CN9501658

The Combined Effects of Radiation and Ultrasound on ICR Mouse Embryos

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ABSTRACT

We have investigated the combined effects of radiation and ultrasound on the embryos of ICR mice. The pregnant ICR mice on day 8 of gestation were irradiated with 1.0W ultrasound after exposure to 1.5Gy radiation immediately or irradiated with time interval of one hour. The incidences of external malformations synergistically increased in the group irradiated with both agents. Especially in the group treated with time interval of one hour, the incidences of external malformations reached to the maximum. The histological examination showed that the frequencies of pyknotic cells in the neural folds of embryos on day 8 of gestation increased synergistically while the frequencies of mitotic cells decreased steeply in the group treated with both agents. We concluded that the combined effects of radiation and ultrasound on external malformations and the histological changes in mouse embryos were synergistic-sensitization effects.

1. INTRODUCTION

There are multiple environmental factors, e.g. physical, chemical and biological agents etc., it is, therefore, important to study the combined effects of these factors from the viewpoints of radiation protection and safety. There was, however, little information on the combined effects of two or more agents.

In this study, we investigated the combined effects of radiation and ultrasound on embryos / fetuses, such as prenatal mortality, external malformation, reduction of fetal body weight and sex ratio. Furthermore, we carried out the histological examination of embryonic organs or tissues.

2. MATERIALS AND METHODS

2-1) EXPERIMENTAL ANIMALS AND MATING PROCEDURE

The closed colony of ICR(Crj:CD-1) mice was used. Female mice with 8 weeks old were placed together with male mice of the same age range in the same cage for only three hours from 6:00 a.m.to 9:00 a. m. and were examined on vaginal plugs at 9:00 p.m. The female mice in which vaginal plugs were found were assumed to have become pregnant at 8:00 a.m., and the day was designated as day 0:00 of gestation(Streffer, 1980; Molles, 1982). The total numbers of mice used in this experiment were 202 dams and 1876 fetuses.

2-2) IRRADIATION WITH GAMMA RAYS AND ULTRASOUND

The pregnant mice on day 8 of gestation were irradiated with a single whole body ^{137}Cs-gamma ray at dose of 1.0 Gy and 1.5 Gy. The ultrasound were irradiated with dose of 1.0W/cm^2 and 1.5W/cm^2 for 10 minutes on the pregnant mice.

In the combined treatment with radiation and ultrasound, the pregnant

mice on day 8:00 of gestation were exposed with 1.5 Gy of radiation and 1.0W/cm^2 of ultrasound immediately or with time interval of one hour.

2-3) OBSERVATION OF EMBRYONIC/FETAL EFFECTS

After irradiation, the pregnant mice were killed on day 18 of gestation. The total numbers of implantation sites, live embryos, dead embryos and fetuses were counted. Live fetuses were removed from the uterus and their external malformations, fetal body weight and sex were examined. The main types of external malformation irradiated at day 8 of gestation observed were excencephaly, anophthalmia and abnormal tail.

2-4) OBSERVATION OF HISTOLOGICAL CHANGE

The pregnant mice on day 8 of gestation were treated with gamma rays and ultrasound. After 6 hours of the treatment, the embryos and their appendices were removed from the uterus and fixed in modified Bouin solution. The histological specimen were made by on ordinary method, 4μm sections were cut and stained with nuclear fast red and crystal violet (F.J. Fraser,1982). The pyknotic and mitotic cells of neural folds of each section were observed by a light microscope of 1500 magnifications.

3. RESULTS

3-1) MORTALITY AND EXTERNAL MALFORMATIONS

The relationships between radiation dose and mortality or frequencies of external malformations are shown in **Fig.1**. The mortality in early stage of embryos increased at dose of 1.0Gy, and the incidence of excencephaly also increased at dose of 1.0Gy.

The relationships between ultrasound dose and mortality or frequencies of external malformations are shown in **Fig.2**. The mortality in early stage of embryos increased at dose of 1.5W/cm^2, and the incidence of excencephaly also increased at dose of 1.5W/cm^2.

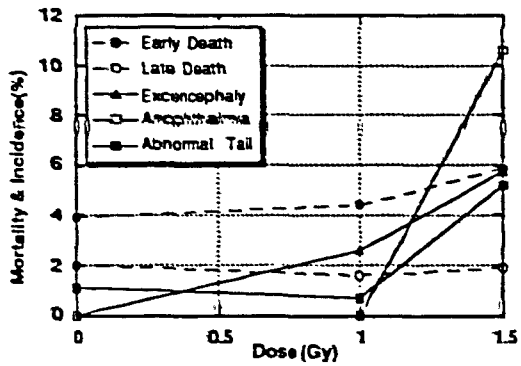

Fig.1 Dose reponses of the mortalities and incidences of external malformation to radiation

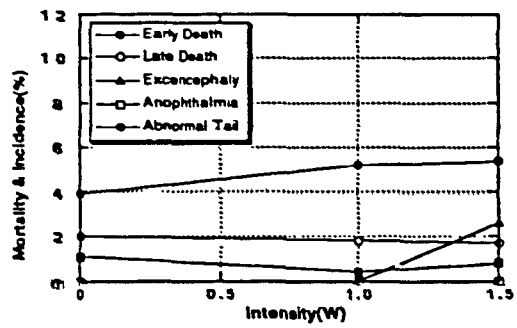

Fig. 2 Dose reponses of the mortalities and incidences of external malformation to ultrasound

3-2) COMBINED EFFECTS OF RADIATION AND ULTRASOUND

There was no significant difference among all groups of embryonic or fetal mortality(**Fig.3**). The incidences of the excencephaly, anophthalmia and abnormal tail in all treated groups had significant difference from the control mice. Especially in the combined irradiation with time interval of one hour, it was evident that the external malformation of

the fetuses were synergistic-sensitized by the combined treatment of radiation (1.5Gy) and ultrasound (1.0W) (Fig.4).

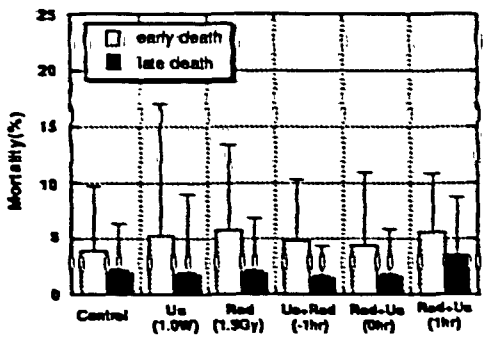

Fig. 3 The combined effects of radiation and ultrasound on mortalities of embyroe & fetus

3-3) EXAMINATION OF EMBRYONIC NEURAL FOLD

With respect to the dose-response relationships of pyknotic and mitotic cells, we observed that the frequencies of pyknotic cells increased with the increasing of the dose of radiation, while the frequencies of mitotic cells decreased (Fig.5). In addition, we observed that the frequencies of pyknotic cells in combined treatment with radiation (1.5Gy) and ultrasound (1.0W) were higher than those in single treatment with radiation or ultrasound, while the frequencies of mitotic cells were reverse. These were markedly observed in the time interval of one hour (Fig.6).

4. DISCUSSION

The threshold dose of mortality and external malformation by radiation were between 0.5 Gy and 1.0 Gy (Fig.1), and that of the ultrasound were between 1.0W and 1.5W (Fig.2).

The combined treatment of radiation (1.5Gy) and ultrasound (1.0W) produced synergistic-sensitization effects on the external malformation of fetuses (Fig.4). The clinical manifest effects were not observed in the mice treated with ultrasound, however, ultrasound modified and enhanced the effects which produced by radiation in this study.

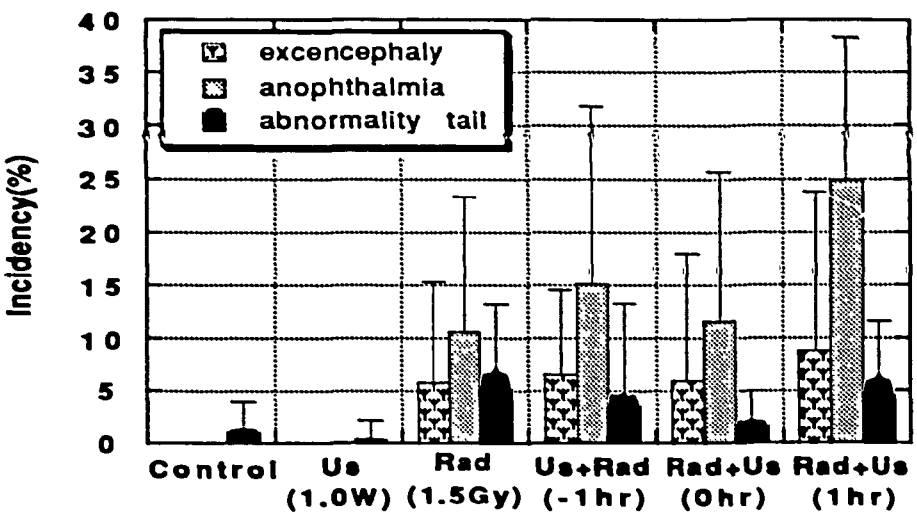

Fig. 4 The conbined effects of radiation and ultrasound on external malformations

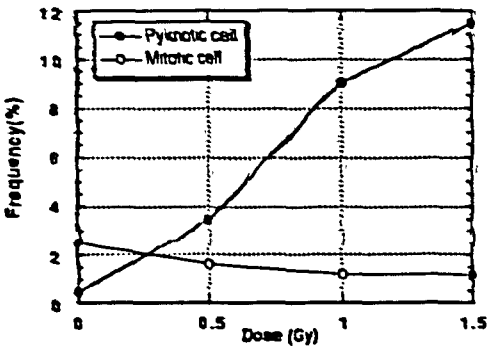

Fig. 5 Dose Responses of Frequencies of Pyknotic & Mitotic Cells to Radiation

The histological observations suggested that the malformation, such as excencephaly might be caused by a large number of pyknotic cells in the neural folds of embryo (Fig.6).

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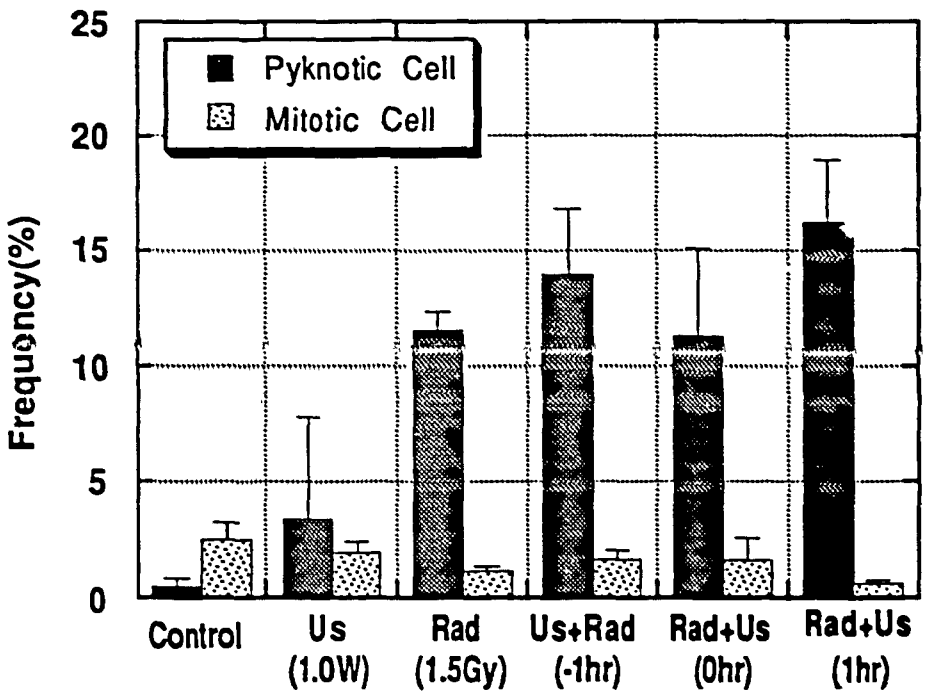

Fig. 6 The combined effects of radiation and ultrasound on the frequencies of pyknotic and mitotic cell