3 - 64 Effect of Carbon-ion Irradiation on Zebrafish Eye Development*

Zhou Rong, Zhang Hong and Si Jing

Heavy ions have become potentially radiotherapeutic tools. However, studies of the effects on development of normal organs were limited. Using a zebrafish model, this study was designed to investigate the potential developmental toxicity in eyes exposed to carbon-ion irradiation. Zebrafish embryos at 12 h post-fertilization (hpf) were irradiated using $^{12}C^{6+}$ion beams at doses of 2, 4, and 8 Gy. The eye size was measurement at 144 hpf. The results suggested that irradiation at high doses may disrupt eye development of zebrafish embryos.

The mean diameter of whole eyes in the control group at 144 hpf was (318.14 ± 13.34) μm. Statistical analysis showed that the diameters of the whole eyes were significantly reduced compared to the other groups after an irradiation dose of 8 Gy (Tukey's test, $P < 0.05$), whereas the differences between the other pairwise groups were not significant. The mean diameter of the lens in the control group at 144 hpf was (118.24 ± 4.99) μm. The diameter of the lens was significantly reduced compared to the other groups after irradiation at a dose of 8 Gy (Tukey's test, $P < 0.05$), but the difference between the other pair wise groups was not significant (Fig. 1).

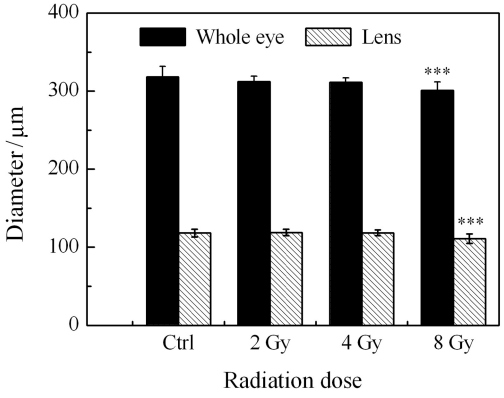

Fig. 1 (color online) $^{12}C^{6+}$ ion irradiation -induced changes in the diameters of whole eyes and lenses at 144 hpf. Each value is expressed as the mean ± SD ($N = 15$). Significant differences between the control and irradiated groups were determined using one-way ANOVA, followed by Tukey's test. $^{***}P < 0.001$ compared with the control.

In our work, significant decreases of diameters of whole eyes and lens was observed in the 8 Gy irradiated group. The developmental toxic effect of carbon-ion irradiation on development was dose-dependent. It was clear from our studies that irradiation at increased doses was a high-risk practice. Microphthalmia was also observed in the zebrafish larvae that experienced high-dose γ-rays (10 Gy) and X-ray (8 Gy) irradiation at the blastula stage and gastrula stage, respectively[1,2]. However, more investigations are needed to further determine the molecular mechanism of carbon-ion irradiation-induced microphthalmia.

References

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3- 65 Effects of Carbon Monoxide-releasing Molecule (CORM-3) on Zebrafish Embryos Induced by X-rays**

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CO is increasingly appreciated as a signal molecule, due to its protective features, such as anti-inflammatory function[1], anti-apoptosis[2]. Carbon monoxide -releasing molecules (CORMs) represent a group of compounds that are capable of modulating physiological functions via liberating CO. Among various CORMs synthetized, CORM-3 is one of the most promising compounds because it is soluble in water and the half-life to liberate CO in saline was 10.6 h, thus imitating the action of CO more closely[3].

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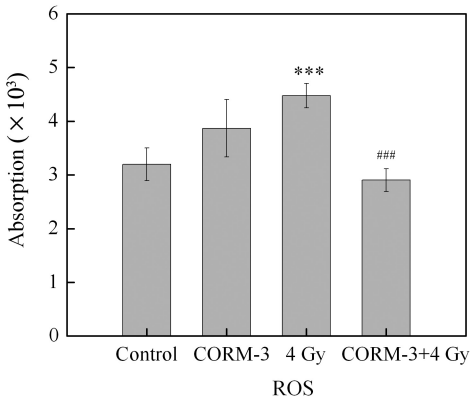

Fig. 1 Changes in the contents of ROS in zebrafish embryos. Each value is expressed as the mean ± SEM ($N = 3$). $^{***}P < 0.001$ compared with the control, $^{###}P < 0.001$ versus the CORM-3+ irradiation group for the irradiation group.

To investigate the effects of CORM-3 on zebrafish embryos induced by X-rays healthy developing zebrafish embryos were selected and divided into four groups as follows: control group (received no further treatment), drug group (raised in 10 μM freshly prepared CORM-3 solution at 4 hpf (hour post-fertilization, hpf), the irradiated group (received 4 Gy X-ray at 4hpf and raised by E3), and irradiated and drug group (raised in 10 μM freshly prepared CORM-3 solution at 4 hpf and received 4 Gy X-ray immediately). The mortality and malformation of the zebrafish embryos were observed until 3 dpf (day post-fertilization, dpf). The results showed that 4 Gy X-ray caused morphological abnormalities in zebrafish embryos, including pericardial sac edema, spinal column curving and tail curvature(Fig. 1). The mortality and malformation rates increased in irradiated group and both can be modified by CORM-3. At 24 hpf, we examined the ROS from each group. Our data showed that the CORM-3 reduced the production of ROS induced by X-ray, thus protecting the oxidative stress-related damage.

References

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3- 66 Toxic Effect of CORM-3 on the Development of Zebrafish Embryos*

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CORM-3, a water soluble transitional metal carbonyls based around ruthenium can release CO activated by light[1]. Although CORM-3 has resulted in promising preclinical data, such as anti-inflammatory, anti-apoptosis and vasodilatory effect, they present a poorly understanding on toxicological profile.

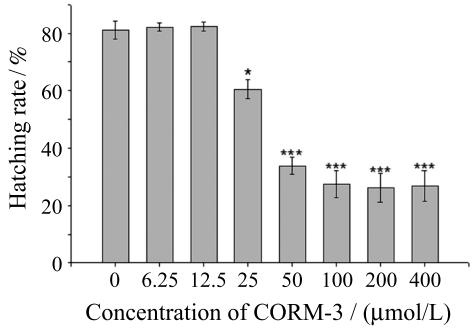

Fig. 1 Hatching rate of zebrafish embryos exposed to different CORM-3 concentrations at 72 h post fertilization. Hatching rate was calculated as number of embryos hatched out /number of live embryos ×100% at 72 h post fertilization. Data are expressed as means ±S.E. from three independent experiments ($^{*}P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001$).

To evaluate the effects of CORM-3 on the development of zebrafish embryos, we selected the zebrafish embryos developing normally at the stage of blastocyst at 4 h post fertilization and transferred into 24-well culture plates with 2 embryos per well and then exposed to freshly prepared CORM-3 solution with E3 (0, 6.25, 12.5, 25, 50, 100, 200 and 400 μmol/L) for 4∼144 hpf for a continuing observation period on the development of zebrafish. Acute endpoints including hatching rate and embryonic/larval mortality were evaluated every 24 h(Fig. 1).

Our data showed that the mortality increased in a concentration-dependent manner. The hatching rate of embryos exposed to CORM-3 was not apparently affected by the low concentration. However, the results showed that the hatching rate decreased in a concentration-dependent manner induced by high concentration after exposed to CORM-3 solution and made a significant difference, indicating that the concentration over 25 μmol/L caused a strong inhibition of embryo hatching, and led to a direct delay of embryos development.