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Research Article

# Chemoprotective effect of cysteamine against the induction of micronuclei by methyl methanesulfonate and cyclophosphamide

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## Abstract

Cysteamine or 2-mercaptoethylamine (MEA) is an aminothiol with a well-known radioprotective action. No specific information is available in the literature about the possible chemoprotective action of MEA against genotoxic chemical agents. This paper presents the results of studies on the ability of MEA to protect mouse bone marrow polychromatic erythrocytes against the induction of micronuclei by alkylating agents such as methyl methanesulfonate (MMS) and cyclophosphamide (CP). We observed that MEA administered intraperitoneally 30 min before or 30 min after the administration of MMS or CP significantly reduced the frequency of micronucleated polychromatic erythrocytes (MNPCEs) induced by the alkylating agents. When MEA was administered in combination with MMS or CP the reduction in the frequency of MNPCEs did not reach statistically significant levels, although it reached values close to significance. With respect to the polychromatic erythrocyte/normochromatic erythrocyte (PCE/NCE) ratio, we observed that MEA did not provide significant protection against the bone marrow toxicity induced by CP.

*Key words:* cysteamine, MEA, micronuclei, amifostine, WR-2721.

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## Introduction

Amifostine or WR-2721 is an organic thiophosphate chemically known as ethanethiol. It was developed during the Cold War as a radioprotectant by the Walter Reed Army Institute-USA (Santini and Giles, 1999). Later studies demonstrated that amifostine selectively protected normal tissue cells in relation to malignant cells both in radiotherapy and chemotherapy, especially when alkylating agents, organoplatinum agents and antracyclins were used (Santini and Giles, 1999; Schuchter, 1997; McCauley, 1997; Foster-Nora and Siden, 1997; Valeriote and Tolen, 1982; List *et al.*, 1996; Murray *et al.*, 2000; Marzatico *et al.*, 2000; De Souza *et al.*, 2000). Mazur and Blawat (1999) observed that amifostine also had a protective effect on mouse bone marrow, significantly reducing the frequencies of micronucleated polychromatic erythrocytes (MNPCEs) induced by cyclophosphamide.

Cysteamine or 2-mercaptoethylamine (MEA) is an aminothiol well known for its radioprotective action (Bacq, 1951). MEA can protect mammalian cells from radiologic phenomena such as cell death (Barendsen, 1964; Eker and Pihl, 1964; Vergroesen *et al.*, 1967; Sinclair, 1967; 1968; Vos, 1969), mitotic delay (Firket and Mathieu, 1966), chromosome aberrations (Yang and Hahn, 1968; Vos and Kaalen, 1968) and depression of DNA synthesis (Honjo, 1963). Santos-Mello (1977) reported that MEA can also inhibit DNA repair (unscheduled DNA synthesis - UDS) as well as the repair of radioinduced chromosome breaks in human lymphocytes. However, in contrast to amifostine, no specific information is available in the literature about the possible chemoprotective action of MEA against genotoxic chemical agents.

In the present paper, we report the results of studies on the possible chemoprotective action of MEA against the induction of MNPCEs in mice by alkylating agents such as methyl methanesulfonate (MMS) and cyclophosphamide (CP).

## Materials and Methods

The experiments were conducted on bone marrow cells of Swiss albino mice aged 8 to 10 weeks and weighing on average 34 g (males) and 30 g (females). The animals were kept in propylene cages, receiving standard ration and water ad libitum, with 52% humidity and exposed to a 12-h light 12-h darkness photophase at room temperature.

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The experiments with MMS were carried out on females and the experiments with CP on males. All solutions were freshly prepared just before use.

### Experiments with MMS and MEA

The animals were divided into six groups of at least six mice per group and treated as follows: group 1 received only saline solution (0.2 mL/animal - negative control), group 2 received MMS, group 3 received MEA, group 4 received MEA 30 min before the administration of MMS, group 5 simultaneously received MEA and MMS, and group 6 received MEA 30 min after the administration of MMS.

MMS (Sigma) and MEA (cysteamine HCl - Sigma) dissolved in physiological saline were administered intraperitoneally at doses of 50 and 150 mg/kg body weight, respectively.

### Experiments with CP and MEA

The animals were also divided into six groups of seven mice per group and treated as described above, except that CP (Genuxal - Asta Medica - 135 mg/kg body weight) dissolved in physiological saline was administered instead of MMS.

### Preparation and scoring

The animals were sacrificed 24 h after the respective treatments and slides were prepared by the methods of Heddle (1973) and Schmid (1975). MNPCE frequencies were determined in 2000 polychromatic erythrocytes (PCE)/animal and bone marrow toxicity was determined by the PCE/normochromatic erythrocyte (NCE) ratio in a total of 1000 erythrocytes per animal.

Data were analyzed statistically by the nonparametric Mann-Whitney test (Siegel, 1956) using the BioEstat 2.0 package (Ayres *et al.*, 2000).

## Results

Tables 1 and 2 show the MNPCE frequencies and the percentages of PCE in relation to the total number of erythrocytes in the bone marrow cells of mice submitted to the various treatments.

Table 3 shows the results of statistical comparison of the groups submitted to the different treatments, which demonstrated that:

- Animals treated with MMS or CP alone showed significantly higher MNPCE frequencies than their respective negative controls.

- MNPCE frequencies were significantly higher in the groups treated with MEA alone compared to their respective negative controls, with no significant difference in percent PCE.

- The group treated with MMS alone did not differ significantly from the control group in terms of percent

**Table 1** - The effect of MEA on the frequencies of MNPCEs in bone marrow of mice after treatment with MMS.

| Treatment | Animal | % PCE[a] | MNPCEs / 1000 PCE[b] |
|---|---|---|---|
| Control$^-$ | 1 | 52.80 | 2.00 |
| | 2 | 54.60 | 1.50 |
| | 3 | 55.00 | 1.00 |
| | 4 | 52.10 | 2.00 |
| | 5 | 51.50 | 2.50 |
| | 6 | 55.40 | 2.00 |
| | 7 | 55.50 | 2.00 |
| | Mean ± S.D. | 53.84 ± 1.67 | 1.86 ± 0.48 |
| MMS (50 mg/kg) | 1 | 48.30 | 25.50 |
| | 2 | 52.00 | 28.00 |
| | 3 | 50.20 | 33.50 |
| | 4 | 54.40 | 21.50 |
| | 5 | 56.60 | 21.50 |
| | 6 | 50.90 | 35.00 |
| | Mean ± S.D. | 52.07 ± 3.00 | 27.50 ± 5.80 |
| MEA (150 mg/kg) | 1 | 59.00 | 6.00 |
| | 2 | 53.80 | 4.00 |
| | 3 | 56.40 | 3.00 |
| | 4 | 59.20 | 2.50 |
| | 5 | 56.60 | 2.50 |
| | 6 | 51.20 | 2.50 |
| | 7 | 58.50 | 3.00 |
| | Mean ± S.D. | 56.39 ± 2.97 | 3.36 ± 1.28 |
| MMS (50 mg/kg) + MEA (150 mg/kg) (30 min before) | 1 | 53.80 | 20.50 |
| | 2 | 52.20 | 16.50 |
| | 3 | 50.40 | 20.50 |
| | 4 | 49.90 | 26.50 |
| | 5 | 51.00 | 21.00 |
| | 6 | 52.50 | 18.50 |
| | Mean ± S.D. | 51.63 ± 1.46 | 20.58 ± 3.35 |
| MMS (50 mg/kg) + MEA (150 mg/kg) (administered together) | 1 | 48.80 | 19.50 |
| | 2 | 54.70 | 18.00 |
| | 3 | 51.30 | 30.50 |
| | 4 | 53.30 | 23.00 |
| | 5 | 54.00 | 19.50 |
| | 6 | 48.30 | 21.00 |
| | Mean ± S.D. | 51.73 ± 2.72 | 21.92 ± 4.53 |
| MMS (50 mg/kg) + MEA (150 mg/kg) (30 min after) | 1 | 44.00 | 12.00 |
| | 2 | 45.50 | 13.50 |
| | 3 | 53.10 | 9.00 |
| | 4 | 51.80 | 12.50 |
| | 5 | 47.50 | 17.50 |
| | 6 | 51.80 | 14.50 |
| | 7 | 55.10 | 20.00 |
| | Mean ± S.D. | 49.83 ± 4.01 | 14.14 ± 3.65 |

[a]1000 erythrocytes/sample were scored.
[b]2000 PCEs/sample were scored.

**Table 2** - The effect of mea on the frequencies of MNPCEs in bone marrow of mice after treatment with CP.

| Treatment | Animal | % PCE[a] | MNPCEs / 1000 PCE[b] |
|---|---|---|---|
| Control - | 1 | 48.90 | 2.00 |
| | 2 | 49.90 | 4.00 |
| | 3 | 57.90 | 3.50 |
| | 4 | 57.60 | 2.50 |
| | 5 | 56.00 | 2.00 |
| | 6 | 55.00 | 3.00 |
| | 7 | 56.20 | 2.50 |
| | Mean ± S.D. | 54.50 ± 3.63 | 2.79 ± 0.76 |
| CP (135 mg/kg) | 1 | 35.90 | 19.50 |
| | 2 | 43.50 | 21.00 |
| | 3 | 38.90 | 23.50 |
| | 4 | 37.60 | 25.50 |
| | 5 | 33.70 | 18.50 |
| | 6 | 33.80 | 17.00 |
| | 7 | 47.70 | 20.00 |
| | Mean ± S.D. | 38.73 ± 5.20 | 20.71 ± 2.93 |
| MEA (150 mg/kg) | 1 | 51.00 | 3.00 |
| | 2 | 56.10 | 3.00 |
| | 3 | 54.50 | 4.50 |
| | 4 | 58.40 | 4.50 |
| | 5 | 54.20 | 3.50 |
| | 6 | 57.70 | 4.50 |
| | 7 | 60.50 | 5.50 |
| | Mean ± S.D. | 56.06 ± 3.15 | 4.07 ± 0.93 |
| CP (135 mg/kg) + MEA (150 mg/kg) (30 min before) | 1 | 38.30 | 17.50 |
| | 2 | 40.40 | 12.00 |
| | 3 | 42.60 | 12.50 |
| | 4 | 49.80 | 8.50 |
| | 5 | 40.80 | 16.00 |
| | 6 | 45.50 | 16.00 |
| | 7 | 43.20 | 12.00 |
| | Mean ± S.D. | 42.94 ± 3.79 | 13.50 ± 3.14 |
| CP (135 mg/kg) + MEA (150 mg/kg) (administered in combination) | 1 | 40.70 | 17.50 |
| | 2 | 35.60 | 18.00 |
| | 3 | 35.00 | 12.50 |
| | 4 | 34.20 | 20.00 |
| | 5 | 35.20 | 9.50 |
| | 6 | 40.00 | 19.50 |
| | 7 | 37.00 | 20.00 |
| | Mean ± S.D. | 36.81 ± 2.56 | 16.71 ± 4.11 |
| CP (135 mg/kg) + MEA (150 mg/kg) (30 min after) | 1 | 35.40 | 12.50 |
| | 2 | 34.50 | 11.50 |
| | 3 | 43.50 | 12.00 |
| | 4 | 43.10 | 8.50 |
| | 5 | 42.60 | 8.50 |
| | 6 | 39.40 | 9.50 |
| | 7 | 39.20 | 16.00 |
| | Mean ± S.D. | 39.67 ± 3.66 | 11.21 ± 2.67 |

[a]1000 erythrocytes/sample were scored.
[b]2000 PCEs/sample were scored.

PCE, indicating that the dose used was not toxic to bone marrow.

- The group treated with CP alone showed a significantly lower percentage of PCE compared to the negative control, indicating toxicity to bone marrow.

- When MEA was administered to the animals 30 min before or 30 min after MMS or CP, MNPCE frequencies were significantly reduced compared to the animals treated with MMS or CP alone.

- When MEA was administered to the mice together with MMS or CP there was a reduction in MNPCE frequency which was close to significance although it did not reach it.

- The maximum reduction in MNPCE frequency occurred when MEA was administered 30 min after MMS or CP.

- At the dose used, MEA did not confer significant protection in terms of toxicity to bone marrow (measured as % PCE), although it showed a chemoprotective action against the induction of MNPCEs caused by CP.

## Discussion

The present results demonstrated that MEA can protect mouse bone marrow PCE against MNPCE induction by alkylating agents such as MMS and CP.

Alkylating agents can bind DNA, causing damage that may result in strand breaks, chromosome breaks, micronucleus formation, and cell death (Mazur and Blawat, 1999; Brookes, 1990; Moore, 1991). The extent of DNA damage induced by alkylating agents depends on thiol concentration in the cell nucleus (Moore, 1991). The protective mechanisms of thiols are not fully known but probably include free radical scavenging, hydrogen donation reactions, and inhibition of alkylating agents when the thiol attracts the positively charged carbonium ions (Mazur and Blawat, 1999). We found no information in the literature about the specific chemoprotective action of MEA against genotoxic chemical products such as alkylating agents. We suggest that the chemoprotection observed here for MEA was due to radical scavenging and hydrogen donation reactions, as reported for the chemoprotective agent WR-1065, the active metabolite of amifostine (Spencer and Goa, 1995).

Mazur and Blawat (1999), reported that amifostine significantly reduced MNPCE frequencies in PCE even when administered half an hour before CP. Spencer and Goa (1995), reported cell protection when amifostine was administered up to one hour after CP therapy. With respect to MEA, we observed a significant reduction in MNPCE frequency even when this aminothiol was administered 30 min before or 30 min after the alkylating agents.

Indeed, amifostine is a pro-drug which is rapidly dephosphorylated by a cell membrane enzyme (alkaline phosphatase) resulting in an active form denoted WR-1065. So far, chemoprotection against alkylating agents has been mainly attributed to WR-1065 (Santini and Giles, 1999;

**Table 3** - Comparison of the various samples by the Mann-Whitney test.

| | PCE | | | Micronuclei | | |
|---|---|---|---|---|---|---|
| | U[b] | Z | P | U[b] | Z | P |
| Control$^-$ X MMS | 11.0 | 1.429 | 0.153 | 0.0 | 3.000 | 0.003[a] |
| Control$^-$ X MEA | 11.0 | 1.725 | 0.084 | 1.5 | 2.939 | 0.003[a] |
| MMS X MMS + MEA (administered 30 min before) | 18.0 | 0.000 | >0.999 | 3.0 | 2.401 | 0.016[a] |
| MMS X MMS + MEA (administered in combination) | 17.5 | 0.080 | 0.936 | 6.0 | 1.921 | 0.055 |
| MMS X MMS + MEA (administered 30 min after) | 15.0 | 0.857 | 0.391 | 0.0 | 3.000 | 0.003[a] |
| Control $^-$ X CP | 0.0 | 3.130 | 0.002[a] | 0.0 | 3.130 | 0.002[a] |
| Control $^-$ X MEA | 19.0 | 0.703 | 0.482 | 6.5 | 2.300 | 0.021[a] |
| CP X CP + MEA (administered 30 min before) | 12.0 | 1.597 | 0.110 | 1.0 | 3.003 | 0.003[a] |
| CP X CP + MEA (administered in combination) | 21.0 | 0.447 | 0.655 | 11.5 | 1.661 | 0.097 |
| CP X CP + MEA (administered 30 min after) | 19.5 | 0.639 | 0.523 | 0.0 | 3.130 | 0.002[a] |

[a]Significant at the 0.05 level of significance (two-sided test).
[b]U, Mann-Whitney U test.

Spencer and Goa, 1995; Shaw *et al.*, 1996). Considering the fact that WR-1065, in turn, can also be metabolized to MEA and others sulphide compounds with reactive sulphydryl groups (Mazur and Blawat, 1999; Spencer and Goa, 1995; Shaw *et al.*, 1996) and on the basis of the present findings, we cannot rule out the possibility that at least part of the chemoprotective action attributed to WR-1065 may be actually due to the action of MEA.

Our results also demonstrate that, even though MEA presented a chemoprotective action in terms of micronucleus induction by MMS and CP, it can produce, on its own, a significant increase in micronuclei compared to the negative control, as previously reported by Mazur (1995). However, this investigator can not explain the reason for these findings. Delrez and Firket (1968) observed that MEA at low concentrations was able to induce chromosome breaks in Chinese hamster cells. Takagy *et al.* (1974), in experiments conducted on Hela cells observed that the genotoxicity of MEA at low concentrations developed gradually over time during which hydrogen peroxide was generated in the medium, with the addition of catalase and peroxidase inhibiting this "paradoxical" effect. Vergroesen *et al.* (1967), demonstrated that thiols with a pK value of less than 10 were toxic at concentrations of 0.1 and 2.0 mM, with the addition of another thiol at high concentrations, the lowering of the pH or the presence of KCN (which has no radioprotective power and has no effect on the radioprotective action of SH compounds), eliminating the toxicity. Whereas the addition of $Na_2S_2O_3$ (which does not penetrate the cell) to the system does not change the toxic condition, a fact probably indicating that this toxicity may be due to a process occurring at the intracellular level, possibly of an oxidative nature. To explain the toxicity of cysteamine, the same authors proposed that, at low concentrations, this compound dissociates into thiol ions (RS-) and H+ ions (at a pK value of 8.3) and that its toxicity may be due to the presence of thiol ions. For the case described in the present paper, cysteamine administered i.p. to mice appears to be eliminated gradually until its concentration is so low that it reaches a point when it favors the triggering of the reaction described above. Finally, we suggest that further studies are needed to establish the most effective MEA dose and time of administration for chemoprotection, since in the present study we only administered one dose (150 mg/kg body weight) 30 min before and 30 min after the alkylating agents.

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