixation, without colchicine addition. Two controls were performed, i.e., with and without the addition of colchicine two or four hours before harvesting.

Metaphase preparations were obtained by the technique of Moorhead *et al.* (1960), modified. Slides were stained with Giemsa. One-hundred metaphases from each culture were analyzed for chromosome aberrations. A total of 2000 cells/individual/treatment were counted for the determination of mitotic index and antimutagenic potential.

Statistical analyses were carried out by the Friedman test. Fisher's exact test analyses were also applied to assess the effects of treatment. Data from the positive control cultures were not included in the statistical analysis.

RESULTS

Wistar rats

There was no significant difference for the number of chromosomal aberrations or mitotic index values between groups (Table I).

Table I - Mitotic index, frequency of chromosome aberrations and numerical alterations in Wistar rat bone marrow cells treated with MBZ; 600 metaphases/treatment.

Treatments (mg/kg b.w.)	MI (%)	CA		POL	END
		Gaps	Breaks		
0	3.2	2	0	4	5
1300	4.0	0	0	5	4
1750	3.5	0	1	1	3
3500	2.6	1	0	1	2
7000	2.8	0	0	1	2
CP	0.8	169	23	-	-

MI, Mitotic index; POL, polyploid cells; END, endoreduplicated cells; CP, cyclophosphamide; CA, chromosome aberrations.

The frequency of cells with polyploidy or endoreduplication (Table I) detected in the different MBZ assays was lower than that obtained for the control group, but the difference was not statistically significant (P = 0.16).

Human peripheral blood lymphocytes

There were no significant differences in the frequency of chromosomal aberrations and of mitotic

index values between control cultures and cultures treated with MBZ (Table II).

Table II - Mitotic index, frequency of chromosome aberrations and numerical alterations in human lymphocytes cultures treated with MBZ; 500 metaphases/treatment.

Treatments (µg/ml)	MI (%)	CA		POL	
		Gaps	Breaks		
0	9.9	7	3	1	0
DMSO (1%)	8.8	7	1	1	0
5	10.6	7	1	1	11*
10	7.3	7	0	1	13*
20	5.9	1	0	5	2
Ara-C	5.7	45	91	-	-

Ara-C, Cytosine arabinoside.

*Statistically greater than the control.

The frequency of cells with endoreduplication (Table II) detected in the treatments with MBZ doses of 5 and 10 µg/ml culture medium was significantly higher ($P < 0.05$) than that obtained for the control.

The mitotic indices obtained for the culture tested for the antimetabolic potential of MBZ were very close to or higher than those obtained for the cultures treated with colchicine, with statistically significant differences both for the 2-h and the 4-h treatments (Fisher exact test, $P < 0.05$) (Table III).

Table III - Effect of mebendazole on mitotic index in human lymphocyte cultures exposed for two and four hours, 12000 metaphases/treatment.

Treatments (µg/ml)	MI (%)	
	2 hours	4 hours
0	2.8	2.2
Colchicine (0.016%)	7.4*	11.9*
5	5.8*	9.7*
10	6.8*	12.9*
20	5.5*	9.2*

*Significantly greater than the control.

DISCUSSION

The literature on mebendazole mutagenicity is very contradictory. Its mutagenic properties were not confirmed by the lethal test in mouse germ cells (Léonard

et al., 1974), but in mouse bone marrow cells, MBZ induced c-mitoses, polyploid cells and sister chromatid exchange (Sinsheimer *et al.*, 1990).

According to Preston *et al.* (1987), if the chemical agent is not potentially toxic and does not change the mitotic index, the doses can be administered up to the limit of drug solubility. Thus, the MBZ doses tested here were 1750, 3500 and 7000 mg/kg b.w. The dose of 1300 mg/kg b.w. was also included in the present tests because it is close to the LD50 reported in the literature for mebendazole (Marsboom, 1973). Cyclophosphamide data obtained at the Cytogenetics and Mutagenesis Laboratory of the Faculdade de Medicina de Ribeirão Preto were used for the positive control.

MBZ did not induce an increase in the frequency of chromosome aberrations in Wistar rat bone marrow cells, and the frequency of polyploid cells and endoreduplicated cells was lower than that observed in the negative control group (Table I). The MBZ used in clinical and veterinary treatments is composed of three polymorphic forms, so that, among other properties, some differences in the chemotherapeutic effect and toxicity of the drug may probably occur and the contradictory results obtained in mutagenicity tests carried out on rodents may be due to the use of drug preparations with different properties (Rodríguez-Caabeiro *et al.*, 1987). Furthermore, the very low water solubility of MBZ prevents its intravenous administration and causes it to be poorly absorbed when administered orally; as a consequence, pharmacokinetic evaluation of the drug has revealed a wide intraindividual variety in different tissues (Prieto *et al.*, 1991).

In human lymphocytes treated *in vitro*, MBZ did not induce an increase in the frequency of chromosome aberrations (Table II) but induced a significant increase in the frequency of endoreduplicated cells and was effective in blocking the cell cycle at metaphase (Table III). The results obtained for the antimetabolic potential of MBZ were indistinguishable from those observed for the positive control, colchicine. The minimum effective concentration of mebendazole was 5 µg/ml, a lower value than that reported by Holden *et al.* (1980).

The induction of antimetabolic activity by benzimidazole derivatives has been noted in several *in vitro* systems and includes cell cycle and cytoskeletal disruption (Holden *et al.*, 1980; Delatour and Richard, 1976). Sbrana *et al.* (1993) observed that thiabendazole induced hypodiploid and polyploid cells in human lymphocytes and reported that mitotic index evaluation, when it reveals increases after exposure to a chemical, indicates the accumulation of mitoses, which also involves disturbances of spindle functions.

Liang and Satya-Prakash (1985) proposed that all agents that interrupt cell division are potential aneuploidy

inductors which may cause serious damage to the human population. Although we detected significant increases in numerical alterations, the frequencies were very low and we did not obtain the same results in the *in vivo* assays.

In conclusion, under the conditions used and the concentrations tested, mebendazole had no clastogenic activity on the systems investigated, but effectively blocked the cell cycle at metaphase in human lymphocytes. Additional studies are needed to determine whether the extremely high doses of MBZ administered for long periods of time for the chemotherapy of parasitic diseases are without risk for humans.

ACKNOWLEDGMENTS

We are grateful to Miss S.A. Neves and Mr. L.A. Costa Jr. for valuable technical assistance. Research supported by CNPq. Publication supported by FAPESP.

RESUMO

O mebendazole é um potente anti-helmíntico. Devido ao seu amplo uso na medicina a determinação do seu potencial mutagênico é de grande importância. A clastogenicidade do MBZ foi avaliada em células da medula óssea de ratos Wistar (1300, 1750, 3500 e 7000 mg/kg de peso corpóreo). A clastogenicidade e o potencial antimitótico do MBZ também foram avaliados em linfócitos do sangue periférico humano em cultura (5, 10 e 20 µg/ml de meio de cultura). Cem metáfases/tratamento foram analisadas para determinar a indução de aberrações cromossômicas e 2000 células/tratamento foram contadas para a determinação do índice mitótico. O MBZ não induziu um aumento significativo na frequência de aberrações cromossômicas, mas os resultados obtidos mostram que essa droga é eficiente em bloquear o ciclo celular em metáfase.

REFERENCES

- Bekhti, A. and Pirotte, J. (1987). Hepatotoxicity of mebendazole. Relationship with serum concentrations of the drug. *Gastroenterol. Clin. Biol.* 11: 701-703.
- Brugmans, H.P., Thienpont, D.C., van Wijngaarden, I., Vanparijs, O.F., Schuermans, V.L. and Lauwers, H.L. (1971). Mebendazole in enterobiasis. Radiochemical and pilot clinical study in 1,278 Subjects. *JAMA* 217: 313-316.
- Delatour, P. and Richard, Y. (1976). Propriétés embryotoxiques et antimitotiques en série benzimidazole. *Thérapie* 31: 505-515.
- Delatour, P., Lorgue, G., Lapras, M. and Richard, Y. (1976). Propriétés embryotoxiques et antimitotiques du parbendazole, du mebendazole et du cambendazole. *C.R. Sc. Paris.* 282: 517-518.
- Ford, C.E. and Hamerton, J.L. (1956). A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. *Stain Technology* 31: 247-251.
- Harris, A. (1979). Pyrexia and mebendazole. *BMJ* 2: 1365.
- Higa, F., Kitsukawa, K., Gaja, M., Tateyama, M., Shikiya, K., Shigenno, Y., Kinjo, F. and Saito, A. (1992). Cytotoxicity of mebendazole against established cell lines from the human, rat and mouse liver. *Arch. Toxicol.* 66: 224-227.
- Holden, H.E., Crider, P.A. and Wahrenburg, M.G. (1980). Mitotic arrest by benzimidazole analogs in human lymphocyte cultures. *Environ. Mutagen.* 2: 67-73.
- Léonard, A., Vandesteena, R. and Marsboom, R. (1974). Mutagenicity tests with mebendazole in the mouse. *Mutation Res.* 26: 427-430.
- Liang, J.C. and Satya-Prakash, K.L. (1985). Induction of aneuploidy by mitotic arrestants in mouse bone marrow. *Mutation Res.* 155: 61-70.
- Marsboom, R. (1973). Toxicology studies on mebendazole. *Toxicol. Appl. Pharmacol.* 24: 371-377.
- Moorhead, P.S., Nowell, P.C., Mellman, W.J., Batipps, D.M. and Hungerford, D.A. (1960). Chromosome preparation of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* 20: 613-616.
- Murray-Lyon, I.M. and Reynolds, K.W. (1979). Complication of mebendazole treatment for hydatid disease. *BMJ* 2: 1111.
- 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 cell. *Mutation Res.* 189: 157-165.
- Prieto, J.G., Justel, A., del Estal, J.L., Barrio, J.P. and Alvarez, A. (1991). Comparative study on gastric absorption of albendazole and mebendazole in rats. *Comp. Biochem. Physiol.* 100: 397-400.
- Rodriguez-Caabeiro, F., Criado-Fornelio, A., Jimenez-Gonzales, A., Guzman, L., Igual, A., Perez, A. and Pujol, M. (1987). Experimental chemotherapy and toxicity in mice of three mebendazole polymorphic forms. *Chemotherapy* 33: 266-271.
- Sbrana, I., Di Sibio, A., Lomi, A. and Scarcelli, V. (1993). C-mitosis and numerical chromosome aberration analyses in human lymphocytes: 10 known or suspected spindle poisons. *Mutation Res.* 287: 57-70.
- Seiler, J.P. (1976). The mutagenicity of benzimidazole derivatives. VI. Cytogenetic effects of benzimidazole derivatives in the bone marrow of the mouse and chinese hamster. *Mutation Res.* 40: 339-348.
- Sinsheimer, J.E., Giri, A.K., Osorio, S., Wise, D.S. and Townsend, L.B. (1990). Comparative mutagenicity and genotoxicity of the antiparasitic drugs, mebendazole, flubendazole e flubendazole oxime. *Mutation and the Environment E:* 225-234.
- Van den Bossche, H., Rochette, F. and Horig, C. (1982). Mebendazole and related anthelmintics. *Adv. Pharmacol. Chemother.* 19: 67-128.
- Whittaker, S.G. and Faustman, E.M. (1992). Effects of benzimidazole analogs on cultures of differentiating rodent embryonic cells. *Toxicology and Applied Pharmacology* 113: 144-151.