4 - 61 Effects of CORM-3 on Developmental Toxicity Induced by Ionizing Radiation in Zebrafish Embryos*

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CORM-3 is a water-soluble CO-releasing carbonyl complex. A low concentration of CO has been proved to be involved in the protection against cell death and oxidative injury, anti-inflammatory and anti-apoptotic effects, control of cell proliferation, tolerance of organ transplantation and neurotransmission[1]. Using a zebrafish model, this study was designed to investigate the protective effects of CORM-3 on the developmental toxicity in zebrafish embryos induced by ionizing radiation with different linear energy transfer (LET). Embryos with healthy developing were divided into four groups as follows, (a) the control (C) group, which received no further treatments after rinsed with E3; (b) CORM-3-pretreated (CORM) group, which did not receive irradiation after exposed to 10 μM CORM-3 at 4 h post-fertilization (hpf); (c) irradiation (IR) groups, which received X-ray or $^{12}C^{6+}$ ion irradiation at 5 hpf and (d) CORM-3-pretreatment + irradiation (CORM+IR) group, which received 10 μmol/L CORM-3, which was freshly prepared by dissolving CORM-3 in E3 culture medium, treatment 1h before X-ray or $^{12}C^{6+}$ ion irradiation. Three repeated experiments were separately carried out. The hatching rate and incidence of mortality were calculated at 72 hpf and 120 hpf respectively.

As Fig. 1 shows, significant increases in mortality were observed in the embryos irradiated with 4 Gy X-ray and $^{12}C^{6+}$ ion beams and both can be modified by CORM-3. The mortality increased significantly in embryos irradiated with X-ray ($P < 0.01$) and $^{12}C^{6+}$ ion beams ($P < 0.001$) at doses of 4 Gy. When CORM-3 was present 1 h before the X-ray irradiation, the mortality was significantly decreased compared with the X-ray irradiation-only group ($P < 0.05$). In contrast, there was no obvious decrease in mortality in the embryos treated with CORM-3 at 1 h before irradiation compared with embryos exposed to $^{12}C^{6+}$ ion irradiation only. After exposure to 4 Gy X-rays or $^{12}C^{6+}$ ion irradiation, significant reduction in hatching rate ($P < 0.05$ for X-ray irradiation and $P < 0.001$ for $^{12}C^{6+}$ ion irradiation) was observed when compared with the control. The hatching rate increased significantly ($P < 0.05$) when the X-ray irradiation occurred in the presence of CORM-3 compared to the X-ray irradiation-only group. However, there was no significant difference in hatching rate between the IR group and IR+CORM-3 group for $^{12}C^{6+}$ ion irradiation. CORM-3 alone in the absence of irradiation had no appreciable effect on either mortality or hatching rate.

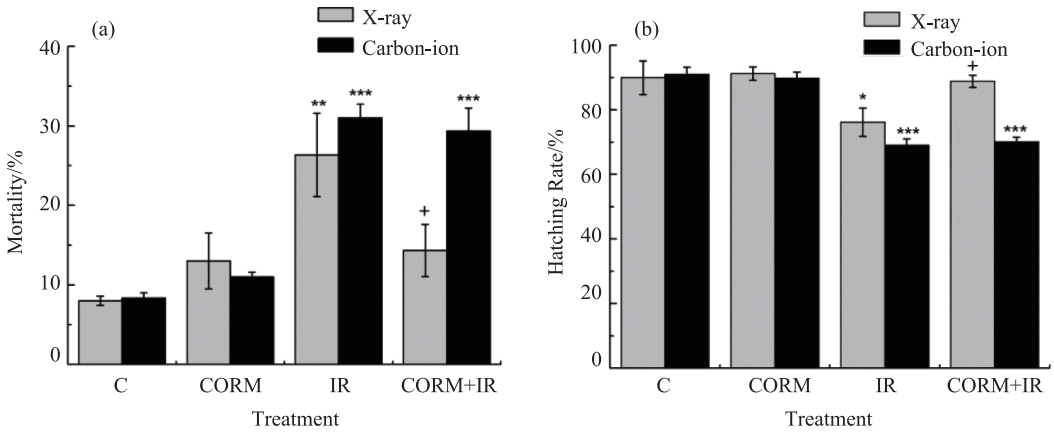

Fig. 1 Changes in mortality (a) and hatching rate (b) of zebrafish induced by X-ray or carbon-ion irradiation with or without pretreatment with CORM-3 at 120 hpf and 72 hpf, respectively. Each value is expressed as the mean ± SEM ($N = 3$). *$P < 0.05$ compared with the C group, **$P < 0.01$ compared with the C group, ***$P < 0.001$ compared with the C group, + $P < 0.05$ versus the CORM + IR group for the IR group.

In zebrafish, mortality is one of the most important indices used to assess the toxicological effects of ionizing radiation[2]. After carbon-ion irradiation, the mortality of zebrafish embryos increased in a dose-dependent manner[2,3]. Our results indicate that CORM-3 has a radioprotective effect on X-ray irradiation-induced develop-

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mental toxicity, but does not have the same effect on carbon-ion irradiation-induced developmental toxicity.

References

[1] L. Wu, R. Wang, Pharmacol Rev., 57(2005)585.

[2] R. Zhou, H. Zhang, Z. G. Wang, et al., Life Sci., 139(2015)114.

[3] J. Si, H. Zhang, Z. H. Wang, et al., Mutat Res, 745-746(2013)26.

4 - 62 Apoptosis in Zebrafish (Danio rerio) Embryos Induced by ^{56}Fe Ion Radiation*

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All living organisms and ecosystems are permanently exposed to ionizing radiation. Of all the types of ionizing radiation, heavy ions such as ^{56}Fe have the potential to cause the most severe biological effects. We therefore examined the effects and potential mechanisms of iron ion irradiation on the induction of apoptosis in zebrafish embryos. Numerous experiments have shown that high-LET heavy ions may cause more DNA breaks than low-LET radiation. When DNA damage is irreparable, cell death occurs. DNA fragmentation is a characteristic feature of apoptosis, which is considered to be one consequence of radiation, via the induction of programmed cell death. TUNEL assay is designed to detect apoptotic cells, which undergo extensive DNA degradation during the late stages of apoptosis. Because of the optical transparency of zebrafish embryos, fluorescent TUNEL assay could allow the detection of apoptosis in zebrafish embryos directly and visually.

Zebrafish embryos at 4 h post-fertilization (hpf) were divided into five groups: a control group; and four groups irradiated with 0.5, 1, 2, and 4 Gy radiation, respectively. The beam energy of Iron particles was 160 MeV/u. The dose rate was 0.5 Gy/min approximately. The extent of apoptosis was examined by performing TUNEL staining (the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling) assay using ApopTag®Fluorescein In Situ Apoptosis Detection Kit (Oncor, Gaithersburg, MD, USA). The method is based on the ability of TdT to label blunt ends of double-stranded DNA breaks independent of a template[1]. The TUNEL intensity within the samples was examined under a fluorescence microscope (Olympus BX51, Tokyo, Japan). The fluorescence was measured and quantified using ImagePro Plus software (Media Cybernetics, Silver Springs, USA). As shown in Fig. 1, there were few TUNEL-positive cells in the control, 0.5, and 1 Gy irradiation groups, whereas the fluorescence signal in embryos was markedly increased following 2 or 4 Gy irradiation (Fig. 1(a)). The relative fluorescence results in zebrafish embryos at 24 hpf showed that cell apoptosis increased in a dose-dependent manner. Iron ion irradiation resulted in slight increases in apoptosis at 0.5 and 1 Gy irradiation, but more significant increases at 2 and 4 Gy irradiation, with an approximately 2.05-fold increase with 2 Gy and 2.87-fold increase with 4 Gy compared with the control group ($P < 0.01$) (Fig. 1(b)).

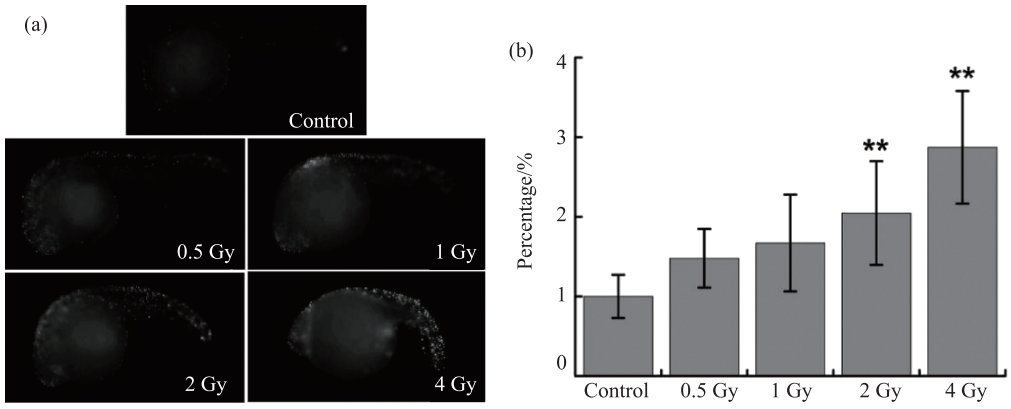

Fig. 1 Detection of iron ion radiation-induced changes in the cell apoptosis within live zebrafish embryos using the TUNEL assay. (a) Representative photographs of TUNEL labeling was emitted from cells undergoing cell apoptosis indicated by a green fluorescence signal on a black background. (b) Radiation dose–response curves of cell apoptosis measured as relative fluorescence within zebrafish embryos at 24 hpf. Each value is expressed as the mean ± SEM. Significant differences between the control and irradiated groups were determined using one-way ANOVA (* $P < 0.05$, ** $P < 0.01$), $N = 10$.