Original article DOI: 10.2478/aiht-2023-74-3692

Different damaging effects of volatile anaesthetics alone or in combination with 1 and 2 Gy gamma-irradiation *in vivo* on mouse liver DNA: a preliminary study

Vesna Benković[1*], Mirta Milić[2*], Nada Oršolić[1], Anica Horvat Knežević[1], Gordana Brozović[3,4], and Nikola Borojević[5]

[1] University of Zagreb Faculty of Science, Department of Biology, Zagreb, Croatia
[2] Institute for Medical Research and Occupational Health, Mutagenesis Unit, Zagreb, Croatia
[3] Sestre Milosrdnice University Hospital, Clinic for Tumours, Department of Anaesthesiology, Reanimatology, and Intensive Care, Zagreb, Croatia
[4] University of Osijek Faculty of Dental Medicine and Health, Osijek, Croatia
[5] National Health Service Foundation Trust, Warrington and Halton Teaching Hospitals, Warrington, United Kingdom

[Received in November 2022; Similarity Check in November 2022; Accepted in March 2023]

As the number of radiotherapy and radiology diagnostic procedures increases from year to year, so does the use of general volatile anaesthesia (VA). Although considered safe, VA exposure can cause different adverse effects and, in combination with ionising radiation (IR), can also cause synergistic effects. However, little is known about DNA damage incurred by this combination at doses applied in a single radiotherapy treatment. To learn more about it, we assessed DNA damage and repair response in the liver tissue of Swiss albino male mice following exposure to isoflurane (I), sevoflurane (S), or halothane (H) alone or in combination with 1 or 2 Gy irradiation using the comet assay. Samples were taken immediately (0 h) and 2, 6, and 24 h after exposure. Compared to control, the highest DNA damage was found in mice receiving halothane alone or in combination with 1 or 2 Gy IR treatments. Sevoflurane and isoflurane displayed protective effects against 1 Gy IR, while with 2 Gy IR the first adverse effects appeared at 24 h post-exposure. Although VA effects depend on liver metabolism, the detection of unrepaired DNA damage 24 h after combined exposure with 2 Gy IR indicates that we need to look further into the combined effects of VA and IR on genome stability and include a longer time frame than 24 h for single exposure as well as repeated exposure as a more realistic scenario in radiotherapy treatment.

KEY WORDS: alkaline comet assay; halothane; ionising irradiation; isoflurane; sevoflurane

Despite its widely known genotoxic and cytotoxic action, ionising radiation (IR) remains standard cancer treatment (1). Through ionisation of molecules, IR generates toxic free radicals, which can result in single and double-strand DNA breaks, incur oxidative damage to sugar and base residues, and cause chromosomal aberrations and mutations without distinguishing between tumour and normal tissues (1, 2). Even though cancer cells are more vulnerable to IR than normal cells due to rapid cell division and inefficient DNA repair, both sustain DNA damage. For this reason, exposure of normal tissues is limited in terms of maximum deliverable dose, which also limits the efficacy of IR therapy (3).

According to the latest version of the guidelines issued by the Royal College of Radiologists (2), a typical IR dose in different radiotherapies (intraoperative, brachytherapy, fractionated and hypofractionated radiotherapy) is 1 or 2 Gy. To optimise the therapeutic effects of these doses and minimise the damage to normal tissue with precision targeting, some patients such as children or those with anxiety issues or claustrophobia are immobilised (4, 5) or put under the general anaesthesia. Anaesthesia is in some cases also used during intraoperative radiotherapy or brachytherapy.

Standard general anaesthesia involves volatile anaesthetics (VAs), such as isoflurane (I), sevoflurane (S), and halothane (H) as an alternative to intravenous application. They have similar mechanisms of action as they enhance the inhibitory activity of postsynaptic channels [gamma-aminobutyric acid (GABA) and glycine] and inhibit the excitatory activity of synaptic channels [glutamate, *N*-methyl-D-aspartate (NMDA), nicotinic acetylcholine, and serotonin] in the central nervous system (6). Although considered safe, VAs have been reported to cause different adverse effects in patients and occupationally exposed personnel, depending on the dose, treatment duration (exposure and number of exposures), and their metabolism and toxicokinetic activity in the liver, kidney, or brain (7–9).

Corresponding author: Mirta Milić, Mutagenesis Unit, Institute for Medical Research and Occupational Health, Mutagenesis Unit, Ksaverska cesta 2, 10001 Zagreb, Croatia, e-mail: *mmilic@imi.hr*

*equal contribution

Earlier studies (7, 10–12) have already described DNA damage and repair in human blood samples for each of the three anaesthetics and IR doses usually applied in therapy, but none has yet addressed single-dose exposure to a combination of VAs and IR, even though IR at sufficient doses has a similar mechanism of action as VA (13) and their combinations might have synergistic effects.

Therefore, the aim of this preliminary study was to investigate the effects of combined exposure to a single treatment with isoflurane, sevoflurane, or halothane alone or in combination with IR at 1 and 2 Gy in terms of liver tissue DNA damage and repair immediately and 2, 6, and 24 h after exposure, considering that the dynamics of DNA damage and repair also depend on the target organ tissue and time since exposure (7, 11–14).

MATERIALS AND METHODS

Chemicals

The inhalation anaesthetics, namely sevoflurane (Sevorane®), isoflurane (Forane®), and halothane (Halothane®) were procured from Abbott Laboratories (Queenborough, UK). Chemicals and reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), unless specified otherwise.

Ethical approval

The study was approved by the Ethics Committee of the University of Zagreb, Faculty of Science, Zagreb, Croatia (approval No. 251-58-508-11-9) and was designed in accordance with the Croatian Animal Protection Act (15), Ordinance on the protection of animals used for scientific purposes (16, 17), and the EU Directive 2010/63/EU (18).

Animals

The study included 240 male Swiss albino mice, aged 60±5 days and weighing 20–25 g. The mice were kept in standard breeding conditions (22±1 °C ambient temperature, 50–70 % humidity, 12-hour photoperiod) and had free access to a standard laboratory diet (Standard Diet GLP, 4RF 1, Mucedola, Settimo Milanese MI, Italy) and tap water. We opted for male mice only, as females are more prone to X-chromosome loss and higher DNA damage after IR (19).

Study design

On the experiment day, the animals were randomly divided into the following 12 groups: control (not exposed to either VA or IR); IR treatment alone with either 1 or 2 Gy; anaesthetic treatment alone with S, I, or H; and combined treatments with either of the three anaesthetics and either IR dose (S+1 Gy, S+2 Gy, I+1 Gy, I+2 Gy; H+1 Gy; H+2 Gy). Each group consisted of four subgroups of five animals according to the time points at which their liver was taken: 0 h (immediately after the exposure), 2 h, 6 h, and 24 h after exposure (Figure 1).

Anaesthesia and radiation

Mouse anaesthesia with S (2.4 vol %), I (1.7 vol %), or H (2.4 vol %), was maintained with an anaesthetic machine (Sulla 800; Dräger, Drägerwerk AG & Co. KGaA, Lübeck, Germany) and a compatible evaporator in an induction chamber with the even oxygen-to-air ratio at a continuous flow of (3 L/min) for 2 h. Anaesthesia was considered achieved when the mice fell into a calm sleep, breathed spontaneously, and stilled their tail.

The mice planned for irradiation alone and irradiation combined with anaesthesia received a single dose of either 1 or 2 Gy (dose rate of 1.88 Gy/min) (^{60}Co source, Theratron Phoenix teletherapy unit, Atomic Energy Ltd., Ottawa, Ontario, Canada). Anaesthesia was given to the animals prior the IR treatment to reflect actual radiotherapy conditions. In other words, the animals were treated with anaesthetics prior to IR.

Sample preparation

Before liver sampling, the animals were killed by cervical dislocation in compliance with relevant national and EU legislation (16–18). Small pieces of freshly resected mice liver tissue taken from the right lobe of each animal in each group were dissected and homogenised mechanically with a freshly prepared and chilled (4 °C) homogenisation buffer (0.075 mol/L NaCl and 0.024 mol/L Na_2EDTA) in the proportion of 1 g of tissue per 1 mL of buffer. Single-cell suspension was prepared by mincing and passing the homogenised sample through a stainless-steel mesh with a pore diameter of 40 μm. The whole procedure of sample preparation was done at 4 °C in order to stop DNA repair in the sample.

Alkaline comet assay

We chose the Comet assay to assess DNA damage and repair because it is a standard method used in human biomonitoring studies after *in vivo* exposure to anaesthetics and IR (9, 19–23), and our reporting adheres to the Minimum Information for Reporting Comet Assay (MIRCA) recommendations (24).

The assay was performed under alkaline conditions as described elsewhere (25, 26) with four agarose layers in total. Briefly, 10 μL of liver cell suspension was mixed with 100 μL of 0.5 % low melting point (LMP) agarose and layered as the third layer onto precoated microscope slides [the first layer consisted of a 1 % normal melting point (NMP) agarose and the second of 0.6 % LMP agarose]. The slides were then covered with coverslips and kept horizontally at 4 °C for 10 min. Then another layer of 0.5 % LMP agarose was placed on top. After solidification and coverslip removal, the slides were immersed in a freshly prepared ice-cold lysis solution (2.5 mol/L NaCl, 100 mmol/L Na_2EDTA, 10 mmol/L Tris-HCl, 1 % pH 10 sodium sarcosinate with 1 % Triton X-100, and 10 % dimethyl sulphoxide) at 4 °C for 2 h. In a freshly prepared

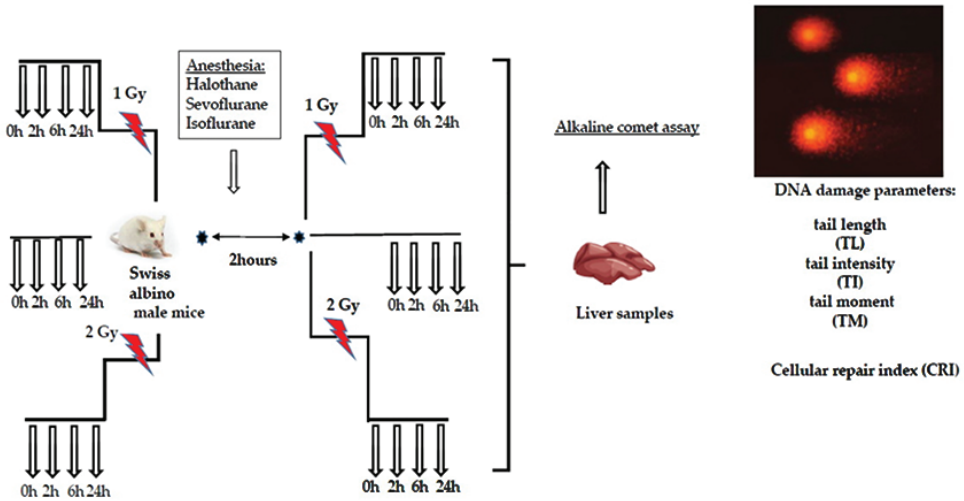

Figure 1 Scheme of the experiment. Male Swiss albino mice (n=240) were divided into 12 groups (control; 1 or 2 Gy irradiated; exposed to only halothane, sevoflurane or isoflurane; exposed to a combination of 1 or 2 Gy and either halothane, sevoflurane or isoflurane) and further each group was divided into 4 subgroups (according to the liver sampling time after the exposure- 0, 2, 6 and 24 hours) with 5 animals in each subgroup. Alkaline Comet assay was used for DNA damage and cellular repair index assessment

electrophoresis solution (300 mmol/L NaOH, 1 mmol/L Na_2EDTA, pH13) at 4 °C, denaturation first (for 20 min, vertical Coplin jars) and then electrophoresis [for another 20 minutes, with changed electrophoresis solution, in a horizontal gel-electrophoresis unit at 25 V (300 mA, 0.8 V/cm)] took place under dim light at 4 °C. Following the electrophoresis, the slides were neutralised three times at five-minute intervals by adding 0.4 mol/L Tris-HCl buffer, pH 7.5, stained with ethidium bromide (20 μg/mL), and examined under a 200× magnification epifluorescence microscope (BX40, Olympus, Tokyo, Japan) linked with a CCD camera to a computer-based image analysis system (Comet Assay IV software, Instem, London, UK). For each animal (sample), 40 randomly selected images of cells were analysed.

Scoring and alkaline assay parameters

A total of 200 comets per group (40 per animal) were analysed for each test point. Tail length (TL, distance of DNA migration from the centre of the nuclear core in μm), tail intensity (TI, the percentage of genomic DNA migration from the nuclear core to the tail during electrophoresis), and tail moment (TM, calculated as the product of TL and TI, in arbitrary units) were the Comet assay parameters used to determine DNA damage.

Cellular DNA repair index (CRI)

To calculate cellular DNA repair index (CRI) we used the formula described elsewhere (27), as follows:

$CRI = [1-(\text{Comet parameter at time t}/\text{Comet parameter at initial time } t_0)] \times 100$

where the "comet parameter" refers to either TL or TI.

Statistical analysis

Statistical analysis was run on Statistica 9.0 (Statsoft, Tulsa, USA) and Statistica 13.5.0.17 packages (TIBCO Software Inc., California, Palo Alto, USA). Before the analysis of 200 comets per each point, we used the analysis of variance (ANOVA) to compare all results for all animals in a group for one measured point. Since the results between them did not significantly differ, they were all combined and the analysis done on 200 comets per group. Means, medians, and standard deviations (SD) were calculated as part of descriptive statistics for TL, TI, and TM. We compared the data for each group with respective controls at 0 h using the Mann-Whitney *U*-test. Control for the single treatment were unexposed mice and for combined treatments mice irradiated with 1 or 2 Gy alone at 0 h. The level of statistical significance was set at $p<0.05$.

RESULTS

In mice exposed to the anaesthetics alone, all three parameters (TL, TI, and TM) were higher than control at nearly all time points (Figures 2a–c). In general, S had the strongest effect on TL; while H and S had the strongest effect on TI and TM. We saw no time-dependent trend for any of the three parameters.

In combination with 1 Gy irradiation, all damage parameter values increased, but with S (in the early hours) and I were generally lower than irradiation alone by the end of measurements, whereas H generally worsened the irradiation effects (Figures 3a–c).

The same is true for H combined with 2 Gy irradiation until hour 24, when TI and TM dropped, whereas all three parameters significantly increased with S by that time (compared to 2 Gy irradiation alone at 0 h). Isoflurane, again, turned out to be the least damaging in such combination.

Considering that we observed some lowering of DNA damage with S and I in combination with 1 and 2 Gy irradiation, we wanted to see whether it would reflect on the level and speed of DNA repair (CRI). Compared to irradiation alone, CRI with I was higher for both TL and TI after 1 Gy exposure at all time points, while a similar effect was seen after 2 Gy exposure in the first six hours. With S,

CRI was in general lower for TL and TI regardless of irradiation dose and sampling time. Repair with H was also generally lower after 1 Gy exposure for both TL and TI. Even though CRI it was higher for TL and TI after 2 Gy exposure, the combination of H and 2 Gy IR caused higher DNA damage than radiation alone, which is important for the evaluation of its effects.

DISCUSSION

Our findings show that a single exposure to any of the three anaesthetics alone caused varying DNA damage in liver cell samples of Swiss albino male mice and that, in general, H caused the highest damage. In combined exposure with IR, S and I demonstrated a slightly protective effect, which was more evident for 1 Gy exposure. For 2 Gy exposure, damage levels were similar to those in irradiated control samples by hour 24, after which they increased significantly.

Although this is a preliminary study and its results need to be explored further as well as include other methods, it gives new evidence about different effects of volatile anaesthetics and their combined use with IR.

It seems that pre-treatment with any of the three VAs can cause some damage to the DNA, most notably halothane. DNA damage always triggers (different) repair mechanisms (in general, there are five) (28). If we consider all TL, TI, TM, and CRI results together, isoflurane lowered the levels of DNA damage in combination with IR, and repair was more efficient than with the other two anaesthetics. Sevoflurane also lowered the DNA damage, but it was followed by lower repair, while halothane caused the highest DNA damage alone and combined with IR, followed by varying repair.

Considering our CRI results and the results of other authors, it seems that sevoflurane and isoflurane have different mechanisms of DNA damage and repair. Sevoflurane has been reported to increase and directly induce more complex DNA damage and is associated with longer and slower DNA repair (more than 24 hours) (11), while isoflurane increases systemic antioxidative status, protecting cells from DNA damage *in vivo* (33). Our results are in line with these reports, especially it comes to isoflurane, at least in the first 24 h, but their mechanism of action and repair seem to differ. We did not look into the damage and repair beyond 24 hours, so we cannot say how would sevoflurane in combination with IR influence DNA damage in the liver over a longer period of time. We can only rely on other animal studies *in vivo* or rare single-exposure human studies, which have demonstrated a similar trend of DNA damage repair in different organs (blood, kidney, and brain in animal studies *in vivo* and blood in human studies) after exposure to similar VAs or a combination of a VA and IR (blood, kidney, and brain in animal studies *in vivo*) (10, 11, 31–33, 35–38). However, none of them observed DNA damage for more than 24 hours from exposure. There is also a study that demonstrated higher DNA damage in the liver cells of Swiss albino mice exposed to combined S and cisplatin (used for radiosensitisation) than to the two administered alone (38), which suggests that sevoflurane has no protective effect against irradiation. On the other hand, a combination of cisplatin and isoflurane had a lower genotoxic effect than cisplatin and isoflurane alone or halothane alone or combined with cisplatin (10).

Respiration is the pathway that eliminates most VAs, but a small amount is metabolised by the liver and then excreted by the kidneys. Of the three VAs investigated in this study, halothane's liver metabolism is 15–20 %, sevoflurane's around 5 %, and isoflurane's up to 0.2 % (39–41). The presence of halothane in the liver this high can also be responsible for its damaging effects. The use of halothane in humans or animals has become less frequent since we learned about its side effects, mostly about its hepatotoxicity through reactive oxidative species (ROS) (42) and its ability to induce enzymes that metabolise it (43). Taking this into account, our findings for halothane were expected. Earlier studies with halothane, isoflurane, and sevoflurane (7, 11, 14, 36, 37) demonstrated a similar pattern in the formation of DNA damage in other organs such as blood, kidney or brain, with at different time points after *in vivo* exposure, which may be owed to different repair or oxidative mechanisms.

Future studies should include combined VA+IR effects on healthy cells surrounding a repeatedly exposed tumour at IR doses lower than the standard 1 or 2 Gy. In addition, they should include both sexes to establish potential differences in DNA damage and repair. We also need to learn more about the mechanisms and modes of action (using histology, oxidative stress biomarkers, Western blotting) and expression of different proteins and enzymes involved in DNA damage and repair, changes in cell proliferation, and the frequency of apoptotic and necrotic processes in the cell. A better insight would also result from a long-time follow up after a single- and multiple-dose exposure to see if DNA damage is completely repaired or eliminated and to determine the sensitivity of different organs after such exposure.

CONCLUSION

In conclusion, single exposure to VAs alone has led to different DNA damage and repair in the liver cells of male mice. In combination with ionising radiation, sevoflurane and isoflurane had slightly protective effects, mostly at 1 Gy exposure. Our findings are in line with current literature regarding DNA damage and repair in the liver and other organ systems, and suggest that both sevoflurane and isoflurane are suitable for combined treatment with IR, but isoflurane is preferable, where its use is possible.

Conflict of interest

None to declare.

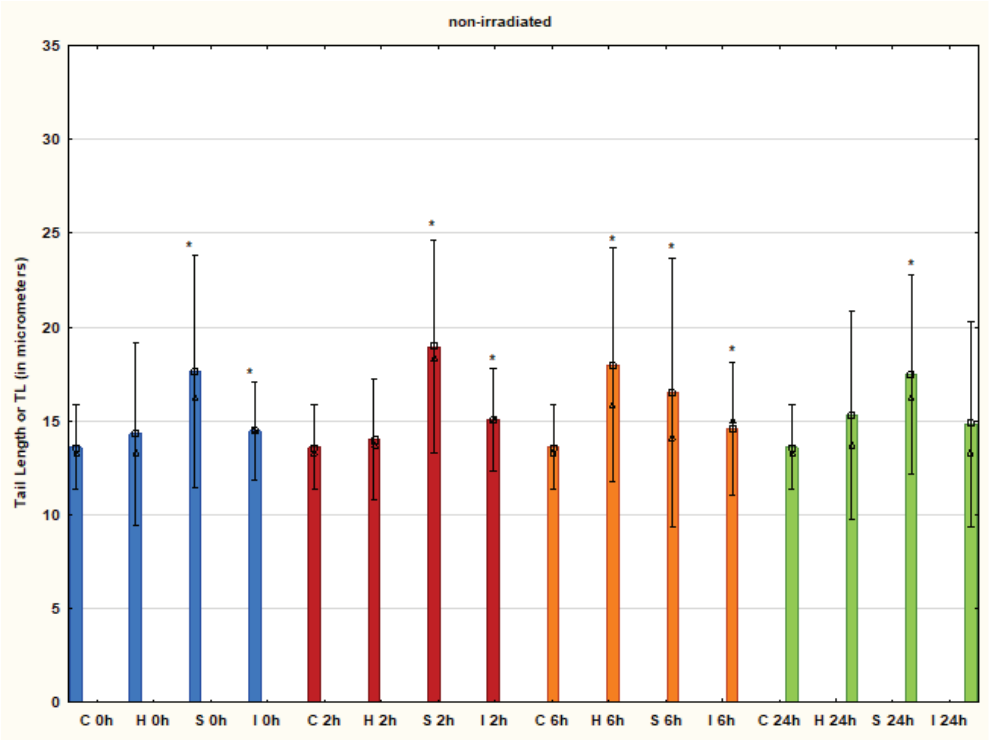

Figure 2a Tail length values in liver cells of non-irradiated male Swiss albino mice anaesthetised solely with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. C-control. Samples were compared to control 0 h (C 0h), statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

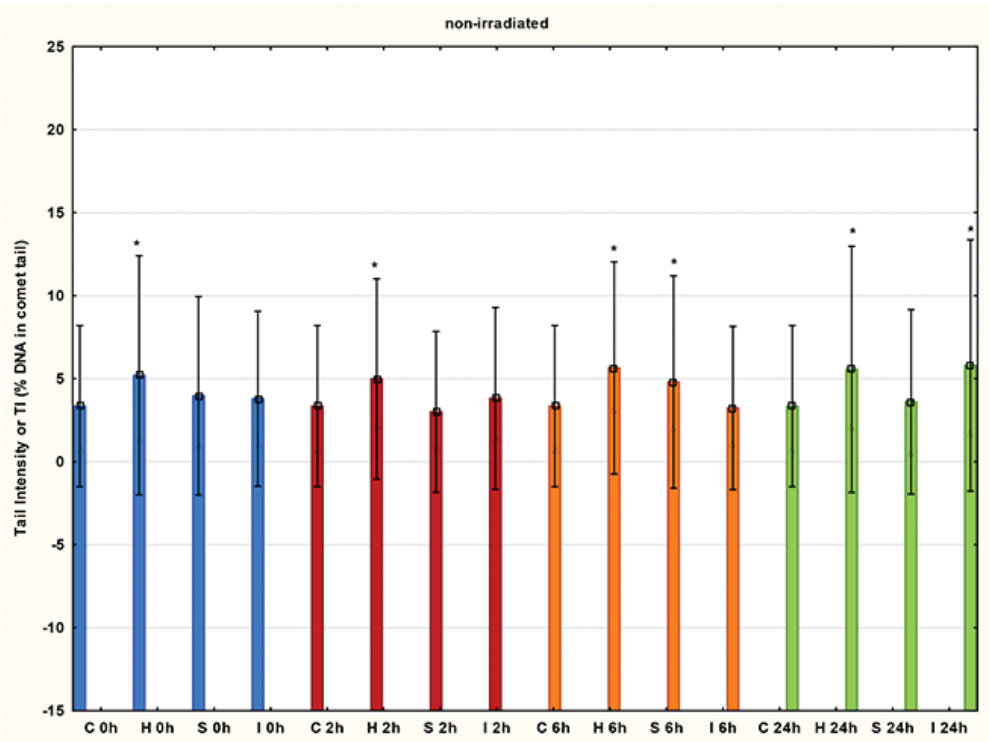

Figure 2b. Tail intensity values in liver cells of non-irradiated male Swiss albino mice anaesthetised solely with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24h after treatment were analysed from five animals per group for 200 comets. C-control. Samples were compared to control 0 h (C 0h), statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

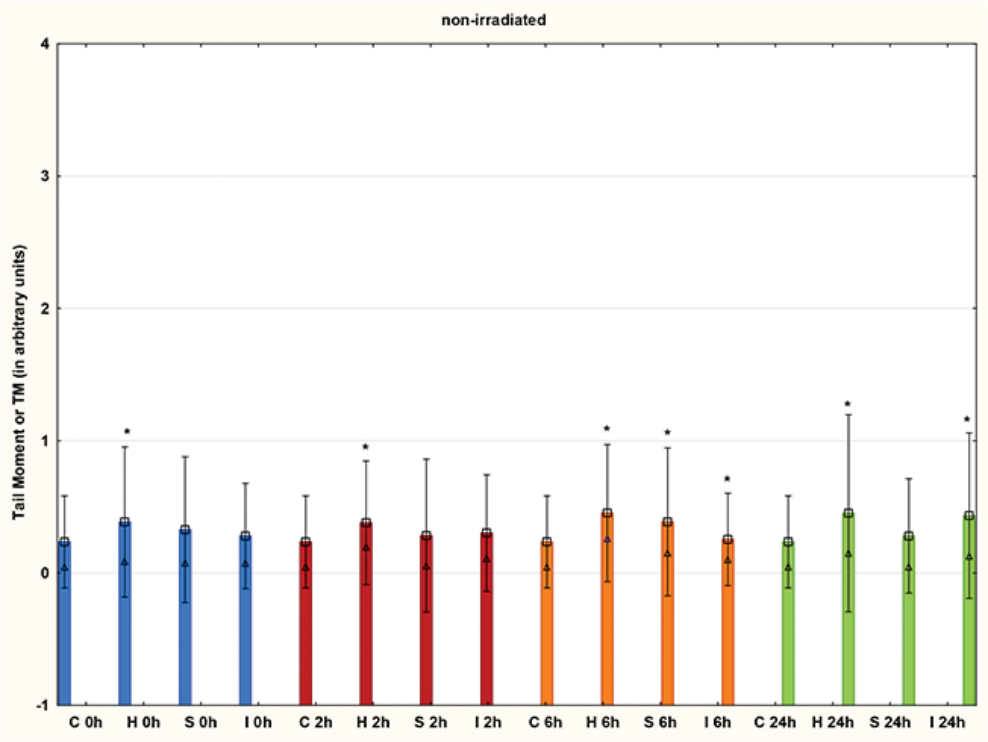

Figure 2c. Tail moment values in liver cells of non-irradiated male Swiss albino mice anaesthetised solely with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. C-control. Samples were compared to control 0 h (C 0h), statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

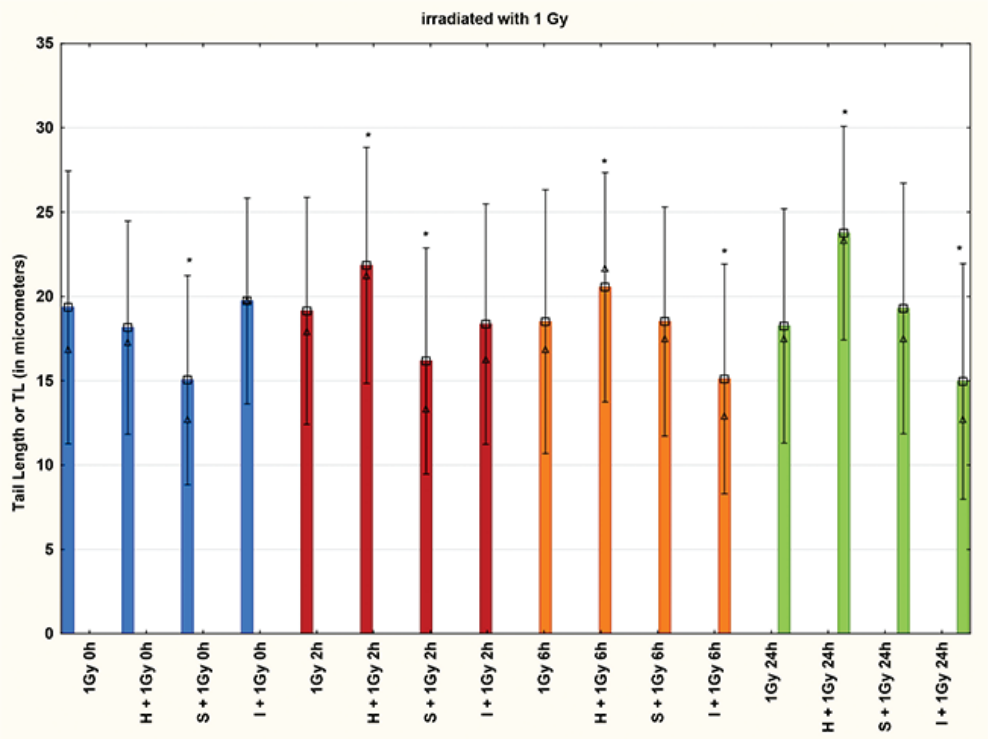

Figure 3a. Tail length values in liver cells of 1 Gy irradiated male Swiss albino mice previously anaesthetised with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. Samples were compared to 1 Gy 0 h, statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

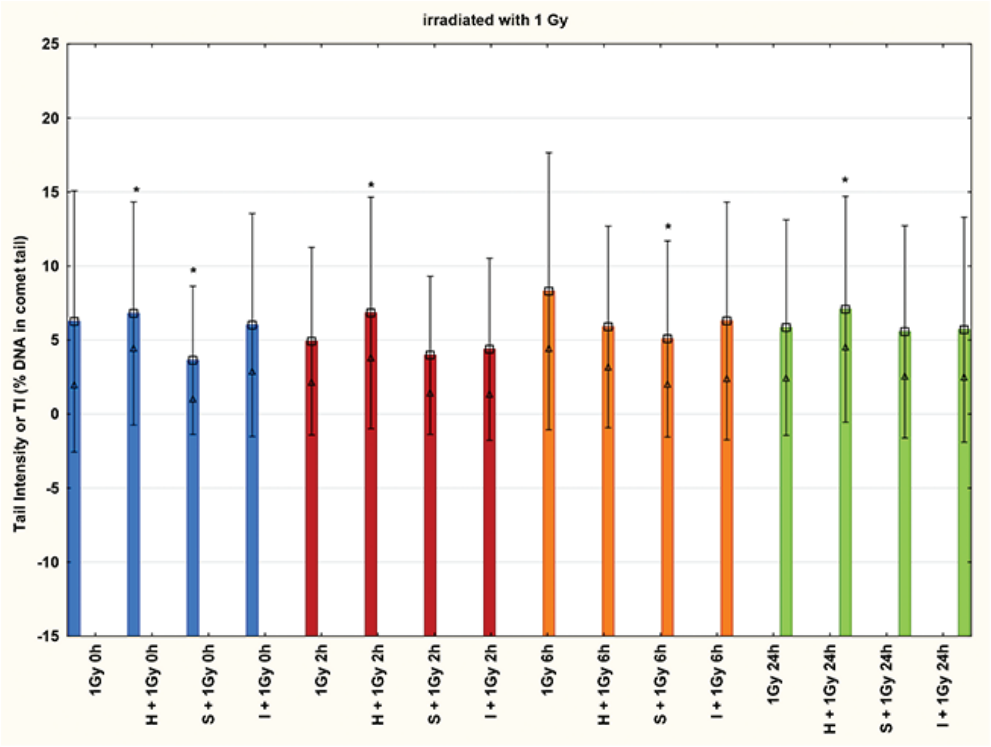

Figure 3b. Tail intensity values in liver cells of 1 Gy irradiated male Swiss albino mice previously anaesthetised with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. Samples were compared to 1 Gy 0 h, statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

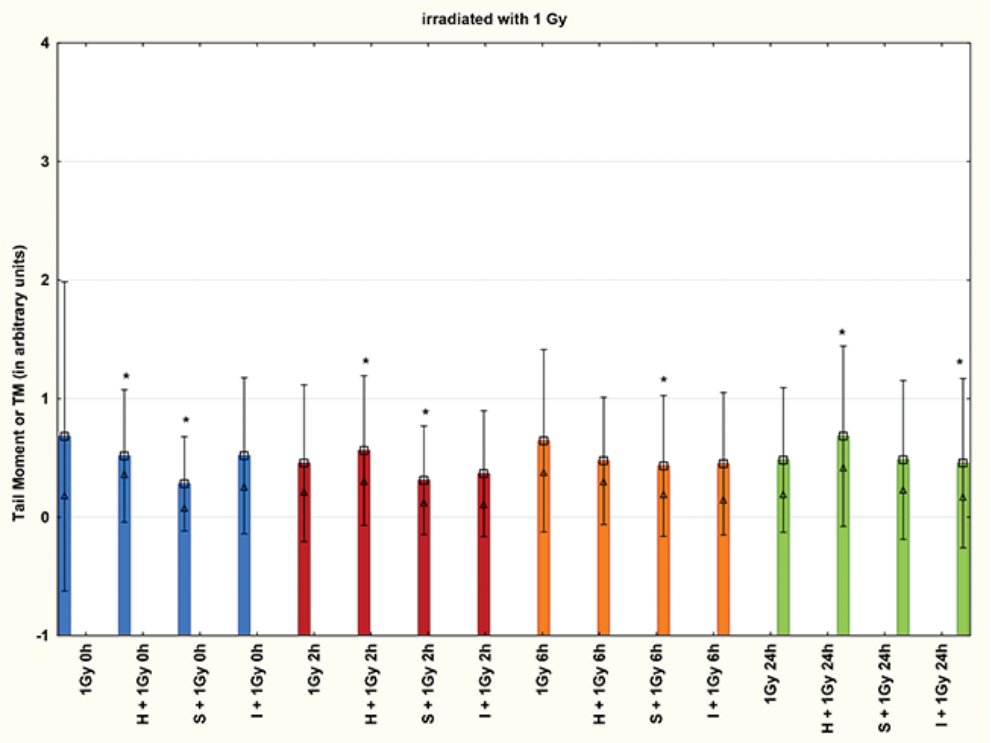

Figure 3c. Tail moment values in liver cells of 1 Gy irradiated male Swiss albino mice previously anaesthetised with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. Samples were compared to 1 Gy 0 h, statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

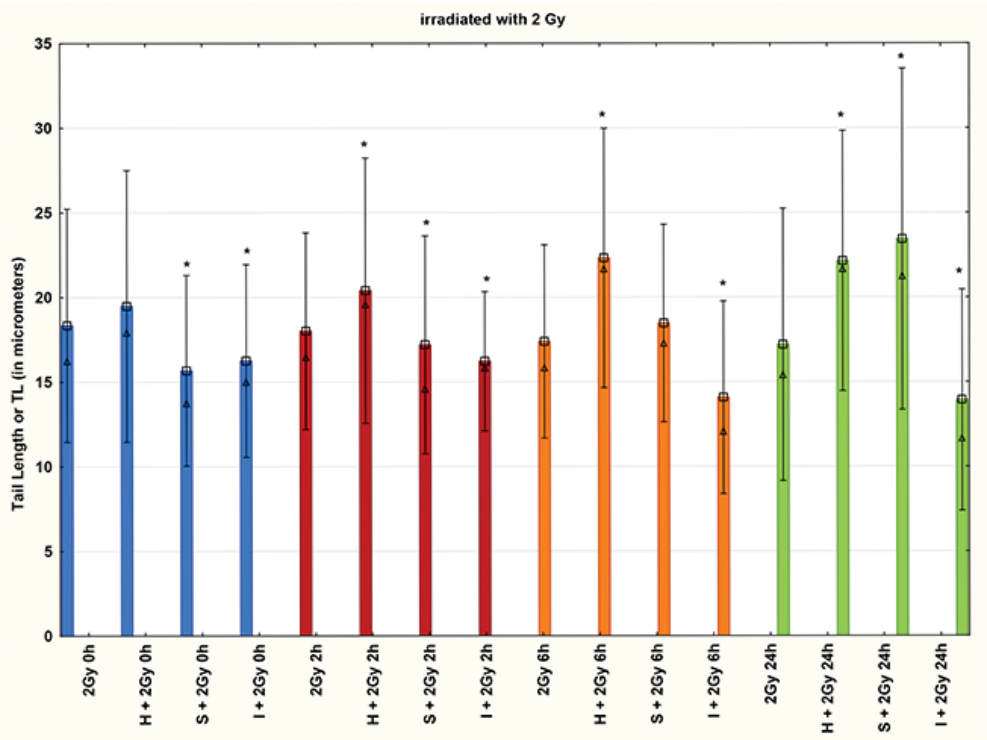

Figure 4a. Tail length values in liver cells of 2 Gy irradiated male Swiss albino mice previously anaesthetised with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. Samples were compared to 2 Gy 0 h, statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range).

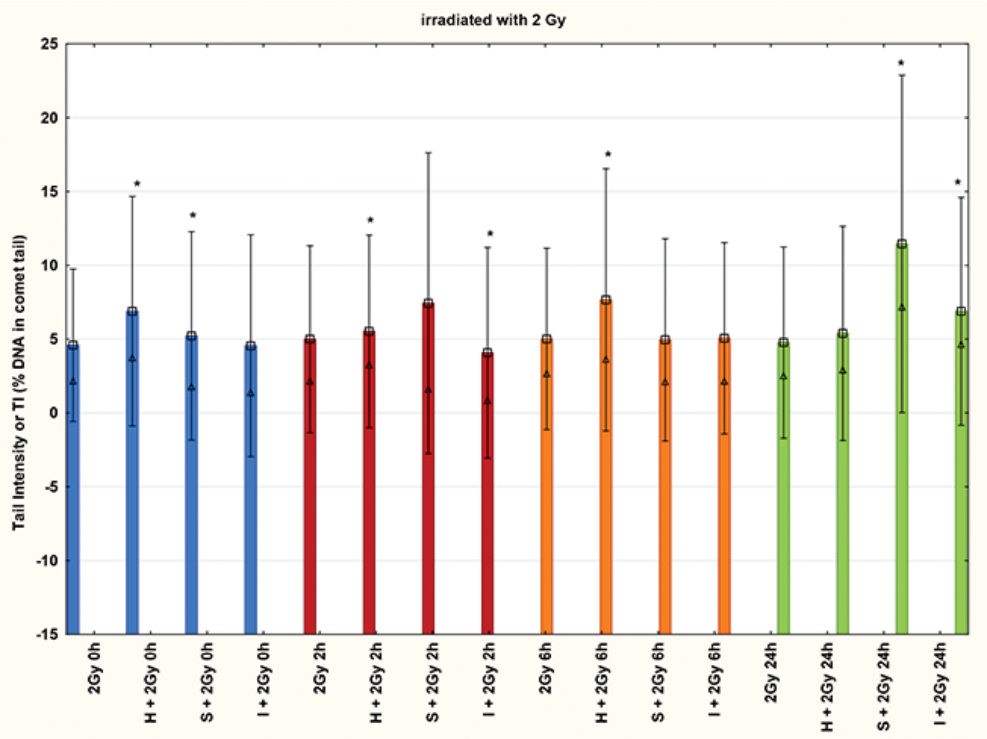

Figure 4b. Tail intensity values in liver cells of 2 Gy irradiated male Swiss albino mice previously anaesthetised with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. Samples were compared to 2 Gy 0 h, statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle), and standard deviation (SD, range)

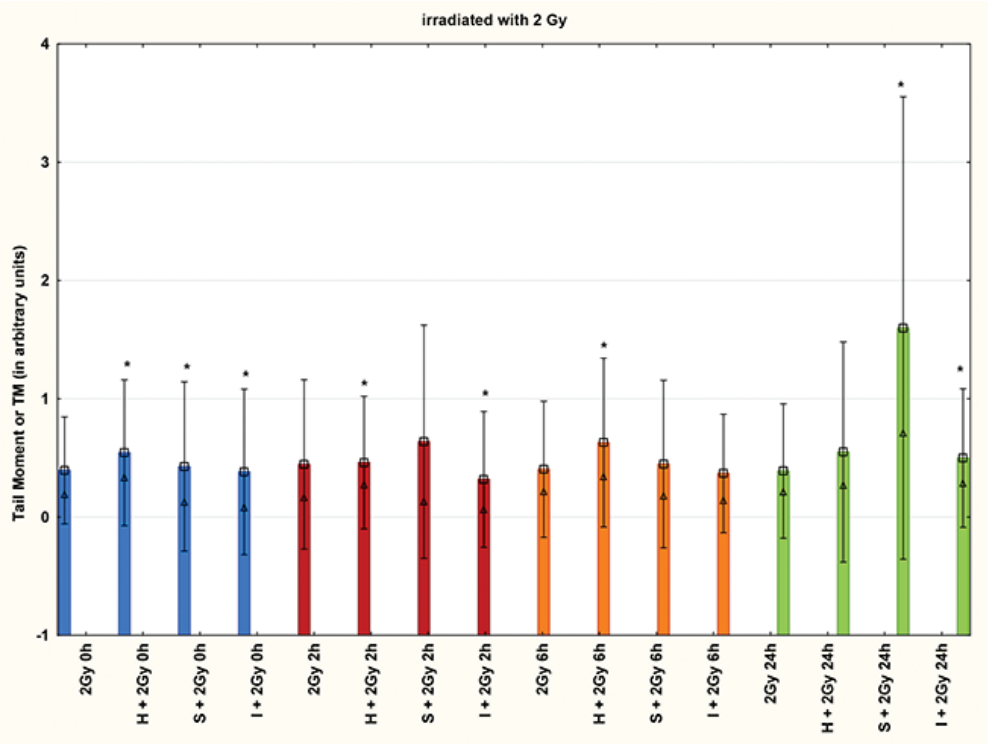

Figure 4c. Tail moment values in liver cells of 2 Gy irradiated male Swiss albino mice previously anaesthetised with halothane (H), sevoflurane (S) or isoflurane (I). Samples taken immediately after (0 h), 2, 6 or 24 h after treatment were analysed from five animals per group for 200 comets. Samples were compared to 2 Gy 0 h, statistical differences (*) were analysed with the Mann-Whitney *U* test. The values represented here are mean (square), median (triangle) and standard deviation (SD, range)

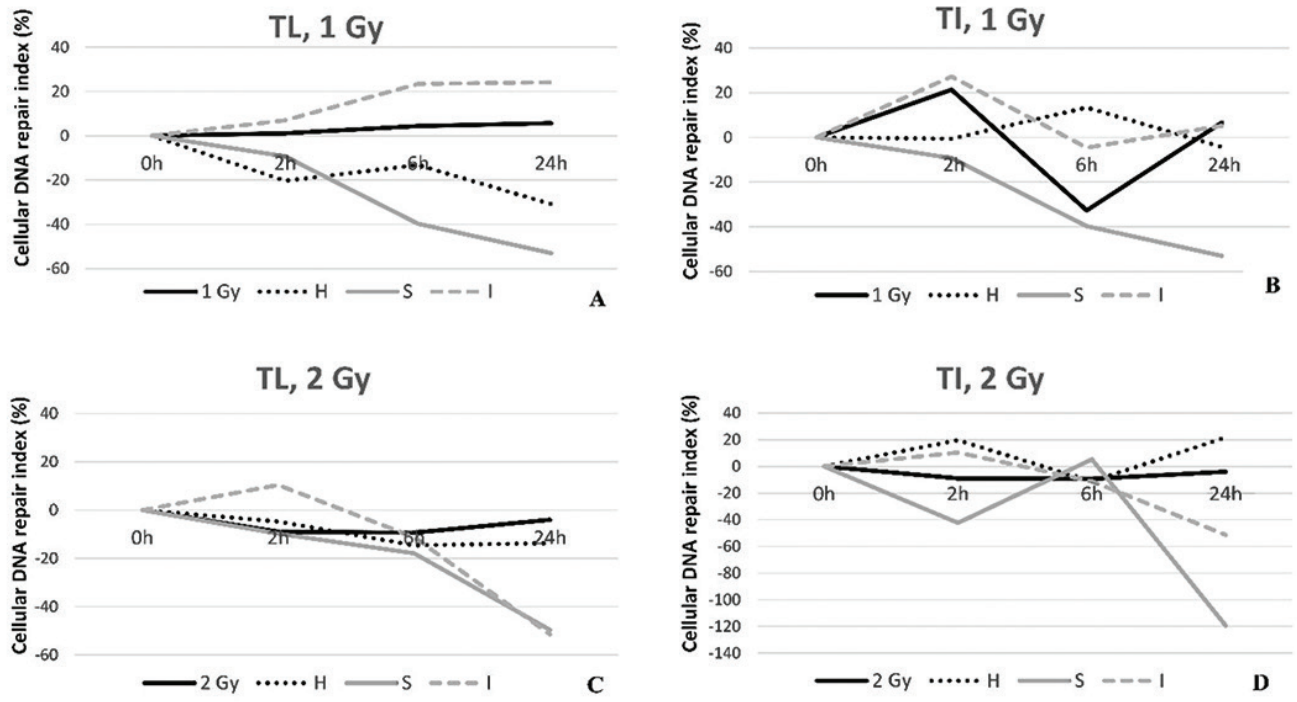

Figure 5 CRI index (percentage of repair) for tail length (TL) and tail intensity (TI) parameter measured in liver cells of mice for 24 hours. 0 h-immediately after, 2 h, 6 h and 24 h after combined exposure to anaesthetics and: 1 Gy (A, B) or 2 Gy (C, D) γ-irradiation (^{60}Co). H-halothane, S-sevoflurane, I-isoflurane

Acknowledgements

This work was supported by the Institute for Medical Research and Occupational Health, Ministry of Science and Education of the Republic of Croatia, and the University of Zagreb.

REFERENCES

1. International Agency for Research on Cancer (IARC). Monographs on the Evaluation of Carcinogenic Risks to Humans. Radiation. Volume 100D, 2012 [displayed 14 August 2022]. Available at https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Radiation-2012
2. The Royal College of Radiologists. Radiology Dose Fractionation. Third edition. London: RCR; 2019 [displayed 14 August 2022]. Available at https://www.rcr.ac.uk/system/files/publication/field_publication_files/brfo193_radiotherapy_dose_fractionation_third-edition.pdf
3. Rafat M, Ali R, Graves EE. Imaging radiation response in tumor and normal tissue. Am J Nucl Med Mol Imaging 2015;5:317–32. PMID: 26269771
4. Arunkumar R, Rebello E, Owusu-Agyemang P. Anesthetic techniques for unique cancer surgery procedures. Best Pract Res Clin Anaesthesiol 2013;27:513–26. doi: 10.1016/j.bpa.2013.09.002
5. Ntoukas SM, Ritchie T, Awrey S, Hodgson DC, Laperriere N, Yee R, Ramaswamy V, Millar BA, Becker N, Tsang DS. Minimizing general anesthetic use in pediatric radiation therapy. Pract Radiat Oncol 2020;10:e159–65. doi: 10.1016/j.prro.2019.12.001
6. Campagna JA, Miller KW, Forman SA. Mechanisms of actions of inhaled anesthetics. N Engl J Med 2003;348:2110–24. doi: 10.1056/NEJMra021261
7. Brozovic G, Orsolic N, Rozgaj R, Kasuba V, Knezevic F, Horvat Knezevic A, Benkovic V, Lisicic D, Borojevic N, Dikic D. DNA damage and repair after exposure to sevoflurane *in vivo*, evaluated in Swiss albino mice by the alkaline comet assay and micronucleus test. J Appl Genet 2010;51:79–86. doi: 10.1007/BF03195714
8. Chiao S, Zuo Z. A double-edged sword: volatile anesthetic effects on the neonatal brain. Brain Sci 2014;4:273–94. doi: 10.3390/brainsci4020273
9. Yılmaz S, Çalbayram NÇ. Exposure to anesthetic gases among operating room personnel and risk of genotoxicity: A systematic review of the human biomonitoring studies. J Clin Anesth 2016;35:326–31. doi: 10.1016/j.jclinane.2016.08.029
10. Brozovic G, Orsolic N, Knezevic F, Horvat Knezevic A, Benkovic V, Sakic K, Borojevic N, Dikic D. The *in vivo* genotoxicity of cisplatin, isoflurane and halothane evaluated by alkaline comet assay in Swiss albino mice. J Appl Genet 2011;52:355–61. doi: 10.1007/s13353-011-0046-0
11. Brozović G, Oršolić N, Rozgaj R, Knežević F, Horvat Knežević A, Maričić M, Krsnik D, Benković V. Sevoflurane and isoflurane genotoxicity in kidney cells of mice. Arh Hig Rada Toksikol 2017;68:228–35. doi: 10.1515/aiht-2017-68-2941
12. Milić M, Rozgaj R, Kašuba V, Jazbec AM, Hrelia P, Angelini S. The influence of individual genome sensitivity in DNA damage repair assessment in chronic professional exposure to low doses of ionizing radiation. In: Chen CC, editor. Selected Topics in DNA Repair. IntechOpen, Croatia 2011 [displayed 14 July 2022]. Available at https:// https://www.intechopen.com/chapters/22725. doi: 10.5772/20814
13. Alcaraz M, Acevedo C, Castillo J, Benavente-Garcia O, Armero D, Vicente V, Canteras M. Liposoluble antioxidants provide an effective radioprotective barrier. Br J Radiol 2009;82:605–9. doi: 10.1259/bjr/30930369
14. Benković V, Borojević N, Šikić D, Horvat Knežević A, Milić M. DNA damage assessment in peripheral blood of Swiss albino mice after combined exposure to volatile anesthetics and 1 or 2 Gy radiotherapy *in vivo*. Int J Radiat Biol 2021;97:1425–35. doi: 10.1080/09553002.2021.1962565
15. Zakon o zaštiti životinja [Animal protection act, in Croatian]. Narodne novine 102/2017.
16. Pravilnik o zaštiti životinja koje se koriste u znanstvene svrhe [Act on protection of animals used for scientific purposes, in Croatian]. Narodne novine 55/2013.
17. Pravilnik o izmjenama pravilnika o zaštiti životinja koje se koriste u znanstvene svrhe [Act on amendments to the Act on protection of animals used for scientific purposes, in Croatian]. Narodne novine 39/2017.
18. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes [displayed 7 March 2023]. Available at https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF
19. Narendran N, Luzhna L, Kovalchuk O. Sex difference of radiation response in occupational and accidental exposure. Front Genet 2019;10:260. doi: 10.3389/fgene.2019.00260
20. Collins A, Milic M, Bonassi S, Dusinska M. The comet assay in human biomonitoring: Technical and epidemiological perspectives. Mutat Res Genet Toxicol Environ Mutagen 2019;843:1–2. doi: 10.1016/j.mrgentox.2019.06.002
21. Xiao C, He N, Liu Y, Wang Y, Liu Q. Research progress on biodosimeters of ionizing radiation damage. Rad Med Protec 2020;1:127–32. doi: 10.1016/j.radmp.2020.06.002
22. Milić M, Ceppi M, Bruzzone M, Azqueta A, Brunborg G, Godschalk R, Koppen G, Langie S, Møller P, Teixeira JP, Alija A, Anderson D, Andrade V, Andreoli C, Asllani F, Bangkoglu EE, Barančoková M, Basaran N, Boutet-Robinet E, Buschini A, Cavallo D, Costa Pereira C, Costa C, Costa S, Da Silva J, Del Bo' C, Dimitrijević Srećković V, Djelić N, Dobrzyńska M, Duračková Z, Dvořáková M, Gajski G, Galati S, García Lima O, Giovannelli L, Goroshinskaya IA, Grindel A, Gutzkow KB, Hernández A, Hernández C, Holven KB, Ibero-Baraibar I, Ot-testad I, Kadioglu E, Kažimirová A, Kuznetsova E, Ladeira C, Laffon B, Lamonaca P, Lebailly P, Louro H, Mandina Cardoso T, Marcon F, Marcos R, Moretti M, Moretti S, Najafzadeh M, Nemeth Z, Neri M, Novotna B, Orlow I, Paduchova Z, Pastor S, Perdry H, Spremo-Potparević B, Ramadhani D, Riso P, Rohr P, Rojas E, Rossner P, Safar A, Sardas S, Silva MJ, Sirota N, Smolkova B, Staruchova M, Stetina R, Stopper H, Surikova EI, Ulven SM, Ursini CL, Valdiglesias V, Valverde M, Vodicka P, Volkovova K, Wagner KH, Živković L, Dušinská M, Collins AR, Bonassi S. The hCOMET project: International database comparison of results with the comet assay in human biomonitoring. Baseline frequency of DNA damage and effect of main confounders. Mutat Res Rev Mutat Res 2021;787:108371. doi: 10.1016/j.mrrev.2021.108371

23. OECD. Test No. 489: *In Vivo* Mammalian Alkaline Comet Assay, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD; 2016 [displayed 14 July 2022]. Available at https://doi.org/10.1787/9789264264885-en
24. Møller P, Azqueta A, Boutet-Robinet E, Koppen G, Bonassi S, Milić M, Gajski G, Costa S, Teixeira JP, Costa Pereira C, Dusinska M, Godschalk R, Brunborg G, Gutzkow KB, Giovannelli L, Cooke MS, Richling E, Laffon B, Valdiglesias V, Basaran N, Del Bo' C, Zegura B, Novak M, Stopper H, Vodicka P, Vodenkova S, de Andrade VM, Sramkova M, Gabelova A, Collins A, Langie SAS. Minimum Information for Reporting on the Comet Assay (MIRCA): recommendations for describing comet assay procedures and results. Nat Protoc 2020;15:3817–26. doi: 10.1038/s41596-020-0398-1
25. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 2004;26:249–61. doi: 10.1385/MB:26:3:249
26. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91. doi: 10.1016/0014-4827(88)90265-0
27. Nair GG, Nair CKK. Protection of cellular DNA and membrane from γ-radiation-induced damages and enhancement in DNA repair by sesamol. Cancer Biother Radiopharm 2010;25:629–35. doi: 10.1089/cbr.2010.0803
28. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen 2017;58:235–63. doi: 10.1002/em.22087
29. Van Allen NR, Krafft PR, Leitzke AS, Applegate RL 2nd, Tang J, Zhang JH. The role of volatile anesthetics in cardioprotection: a systematic review. Med Gas Res 2012:2(1):22. doi: 10.1186/2045-9912-2-22
30. Kharasch ED, Frink Jr EJ, Zager R, Bowdle TA, Artru A, Nogami WM. Assessment of low-flow sevoflurane and isoflurane effects on renal function using sensitive markers of tubular toxicity. Anesthesiology 1997;86:1238–53. doi: 10.1097/00000542-199706000-00004
31. Braz MG, Mazzoti MÁ, Giacobino J, Braz LG, de Assis Golim M, Ferrasi AC, de Carvalho LR, Braz JR, Salvadori DM. Genotoxicity, cytotoxicity and gene expression in patients undergoing elective surgery under isoflurane anesthesia. Mutagenesis 2011;26:415–20. doi: 10.1093/mutage/geq109
32. Braz MG, Braz JG, Braz JR, Pierine DT, Correa CR, Ferreira AL, Carvalho LR, Yeum KJ, Salvadori DM. Comparison of oxidative stress in ASA physical status I patients scheduled for minimally invasive surgery under balanced or intravenous anesthesia. Minerva Anestesiol 2013;79:1030–8. PMID: 23598734
33. Rocha TL, Dias-Junior CA, Possomato-Vieira JS, Gonçalves-Rizzi VH, Nogueira FR, de Souza KM, Braz LG, Braz MG. Sevoflurane induces DNA damage whereas isoflurane leads to higher antioxidative status in anesthetized rats. Biomed Res Int 2015;2015:264971. doi: 10.1155/2015/264971
34. Hoetzel A, Schmidt R. Regulatory role of anesthetics on heme oxygenase-1. Curr Drug Targets 2010;11:1495–503. doi: 10.2174/1389450111009011495
35. Karabiyik L, Sardaş S, Polat U, KocabaS NA, Karakaya AE. Comparison of genotoxicity of sevoflurane and isoflurane in human lymphocytes studied *in vivo* using the comet assay. Mutat Res 2001;492:99–107. doi: 10.1016/s1383-5718(01)00159-0
36. Benković V, Oršolić N, Knežević AH, Borojević N, Brozović G, Milić M. Kidney cell DNA damage caused by combined exposure to volatile anesthetics and 1 Gy or 2 Gy radiotherapy dose *in vivo*. Arh Hig Rada Toksikol 2022;73:62–70. doi: 10.2478/aiht-2022-73-3600
37. Benković V, Milić M, Oršolić N, Horvat Knežević A, Brozović G, Borojević N. Brain DNA damaging effects of volatile anesthetics, 1 and 2 Gy gamma-irradiation *in vivo*: Preliminary results. Toxicol Ind Health 2023;39:67–80. doi: 10.1177/0748233722114559
38. Brozovic G, Orsolic N, Knezevic F, Horvat Knezevic A, Benkovic V, Vrdoljak DV, Saric A. Evaluation of DNA damage *in vivo* induced by combined application of cisplatin and sevoflurane. Eur J Anaesthesiol 2008;25:642–7. doi: 10.1017/S0265021508004171
39. Davis PJ, Bosenberg A, Davidson A, Jimenez N, Kharasch E, Lynn AM, Tofovic SP, Woelfel S. Pharmacology of pediatric anesthesia. In: Davis PJ, Cladis FP, Motoyama EK, editors. Smith's Anesthesia for Infants and Children. 8th ed. Elsevier, Mosby 2011. p. 179–61.
40. Edgington TL, Muco E, Maani CV. Sevoflurane. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Updated 2022 Jun 19 [displayed 14 July 2022]. Available at https://www.ncbi.nlm.nih.gov/books/NBK534781
41. Martin Jr. JL, Njoku DB. Metabolism and toxicity of modern inhaled anesthetics. In: Miller´s anesthesia. 6th ed. Philadelphia: Elsevier; 2005. p. 231–72.
42. Tanko B, Molnar L, Fuksedi B, Molnar C. Occupational hazards of halogenated volatile anesthetics and their prevention: review of the literature. J Anesth Clin Res 2014;5:7. doi: 10.4172/2155-6148.1000426
43. Gyorfi MJ, Kim PY. Halothane Toxicity. Treasure Island (FL): StatPearls Publishing; 2022 [displayed 14 July 2022]. Available at https://www.ncbi.nlm.nih.gov/books/NBK545281/
44. Alleva R, Tomasetti M, Solenghi MD, Stagni F, Gamberini F, Bassi A, Fornasari PM, Fanelli G, Borghi B. Lymphocyte DNA damage precedes DNA repair or cell death after orthopedic surgery under general anaesthesia. Mutagenesis 2003;18:423–8. doi: 10.1093/mutage/geg013
45. Kadioglu E, Sardas S, Erturk S, Ozatamer O, Karakaya AE. Determination of DNA damage by alkaline halo and comet assay in patients under sevoflurane anesthesia. Toxicol Ind Health 2009;25:205–12. doi: 10.1177/0748233709106445
46. Szyfter K, Szulc R, Mikstacki A, Stachecki I, Rydzanicz M, Jaloszyński P. Genotoxicity of inhalation anaesthetics: DNA lesions generated by sevoflurane *in vitro* and *in vivo*. J Appl Genet 2004;45:369–74. PMID: 15306730
47. Wiesner G, Schiewe-Langgartner F, Lindner R, Gruber M. Increased formation of sister chromatid exchanges, but not of micronuclei, in anaesthetists exposed to low levels of sevoflurane. Anaesthesia 2008;63:861–4. doi: 10.1111/j.1365-2044.2008.05498.x
48. Braz MG, Braz LG, Barbosa BS, Giacobino J, Orosz JE, Salvadori DM, Braz JR. DNA damage in patients who underwent minimally invasive surgery under inhalation or intravenous anesthesia. Mutat Res 2011;726:251–4. doi: 10.1016/j.mrgentox.2011.09.007
49. Pokhrel LR, Grady KD. Risk assessment of occupational exposure to anesthesia Isoflurane in the hospital and veterinary settings. Sci Total Environ 2021:783:146894. doi: 10.1016/j.scitotenv.2021.146894

Različiti učinci samih hlapljivih anestetika ili u kombinaciji s gama-zračenjem od 1 i 2 Gy *in vivo* na DNA mišje jetre: preliminarno istraživanje

Kako se broj radioterapijskih i radioloških dijagnostičkih postupaka iz godine u godinu povećava, tako raste i primjena hlapljivih anestetika za opću anesteziju. Iako se smatralo sigurnim, izlaganje hlapljivim anesteticima može izazvati različite štetne učinke, a u kombinaciji s ionizirajućim zračenjem može izazvati i sinergijske učinke. Međutim, malo se zna o oštećenju DNA koje uzrokuje ova kombinacija u dozama primijenjenima u jednom izlaganju u radioterapiji. Kako bismo saznali više o tome, alkalnim komet-testom analizirali smo oštećenje DNA i odgovor na popravak u jetrenom tkivu muških Swiss albino miševa nakon izlaganja samo izofluranu, sevofluranu ili halotanu, odnosno u kombinaciji sa zračenjem od 1 ili 2 Gy. Uzorci su uzeti odmah (0 h) te 2, 6 i 24 sata nakon izlaganja. U usporedbi s kontrolom, najveća oštećenja DNA utvrđena su u miševa koji su primili halotan, sam ili u kombinaciji sa zračenjem od 1 ili 2 Gy. Sevofluran i izofluran pokazali su zaštitne učinke nakon izlaganja zračenju od 1 Gy, a pri 2 Gy prve nuspojave pojavile su se 24 sata nakon izlaganja. Iako učinci hlapljivih anestetika ovise o metabolizmu jetre, otkrivanje nepopravljenog oštećenja DNA 24 sata nakon kombinirane izloženosti sa zračenjem od 2 Gy upućuje na to da trebamo nastaviti istraživati kombinirane učinke hlapljivih anestetika i ionizirajućega zračenja na stabilnost genoma i obuhvatiti šire razdoblje nakon jednokratne izloženosti (duže od 24 sata). Također treba obuhvatiti višekratna izlaganja kao realističniji scenarij u liječenju radioterapijom.

KLJUČNE RIJEČI: alkalni komet test; halotan; ionizirajuće zračenje; izofluran; sevofluran