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Oriental Pharmacy and Experimental Medicine 2006 **6(3)**, 245-251

Radiation effects to acupuncture in mice embryos

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SUMMARY

We examined the radioprotection effects of acupoint (acupuncture point) stimulation during organogenesis stages of ICR mice. Pregnant mice received 1.5 Gy whole body X-irradiation on day 8 of gestation, which is the early stage of organogenesis. The embryonic death rate and teratogenesis rate by radiation were examined. Electroacupuncture to the leg acupoints and/or transcutaneous stimulation to the back acupoints on the pregnant mice showed no protective effect against irradiation on embryonic or fetal death rate. On the contrary, the strong stimulation resulted in increase in the mortality after irradiation rather than protection. However acupoint stimulation to the pregnant mice never showed harmful effects by itself on embryos. It tended to reduce the skeletal malformations induced by X-ray irradiation. We suspect that acupoint stimulation removed the cells injured by irradiation during embryonic development, resulting in an increase in embryonic death rate and reduction in skeletal anomalies.

Key words: Acupoint stimulation; X-ray irradiation; Embryonic effect; Mortality; Malformation

INTRODUCTION

Radiation is harmful to organisms, especially to dividing cells. On the other hand, radiation has an essential role in medicine, industry, agriculture and research fields. It is important to protect organisms from harmful effects of irradiation in order to make good use of radiation. To achieve such a purpose, radioprotectors are used in the clinic and in space development. The sulfhydryl compounds, cysteine and cysteamine were discovered early as radioprotectors but are toxic. It is useful to explore compounds that reduce the biological damages of radiation without undesirable side effects. At present, amifostin (WR-2721) appears to be the best for radiotherapy among synthesized compounds (Yuhas *et al.*, 1980). Practically, the recovery medicine from radiation damages may be more necessary.

Electroacupuncture was able to help recovery from the harmful effects of X-irradiation on the leukocytes and plasma protein (Hau, 1984).

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Moxibustion revealed the same radioprotective effects in irradiated rats (Hau, 1981; Wu *et al.*, 1986). Furthermore the effect of moxibustion on cellular immunocompetence of γ-irradiated mice proved by Hau *et al.* (1989). The purpose of this study is to investigate whether electric stimulation of acupuncture points shows radioprotective and recovery effects on irradiated animals.

Gu *et al.* have examined the radioprotective effect of various substances during organogenesis of ICR mice (Gu *et al.*, 2000, 2001). Embryos and fetuses are more sensitive to radiation than children or adults. Among somatic effects of radiation other than cancer induction, developmental effects on the embryo and fetus are of great concern. The developmental effects are classified into two types: (1) lethal effect: death at various developmental periods before birth, and (2) malformation: congenital anomalies of structure. Gu *et al.* established the sensitive assay system to radiation effects during embryonic development. They irradiated pregnant mice on day 8 of gestation and investigated mortality of embryos and malformations of live fetuses (Gu *et al.*, 2000, 2001). We employed this assay system in order to examine the effects of acupuncture stimulation on embryonic development in irradiated pregnant mice.

MATERIALS AND METHODS

Animals and mating procedure

8 weeks old ICR mice were purchased from SLC (JAPAN SLC Inc.) and housed at a temperature of 22 - 25°C and a relative humidity of 50 - 70% with a 12 h light-dark cycle. The mice were given free access to food and water. One or two female mice of 9 - 13 weeks old and one male mouse of 9 - 15 weeks old were mated for 3 hours. After mating, the time when vaginal plug was recognized was defined as time zero (0 h) of gestation. The animal committee of the Suzuka University of Medical Science approved the experimental protocol and procedures.

X-ray irradiation

On 8th day of gestation (192 h), the pregnant mice were placed in a special cage for X-ray exposure. They were treated with a single whole body irradiation of 1.5 Gy with dose rate of 0.35 Gy/min by Phillip X-irradiation equipment (225 kV).

Electric stimulation of acupoints

The pregnant ICR mice were divided into the following groups. C: control (fixation only), LB: electroacupuncture (EA) treatment on the leg and strong transcutaneous stimulation on the back, X: X-irradiation. The X + MBL and X + LB groups were both X-irradiation with EA on the leg and transcutaneous stimulation on the back groups, however they were distinguished according to stimulation intensity on the back. X + MBL group was treated with mild stimulaton that gave mild twitching. X + LB group was treated with strong stimulation that gave severe twitching. X + B: X-irradiation with strong transcutaneous stimulation on the back, X + L: X-irradiation with EA on the leg. For transcutaneous stimulation on the back, the pregnant mice were fixed on a plate and stimulated just before X-irradiation. The surface electrodes were pasted at the GV8 acupoint (under the external occipital protuberance) and the GV16 acupoint (between the spinous processes of T 9 and T 10) after depilation, and the mice received the stimulation with 14 mA, 60 Hz, for 30 min. For EA on the leg, mice were fixed in a plastic device, and acupuncture needles were inserted at the unilateral ST 36 acupoint (lower lateral side of the knee) and the SP 6 acupoints (upper medial side of the unkle joint). The legs were stimulated alternately daily. The stimulation condition was 5 mA, 2 Hz, 10 min with an intensity that caused the muscle to twitch considerably. The EA on the leg was provided from the third to the 13th pregnant day, except on the irradiation day (8th day).

Items tested

The treated mice were sacrificed on the day 18 of

gestation and the total number of corpus luteum in the ovaries, implantation sites, dead embryos, and live and dead fetuses were counted. The live fetuses were taken out and examined for gross external malformations under a dissecting microscope. The body weight and sex of each live fetus were also determined. Then skeletal malformations were examined after staining with arizalinred and Alcian blue (Jacobson, 1965).

The litters were used as the experimental unit in order to avoid the litter effect. The average fetal effects to irradiation and acupoint stimulaton were calculated based on the death rate and malformation rate of each litter. We used Tukey method for the fetal body weight and Steel-Dwass method as a nonparametric test for other experimental items.

RESULTS

Preimplantation death

The preimplantation death rate corresponds to mortality between conception and 4.5 days postconception (Russell *et al.*, 1954). The number of preimplantation death in each mouse was calculated by subtracting the number of embryos and fetuses from the number of corpora lutea in each pregnant mouse. There was a significant difference between the non-irradiated groups and some of the irradiated groups. Notice that preimplantation death was caused before irradiation. If dead embryos by radiation were absobed early, they were probably counted as preimplantation deaths.

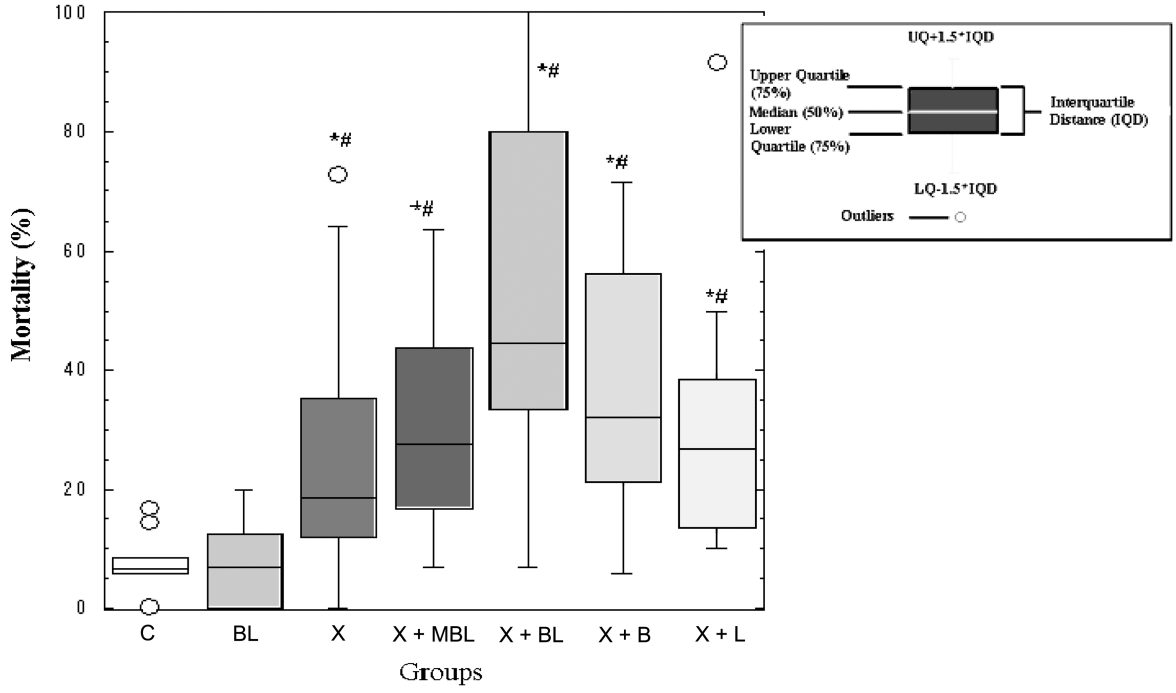

Fig. 1. Embryonic death of ICR mice irradiated at organogenesis period. The control and treatment groups were detected with statistical significance ($p < 0.05$) in comparison with control and sham control groups by Wilcoxon test. C: control (fixation only), LB: EA treatment on the leg and strong transcutaneous stimulation on the back, X: X-irradiation. The X + MBL and X + LB groups were both X-irradiation with EA on the leg and transcutaneous stimulation on the back groups, however they were distinguished according to stimulation intensity on the back. X + MBL group was treated with mild stimulaton that gave mild twitching. X + LB group was treated with strong stimulation that gave severe twitching. X + B: X-irradiation with strong transcutaneous stimulation on the back, X + L: X-irradiation with EA on the leg. *Statistically significant ($P < 0.05$) from the control group (C). #Statistically significant ($P < 0.05$) from the acupoint stimulation group (BL).

Embryonic death

Implantation sites, placental remnants and absorption embryos were considered to be embryonic deaths, which are deaths after the implantation. Embryonic death is caused from 4.5 days to 13.5 days postconception (Russell *et al.*, 1954). The result of embryonic death rate caused by X-ray exposure on 8 days after conception is shown in Fig. 1. The frequencies of embryonic death in the un-irradiated groups were 7 to 8%, representing spontaneous embryonic death rate (Fig. 1, C and BL groups). The frequency of embryonic death rate in the irradiated groups significantly elevated. The combination of strong stimulation on the back and stimulation on the legs significantly raised the embryonic mortality by X-irradiation (Compare X group and X + BL group), and in other groups slightly increased the embryonic death rate (X + MBL, X + B and X + L groups). As a detail of embryonic deaths, implantation sites were major in the non-irradiated groups, while placental remnants were major in the irradiated groups. Because of the high embryonic death rate of X + BL group, the rate of live fetuses in that group was lower (41%) than those of other irradiated groups (60% to 68%).

Fetal death

Dead fetuses with recognizable eyelids, which were caused 13.5 days postconception, were defined as fetal deaths. There was no significant difference in the fetal death rate between the irradiated and the unirradiated groups.

Fetal body weight

The body weight of the irradiated groups was lower than that of the unirradiated groups. There was no significant difference among the irradiated groups. Acupoint stimulation tended to decrease the body weight of the irradiated groups (X vs. X + MBL, X + BL, X + B, X + L groups), however, the stimulation slightly increased that of the unirradiated groups (C vs. BL).

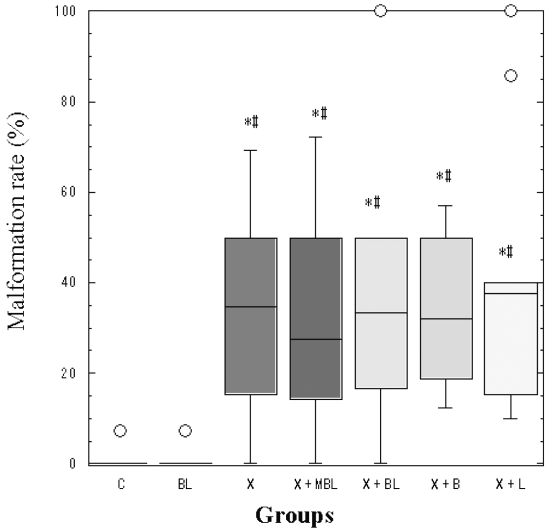

Fig. 2. Malformation incidence of ICR mice irradiated at organogenesis period. Each treatment groups were detected with statistical significance ($P < 0.05$) among all dose groups by Wilcoxon test.
C: control (fixation only), LB: EA treatment on the leg and strong transcutaneous stimulation on the back, X: X-irradiation. The X + MBL and X + LB groups were both X-irradiation with EA on the leg and transcutaneous stimulation on the back groups, however they were distinguished according to stimulation intensity on the back. X + MBL group was treated with mild stimulaton that gave mild twitching. X + LB group was treated with strong stimulation that gave severe twitching. X+B: X-irradiation with strong transcutaneous stimulation on the back, X + L: X-irradiation with EA on the leg. *Statistically significant ($P < 0.05$) from the control group (C). #Statistically significant ($P < 0.05$) from the acupoint stimulation group (BL).

External malformation

External malformations observed in live fetuses on day 18th of gestation are shown in Fig. 2. The exencephaly (brain protrudes outside the skull) and clept plate were frequently observed in the live fetuses. Hydrocephalia, non face, open eye, anophthalmos, abdominal hernia, aproctia and abnormalies of tail were also observed. Total numbers of malformation in the irradiated groups were much higher than those in the unirradiated groups. There was not a significant difference in the external malformation rate between the X-ray group

and the X-ray + acupoint stimulation groups. However, compared to the X group, the number of fetuses having more than one malformation was reduced in X + MBL and X + B, especially in X + L group. However, we compared X + B group with X group in particular did not have significant difference by incidence of malformation rate.

Skeletal anomalies

Numbers of live fetuses bearing skeletal anomalies on day 18 of gestation are presented in Fig. 3. Full rib was observed in the control group (C). Full rib and unossified rib were observed in the unirradiated BL group. Acrania, bipartite ossification of strnebra, incomplete ossification of rib, fused rib, absent rib, unossified sternebra and abnormality of vertebrae were observed in the irradiated groups. The anomaly rate of the X + MBL, X + B and X + L groups was lower than that of the X group. Although the fetuses bearing more than one skeletal anomalies were observed in the X and the X + BL groups, such fetuses were not detected in the X + MBL, X + B and X + L groups (Data not shown). Therefore, the total malformation events caused by irradiation in acupoint stimulation groups were considerably low except in X + BL group. Especially, there was not a significant difference between the unirradiated groups and the X + L group.

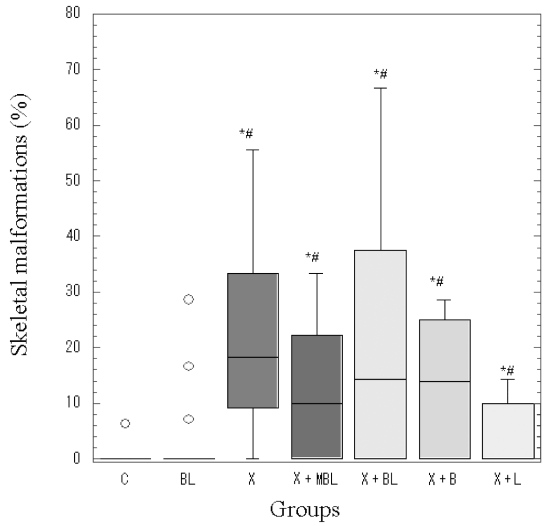

Fig. 3. Skeletal malformations in cidence of ICR mice irradiated at organogenesis period. Each treatment groups were detected with statistical significance ($P < 0.05$) among all dose groups by Wilcoxon test. C: control (fixation only), LB: EA treatment on the leg and strong transcutaneous stimulation on the back, X: X-irradiation. The X + MBL and X + LB groups were both X-irradiation with EA on the leg and transcutaneous stimulation on the back groups, however they were distinguished according to stimulation intensity on the back. X + MBL group was treated with mild stimulaton that gave mild twitching. X + LB group was treated with strong stimulation that gave severe twitching. X + B: X-irradiation with strong transcutaneous stimulation on the back, X + L: X-irradiation with EA on the leg.

DISCUSSION

Radiation produces catastrophic effects on developing embryos and fetuses. The study of embryonic effects by irradiation is not only the concern of medical science but also has a broader social implication. It was reported that some substances, such as propolis, guarana and β-glucan, showed radioprotective effects on embryos (Gu *et al.*, 2001, 2005; Takagi *et al.*, 2005). In this study, we investigated radioprotective and recovery effects of acupoint stimulation on irradiated embryos.

Russel and Russel (1954) divided the total developmental period in utero into 3 stages: preimplantation, organogenesis and fetal stage. Here, the mice were irradiated at day 8 of gestation, which corresponds to the major organogenesis stage, especially neurogenesis stage. The developmental effects on the embryos are classified into 3 categories: lethal effects, malformations and growth disturbances. During organogenesis, the principal effect of radiation is known to be a variety of anomalies and embryonic death due to severe anomalies.

In this study, preimplantation death was caused prior to X-irradiation, therefore the preimplantation death rate was not influenced by irradiation. In the case of the early absorbance of dead embryos by irradiation, however, the implantation death rate

of irradiated groups seemed to be higher than that of unirradiated groups. Irradiation on the 8th day of gestation significantly elevated the embryonic death, providing 26% of implantation embryos. Acupoint stimulation did not show beneficial effects on embryonic mortality in the irradiated mice. On the contrary, strong stimulation (X + BL group) raised embryonic death rate, suggesting that severe stimulation immediately before irradiation was enhanced radiation damage. However such stimulation on pregnant mice did not show any harmful effects itself on the embryos (BL group). The significant differences in fetal death rate between the irradiated and unirradiated groups were not detected, probably because major mortality was due to embryonic deaths. Embryos exposed during early organogenesis also exhibit an intrauterine growth retardation. This was expressed as a weight reduction of fetuses. Acupoint stimulation did not show any significant effects on the fetal body weight.

One of the purposes of this study was to investigate the effect of acupoint stimulation on teratogenesis by radiation. During organogenesis, most of the embryonic cells are in the differentiating stage and especially sensitive to radiation. As shown in Fig. 2 and 3, 1.5 Gy X-irradiation brought a high incidence of malformations. Acupoint stimulation did not show any beneficial effects on the external malformation rate of irradiated mice. Here, the malformation rate indicates the frequency of fetuses bearing at least one anomaly in the total live fetuses, calculated in each litter. The acupoint stimulation reduced the frequency of malformation events, because the fetuses having more than one anomaly were not detected in the acupoint stimulation groups. Acupuncture stimulation tended to lower the skeletal malformation rate of irradiated mice, however significant differences were not detected. The rate of fetuses bearing plural malformations was not detected in X + MBL, X + B and X + L groups, indicating acupuncture diminished the severity of skeletal anomalies. Especially, the significant difference was not shown between the X + L group and the unirradiated groups. It is suggested that acupoint stimulation might remove the damaged cells by irradiation, resulting in the higher mortality of irradiated embryos and a lower incidence of malformation. It is necessary to investigate the apoptosis frequencies of X-ray + acupoint stimulation in order to test this hypothesis. And further studies about appropriate stimulation periods and conditions of acupuncture are needed to define the acupoint effects on irradiated embryos.

REFERENCES

Gu Y, Hasegawa T *et al.* (2000) Study of the radioprotective effects of TMG on teratogenic malformations on irradiated mice. *Nippon Igaku Hoshasen Gakkai Zasshi* **60**, 845-855.

Gu Y, Hasagawa T *et al.* (2001) A study of the radioprotection effect of guarana (Paullinia cupana) on the fetuses of ICR mice. *J. Korea Asso. Radiat. Prot.* **26**, 347-356.

Gu Y, Hasagawa T *et al.* (2001) The Combined effects of MRI and X-rays on ICR mouse embryos during organogenesis. *J. Radiat. Res.* **42**, 265-272.

Gu Y, Takagi Y *et al.* (2005) Enhancement of Radioprotection and Anti-Tumor Immunity by Yeast-Derived β-Glucan in Mice. *J. Med. Food* **8**, 154-158.

Hau DM. (1981) Effect of differential exposures of X-irradiation on hematological components in guinea pigs. *Chinese J. Radiol.* **6**, 352-364.

Hau DM. (1984) Effects of electroacupuncture on leukocytes and plasma protein in the X-irradiated rats. *Am. J. Chin. Med.* **12**, 106-114.

Hau DM, Wu JC *et al.* (1989) Effects of moxibustion on cellular immunocompetence of ã-irradiated mice. *Am. J. Chin. Med.* **17**, 157-163.

Jacobson L. (1965) Low dose embryonic X-irradiation in mice and some seasonal effects in the prenatal period. *Radiat. Res.* **25**, 611-625.

Russell LB, Russell WL. (1954) An analysis of the changing radiation response of the developing mouse embryo. *J. Cell Physiol.* **43**, 1030-1149.

Takagi Y, Choi I *et al.* (2005) Immune activation and radioprotection by propolis. *Am. J. Chin. Med.* **33**, 231-

240.

Wu JC, Hau DM *et al.* (1986) Effects of electroacupuncture on cellular immunocompetence of γ-irradiated mice. *Acup. Res. Quart* **10**, 77-84.

Yuhas, JM, Spellman JM *et al.* (1980) The role of WR2721 in radiotherapy and/or chemotherapy. *In Brady L(ed): Radiation Sensitizers Masson New York* 303-308.