Protective role of isoflurane after combined exposure with 1 or 2 Gy of ionizing radiation assessed with alkaline comet assay *in vivo*

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INTRODUCTION

Cancer yearly increase is connected also with radiotherapy increase, and younger patients such as children are also not spared from the yearly cancer and radiotherapy increase. To ease their pain and to make them comfortable during the radiotherapy, and also the lower the number of the dose treatment, by precise localization of the treatment, sometimes it is necessary to use the volatile anaesthetics as part of general anaesthesia [1] . In past, those anaesthetics were considered safe, but there are new findings demonstrating that this is not always true [2,3] . Isoflurane is a fluorinated ether with general anesthetic and muscle relaxant activities. Although the exact mechanism of action has not been established, inhaled isoflurane appears to act on the lipid matrix of the neuronal cell membrane, which results in disruption of neuronal transmission [4] . We decided to use the most used VA isoflurane to examine animal model *in vivo* conditions whether there is synergistic effect in combined exposure to isoflurane and to 1 or 2 Gy of ionizing radiation, the doses usually used in fractioned radiotherapy.

MATERIALS AND METHODS

Healthy Swiss male albino mice 60 days old, cca. 25 grams of body weight, we exposed them for 2 hours to 1.7% of inhaled isoflurane mixture with oxygen and air and afterwards some of them were also exposed to 1 or 2 Gy of ionizing radiation and were sacrificed immediately after exposure, 2, 6 and 24 hours from the exposure (Figure 1). We had 5 animals per group and radiation source was cobalt, with the dose rate of 1.88 Gy/minute on the Theratron Phoenix teletherapy unit, Atomic Energy Ltd., at the Clinical Hospital "Sveti Duh", Zagreb, Croatia. Study was approved by Ethical Committee of the Faculty of Science (University of Zagreb, Croatia) and was designed in accordance with the relevant Croatian guidelines: The Animal Protection Act (OG 102/17.) and the Ordinance on the protection of animals used for scientific purposes (OG 55/13; 39/17). We measured primary DNA damage level using alkaline comet assay [5], well established method for *in vivo* genotoxicity assessment according to OECD guidelines on 200 comets per each time point and treatment [6]. DNA damage was assessed using fluorescence microscope Olympus BX 40 connected with CCD camera with Comet Assay IV software and with parameters of tail length, tail intensity and tail moment. We took single cell suspension of blood or liver samples, as compartments firstly affected by the exposure (blood-inhalation, liver-metabolisation).

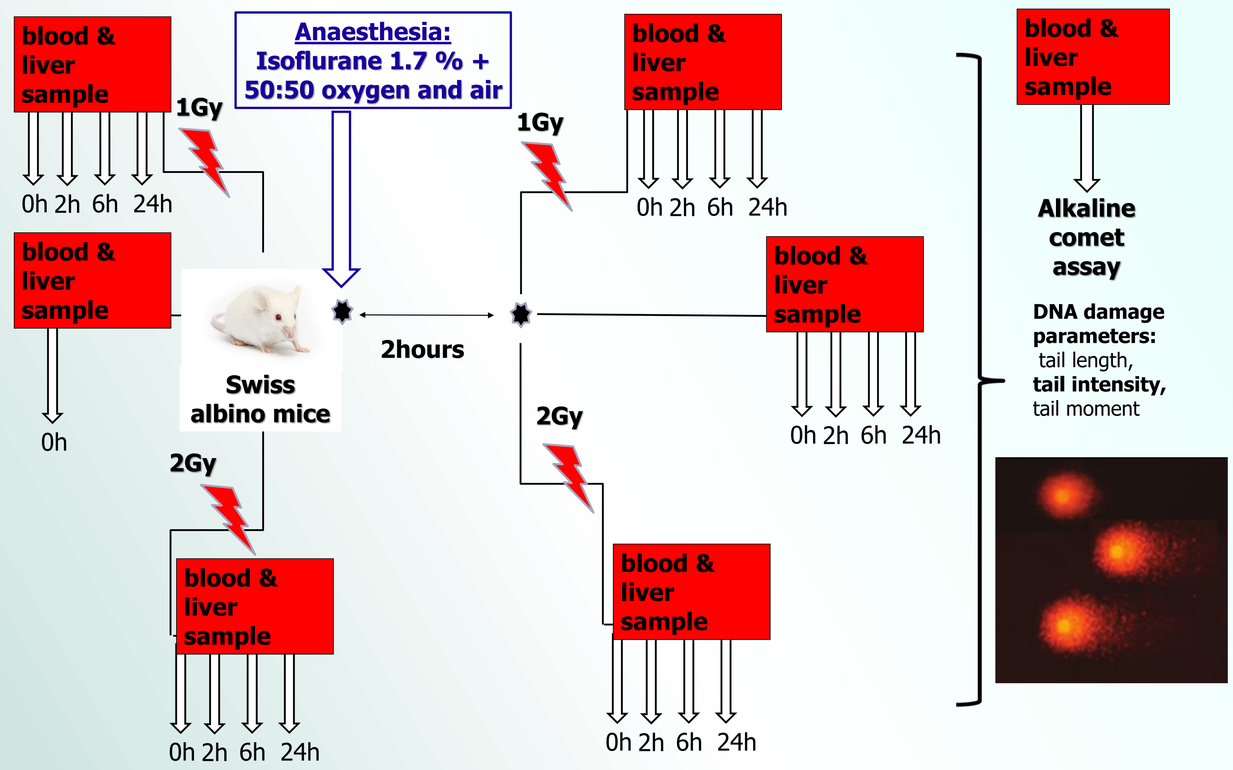

Figure 1. Sheme of the experiment

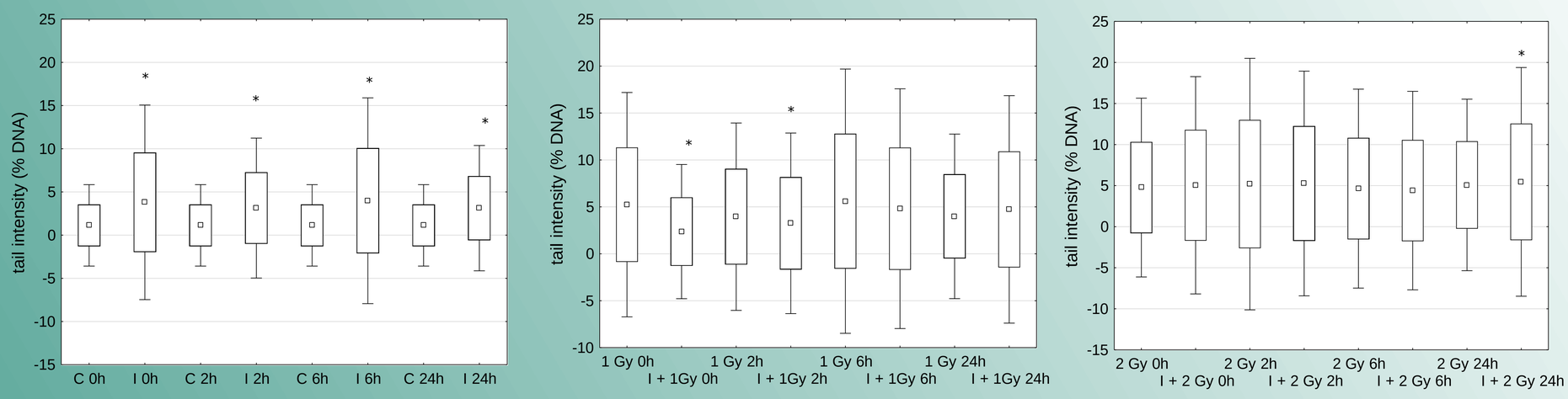

Figure 2. Tail intensity value in blood cells of Swiss albino mice treated with anesthetic isoflurane (I) alone or in combination with irradiation of 1 or 2 Gy. Samples were taken immediately after (0h), 2 hours (2h), 6 hours (6h) and 24 hours (24h) after irradiation. C-control, *statistically different form control of the same time point, ▫ Mean ▯ Mean±SD ┬ Mean±1,96*SD

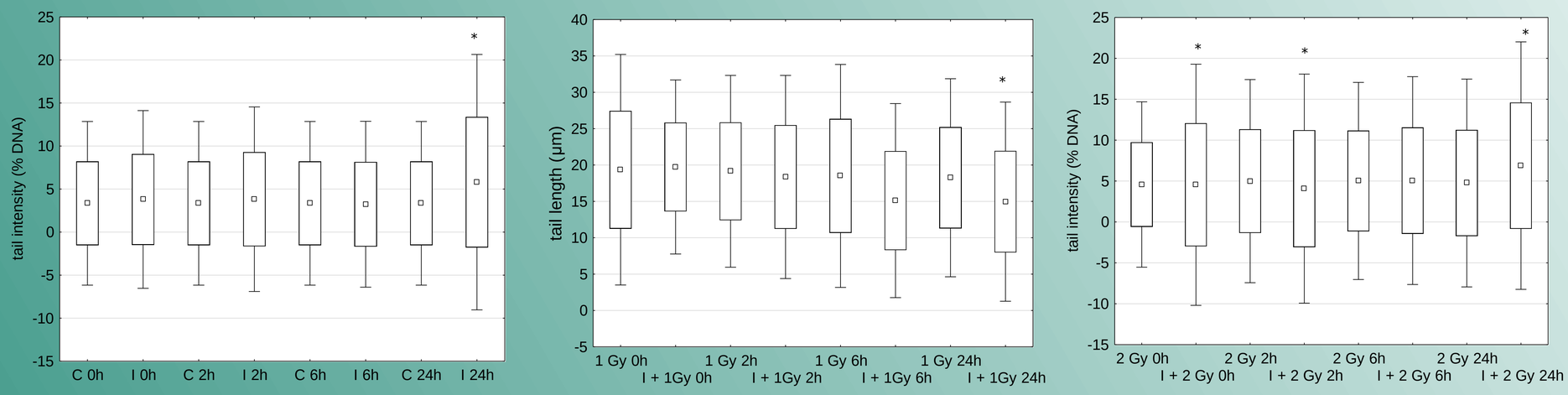

Figure 3. Tail intensity value in liver cells of Swiss albino mice treated with anesthetic isoflurane (I) alone or in combination with irradiation of 1 or 2 Gy. Samples were taken immediately after (0h), 2 hours (2h), 6 hours (6h) and 24 hours (24h) after irradiation. C-control, *statistically different form control of the same time point, ▫ Mean ▯ Mean±SD ┬ Mean±1,96*SD

RESULTS

Both single exposure to isoflurane or to either of the two doses of ionising radiation caused significantly higher DNA damage levels when compared to the control samples, with isoflurane and 1 Gy ionizing radiation having similar levels, while 2 Gy exposure caused the highest DNA damage levels. Surprisingly, combined exposure of isoflurane and ionizing radiation caused lower levels of DNA damage when compared to only radiated samples.

CONCLUSIONS

These findings implicate that isoflurane can cause protective effect and lower level of DNA damage and should be used in radiotherapy. The mechanism by which isoflurane caused this effect is probably adaptive response, by activating DNA repair mechanisms and the levels of scavengers of reactive free oxygen radicals, covering on that way both types of which IR exposure can cause DNA damage effects (indirect and direct IR effect on DNA).

LITERATURE

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