Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 11
ISSN: 2321–3647(online)

Scarus ghobban Fish mucus methanolic extract attenuates UV-B radiation induced immune suppression reactions analyzed through cytokine IL-2 on Human Keratinocytes.

Shankarlal S*[1], Natarajan E[1]
1. Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, India

ABSTRACT

Ultraviolet-B radiation affects the surface layer of the skin mainly Keratinocytes which leads to harmful biological disorder. This study explored the effect of Methanolic extracts of Fish Mucus (FME) from parrot fish *Scarus ghobban* against UV-B radiation treated Cultured Human Keratinocytes (HaCaT). HaCaT cells treated with FME for 24hrs followed by UV-B irradiation for 15mJ/cm^2. After the irradiation, the HaCaT Cells were assayed for IL-2 cytokine. UV-B radiation induces an increase in cytokines, when ti is incorporated with the FME the concentration of cytokines reduced to normal. This study reveals that FME may be a photoprotective agent.

Keywords: Keratinocytes, UV-B, FME.

*Corresponding Author Email shankarlalmarine@gmail.com
Received 16 September 2014, Accepted 28 September 2014

Please cite this article as: Shankarlal S *et al*.,*Scarus ghobban* Fish mucus methanolic extract attenuates UV-B radiation induced immune suppression reactions analyzed through cytokine IL-2 on Human Keratinocytes. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Fish mucus is known to contain many biologically active peptides and proteins to enable several common biological functions such as respiration, ionic and osmotic regulation, communication, reproduction, and diseases protection[1, 2, 3]. Some parrotfish are known to eat algae and hard coral[4], which can contain MAAs (UV absorbing compounds) [5]. The biological effect of ultraviolet radiation is due to its spectral distribution Solar ultraviolet radiation consists of UV-C (wavelength, below 280 nm), UV-B (280-320 nm), and UVA (320-400 nm) [6]. Because the incidence of UV-B induced diseases continues to rise, the development of photoprotective agents against UV-B exposure has become an important subject[7]. Sun screening compounds were found in the external mucus of fish [8]. Immunological studies with skin cancer patients have indicated that the immune suppression induced by UVB radiation is a major risk factor in skin cancer development[9]. Studies have shown that UV radiation induces cytokines in human keratinocytes. Such cytokines create an inflammatory environment that supports the progression of carcinogenic cells[10]. The UVB (280–320 nm) component of solar ultraviolet radiation has been shown to suppress the immune system and act as a tumor initiator, tumor promoter and co-carcinogen[11, 12, 13]. UV does not only have the ability to influence the release of cytokines but that it can also interfere with the biological activity of immunomodulatory mediators[14].

MATERIALS AND METHODS

Culture of Human Keratinocyte (HaCaT) Cell Line

HaCaT cells were grown in DME/HAMS F-12 medium containing 10% FBS, 10,000 IU/ml penicillin and 10,000 µg/ml streptomycin in a 25 cm^2 culture flask in a CO_2 incubator at 37°C and 5% CO_2 under controlled humidified atmosphere. Once the cells reached ~90% confluency, they were trypsinized using trypsin (0.05%) – EDTA (0.54 mM) solution, washed thoroughly with media and subcultured into a 75-cm^2 culture flask for expansion. This process was repeated twice till the cells attained a consistent growth phase. Once after the cells attained consistent growth phase, they were trypsinized at 80% confluency and then utilized for the assay.

Experimental Design

Extraction of FME from the Parrot fish *Scarus ghobban*, the FME were divided into 6 groups, 30 minutes before irradiation test doses (3 µg/ml and10µg/ml) of FME were added.

Group 1: Normal keratinocytes

Group 2: UV-B irradiated keratinocytes

Group 3: FME (3µg/ml)

Group 4: FME (10µg/ml)

Group 5: UV-B irradiated with FME (3µg/ml)

Group 6: UV-B irradiated with FME (10µg/ml)

Irradiation procedure

For UV-B irradiation, cells were irradiated in 35-mm Petri dishes containing 2 mL of PBS and covered with a UV permeable membrane to prevent contamination. A battery of TL 20 W/20 fluorescent tubes served as a UV-B source, which had a wavelength range set at 280 to 320 nm, peaked at 312 nm and an intensity of 2.2 mW/cm^2 for 7 minutes. The total UV-B irradiation was 15 mJ/cm^2. After irradiation, the keratinocytes were kept at room temperature for 30 minutes and then subjected to biochemical assays. Sources of UVR are characterized in radiometric units. The terms dose (J/m2) and dose rate (W/m2) pertain to the energy and power, respectively, striking a unit surface area of an irradiated object (Jagger, 1985). UV-B radiation emitted by the narrowband TL20/W01 IS 2.3WATT. The tube emits radiation in the range of 280nm-320nm, peaked at 312nm.

ELISA for cytokine estimation

ELISA, or Enzyme-Linked Immunosorbent Assay, is a method for the quantitative or qualitative detection of analytes (in our case the cytokine IL-2). This technique is based on the specific binding between an antigen and its antibody, and is therefore highly specific. We use sandwich-ELISA Kits. The antibodies for the cytokine of interest are coated on each well of a 96-well polystyrene plate. When the serum or plasma is added, the cytokines will strongly interact with their antibodies and form very strong complexes. After washing steps to remove interfering molecules and unbound cytokines, we add secondary antibodies, which will bind to the cytokines and which are conjugated with an enzyme. This enzyme will therefore be indirectly attached to the cytokines. Sometimes, the secondary antibodies are conjugated to a biotin molecule. Biotin has the feature to have a powerful interaction with streptavidin. The streptavidin carries the enzyme responsible for the conversion of the substrate (chromogen) added in the last step, resulting in colour development. The intensity of the colour is directly related to the amount of cytokines bound to the antibodies, and is measured by the spectrophotometer. This allows us to measure the concentration of the cytokine in the solution. The classical procedure of sandwich-ELISA is followed. The enzyme often used is Horseradish peroxidase (HRP). It catalyzes the oxidation of a substrate by hydrogen peroxide, which leads to a blue colour. The most common substrate is tetramethylbenzidine (TMB). To stop this enzymatic reaction, sulphuric acid is added

since it denaturates HRP. The colour changes from blue to yellow, with a maximum absorption at 450 nm.

RESULTS AND DISCUSSION

Enzyme-linked Immunosorbent assay (ELISA) for IL-2 Measurement

IL-2 Production in Keratinocyte Cells

Table 1 Effect of FME on cytokine induced by UV-B radiation in HaCaT Cells.

Treatment	IL-2		Average	S.D
Control	382.5	400.52	391.51	12.74206
3µg of FME	300.42	310.822	305.621	7.355325
10µg of FME	215.79	220.2	217.995	3.118341
UV-B alone	150.42	116.72	133.57	23.8295
UV-B WITH 3µg of FME	166.38	108.53	137.455	40.90613
UV-B WITH 10µg of FME	171.42	195.32	183.37	16.89985

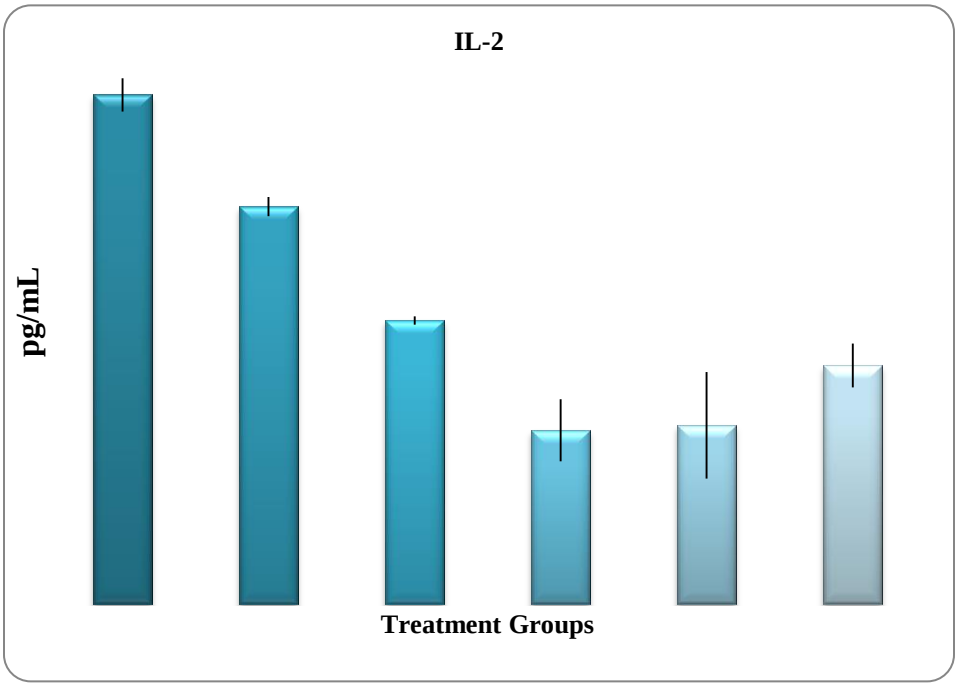

Figure 1 Effect of FME on cytokine induced by UV-B radiation in HaCaT Cells.

The constitutive productions of cytokines and other soluble factors are low in human keratinocytes, but various stimuli, including endotoxins and UV, can trigger the expression of proinflammatory cytokines[16]. In the present study, UV-B radiation increases the concentrations of cytokine IL-2 in HaCaT Cells, the concentration of cytokines were decreased when it is pretreated with FME with UV-B radiation. This result clearly reveals that the methanolic extract of fish mucus FME clearly attenuates the expression of cytokines in Human Keratinocytes. The FME may be used as a photoprotective agent.

CONCLUSION

The present study revealed that methanolic extracts of Parrot fish *Scarus ghobban* mucus (FME) attenuates the effect of UV-B radiation induced inflammatory response in the human keratinocytes. The FME absorb the UV-B radiation effectively and reduce the effect of UV-B radiation.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the University Grant Commission UGC-RGNF Scheme for providing financial assistance in the form of Senior Research Fellowship and then to Pondicherry Centre of Biological Sciences, Pondicherry for invitro cell culture and analysis of cytokine measurement by ELISA.

REFERENCES

1. Handy R.D, Eddy F.B, Romain G. In vitro evidence for the ionoregulatory role of rainbow trout mucus in acid, acid aluminium and zinc toxicity. J. Fish Biol. 1989; 35, 737– 747.
2. Shephard KL. Functions for fish mucus. Rev. Fish Biol. Fish. 1994;4, 401– 429.
3. Aranishi F, Nakane M. Epidermal proteinases in the European eel. Physiol. Zool. 1997; 70 (5), 563– 570.
4. Bruggemann JH. Parrotfish grazing on coral reefs, a trophic novelty. PhD thesis, University of Groningen. 1994.
5. Klisch M, Sinha RP, Richter PE, Hader DP. Mycosporine-like amino acids (MAAs) protect against UV damage in Gyrodinium dorsum Kofoid. J. Plant Physiol. 2001; 158:1449–1454.
6. Matsumura Y Ananthaswamy HN. Toxic effects of ultraviolet radiation on the skin. Toxicol Appl Pharmacol. 2004; 195(3):298-308.
7. Cockell CS, Knowland J. Ultraviolet radiation screening compounds. Biol. Rev. Camb. Philos. Soc., 1999; 74, 311–345.
8. Zamzow JP, Losey GS. Ultraviolet radiation absorbance by coral reef fish mucus: photo-protection and visual communication. Environ Biol Fishes 2002; 63:41–47.
9. Yoshikawa T, Rae V, Bruins-Slot W, Van den Berg J.W, Taylor J.R, Streilein J.W. Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. J. Invest. Dermatol. 1990; 95, 530–536.

10. Ravi R, Piva TJ. The Role of Furin in the Development of Skin Cancer. In D. P. Vereecken (Ed.) Highlights in Skin Cancer. Intech. 2013.
11. Kripke, M.L. Photoimmunology. Photochem. Photobiol. 1990; 52, 919–924.
12. Katiyar S.K, Korman N.J, Mukhtar H, Agarwal R. Protective effects of silymarin against photocarcinogenesis in a mouse skin model. J. Natl. Cancer Inst. 1997; 89, 556–566.
13. Donawho C.K, Kripke M.L. Evidence that the local effect of ultraviolet radiation on the growth of murine melanomas is immunologically mediated Cancer Res. 1991; 51, 4176–4181.
14. Aragane Y, Schwarz A, Luger T. A, Ariizumi K, Takashima A, Schwarz T. J. Immunol. 1997; 158, 5393–5399.
15. Jagger J. Solar-UV Actions on Living Cells. New York: Praeger. 1985.
16. Yoshizumi M, Nakamura T, Kato M, et al., Release of cytokines/chemokines and cell death in UVB-irradiated human keratinocytes, HaCaT, Cell Biology International, 2008;. 32(11): 1405–1411.