Article

Toxicology and Industrial Health

Toxicology and Industrial Health
2023, Vol. 0(0) 1–14

DOI: 10.1177/07482337221145599
journals.sagepub.com/home/tih
SAGE

Brain DNA damaging effects of volatile anesthetics and 1 and 2 Gy gamma irradiation *in vivo*: Preliminary results

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

Abstract

Although both can cause DNA damage, the combined impact of volatile anesthetics halothane/sevoflurane/isoflurane and radiotherapeutic exposure on sensitive brain cells *in vivo* has not been previously analyzed. Healthy Swiss albino male mice (240 in total, 48 groups) were exposed to either halothane/sevoflurane/isoflurane therapeutic doses alone (2 h); 1 or 2 gray of gamma radiation alone; or combined exposure. Frontal lobe brain samples from five animals were taken immediately and 2, 6, and 24 h after exposure. DNA damage and cellular repair index were analyzed using the alkaline comet assay and the tail intensity parameter. Elevated tail intensity levels for sevoflurane/halothane were the highest at 6 h and returned to baseline within 24 h for sevoflurane, but not for halothane, while isoflurane treatment caused lower tail intensity than control values. Combined exposure demonstrated a slightly halothane/sevoflurane protective and isoflurane protective effect, which was stronger for 2 than for 1 gray. Cellular repair indices and tail intensity histograms indicated different modes of action in DNA damage creation. Isoflurane/sevoflurane/halothane preconditioning demonstrated protective effects in sensitive brain cells *in vivo*. Owing to the constant increases in the combined use of radiotherapy and volatile anesthetics, further studies should explore the mechanisms behind these effects, including longer and multiple exposure treatments and *in vivo* brain tumor models.

Keywords

cellular DNA repair index, DNA damage, ionizing radiation, volatile anesthetics, tail intensity, protective effect

Received 21 April 2022; Revised 10 November 2022; Accepted 25 November 2022

Introduction

The American Cancer Society listed brain tumors as one of the top 10 causes of tumor-related deaths, with a 26% and 21% distribution in the 0–14 and 15–19 age categories, respectively, in the USA in 2012–2016 (Rasheed et al., 2021). Among the risk factors for brain tumors is ionizing radiation (IR), a well-known carcinogen classified into Group 1 (IARC, 2012; Rasheed et al., 2021). IR is also used in radiotherapy (RT) as the gold standard for brain tumor treatments (Wujanto et al., 2021), usually at a 2 or 1 gray (Gy) dose (TRCR, 2019). Recent studies revealed also that the prefrontal cortex and hippocampus are the most radiation-sensitive brain regions (Baluchamy et al., 2012; Kovalchuk and Kolb, 2017; Loganovsky and Yuryev, 2004; Makale et al., 2017).

[1]Faculty of Science, University of Zagreb, Zagreb, Croatia
[2]Mutagenesis Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia
[3]Department of Anesthesiology, Reanimatology and ICU, University Hospital for Tumors, Sestre Milosrdnice University Hospital Centre, Zagreb, Croatia
[4]Faculty of Dental Medicine and Health, University of Osijek, Osijek, Croatia
[5]Warrington and Halton Teaching Hospitals NHS Foundation Trust, Warrington, UK

Vesna Benković and Mirta Milić equal contribution.

Corresponding author:
Mirta Milić, Mutagenesis Unit, Institute for Medical Research and Occupational Health, Ksaverska cesta 1, Zagreb 10 001, Croatia.
Email: mmilic@imi.hr

IR exposure should be kept to a minimum when combined with general anesthesia (such as volatile anesthetics or VAs), which can be beneficial in lowering the necessary number of doses, providing fast sedation, and maintaining stable conditions in patients (Borras et al., 2018; Brozović et al., 2009, 2010, 2011, 2017; IARC 2012; TRCR 2019). There is a yearly increase in VA-RT (also in VA-surgery, VA-immediate radiation, VA-intraoperative-RT, or VA-brachytherapy) (Arunkumar et al., 2013; McMullen et al., 2015; Ntoukas et al., 2020) in both children and adults with anxiety issues, usually associated with the required use of masks during radiation/simulation, long waiting period during the procedure in a cold dark room, or claustrophobia. Isoflurane (I), sevoflurane (S), and halothane (H), as the most frequently used VAs, have similar mechanisms of action: enhancing the inhibitory activity of postsynaptic channels (gamma-aminobutyric acid (GABA) and glycine) and inhibiting the excitatory activity of synaptic channels (glutamate, N-methyl-D-aspartate (NMDA), nicotinic acetylcholine, and serotonin) in the central nervous system (Campagna et al., 2003). Although VA use is considered safe, increasing evidence in recent decades about VAs' damaging effects in *in vitro* and *in vivo* studies conducted in neonatal animals, and also in occupational personnel/patients, has raised concerns about VA + IR safety (Brozović et al., 2009, 2010, 2011, 2017; IARC, 2012). *In vitro* VA can change protein and gene expression, leading to inflammation and apoptosis; and preclinical rodent and non-human primate studies pointed out the possibility of neurodegeneration with possible cognitive sequelae or the induction of brain plasticity together with long-term cellular and molecular changes leading to behavioral and/or cognitive consequences in neonates, children, or adults even after a month from the initial exposure (Andropoulos, 2018; De Hert and Moerman, 2015; Drobish et al., 2016; Stenroos et al., 2021). VAs can also increase blood–brain barrier permeability (Tétrault et al., 2008) thus leaving the brain tissue exposed to peripheral inflammatory responses. VA adult exposure also affects their offsprings' brain development (Ju et al., 2019).

There are only 2 studies on the combined VA + IR effects (Benković et al., 2021, 2022), and none were performed on the brain, so we wanted to further investigate their effects on DNA damage in the IR-sensitive brain region. As the dynamics of DNA damage and repair also depend on the target tissue organ and the time from the exposure, we included multiple time points following exposure.

Methods

Unless otherwise specified, all chemicals and reagents were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Inhalation anesthetics S (Sevorane®), I (Forane®), and H (Halothane®) were provided by Abbott Laboratories LTD (Queenborough, UK).

The study was approved by the Ethics Committee of the Faculty of Science, University of Zagreb (No. 251-58-508-11-9) and was designed in accordance with relevant Croatian guidelines: Animal Protection Act (OG 102/17), the Ordinance on the protection of animals used for scientific purposes (OG 55/13, 39/17), and EU Directive 2010/63/EU (EU, 2010).

Brain proliferation, metabolism, and survival depend also on sex hormones (females more prone to X-chromosome loss and higher DNA damage) (Behl, 2002; Narendran et al., 2019; Schmitz-Feuerhake et al., 2016; Silasi et al., 2004). Drug metabolism, target organ, and hormonal balance also influence VA effects, so adult Swiss male animals were selected (Brozović et al., 2010; Chiao and Zuo, 2014; Yılmaz and Çalbayram, 2016). In this study, we used three VAs at appropriate concentrations for mice anesthesia. These concentrations, also used in previously published studies (Brozović et al., 2009, 2010, 2011, 2017; Benković et al., 2021, 2022), were determined according to the VA oil/gas partition coefficient and minimal alveolar concentration (MAC). Minimal alveolar concentration is the percentage necessary to prevent movement in 50% of patients during skin incision (and in rodents corresponding to the dose where 50% of animals lose a motor response to a noxious stimulus) according to the guidelines for anesthesia and analgesia in laboratory animals (American College of Laboratory Animal Medicine Series, 2008; Navarro et al., 2021). We should also say that the doses used here have the same effects as the doses used in human anesthesia- so the term "corresponding animal doses to human therapy" is the term used in laboratory animal anesthesia guidelines to refer how the doses are chosen in order to get the same effect as in humans (classified Stage 3-Surgical Anesthesia) (Siddiqui and Kim, 2020).

DNA damage and repair dynamics were estimated using the alkaline comet assay, and damage was monitored immediately and 2, 6, and 24 hours (h) after

the exposure (in controls, in only irradiated samples, in only anesthetized mice, in mice exposed to combined anesthetic, and radiation exposure) as in previous studies (Brozović et al., 2010; Milić et al., 2011,2021; Neri et al., 2015; Chiao and Zuo, 2014; OECD-TG 489, 2016).

Mice (Department of Biology, Faculty of Science, University of Zagreb, Croatia; 22 ± 1°C, humidity 50–70%, 12/12 h photoperiod; Standard Laboratory Diet GLP-4RF1-Mucedola, Settimo Milanese MI, Italy; water *ad libitum*) with 20–25 g bodyweight and 60 ± 5 days old randomly divided into 48 groups (n = 5). Except for the control and only-IR groups, VA groups were anesthetized in an induction chamber connected to an anesthetic machine (Sulla 800; Dräger, Velbert, Germany) with a compatible evaporator (S-2.4 vol%, I-1.7 vol%, H-2.4 vol%; in a 50:50 mixture of oxygen and air (3 L/min) in continuous flow for 2h), with acceptable deep anesthesia corresponding to the classified Stage 3–Surgical Anesthesia (Siddiqui and Kim, 2020). Afterward, combined-treatment and only-IR groups were irradiated with 1 or 2 Gy gamma (ɣ)-radiation (^{60}Co source, Theratron Phoenix teletherapy unit, Atomic Energy Ltd., Ontario, Canada; 1.88 Gy/min) (Table 1).

Animals were sacrificed by cervical dislocation according to laboratory animal legislation (OG 55/13, 39/17). Small pieces of freshly resected mouse frontal lobe brain tissue were mechanically homogenized using fresh chilled homogenization buffer (0.075 M NaCl, 0.024 M Na_2EDTA, 4°C), with the ratio 1 g tissue/1 mL buffer. For each animal and sample, a 10 µL single-cell suspension homogenate at 4°C immersed immediately into agarose gel was put on a microscopic slide and a comet assay was carried out under alkaline standardized conditions (Brozović et al., 2017; Benković et al., 2022). Sample gels passed through 2 h lysis, 20 min denaturation, 20 min electrophoresis (0.8 V/cm), neutralization, and ethidium bromide staining (20 µg/mL, 10 min). In each group, 200 comets were examined by an epifluorescence microscope (B×40, Olympus, Tokyo, Japan, ×200 magnification) with a CCD camera connected to a computer-based image analysis system (Comet Assay IV software, Instem, London, UK). The tail intensity (TI) parameter was determined as linearly related to the DNA break frequency over a wide range of damaged DNA (OECD-TG 489, 2016), defined as % of migrated genomic DNA in the comet tail.

For cell repair efficiency and possible influence on faster/slower/delayed repair, a cellular DNA repair index (CRI) defined as % decrease from the initial value of the parameter due to repair was calculated from the TI parameter medians according to the formula of Nair and Nair (2010).

$$\mathrm{CRI} = \left[1 - \left(\frac{TI\ at\ time\ \mathrm{t}}{TI\ at\ initial\ time\ t0}\right)\right] x100.$$

In statistical analysis (Statistica 13.5.0.17 (TIBCO Software Inc., Palo Alto, CA, USA), groups were compared by the non-parametric Kruskal–Wallis test,

Table 1. Design of the experiment. A total of 240 male Swiss albino mice were divided in 48 groups of 5 animals each. The samples of mouse brain were taken at time periods: 0 h, 2 h, 6 h and 24 h from the exposure for alkaline comet assay. Totally 200 comets (40 comets per animal) from each tested group were analysed.

	Control	Halothane (2.4% vol.)	Sevoflurane (2.4% vol.)	Isoflurane (1.7% vol.)
		Non-Irradiated		
Time points	0 h (n = 5)	0 h (n = 5)	0 h (n = 5)	0 h (n = 5)
	2 h (n = 5)	2 h (n = 5)	2 h (n = 5)	2 h (n = 5)
	6 h (n = 5)	6 h (n = 5)	6 h (n = 5)	6 h (n = 5)
	24 h (n = 5)	24 h (n = 5)	24 h (n = 5)	24 h (n = 5)
		Irradiated 1 Gy		
Time points	0 h (n = 5)	0 h (n = 5)	0 h (n = 5)	0 h (n = 5)
	2 h (n = 5)	2 h (n = 5)	2 h (n = 5)	2 h (n = 5)
	6 h (n = 5)	6 h (n = 5)	6 h (n = 5)	6 h (n = 5)
	24 h (n = 5)	24 h (n = 5)	24 h (n = 5)	24 h (n = 5)
		Irradiated 2 Gy		
Time points	0 h (n = 5)	0 h (n = 5)	0 h (n = 5)	0 h (n = 5)
	2 h (n = 5)	2 h (n = 5)	2 h (n = 5)	2 h (n = 5)
	6 h (n = 5)	6 h (n = 5)	6 h (n = 5)	6 h (n = 5)
	24 h (n = 5)	24 h (n = 5)	24 h (n = 5)	24 h (n = 5)

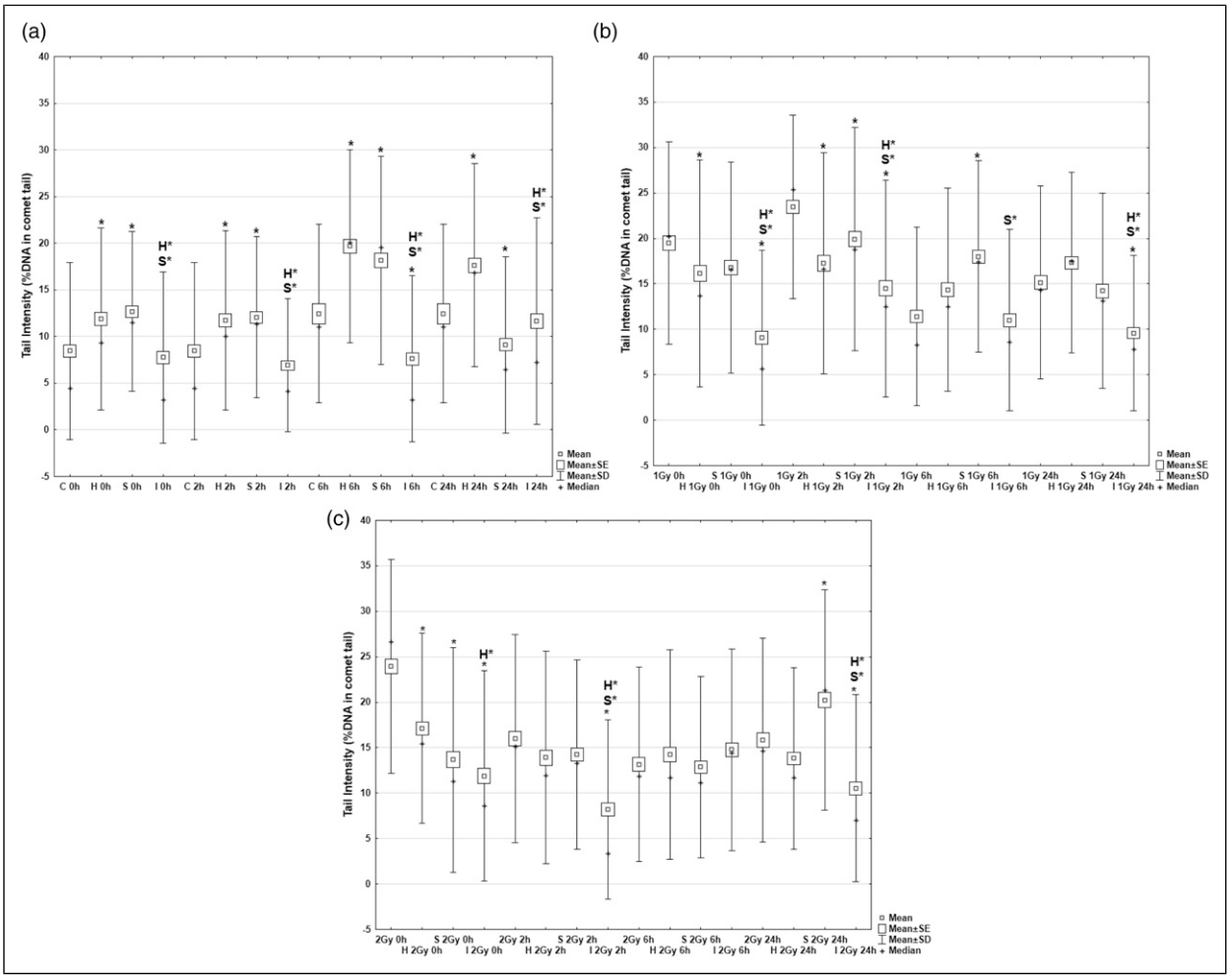

Figure 1. (a) Tail intensity values of brain cells of non-irradiated mice: control samples (non-exposed) (C); after only halothane (H)/sevoflurane (S)/isoflurane (I) exposure. Data demonstrate values for mean, median, standard error (SE), and standard deviation (SD). Samples were taken immediately after exposure (0 h) and 2 h, 6 h, and 24 h from the exposure. *-p values represent significant differences from C, $p \leq 0.05$; H* and S* letters also indicate significant H and S difference from I sample. (b) Tail intensity values of brain cells of 1 Gy-irradiated mice: control samples (1 Gy); after VA+1 Gy combined exposure to halothane (H)/sevoflurane (S)/isoflurane (I) exposure. Data demonstrate values for mean, median, standard error (SE), and standard deviation (SD). Samples were taken immediately after exposure (0 h) and 2 h, 6 h, and 24 h from the exposure. *-p values represent significant differences from only 1 Gy samples, $p \leq 0.05$; H* and S* letters also indicate significant H and S difference from I sample. (c) Tail intensity values of brain cells of 2 Gy-irradiated mice: control samples (2 Gy); after VA+2 Gy combined exposure to halothane (H)/sevoflurane (S)/isoflurane (I) exposure. Data demonstrate values for mean, median, standard error (SE), and standard deviation (SD). Samples were taken immediately after exposure (0 h) and 2 h, 6 h, and 24 h from the exposure. *-p values represent significant differences from only 2 Gy samples, $p \leq 0.05$; H* and S* letters also indicate significant H and S difference from I sample.

with significance at $p \leq 0.05$. The TI parameter was expressed as mean, median, standard error (SE), and standard deviation (SD), used for CRI; and DNA (TI) damage distribution was represented in histograms.

Results

TI results are represented in Figure 1(a) (control and samples from mice exposed to only VAs), Figure 1(b) (1 Gy and VAs+1 Gy samples), and Figure 1(c) (2 Gy and VAs+2 Gy samples). Considering all time points and all three types of exposures, I had protective effects and caused lower DNA damage levels when compared to control or in combined exposures when compared to only irradiated samples. S demonstrated elevated DNA damage levels when compared to the control but also protective (lower DNA damage than irradiated samples) in combined IR exposure. H had higher DNA damage levels

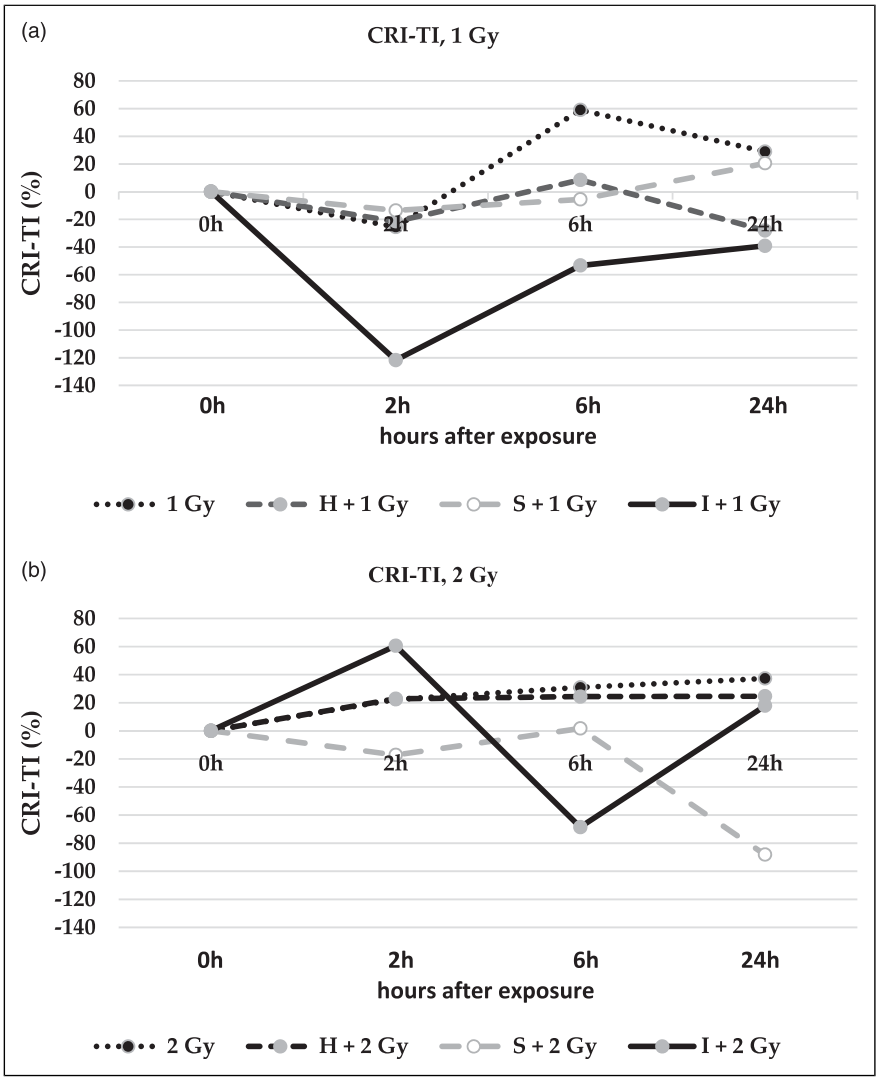

Figure 2. Cellular DNA repair index (percentage of repair, CRI) of the tail intensity (TI) parameter of brain cells of Swiss albino mice exposed to combined treatment with halothane (H), sevoflurane (S), or isoflurane (I) and 1 Gy (a) or 2 Gy (b) irradiation, immediately (0 h) and 2 h, 6 h, and 24 h (hours) after exposure; control only irradiated with 1 Gy (a) or 2 Gy (b). The lower values obtained in the combined treatment indicate that repair was slower and less efficient than in the control samples, and all values higher than the control indicate that repair was faster and more efficient than in the control samples.

in only VA exposure and in VA + IR samples demonstrated similar levels of damage as irradiated controls.

VA+1 Gy showed no faster DNA damage repair than 1 Gy samples (CRI results, Figures 2(a) and (b)). At 2 h after exposure, H (for 60%) and S (for 70%) had a slower repair rate than 1-Gy 2 h sample. I stopped/delayed the repair ignition up to 2 h from the exposure. Afterward, following a similar though slower curve as the IR sample, I started to repair the damage. These results suggest the influence of VA on cell proliferation and mitotic index.

2 Gy-exposure (CRI results, Figure 2(b)) demonstrated a faster DNA repair rate for I when compared to 2 Gy, but only up to the time point of 2 h, which was followed by a delay in repair. At 6 h, the I-repair process started and almost reached the values of the 2 -Gy 24 h sample. The repair curve for H2 Gy and 2 Gy were similar up to 6 h, and afterward, H-treated samples showed slightly decreased repair. The S-treatment demonstrated a small delay of up to 2 h of 20%. Afterward, S started repair that continued up to 6 h, which was followed by a renewed rapid repair decrease.

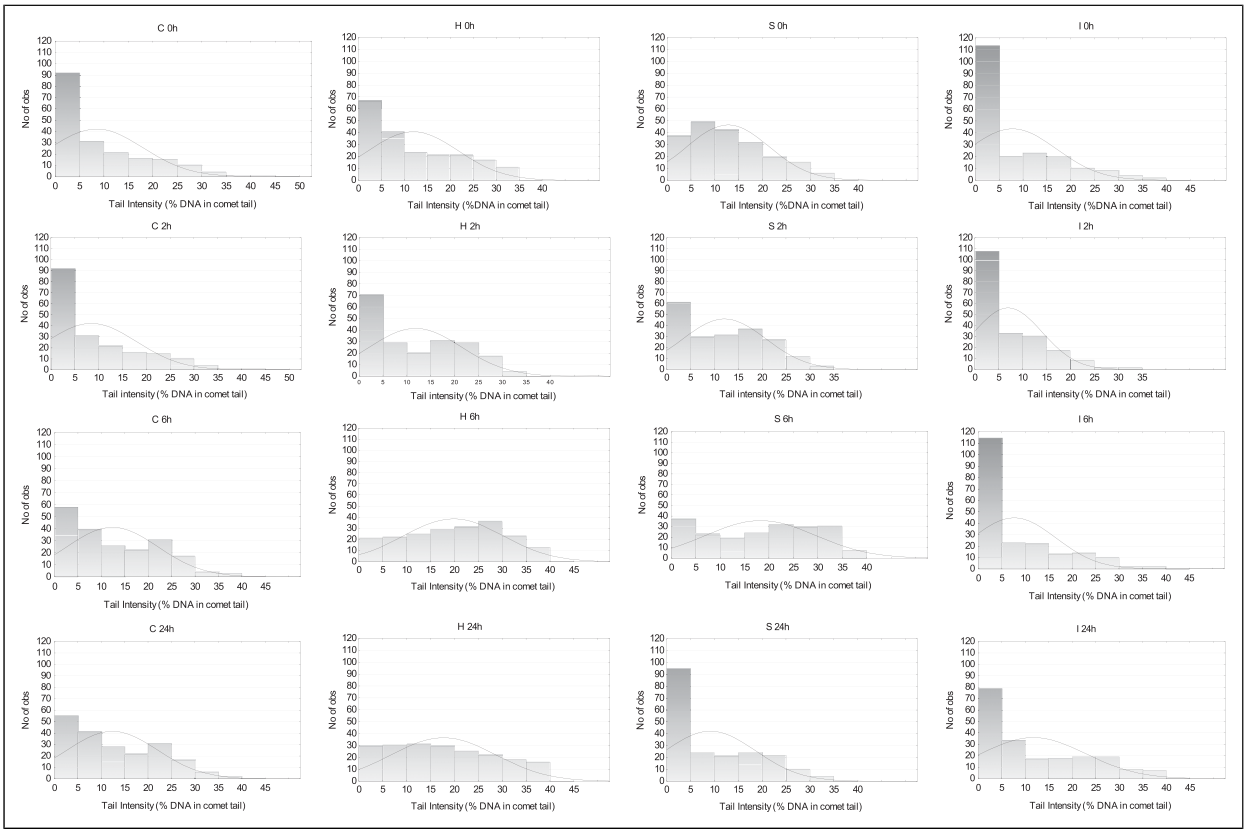

Figure 3. Histogram of mice brain cells' tail intensity (% DNA in comet tail) values of time points 0 h, 2 h, 6 h, and 24 h of control or halothane (H)/sevoflurane (S)/isoflurane (I) exposure, No of obs—number of observations for each category (0–5, 5–10, 10–15, 15–20, 20–25, 25–30, 30–35, 35–40, 40–45, 45–50 % DNA in comet tail) of DNA damage measured as tail intensity.

These repair results indicated an influence on the decrease of repair and showed a protective effect (considering the amount of DNA damage induced when compared to only irradiated samples). The next step was to determine whether there were any other changes in the DNA damage distribution that could explain DNA repair delay processes. This includes changes in the number of cells or time for cells to start apoptosis (apoptosis could not be measured by comet assay) or changes in a different damage distribution that could not be detected from the mean and median values. Damage distributions for time points 0, 2, 6, and 24 h are represented as histograms in Figure 3 (H/S/I exposure), Figure 4 (H/S/I + 1 Gy), and Figure 5 (H/S/I + 2 Gy). I and C (control) had a similar histogram for all time points. I treatment showed a very low number of cells (Nc) with damage exceeding 5%. H and S caused a higher number of damaged cells. At the 6 -h time point, both H and S had a similar Nc with small and higher damage levels. That rate was also similar at 24 h for H, while for S, the Nc with higher damage decreased and the Nc with less damage increased significantly. This means that S-treated cells had more complex damage that after the first repair in the first 6 h was left as smaller or simpler DNA damage to be repaired in the next round. In our previous studies *in vivo*, S started to repair damage in the first 6 h, so these findings and theory would be in accordance (Brozović et al., 2009, 2010, 2011, 2017; Benković et al., 2021, 2022).

Figure 4 (VA+1 Gy) shows that H and S demonstrated a similar distribution of all types of damaged cells. Numbers for both H and S were slightly lower than in IR-only cells at the 0 h time point, while at the 2 h time point, H and S maintained those values and distribution, likely due to the influence on cell cycle stopping in proliferation and repair. In the meantime, IR-only cells had an increase in the highly damaged Nc, demonstrating the start of the repair process. The process for IR-only cells was nearly complete at the 6 h time point, but looking at 24 h time point, there was still an unrepaired part of DNA damage. At the 2 h time point, H and S repaired

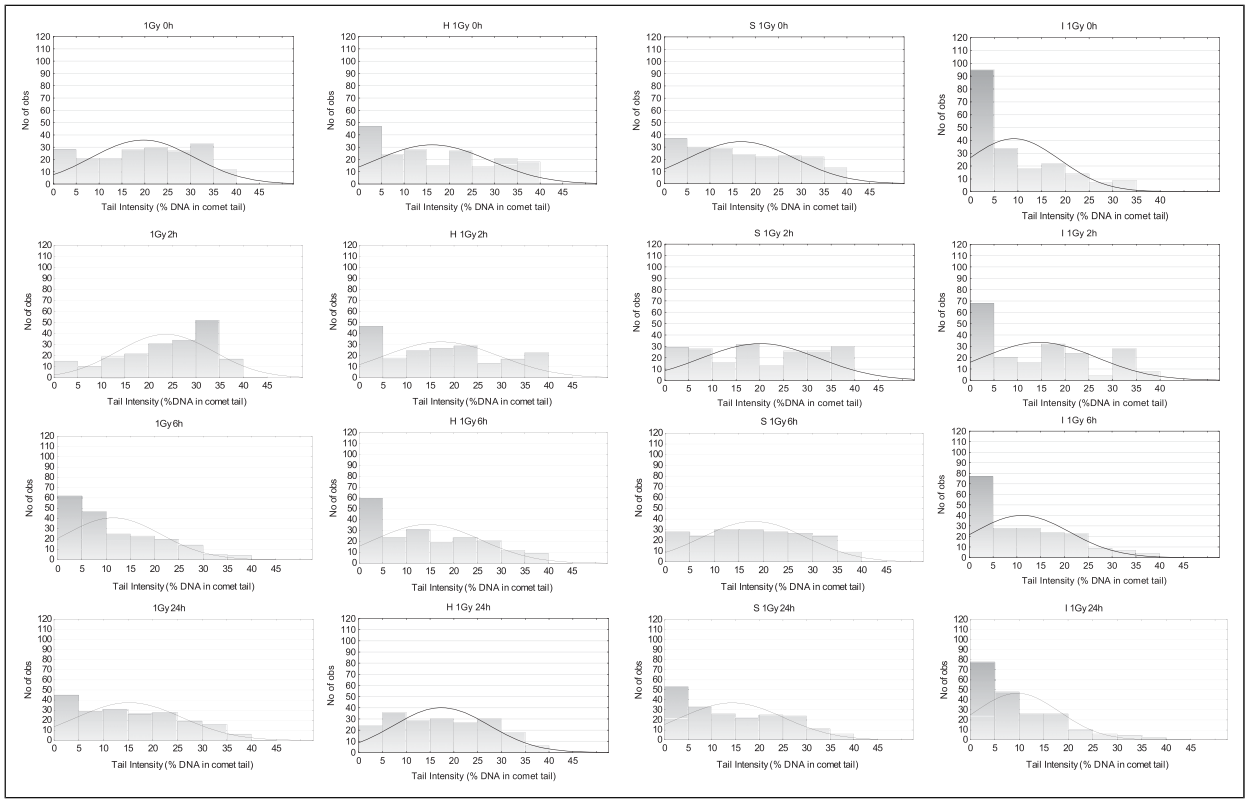

Figure 4. Histogram of mice brain cells' tail intensity (% DNA in comet tail) values of time points 0 h, 2 h, 6 h, and 24 h after 1 Gy radiation, or halothane (H)/sevoflurane (S)/isoflurane (I) + 1 Gy exposure, No of obs—number of observations for each category (0–5, 5–10, 10–15, 15–20, 20–25, 25–30, 30–35, 35–40, 40–45, 45–50 % DNA in comet tail) of DNA damage measured as tail intensity.

highly damaged cells, while at the 24 h time point, they continued to repair cells with lower levels of DNA damage. On the contrary, I had a higher amount of slightly damaged cells at the 0 h time point, 3 times more than in control cells, though the number of slightly damaged cells decreased gradually to the 24 h time point, demonstrating that no newer damage appeared, as was the case in H or S.

Figure 5 (VA+2 Gy) demonstrates that an increase of more damaged 2 Gy cells was seen immediately (0 h), and the damage was repaired during the next 2 and 6 h, with a similar Nc remaining in all of the damaged categories, like the 1 Gy samples. For the H-treatment, there was a higher Nc with medium level damage at 0 h (5–25% damage), and these values decreased through the other time points, with a small increase in more damaged cells and an increase in slightly damaged cells. S demonstrated a similar pattern, though the highest damage was recorded at the 2 h. On the contrary, I demonstrated a similar pattern as in 1 Gy, with a wider distribution of damaged cells.

Discussion

The brain is a radiation-sensitive organ, although the cognitive, molecular, and histopathological level effects depend on the dose received and the exposure period. This was particularly evident after exposure to dose levels of ≥1 Gy *in vivo* but also in human patients (Baluchamy et al., 2012; Loganovsky and Yuryev, 2004; Mizumatsu et al., 2003). Harmful IR effects are predominantly attributable to reactive oxygen species (ROS) creation and its interaction with organic molecules (Von Sonntag, 1994).

The brain represents only 2.5% of the total whole-body mass, accounts for 15% of heart output, and consumes more than 50 mL O_2 per minute (over 20% of the general oxygen consumption by a human at rest) (Loganovsky and Yuryev, 2004). The frontal part (analyzed in this study) has a significantly higher blood circulation and therefore a higher oxygen organ/tissue level directly correlating with increasing radiation effects (cellular structures intensified peroxidation, further IR-exposure neuron damage) (Coggle,

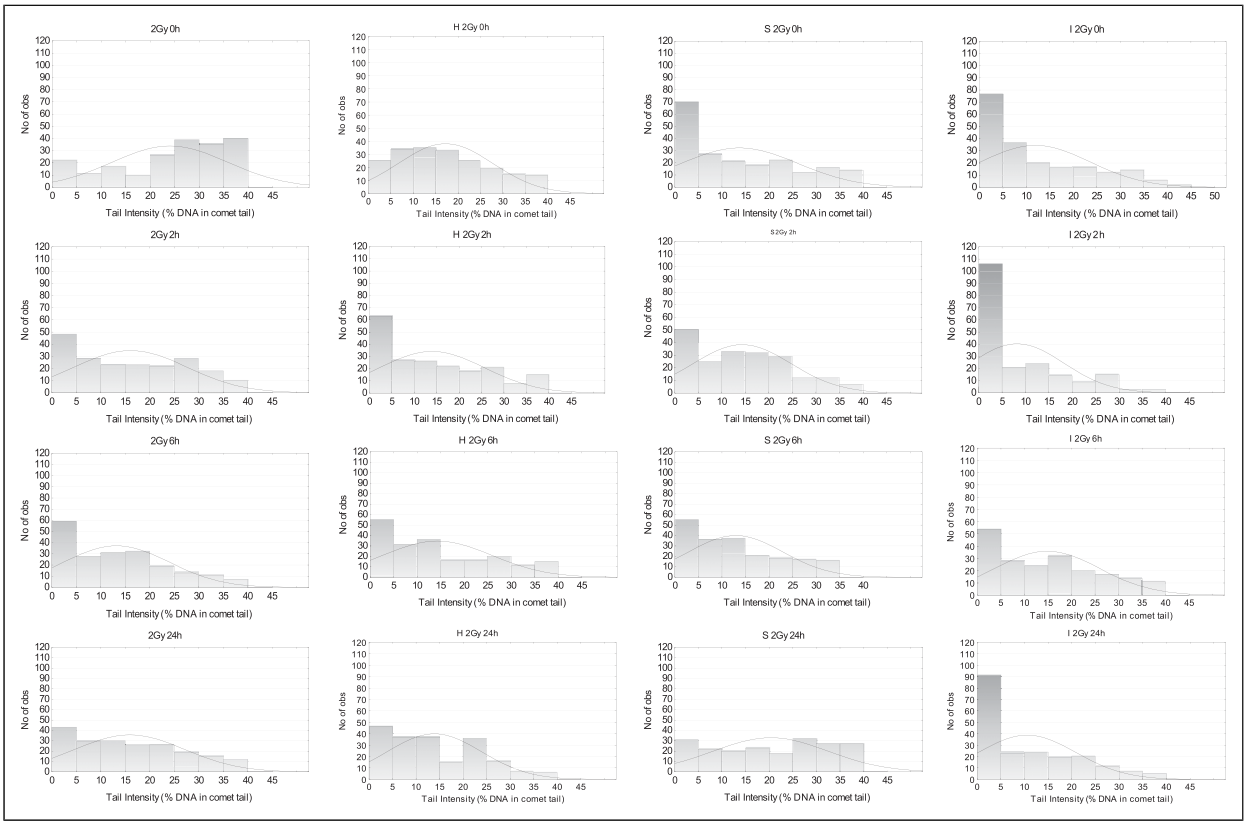

Figure 5. Histogram of brain cells' tail intensity values of time points 0 h, 2 h, 6 h, and 24 h after 2 Gy radiation, or 2 Gy+ halothane (H)/sevoflurane (S)/isoflurane (I) exposure, No of obs—number of observations for each category (0–5, 5–10, 10–15, 15–20, 20–25, 25–30, 30–35, 35–40, 40–45, 45–50 % DNA in comet tail) of DNA damage measured as tail intensity.

1983). The brain is not rich in antioxidant defenses (Floyd and Carney, 1992, 1993), has lower catalase activity (10% of the liver) (Marklund et al., 1982), and, in adults, represents low-proliferative post-mitotic tissue linked with the non-cancer morbidity and mortality increase as in the Chernobyl accident (Loganovsky and Yuryev, 2004). The mouse brain repair half-time after x/ɣ IR-exposure of 7.5–15 Gy is 45 min (most single-strand breaks (SSBs) could be repaired within 90 min with no repair time for double-strand breaks (DSBs)) (Olive, 1999; Zheng and Olive, 1996).

Part of DNA damage may be lost due to apoptosis increase. Since the comet assay cannot measure apoptosis levels, to exclude a bias of IR-apoptosis induction on the results, we examined other published papers. *In vitro* doses ≥2 Gy can cause apoptosis, while *in vivo*, this occurs at ≥6 Gy (Chatterjee et al., 2018; Lagroye and Poncy, 1997). Doses of 1 and 2 Gy can cause a decrease in neuron proliferation (Hudson et al., 2011), whereas 2 Gy can cause significant DNA damage (Chatterjee et al., 2018; Nairy et al., 2015) with more than 90% of proliferating (tumor) cells in the adult mice brain going into apoptosis and can lower the rate of tumor cell repair (Zhao et al., 2019) with most of SSB repaired within 48 h of exposure (El-Nahas et al., 1993; Mut et al., 2004) and residual damage considered to be DSB (Benitez-Bribiesca and Sanchez-Suarez, 1999; Mut et al., 2004; Olive, 1998; Zhang et al., 2014). This approach is also used in RT.

The VA-preconditioning neuroprotection effect is a shared VA feature, mediated in a concentration-dependent manner by glutamate transporter activity modification (after 15 min, maximum 5 h after brain ischemia), adenosine triphosphate (ATP)–dependent potassium channel activation, nitric oxide synthase upregulation, excitotoxic stressors and cerebral metabolic rate reduction, augmentation of peri-ischemic cerebral blood flow, and anti-apoptotic factor upregulation (Wang et al., 2007).

Although VAs should have similar neuroprotection mechanisms at least within the first 5 h of brain damage,

these drugs can have significantly different effects on many aspects influenced by the rate of metabolization (H = 15–20%, S = 5%, I < 0.2%) (Davis, 2011; Gyorfi and Kim, 2021; Martin and Njoku, 2005), metabolites, and their presence within the body until excretion, diverse aspects of cerebral vasodilating potency (H-the highest, S and I similar), and therefore different influences on the brain receptors after inhalation and on different percentage and the velocity of ROS creation (Chen et al., 2019; Drummond and Patel, 2000).

H-neuroprotection effects are much lower than other volatile and intravenous anesthetics (Haelewyn et al., 2003; Kobayashi et al., 2007; Schifilliti et al., 2010). Due to its high hepatotoxicity and mortality rate, H has been replaced by other VAs, but is still in common use in many developing countries (Schifilliti et al., 2010). In our study, it had the least protective and the most damaging effect in a single exposure. H-elevated ROS and liver ROS removal enzyme levels were seen in anesthetized patients and occupationally exposed workers (Tankó et al., 2014). Added *in vitro* and *vivo*, H does not directly interact with genome DNA but causes changes in intracellular signaling pathways, oxidative biotransformation in the liver, and antibody-mediated reaction, triggering irreversible concentration correlated DNA damage effects (from 1.5 up to 100 mM) (Brozović et al., 2011; Jaloszyński et al., 1999; Karabiyik et al., 2001; Szyfter et al., 2004; Topouzová-Hristova et al., 2007).

S induces anti-excitotoxic properties during oxygen–glucose deprivation; decreases ROS generation during re-oxygenation (Schifilliti et al., 2010; Zheng and Zuo, 2003), without the influence on the neuronal plasma membrane and cell viability *in vitro* and *in vivo* (Dong et al., 2021), an anti-proliferative effect at 2 mM in C6 glioma cells (Argano et al., 2019; O'Leary et al., 2000), and cognitive impairment in young mice; and influences neurogenesis *in vitro* (Yi et al., 2016; Zhang et al., 2013, 2019) and micro-RNA *in vivo* (Edgington et al., 2021; Gentz and Malan Jr, 2001; Shao and Xia, 2019; Yang et al., 2018).

In our study we used preconditioning with volatile anesthetics. I-2 h preconditioning in other studies demonstrated a reduction in neuronal cell death *in vitro*, improvement of long-term neurological outcomes and dose-dependent neuroprotection *in vivo* during reperfusion after oxygen–glucose deprivation or brain ischemia reduced brain injury in rats (Kitano et al., 2007; Lee et al., 2008; Li and Zuo, 2009). It also increases antioxidative status and cell viability in Wistar rats and plasma antioxidant capacity in patients undergoing minimally invasive surgery (Braz et al., 2013; Rocha et al., 2015). Protection is connected with heme oxygenase-(HO-)1-modulation, anti-inflammatory and antioxidative effects (Hoetzel and Schmidt, 2010; Schmidt et al., 2007), microglial activation (Li et al., 2011; Sun et al., 2015), and apoptosis reduction (Lehnardt et al., 2008; Li et al., 2011; Sun et al., 2015). Compared to other VAs, I-maintained anesthesia demonstrated better neuroprotection and lower cerebral blood flow without electroencephalogram (EEG) during focal brain ischemia *in vivo* (Drummond and Patel, 2000) and among patients when compared to H-patients (Michenfelder et al., 1987). I increased both the hydrophilic and total antioxidant capacity in human patient plasma and *in vivo*, with no systemic DNA breaks or oxidative DNA damage in clinical studies while the same effect was not seen for S *in vivo* (Braz et al., 2011a, 2011b, 2013; Rocha et al., 2015). S-repeated exposure *in vivo* had a longer DNA damage removal time (more than 24 h) when compared to isoflurane (6 h), probably due to I-caused-simpler-DNA-damage and S-more-complex-DNA-damage type forms (Brozović et al., 2017). VA exposure caused elevated DNA damage levels in adult brain cells *in vitro* and *in vivo* in both a dose- and time-dependent manner (Andropoulos, 2018; Brozović et al., 2009, 2010, 2011, 2017; Benković et al., 2021, 2022; McCann and Soriano, 2019; Rocha et al., 2015). I at 2.5% and 3% suppressed neuronal activities much higher than 1.5% and 2% in adult male Wistar rats (Tsurugizawa et al., 2016), while prolonged-4 h-2.4%-exposure in 60-day-old-rats decreased progenitor neuronal cell proliferation but increased neuronal differentiation (Stratmann et al., 2009, 2010). At 4.8% or higher, S decreased proliferation and increased apoptosis in developing brains with high proliferation potential, and prolonged-6 h-4.1%-S-treatment caused neuroapoptosis and autophagy (a prosurvival mechanism) *in vitro* and *in vivo* (Chen et al., 2013; Yang et al., 2018; Zhou et al., 2016).

Conclusions

2 h preconditioning demonstrated differences in neuroprotection, with I causing the least damage after single 1 or 2 Gy IR, and with 24 h sufficient for the repair of most damage. VA + IR impact should be further explored to avoid short-term and late-term unrepairable effects and safety. This is particularly

important for children and adult RT and during VA-animal treatments in veterinary procedures.

Acknowledgments

We thank Prof Makso Herman for English editing.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Institute for Medical Research, Ministry of Science and Education of the Republic of Croatia, and the University of Zagreb, Faculty of Science, Department of Biology.

ORCID iD

Mirta Milić https://orcid.org/0000-0002-9837-7185

References

Andropoulos DB (2018) Effect of anesthesia on the developing brain: infant and fetus. *Fetal Diagnosis and Therapy* 43(1): 1–11. DOI: 10.1159/000475928

American College of Laboratory Animal Medicine Series (2008) In: Fish RE, Brown MJ, Danneman PJ, et al. (eds) *Anesthesia and Analgesia in Laboratory Animals*. 2nd edition. Academic Press (Elsevier). DOI: 10.1016/B978-0-12-373898-1.50037-1

Argano M, De Maria R, Vogl C, et al. (2019) Canine mammary tumour cells exposure to sevoflurane: effects on cell proliferation and neuroepithelial transforming gene 1 expression. *Veterinary Anaesthesia and Analgesia* 46(3): 369–374. DOI: 10.1016/j.vaa.2018.12.006

Arunkumar R, Rebello E and Owusu-Agyemang P (2013) Anaesthetic techniques for unique cancer surgery procedures. *Best Practice and Research Clinical Anaesthesiology* 27(4): 513–526. DOI: 10.1016/j.bpa.2013.09.002

Baluchamy S, Ravichandran P, Ramesh V, et al. (2012) Reactive oxygen species-mediated tissue damage in high energy proton irradiated mouse brain. *Molecular and Cellular Biochemistry* 360(1–2): 189–195. DOI: 10.1007/s11010-011-1056-2

Behl C (2002) Oestrogen as a neuroprotective hormone. *Nature Reviews. Neuroscience* 3: 433–442. DOI: 10.1038/nrn846. PMID: 12042878.

Benitez-Bribiesca L and Sanchez-Suarez P (1999) Oxidative damage, bleomycin, and gamma radiation induce different types of DNA strand breaks in normal lymphocytes and thymocytes. A comet assay study. *Annals of the New York Academy of Sciences* 887: 133–149. DOI: 10.1111/j.1749-6632.1999.tb07928.x

Benković V, Borojević N, Šikić D, et al. (2021) DNA damage assessment in peripheral blood of Swiss albino mice after combined exposure to volatile anesthetics and 1 or 2 Gy radiotherapy *in vivo*. *International Journal of Radiation Biology* 97(10): 1425–1435. DOI: 10.1080/09553002.2021.1962565

Benković V, Oršolić N, Knežević AH, et al. (2022) Kidney cell DNA damage caused by combined exposure to volatile anaesthetics and 1 Gy or 2 Gy radiotherapy dose *in vivo*. *Arhiv za Higijenu Rada i Toksikologiju* 73(1): 62–70. DOI: 10.2478/aiht-2022-73-3600

Borras JM, Grau C, Corral J, et al. (2018) Estimating the number of fractions by tumour site for European countries in 2012 and 2025: an ESTRO-HERO analysis. *Radiotherapy and Oncology : Journal of the European Society for Therapeutic Radiology and Oncology* 126(2): 198–204. DOI: 10.1016/j.radonc.2017.11.009

Braz MG, Braz LG, Barbosa BS, et al. (2011a) DNA damage in patients who underwent minimally invasive surgery under inhalation or intravenous anesthesia. *Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis* 726(2): 251–254. DOI: 10.1016/j.mrgentox.2011.09.007

Braz MG, Mazoti MÁ, Giacobino J, et al. (2011b) Genotoxicity, cytotoxicity and gene expression in patients undergoing elective surgery under isoflurane anaesthesia. *Mutagenesis* 26(3): 415–420. DOI: 10.1093/mutage/geq109

Braz MG, Braz LG, Braz JR, et al. (2013) Comparison of oxidative stress in ASA physical status I patients scheduled for minimally invasive surgery under balanced or intravenous anesthesia. *Minerva Anestesiologica* 79(9): 1030–1038. PMID: 23598734.

Brozović G, Orsolic N, Knezevic F, et al. (2009) Genotoxicity and cytotoxicity of cisplatin treatment combined with anaesthetics on EAT cells *in vivo*. *Onkologie* 32(6): 337–343. DOI: 10.1159/000218066

Brozović G, Orsolic N, Rozgaj R, et al. (2010) DNA damage and repair after exposure to sevoflurane *in vivo*, evaluated in Swiss albino mice by the alkaline comet assay and micronucleus test. *Journal of Applied Genetics* 51(1): 79–86. DOI: 10.1007/BF03195714

Brozović G, Orsolic N, Knezevic F, et al. (2011) The *in vivo* genotoxicity of cisplatin, isoflurane and halothane evaluated by alkaline comet assay in Swiss albino mice. *Journal of Applied Genetics* 52(3): 355–361. DOI: 10.1007/s13353-011-0046-0

Brozović G, Oršolić N, Rozgaj R, et al. (2017) Sevoflurane and isoflurane genotoxicity in kidney cells of mice. *Arhiv za Higijenu Rada i Toksikologiju* 68(3): 228–235. DOI: 10.1515/aiht-2017-68-2941

Campagna JA, Miller KW and Forman SA (2003) Mechanisms of actions of inhaled anesthetics. *The New England Journal of Medicine* 348(21): 2110–2124. DOI: 10.1056/NEJMra021261. PMID: 12761368.

Chatterjee J, Nairy RK, Langhnoja J, et al. (2018) ER stress and genomic instability induced by gamma radiation in mice primary cultured glial cells. *Metabolic Brain Disease* 33(3): 855–868. DOI: 10.1007/s11011-018-0183-9

Chen G, Gong M, Yan M, et al. (2013) Sevoflurane induces endoplasmic reticulum stress mediated apoptosis in hippo-campal neurons of aging rats. *Plos One* 8: e57870.

Chen J, Jiang S, Wang J, et al. (2019) A comprehensive review of cytochrome P450 2E1 for xenobiotic metabolism. *Drug Metabolism Reviews* 51(2): 178–195. DOI: 10.1080/03602532.2019.1632889

Chiao S and Zuo Z (2014) A double-edged sword: volatile anesthetic effects on the neonatal brain. *Brain Sciences* 4(2): 273–294. DOI: 10.3390/brainsci4020273

Coggle JE (1983) *Biological Effects of Radiation*. London: Taylor Francis Ltd.

Davis PJ (2011) Pharmacology of pediatric anesthesia. In: Davis PJ, Cladis FP, Motoyama EK (eds) *Smith's Anesthesia for Infants and Children*. 8th edition. Elsevier Inc. All, 179–261.

De Hert S and Moerman A (2015) Sevoflurane. *F1000 Research* 4(F1000 Faculty Rev): 626. DOI: 10.12688/f1000research.6288.1. PMID: 26380072; PMCID: PMC4560253.

Dong Y, Liang F, Huang L, et al. (2021) The anesthetic sevoflurane induces tau trafficking from neurons to microglia. *Communications Biology* 4(1): 560. DOI: 10.1038/s42003-021-02047-8

Drobish JK, Gan ZS, Cornfeld AD, et al. (2016) From the cover: volatile anesthetics transiently disrupt neuronal development in neonatal rats. *Toxicological Sciences* 154(2): 309–319. DOI: 10.1093/toxsci/kfw164

Drummond J and Patel P (2000) Chapter 17: cerebral physiology and the effects of anesthetics and techniques. In: Miller R (ed) *Anesthesia*. 1. Philadelphia: Churchill Livingstone, 695–733.

Edgington TL, Muco E and Maani CV (2021) Sevoflurane. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; PMID: 30521202.

El-Nahas SM, Mattar FE and Mohamed AA (1993) Radioprotective effect of vitamins C and E. *Mutation Research. Fundamental and Molecular Mechanisms of Mutagenesis* 301(2): 143–147. DOI: 10.1016/0165-7992(93)90037-v

Floyd RA and Carney JM (1992) Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. *Annals of Neurology* 32: S22–S27.

Floyd RA and Carney JM (1993) The role of metal ions in oxidative processes and aging. *Toxicology and Industrial Health* 9(1–2): 197–214. DOI: 10.1177/0748233793009001-214

Gentz BA and Malan TP Jr (2001) Renal toxicity with sevoflurane: a storm in a teacup? *Drugs* 61(15): 2155–2162. DOI: 10.2165/00003495-200161150-00001

Gyorfi MJ and Kim PY (2021) Halothane toxicity. [Updated 2021 Apr 20]. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https://www.ncbi.nlm.nih.gov/books/NBK545281/

Haelewyn B, Yvon A, Hanouz JL, et al. (2003) Desflurane affords greater protection than halothane against focal cerebral ischaemia in the rat. *British Journal of Anaesthesia* 91(3): 390–396. DOI: 10.1093/bja/aeg186

Hoetzel A and Schmidt R (2010) Regulatory role of anesthetics on heme oxygenase 1. *Current Drug Targets* 11(12): 1495–1503. DOI: 10.2174/1389450111009011495

Hudson D, Kovalchuk I, Koturbash I, et al. (2011) Induction and persistence of radiation-induced DNA damage is more pronounced in young animals than in old animals. *Aging* 3(6): 609–620. DOI: 10.18632/aging.100340

IARC-International agency for research on cancer (2012) *Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100D. Radiation*. IARC.

Jaloszyński P, Kujawski M, Wasowicz M, et al. (1999) Genotoxicity of inhalation anesthetics halothane and isoflurane in human lymphocytes studied *in vitro* using the comet assay. *Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis* 439(2): 199–206. DOI: 10.1016/s1383-5718(98)00195-8

Ju LS, Yang JJ, Xu N, et al. (2019) Intergenerational effects of sevoflurane in young adult rats. *Anesthesiology* 131(5): 1092–1109. DOI: 10.1097/ALN.0000000000002920

Karabiyik L, Sardaş S, Polat U, et al. (2001) Comparison of genotoxicity of sevoflurane and isoflurane in human lymphocytes studied in vivo using the comet assay. *Mutation Research. Fundamental and Molecular Mechanisms of Mutagenesis* 492(1–2): 99–107. DOI: 10.1016/s1383-5718(01)00159-0

Kitano H, Young JM, Cheng J, et al. (2007) Gender-specific response to isoflurane preconditioning in focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism* 27(7): 1377–1386. DOI: 10.1038/sj.jcbfm.9600444

Kobayashi M, Takeda Y, Taninishi H, et al. (2007) Quantitative evaluation of the neuroprotective effects of thiopental sodium, propofol, and halothane on brain ischemia in the gerbil: effects of the anesthetics on ischemic depolarization and extracellular glutamate concentration. *Journal of Neurosurgical Anesthesiology* 19(3): 171–178. DOI: 10.1097/ANA.0b013e318051743d

Kovalchuk A and Kolb B (2017) Low dose radiation effects on the brain - from mechanisms and behavioral outcomes

to mitigation strategies. *Cell Cycle* 16(13): 1266–1270. DOI: 10.1080/15384101.2017.1320003.

Lagroye I and Poncy JL (1997) The effect of 50 H z electromagnetic field radiation on the formation of micronuclei in rodent cell lines exposed to gamma radiation. *International Journal of Radiation Biology* 72(2): 249–254. DOI: 10.1080/095530097143473

Lee JJ, Li L, Jung HH, et al. (2008) Postconditioning with isoflurane reduced ischemia-induced brain injury in rats. *Anesthesiology* 108(6): 1055–1062. DOI: 10.1097/ALN.0b013e3181730257

Lehnardt S, Schott E, Trimbuch T, et al. (2008) A vicious cycle involving release of heat shock protein 60 from injured cells and activation of toll-like receptor 4 mediates neurodegeneration in the CNS. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 28(10): 2320–2331. DOI: 10.1523/JNEUROSCI.4760-07.2008

Li L and Zuo Z (2009) Isoflurane preconditioning improves short-term and long-term neurological outcome after focal brain ischemia in adult rats. *Neuroscience* 164: 497–506. DOI: 10.1016/j.neuroscience.2009.08.011

Li Y, Si R, Feng Y, et al. (2011) Myocardial ischemia activates an injurious innate immune signaling via cardiac heat shock protein 60 and Toll-like receptor 4. *The Journal of Biological Chemistry* 286(36): 31308–31319. DOI: 10.1074/jbc.M111.246124

Loganovsky KN and Yuryev KL (2004) EEG patterns in persons exposed to ionizing radiation as a result of the Chornobyl accident. Part 2: quantitative EEG analysis in patients who had acute radiation sickness. *The Journal of Neuropsychiatry and Clinical Neurosciences* 16(1): 70–82.

Makale MT, McDonald CR, Hattangadi-Gluth JA, et al. (2017) Mechanisms of radiotherapy-associated cognitive disability in patients with brain tumours. *Nature Reviews. Neurology* 13(1): 52–64. DOI: 10.1038/nrneurol.2016.185

Marklund SL, Westman NG, Lundgren E, et al. (1982) Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Research* 42(5): 1955–1961. PMID: 7066906.

Martin JL Jr and Njoku DB (2005) Metabolism and toxicity of modern inhaled anesthetics. In: *Miller´s Anesthesia*. 6th edition. Philadelphia, USA: Elsevier, 231–264.

McCann ME and Soriano SG (2019) Does general anesthesia affect neurodevelopment in infants and children? *BMJ : British Medical Journal/British Medical Association* 367: l6459. DOI: 10.1136/bmj.l6459

McMullen KP, Hanson T, Bratton J, et al. (2015) Parameters of anesthesia/sedation in children receiving radio-therapy. *Radiation Oncology* 10: 65. DOI: 10.1186/s13014-015-0363-2

Michenfelder JD, Sundt TM, Fode N, et al. (1987) Isoflurane when compared to enflurane and halothane decreases the frequency of cerebral ischemia during carotid endarterectomy. *Anesthesiology* 67(3): 336–340. DOI: 10.1097/00000542-198709000-00010

Milić M, Rozgaj R, Kašuba V, et al. (2011) The Influence of Individual genome sensitivity in DNA damage repair assessment in chronic professional exposure to low doses of ionizing radiation. In: Chen CC (ed) *Selected Topics in DNA Repair*. IntechOpen, Available from: https://www.intechopen.com/books/selected-topics-in-dna-repair/the-influence-of-individual-genome-sensitivity-in-dna-damage-repair-assessment-in-chronic-profession

Milić M, Ceppi M, Bruzzone M, et al. (2021) 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. *Mutation Research. Reviews in Mutation Research* 787: 108371. DOI: 10.1016/j.mrrev.2021.108371

Mizumatsu S, Monje ML, Morhardt DR, et al. (2003) Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. *Cancer Research* 63(14): 4021–4027. PMID: 12874001.

Mut M, Oge K, Zorlu F, et al. (2004) Effects of ionizing radiation on brain tissue surrounding arteriovenous malformations: an experimental study in a rat caroticojugular fistula model. *Neurosurgical Review* 27(2): 121–127. DOI: 10.1007/s10143-003-0316-3

Nair GG and Nair CK (2010) Protection of cellular DNA and membrane from γ-radiation-induced damages and enhancement in DNA repair by sesamol. *Cancer Biotherapy & Radiopharmaceuticals* 25(6): 629–635. DOI: 10.1089/cbr.2010.0803

Nairy KR, Bhat NN, Joseph P, et al. (2015) Studies on electron beam induced DNA damage and repair kinetics in lymphocytes by alkaline comet assay studies on electron beam induced DNA damage and repair kinetics in lymphocytes by alkaline comet assay. *International Journal of Radiation Research* 13: 213–220. DOI: 10.7508/ijrr.2015.03.003

Narendran N, Luzhna L and Kovalchuk O (2019) Sex difference of radiation response in occupational and accidental exposure. *Frontiers in Genetics* 10: 260. DOI: 10.3389/fgene.2019.00260

Navarro KL, Huss M, Smith JC, et al. (2021) Mouse anesthesia: the art and science, *ILAR Journal* 62(1–2): 238–273. DOI: 10.1093/ilar/ilab016

Neri M, Milazzo D, Ugolini D, et al. (2015) Worldwide interest in the comet assay: a bibliometric study. *Mutagenesis* 30(1): 155–163. DOI: 10.1093/mutage/geu061

Ntoukas SM, Ritchie T, Awrey S, et al. (2020) Minimizing general anesthetic use in pediatric radiation therapy. *Practical Radiation Oncology* 10(3): e159–e165. DOI: 10.1016/j.prro.2019.12.001

OECD Guidelines for the Testing of Chemicals, Section 4 (2016) *Test No. 489: In Vivo Mammalian Alkaline Comet*

Assay. Paris: OECD Publishing. Available Online: https://www.oecd-ilibrary.org/docserver/9789264264885en.pdf?expires=1626873191&id=id&accname=guest&checksum=A78AF4218D9A95A23036CB52308C0BFA

O'Leary G, Bacon CL, Odumeru O, et al. (2000) Antiproliferative actions of inhalational anesthetics: comparisons to the valproate teratogen. *International Journal of Developmental Neuroscience : The Official Journal Of The International Society for Developmental Neuroscience* 18(1): 39–45. DOI: 10.1016/s0736-5748(99)00109-4

Olive PL (1998) The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. *Radiation Research* 150(Suppl): S42–S51. PMID: 9806608.

Olive PL (1999) DNA damage and repair in individual cells: applications of the comet assay in radiobiology. *International Journal of Radiation Biology* 75(4): 395–405. DOI: 10.1080/095530099140311

Rasheed S, Rehman K and Akash MSH (2021) An insight into the risk factors of brain tumors and their therapeutic interventions. *Biomedicine & Pharmacotherapy = Biomédecine & Pharmacothérapie* 143: 112119. DOI: 10.1016/j.biopha.2021.112119

Rocha TL, Dias-Junior CA, Possomato-Vieira JS, et al. (2015) Sevoflurane induces DNA damage whereas isoflurane leads to higher antioxidative status in anesthetized rats. *BioMed Research International* 264971. 1–6. DOI: 10.1155/2015/264971

Schifilliti D, Grasso G, Conti A, et al. (2010) Anaesthetic-related neuroprotection: intravenous or inhalational agents? *CNS Drugs*. 24(11): 893–907. DOI: 10.2165/11584760-000000000-00000

Schmidt R, Tritschler E, Hoetzel A, et al. (2007) Heme oxygenase-1 induction by the clinically used anesthetic isoflurane protects rat livers from ischemia/reperfusion injury. *Annals of Surgery* 245(6): 931–942. DOI: 10.1097/01.sla.0000256891.45790.4d

Schmitz-Feuerhake I, Busby C and Pflugbeil S (2016) Genetic radiation risks - a neglected topic in the low dose debate. *Korean Journal Environmental Toxicology* 0. DOI: 10.5620/eht.e2016001. Published online 2016 January 20.

Shao CZ and Xia KP (2019) Sevoflurane anesthesia represses neurogenesis of hippocampus neural stem cells via regulating microRNA-183-mediated NR4A2 in newborn rats. *Journal of Cellular Physiology* 234(4): 3864–3873. DOI: 10.1002/jcp.27158

Siddiqui BA and Kim PY (2020) Anesthesia stages. In: *StatPearls* [Internet]. Update: April 29, 2020.

Silasi G, Diaz-Heijtz R, Besplug J, et al. (2004) Selective brain responses to acute and chronic low-dose X-ray irradiation in males and females. *Biochemical and Biophysical Research Communications* 325(4): 1223–1235. DOI: 10.1016/j.bbrc.2004.10.166

Stenroos P, Pirttimäki T, Paasonen J, et al. (2021) Isoflurane affects brain functional connectivity in rats 1 month after exposure. *NeuroImage* 234: 117987. DOI: 10.1016/j.neuroimage.2021.117987

Stratmann G, Sall JW, May LD, et al. (2009) Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. *Anesthesiology* 110(4): 834–848. DOI: 10.1097/ALN.0b013e31819c463d

Stratmann G, Sall JW, May LD, et al. (2010) Beyond anesthetic properties: the effects of isoflurane on brain cell death, neurogenesis, and long-term neurocognitive function. *Anesthesia and Analgesia* 110(2): 431–437. DOI: 10.1213/ANE.0b013e3181af8015

Sun M, Deng B, Zhao X, et al. (2015) Isoflurane preconditioning provides neuroprotection against stroke by regulating the expression of the TLR4 signaling pathway to alleviate microglial activation. *Scientific Reports* 5: 11445. DOI: 10.1038/srep11445

Szyfter K, Szulc R, Mikstacki A, et al. (2004) Genotoxicity of inhalation anaesthetics: DNA lesions generated by sevoflurane *in vitro* and *in vivo*. *Journal of Applied Genetics* 45(3): 369–374. PMID: 15306730.

Tankó B, Molnár L, Fülesdi B, et al. (2014) Occupational hazards of halogenated volatile anesthetics and their prevention: review of the literature. *Journal of Anesthesia & Clinical Research* 5(7): 426. DOI: 10.4172/2155-6148.1000426

Tétrault S, Chever O, Sik A, et al. (2008) Opening of the blood-brain barrier during isoflurane anaesthesia. *The European Journal of Neuroscience* 28: 1330–1341. DOI: 10.1111/j.1460-9568.2008.06443.x

Topouzová-Hristova T, Hazarosova R, Bandreva B, et al. (2007) Halothane does not directly interact with genome DNA of A549 cells. *Folia Biologica (Praha)* 53(5): 176–182.

TRCR-The Royal College of Radiologists (2019) Radiology dose fractionation. 3rd edition. London: The Royal College of Radiologist BFCO(19)3, Available online: www.rcr.ac.uk (updated on 2 December 2020, accessed on 2 August 2021).

Tsurugizawa T, Takahashi Y and Kato F (2016) Distinct effects of isoflurane on basal BOLD signals in tissue/vascular microstructures in rats. *Scientific Reports* 6: 38977. DOI: 10.1038/srep38977

Von Sonntag C (1994) Radiation chemistry in the 1990s: pressing questions relating to the areas of radiation biology and environmental research. *International Journal of Radiation Biology* 65: 19–26. DOI: 10.1080/09553009414550031

Wang C, Jin Lee J, Jung HH, et al. (2007) Pretreatment with volatile anesthetics, but not with the non immobilizer 1, 2-dichlorohexafluorocyclobutane, reduced cell injury in rat cerebellar slices after an in vitro simulated ischemia. *Brain Research* 1152: 201–208. DOI: 10.1016/j.brainres.2007.03.030

Wujanto C, Vellayappan B, Chang EL, et al. (2021) Radiotherapy to the brain: what are the conse-quences of this age-old treatment? *Annals of Palliative Medicine* 10(1): 936–952. DOI: 10.21037/apm-20-856

Yang L, Shen Q, Xia Y, et al. (2018) Sevoflurane-induced neurotoxicity is driven by OXR1 post-transcriptional downregulation involving hsa-miR-302e. *Molecular Medicine Reports* 18(5): 4657–4665. DOI: 10.3892/mmr.2018.9442

Yi X, Cai Y, Zhang N, et al. (2016) Sevoflurane inhibits embryonic stem cell self-renewal and subsequent neural differentiation by modulating the let-7a-Lin28 signaling pathway. *Cell and Tissue Research* 365: 319–330. DOI: 10.1007/s00441-016-2394-x

Yılmaz S and Çalbayram NÇ (2016) Exposure to anesthetic gases among operating room personnel and risk of genotoxicity: a systematic review of the human biomonitoring studies. *Journal of Clinical Anesthesia* 35: 326–331. DOI: 10.1016/j.jclinane.2016.08.029

Zhang J, Cui F, Li L, et al. (2014) Contrasting effects of Krüppel-like factor 4 on X-ray-induced double-strand and single-strand DNA breaks in mouse astrocytes. *Cell Biochemistry and Function* 32(3): 241–248. DOI: 10.1002/cbf.3007

Zhang L, Yan J, Liu Q, et al. (2019) LncRNA Rik-203 contributes to anesthesia neurotoxicity via microRNA-101a-3p and GSK-3β-mediated neural differentiation. *Scientific Reports* 9(1): 6822. DOI: 10.1038/s41598-019-42991-4

Zhang Y, Dong Y, Zheng H, et al. (2013) Sevoflurane inhibits neurogenesis and the Wnt-catenin signaling pathway in mouse neural progenitor cells. *Current Molecular Medicine* 13(9): 1446–1454. DOI: 10.2174/15665240113139990073

Zhao H, Zhuang Y, Li R, et al. (2019) Effects of different doses of X-ray irradiation on cell apoptosis, cell cycle, DNA damage repair and glycolysis in HeLa cells. *Oncology Letters* 17(1): 42–54. DOI: 10.3892/ol.2018.9566

Zheng H and Olive PL (1996) Reduction of tumor hypoxia and inhibition of DNA repair by nicotinamide after irradiation of SCCVII murine tumors and normal tissues. Cancer Research 56(12): 2801–2808. PMID: 8665517.

Zheng S and Zuo Z (2003) Isoflurane preconditioning reduces purkinje cell death in an in vitro model of rat cerebellar ischemia. *Neuroscience* 118(1): 99–106. DOI: 10.1016/s0306-4522(02)00767-4

Zhou YF, Wang QX, Zhou HY, et al. (2016) Autophagy activation prevents sevoflurane-induced neurotoxicity in H4 human neuroglioma cells. *Acta Pharmacologica Sinica* 37(5): 580–588. DOI: 10.1038/aps.2016.6