Indian Journal of Experimental Biology
Vol. 24, December 1986, pp 767-770

Role of 2′-Mercaptopropionylglycine (MPG) against Toxicity of Cyclophosphamide in Normal and Tumour-bearing Mice

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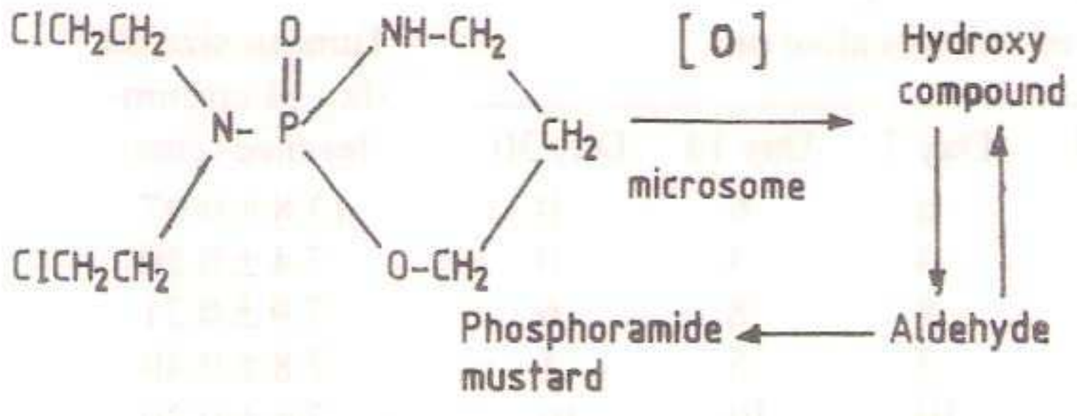
Received 1 *October* 1985; *revised* 15 *July* 1986

Protective effect of MPG against the side effects of cyclophosphamide was studied in normal as well as tumour bearing mice. It has been noticed that MPG treatment improved the cyclophosphamide induced toxicities such as ruffling of hair, epilation and diarrhoea and enhanced survival rate against cyclophosphamide induced mortality. The leucocytopenia and increased serum alkaline phosphatase and serum glutamate pyruvate transaminase levels induced by cyclophosphamide toxicity were also found to be corrected by MPG treatment. Experiments with different doses of MPG and cyclophosphamide, in ascites tumour bearing mice indicated that MPG repairs the cyclophosphamide induced lethal damages while retaining the anticancer property of cyclophosphamide.

Most of the anticancer drugs currently used in therapy are cytotoxic to normal cells leading to unwanted side effects. Therapeutically effective doses of many anticancer drugs may produce irreversible changes in normal tissues which may prove toxic to the patients. Therefore, a search for a compound which can reduce the harmful side effects of anticancer drugs in normal tissues is necessary. The problem of finding a substance which combines a high degree of protection to normal tissues with a minimum or no protection to tumour tissues has been of great interest. Several investigators are working on with a view to find out a suitable drug against cytotoxic effects of anticancer drugs to normal tissues[1-5]. In the present study a synthetic −SH compound, 2′-mercaptopropionylglycine (MPG) has been tested against the toxicity of cyclophosphamide in normal as well as in tumour bearing mice.

Cyclophosphamide, an alkylating agent, is a broad-spectrum anticancer drug which gets activated in the liver by microsomal enzyme system in the presence of oxygen and reduced NADPH. Out of the resulting breakdown products, phosphoramide mustard is the major alkylating agent, which is supposed to be further activated at the site of action and converted to the proper cytostatic agent[6].



The MPG is a sulphydryl derivative and its chemical structure is as given below:-

$$CH_3-CH(SH)-CONH-CH_2-COOH$$

MPG due to its −SH group has an important physiological function in the human body as an anti-toxic agent and is an enzymatic activator[7]. MPG is a potent radioprotector and effective at a nontoxic dose of 20 mg/kg body weight, far below its toxic dose, i.e. 2100 mg/kg body weight[8]. The present study was undertaken with a view to find out the modifying properties of MPG treatment against toxicity of cyclophosphamide in normal as well as tumour bearing mice.

Materials and Methods

Adult male Swiss albino mice weighing 25 ± 4 g were selected from an inbred colony, maintained on standard mice feed and water *ad libitum*. MPG was received from Santen Pharmaceutical Co., Osaka, Japan and dissolved in double distilled water and *p*H was adjusted to 6.5 by using dilute NaOH solution. MPG was injected (ip) 20 to 30 min before cyclophosphamide treatment at the dose of 20, 50, 100 and 200 mg/kg body weight. The cyclophosphamide (Endoxan-ASTA; Khandelwal Laboratories Pvt Ltd, Bombay) was administered (ip) at different doses of 50, 100, 200 and 500 mg/kg body weight, with and without MPG pre-treatment (Table 1).

The Dalton's lymphoma tumour cells grown in ascites form were collected from mice and washed two to three times with normal physiological saline. These cells further resuspended in normal physiological saline to adjust the concentration to 1 million cells/ml. One ml of this solution was injected (ip) to the animals. After 7 days of tumour cells injection, the animals

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were treated with cyclophosphamide with or without MPG. The mortality data were recorded during 30 days and tumour volume was measured on day 14. The blood for biochemical and microscopic tests was collected from caudal vein at two different intervals i.e. on day 3 and 7. Total leucocyte counts were performed using Neubauer haemocytometer. The serum alkaline phosphatase and serum glutamate pyruvate transaminase levels were determined by 4-aminoantipyrine method and dinitrophenyl hydrazine methods, respectively[9].

Results

The cyclophosphamide induced toxic manifestations such as ruffling of hair, epilation and diarrhoea were less severe in the case of MPG protected animals. Single dose of 500 mg/kg body weight of cyclophosphamide was found to be highly toxic to animals as most of the animals died by day 7, whereas animals treated with cyclophosphamide and MPG survived up to day 30 (Table 1).

Moreover the pharmacologically effective dose of MPG has been non-toxic and even 10 times higher doses were found to be quite safe. No mortality was recorded in any of MPG-treated group up to 14 days, even at a dose of 200 mg/kg body weight up to 7 days (Table 1). In another experiment the animals treated with cyclophosphamide at the dose of 200 and 500 mg/kg body weight on alternate days, showed highest mortality during 7 days, whereas in case of MPG pretreated animals (daily for 14 days) survival was higher on day 7 in comparison to control group (only cyclophosphamide treated group) (Table 1). These results indicated that MPG has been protective against the cyclophosphamide induced mortality and side effects, even at repeated dose levels (Table 1).

In order to study whether the cyclophosphamide treatment with MPG retains the anticancer effect, a group of experiments were conducted and results presented in Table 2. Dalton's lymphoma cells (1 million cells) injected animals (without any drug treatment) showed distended abdomen by day 10 onwards: 50% animals were dead by day 14 and 100% mortality was recorded by day 20, whereas cyclophosphamide treatment at the dose of 50 mg/kg body weight (from day 7 to 21 daily) also resulted in mortality of animals approximately at the same ratio as in case of without any drug treatment. Only one animal out of 20 survived on day 30 in this group, while in case of MPG pretreatment all the animals survived up to day 30 and all the animals were disease free (Table 2). The above experiment was repeated with a higher dose of cyclophosphamide (up to 500 mg/kg body weight) causing further toxicity, which could be prevented at least partially by MPG treatment.

The cyclophosphamide treatment caused reduction in the number of total leucocyte in mice on days 3

Table 1—Cyclophosphamide Induced Mortality in Normal Mice and Its Modification by MPG

[Ten animals were used in each test, MPG was injected before 30 min of cyclophosphamide injection]

Sl. No.	Cyclophosphamide dose (mg/kg body wt)	MPG dose (mg/kg body wt)	No. of animals alive on					
			Day 1	Day 3	Day 7	Day 14	Day 30	(% survival)
A	500 × 1	Nil	10	7	2	0	0	—
B	Nil	20 × 7 (Daily)	10	10	10	10	10	100%
C	Nil	100 × 7 (do)	10	10	10	10	10	100%
D	Nil	200 × 7 (do)	10	10	10	10	10	100%
E	500 × 1	20 × 7 (do)	10	10	10	10	10	100%
F	200 × 7 (alt. days)	Nil	10	10	1	0	0	—
G	200 × 7 (do)	20 × 14 (do)	10	10	10	8	8	80%
H	500 × 7 (do)	Nil	10	9	0	0	0	—
I	500 × 7 (do)	20 × 14 (do)	10	10	7	2	0	—

Table 2—Survival Rate of Ascitis Bearing Animals with Cyclophosphamide Treatment and its Modification by MPG

[Ten animals were used in each set of experiment. The drug treatment started 7 days after the injection of Dalton's lymphoma cells. MPG was injected 30 min before cyclophosphamide injection]

Sl. No.	Cyclophosphamide dose (mg/kg body wt)	MPG dose (mg/kg body wt)	No. of animals alive on					Tumour size on day 14 circumferance (cms)
			Day 1	Day 3	Day 7	Day 14	Day 30	
A	Nil	Nil	10	9	6	6	0	13.8 ± 0.07
B	100 × 14 (daily)	Nil	10	8	3	3	0	7.4 ± 0.20
C	100 × 14 (do)	20 × 14(daily)	10	10	8	8	6	7.9 ± 0.21
D	150 × 14 (do)	Nil	10	9	7	5	1	7.8 ± 0.40
E	50 × 14 (do)	20 × 14 (do)	10	10	10	10	10	7.6 ± 0.20
F	Control							7.5 ± 0.28

and 7, whereas the leucocyte counts in most of the MPG-treated groups were found higher as compared to only cyclophosphamide treated groups. The values were statistically significant (Table 3).

The serum alkaline phosphatase and serum glutamate pyruvate transaminase levels were elevated in cyclophosphamide treated group as compared to normal. MPG treated group showed slight reduction of these parameters as compared to cyclophosphamide treated group (Table 4 & 5). The values were found to be statistically significant.

Discussion

In the present study the treatment of MPG reduced the cyclophosphamide induced toxicity. The general health characters of MPG-treated group were better than the cyclophosphamide alone treated ones. The repeated doses of cyclophosphamide and MPG treatment also resulted in a higher survival rate in comparison to only cyclophosphamide treated group which indicates that higher doses of cyclophosphamide can be used under the influence of MPG. The repeated doses of cyclophosphamide, (200 mg/kg body weight, alternate days) showed 100% mortality during 7 days whereas MPG pretreatment provides protection against this, resulting 80% survivability in day 30. Since gastrointestinal epithelium and the bone marrow usually represents most life-threatening toxic reactions during cancer chemotherapy, the early death of cyclophosphamide treated animals may be due to the gastrointestinal syndrome. Hopkins *et al.*[10] and Hopkins and Looney[11] reported that the gastrointestinal mucosa in the rat recovers in 4 to 5 days following one single ap-

Table 3—Total Leucocyte Count/mm^3 of Ascitis Bearing Mice with Cyclophosphamide Treatment and Its Modification by MPG

[Drug treatment started 7 days after the injection of Dalton's Lymphoma cells. MPG was administered 30 min. before cyclophosphamide injection]

Sl. No.	Tumour Injection on −7 day	Cyclophosphamide dose (mg/kg body wt)	MPG dose (mg/kg body wt)	Total lymphocyte count cells/mm^3			
				Day 3 (mean ± SE)	*P* Value	Day 7 (mean ± SE)	*P* Value
A	−	500 × 1	--	2,125 ± 180		3,025 ± 51	
B	−	500 × 1	20 × 7 (daily)	2,765 ± 160	(>0.001)	3,635 ± 128	(>0.001)
C	−	200 × 7 (alt.days)	--	4,000 ± 41		4,200 ± 76	
D	−	200 × 7 (")	20 × 14 (do)	4,400 ± 104	(>0.001)	4,100 ± 141	(<0.1)
E	−	500 × 7 (")	--	4,200 ± 256		Animal died	
F	−	500 × 7 (")	20 × 14 (do)	4,500 ± 321	(<0.1)	Animal died	
G	+	100 × 14 (")	--	4,100 ± 100		1,870 ± 104	
H	+	100 × 14 (")	20 × 14 (do)	4,000 ± 104	(<0.1)	2,800 ± 76	(>0.001)
I	+	50 × 14 (")	--	3,800 ± 64		3,000 ± 210	
J	+	50 × 14 (")	20 × 14 (do)	3,900 ± 50	(>0.05)	3,100 ± 125	(<0.01)
K	+	--	--	3,000 ± 64		2,800 ± 217	
L	−	--	--	4,100 ± 52			

−, No tumour cells; +, Tumour cell injected; --, No drug treatment.

Table 4—Serum Alkaline Phosphatase Levels (KA units) in Ascites Bearing Mice with Cyclophosphamide Treatment and its Modification by MPG

Sl. No.	Tumour injection on-7 day	Endoxan dose mg/kg body wt	MPG dose mg/kg body wt	Serum alkaline phosphatase levels			
				Day 3 (Mean ± SE)	*P* Value	Day 7 (Mean ± SE)	*P* Value
A	−	500 × 1	--	6.05 ± 0.12	>0.001	6.4 ± 0.15	>0.001
B	−	500 × 1	20 × 7	4.2 ± 0.14	>0.05	4.05 ± 0.06	>0.001
C	−	200 × 7 (Alt. days)	20 × 14	2.5 ± 0.16	<0.1	2.8 ± 0.12	<0.1
D	−	200 × 7 (")	--	5.5 ± 0.27	>0.001	Animal died	—
E	−	500 × 7 (")	20 × 14	4.1 ± 0.25	>0.05	Animal died	—
F	−	500 × 7 (")	--	7.2 ± 0.22	>0.001	Animal died	—
G	+	100 × 14 (daily)	20 × 14	3.4 ± 0.16	<0.1	4.1 ± 0.16	>0.01
H	+	100 × 14 (")	--	3.6 ± 0.06	>0.05	4.6 ± 0.08	>0.001
I	+	50 × 14 (")	20 × 14	3.6 ± 0.13	>0.05	2.6 ± 0.16	<0.1
J	+	50 × 14 (")	--	4.2 ± 0.18	>0.05	3.6 ± 0.26	>0.01
K	+	—	--	4.1 ± 0.12	>0.01	4.8 ± 0.08	>0.001
L	−	—	--	3.1 ± 0.14	—	—	—

−, No tumour cells; +, Tumour cell injected; --, No drug treatment

Table 5 – Serum Glutamate Pyruvate Transaminase Levels (IU/Liter) in Ascites Bearing Mice with Cyclophosphamide Treatment and Its Modification by MPG

Sl. No.	Tumour injection on-7 day	Cyclophosphamide dose (mg/kg body wt)	MPG dose (mg/kg body wt)	Serum glutamate pyruvate transminase level			
				Day 3 (Mean ± SE)	*P* Value	Day 7 (Mean ± SE)	*P* Value
A	–	500 × 1	--	6.22 ± 0.09	> 0.001	5.8 ± 0.18	> 0.001
B	–	500 × 1	20 × 7	5.8 ± 0.21	> 0.001	6.0 ± 0.06	> 0.001
C	+	100 × 14	20 × 14	3.05 ± 0.10	< 0.1	3.0 ± 0.21	< 0.1
D	+	100 × 14	--	3.85 ± 0.95	> 0.001	4.0 ± 0.17	> 0.001
E	+	50 × 14	20 × 14	2.25 ± 0.15	> 0.05	2.0 ± 0.10	> 0.001
F	+	50 × 14	--	5.2 ± 0.20	> 0.001	6.0 ± 0.12	> 0.001
G	+	--	--	5.2 ± 0.20	> 0.001	5.6 ± 0.14	> 0.001
H	–	--	--	2.8 ± 0.09	—	—	—

–, No tumour cells; +, Tumour cell injected; --, No drug treatment.

proximately LD/10 dose of cyclophosphamide while bone marrow recovers in 10 to 11 days. Thus the death occured during 7 days period in the present experiment can be attributed to the gastrointestinal syndrome. The enhanced survival rate in normal animals by MPG against cyclophosphamide induced mortality may be due to the result of protection given by MPG to the gastrointestinal epithelium and hematopoietic organs of mice against cyclophosphamide induced toxicity.

The survival rate of MPG-treated ascites bearing animals revealed that MPG treatment did not produce adverse effect on anticancer property of the cyclophosphamide which suggests that higher doses of cyclophosphamide can be used safely in the treatment of tumour in presence of MPG. A lower level of serum alkaline phosphatase and serum glutamate pyruvate transaminase in MPG-treated group in comparison to ascites cell injected with or without cyclophosphamide treated groups revealed protection by MPG against the liver necrosis in normal as well as tumour bearing animals.

The total leucocyte count reduced considerably after cyclophosphamide treatment on day 3. Looney and Hopkins[11] also reported that circulating lymphocytes declined rapidly and reached lowest level on day 5 and also a similar decline in polymorphonuclear leucocytes with a nadir on day 4. The higher number of total leucocyte in MPG-protected group in the present study may be due to the protection of hematopoietic organs by MPG. Similar results were reported in case of MPG protection against Gamma Rays induced leucopenia in mice[12].

The exact mechanism by which MPG protects the animal against the cyclophosphamide induced toxicity is not clearly understood. Autoradiographic studies indicate that MPG inhibits mitosis temporarily during early hours. The temporary inhibition of mitosis may allow time for repair processes to act before cyclophosphamide induced structural defects are replicated.

The present studies clearly indicate that MPG protects the toxic manifestation of the cyclophosphamide, while retaining the anticancer effects of cyclophosphamide. Therefore MPG is a useful adjuvant drug against the treatment of cancer.

Acknowledgement

The financial support by CSIR, New Delhi and technical assistance by Miss. Mariyamma are acknowledged. The authors are thankful to Dr.V.P. Gopinath for his comments.

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