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ASSESSMENT OF THE RADIOPROTECTIVE EFFECTS OF AMIFOSTINE AND MELATONIN ON HUMAN LYMPHOCYTES IRRADIATED WITH GAMMA-RAYS *IN VITRO**

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Radioprotective effects of amifostine and melatonin as well as their ability to modulate the level of spontaneous and γ-irradiation-induced genetic changes on human peripheral blood lymphocytes were investigated using the cytokinesis-block micronucleus (CBMN) assay and sister chromatid exchange (SCE). Parallel blood samples were pre-treated with amifostine, melatonin and their combination for 30 minutes. Negative controls were also included. After the treatment with radioprotectors, one blood sample of each experimental group was exposed to γ-rays from a ^{60}Co source. The radiation dose absorbed was 2 Gy. Our research confirmed the radioprotective effects of both chemicals *in vitro,* with no significant genotoxicity. Pre-treated irradiated blood samples showed a decrease in the total number of micronuclei (MN) and in the number of cells with more than one MN. They also showed significantly lower mean SCE values. This study shows that it is possible combine these radioprotectors by adjusting the doses of amifostine to achieve the best radioprotective effect with as few side effects as possible. However, further *in vitro* and clinical studies are needed to clarify their mechanisms of action and possible interactions.

KEY WORDS: *ionising radiation, peripheral blood, radioprotectors*

Recent technological advancement has made it possible to produce high therapeutical doses of radiation and/or chemotherapy which have increased the chances to cure a patient or prolong the emission of a number of neoplasms (1).

Ionising radiation causes a variety of changes in the living cell, which depend on the absorbed dose, duration of exposure, interval of exposure, and susceptibility of the tissue. Ionising radiation forms radicals in the DNA (direct effect) and in the surrounding water molecules of the hydration shell of the DNA (indirect effect), which in turn destroy DNA (2).

The use of ionising radiation in cancer therapy may lead to transient and/or permanent injury to normal tissues within the treatment field. The magnitude of damage depends both on the volume of tissue irradiated and the dose of radiation delivered (3). To increase the therapeutic index of radiation therapy, various modes of radioprotection have been developed that selectively reduce cytotoxic effects to normal tissues (4).

Because radiation-induced cellular damage is attributed primarily to the harmful effects of free radicals, molecules with radical scavenging properties are particularly promising as radioprotectors (5, 6).

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However, most of them produce serious side effects, and some are considered to be toxic at the doses required for radioprotection (7).

The most effective compound studied for its radioprotective efficacy is amifostine or S-2-(3-amino-propylamino) ethylphosphorothioic acid (synonyms: WR-2721, ethyol). According to preclinical studies, amifostine protects normal tissues from the cytotoxic effects of radiotherapy. It is a pro-drug that is dephosphorylated in the tissue by alkaline phosphatase to a free thiol, the active metabolite WR-1065 (8-10). The protection of cell damage by WR-1065 is thought to occur through scavenging oxygen-derived free radicals (induced by ionising radiation and certain types of chemotherapy) and hydrogen donation to repair damaged target molecules (11). Although amifostine is the best-known radioprotector and cytoprotector that has been incorporated into general oncology practice, it still has a number of undesirable side effects, severe enough to limit the amount of the drug tolerated (12, 13). Moreover, the data regarding its action at the cellular level remain unclear (4).

In the last decade there have been reports on the radioprotective ability of melatonin, an endogenous compound mainly synthesised by the pineal gland in the human brain. Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitously acting molecule with several functions. It is highly lypophilic and somewhat water-soluble as well. The widespread cellular distribution of melatonin may allow it to interact with all molecules, thereby reducing oxidative damage to molecules both in lipid and aqueous environments of the cell. It was reported that melatonin directly scavenges the highly toxic hydroxyl radicals both *in vitro* and *in vivo*, as well as several other reactive species such as singlet oxygen, peroxynitrite anion (5, 6). Its free radical–scavenging capacity is mediated by electron donation (14). Results of different studies indicate that both the acute and chronic toxicity of melatonin is extremely low (5, 6).

This study investigated the effects of amifostine and melatonin on the extent of spontaneous as well as radiation-induced DNA damage in human peripheral lymphocytes irradiated with γ-rays *in vitro*. DNA damage was estimated using two well-established biomarkers (15): the cytokinesis-block micronucleus assay (CBMN) and the sister chromatid exchange (SCE) analysis. In addition to the assessment of DNA and chromosome damage, we also analysed lymphocyte proliferation *in vitro*.

MATERIAL AND METHODS

The study was performed on peripheral blood (V=40 mL) obtained from a healthy non-smoking male donor (aged 25 years) by venipuncture into heparinised tubes (Becton Dickinson, USA).

Parallel samples (V=5 mL) were pre-treated for 30 minutes with amifostine (7.7 mmol L^{-1}, Sigma), melatonin (2 mmol L^{-1}, Sigma) and their combination. Negative controls (untreated samples) were also included. After the treatment with radioprotectors, one blood sample from each experimental group was exposed to γ-rays from a ^{60}Co source (Alcyon, CGR-MeV) at room temperature. For this purpose, vacutainer containing blood sample was mounted on an acrylic phantom (dimensions: 20 cm x 20 cm x 15 cm) at the depth of 5.5 cm and was placed transversally to the axis of irradiation. The radiation field was 15 cm x 15 cm, and the distance between the surface of the phantom and the source of radiation was 80 cm. Total exposure to radiation lasted 1.24 min and the absorbed dose was 2 Gy. Other radioprotector-treated blood samples were not irradiated, but they were handled in the same manner. Amifostine and melatonin were used in their therapeutic concentrations. The radiation dose of 2 Gy was chosen because it is the standard fraction applied daily in fractionation regimens in radiotherapy.

Lymphocyte cultures for the CBMN assay and the SCE analysis were established from each blood sample.

CBMN assay was performed using a standard protocol, as described by *Fenech and Morley* (16). The frequency of MN induction was evaluated by scoring 1000 binucleated lymphocytes per sample. Slides were analysed blind at 1000x magnification. Cell were scored according to the criteria outlined by *Fenech et al.* (17). The data are expressed as the number of MN per 1000 binucleated cells as well as the frequency of binucleated cells containing one or more MN. To provide data regarding proliferation kinetics, the frequencies of mono-, bi-, tri- and tetranucleate cells per each treatment group were determined. A nuclear division index (NDI) was calculated according to the formula proposed by *Eastmond and Tucker* (18) as follows: $NDI=(1xM_1+2xM_2+3xM_3+4xM_4)/N$, where M_1 to M_4 represent the number of cells with one to four nuclei and N is the total number of cells scored. In our study N was 1000 cells. The statistical significance of the differences between the observed

MN frequencies was tested using the χ^2-test. The level of statistical significance was set at $p<0.05$.

The SCE analysis was performed according to the standard protocol (19). A total of 100 well-spread, second-division metaphases (50 per replicate) were examined to determine the SCE frequency per sample. Every point of exchange was counted as an SCE. Exchanges at the centromere were included only when twisting at this point could be ruled out. The results of SCE were expressed as mean±SD for each analysed sample. The data were analysed using the Statistica 5.0 (StatSoft) package. Multiple comparisons between groups were done by means of ANOVA with the Scheffé post-hoc test and considered significantly different when p values were less than 0.05.

In addition to SCE examination, the BrdU differential staining technique was used to assess the effects of ionising radiation and radioprotectors on cell replication. For each sample the proportion of the first (M_1), second (M_2) and third (M_3) metaphases was determined in 100 consecutive metaphases. The proliferation rate index (PRI) was calculated as follows: $PRI=(1M_1+2M_2+3M_3)/100$ (20). Statistical significance of data obtained for cellular kinetics was tested using the χ^2-test.

RESULTS

CBMN assay

The background level of MN in blood donor was 7 MN per 1000 binucleated cells (Table 1). Amifostine in the concentration of 7.7 mmol L^{-1} and melatonin in the concentration of 2 mmol L^{-1} induced a similar number of MN in non-irradiated blood samples. Although slightly elevated with respect to the control sample, the difference in MN was not statistically significant. Moreover, combined application of both radioprotectors in the non-irradiated blood sample did not cause any measurable changes (Table 1). These results suggest that both chemicals in tested concentrations did not induce significant damage to lymphocyte genome *in vitro*.

Table 1 shows that γ-irradiation (2 Gy) increased the number of MN in exposed lymphocytes over 12-fold (89 MN per 1000 binucleated cells). In blood samples irradiated in the presence of single amifostine and melatonin, as well as their combination, protective effects were observed. Both compounds caused a clear reduction in frequency of radiation-induced MN. Moreover, the proportions of cells containing more than one MN were also significantly decreased (Table 1). According to our observations, single melatonin offers better protective effects to γ-irradiated human lymphocytes *in vitro* than single amifostine. However, the best radioprotective effects *in vitro* were observed when the combination of both substances was added to blood samples prior to irradiation. Differences between the total number of MN recorded after treatments with single melatonin and combination of amifostine and melatonin in irradiated samples were not statistically significant (Table 1).

Table 1 *Results of the micronucleus assay of blood samples irradiated with gamma-rays (2 Gy) after in vitro pre-treatment with radioprotectors amifostine (7.7 mmol L^{-1}), melatonin (2 mmol L^{-1}), and their combination. Non-irradiated samples treated with the same radioprotectors were studied simultaneously, and corresponding negative controls were also included.*

	Number of cells		Number of cells with			ΣMN	Number of cells				NDI
	without MN	with MN	1 MN	2 MN	3 MN		M_1	M_2	M_3	M_4	
IRRADIATED SAMPLES (absorbed dose = 2 Gy)											
Amifostine	959	41*	33	6	2	51*	109	719	86	86	2.15
Melatonin	967	33*	31	2	-	35*	112	746	75	67	2.10
A + M	970	30*	26	4	-	34*	124	755	64	57	2.07
Control	927	73*,↑	57	16	-	89*,↑	120	759	59	62	2.06
NON-IRRADIATED SAMPLES											
Amifostine	987	13	13	-	-	13	57	804	64	75	2.16
Melatonin	988	12	10	1	-	12	92	748	79	81	2.15
A + M	993	7	7	-	-	7	148	715	54	83	2.07
Control	993	7	7	-	-	7	105	752	63	80	2.12

MN - micronucleus; M_1 to M_4 represents the number of lymphocytes with one to four nuclei; NDI (Nuclear Division Index); $NDI=(1M_1+2M_2+3M_3+4M_4)/1000$ cells counted in total. * - significantly increased compared to non-irradiated sample pre-treated with the same radioprotector; ↑ - significantly increased compared to other irradiated samples ($p<0.05$; χ^2 – test).

NDI was calculated to assess the effects of radioprotectors on the proliferative capacity of irradiated human lymphocytes. The values of NDI in negative controls pre-treated with single amifostine or melatonin were slightly higher than in control sample, suggesting enhanced proliferation of lymphocytes. However, it was observed that γ-radiation at a dose of 2 Gy caused a delay in cell proliferation, as manifested by a change in relative numbers of M_1 to M_4 cells. Our results indicate that radioprotectors modulate the proliferative activity, especially when added in combination. The same value of NDI in γ-irradiated and non-irradiated lymphocytes pre-treated with amifostine and melatonin may reflect a successful overcome of cytotoxic effects caused by ionising radiation (Table 1).

SCE analysis

In this study we also wanted to see whether radioprotectors, alone or in combination, induced any changes in the incidence of SCE in non-irradiated and γ-irradiated human lymphocytes *in vitro*. The results of SCE analysis are shown in Table 2 and Figure 1.

The background level of SCE in 100 metaphases was 4.01±1.40 in a range of 1 SCE to 8 SCE per cell (Table 2). Our results demonstrate that the tested doses of amifostine and melatonin do not induce genotoxic response in non-irradiated human lymphocytes *in vitro*. Namely, the frequencies of SCE in blood samples pre-treated with amifostine or melatonin alone did not differ significantly from SCE frequences recorded in the control sample. We also observed that the combination of both radioprotectors led to a drop in SCE frequency (3.73±1.54; range 1 SCE to 7 SCE per cell) with respect to samles pretreated with either radioprotector alone and to negative control. However, this difference was statistically significant only with the sample pre-treated with melatonin. These results indicate that the combination of both radioprotectors reduced the amounts of free radicals and even diminished the baseline DNA damage present at the moment of blood sampling.

As reported in Table 2, γ-irradiation (2 Gy) caused a significant increase in SCE frequency (6.97±2.24) and range (3 SCE to 16 SCE per cell) in the exposed lymphocytes compared to non-irradiated negative control. The addition of either radioprotector alone or in combination before γ-irradiation significantly reduced SCE frequency. According to our results, the best radioprotective effects on γ-irradiated lymphocytes *in vitro* were observed in the sample pre-treated with the combination of both chemicals. However, the differences between mean SCE frequencies obtained for single radioprotectors and their combination were not statistically significant (Table 2). Figure 1 shows the distribution of SCE in all analysed samples, confirming the above observations.

The results of the cell-cycle analysis show that γ-irradiation slowed down the lymphocyte cell cycle *in vitro,* while both radioprotectors altered this effect to some extent. The delay in cell turnover times was

Table 2 *Frequency of sister chromatid exchanges (SCE) and cell cycle kinetics in human peripheral blood lymphocytes irradiated with gamma-radiation after pre-treatment with amifostine, melatonin, and their combination in vitro. Non-irradiated samples treated with the same radioprotectors were studied simultaneously, and corresponding negative controls were also included.*

	SCE in 100 cells (mean±SD)	SCE range	Number of cells in			PRI
			M_1	M_2	M_3	
IRRADIATED SAMPLES (absorbed dose = 2 Gy)						
Amifostine	5.00±1.65	2-9	23	60	17	1.94
Melatonin	5.16±1.45	3-8	22	67	11	1.89
A + M	4.72±1.73	1-9	25	58	17	1.92
Control	6.97±2.24↑	3-16	28	52	20	1.92
NON-IRRADIATED SAMPLES						
Amifostine	4.05±1.34	1-8	22	62	16	1.94
Melatonin	4.42±1.72	1-9	19	59	22	2.03
A + M	3.73±1.54*	1-7	17	65	18	2.01
Control	4.01±1.40	1-8	15	64	21	2.06

SCE frequency was scored in 100 cells per sample. Proliferation Rate Index, (PRI) was determined using the formula PRI = $(1M_1 + 2M_2 + 3M_3)/100$ where M_1, M_2, and M_3 corresponded to the number of cells in first, second and third in vitro mitotic division. ↑- significantly increased with respect to other irradiated samples. *- significantly decreased compared to non-irradiated sample pre-treated with melatonin; ($p<0.05$; ANOVA, post-hoc Scheffé test).

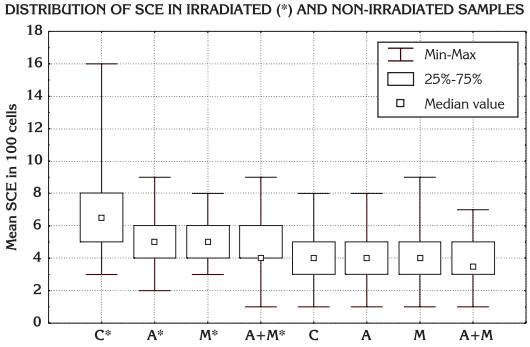

Figure 1 *Distribution of mean SCE in blood samples pre-treated with amifostine (A), melatonin (M), and their combination (A+M). (*) Irradiated samples; (C) negative controls.*

manifested as a change in the relative frequencies of M_1, M_2 and M_3 metaphases (Table 2). Amifostine given alone before irradiation slightly enhanced the proliferation of lymphocytes, whereas melatonin slowed it down, but not significantly. In combination, the two radioprotectors did not significantly affect the proliferation of treated lymphocytes compared to control. As shown in Table 2, their effects on the cell cycle did not significantly differ either between them or with respect to the control. Amifostine induced a slight, but statistically not significant delay in the first and second division *in vitro*, compared to other samples analysed (Table 2).

DISCUSSION

There is a continued interest and need to identify and develop non-toxic radioprotective compounds. An efficient radioprotector could prove useful in occupational and therapeutic settings where ionising radiation is used or where exposure occurs; after nuclear accidents which leave radioactivity in the environment; and during space travel to protect astronauts from the effects of high doses of radiation associated with solar flares (21).

This study focused on two compounds, amifostine and melatonin, and their ability to modify the level of spontaneous and γ-irradiation-induced genetic changes on human peripheral blood lymphocytes. The main result of our research was the confirmation of the radioprotective effects of both chemicals *in vitro,* with no significant genotoxicity, as revealed using two well-established and sensitive biomarkers: the CBMN assay and the SCE analysis.

Our goal was also to evaluate the cytogenetic effects of single exposure to γ-radiation of 2 Gy, because it is a standard fraction applied daily to cancer patients in fractionation regimens in radiotherapy. This study confirmed the ability of γ-radiation to increase the incidence of MN in exposed human lymphocytes, as reported previously in many other *in vitro* and *in vivo* investigations (22-26).

Reports on SCE induction after exposure to ionising radiation *in vivo* and *in vitro* are contradictory. Although ionising radiation is known to damage DNA, it was observed early on that it is a poor inducer of SCEs. The extent of stimulation of SCE by *in vitro* irradiation varies from no increase to levels as high as two to three times higher than the baseline level (27). Our results are in good agreement with these observations: single *in vitro* exposure of human lymphocytes to γ-irradiation of 2 Gy in our study nearly doubled the mean SCE frequency measured in the non-irradiated sample. Our result (6.97±2.24) is even lower than the level recorded in previous study by *Iijima and Morimoto* (28) (8.6±0.6), who also exposed human lymphocytes to the same dose of γ-rays. These data suggest that γ-irradiation does not induce long-lived lesions in the DNA of G_0 lymphocytes that can be expressed as SCE in subsequent mitoses.

We noticed that γ-irradiation caused delays in different phases of the cell cycle. These observations are consistent with earlier reports (28, 29). In general, different forms of ionising radiation delay lymphocyte cell cycle. *Purrot et al.* (30) have estimated that after exposure to radiation, lymphocytes suffer an average mitotic delay of about 1 h per gray. The data from previous studies indicate that mutagens that cause larger inductions of SCE also induce long cell-cycle delays (31) as well as that higher PRI correlates with lower SCE frequency (20). These delays in cell cycle allow the cell to repair DNA and try to reduce the adverse effects of radiation (32). Our results confirm these observations.

To our knowledge, there have been no published studies investigating possible synergistic radioprotective effects of amifostine and melatonin using cytogenetic biomarkers. While the cytogenetic effects of melatonin were studied previously, the reports on amfiostine effects on the cellular level are limited. Melatonin was found to be an efficient radioprotector when studied on animal and human models *in vitro* and *in vivo*. *Badr et al.* (14) observed that MN frequency in polychromatic erythrocytes and the number of chromosomal

aberrations in spermatogonia and spermatocytes were significantly reduced in melatonin-pre-treated irradiated mice compared to those treated with diluent only. *Vijayalaxmi et al*. (33-35) reported a significant decrease in genetic damage, as well as lower MN frequency in human blood lymphocytes pre-treated with melatonin and exposed to 1.5 Gy γ-radiation. Moreover, their results suggest that melatonin at the dose levels 0.05 mmol L^{-1} to 1.0 mmol L^{-1} did not significantly increase SCE frequency (35). In our study, we used a higher concentration of melatonin (2 mmol L^{-1}) and a higher radiation dose (2 Gy) than reported in previous *in vitro* studies. Even so, it confirmed the significant radioprotective effects and no genotoxicity of melatonin. Moreover, our results for SCE (mean SCE:4.42±1.72) are very similar to those obtained by *Awara et al*. (36) (mean SCE: 4.90±0.37), who investigated possible anti-mutagenic effects of melatonin on carbamazepine-treated human peripheral lymphocytes.

Even though many other substances with assumed cytoprotective effects are the subjects of laboratory and/or clinical studies, at the moment there is no ideal protective strategy to be universally employed in patients receiving radio- or chemotherapy. Many recent clinical studies have shown that amifostine is a useful radioprotector (4, 5, 9-13). However, no data regarding its possible genotoxic effects are available. The results of our study *in vitro* indicate that exposure to a single dose of amifostine is not associated with significant genotoxicity in human peripheral lymphocytes. No statistically significant increase in the frequency of SCE and MN were found in treated samples as compared with control. Furthermore, our results showed noteworthy radioprotective effects of amifostine when applied prior to γ-irradiation. Similar results were reported by *Littlefield and Hoffman* (37), who observed that amifostine metabolite WR-1065 caused a significant decrease in chromosome aberrations and micronuclei in G_0 human lymphocytes irradiated with X-rays and neutrons.

Although amifostine was tolerated well in many clinical trials, it is expensive, its use is limited to clinical settings because it must be given intravenously, and it has various undesirable side effects (nausea, vomiting, flushes, mild somnolence, hypocalcaemia and hypotension) (10, 21).

We believe that combined use of amifostine and melatonin (or other radioprotectors) would in some circumstances prove beneficial and might overcome a part of undesired side effects produced by amifostine alone. The results of our study, where amifostine and melatonin were added simultaneously to blood samples prior to the γ-irradiation, indicate that both compounds act synergistically to reduce DNA damage. Therefore, in some conditions, it would be reasonable to administer them in combination, exploiting their best radioprotective properties, since each acts as very specific antioxidant. An important advantage of amifostine over melatonin is its differential and selective uptake only in normal, but not in tumor cells (4, 12). In contrast, melatonin, as amphiphilic molecule, can enter any cell type and even cell compartment (38). The combination of these substances could also be beneficial because amifostine itself does not reduce anti-tumor activity of other drugs (10); whereas some studies indicate that melatonin itself has an antitumour effect, as it slows down the cell cycle (39). Another feature that possibly increases the efficiency of melatonin in reducing oxidative stress is that the second and third generation of its metabolites seem to be efficient scavengers (40). Moreover, melatonin may also influence DNA repair enzymes directly and indirectly stimulate intracellular signals to activate genes responsible for protein synthesis in DNA repair (5).

In conclusion, the data obtained in our study suggest that amifostine and melatonin prevent DNA damage inflicted by γ-radiation in human peripheral blood lymphocytes *in vitro*, if given before exposure. They also suggest that amifostine doses should be adjusted for optimal radioprotective effect in healthy cells, with as few side effects in cancer patients as possible. Despite its limitations, this study has produced new data that contribute to the growing body of evidence that both amifostine and melatonin are effective radioprotectors. However, before they are approved for clinical use, further experimental studies using different cytogenetic and molecular biomarkers at the same time and well designed clinical studies are needed to clarify the exact mechanisms of their radioprotective action and possible interactions.

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Sažetak

RADIOPROTEKTIVNI UČINCI AMIFOSTINA I MELATONINA NA LJUDSKE LIMFOCITE IZLOŽENE γ-ZRAČENJU U UVJETIMA *IN VITRO*

Primjena zračenja u liječenju zloćudnih bolesti (radioterapija) značajno pridonosi preživljenju bolesnika, ali izaziva i niz neželjenih učinaka na zdrave stanice i tkiva. Nuspojave ionizirajućeg zračenja mogu se značajno smanjiti s pomoću kemijskih spojeva s antioksidativnim učinkom koji djeluju kao 'hvatači' slobodnih radikala i štite vrlo osjetljivu molekulu DNA. Među spojeve s pretpostavljenim ili dokazanim radioprotektivnim učincima ubrajaju se amifostin i melatonin, koji su predmet istraživanja ovog rada. U literaturi nema dovoljno podataka o njihovoj genotoksičnosti ni međusobnim interakcijama. Stoga smo primjenom mikronukleusnog testa i analize izmjena sestrinskih kromatida (SCE) u uvjetima *in vitro* istražili djelovanje amifostina i melatonina na genom neozračenih i ozračenih ljudskih limfocita periferne krvi. Pojedinačno ili u kombinaciji, amifostin i melatonin dodavani su u uzorke pune krvi 30 minuta prije jednokratnog ozračivanja γ-zrakama izvora ^{60}Co. Doza zračenja iznosila je 2 Gy, a koncentracije radioprotektora odgovaraju onima prije upotrebljavanim u kliničkoj primjeni ili u preliminarnim istraživanjima na ljudskoj populaciji. Ozračena krv kultivirana je 72 h u uvjetima *in vitro* prema standardnim protokolima za mikronukleusni test i test izmjena sestrinskih kromatida. Učinci amifostina i melatonina usporedo su istraživani i na kontrolnim, neozračenim uzorcima krvi. Dobiveni rezultati upućuju na značajno smanjenje ukupnog broja mikronukleusa i smanjenje udjela stanica s više od jednog mikronukleusa te sniženje ukupnog broja i raspona izmjena sestrinskih kromatida u pretretiranim uzorcima krvi. Potvrđen je vrlo dobar radioprotektivni učinak svakog spoja testiranog posebno, a ujedno je utvrđeno da oba spoja sinergistički djeluju na snižavanje razina oštećenja izazvanih u genomu limfocita pod utjecajem γ-zraka. S obzirom na to da primjenom citogenetičkih testova nije dokazana genotoksičnost navedenih radioprotektora za ljudske limfocite u uvjetima *in vitro*, dobiveni rezultati govore u prilog daljnjih istraživanja ovih spojeva i njima srodnih tvari u uvjetima *in vivo* te njihove moguće zajedničke primjene u kliničkoj praksi.

KLJUČNE RIJEČI: *ionizirajuće zračenje, periferna krv, radioprotektori*

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