Protective Activity of Fish Peptides Fraction in Optimized Model of UV-B Irradiated Mouse Fibroblasts †

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Abstract: The aim of this study was to evaluate the photoprotective effect of fish bone-derived peptides (FBDPs) in a culture of UV-B-irradiated mouse fibroblasts and their anti-inflammatory activity in a model of stimulated human macrophages. The results showed that cell viability of FBDPs-treated and irradiated fibroblasts was higher than that of irradiated cells, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and lactate dehydrogenase (LDH) assay, after 25 and 60 s of treatment. Moreover, secretion of interleukin 1-beta (IL-1β) was decreased in FBDPs-treated inflamed macrophages, as determined by ELISA assay.

Keywords: fish by-product; peptides; skin photoprotection; cytotoxicity; cytokines

1. Introduction

Ultraviolet-B (UV-B) exposure induces cell structure and function deterioration through accumulated mutations due to excessive production of reactive oxygen species, which result in an alteration of cell cycle progression and dysregulation of matrix metalloproteinases (MMPs) [1]. Destruction of skin connective tissue was reported after prolonged exposure to UV radiation, which triggers cytokines and growth factor receptors activation, leading to MMP overexpression [2]. In this study, we have evaluated the biologic activity of fish bone-derived peptides (FBDPs) in an optimized model of UV-B-irradiated fibroblasts by cell viability tests and cell morphology observations. Moreover, their influence on pro-inflammatory cytokine secretion in THP-1 cells was investigated.

2. Materials and Methods

FBDPs were extracted from a Hypophthalmichthys molitrix skeleton by papain digestion, isolated by centrifugal ultrafiltration and fractionated by size-exclusion chromatography. They were separated according to their molecular weight using tricine gel electrophoresis and to their hydrophobicity by cation-exchange chromatography. Several exposure times and doses were tested to optimize the experimental model of UV-B-irradiated mouse fibroblasts from the NCTC clone L929 cell line. Pretreatment and co-treatment with cytocompatible concentrations of FBDPs were assessed by MTT and LDH cell viability tests [3] and cell morphology observations. Specific
secretion of IL-1β in FBDPs-treated THP-1 macrophages was analyzed in harvested conditioned media using an ELISA kit.

3. Results

A heterogeneous mixture of both hydrophilic and hydrophobic (oligo)peptides with an average molecular weight of 7.1 kDa was obtained. In vitro tests showed that the viability of irradiated cells decreased in a time-dependent manner, while FBDPs-treated fibroblasts showed higher viability by ~10, 5 and 3% at 25, 60 and 90 s of irradiation, respectively (Figure 1). Additionally, FBDPs-treated macrophages presented an inhibition of IL-1β secretion, compared to that quantified in inflamed cells.

![Cell viability of fish bone-derived peptide (FBDPs)-treated L929 fibroblasts at different UV-B exposure times, determined by MTT assay (* p < 0.05, ** p < 0.01, compared to irradiated control).](image)

4. Conclusions

Biotechnologies applied to fish by-products yielded valuable peptides with photoprotective activity demonstrated by their ability to increase the cell viability in fibroblasts exposed to UV-B and to inhibit cytokine secretion in inflamed macrophages.

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References


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