Effects of Iodine Contrast Media on the Bone Marrow Colony Forming Units (CFUs) Following X-ray Irradiation in Mice

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The combined effects of an iodine contrast medium and X-rays on the number of colony forming units (CFUs) in mice bone marrow were examined in vitro and in vivo. No changes were observed in the number of CFUs per 10^5 nucleated bone marrow cells, when the contrast medium alone was added to bone marrow cell suspensions at concentrations of 0.5 and 5.0%. X-irradiation of the suspensions above a dose of 50 R reduced the number of CFUs per 10^5 cells. The irradiation of the suspensions in the presence of the contrast medium produced further decrease in the number of CFUs per 10^5 cells, depending on the concentration of the medium used. No enhancement of the X-ray effect was observed when mice were injected with the contrast medium at a dosage 10 times higher than that used in X-ray diagnosis in man and irradiated immediately. Thus, the iodine contrast medium sensitized hematopoietic stem cells to X-rays in vitro but did not in vivo.

INTRODUCTION

Iodine contrast media have been reported to cause chromosome aberrations1-3) and sister chromatid exchanges4) in mammalian cells and human lymphocytes in culture. The presence of the iodine contrast medium above a certain concentration at the time of irradiation enhanced the chromosome aberrations2,4), sister chromatid exchanges5), the frequency of micronuclei2,3,6), the cell killing6-9) and the yield of DNA single-strand breaks6) induced by X-rays. The iodine contrast medium has also been found to be responsible for the in vivo cytogenetic damage observed in blood lymphocytes in patients who underwent angiography2-4,10-13). Cell fractionation studies suggested cell uptake and binding of significant amounts of contrast medium by all subcellular components, particularly the nuclear fraction14). Animals which had received X-irradiation retained a significantly larger amount of ^121I-labelled contrast medium in the kidney, liver, spleen and plasma compared to animals which had received ^125I-labelled contrast medium with no prior X-irradiation15). The medium was considered to act by increasing the absorbed dose through the photoelectric effects of X-rays2,3,6,8), although other mechanisms could not be rigorously excluded2,3,7,8).

Radiosensitization due to the contrast medium may be observed in other tissues once their
cells are in contact with sufficient concentration of the medium. Thus, it is important to examine whether or not the radiosensitive tissues such as the hematopoietic system sustain more damage after X-ray irradiation when the contrast medium is present. However no analysis has been undertaken on the combined effects of X-rays and the contrast medium on the hematopoietic tissue in vivo. This paper describes the influence of the contrast medium on the hematopoietic stem cells when combined with X-rays, not only in vitro but also in vivo by using colony forming units (CFUs) assay.

**MATERIALS AND METHODS**

Normal male 8 to 10 week-old ICR/JCL mice were used. The iodine contrast medium employed was Conraxin-H (Takeda Pharmaceutical Co., Ltd., Osaka). Chemically, it consisted of an 80% solution of methylglucamine and sodium salt of iodamide (3-acetamidomethyl-5-acetamido-2,4,6-triiodobenzoic acid).

X-irradiation was carried out with a machine (Shimadzu Seisakusho Co., Ltd., Kyoto) operated at 200 kVp and 20 mA, filtered through 0.5 mm Cu and 0.5 mm Al, with a HVL of 1.2 mm Cu.

For in vitro studies, marrow cells, collected from femora by forcing cold isotonic solution through the shaft with a 26-gauge needle, were suspended in 2 ml of Hank's solution, and nucleated cells were counted with a hemocytometer. Conraxin-H was added to the bone marrow cell suspensions at final concentrations of 0.5 and 5.0% (w/v). The cell suspensions were kept for 20 minutes at 0°C before being irradiated with 50, 100 and 200 R. Immediately after irradiation, the cell suspension was injected intravenously to supralethally (800 R) irradiated mice, and on the 10th day the number of CFUs per 10^5 nucleated cells was examined according to Till et al.\(^\text{16}\). The effects of the contrast medium (0.5 and 5.0%) or X-ray alone were also investigated in the same way as above.

For the in vivo studies, each 6 to 8 mice were injected intravenously with 0.3 ml of Conraxin-H, which corresponded to a dosage 10 times higher than that used in X-ray diagnosis in man. After 5 to 10 minutes, mice were exposed to 25 and 200 R of X-rays. The number of nucleated cells and CFUs in the femoral marrow was examined at 1, 3 and 14 days after treatment with the contrast medium, with or without X-rays, according to protocols described above.

The significance of the effects was evaluated by the t-test.

**RESULTS**

The results obtained from the in vitro studies are shown in Fig. 1. The number of CFUs per 10^5 cells was 9.5 ± 1.9 in the control group, and 9.7 ± 2.4 and 9.2 ± 1.5 for mice treated with 0.5 and 5.0% of the contrast medium alone, respectively. Therefore, no significant decrease in the number of CFUs was found with the addition of the contrast medium alone. A decrease in the number of CFUs was evident with the increase of X-ray dosage. It was significant even with an exposure to 50 R of X-rays (p<0.01). Irradiation in the presence of the contrast medium
produced further decrease in the number of CFUs. At 0.5%, a tendency to further decrease in the number of CFUs compared to radiation exposure alone was observed only with the X-ray exposure to 200 R (p<0.05). At 5%, further decrease in the number of CFUs per 10^5 cells was observed when the cells were irradiated with 100 and 200 R of X-rays (p<0.01), but no further significant decrease was noted after 50 R.

Fig. 2 shows the results obtained from the in vivo studies. No change in the number of CFUs per femoral marrow was observed at 1 and 3 days after injection of the medium alone. A slight decrease observed at 14 days was not significant statistically. In vivo exposure of mice to 200 R of X-rays produced a marked decrease in the number of CFUs per femoral marrow at 1 and 3 days after exposure (p<0.01). At 14 days after exposure, apparent recovery was observed in the number of CFUs up to a level significantly below normal (p<0.01). When mice were exposed to 200 R of X-rays just after injection of the contrast medium, no enhancement of X-ray effect was found.

Fig. 1. Effects of in vitro X-ray irradiation with (▲ - ▲ 0.5%; ○ - ○ 5%) or without (○ - ○) the contrast medium on the number of femoral CFUs per 10^5 nucleated cells.
Fig. 2. Effects of in vivo 200 R X-ray irradiation with (● - -●) or without (○ - ○) the contrast medium on the number of femoral CFUs. Unirradiated mice with (□ - -□) or without (■ - -■) contrast medium served as controls.

DISCUSSION

Iodine contrast media are used because they absorb X-rays much more strongly than the body fluid into which they are administered. This property was considered to be the main cause of radiosensitizing effects observed in various cells²,³,⁶,⁸). Any cells that are irradiated while suspended in contrast medium will absorb a larger dose than when irradiated in the body fluid alone. As hematopoietic tissue has a high radiation sensitivity, it is important to ascertain whether or not X-ray damage to this tissue is enhanced by the addition of the contrast medium.

In both in vivo and in vitro experiments, the contrast medium alone induced no change in the number of CFUs. The presence of the contrast medium in bone marrow cell suspensions at the time of in vitro irradiation enhanced X-ray induced killing of femoral CFUs, depending on the concentration of the medium used. From these results, it is considered that hematopoietic stem cells can be sensitized when they are in direct contact with a high enough concentration of the contrast medium. However, administration of the contrast medium to mice just before irradiation with X-rays failed to enhance the radiation response of femoral CFUs. In in vivo experiments, the contrast medium was injected into mice at a dosage 10 times higher than that used in man. Nevertheless, actual concentration of the contrast medium in the hematopoietic tissues of mice may be insufficient to enhance the effects of X-irradiation. The marrow-blood barrier may prevent the contrast medium from entering into the hematopoietic area¹⁷). This may explain why the contrast medium sensitizes hematopoietic stem cells to X-rays in vitro but not
in vivo in our present experiments. One explanation for the chromosome damage detected in peripheral lymphocytes of patients after X-ray angiography may be that these cells are circulating in the blood in direct contact with a higher concentration of iodine contrast media than in the bone marrow. Assay of CFUs in the spleen, which reportedly retains significant amount of contrast medium\textsuperscript{(15)}, appeared to be an appropriate subject.

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