

In vitro and *in vivo* tests in humans confirm that the antimalarial drug mefloquine is not mutagenic

Cesar Koppe Grisolia¹, Catarina Satie Takahashi² and Iris Ferrari¹

ABSTRACT

The antimalarial drug mefloquine has till now only been evaluated for mutagenicity using nonhuman systems. In the present study, mefloquine was tested in human lymphocytes *in vitro* for chromosomal aberrations (C.A.) and sister chromatid exchanges (SCE), and in human lymphocytes *in vivo* from patients under mefloquine antimalarial therapy living in the Amazon gold panning areas (Brazil). The data show a decrease in mitotic index at the highest concentration (12 µg/ml), representing a cytotoxic effect in the *in vitro* treatment, for both 48- and 72-hour cultures. From the results obtained from *in vivo* human therapy, it can be concluded that this compound induces a significant decrease in lymphocyte proliferation, also found *in vitro*. In both situations, mefloquine did not show any evidence of an increase in C.A. and SCE in human lymphocytes.

INTRODUCTION

The continuing existence and spread of malaria have given rise to an increasing number of drug resistant *Plasmodium falciparum* strains, especially in Southeast Asia, Africa and South America. Chloroquine, a 4-aminoquinoline, was developed as a substitute for quinine in the treatment of malaria during World War II. However, chloroquine resistant malaria is now spreading rapidly throughout malaria endemic areas, which has provoked an intensive search for new drugs and the reappraisal of older ones (Krogstad *et al.*, 1987 and Martin *et al.*, 1987).

Mefloquine, a 4-quinolinemethanol, was developed in the Walter Reed Army Research Institute (USA), and it is the only drug proven to be effective against multiple drug-resistant malaria. Laboratory

studies indicate that a single oral dose of mefloquine administered to man has a prolonged antimalarial activity, and significant blood schizonticidal activity was observed in serum samples collected from individuals 28 days after they had received 1000 mg of this drug (Threnholme *et al.*, 1975 and Martin *et al.*, 1987).

Mefloquine does not significantly bind to DNA (Davidson *et al.*, 1975). Schupbach (1979) showed no mutagenic activity of mefloquine in three tester strains TA1535, TA1537 and TA1538 of *Salmonella typhimurium*. Grisolia and Takahashi (1994) did not find any clastogenic effect of mefloquine on bone marrow cells of Wistar rats, at different doses and treatment times. However, tests of clastogenic activity in human lymphocytes have not been done, *in vitro* or in patients undergoing therapy.

MATERIAL AND METHODS

Mefloquine hydrochloride was obtained from the Roche Company of Brazil, with a purity of 98.5%,

¹ Departamento de Genética e Morfologia, Instituto de Biociências, Universidade de Brasília, 70910-900 Brasília, DF, Brasil. Send correspondence to C.K.G.

² Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14049-901 Ribeirão Preto, SP, Brasil.

May 5, 1988 batch. The chemical composition is alpha-(2-piperidyl)-2-8-bis(trifluoromethyl-4-quinoline methanol) and the MW is 414.77.

***In vitro* tests**

Five independent cultures of human lymphocytes were set up for each experimental *in vitro* test. Blood samples (15 ml) were obtained from healthy non-smoking adults who were not on medication (three males and two females, 25-32 years old).

Chromosomal aberration test: Mefloquine was dissolved in three drops of dimethyl sulfoxide (DMSO, Merck), followed by further dilution with distilled water, thus yielding a final concentration of 3.0, 6.0 and 12.0 µg/ml. Treatment was initiated six hours after incubation at 37°C and cultures were then returned to the incubator for 42 hours. One hundred metaphases were examined for structural and numerical chromosomal aberrations, per dose.

Sister chromatid exchange test: The same doses were added to lymphocyte cultures, after six hours of incubation in the presence of bromodeoxyuridine (G₁ phase), the cultures were returned to the incubator for 66 hours at 37°C. Thirty metaphase cells in the second cycle were examined per dose for SCE scoring.

***In vivo* test**

The participants in the study were patients with *P. falciparum* in the blood and were undergoing mefloquine therapy. They came from gold panning areas in the Brazilian Amazon forest; their age group was 27-53 years and they had similar social and economic backgrounds. Moreover, they belonged to a heterogeneous sample, which included smokers, alcohol consumers, and individuals who had had other malaria infections before, but who had never taken mefloquine. In Brazil, the use of mefloquine is restricted by the Health Ministry, in order to avoid *P. falciparum* resistance, as was the case with chloroquine. Each patient was given a single dose of 4 X 250 mg tablets of mefloquine (Larimar - Roche). Two blood samples (10 ml) were collected from each patient, one prior to treatment and the other three days afterwards. The blood samples were incubated for chromosomal aberration (48 hours) and sister chromatid exchange analysis (72 hours) at 37°C.

The lymphocyte cultures followed Moorhead's *et al.* (1960) technique, modified by the Cytogenetics Laboratory of the Faculty of Medicine of Ribeirão Preto (FMRP-USP); using 80% RPMI medium (Gibco) with

20% normal serum, phytohemagglutinin (prepared in the Cytogenetics Laboratory of FMRP-USP, 0.02 mg/ml medium), streptomycin (CEME, 0.01 mg/ml) and penicillin (Fontoura Wyeth SA, 0.005 mg/ml). For sister chromatid exchange (SCE) analysis, 0.06 ml of bromodeoxyuridine (Sigma, 10 µg/ml) was added to each 10 ml culture. SCEs were visualized by a modification of the technique of Perry and Wolff (1974) and Korenberg and Freedlender (1974). One hundred cells were examined from each patient in the chromosomal aberration test, and 30 second-cell-cycle metaphases for SCE studies, before and after mefloquine therapy.

RESULTS

Chromosome analysis showed that mefloquine had no clastogenic activity in human lymphocytes *in vitro*. There was a small frequency of chromatid-type gaps and breaks, and a cytotoxic effect at 12 µg/ml, demonstrated by a significant decrease in mitotic index (Table I). Doses of 24 and 48 µg/ml induced an inhibition of lymphocyte proliferation.

Mefloquine also did not increase sister chromatid exchanges (Table II) (Wilcoxon test, $P = 0.1088$); however, a delay was observed in the cell cycle of lymphocytes treated with mefloquine for 72 hours. The proliferation index: $[PI = (M_3 - M_1) + 1]$, was used for comparison of the different doses in the five samples. The 12 µg/ml dose showed a significant delay in lymphocyte cell cycle for all five samples (Table II).

In patients undergoing mefloquine therapy, chromatid and isochromatid-type gaps and breaks were similar before and after a 72 hour-treatment, and there were no significant changes in mitotic indices (Table III).

For SCE scoring, only cultures from two patients (numbers 1 and 3) grew 72 hours after mefloquine treatment. These data are in accordance with Table III, which shows a delay in lymphocyte cell cycle at the highest *in vitro* treatment dose. Therefore, Table IV shows data pertaining to only two patients, and the SCE frequencies before and after mefloquine chemotherapy cannot be compared.

DISCUSSION

Cytogenetic studies of chromosomal aberrations and sister chromatid exchanges in human

Table I - Chromosome aberrations in human blood lymphocytes treated *in vitro* with mefloquine. One hundred metaphases were analyzed per treatment for C.A. and 1000 cells scored for MI.

Samples ($\mu\text{g/ml}$)	Sex	Chromatid-type aberrations		MI%
		Gaps	Breaks	
Control	M	-	1	3.65
DMSO		-	1	3.68
3.0		2	1	3.64
6.0		-	-	4.38
12.0		-	-	3.57
Control	M	-	-	3.92
DMSO		2	3	4.82
3.0		-	-	3.21
6.0		1	3	3.61
12.0		-	-	1.87*
Control	F	1	-	4.58
DMSO		1	2	5.26
3.0		1	1	5.10
6.0		-	1	3.70
12.0		-	1	1.54*
Control	M	2	4	7.96
DMSO		1	2	4.85
3.0		3	3	5.90
6.0		1	3	6.78
12.0		3	4	3.75*
Control	F	3	2	4.20
DMSO		1	4	5.20
3.0		4	1	2.25
6.0		-	4	2.12
12.0		1	5	1.53*

C.A. = chromosomal aberrations, MI = mitotic index.

DMSO = dimethyl sulfoxide.

*P < 0.05, t-test for paired samples.

lymphocytes are widely employed in the evaluation of mutagenicity in individuals occupationally or accidentally exposed to chemicals.

In the case of human consumption of medicinal substances, this type of research should be conducted by analyzing the genotoxic potential in human cells. Exposure on the part of risk groups to suspect chemical compounds should be biologically monitored in order to evaluate risk, taking into consideration

lifestyle, habits such as smoking and drinking alcoholic beverage or contact with other chemical compounds.

In Brazil, gold panners find themselves in a precarious social and economic situation, in which they coexist with diseases of the Amazon forest, such as tropical forest viroses; they come into contact with mercury used in gold panning sites; they drink alcoholic beverages; they smoke and they practice self-medication.

In view of the interference of so many factors, it is very difficult to carry out a risk evaluation of a given chemical compound for genotoxicity. To that end, it is necessary to have data available on the compound being analyzed under several different biological systems. In light of the present data, mefloquine is not clastogenic in human lymphocytes treated *in vitro* nor in lymphocytes of individuals undergoing therapy with this antimalarial drug.

According to Desjardins *et al.* (1979), mefloquine is rapidly absorbed when orally administered and spreads throughout the body, having a 13-day plasmatic half-life. It has a high level of affinity for the lungs, liver and lymphoid tissue and it is excreted mainly through faeces. Toxicological tests performed in rats and dogs have shown that administering up to 5 mg/kg/day for 28 consecutive weeks causes lymphocytopenia in rats, whereas in dogs it induces diarrhea, depletion of lymphoid organs and inflammatory reactions in the liver, with vacuolar degeneration (Rozman and Canfield, 1979).

Our data show cytotoxic effects of mefloquine in lymphocytes of human peripheral blood in cultures of 48 and 72 hours, in which doses above 12 $\mu\text{g/kg}$ of medium caused strong inhibition of cell proliferation. Likewise, blood samples collected from patients undergoing mefloquine therapy have also shown such effects. While all control cultures, that is, those of patients prior to therapy, had good-quality growth in 72-hour cultures, after therapy only two samples showed normal growth. It is probable that, when patients' lymphocytes were cultivated after treatment, small amounts of plasmatic mefloquine were included in the cultures, thus exerting a cytotoxic effect *in vitro*. In addition, according to Rozman and Canfield (1979), data from laboratory animals have shown that mefloquine exerts toxic effects on lymphocytes.

In conclusion, we show that mefloquine does not present a genotoxic effect and that, even at higher doses, cytotoxic, but not clastogenic effects are found. In order to fight *P. falciparum*, patients take doses of mefloquine that exert adverse effects on the proliferation of leukocytes when cultivated *in vitro* but chromosomal aberrations are not found.

Table II - Sister chromatid exchanges (SCE) test in human lymphocytes treated *in vitro* with mefloquine.

Samples ($\mu\text{g/ml}$)	Sex	SCE	M ₁ %	M ₂ %	M ₃ %	PI	Cells scored
		$\bar{X} \pm \text{SD}$					
Control	M	8.36 \pm 0.45	41	32	27	0.86	30
DMSO		11.40 \pm 0.77	59	27	14	0.55	30
3.0		11.60 \pm 0.56	49	29	22	0.73	30
6.0		11.33 \pm 0.81	73	21	6	0.33*	18
12.0		9.75 \pm 2.01	81	12	7	0.26*	4
Control	M	7.66 \pm 0.57	54	20	20	0.66	30
DMSO		7.00 \pm 0.47	59	23	18	0.59	30
3.0		6.40 \pm 0.38	56	30	14	0.58	30
6.0		7.19 \pm 0.59	40	33	27	0.87	26
12.0		7.93 \pm 0.52	70	23	7	0.37*	15
Control	F	9.40 \pm 0.60	46	39	15	0.69	30
DMSO		8.23 \pm 0.53	38	35	27	0.89	30
3.0		8.56 \pm 0.46	49	22	29	0.80	30
6.0		8.20 \pm 0.52	53	36	11	0.58	30
12.0		8.00 \pm 0.58	72	22	6	0.34*	30
Control	M	11.93 \pm 0.51	55	29	16	0.61	30
DMSO		11.93 \pm 0.61	52	32	16	0.64	30
3.0		12.80 \pm 0.83	49	32	19	0.71	30
6.0		11.41 \pm 0.63	56	32	12	0.56	28
12.0		11.31 \pm 0.74	67	24	9	0.42*	22
Control	F	7.57 \pm 0.59	53	27	20	0.67	30
DMSO		8.25 \pm 0.53	50	31	19	0.69	30
3.0		8.43 \pm 0.46	44	29	27	0.83	30
6.0		9.23 \pm 0.41	51	35	14	0.63	30
12.0		8.03 \pm 0.61	59	27	14	0.47*	30

M₁%, M₂%, M₃% = percentage of metaphases at the 1st, 2nd, 3rd cell cycle, respectively.

PI = proliferative index.

DMSO = dimethyl sulfoxide.

*P < 0.05, X².

Table III - Chromosomal aberrations and mitotic index (MI) in human lymphocytes of patients before and after mefloquine therapy. Thirty metaphase cells were analyzed before and after treatment, respectively.

Patients	Sex	Before					After				
		Gaps		Breaks		MI%	Gaps		Breaks		MI%
		IC	C	IC	C		IC	C	IC	C	
1	M	1	3	0	1	6.42	1	1	1	2	4.56
2	F	0	2	0	1	1.23	0	1	0	0	4.84
3	M	2	2	1	3	1.83	0	1	1	1	6.66
4	M	0	1	2	4	1.00	0	2	0	2	2.61
5	M	0	1	1	1	2.24	0	1	1	3	1.81

C = chromatid.

IC = isochromatid.

Table IV - Sister chromatid exchanges (SCE) and proliferative index (PI) in lymphocytes derived from mefloquine treated patients. Thirty metaphase cells were analyzed before and after treatment, respectively.

Patients	Before					After				
	SCE/Cell	M ₁ %	M ₂ %	M ₃ %	PI	SCE/Cell	M ₁ %	M ₂ %	M ₃ %	PI
1	12.5 ± 0.7	41	33	26	0.85	14.6 ± 0.6	37	41	22	0.85
3	13.0 ± 1.0	52	35	13	0.61	8.8 ± 0.5	55	33	12	0.57

M₁%, M₂%, M₃% = percentage of metaphases at the 1st, 2nd and 3rd cell cycle, respectively.

ACKNOWLEDGMENTS

Project supported by the National Research Council (CNPq-Brazil) and the Brazilian Ministry of Education (CAPES-PICD). The authors thank to the Malaria Division of The Brazilian Ministry of Health, A.T. Natarajan from Leiden University (Netherlands) for precious comments and Mrs. Isabel K.F. de Miranda Santos (CENARGEN-EMBRAPA) for technical review.

Publication supported by FAPESP.

RESUMO

Este trabalho avalia o antimalárico mefloquina a nível de mutagenicidade em linfócitos humanos tratados *in vitro* e *in vivo*. Nos tratamentos *in vitro*, a mefloquina produziu efeitos citotóxicos, observados através de reduções nos índices mitóticos, mas não houve aumentos nas frequências de aberrações cromossômicas e trocas entre cromátides irmãs. *In vivo*, verificou-se redução no índice proliferativo dos linfócitos, indicando também efeitos tóxicos da mefloquina, mas sem evidências de mutagenicidade.

REFERENCES

- Davidson, M.W., Griggs Jr., B.G., Boykin, D.W. and Wilson, W.D.** (1975). Mefloquine, a clinically useful quinolinemethanol antimalarial which does not significantly bind to DNA. *Nature* 254: 632-634.
- Desjardins, R.E., Pamplin III, C.L., von Bredow, J., Barry, K.G. and Canfield, J.** (1979). Kinetics of a new antimalarial, mefloquine. *Clin. Pharmacol. Ther.* 26: 372-379.
- Grisolia, C.K. and Takahashi, C.S.** (1994). Assessment of antimalarial drugs chloroquine and mefloquine with NaNO₂ and HgCl₂ in rodents. *Mutat. Res.* 305: 151-156.
- Korenberg, J.R. and Freedlender, E.F.** (1974). Giemsa technique for detection of sister chromatid exchanges. *Chromosoma* 48: 355-360.
- Krogstad, D.J., Guzman, I.Y., Kyle, D.E., Oduola, A.M.J., Martin, S.K., Milhous, W.K. and Schlesinger, P.H.** (1987). Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. *Science* 238: 1283-1285.
- Martin, S.K., Oduola, A.M.J. and Milhous, W.K.** (1987). Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. *Science* 235: 899-901.
- Moorhead, P.S., Norwell, P.C., Mellman, W.J., Batipps, D.M. and Hungerford, D.A.** (1960). Chromosome preparation of leukocyte culture from human peripheral blood. *Exp. Cell Res.* 20: 613-616.
- Perry, P. and Wolff, S.** (1974). New giemsa method for the differential staining of sister chromatid exchanges. *Nature* 251: 156-158.
- Rozman, R.S. and Canfield, C.J.** (1979). New experimental antimalarial drug. *Adv. Pharmacol. and Chemotherapy* 16: 1-43.
- Schupbach, M.E.** (1979). Mutagenicity evaluation of the two antimalarial agents chloroquine and mefloquine, using a bacterial fluctuation test. *Mutat. Res.* 68: 41-49.
- Threnholme, G.M., Williams, R.L., Desjardins, R.E., Frischer, H., Carson, P.E. and Rieckmann, K.H.** (1975). Mefloquine WR 142,490 in the treatment of human malaria. *Science* 235: 792-794.

(Received October 20, 1994)