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Etoposide Protects Mice from Radiation-induced Bone Marrow Death

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Etoposide is known to inhibit the activity of topoisomerase II, and to possess radiosensitizing effects. In this paper we show that pretreatment of mice with etoposide one day before whole-body irradiation had a protective effect against radiation-induced bone marrow death. The LD50/30 of mice given radiation alone was 8.26 Gy while that of mice given etoposide one day before whole-body irradiation was 10.35 Gy. The number of endogenous colony-forming units surviving in whole body-irradiated mice was significantly increased by pretreatment with etoposide.

Key words: Etoposide — Radioprotection — Bone marrow toxicity

The dose-limiting factor of etoposide, which is being used increasingly in the treatment of malignancy, is myelosuppression, such as leukocytopenia and thrombocytopenia. Combination of fractionated WBI and high-dose etoposide with bone marrow transplantation has also been used for advanced hematologic malignancies.[1-3] However, there are no published data describing the effects on bone marrow toxicity of the combined modality, even though both radiation and etoposide cause hematological suppression. In this communication, we report that pretreatment of mice with etoposide protected animals from radiation-induced bone marrow death.

The animals were 8- to 12-week-old C3H/HeMsNrsf-ICR female mice. They were produced and maintained in our SPF (specific pathogen-free) facilities at the National Institute of Radiological Sciences. Etoposide (Nihon Kayaku Ltd., Tokyo) was diluted with Ringer's solution immediately before ip injection, and 30 μg/g of etoposide in a volume equal to 0.01 ml/g body weight was used throughout these experiments. This amount of etoposide was approximately one-half of LD50 and caused no apparent toxicity in our experimental animals.

For WBI, mice were put in lucite holders without anesthesia, and received single doses of γ-rays from a ^{137}Cs unit with a dose rate of 0.96 Gy/min. Eight mice were used for each point and the observation period was 30 days. In the endogenous spleen colony assay,[4] the spleen was removed and fixed in Bouin's solution 9 days after irradiation. The number of spleen colonies on the surface of the spleen was macroscopically counted.

The time course of the combined modality was investigated by employing various time intervals between WBI and etoposide treatments (Fig. 1). The radiation dose used here was 7.7 Gy, which killed all mice by day 30. When etoposide was administered one day after WBI or later, no change in survival rate was observed. In contrast, administration of etoposide prior to WBI protected the mice from radiation-induced bone marrow death: eight out of eight mice (100%) survived when etoposide was administered one day before WBI. Less effective protection was observed when the time interval between etoposide and WBI was prolonged up to 9 days.

Figure 2 shows radiation dose-mortality relationships (LD50/30) after the combination modality in which mice received etoposide one day before various doses of WBI. The LD50/30 of mice given radiation alone was 8.26 Gy (7.98–8.53 Gy, 95% confidence) while that of mice given etoposide one day before WBI was 10.35 Gy (10.01–10.68 Gy). The dose modification factor (DMF) was 10.35/8.26, i.e., 1.24.

The effects of the combination modality on the hematopoietic system were investigated by endogenous spleen colony-forming assay. Etoposide was administered one day before WBI. As shown in Fig. 3, etoposide pretreatment resulted in an upward shift of the radiation dose-response curve, with a minimal change in D_0 (radiation dose required to reduce the colony number by 1/e); D_0 was 0.98 (0.81–1.16) Gy for radiation alone and 0.82 (0.67–0.99) Gy for etoposide-pretreated mice.

The present experiments have clearly demonstrated that a larger number of endogenous colony-forming units survived in mice that received etoposide one day before radiation than in mice that received radiation alone.

Etoposide interacts with topoisomerase II, an enzyme that catalyzes alterations in the topology of DNA through an ATP-dependent strand-passing reaction. This enzyme produces an enzyme-bridged DNA strand break

Abbreviations: WBI, whole-body irradiation; CFU-S, colony-forming units in spleen.

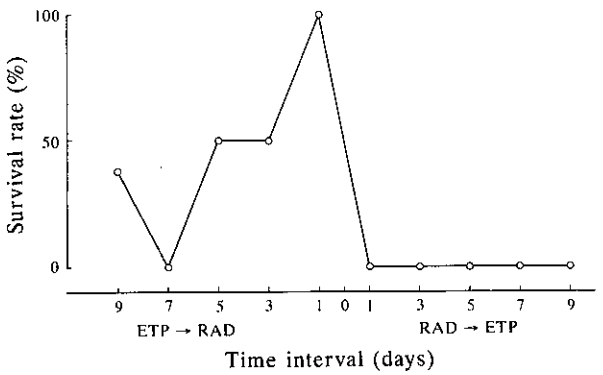

Fig. 1. Effect of timing in the combination modality of WBI and etoposide. Mice received an ip administration of 30 μg/g etoposide either before or after 7.7 Gy WBI. Eight mice were used at each point, and survival rates were determined at 30 days after irradiation. No mice survived after WBI without etoposide.

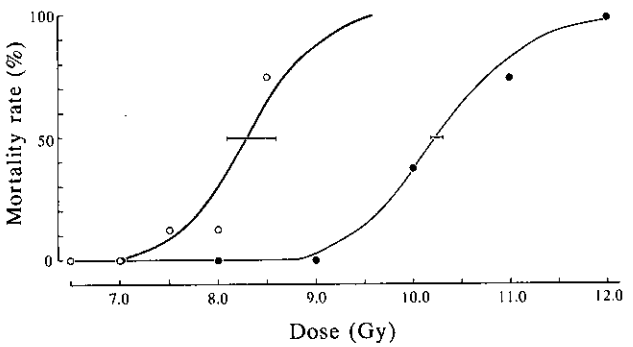

Fig. 2. Radiation dose-mortality relationship. Mice received an ip administration of 30 μg/g etoposide one day before various doses of WBI. Eight mice were used at each point, and mortality rates were determined at 30 days after irradiation. LD50/30 values were calculated by probit analysis. Open and closed circles represent the radiation alone control group and the etoposide plus radiation group, respectively.

which has been termed "the cleavable complex."[5-7] Etoposide stabilizes the cleavable complex and results in both increased DNA strand breaks and inhibition of rejoining.[5-8] Topoisomerase II seems to play a positive role in rejoining of radiation-induced double-strand breaks, and etoposide may suppress repair of DNA damage caused by radiation.[9] Cell cycle distribution is also modified by etoposide.[10, 11] Low and high concentrations of etoposide arrest cells at G_2 and S phase, respectively. These activities of etoposide, except S phase arrest, imply that cells exposed to etoposide could be sensitized to radiation. The combination of radiation and etoposide showed a significant enhancement of *in vitro* cell killing as compared with radiation alone.[12] Etoposide seems to inhibit repair of potentially lethal radiation damage. Clinical studies suggest that etoposide may be the agent responsible for enhanced radiation response in patients with small cell lung cancer.[13, 14]

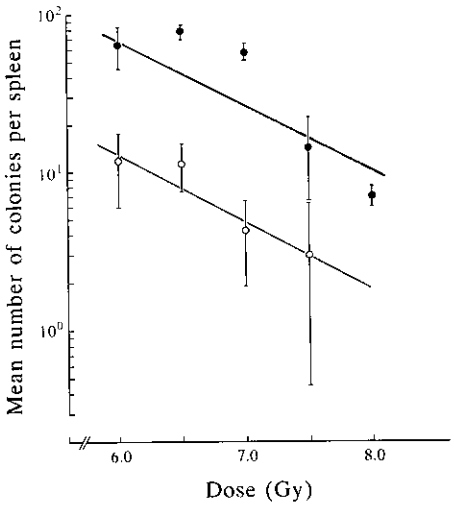

Fig. 3. Survival curve of endogenous CFU-S. Mice received either WBI alone (○) or 30 μg/g etoposide one day before WBI (●). Symbols and bars are mean and 95% confidence limits, respectively.

This is the first report to describe the radioprotective activity of etoposide. Some other cytotoxic agents including cytosine arabinoside, methotrexate, chlorambucil and cyclophosphamide are known to reduce radiation toxicity when administered to mice prior to WBI.[15] These agents do not change the radiosensitivity of the hematopoietic stem cells, but accelerate regeneration of the cells surviving radiation damage.

The finding that endogenous CFU-S formation was increased by administration of etoposide implies the following possibilities for the underlying mechanism of the protection: 1) etoposide increases CFU-S, 2) regeneration of CFU-S is accelerated by etoposide, and 3) the cell cycle of CFU-S is modified by etoposide to a more resistant distribution. We are now investigating these possibilities.

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