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Preventive effects of melatonin and amifostine on irradiated rats with experimental periodontitis

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Abstract

Background The aim of this study was to investigate the preventive effects of amifostine and melatonin oxidatively, biochemically and histomorphometrically in rats with radiotherapy-induced experimental periodontitis.

Methods 40 female Sprague-Dawley rats were divided into 5 groups: Control, experimental periodontitis (Ep), Ep + radiotherapy (Ep + Rt), Ep + Rt + amifostine (Ep + Rt + Ami), Ep + Rt + melatonin (Ep + Rt + Mel). The day after induction of periodontitis by ligature, a single dose of 5 Gy radiotherapy was administered. On the same day, treatments with amifostine (200 mg/kg) for 3 days and melatonin (10 mg/kg) for 15 days were started. By after 23 days of experiment, periodontal bone loss was measured by histomorphometry. RANKL, OPG and Caspase-3 activities were analyzed immunohistochemically and inflammatory cytokine (IL-1β, IL-10, IL-6, TNF-α) levels and oxidative stress (TOS/TAS) were analyzed biochemically in tissue homogenates.

Results It was observed that there was a significant difference in many biochemical parameters and oxidative stress levels between the control group and Ep + Rt ($p < 0.05$). Alveolar bone destruction in the melatonin prophylaxis group was observed to be close to control ($p > 0.05$). Melatonin significantly improved biochemical, histochemical, apoptotic and bone loss levels in irradiated experimental periodontitis rats ($p < 0.05$). When comparing the two drug groups (Ep + Rt + Ami and Ep + Rt + Mel), no statistically significant difference was found at any parameter level ($p > 0.05$).

Conclusion Both melatonin and amifostine can significantly limit RT-induced periodontal bone loss by suppressing inflammatory stress, apoptotic mechanisms, and RANKL-related osteoclastic activity. Given the limited side effects of melatonin, it may be an alternative to amifostine.

Keywords Periodontitis, Radiotherapy, Amifostine, Melatonin, Alveolar Bone, Rat

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Introduction

Periodontitis is a multifactorial, chronic, inflammatory disease characterized by inflammation of the teeth and supporting tissues along with attachment and bone loss [1]. The main pathological feature of periodontal disease is inflammation. Worldwide, the rate of being affected by periodontitis of varying degrees is more than 50% [2, 3].

Cancer, another important health problem worldwide, is a disease with approximately 20 million new cases and about half of these resulting in death. Head and neck cancers constitute approximately one in 10 cases of all cancers [4]. One of the methods that has been used for years in the treatment of head and neck cancer is radiotherapy (RT) [5]. RT aims to increase the person's survival and quality of life by causing less damage to normal tissues close to the tumor tissue [6]. However, some toxic effects also occur in adjacent healthy tissues with RT. In head and neck RT, the craniofacial skeleton in particular is also affected. Some changes occur in the bone and soft tissue. Collagen synthesis increases and fibrosis occurs [7]. With these changes, hypoxia, hypocellularity and hypovascularity occur in the tissue. The ability to regenerate and remodel the affected bone and soft tissue decreases, resulting in an increased risk of infection and necrosis. Osteoradionecrosis, dental caries and periodontal problems are the most common side effects [8]. Moreover, after irradiation, damage is almost inevitable with increased osteoclastic activity in bone tissue [9, 10].

Many agents have been studied to reduce and limit the adverse effects of RT, and one of the prominent agents is amifostine (Ami). Ami is a cytoprotective adjuvant that includes DNA-binding chemotherapeutic agents used in cancer RT and chemotherapy [11]. Ami is thought to protect normal tissues through Warburg-type effects [12]. Although Ami is approved for the prevention of dry mouth [11, 13], some studies have focused on the beneficial effects of Ami on bone protection [14–18].

Melatonin (Mel) is an indole amine produced in the human body by many sources, mainly the pineal gland [19]. The therapeutic effects of Mel on experimental periodontitis have been investigated and have been shown to prevent periodontal destruction [20] and significantly reduce oxidative damage to periodontal tissues [21]. Various experimental studies have proven that Mel also has radioprotective effects on irradiated tissues [22–24].

Ami unfortunately has many serious side effects such as nausea, emesis, transient hypotension [25]. However, no notable side effects of Mel administration have been reported in clinical and animal studies. Although there are numerous literature evidence for the radioprotective efficacy of both Ami and Mel [12, 23, 24, 48], studies on their preventive or therapeutic efficacy in radiotherapy-related periodontal tissue destruction are quite limited. In light of all this information, this study was planned based on the hypothesis that systemic Ami and Mel administration would limit RT-related periodontal tissue destruction. To our knowledge, our study is the first to investigate the periodontal preventive efficacy of Ami on the basis of inflammatory and oxidative parameters and compare it with Mel.

Materials and methods

Ethics approval

The experimental protocol of this study was reviewed by the Recep Tayyip Erdogan University (RTEU) Animal Experiments Ethics Committee and with the approval of the ethics committee numbered 2023/23, the experimental study was carried out in accordance with the rules specified in the ethics committee directive, considering the welfare and quality of life of the experimental animals. RTEU Scientific Research Projects Unit contributed to the study, project number TSA-2024-1606.

Supply and housing of animals

40 female Sprague-Dawley rats, aged 4–6 months, with an average weight of approximately 200–250 g, were obtained from RTEU Experimental Animal Center. All animals received humane care as specified in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. The rats were randomly distributed into cages of 8, housed in an environment with unlimited access to food and water (ad libitum), a room temperature of 21 ± 2 °C, a relative humidity of 58%, and a photoperiod of 6:00–19:00 h. The animals were allowed to adapt to environmental conditions for 7 days. The experiment was conducted at the RTEU Experimental Animal Center for 23 days completed in May 2023.

Study groups

The animals were randomly divided into 5 groups: Control, Experimental periodontitis (Ep), Ep + Radiotherapy (Ep + Rt), Ep + Rt + Amifostine (Ep + Rt + Ami), Ep + Rt + Melatonin (Ep + Rt + Mel).

Experimental periodontitis

To induce periodontal tissue destruction, anesthesia was first achieved with intraperitoneal injections of xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) 10 mg/kg and ketamine hydrochloride (Ketalar, Pfizer, Istanbul, Turkey) 40 mg/kg. Subsequently, a 3.0 silk suture was passed to the cervical region of the right and left lower first molars in a sub-paramarginal position and knotted on the mesial side, and the suture was kept in place for 15 days to create experimental periodontitis [21, 22, 26]. The suture, due to its structure and position, is designed to cause plaque accumulation, inflammatory changes, and ultimately periodontitis.

Radiotherapy, amifostine and melatonin administration protocol

Single dose (5 Gy) RT was applied the day following the induction of experimental periodontitis (day 9). On the same day, the first systemic Ami was administered intraperitoneally at 200 mg/kg, 30 min before the single dose of radiation [23, 24]. Ami application was repeated with the same method for the following 2 days (days 10 and 11). The first application of Mel was performed 30 min before single-dose RT, similar to Ami application, at a dose of 10 mg/kg for 15 days. Mel was freshly dissolved in physiological saline solution (0.01%) and applied at night (23:00) [22, 26]. Saline was administered intraperitoneally at 11:00 pm as a single daily dose of 10 mg/kg to the control and Ep+Rt groups for 15 days and to the Ep+Rt+Ami group for the remaining 12 days.

Rats were irradiated with 6 MV by isocentric method from a 10 cm x 20 cm anterior-posterior area using a 1 cm bolus and gantry angles of 0 and 180 degrees. RT planning was performed using photon irradiation 6 MV (X-ray), a linear accelerator (Elekta Synergy; Elekta, Crawley, United Kingdom) at a dose rate of 4 Gy/min, and the CMS XiO planning system (version 13.2) [27]. Rats received total cranial irradiation with a single 5 Gy dose according to our previous study [28] (Fig. 1).

Sample collection and preparation

On the 23rd day following the completion of the experiment, anesthesia was provided in all groups as described, and intracardiac left ventricular blood samples were collected with 10 ml syringes. Collected blood samples were centrifuged at 1739 g for 15 min at +4 °C to obtain serum samples. These samples were stored at −80 °C in a freezer until analysis. Euthanasia was performed by decapitation. Mandibular tissues containing the first molar teeth were removed along with the surrounding tissues. Right

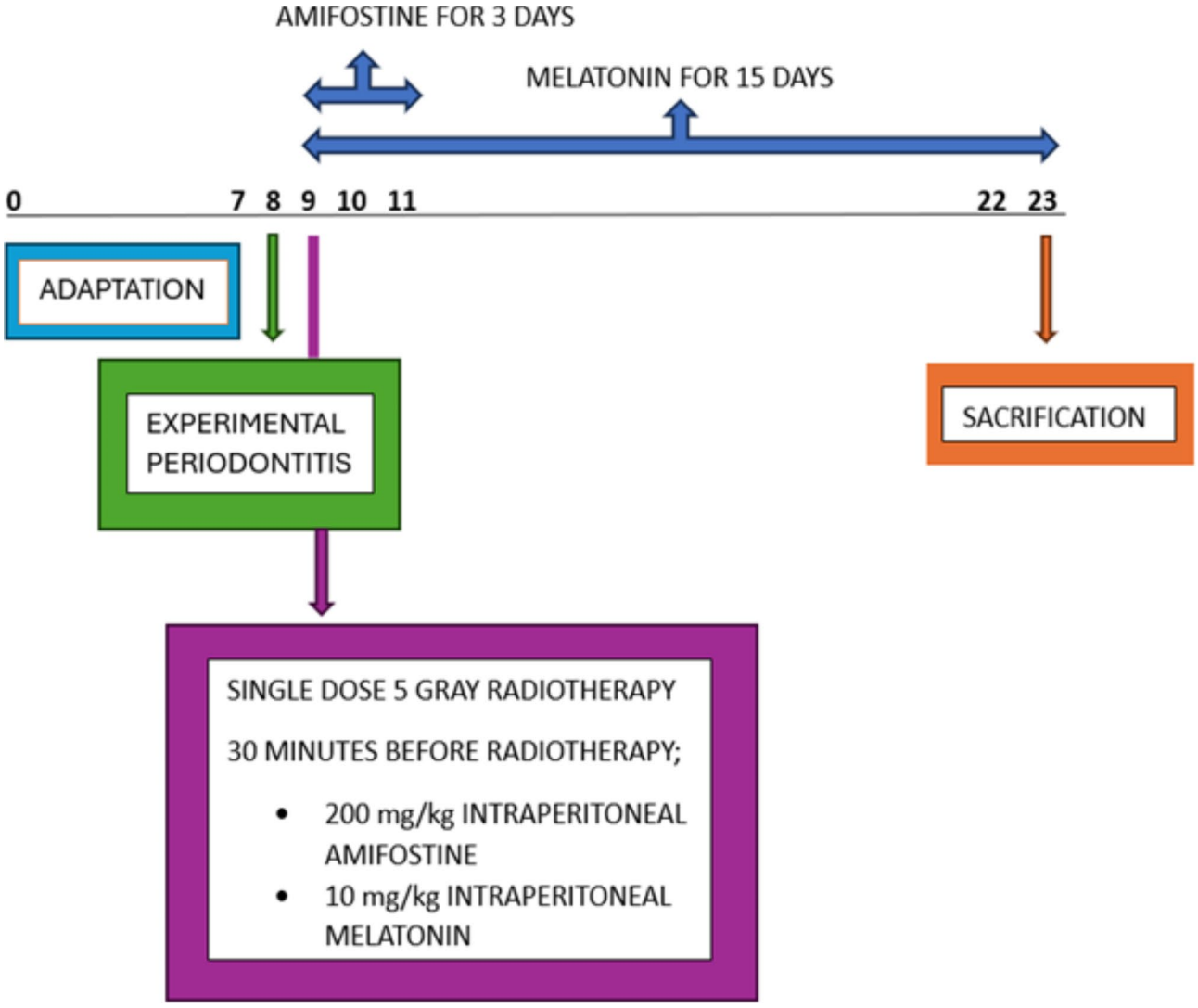

Fig. 1 Experimental Scheme

mandible samples were transferred to containers containing 10% neutral formaldehyde solution to be used in histological (histomorphometric and immunohistochemical) analyses. Left mandibular gingival tissues were placed in containers to be used in biochemical analyses. Both histological and biochemical examinations were performed by two expert researchers who were blinded to the study group allocations (Histology: TM, LT; Biochemistry: KA, AY). RANKL, osteoprotegerin (OPG) and Caspase pozitivities were evaluated in immunohistochemical examinations. RANKL/OPG ratio was calculated. IL(Interleukin)-1(beta)β, IL(Interleukin)-10, IL(Interleukin)-6, Tumor necrosis factor alpha (TNF-α), Bone Alkaline Phosphatase (b-ALP), Total Oxidative Status (TOS) and Total Antioxidant Status (TAS) levels were examined biochemically by ELISA and spectrophotometric methods. IL-1β/IL-10 and TOS/TAS ratios were calculated.

Histological analyses

Determination of alveolar bone loss

Histological monitoring of the tissues surrounding the right mandibular first molar region and embedding them in paraffin blocks were performed as detailed in our previous studies [22, 26, 29]. Serial sections of 4–5 µm thickness were taken from the paraffin blocks using a rotary microtome (Leica RM2255, Lecia Biosystems, Germany) in the buccolingual direction using the long axis of the first molar tooth as a guide. Five non-adjacent sections were randomly selected from each block to be used in morphometric analyses. Alveolar bone levels on the buccal and lingual sides of the teeth were measured as the distance between the cemento-enamel junction (CEJ) and the alveolar bone crest (BC) and the furcation roof (FR) and the furcation alveolar crest (FAC) (Fig. 2C). All histomorphometric measurements were performed independently by 2 histologists (TM, LT) at the Histology Research Laboratory of the Department of Histology and Embryology of the RTEU Faculty of Medicine using an Olympus triocular BX51 TF (Olympus Corp., Tokyo, Japan) microscope with an Olympus DP72 camera attachment (Olympus Corp., Tokyo, Japan) Morphometric measurements were performed using the arbitrary probe of the CellsSens computer-based program (Olympus Corp., Tokyo, Japan), which is compatible with the Olympus DP72 camera (Fig. 2).

Immunohistochemical analysis

Serial sections of 1–2 µm thickness were cut from paraffin blocks of right mandibular tissue using a rotary microtome (Leica RM2255, Lecia Biosystems, Germany) in the buccolingual direction, using the long axis of the first molar as a guide. Immunohistochemistry kits containing RANKL primary antibody (Abcam, United Kingdom), osteoprotegerin (OPG) primary antibody (Abcam, United Kingdom) and Caspase-3 primary antibody (Abcam, United Kingdom) and compatible secondary antibodies were used on the sections obtained. After incubation of the sections according to the manufacturer's instructions, the tissues were stained with Harris Haematoxylin (Merck GmbH, Darmstadt, Germany). Tissues were examined by light microscopy (Olympus Co, BX51, Japan) and photographed by digital camera (Olympus Co, DP71, Japan). For each mandibular first molar, RANKL-positive, OPG-positive and Caspase-3-positive stained cells were counted in 3 different sections and 3 different areas from the furcation region in each section. The measurement was performed by two

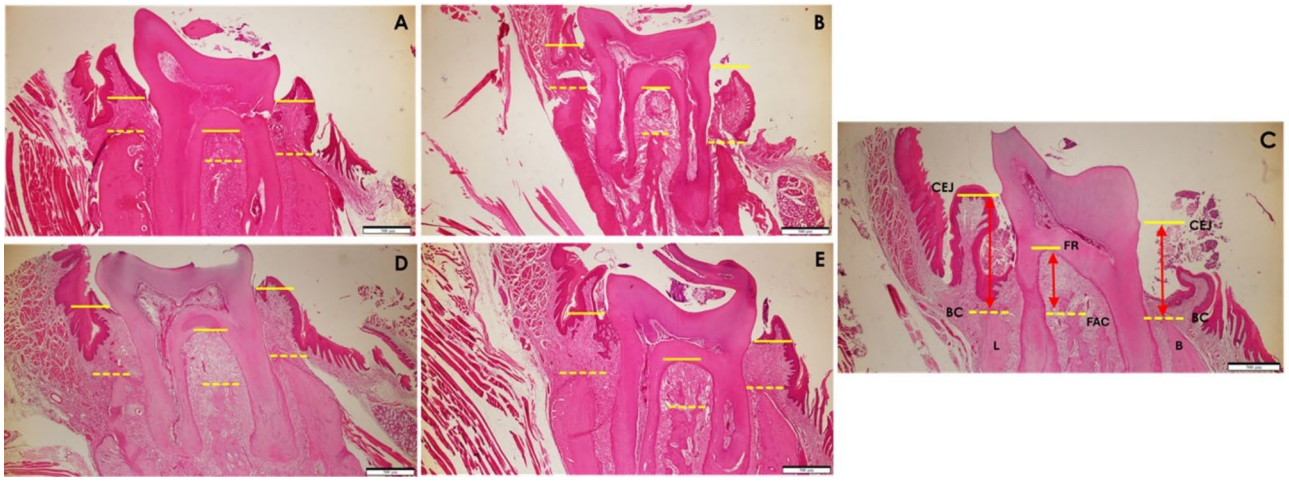

Fig. 2 Evaluation of alveolar bone loss via histological sections (Signifiers for groups and measurements in the hematoxylin and eosin staining of the sections are as follows: **A**: Control, **B**: Ep, **C**: Ep + Rt, **D**: Ep + Rt + Ami, **E**: Ep + Rt + Mel; yellow dashed line: BC (FAC), yellow line: CEJ (FR), red arrow: CEJ-BC (FR-FAC); CEJ: cemento-enamel junction, BC: alveolar bone crest, FR: furcation roof, FAC: furcation alveolar crest; CEJ-BC: distance between the cemento-enamel junction and the bone crest; FR-FAC: distance between the furcation roof and the furcal alveolar crest; B: bukkal side, L: lingual side)

experienced researchers (TM, LT) who were blinded to the groups, similar to previous studies [22, 26].

Semiquantitative analysis

Cells showing RANKL, OPG and Caspase-3 positivity were analysed semiquantitatively [30]. A total of 9 different areas from 3 different sections of each rat were evaluated by double-blind histopathologists (TM, LT) under ×40 objective magnification. Scoring was done according to positive cell detection in the range of 0–4 [30].

Biochemical analyses

Preparation of tissue homogenates

The left mandibular tissue samples were washed with cold phosphate buffered saline (pH: 7.4), dried and 100 mg of each was weighed and placed in eppendorf tubes. Then 1 mL phosphate buffered saline was added to each tube. All samples were homogenised using a homogeniser (Tissue Lyser II, Qiagen, Germany) at a frequency of 30 Hz for 5 min. Tissue homogenates were centrifuged (Thermo Scientific, Heraeus Multifuge, Waltham, Massachusetts, USA) at 3000 g for 15 min at 4 °C. The supernatants obtained were used to analyse the levels of TOS, TAS, IL-1β, IL-10.

Analysis of tissue IL-1β and IL-10 and serum TNF-α, IL-6 and b-ALP levels by ELISA Kit

Tissue IL-1β (catalogue number: E-EL-R0012) and IL-10 (catalogue number: E-EL-R0016) levels and serum TNF-α (catalogue number: E-EL-R2856), IL-6 (catalogue number: E-EL-R0015) and b-ALP (catalogue number: E-EL-R1109) levels were analysed using rat-specific ELISA kits (Elabscience, Houston, Texas, USA) according to the manufacturer's workflow principles. Results were expressed as pg/mL. Tissue levels were expressed numerically by dividing by grams of tissue.

Determination of TOS and TAS levels and calculation of oxidative stress Index (OSI)

The TOS and TAS levels of the supernatants obtained from the mandibular tissues were calculated by an automated method developed by Erel [31, 32], which works on the principle of light absorption and allows the measurement of the total amounts of the relevant molecules as a whole. The percentage ratio of TOS/TAS was calculated as OSI = [(TOS (μmol H2O2 equivalent/L)/TAS (μmol Trolox equivalent/L)] [33].

Statistical analysis

All data obtained as a result of quantitative and semi-quantitative analyses were evaluated using the SPSS 29.0 statistical program (IBM Corp., Armonk, NJ, USA) with Kruskal-Wallis test, post hoc Bonferroni, Spearman correlation analysis. Semi-quantitative analyses were calculated as median, 25% and 75% interquartile ranges. The type 1 error level of 5% was accepted for statistical significance.

Results

Morphometric results (alveolar bone loss)

On both the buccal and lingual sides, alveolar bone loss was statistically significantly higher in the Ep, Ep+Rt and Ep+Rt+Ami groups compared to the control group ($p<0.05$). It was observed that Mel treatment could significantly limit bone loss in rats with periodontitis and RT ($p<0.05$). When the results were evaluated in the furcal region, the differences between the groups were similar. In contrast, the destruction in the Ep+Rt+Ami group was higher than in the control group, but not statistically significantly (Fig. 3).

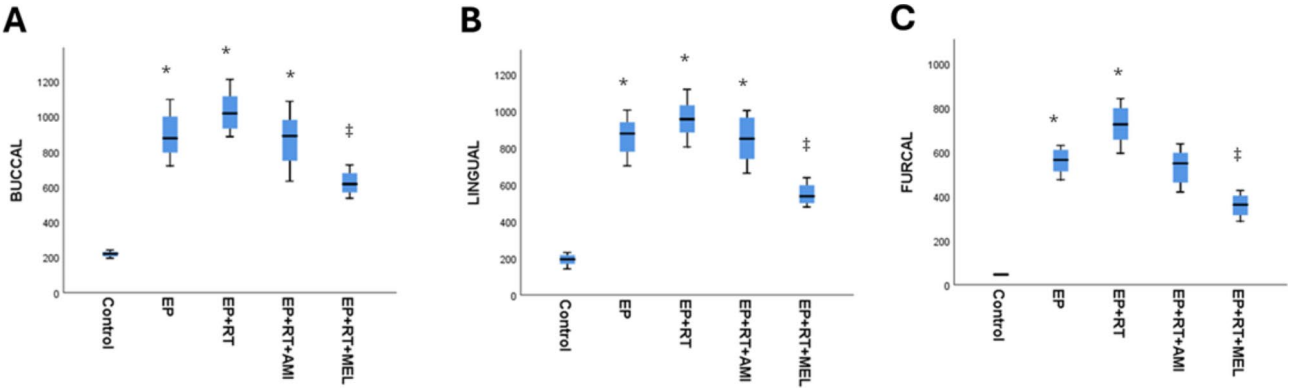

Fig. 3 Comparison of Alveolar Bone Loss Results between Groups in Buccal, Lingual and Furcal Regions (**A**: bone loss in the buccal region, **B**: bone loss in the lingual region, **C**: bone loss in the furcal region; EP: Experimental Periodontitis, RT: Radiotherapy, AMI: Amifostine, MEL: Melatonin. The values were expressed as mean ± SD. Footnote symbols (*, ‡) signify statistically significant differences between the groups. (*) intergroup, compared to Control; (‡) intergroup, compared to EP + RT. Statistically significant difference ($p<0.05$). Attachment loss is expressed in μm.)

Immunohistochemical results

Tissue RANKL, OPG and caspase-3 results

Semi-quantitative staining of histological sections revealed that RANKL, OPG and Caspase-3 positive cells were significantly different in the Ep, Ep+Rt+Ami and Ep+Rt+Mel groups compared to the control group. While RANKL and Caspase-3 scores showed significant differences between the control and Ep+Rt groups, the difference in terms of OPG was not significant ($p=0.088$). For all three scores, it was observed that there was a statistically significant difference between the Ep+Rt, Ep+Rt+Ami and Ep+Rt+Mel groups. While a significant difference was found between the Ep group and Ep+Rt+Mel at the three parameter levels, no significant difference was found between Ep+Rt+Ami and the OPG level (Fig. 4; Table 1).

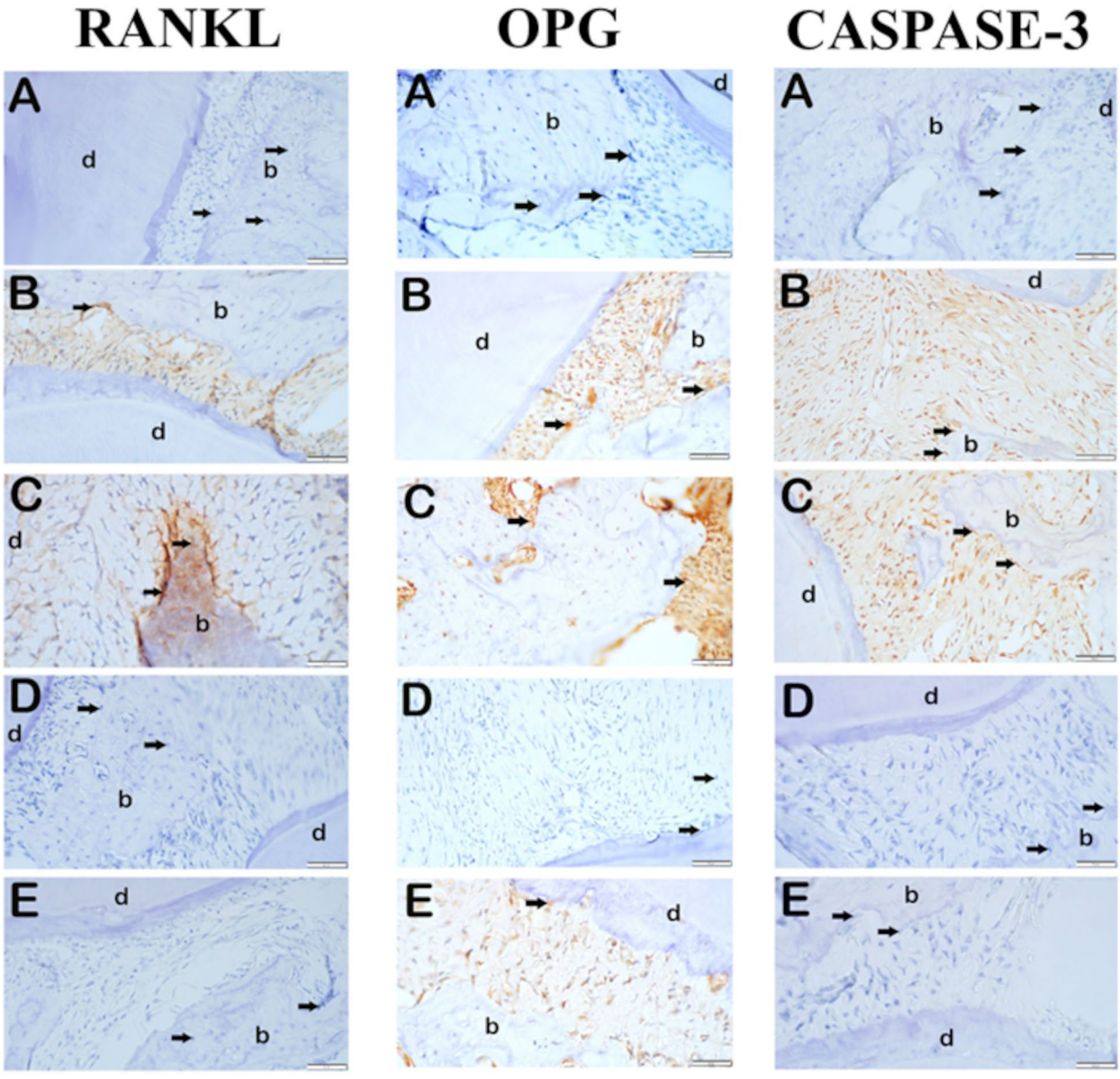

Fig. 4 RANKL, OPG and Caspase-3 positive cells observed in histological sections (Signifiers for groups, experimental procedures, and measurements in the identification of RANKL, OPG and Caspase-3 positive cells via immunohistochemical staining on sections are as follows: **A**: Control, **B**: Ep, **C**: Ep+Rt, **D**: Ep+Rt+Ami, **E**: Ep+Rt+Mel; Ep: Experimental periodontitis, Rt: Radiotherapy, Ami: Amifostine, Mel: Melatonin; b: bone, d: dentin; black arrow: immune positive cells)

Table 1 Median, minimum and maximum values and differences between groups according to the scoring system (EP: experimental periodontitis, RT: Radiotherapy, AMI: Amifostine, MEL: melatonin. X(y-z): median(minimum-maximum); a: intergroup, compared to control; b: intergroup, compared to EP; c: intergroup, compared to EP + RT. statistically significant difference ($p < 0.05$).)

	RANKL Immune Positivity	OPG Immune Positivity	Caspase-3 Immune Positivity
Control	0(0–1)	0(0–1)	0(0–1)
EP	2(2–3) [a]	1(1–1) [a]	2(2–3) [a]
EP + RT	2(2–3) [a]	1(1–1)	2(2–3) [a]
EP + RT + AMI	1(1–1) [a, b, c]	1(1–2) [a, c]	1(1–1) [a, b, c]
EP + RT + MEL	1(1–1) [a, b, c]	2(1–2) [a, b, c]	1(1–1) [a, b, c]

Biochemical results

Tissue IL-1β, IL-10, IL-1β/IL-10 and serum TNF-α, IL-6, b-ALP

For all these biochemical parameters, a statistically significant difference was found in the Ep + Rt group compared to the control group. Compared to the control group, the IL-10 and IL-1β/IL-10 results in the Ep group were significantly different; the IL-1β level was almost significantly different ($p = 0.058$). A significant difference in IL-1β, IL-1β/IL-10 and IL-6 levels was observed between Ep + Rt and Ep + Rt + Mel. Between Ep + Rt and Ep + Rt + Ami, a statistically significant difference was observed only at the IL-1β level (Fig. 5A, B, E, G, H and I).

Tissue TOS, TAS and OSI

When looking at the changes in TOS and OSI levels in general, the difference in the Ep and Ep + Rt groups compared to the control group was statistically significant. At the same time, a statistical difference was observed between the Ep + Rt group and Ep + Rt + Mel at the TOS and OSI levels. At the TAS level, a near significant difference was found only between the control group and Ep + Rt + Mel ($p = 0.054$) (Fig. 5C, D and F).

An additional file shows this in more detail [see Additional file 1 (Fig. 5A, B, C, D, E, F, G, H and I)].

Discussion

The present study showed that RT increased the periodontal destructive effects in rats with experimental periodontitis through the mechanism of increasing oxidative parameters and RANKL expression. In addition, the destructive efficacy of RT was confirmed by inducing cellular apoptosis. It was shown for the first time in a comparative manner that prophylactically administered systemic Ami and Mel prevented increased bone destruction in RT-related periodontitis.

Considering that periodontitis is an inflammatory disease, pro-inflammatory cytokines, RANKL and oxidative stress are prominent markers of bone loss. In RT, the underlying destructive mechanism is apoptosis and oxidative stress. So, the common factor in both cases is oxidative stress. Oxidative stress occurs when the oxidant/antioxidant balance disturbed in favour oxidants. When this balance is disturbed, a transition from physiological to pathological processes occurs. In our study, we compared the OSI, which is the ratio of oxidative status to antioxidant capacity. Thus, the method developed by Erel [31, 32] allows a more practical and accurate interpretation of oxidative stress [33]. Similar to our results, other studies have shown that oxidative stress increases more significantly in RT-induced [28] or unrelated [34] models of periodontitis. Oxidative stress contributes to inflammatory and apoptotic mechanisms [22]. Therefore, these complex and intertwined mechanisms should be considered as a whole in the pathogenesis of tissue destruction.

One of the fundamental mechanisms of periodontal tissue destruction is the disruption of the balance between anti-inflammatory and pro-inflammatory cytokines [35, 36]. The role of pro-inflammatory molecules such as TNF-α, IL-1β and IL-6 in the pathogenic mechanism is noteworthy, as they promote alveolar bone loss, one of the indicators of periodontal disease [17, 21, 22, 37]. With the inclusion of RT, the destruction at the level of the inflammatory process is further intensified [7, 28, 38]. In our study, we showed this exacerbation with increased tissue IL-1β/IL-10 and serum TNF-α and IL-6. In support of this, in a previous study using similar methodology [28], we showed that RT-induced inflammatory stress and periodontal tissue destruction can be significantly limited by systemic Mel treatment. Moreover, many other animal studies have found that Mel significantly improves the levels of some of the key inflammatory and oxidative markers associated with periodontal tissue destruction, such as TNF-α [21, 22, 37], IL-1β [17, 21, 22, 37], MDA [21, 22, 39], GSH [21, 39].

Bone formation and resorption are intertwined processes in normal tissue. The enzyme ALP is one of the most commonly used biochemical parameters in bone mineralisation [30]. ALP is released by many structural cells, particularly osteoclasts, as an indicator of bone formation [40]. In our study, ALP levels decreased in the RT and periodontitis groups, and increased in the treatment groups to levels approaching those of the control group. Similarly, an increase in b-ALP levels was observed after Mel administration in the periodontitis rat model of Arabacı et al. [20]. Margulies et al. [41] investigated the effects of Ami application on ALP expression levels after irradiation in vitro and found that Ami selectively conferred significant radioprotection in osteoblast cells. In several rat studies [42, 43] using the ALP assay to test the osteogenic potential of calvarial osteoblast cells, it was observed that pretreatment with Ami resulted in a slight but statistically significant increase in ALP production

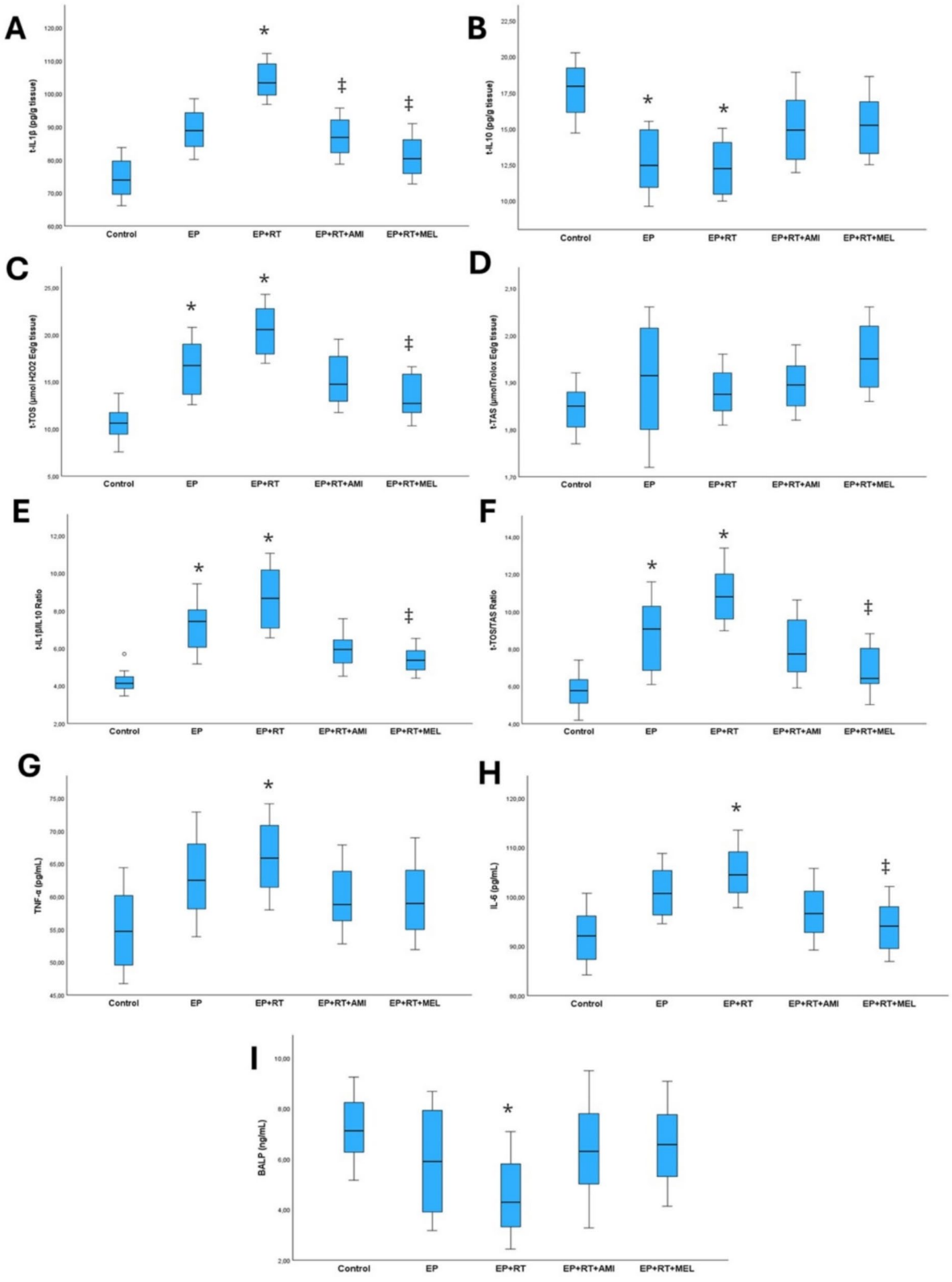

Fig. 5 Comparison of Biochemical Parameter Results Between Groups (EP: Experimental Periodontitis, RT: Radiotherapy, AMI: Amifostine, MEL: Melatonin. **A**: IL-1β levels, **B**: IL-10 levels, **C**: TOS levels, **D**: TAS levels, **E**: IL-1β/IL-10 ratio levels, **F**: TOS/TAS ratio levels, **G**: TNF-α levels, **H**: IL-6 levels, **I**: b-ALP levels. Footnote symbols (*, ‡) signify statistically significant differences between the groups. (*) intergroup, compared to Control; (‡) intergroup, compared to EP + RT; statistically significant difference ($p < 0.05$).)

compared to irradiated cells. However, prophylactic Ami still significantly decreased ALP production compared to the control group (2.5x reduction) ($p<0.05$) [42]. In this study [42], even with fractionation, Ami could not completely block the inhibitory effect of ionising radiation on normal osteoblast osteodifferentiation as measured by ALP production. This result suggests that it may be caused by the administration of Ami at a different dose than in our study, or by different dose and type of RT and/or fractionation.

Molecules that play a fundamental role in periodontal bone destruction are RANKL, RANK and OPG. While RANKL plays a role in bone destruction by stimulating osteoclastic differentiation and activation, OPG has the opposite biological effect, causing a decrease in osteoclastic activity [44]. Pro-inflammatory cytokines with the inflammatory response inherent to periodontitis and/or RT, induce osteoclastogenesis by reducing OPG production in stromal cells and osteoblasts and stimulating RANKL expression [45]. Thus, in both cases, the process of bone destruction begins. Our results confirmed this process, both with the increase in the number of RANKL-positive stained cells in the Ep and Ep+Rt groups and with the results of our morphometric measurements. The fact that OPG, a marker of bone formation, also increased slightly suggests that bone formation and destruction are an integral whole. Furthermore, considering our experimental duration, it would not be surprising to see an increase in early results for this parameter. In a number of animal studies [20, 26, 37] RANKL and/or the RANKL/OPG ratio have been shown to reach near normal levels with Mel. In addition to these studies, other studies [22, 28, 29, 39, 46] have found a significant reduction in the level of alveolar bone loss with Mel supplementation. Although Ami has been approved by the FDA for use in xerostomia and mucositis [11, 13], it has become a promising drug to counteract the destructive effects of radiotherapy on bone tissue [15, 18, 41]. In this context, many studies suggest that Ami may be effective in repairing and healing the destruction that occurs in bone tissue [14–18]. In many animal studies examining bone and tissue mineral density [14–16], Ami showed a statistically significant improvement compared with groups receiving RT, and it was concluded that no difference was observed between Ami and control groups in this respect. Some studies [17, 18, 47] have also looked at osteocyte number, osteoid volume and bone volume. Mandibles treated with Ami had statistically significantly higher osteocyte numbers and bone volume/tissue volume ratio compared to irradiated mandibles [47]. However, when we reviewed the literature, we found only one study that showed a protective effect of Ami on bone tissue at the level of RANKL and/or OPG. This study by Zhang et al. [48] showed that RANKL/OPG levels increased with RT and that Ami had a protective effect by inhibiting osteoclast differentiation. The radioprotective effect of Ami may be related to its suppression of DNA damage and reactive oxygen species release [12, 48]. However, it is clear that further studies are needed to prove the effect of Ami on bone metabolism through these parameters.

RT not only affects tumour tissue, it also damages healthy tissue. It acts on bone tissue mainly through fibrosis and apoptosis mechanisms. One of the most commonly used apoptosis indicators is Caspase [49]. Caspase-3 is a cysteine protease activated during apoptosis [49]. Active Caspase indicates the active and acute period of the detected cells. In this way, it will be possible to detect cell death more accurately. In our study, the rate of active cells with Caspase-3 positivity increased in the irradiated groups and decreased in both treatment groups with a statistical significance close to the control group. This clearly demonstrated the effect of RT on apoptosis and the antiapoptotic effect of drugs. Sola et al. [50] aimed to test Mel's protective properties by culturing gingival cells from Wistar rats. Mel has been shown to have antioxidant and antiapoptotic effects. In another study carried out by our team [22], it was observed that the apoptotic activity decreased with the administration of Mel to rats with periodontitis. However, in the animal study by Yuce et al. [46], which used a similar methodology to produce periodontitis and included diabetes, it was found that alveolar bone loss decreased with Mel application in rats with diabetes and periodontitis, supporting our results; it was observed that it had no effect on iNOS, IL-1β and bax levels. The reason for this difference may be that RT is more dominant at the level of apoptosis compared to diabetes and has a clearer effect on a marker such as the bax gene. In an in vitro study [41], the radioprotective activity of Ami was demonstrated with Caspase-3 in cell culture. In an experimental animal model [51], Ami was shown to increase Caspase-3 expression in the colon tissue of irradiated mice. However, there are no studies investigating the antiapoptotic effect of Ami on irradiated alveolar bone tissue.

In literature, there were relatively few studies comparing Ami and Mel in bone tissue. In an animal study by Çakir et al. [23], which is the most methodologically similar to our study, the biomechanical effect of Mel on irradiated bone tissue was found to be similar to that of Ami. However, this shows an effect on bone mineral density at the biomechanical level. Our study is the first to investigate the effects of Ami and Mel on RT and periodontitis-induced damage in irradiated bone tissue from a biochemical, histochemical and morphometric perspective. Topkan et al. [24] reported that Mel had a more pronounced radioprotective effect than Ami in preventing radiation-induced epiphyseal growth plate damage

in rats; however, combined use did not provide any additional benefit.

The results of this experimental study showed that prophylactic use of Ami and Mel ameliorated the adverse effects of periodontitis and RT. If we compare these two agents, Mel can be considered an important alternative to Ami in terms of periodontal radioprotective efficacy, thanks to its strong antioxidant and immunomodulatory properties, and considering its very low side effect profile. In this context, and taking into account factors such as patient comfort and cost of treatment, it can be said that our findings can form the basis for new and more extensive experimental and clinical studies in the future.

However, there are limitations to this comprehensive study. Experimental periodontitis occurs earlier and is more rapid and destructive than human periodontitis [52]. Therefore, a definitive attribution of our results to human periodontitis may be misleading. Secondly, although the inclusion of only female rats was necessary for standardization, it may not be correct to generalize the results to both sexes. Thirdly, in our study, the radiation therapy was given as a single dose. Fractionation and/or dose differences may be valuable in diversifying current results. Finally, unfortunately, there is no consensus on protocols for the administration of Mel and Ami in experimental animal models.

Conclusion

This experimental study showed that both Mel and Ami can significantly limit RT-induced periodontal inflammatory stress and bone loss by suppressing RANKL-related osteoclastic activity and oxidative stress. In this context, Mel may be an important alternative to Ami in terms of radioprotective activity.

Abbreviations

RT	Radiotherapy
Ami	Amifostine
Mel	Melatonin
RTEU	Recep Tayyip Erdogan University
OPG	Osteoprotegerin
IL	Interleukin
TNF-α	Tumor necrosis factor alpha
b-ALP	Bone Alkaline Phosphatase
TOS	Total Oxidative Status
TAS	Total Antioxidant Status
CEJ	Cemento-enamel junction
BC	Bone crest
FR	Furcation roof
FAC	Furcation alveolar crest
FDA	Food and Drug Administration

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-024-05251-0.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

Supplementary Material 13

Supplementary Material 14

Supplementary Material 15

Supplementary Material 16

Supplementary Material 17

Supplementary Material 18

Supplementary Material 19

Supplementary Material 20

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Author contributions

N.Y.: Conceptualization, methodology, writing, prepared all figures; O.K.: Conceptualization, methodology, editing, S.Y.R.: Radiotherapy protocol; T.M.: Histological analyses; K.A.: Biochemical analyses; L.T.: Histological analyses; A.Y.: Biochemical analyses. All authors reviewed the manuscript.

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Data availability

Data is provided within the manuscript and supplementary information files.

Declarations

Ethics approval and consent to participate

This study was approved by the RTEU Local Ethics Committee with the decision letter 2023/23 on 25.04.2023.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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