

REPAIR OF UV LIGHT-INDUCED DNA DAMAGE

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Aim

Evaluate whether DNA repair, in UV-irradiated Medaka eleutheroembryos, could be enhanced through topical application of a preparation containing DNA repair enzymes, amino acids, teprenone and Zn⁺ (EZ). In order to assure nuclear delivery, each ingredient was encapsulated individually into liposomes.

Results

We assayed endogenous DNA repairing mechanisms in cells from Medaka fish embryos by measuring the reduction of cyclobutane-pyrimidine dimers (CPDs), after UV irradiation. By comparing the amount of CPDs formed immediately after UV light irradiation on cells treated with a control formulation (EZ minus active ingredients) and cells treated with EZ, we observed a significant decrease (36%) in the formation of CPDs.

p53 helps preventing genome mutation, due to its crucial role in regulating cellular responses to various DNA-damaging agents, including UV radiation. p21 is directly linked to p53 because its expression is tightly controlled by the protein p53. We also studied the effect of UV light in the expression levels of p53 and p21 by comparing samples with or without irradiation. We found that the expression levels of p53 and p21 did not change in embryos not irradiated or embryos irradiated with UV light at t=0 minutes or t=15 minutes indicating that at 15 min a p53-mediated response is not yet active. On the other hand, we observed that EZ treatment reduced the endogenous level of p53, allowing for an early damage response to UV light (t=15 min after UV irradiation) increasing the levels of p53. This early response induced by EZ treatment provoked in turn an increase in p21 expression of 130% as early as 15 min after irradiation.

c-Fos is required for excision repair processes triggered by DNA lesions produced by UV radiation. Therefore, we measured c-Fos expression level in control embryos and embryos treated with EZ, exposed or not to UV light. Results show that c-Fos does not significantly increase 15 minutes after UV radiation in control embryos. On the the contrary, 15 minutes after UV radiation c-Fos is overexpressed in embryos previously treated with EZ.

In addition, we measured cell cycle immediately after irradiation with UV light and 15 minutes post irradiation, and we found that there were no significant changes in cell distribution in each cell cycle phase. Furthermore, we measured cell cycle immediately after UV light irradiation on cells treated with the control preparation and embryos treated with EZ, and we did not observe any significant changes in cell distribution further indicating that the above gene expression changes detected, were not a consequence of changes in the cell cycle.

Conclusions

EZ protects cells against UV light-induced damage by reducing the amount of CPDs in the DNA and triggers the endogenous DNA repair mechanisms that involve the action of p53, p21 and c-Fos.

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