CHAPTER 16

PROTECTION BY CHEMICALS AGAINST RADIATION-INDUCED BYSTANDER EFFECTS

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Abstract: The purpose of this work was to study possible mechanisms of radiation-induced bystander effect proceeding from its modification using different radioprotective substances: melanin, melatonin and α-tocopherol. All substances were able to statistically significant decrease the damaging effect of bystander factor from irradiated cells on non-irradiated. The protective effect against bystander irradiation was much less than against direct irradiation. Melatonin showed the best protective effect against both direct and bystander irradiations, and vitamin E – the least. According to the results, bystander factor may have physical component and oxidative nature.

Keywords: radiation-induced bystander effect; melanin; melatonin; α-tocopherol (vitamin E); HPV-G cells; radioprotection

Introduction

In April, 2006, 20 years have been passed since the explosion of the fourth block of Chernobyl nuclear power plant. This accident affected millions of people, and large territories were contaminated by radionuclides. As a result, background radiation levels increased, and people from contaminated territories are living constantly in low dose radiation conditions. The situation became more complicated after the discovery of non-direct radiation effects, which were not taken into account in procedures of radiation dose evaluation. Bystander effect is one of such indirect radiation effects.

The radiation-induced bystander effect is a phenomenon whereby the cellular damage is expressed in unirradiated neighboring or bystander cells,

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connected or not to an irradiated cell or cells. The mechanism of this transfer of damage from irradiated cells to non-irradiated neighbours is still unknown. There is evidence that the bystander effect may have at least two separate pathways: through gap junctions (Nagasawa and Little, 1992; Deshpande et al., 1996; Lorimore et al., 1998) or by cell-culture mediated factors (Mothersill and Seymour, 1997). The nature of these factors remains unknown, but it was hypothesised that it may be a protein. A series of studies (Lehnert and Goodwin, 1997; Narayanan et al., 1999) suggest a mechanism in which the irradiated cells secrete cytokines or other factors that act to increase intracellular levels of reactive oxygen species in unirradiated cells. Barcellos-Hoff and Brooks (2001) have also hypothesised that TGFβ1, an extracellular sensor of damage, may also be involved in the bystander effect. Another possible mediator of the bystander effect is the apoptosis inducing factor, secreted by mitochondria in response to oxidative stress (Kroemer, 1997).

Both high LET alpha-particles and low LET γ-irradiation have been shown to induce this effect; however, it remains unclear whether the same signal is involved for both types of radiation. Also recently, bystander effect was induced using UV- (Dahle et al., 2005) and laser (Mosse et al., 2006) irradiation.

This effect has been studied extensively since 1992 by many researchers, but to date there have been no any published reports on the use of radioprotectors in modifying bystander responses. All studies are focused on understanding possible mechanisms of bystander effects and the nature of bystander factor. Meanwhile, modification of this phenomenon may help to understand its possible mechanisms, proceeding from the properties of modifying substances.

We used three different radioprotective substances to modify radiation-induced bystander effect – melanin, melatonin and α-tocopherol. All these substances were shown to have antioxidant properties. Melanin is a photoprotective pigment, which is also able to take-up and retain for a long period many xenobiotics and to convert all types of physical energy into heat. Melatonin is a neurohormone, which is involved in reproductive physiology, control of circadial rhythms, immune function and cancer growth. α-Tocopherol (vitamin E) participates in stabilisation of biological membranes and prevents many deceases. Its main protective effect is connected with reparation processes. All these substances are non-toxic, widespread, effective in low concentrations and able to reduce genetic effects of radiation.

The aim of this study was to assess the direct and bystander effect of low level γ-radiation on human keratinocytes, immortalised with HPV-virus *in vitro* and the possibility to modify these effects using radioprotective substances.

Materials and Methods

CELL CULTURE

The HPV-G cell line is a human keratinocyte line, which has been immortalised by transfection with the HPV virus, rendering the cells p53 null. They grow in culture to form a monolayer, display contact inhibition and gap junction intracellular communication.

HPV-G cells were cultured in Dulbecco's MEM: F12 (1:1) medium supplemented with 10% Foetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine and 1 μg/mL hydrocortisone. The cells were maintained in an incubator at 37°C, with 95% humidity and 5% carbon dioxide and routinely subcultured every 8–10 days. When 80–100% confluent, the medium was poured from the flask and replaced with 1:1 solution of versene (1nM solution) to trypsin (0.25% in Hank's Balanced Salt Solution) (Gibco, Irvine, UK) after washing with sterile PBS. The flask was placed in the incubator at 37 degrees Celsius for about 11 min until the cells started to detach. The flask was then shaken to ensure that all cells had been removed from the base of the flask. The cell suspension was added to an equal volume of DMEM F12 medium to neutralise the trypsin. From this solution new flasks could be seeded at the required cell quantity.

Radioprotective Substances

MELANIN

Melanin was isolated from animal hair by Belarus Pharmaceutical Association (Minsk). By analysis, it was determined to be eumelanin. Both ortochinoid and indolic fragments were present. Melanin was added to the cell medium at 10 mg/L concentration 30 min–1 h before irradiation for directly irradiated cells and 1 h after irradiation to the irradiated cell-culture medium (ICCM) before filtration for bystander recipient cells.

MELATONIN

Melatonin (*N*-Acetyl-5-methoxytryptamine) was received from Sigma (Germany) as white powder, synthetic. Melatonin was added to the cell medium at 10 mg/L 30 min–1 h before irradiation for directly irradiated cells and 1 h after irradiation to the ICCM before filtration for bystander recipient cells.

α-TOCOPHEROL

α-Tocopherol water-soluble analogue – Trolox was received from Sigma (Germany) as a brown liquid, synthetic. Tocopherol was added to the cell

medium at 2 μg/mL concentration 30 min–1 h before irradiation for directly irradiated cells and 1 h after irradiation to the ICCM before filtration for bystander recipient cells.

Y-IRRADIATION

HPV-G cells were treated 12–24 h after plating in culture flasks, size 25 cm^2. By this time they had attached to the bottom of the flask. The dose was delivered at room temperature using a ^{60}Cobalt teletherapy source, delivering approximately 1.9 Gy/min at a source-to-cell distance of 80 cm. The control cultures were removed from the incubator and brought to the ^{60}Co teletherapy unit with the irradiated cultures but were not irradiated. All cells in the flasks received the same dose of 0.5 Gy. Once irradiated, the cells were immediately replaced in the CO_2 incubator und left undisturbed before analysis.

CLONOGENIC ASSAY

The cell suspension after dilution was counted using a Coulter counter (Coulter Z1). Appropriate cell numbers were plated according to the Puck and Marcus (1956) technique in 5 mL medium in 25 cm^2 NUNC flasks. There were three types of flasks: direct irradiation, bystander donor and bystander recipient. Bystander donor flasks were very heavily seeded with cells (0.5×10^6 cells per flask) in order to produce the bystander factor into the medium after irradiation. Bystander recipient flasks were set up with the ordinary cloning number (300 cells per flask) and received no treatment except the bystander medium (ICCM) from the bystander donor flasks. The direct irradiation flasks were ordinary survival measurement (seeded with 300 cells per flask), after irradiation they received no further treatment. Each of three types of flasks had four sets in triplicate: control, melanin, irradiated cells and irradiated cells with melanin added.

After seeding cells, the flasks were left at 37°C in the incubator to attach for 12 h. Then bystander donor and directly irradiated flasks were irradiated and replaced back in the incubator at 37°C for 1 h. The medium from bystander donor flasks was removed and the radioprotective substance added in appropriate concentration for 30–60 min. Further, the medium was filtered through NALGENE 0.22 μm sterile syringe filters (to ensure that no cells were present in the medium) and used to replace the medium from bystander recipient flasks. Then all flasks were returned to the incubator and left untouched for 9–10 days (until colonies were visible) and then stained with carbol fuchsin and colonies were counted and surviving fraction calculated.

The data are presented as mean ± standard error in all cases. Significance was determined using the *t*-test.

For the clonogenic assay experiments, plating efficiency is the proportion of in vitro plated cells that form colonies and it is calculated as a percentage of the final number of colonies counted over the initial number of cells plated.

Surviving fraction of cells is calculated from the plating efficiency of the irradiated cells divided by the plating efficiency of the control cells (it is expressed as a percentage of the control plating efficiency).

MICRONUCLEUS ASSAY

The cell suspension after dilution was counted using a Coulter counter (Coulter Z1). For direct irradiation and bystander recipient experiments, about 6,000 cells were plated on glass coverslips (diameter 23 mm) in Petri dishes (diameter 60 mm) in 1 mL of medium for 6 h to attach. Then another 5 mL of cell-culture medium were added to the dishes. Bystander donor cells were plated in 5 mL of medium in 25 cm^2 NUNC flasks and were very heavily seeded with cells (0.5×10^6 cells/flask) in order to produce the bystander factor into the medium after irradiation. Each of three types of flasks had 4 sets in triplicate: control, melanin, irradiated cells and irradiated cells with melanin added.

After seeding, cells were left at 37°C in the CO_2 incubator to attach for 12 h. Then bystander donor and direct flasks were irradiated and replaced back in the incubator at 37°C for 1 h. Later, the medium from bystander donor flasks was removed and the radioprotective substance was added in appropriate concentration for 30 min. Then ICCM was filtered through NALGENE 0.22 µm sterile syringe filter to ensure that no cells were present in the medium and no melanin added to recipient flasks thus showing the usual protective effect. This sterile filtered ICCM medium was used to replace the medium from bystander recipient cells. The direct irradiation cells were exposed to the radioprotective substance 30–60 min before irradiation and then left untouched as a direct irradiation test sample.

Then cells were moved back to incubator. After 1–1.5 h cytochalasin B was added at 7 µg/mL concentration, and the cells were incubated for 24 h. After this the cell-culture medium was removed, the cells were washed with PBS and fixed with chilled Karnua solution (1 part of glacial acetic acid and 3 parts of methanol, 10–15 mL three times for 10–20 min). Later coverslips were dried and stained by 10% Giemsa solution. Using mounting medium (Sigma), coverslips were attached to the microscope slides.

The micronuclei count was carried out under inverted microscope (×400). Micronuclei were counted only in binucleated cells (1,000 binucleated cells per flask).

All the data is calculated as the micronuclei number recorded per 1,000 binucleated cells (micronuclei were analyzed only in binucleated cells).

SPECTROMETRY

The absorbance of intact filtered culture medium, culture medium with melanin added and filtered culture medium with melanin added has been analyzed using a Perkin Elmer Lamda 900 UV/VIS/NIR Spectrometer. This spectrometer is a double-beam, double monochromator ratio recording system with pre-aligned tungsten-halogen and deuterium lamps as sources. The wavelength is from 175 to 3300 nm with an accuracy of 0.08 nm in the UV-visible region and 0.3 nm in the NIR region guaranteed.

Results and Discussions

CLONOGENIC EXPERIMENTS

Figure 1 presents the results obtained for clonogenic assay for four direct irradiation and bystander recipient sets of flasks: control, control with melanin, cells irradiated at 0.5 Gy and cells irradiated at 0.5 Gy with melanin added.

Figure 1 shows a significant decrease in the survival of directly irradiated or bystander recipient HPV-G cells irradiated at 0.5 Gy (108 ± 3.5 and 119 ± 0.6) and irradiated at 0.5 Gy with melanin added (123 ± 1.5 and 130 ± 1.2) compared to controls (146 ± 3.2 and 147 ± 2.2). Addition of melanin to the medium of irradiated cells shows a significant increase in the number of colonies compared with cells irradiated without melanin in the medium ($t = 3.86$, $P < 0.01$). And addition of melanin to the ICCM before transfer to recipient

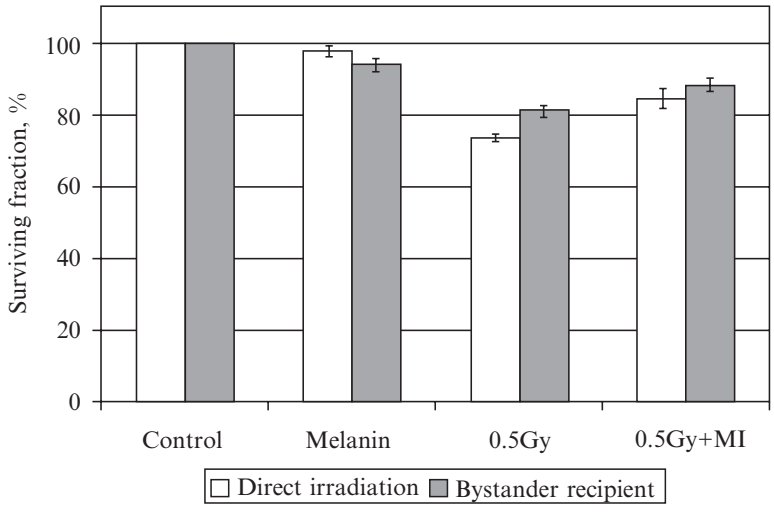

Fig. 1. The surviving fraction of direct irradiated and bystander recipient HPV-G cells (melanin added before irradiation).

cells shows not as significant an increase in the number of colonies compared with cells treated with ICCM without melanin ($t = 3.30$; $P < 0.01$). It can be clearly seen in Fig. 1, where the difference in survival between directly irradiated cells with and without melanin and bystander recipient cells treated with and without melanin is especially evident. Melanin treatment alone was not found to alter the survival of HPV-G cells (143 ± 1.2 and 138 ± 1.2; $P > 0.05$).

These results suggest that melanin is capable of decreasing low-dose radiation effects both direct and bystander cells, but it is more effective after direct irradiation.

Earlier it was shown (Mothersill and Seymour, 1997) that medium alone, irradiated in the absence of cells had no effect on survival of unirradiated cultures. This would seem to exclude the possibility that hydrolysis of medium to give radicals is involved. The time over which the effect persists also excluded any possibility that short-lived species are causing the cell death.

In these experiments, melanin was added to bystander donor cells before irradiation and filtration. It was important to insure that melanin was not present in the medium after filtration so that it could not influence bystander recipient cells, thus showing the usual radioprotective effect. The absorbance of intact culture medium, medium with melanin added and filtered medium with melanin added was compared. Figure 2 shows that filtered medium is identical to culture medium, indicating that the melanin is no longer present in the medium after filtration.

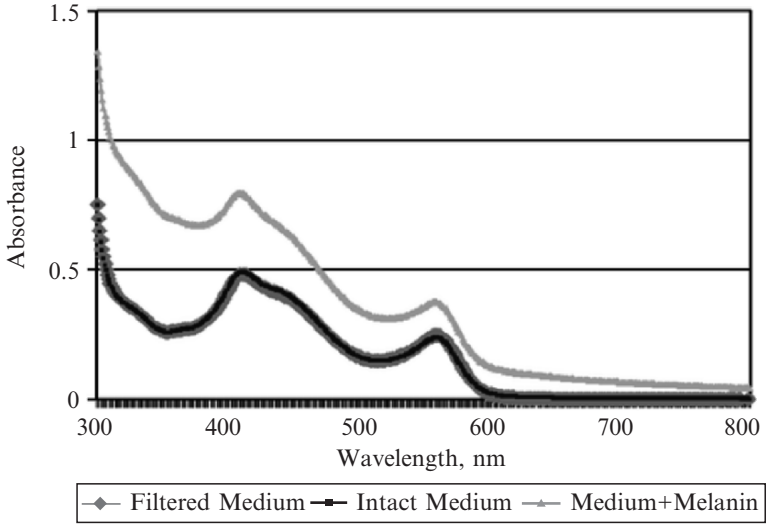

Fig. 2. Absorbance spectra of HPV-G cell-culture intact medium, medium containing melanin and filtered medium containing melanin.

Also it was supposed that addition of melanin before irradiation to bystander donor cells decreases the effect of direct irradiation and that is why a decreased bystander effect was observed. In order to further define the effect, melanin was added after irradiation. To show that melanin could decrease bystander effect in recipient cells, melanin was added to the ICCM 1 h after irradiation (when the bystander factor was produced) before filtration and transfer to recipient cells. The results are presented in Fig. 3.

Figure 3 shows that direct irradiation and irradiated bystander medium clearly reduces the survival of HPV-G cells compared with controls. No cytotoxic effect of melanin on HPV-G cells was observed. Addition of melanin to directly irradiated cells clearly reduces the radiation effects, significantly increasing survival of HPV-G cells ($t = 7.55$; $P < 0.01$). And addition of melanin to the ICCM after irradiation increased the survival of HPV-G cells compared to cells treated with ICCM without melanin, but the effect was not as significant ($t = 2.54$; $P < 0.05$).

In previous cases when melanin was added before irradiation $P < 0.01$, but when melanin was added after irradiation to ICCM, $P < 0.05$. This shows that addition of melanin before irradiation may influence bystander donor cells and protect them from direct irradiation, producing not as much of the damaging bystander factor. When added to ICCM, bystander factor was formed and melanin was not in contact with bystander donor cells.

The data show that melanin clearly decreases direct irradiation effects and not as clearly bystander effects. In bystander cells, the effect of melanin

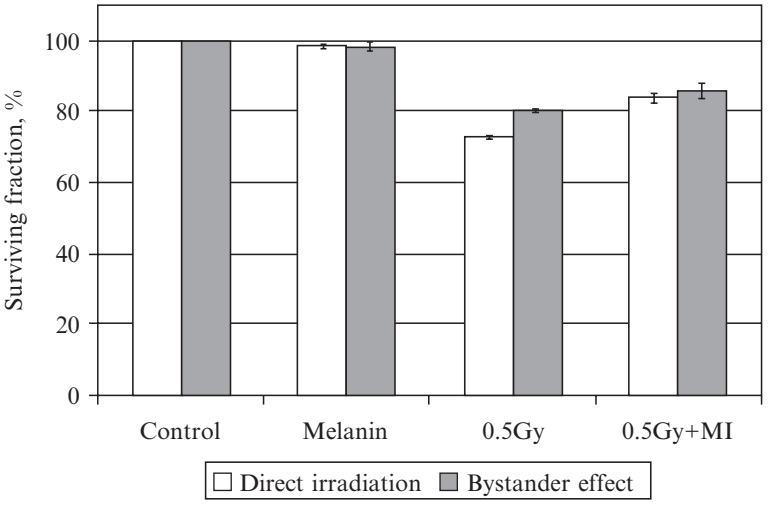

Fig. 3. The surviving fraction of direct irradiated and bystander recipient HPV-G cells (melanin added after irradiation).

depends of the time of its addition to the medium – if it was added before irradiation, it decreases the bystander effect significantly, but if it was added after irradiation before filtration, the decrease is less significant. The radioprotective action of melanin was not as effective for bystander cells as for directly irradiated cells.

As melanin can absorb all types of physical energy, the result indicates that the bystander signal may have a physical nature (component). At the same time, melanin has very effective antioxidant properties. We decided to compare the effect of melanin with another effective antioxidant – melatonin.

Figure 4 presents the results of the effect of melatonin on survival of directly irradiated and ICCM exposed HPV-G cells. Melatonin was added before irradiation to directly irradiated cells and after irradiation to bystander donor cells.

The results from Fig. 4 show that there is no significant influence of melatonin on HPV-G ($P > 0.05$). Thus, no cytotoxic or proliferation inducing effect of melatonin was observed – the survival of control cells with melatonin added is very close to controls.

The data shows a significant decrease in survival of HPV-G cells irradiated at 0.5 Gy (26% compared to control, $P < 0.01$) and after bystander donor medium transfer (20%, $P < 0.01$). Addition of melatonin to the medium of directly irradiated cells results in a significant increase in survival on 12% compared to cells irradiated without melatonin present in the medium ($t = 4.84$; $P < 0.01$), although it is still less than in control. And for

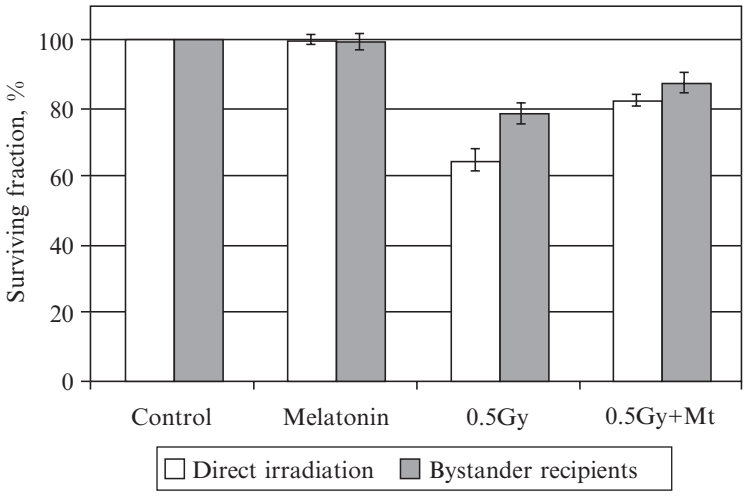

Fig. 4. The surviving fraction of direct irradiated and bystander recipient HPV-G cells (melatonin added after irradiation).

bystander experiments, addition of melatonin also increased the survival of bystander recipient HPV-G cells, but not as significant as in direct irradiation experiments – on 9% ($t = 2.16$; $P < 0.05$).

Thus, melatonin can reduce the damaging effect of the bystander factor after its production, but again not as effective as the protection against the damaging effect of direct irradiation. Both, melanin and melatonin were able to decrease bystander effect, possibly generally because of their antioxidant effect.

In our further study we used vitamin E, which is also very effective antioxidant substance, but it acts in a different pathway – by influencing reparation processes. Also, melanin and melatonin have very close chemical structure, so it was important to use different substance. The results obtained for four sets of flasks: control, control with tocopherol, cells irradiated at 0.5 Gy and cells irradiated at 0.5 Gy with tocopherol added are presented in Fig. 5. Vitamin E was added to the medium of bystander donor cells after irradiation.

Data presented in Fig. 5 show the effect of vitamin E on the survival of HPV-G cells after irradiation. Cells irradiated at 0.5 Gy have clearly lower survival compared with control cells ($P < 0.01$). Addition of tocopherol to the medium of non-irradiated cells does not have any cytotoxic effect.

The survival of irradiated cells with tocopherol added to the medium is lower, than controls, but higher, than the survival of cells irradiated without tocopherol ($t = 4.92$; $P < 0.01$). And addition of vitamin E to the ICCM increased the survival of bystander recipient HPV-G cells, but not as significantly as in direct irradiation experiments ($t = 2.12$; $P < 0.05$).

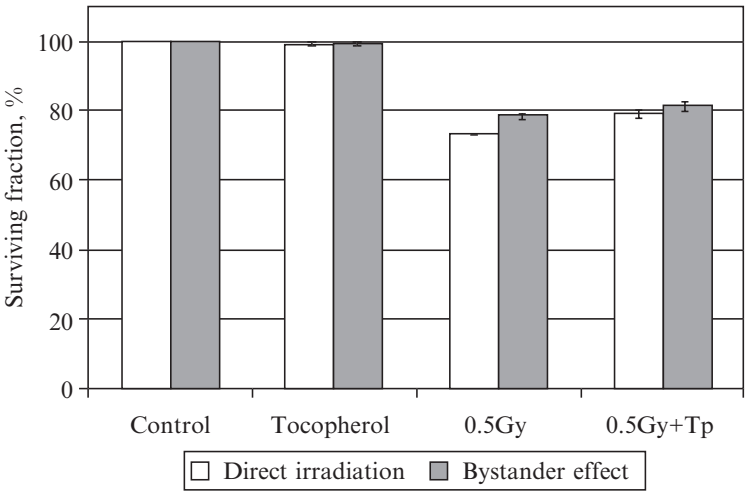

Fig. 5. The surviving fraction of direct irradiated and bystander recipient HPV-G cells treated with α-tocopherol (tocopherol added before irradiation).

Here again we observe the same picture as for melanin and melatonin: α-tocopherol is able to protect HPV-G cells against direct radiation and bystander factors, but its protection against bystander factors is less significant and the least effective between all examined substances. The best protection against bystander factor for both direct and bystander clonogenic experiments showed melatonin.

MICRONUCLEUS EXPERIMENTS

The micronucleus assay currently is widely used in genetic monitoring of populations and for evaluation of the mutagenic effects in vitro. This assay is also used to evaluate individual sensitivity to physical and chemical mutagens. The purpose of the micronucleus test used in the present research was to detect if all substances could modify chromosome structure and segregation in such a way as to lead to induction of micronuclei in interphase cells. HPV-G cells are very sensitive to micronucleus assay.

The results of melanin influence on micronuclei frequency using cytochalasine B block in HPV-G cells irradiated at 0.5 Gy are presented in Fig. 6. The micronuclei frequency in the controls is comparatively low and indicates the level of spontaneous mutagenesis. The number of the cells with 2 and 3 micronuclei is much lower than the number of the cells with 1 micronucleus.

As it can be seen from Fig. 6, the average micronuclei frequency increased in irradiated cells and after bystander donor medium transfer (230.00 ± 10.87

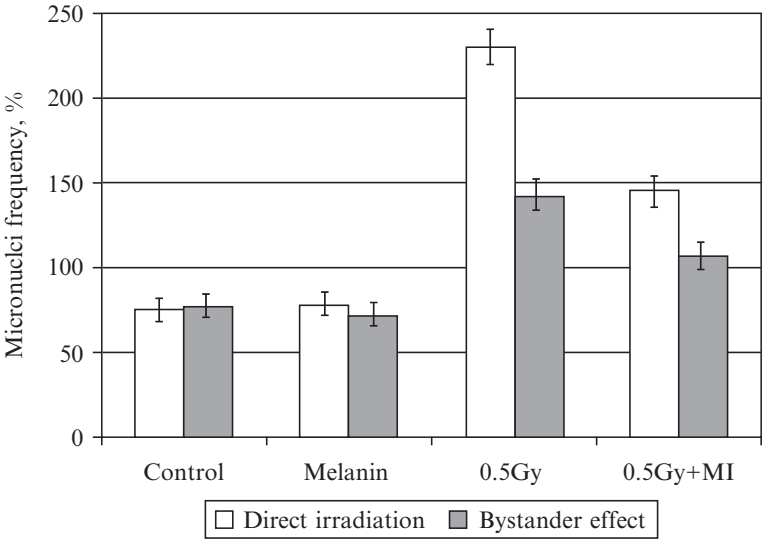

Fig. 6. The effect of melanin on micronuclei frequency of direct irradiated and bystander recipient HPV-G cells (melanin added after irradiation).

and 149.67 ± 9.21, correspondingly) compared with control (75.33 ± 6.81 and 76.67 ± 6.87). Melanin was not found to induce micronuclei – after addition of melanin to the non-irradiated cells the micronuclei frequency was very close to the control (78.00 ± 6.92 and 72.00 ± 6.67, correspondingly; the difference is not significant). This proves that melanin does not have any toxic effect on the cells and that it has no micronuclei inducing or suppressing ability.

After addition of melanin to the medium of the directly irradiated cells the micronuclei frequency (144.00 ± 9.07) was higher, than in the controls, but much less than in irradiated cells ($t = 6.08$; $P < 0.01$). And after addition of melanin to ICCM after irradiation before filtration and transfer to bystander recipient cells the micronuclei frequency (114.01 ± 8.21) was again higher, than in the controls, but less than in cells treated with ICCM without melanin ($t = 2.89$; $P < 0.01$).

The data shows highly significant total micronuclei frequency ($P < 0.01$) for both direct and bystander effects of radiation. If we look at the total number of cells with micronuclei, for directly irradiated cells the addition of melanin to the medium again significantly reduces the number of cells with micronuclei compared to cells irradiated without melanin ($t = 5.35$; $P < 0.01$). But the number of micronuclei cells in bystander recipient cells treated with ICCM + melanin added is not as significant if compared with cells treated with ICCM without melanin ($t = 2.44$; $P < 0.05$). The same situation occurs for the number of the cells with 1 micronucleus – for direct irradiation the difference is highly significant ($t = 4.72$; $P < 0.01$), but it is slightly significant for bystander cells ($t = 2.12$; $P < 0.05$).

This suggests that melanin is able to protect directly irradiated cells and partially to modify radiation-induced bystander effect (by both preventing the decrease in cell survival and increase in micronuclei frequency), suppressing the signals, transferring damage from irradiated cells to non-irradiated cells. And again, as for the clonogenic assay, the protection of HPV-G cells against bystander factors is slightly significant, in contrast to protection against direct irradiation.

Figure 7 presents results obtained after studying the influence of melatonin on the radiation-induced bystander effect using the micronuclei test.

Table 1 shows that radiation influence and irradiated bystander medium clearly induces micronuclei formation in HPV-G cells. As previously shown with melanin, the average total micronuclei frequency in control cells (75.33 ± 6.81 and 76.67 ± 6.87, respectively) is lower than the average total micronuclei frequency in treated cells (230.00 ± 10.87 and 149.67 ± 9.21, respectively). Compared to controls, no micronuclei inducing activity of melatonin was found – the average micronuclei frequency is very close to the control (74.67 ± 6.79 and 76.00 ± 6.84 – the difference is not significant). This means that melatonin has no cytotoxic effect on HPV-G cells.

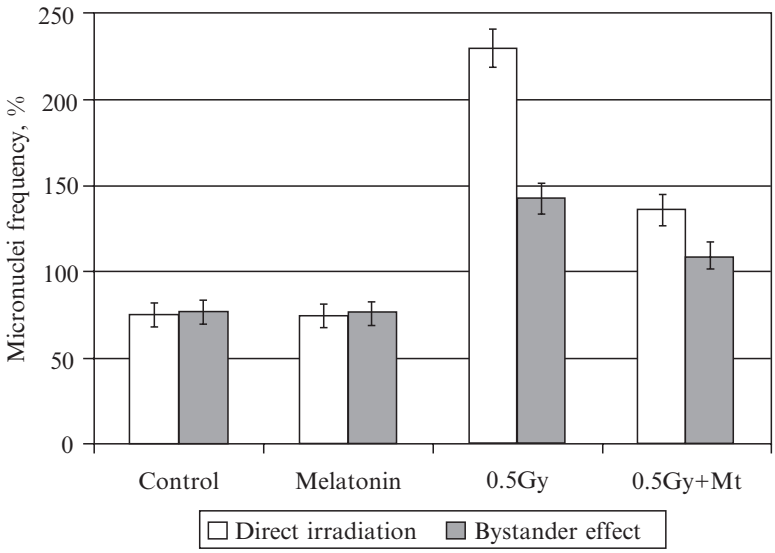

Fig. 7. The effect of melatonin on micronuclei frequency of direct irradiated and bystander recipient HPV-G cells (melatonin added after irradiation).

The results indicate that the average micronuclei frequency of the irradiated cells with melatonin added and after bystander donor medium transfer to recipient cells with melatonin added (136.00 ± 8.85 and 106.00 ± 7.95) is higher than in the control, but lower, than in cells irradiated or treated with ICCM without melanin added ($t = 6.7$ and $t = 3.59$; $P < 0.01$ in both cases). As the protective effect of melatonin is highly significant in both cases, it is again more effective for directly irradiated cells.

Figure 8 presents data obtained in study of the effects of α-tocopherol on micronuclei frequency of directly irradiated and bystander recipient HPV-G cells.

The data from Fig. 8 show that the number of micronuclei in control cells (spontaneous micronuclei frequency) is comparatively low. The micronuclei frequency in control cells with tocopherol added is very close to control (the difference is not significant). Thus, α-tocopherol doesn't have cytotoxic effect on HPV-G cells.

Irradiation at 0.5 Gy and ICCM transfer has a very high micronuclei inducing ability: the number of micronuclei in treated cells is four times higher than in controls. Addition of α-tocopherol to the medium before irradiation significantly reduces micronuclei frequency compared with cells irradiated without α-tocopherol ($t = 5.05$; $P < 0.01$). And micronuclei frequency in bystander recipient cells treated with ICCM with vitamin E

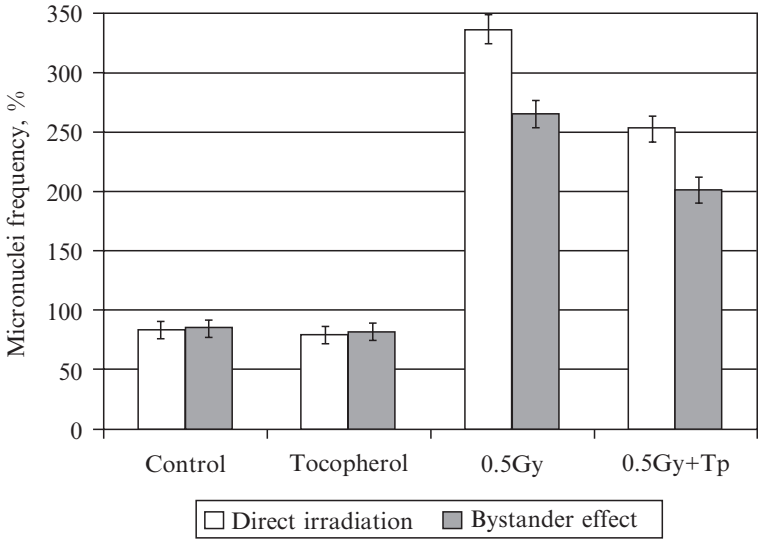

Fig. 8. The effect of vitamin E on micronuclei frequency of direct irradiated and bystander recipient HPV-G cells (vitamin E added after irradiation).

added is higher, than in controls, but lower, than in recipient cells treated with ICCM without vitamin E added ($t = 2.13$, $P < 0.05$). Again Vitamin E shows less effectiveness of protection against bystander factors compared with directly irradiated cells.

The results suggest that α-tocopherol is able to reduce the effects of direct γ-irradiation in human keratinocyte cells, but is less effective against bystander factors. In previous studies, the same effect was observed with melanin and melatonin.

^{60}Co irradiation source emits rays with an energy of 1.25 MeV. Thus, most of the interactions of these rays with cells are Compton interactions. These interactions generate many free radicals, which play a very important role in cell damage following irradiation.

All three radioprotective substances used in the present research were shown to have antioxidant effects and are able to neutralize free radicals. This is possibly the main mechanism of their radioprotective action.

For both, direct irradiation and the bystander effect, α-tocopherol is the less-effective protector against radiation damage. And melatonin appears to provide most effective protection for HPV-G cells against radiation. At the same time, all the substances have more effective protection against direct radiation damage compared with bystander damage. The same was observed for micronuclei assay.

A possible explanation of such different protective effect after direct and bystander irradiations is that in direct irradiation experiments the radioprotective substances were directly in contact with cells since irradiation and were able to protect them against all kinds of damaging factors, starting from energy input (some of these substances may convert all types of energy into heat) and concluding with emerging free radicals. In bystander experiments, the substances were not in contact with cells until added to the ICCM 1 h after irradiation when the damaging factor was already produced. So melanin/melatonin/tocopherol could neutralise only some of the damaging factors (e.g. long-living free radicals) and are unable to protect against the main damaging factor (bystander factor), the nature of which is still unknown.

It is not known the mechanism by which they reduce bystander effect – if they neutralise free radicals or it is possibly another mechanism or factor. But it is definitely clear that these substances are not toxic or stimulating to the cells, increasing their survival or viability or decreasing micronuclei frequency.

Conclusions

The analysis of the effects of radioprotective substances on survival and micronuclei frequency of HPV-G cells after direct and bystander low dose radiation influence allows us to conclude that:

- All substances does not have any cytotoxic effect on HPV-G cells;
- The absorption spectrum of the filtered medium, which contained melanin is identical to one of the intact culture medium. This means that melanin is not present in the filtered medium, so it cannot directly protect recipient cells except by reducing the bystander factor;
- Substances are able to significantly increase the survival of HPV-G cells in vitro after direct low dose radiation influence;
- Added to ICCM before filtration, substances perform protective effect against low doses of radiation (decreasing bystander effect), but not as significant as the effect against direct irradiation;
- The results of micronuclei test show that all substances are able to decrease the level of micronuclei frequency after direct low dose radiation influence and ICCM transfer. They have more effective protective effect in directly irradiated cells than in bystander recipient cells;
- The observed effect of decreased bystander effect may be due to the ability to absorb all types of physical energy, to take-up and retain xenobiotics (as sieves) or because of the antioxidant effect of substances.

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