Bull. Environ. Contam. Toxicol. (1992) 49:395–401

Environmental Contamination and Toxicology

In Vitro Effect of Glutathione on Mitomycin-C in Human Lymphocytes

D. Geetanjali, P. Rita, and P. P. Reddy

Institute of Genetics, Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad-500 016, Andhra Pradesh, India

Mitomycin-C (MMC) is a potent antibiotic and anticancer drug known to cause mutagenic effects (Gebhart 1980). The mutagenic potential of MMC induced micronuclei in Swiss albino mice using the invivo bone-marrow micronucleus test was evaluated in the presence of glutathione (Rita et al. 1991). The lymphocytes were treated with 0.2 ug/ml of MMC and 2.5, 5.0, 10.0, and 20.0 ug/ml glutathione (GSH) for 24, 48 and 72 hrs. There was significant increase in the frequency of chromosomal aberrations in cells treated with MMC alone, however the incidence of chromosomal aberrations reduced in cells treated with MMC and GSH in combination. The results indicate that GSH plays a protective role in the presence of MMC.

MMC has shown antitumor activity in a wide variety of clinical and experimental cancers (Crooke and rander 1976, Doll et al. 1985). It produces specific locus mutations in spermatogonia of male mice (IARC 1976) and induce sister chromatid exchanges in bone marrow and testes of rats, mice and human lymphocytes with invivo treatment. Investigation on the mechanism of carcinogenic and mutagenic action of MMC is based on its conversion to toxic metabolites and its ability to damage DNA (Doll et al. 1985). Glutathione plays an important role in the process of antimutagenesis and anticarcinogenesis (Ketterer 1988). It has been reported that it is antimutagenic in vitamin-A deficiency rats treated with benzo(a) pyrene (Alzieu et al. 1987). Lester and Leishel (1988) found that GSH detoxified methyl parathion and azinophosmethyl in invivo investigation. In the present study the mutagenic potential of MMC is evaluated in the presence of GSH in vitro human lymphocytes.

Send reprint requests to D.Geetanjali at the above address.

MATERIALS AND METHODS

Mitomycin-C (100% purity, CAS No,66F 0494) and Glutathione (100% purity, CAS No. G 4251) were obtained from sigma chemical company (St. Louis, MO,U.S.A.) for the present study.

Intravenous blood was collected from healthy human donor (male) under aseptic conditions. Lymphocyte cultures were initiated by adding 0.3 ml of whole blood to RPMI 1640 medium supplemented with human AB serum, (25%) phytohemagglutinin (0.5%) and dicrystacine (0.25%). Four sets of cultures were maintained. First set of cultures were treated with 0.2 ug/ml of MMC and four concentrations of GSH i.e. 2.5, 5.0, 10.0 and 20.0 ug/ml, second set of cultures received four concentrations of GSH only, third set of cultures were treated with 0.2 ug/ml MMC and fourth set of cultures were treated with an equal volume of distilled water (control). The chemicals were treated to the cultures at different time intervals for 24, 48 and 72 hrs of duration. The cultures were terminated by adding 0.1 ug/ml of colchicine, two hours before harvesting the cultures, to arrest the cell cycle at metaphase. All the cultures were harvested by the method of Moorhead et al. (1960).

400 metaphases were scored for chromosomal aberrations for each concentration and in each set of experiment. The experiment was repeated twice for chromosomal aberrations. The aberration frequencies were similar in both experiments. Various types of chromosomal like chromatid gaps, breaks, deletions, chromatid fragments and isochromatid gaps and breaks were recorded. Statistical analysis of the data was made by using χ^2 test for chromosomal aberrations.

RESULTS AND DISCUSSION

The results on the incidence of chromosomal aberrations after the treatment with 2.5, 5.0, 10.0 and 20.0 ug/ml of GSH for 24,48 and 72 hrs. are given in table I. Chromatid breaks, gaps, deletions, fragments and isochromatid gaps and breaks were observed. There was a dose dependent increase at all intervals in the frequency of chromosomal aberrations. However the statistical analysis reveals that the increase in the frequency chromosomal aberrations was not significant when compared to control. Table 2 shows a statistically significant increase in the chromosomal aberrations in cultures treated with MMC between control at all time intervals. The data in table 3 shows that there was no statistically significant increase in the incidence of chromosomal aberrations treated with MMC (0.2 ug/ml) and GSH (2.5, 5.0, 10.0 and 20.0 ug/ml) simultaneously when compared to control.

Table 1. Frequency of chromosomal aberrations in human lymphocytes after treatment with glutathione for 24, 48 and 72 hrs. Values given in parenthesis are percentages.

Concentrations of glutathione ug/ml	No.of metaphases screened	Gaps		Breaks		Chromatid fragments	Deletions	Total No.of aberrations	
		Chromatid	Iso chromatid	Chromatid	Iso chromatid			+ gaps	- gaps
24 hrs.									
Control	400	4(1.0)	0.0	2(0.5)	0.0	0.0	0.0	6(1.5)	2(0.50)
2.5	400	6(1.5)	0.0	4(1.0)	0.0	0.0	0.0	10(2.5)	4(1.00)
5.0	400	6(1.5)	0.0	4(1.0)	0.0	0.0	0.0	10(2.5)	4(1.00)
10.0	400	7(1.75)	0.0	6(1.5)	0.0	0.0	0.0	13(3.25)	6(1.50)
20.0	400	6(1.50)	0.0	5(1.25)	0.0	2.0(0.50)	0.0	13(3.25)	7(1.75)
48 hrs.									
Control	400	4(1.0)	0.0	3(0.75)	0.0	0.0	0.0	7(1.75)	3(0.75)
2.5	400	5(1.25)	0.0	5(1.25)	0.0	0.0	0.0	10(2.50)	5(1.25)
5.0	400	6(1.50)	0.0	5(1.25)	0.0	0.0	0.0	11(2.75)	5(1.25)
10.0	400	7(1.75)	0.0	6(1.50)	0.0	1.0(0.25)	0.0	14(3.50)	7(1.75)
20.0	400	7(1.75)	0.0	7(1.75)	0.0	2.0(0.50)	0.0	16(4.0)	9(2.25)
72 hrs.									
Control	400	5(1.25)	0.0	3(0.75)	0.0	0.0	0.0	8(2.0)	3(0.75)
2.5	400	6(1.50)	0.0	4(1.0)	0.0	0.0	0.0	10(2.50)	4(1.0)
5.0	400	8(2.0)	0.0	6(1.50)	0.0	0.0	0.0	14(3.50)	6(1.50)
10.0	400	7(1.75)	0.0	6(1.50)	0.0	1.0(0.25)	1.0(0.25)	15(3.75)	8(2.0)
20.0	400	7(1.75)	0.0	5(1.25)	1.0(0.25)	1.0(0.25)	2.0(0.50)	16(4.0)	9(2.25)

400 metaphases were scored for each concentration.

Table 2. Frequency of chromosomal aberrations in human lymphocytes after treatment with mitomycin-C for 24, 48 and 72 hrs. Values given in parenthesis are percentages.

Mitomycin-c ug/ml	No.of metaphases screened	Gaps		Breaks			Deletions	Total No.of aberrations	
		Chromatid	Iso chromatid	Chromatid	Iso chromatid	chromatid fragments		+ gaps	- Gaps
24 hrs.									
Control	400	4(1.0)	0	2(0.50)	0	0	0	6(1.5)	2(0.50)
0.2	400	10(2.50)	6(1.50)	8(2.00)	4(1.00)	4(1.00)	4(1.00)	38(9.5)*	22*(5.5)
48 hrs.									
Control	400	4(1.0)	0	3(0.75)	0	0	0	7(1.75)	3(0.75)
0.2	400	12(3.00)	6(1.50)	9(2.25)	5(1.25)	8(2.00)	3(0.75)	43(10.75)*	25*(6.25)
72 hrs.									
Control	400	3(0.75)	0	4(1.00)	0	0	0	7(1.75)	4(1.00)
0.2	400	14(3.50)	8(2.0)	10(2.50)	6(1.50)	8(2.00)	5(1.25)	51(12.75)*	29*(7.25)

* Significant at 5% level ($P < 0.05$)
Control - Distilled water.

Table 3. Comparison of the frequencies of chromosomal aberrations in human lymphocytes after treatment with mitomycin-C and glutathione at 72 hrs. Values given in parenthesis are percentages.

GSH ug/ml	MMC ug/ml	No.of metaphases screened	Gaps		Breaks		Chromatid fragments	Deletions	Total No.of aberrations	
			Chromatid	Iso chromatid	Chromatid	Iso chromatid			+ gaps	- gaps
Control	0	400	5(1.25)	0.0	3(0.75)	0.0	0.0	0.0	8(2.0)	3(0.75)
2.5	0	400	6(1.50)	0.0	4(1.0)	0.0	0.0	0.0	10(2.50)	4(1.00)
5.0	0	400	8(2.00)	0.0	6(1.50)	0.0	0.0	0.0	14(3.50)	6(1.50)
10.0	0	400	7(1.75)	0.0	6(1.50)	0.0	1.0(0.25)	1.0(0.25)	15(3.75)	8(2.00)
20.0	0	400	7(1.75)	0.0	5(1.25)	1.0(0.25)	1.0(0.25)	2.0(0.50)	16(4.0)	9(2.25)
2.5	0.2	400	6(1.50)	0.0	4(1.0)	0.0	0.0	0.0	10(2.50)	4(1.00)
5.0	0.2	400	7(1.75)	0.0	4(1.0)	0.0	1.0(0.25)	0.0	12(3.00)	5(1.25)
10.0	0.2	400	7(1.75)	1.0(0.25)	5(1.25)	1.0(0.25)	1.0(0.25)	0.0	15(3.75)	7(1.75)
20.0	0.2	400	8(2.00)	1.0(0.25)	6(1.50)	1.0(0.25)	1.0(0.25)	1.0(0.25)	18(4.50)	10(2.50)

The results indicate that GSH plays a protective role in process of chromosomal damage caused by MMC human lymphocyte cultures. Earlier the bio antimutagenic property of L-Cysteine, one of the metabolites of GSH have already proved in invitro system (Speit et al. 1980, Inoue et al. 1985) and in mice bone marrow micronucleus test against an antiamoebic drug (Ghaskadbi et al. 1987). Further Rita et al. (1991) showed that the mutagenicity induced by MMC was reduced with the GSH in vivo bone marrow micronucleus test of mice. The biochemical mechanism of GSH involved in the protection of MMC mutagenicity was not known.

However, GSH derivatives undergo transformation within the detoxifying system of the liver. They are further metabolized to cysteine and mercapturic acid. Ketterer (1988) reviewed the ability of GSH to detoxify electrophiles which have an important role in the process of mutagenesis and carcinogenesis. It is a hypothesis that sulphohydryl group present in GSH brings about biochemical changes to protect DNA from the free radical attack and lead to elevation of DNA repair. Cysteine which is derived from GSH transformation is known to stimulate the biosynthesis of polyamines (Acuff and Smith 1983) and polyamines are reported to stabilize DNA (Tabor and Tabor, 1984). Probably cysteine a byproduct of GSH is involved in the protection against the mutagenic action of MMC in invitro human lymphocyte cultures.

REFERENCES

Acuff RV, Smith JT (1983) The influence of cysteine and methioine supplements on polyamines biosynyhesis in rat. J Nutr 113: 2295-2299.

Alzieu P, Cassand P, Colin C, Grolier P, Narbonne JF (1987) Effect of vitamin-A, C and glutathione on the mutagenicity of Benzo(a)pyrene medicated by S9 from vitamin-A deficient rats. Mut Res 192: 227-237.

Crooke ST, Bradner WT (1976) Mitomycin-C a review. Cancer Treat Rev 3: 121-139.

Doll DC, Wiers RB, Jsseli BP (1985) Mitomycin ten years after approval for marketing. J Clin Oncol 3: 276-286.

Gebhart EB, Windol P, Wopfnur F (1980) Chromosome studies on lymphocytes of patients under cytostatic therapy. II. Studies using the Budr labelling techniquee in cytostatics interval therapy. Hum Genet 51: 167-170.

Ghaskadbi S, Pavaskar SV, Vaidya VG (1987) Bioantimutagenic effect of L.Cysteine on diiodohydroxyq-uinoline-induced micronuclear in Swiss albino mice. Mut Res 187: 219-222.

IARC monographs (1976) Mitomycin in IARC monographs on the evaluation of the carcinogenic risk of chemicals to man : some naturally occuring substances. International Agency for Research on Cancer Lyon 10: 171-179.

Inoue K, Shibata T, Kosaka H, Uozami M. Tsuda S, Abe T (1985) Induction of sister-chromatid exchanges by N-nitrosocimetidine in cultured human lymphocytes and its inhibition by chemical compounds. Mut Res 56: 117-121.
Ketterer Brain (1988) Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. Mut Res 202: 343-361.
Lester G, Leishel W (1988) The role of glutathione in the detoxification of the insecticides methyl parathion and azinophosmethyl in the mice. Toxicol Appl Pharmacol 96:168-174.
Moorhead PS, Nowell PC, Mellmann WJ, Battips DM, Hunger ford DA (1960) chromosome preparations of leucocytes cultured from human peripheral blood. Exp Cell Res 20: 613-616.
Rita P, Geetanjali D, Reddy P P (1991) Effect of glutathione on mitomycin-C induced micronuclei in bone marrow erythrocytes of swiss albino mice. Mut Res 260: 131-135.
Speit G, Wick C, Wolf M (1980) Induction of sister chromatid exchanges by hydroxylamine, hydralazine and isoniazid and their inhibition by cysteine. Hum Genet 54: 155-158.
Tabor CW, Tabor H (1984) Polyamines. Ann Rev Biochem 53: 749-790.

Received March 26, 1991; accepted April 9, 1992.