Contribution ID: **13** Type: **not specified**

Comparative study of the effects induced by innovative radiotherapy treatments in zebrafish

Conventional radiotherapy (RT) is based on the administration of photon beams in the form of low Linear Energy Transfer (LET) radiation (X-rays), which deposit a relatively small amount of energy on the target and disperses it to the surrounding healthy tissue, due to scattering phenomena, differently from high LET radiation (protons), which is delivered directly on the target with minimal dispersion on the close non irradiated tissues. The increased effectiveness of charged particles compared to photons is quantified by the Relative Biological Effectiveness (RBE), that represents a key parameter to compare different radiation qualities for prescribed doses. In recent years, it is grown the necessity of RT protocols improvement. Hence, the therapeutic use of proton beams, alone or in combination with radiomodifiers, requires the development of high performance and reliable in vivo models for preclinical research. In this scenario, the zebrafish embryo is a powerful and versatile model for assessing the effect of ionizing radiation with different LET values. We previously showed the radioprotective role of curcumin in combination with conventional X-rays, by the analysis of mortality, morphological alterations, and identifying the molecular mechanism underlying this biological effect in zebrafish. Based on this, distinct batches of 24 hours post-fertilization (hpf) zebrafish embryos were exposed to protons at the same X-rays dose range (0-15 Gy). Sister batches were pre-treated from 6 to 24 hpf with curcumin, at concentrations of 2,5 and 5 μM, and subsequently irradiated using the abovementioned dose range. Moreover, batches of 6 hpf embryos were either used as untreated controls or subjected to single treatment with curcumin following the same experimental setting used in the combined treatment. Treated and control embryos were carefully examined by daily stereomicroscope observation until 120 hpf, to estimate the mortality rate, developmental delay, morphometric parameters, hatching and heart rate values. Among these, the pericardial edema (PE) measurement was used to calculate a Protection Rate (PR) value in combined vs single treatments. Our experiments, comparing protons vs photons beam as single treatments at the isodose, showed that the damage inflicted by protons is greater than that inflicted by photons, in terms of malformed embryos percentage and PE/spinal curve (SC) incidence. Moreover, curcumin pre-treatment produces a major recovery in combination with protons than with photon irradiation. A PR value has been calculated in terms of PE diameter ratio at 72 and 96 hpf for both curcumin concentration, showing better protection performance using higher curcumin concentration.
Keywords: zebrafish; radiations; curcumin.

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