MELANIN DECREASES REMOTE CONSEQUENCES OF LONG-TERM IRRADIATION.

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INTRODUCTION

Radiocontamination of biosphere results in chronic influence of ionizing radiation in low doses on large groups of living organisms, including human populations.

Earlier we have revealed that melanin was able to reduce the percentage of different mutation types induced by acute irradiation in animals (Drosophila, mice)(1,2). Our experimental data have also shown that melanin decreases the mutation load accumulated in Drosophila populations as a result of X-ray irradiation for 115 generations (3). The investigation of melanin possibility to influence chronic irradiation effects was very important and urgent. It was interesting to study melanin action in human cells too.

METHODS

The influence of melanin isolated from animal hair on genetic effects of irradiation in mice and human lymphocytes has been studied. Mice males of 2.5 months and 22g weight were used. The starch gel or melanin suspension in it were injected into stomach every day with a special needle. Melanin was supplied in concentrations from 0.3 to 30 mg/kg. Mice were exposed to 1-3 Gy of γ-rays of Cs^{137} at the dose rate of 0.007Gy/h (chronic irradiation) and 420Gy/h (acute one). Animals were killed 2.5-3.0 months later when the exposure was stopped. This interval was necessary for repairing irradiated spermatogonia. The levels of reciprocal translocations in metaphase of spermatocytes were analysed cytologically by the method (4) which is a modified Ivens' method (5).

Human cells were cultured according to a standard method. The culturing time was 52h at 37°C. Colchicin was injected 2 hours before cells fixation with ethyl alcohol and glacial acetic acid mixture. Blood was taken from practically healthy people of 25-45 years old in special medical hospital.

Melanin was added to culture media at G_1 and G_2 stages in the following concentrations: 0.1; 0.3; 1.0; 3.0; 10.0; 30.0 mg/l. Human cells were exposed to acute irradiation (0.5Gy of γ-rays) 40 min after melanin injection. The cytological test included dicentric-ring- and fragment- analysis.

RESULTS AND DISCUSSION

Investigations of melanin influence on spontaneous mutation level in mice has demonstrated that melanin itself doesn't possess a mutagenic activity in all concentrations used, even being supplied for 30 days.

Melanin in all concentrations was shown to reduce effectively mutagenic action of acute γ-irradiation. The melanin influence on genetic effect of chronic irradiation was even more effective. The data presented in fig.1 show that the pigment in the concentration of 3 mg/kg greatly reduced the percentage of induced mutations at different doses of chronic irradiation. The same effects were shown if melanin was supplied in other concentrations - it was revealed that melanin activity doesn't depend on concentration used.

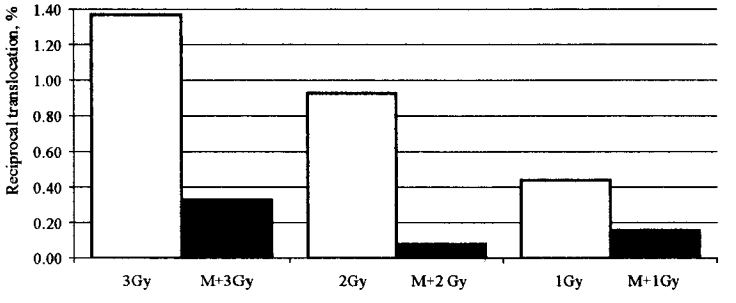

Figure 1. The melanin influence on chronic irradiation induced mutation frequency in mice germ cells (M - melanin).

It's very difficult to compare antimutagenic activity of melanin under acute and chronic irradiation because in the first case one injection of melanin has been used, but in the second case melanin has been injected many times (once a day for 10-20 days). Nevertheless it is possible to draw a conclusion that melanin is no less and even more effective under chronic irradiation than under acute one.

Radioprotective action of this pigment is connected with its high ability to accept and to give back electrons and with anti-radical activity (6,7). It's clear that when low-dose irradiation is used, the possibility for melanin to catch free radicals or electrons is better.

The investigations of melanin action in human lymphocytes have shown, that the aberration frequency in intact cells (control) was ranging from 0.42±0.19 to 1.0±0.3%. These values agree with the literature data. It means that melanin doesn't increase the control level of aberration and has no mutagenic ability.

The study on radioprotective action of melanin has demonstrated, that it is effective in reducing of aberration frequencies, induced by radiation. It was shown, that melanin in all used concentrations (from 0.3 to 30 mg/l) was able to decrease the mutation level (fig.2), but not so effectively as in mice. Strict concentration effect correlation was not observed either in human cells or in mice. There are some proofs that only small amount of melanin can penetrate into cells, and melanin quantity inside cells doesn't increase with rise in outside melanin concentration - this fact can explain absence of such correlation.

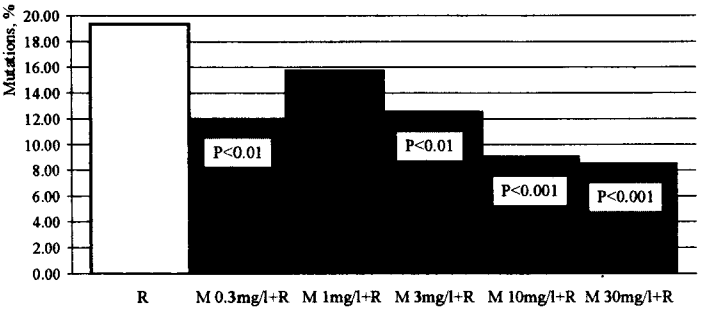

Figure 2. The influence of melanin on radiation induced mutation frequency in human lumphocytes (R - radiation, M - melanin).

Antimutagenic effect of melanin has been revealed to be the same under irradiation at G_2 stage as at G_1 one. These results demonstrate that melanin action doesn't depend (or little depends) on the repair system. The same conclusion was drawn earlier, when melanin had been investigated in drosophila and mice (1, 2).

Complete toxicological tests have been conducted. Melanin could be used in medicine for people protection against genetic consequences of long-term irradiation. We are ready to present this pigment for clinical tests.

CONCLUSIONS

The pigment melanin is not toxic and doesn't possess a mutagenic activity. It reduced the level of mutations, induced by chronic irradiation with low dose rate even more effectively than by acute one. Melanin radioprotective effect is more pronounced in vivo (drosophila, mice), than in vitro (human lymphocytes). Melanin could be used in medicine for people protection.

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