# COMBINED EXPOSURE TO HALOTHANE AND 1 OR 2 GY IONIZING RADIATION CAUSES SYNERGISTIC EFFECT IN DNA DAMAGE IN BOTH BLOOD AND LIVER OF SWISS ALBINO MICE

*Vesna Benković[1], Nikola Borojević[2], Nada Oršolić[1], Gordana Brozović[3], Anica Horvat Knežević[1], Mirta Milić[4]*

*[1]Faculty of Science, University of Zagreb, Zagreb, Croatia*

*[2]Warrington and Halton Teaching Hospitals NHS Foundation Trust, Lovely Ln, Warrington WA5 1QG, United Kingdom*

*[3]Department of Anesthesiology, Reanimatology and ICU, University Hospital for Tumours, Sestre milosrdnice University Hospital Centre, Zagreb, Croatia, Faculty of Dental medicine and Health, University of Osijek*

*[4]Institute for Medical Research and Occupational Health, Mutagenesis Unit Zagreb, Croatia*

## INTRODUCTION

Patient immobilization by general volatile anesthesia (VA) during medical radiology treatment is sometimes necessary and thus far it has had quite an annual increase [1]. With ionizing radiation exposure there will be for sure some level of DNA damage since IR is a well-known genotoxic and cytotoxic agent, although the doses used are maintained at the minimum, with good localization to spare as much as possible healthy tissue and organs that do not need to be exposed [2]. Recently, there is a growing number of studies demonstrating that volatile anesthetics can also cause DNA damage effect in patients, and in occupationally exposed personnel. Halothane(2-bromo-2-chloro-1,1,1-trifluoroethane) is a non-flammable, halogenated, hydrocarbon general inhalation anesthetic. It provides quite fast induction of anesthesia by depressing the central nervous system, thereby producing a reversible loss of consciousness and sensation [3,4]. Since there are no studies on IR and VA combined effects, we decided to use animal model and check whether there are elevated levels of DNA damage after combined exposure using animal model.

## MATHERIALS AND METHODS

Swiss albino male mice three months old, with body weight 20-25 grams, were divided in experimental groups (n = 5). Mice were anaesthetized by inhaling 2.4% halothane mixture with oxygen and air for 2 hours and then were exposed to either 1 or 2 Gy of ionizing radiation ($^{60}$Co source, Theratron Phoenix teletherapy unit, Atomic Energy Ltd)., in at the Clinical Hospital "Sveti Duh", 1.88Gy/min doze rate). Blood samples were collected from the tail vein and liver samples after sacrificing the animals: immediately, 2, 6 and 24 hours after irradiation (Figure 1). 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 and EU Directive 2010/63/EU. The alkaline comet assay was carried out as described by Singh et al. [5]. The slides were stained with ethidium bromide (20 μgmL$^{-1}$) and examined under magnification 200x, using an epifluorescence microscope. 200 comet per group were scored The statistical significance of the results obtained in the comet assay was studied by the statistical program STATISTICA9.0 (StatSoft, Dell, Tulsa, USA) using an analysis of variance with post-hoc Scheffé test modification. The cellular DNA repair index (CRI) for the quantification of the effectiveness of DNA repair using the TI parameter was calculated according to the formula by Nair & Nair [6].

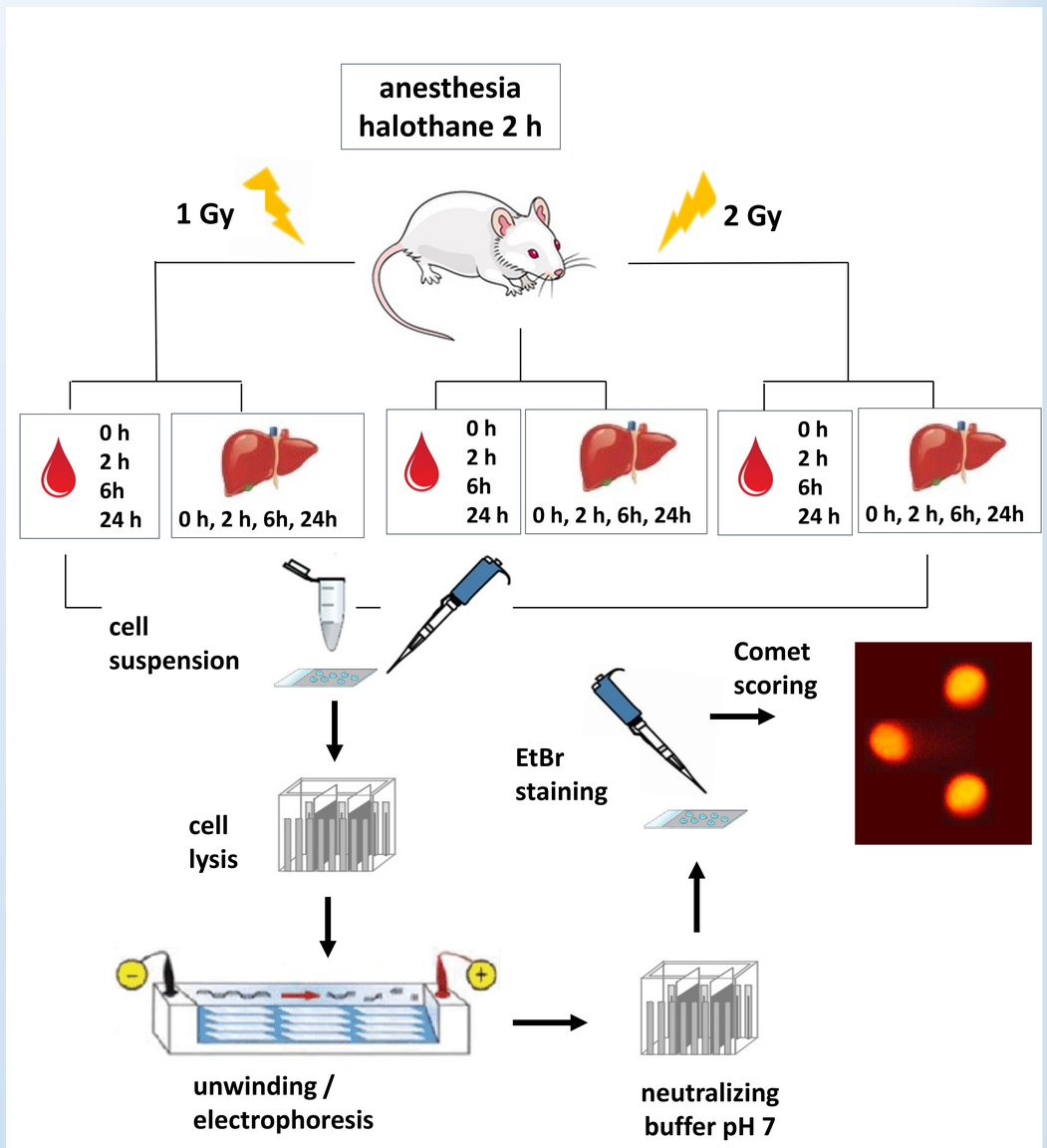



Figure 1. Scheme of experiment

## RESULTS

In blood cells halothane induced statistically increased DNA damage compared to control 2 h, 6 h and 24 h after treatment alone and in combination with 1 Gy irradiation, while in combination with 2 Gy statistically higher DNA damage was observed immediately after, 6 h and 24h after treatment(Figure 2). In liver cells increased DNA damage was observed only 6 h and 24 h in nonirradiated animals and in combined treatment with 2 Gy irradiation after 6h (Figure 3). Halothane blocked DNA repair after 2 h in blood cells (Figure 4).

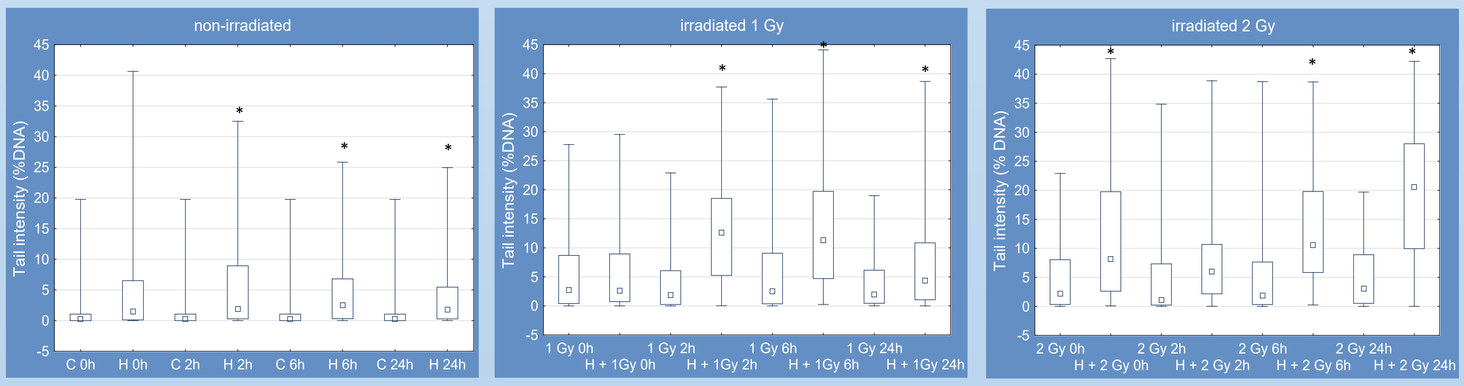



**Figure 2.** Tail intensity value in blood cells of Swiss albino mice treated with anesthetic halothane (H) alone or in combination with irradiation of 1 or 2 Gy. Samples were taken immediately after (0h), 2 hours (2h), 6 hours (6h) and 24 hours (24h) after irradiation. C-control, *statistically different form control of the same time point, Median 25%-75% Min-Max

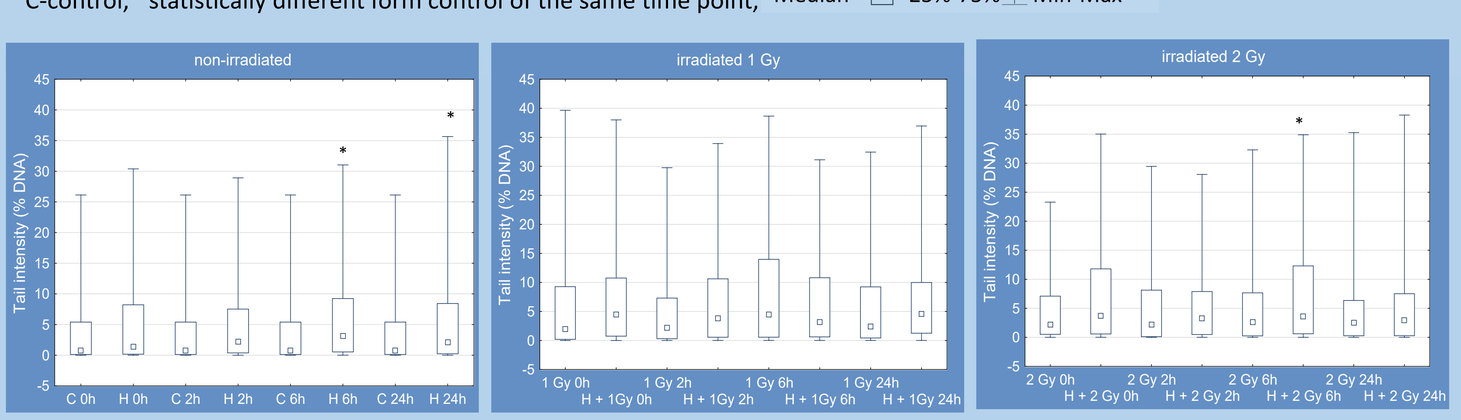
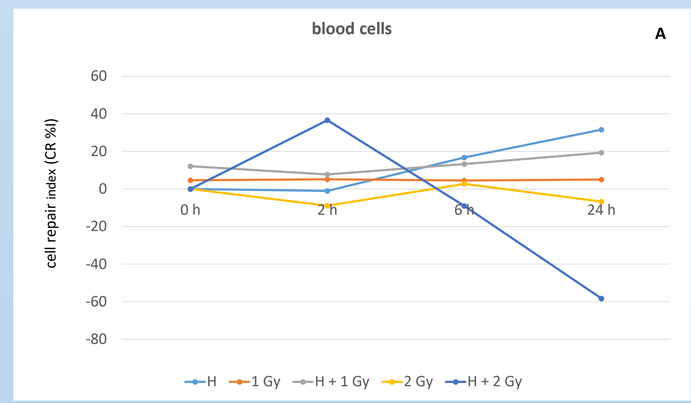



**Figure 3**. Tail intensity value in liver cells of Swiss albino mice treated with anesthetic halothane (H) alone or in combination with irradiation of 1 or 2 Gy. Samples were taken immediately after (0h), 2 hours (2h), 6 hours (6h) and 24 hours (24h) after irradiation. C-control, *statistically different form control of the same time point, Median 25%-75% Min-Max



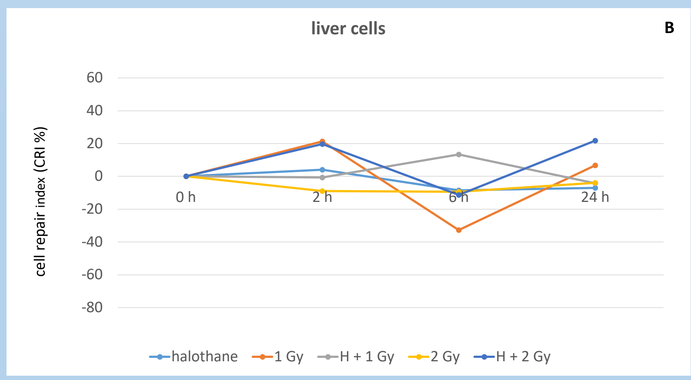




**Figure 4.** Cellular DNA repair index (percentage of repair) of the tail intensity parameter for exposure to halothane (H) and combined exposure to halothane and 1 Gy (H + 1 Gy) or 2 Gy (H + 2 Gy) irradiation, immediately (0h), 2h, 6h and 24h (hours) after exposure in blood (A) and liver cells (B).

## CONCLUSION

Both halothane and IR demonstrated elevated levels of DNA damage, and that combined treatment caused synergistic effect that increased with the dose of radiation and was not repaired not even after 24 hours from the exposure. These data are confirming the concern about safety of combined VA and IR exposure, and calling for further investigation on the safety and proper use of the type of the anesthetic necessary used during radiotherapy.

## REFERENCES

[1] Brunt AM, Haviland JS, Wheatley DA, Sydenham MA, Alhasso A, et al. 2020. Hypofractionated breast radiotherapy for 1 week versus 3 weeks (FAST-Forward): 5-year efficacy and late normal tissue effects results from a multicentre, non-inferiority, randomised, phase 3 trial. Lancet 395(10237):1613-1626

[2] The Royal College of Radiologists. 2019. Radiology dose fractionation, third edition.London: The Royal College of Radiologist, BFCO(19)3.

[3] Gyorfi MJ, Kim PY. 2020. Halothane Toxicity. StatPearls [Internet]. Treasure Island (FL). StatPearls Publishing.

[4] Campagna JA, Miller KW, Forman SA. 2003. Mechanisms of actions of inhaled anesthetics. N Engl J Med. 348(21), 2110-2124.

[5]SinghNP,McCoyMT,TiceRR,SchneiderLL.Asimpletechniqueforquantitationoflowlevelsof DNAdamageinindividualcells.ExpCellRes1988;75:184-191.

[6] Nair GG, Nair CK. 2010. Protection of cellular DNA and membrane from γ-radiation-induced damages and enhancement in DNA repair by sesamol. Cancer Biother Radiopharm. 25(6): 629-635.