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The Myeloprotective Effect of Medroxyprogesterone Acetate in an Irradiated Animal Model

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Background: In this study we evaluated the effect of medroxyprogesterone acetate (MPA) and its major ingredients on protection of the hematopoietic organs against radiation damage.
Method: One group of mice was given saline as placebo and the other groups were given MPA. Mice were injected with MPA (10 mg/kg) or saline 10 days before or after a single 8 Gy whole body cobalt irradiation. On day 14 the mice were sacrificed and their bone marrow transplanted to recipient mice. Ten days after the transplantation, spleen colony formation was investigated in mice.
Results: Administration of MPA with irradiation increased the formation of the spleen colony. Statistically significant enhancement of the spleen colony formation was found in mice treated with MPA repeatedly, as compared with those treated with placebo ($P < 0.001$). No significant difference in Spleen Colony Forming Unit (CFU-S) numbers was observed between pre-and post-radiotherapy administration of MPA ($P = 0.216$).
Conclusion: It is an important observation that no significant difference was observed in CFU-S numbers between pre- and post-irradiation administration of MPA.

Key words: radiotherapy – myeloprotective effect – medroxyprogesterone acetate

INTRODUCTION

Myelosuppresion is one of the major side effects of radiotherapy and/or chemotherapy in the treatment of solid tumors. It is well known that exposure of mice bone marrow to 7 Gy of X-rays kills most cycling hematopoietic cells. In the early years of research, a variety of immune-modulating agents (e.g. Corynebacterium parvum) were shown to be capable of stimulating hemopoiesis at the stem and committed progenitor cell levels. However, adverse side effects have been associated with these agents due to their potentially infectious capabilities, antigenic properties and undefined chemical constituents (1–3). In subsequent years, many similar agents were tested and found to be effective radioprotectors, such as glucans and amifostine (WR2721) (4,5). Amifostine is perhaps the most effective of these agents, but the toxicity assessment conducted in humans has shown that the dose limiting toxicity is hypotension.

In clinical and animal trials, the myeloprotective effect of medroxyprogesterone acetate (MPA) is shown when used in combination with radiotherapy or chemotherapy (6–9). MPA with its androgenic specification provides bone marrow stimulation. MPA has been shown to possess the ability to increase megakaryocytopoiesis and the hematopoietic inductive microenvironment (10). The aim of this study is to examine whether the myeloprotective effect of MPA is related to the timing of its administration, namely before (pre) or after (post) irradiation.

SUBJECTS AND METHODS

MICE

10- to 12-week-old male C3H/Sed mice were used in all experiments. All mice were quarantined and acclimatized to laboratory conditions for 2 weeks before experimentation.

MPA

MPA was administered intramuscularly for 10 days (10 mg/kg/daily) to the mice. It was diluted in saline and administered in 0.5 ml injections.

IRRADIATION

^{60}Co source was used to administer bilateral total body gamma radiation. Mice were placed in ventilated containers and irradiated at a dose rate of 105 cGy/min. A single dose of 8 Gy was given to the whole body.

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Figure 1. Spleen colonies.

Experimental Groups

Donor mice

There are 10 mice in each group

- Group I, no MPA, saline or irradiation was received
- Group II, 10 mg/kg/day MPA was intramuscularly injected for 10 days; on the 10th day, mice were irradiated to the whole body
- Group III, 10 mg/kg/day saline was injected for 10 days; on the 10th day, mice were irradiated to the whole body
- Group IV, mice were irradiated to the whole body; 10 mg/kg/day MPA was intramuscularly injected 10 days after irradiation.
- Group V, mice were irradiated to the whole body; 10 mg/kg/day saline was intramuscularly injected 10 days after irradiation.

On day 14, donor mice were sacrificed by cervical dislocation. Both femora were removed after treatment, cells from the bone marrow were aspirated after washing out with a balanced saline solution of Hanks under aseptic conditions. Each aspiration was diluted with balanced saline solution of Hanks and counted with Thoma's slice under light microscope. Each aspiration was diluted down to 10^6 colonies/0.5 ml of bone marrow cells and made a single cell suspension.

Recipient mice

All received an 8 Gy single dose to the whole body 24 h before intravenous injection of 10^6 colonies/0.5 ml of bone marrow cells to produce CFU-S (11). Five groups of 10 recipient mice for each radiation dose were engrafted.

- Group VI, bone marrow of donor group I was transplanted to group VI
- Group VII, bone marrow of donor group II was transplanted to group VII
- Group VIII, bone marrow of donor group III was transplanted to group VIII
- Group IX, bone marrow of donor group IV was transplanted to group IX
- Group X, bone marrow of donor group V was transplanted to group X

Table 1. CFU-S numbers of all groups

	Group VI	Group VII	Group VIII	Group IX	Group X
No. of mice	10	10	10	10	10
Minimum	13	9	2	7	1
Maximum	22	16	7	15	9
Mean	16.6	12.3	4	10.9	3.6
Standard deviation	2.8363	2.4060	1.6330	2.6854	2.7162

Spleen Colony Forming Unit (CFU-S) Assay

Pluripotent hemopoietic stem cell recovery was evaluated using the CFU-S assay. Ten days after transplantation, the recipient mice were sacrificed by cervical dislocation. Their spleens were removed, fixed in Bouin's solution, and the number of grossly visible spleen colonies counted (Fig. 1).

Statistics

One-way ANOVA was used to determine statistical difference in CFU-S data.

RESULTS

CFU-S values were obtained by counting colonies formed on the surface of mice spleen, which had undergone total body irradiation and bone marrow transplantation. CFU-S values by groups are given in Table 1.

In the control group, CFU-S numbers observed were 14–22. Mean value of CFU-S was 16.6 with a standard deviation of 2.8363. Groups VII and IX, which were given high doses of MPA (10 mg/kg/daily for 10 days) had mean CFU-S values of 12.3 and 10.9, respectively. Groups VIII and X were given saline as placebo and radiotherapy. The mean CFU-S values for these groups were 4.0 and 3.6, respectively.

CFU-S values obtained from Group VI were compared with CFU-S values obtained from other groups. In groups subjected to radiotherapy, CFU-S values were found to be significantly lower in comparison with the control groups ($P < 0.001$). Groups given MPA and saline were also compared. CFU-S values of groups given MPA were found to be significantly higher than those given saline ($P < 0.001$). When Groups VII and IX were compared, the difference between them was not statistically significant. Likewise, the difference between Groups VIII and X was not statistically significant.

DISCUSSION

MPA, with its androgen steroid specification, has stimulatory effects on the bone marrow. Benefiting from this specification

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of MPA, it is used in clinical and preclinical trials to prevent myelosuppression resulting from radiotherapy or chemotherapy.

Clinical studies have consistently demonstrated significant differences in the hematological toxicity in favor of patients who received MPA added to chemotherapy. In 1977, patients with advanced breast cancer received FAC (5-fluorouracil, adriamycin, cyclophosphamide) or FAC + MPA. Leukocyte and platelet counts were significantly higher in patients receiving FAC + MPA than in those receiving FAC alone (12). Wills has given high dose MPA (500 mg/daily) to a group of breast cancer patients who have been treated with CMF (cyclophosphamide, methotrexate, 5-fluorouracil). When white blood cell (WBC) and platelet counts of patients were compared, WBC counts of patients given MPA were found to be significantly higher (8).

In the bone marrow, MPA inhabits mitotic activity and differentiation from stem cells. With this activity it protects stem cells by keeping them at G0 phase. This prevents damage to these cells caused by cytotoxic agents. Amadori et al. (6) divided head and neck cancer patients into two groups. To one of these groups they administered chemotherapy three times. To the other group they added 1000 mg/daily of MPA. The second group took MPA 14 days prior to chemotherapy and continued for 90 days. On the 14th day, CFU-GM (colony forming unit granulocyte macrophage) was observed. Thus, Amadori showed the stimulating effect of MPA in stem cells.

Kaibara et al. showed that thromboembolic complications were prevented by MPA (13). Aydin et al. administered MPA 1000 mg daily to patients who developed neutropenia after receiving their first chemotherapy, which continued to the second. One week after chemotherapy they evaluated the level of GM-CSF (granulocyte macrophage colony stimulating factor) by bone marrow aspiration. In patients given MPA, a decrease in secretion of endogenous cytokines was observed. Erythrocytes and platelets were not affected (14). On the other hand, Cetin et al. observed that MPA had no effect on the mitotic activity of the bone marrow in patients who received cytotoxic chemotherapy (15).

In our study, comparison was made between groups given high doses of MPA and other groups given saline. The bone marrow was protected from myelosuppression in groups receiving MPA. These data seem to confirm the myeloprotective effect of MPA previously hypothesized. On the other hand, its myeloprotective effects did not differ between pre- and post-irradiation groups ($P > 0.05$).

In previous clinical and animal trials showing the myeloprotective effects of MPA, we did not find any remarks regarding the timing of its application. In this study we have shown that the myeloprotective specification of MPA remains unchanged whether it is given before or after irradiation.

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