Protective effects of polypeptide from *Chlamys farreri* on Hela cells damaged by ultraviolet A[1]

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ABSTRACT

AIM: To study the protective effect of polypeptide isolated from *Chlamys farreri* (PCF) on Hela cells damaged by ultraviolet A (UVA) *in vitro*. **METHODS:** Cell proliferation was determined by MTT method; intra-cellular free calcium $[Ca^{2+}]_i$ and rates of apoptosis and death were measured by flow cytometry (FCM). **RESULTS:** PCF (0.5 %-2 %) enhanced the activities of glutathione peroxidase (GSH-px), superoxide dismutase (SOD), and catalase (CAT), and stimulated cell proliferation. The concentration of $[Ca^{2+}]_i$ was increased while the amounts of MDA and the rates of apoptosis and death of the cells were decreased. The differences between the PCF groups and control group were significant ($P<0.05$, $P<0.01$). **CONCLUSION:** PCF protected Hela cells against damage by UVA via its anti-oxidative mechanisms.

INTRODUCTION

As the ozone is becoming thinner and thinner, we receive more and more ultraviolet that includes A, B, and C bands. Many investigators have studied the oxidative injuries of ultraviolet on skin and cells[1]. As ultraviolet A (UVA) is more intense than other bands of ultraviolet, it can easily penetrate the epidermis and get to the dermis. It causes more damages on the skin than other bands of ultraviolet[2].

Natural antioxidants nowadays can inhibit the oxidative damages caused by ultraviolet and mainly come from plants and herbs[3]. We seldom see the reports of polypeptides as antioxidants especially those from marine products. The oxidative models were mainly animals, human beings, and some kinds of cells such as fibroblast, keratinocyte, and others[4]. We have not read any report on the investigation of oxidative model on Hela cells. So we isolated polypeptide from *Chlamys farreri* (PCF), a new marine active material, and established the oxidative damage models of Hela cells to study the protective effect of PCF on them and to explore the anti-oxidative mechanisms of PCF.

MATERIALS AND METHODS

Reagents PCF: purified and analyzed by HPLC (M_r: 800-1000, purity >96 %), stored at 4 °C, isolated by our topic group using biological engineering technique. Hela cells: purchased from Shandong Academy of Medical Sciences. Radiometer and UVA light source: purchased from Beijing Normal University. Enzyme test kits including glutathione peroxidase (GSH-px), superoxide dismutase (SOD), and catalase (CAT)

were purchased from Nanjing Jiancheng Bioengineering Institution, lot number: 20001102. Apoptosis and $[Ca^{2+}]_i$ kits were purchased from Beijing Zhongshan Co, lot number: 200010302.

Effects of PCF on the proliferation of Hela cells damaged by UVA Hela cells were randomly divided into five groups. They were control group, model group, 0.5 % PCF group, 1 % PCF group, and 2 % PCF group. The cells in logarithmic growth phase were adjusted cell number to $1 \times 10^8 L^{-1}$, then put into 24-well plate, triplicate well a group with 1 mL as final volume per well. After incubation for 24 h in 5 % CO_2, at 37 °C, in RPMI-1640, PCF was added to culture media to concentrations of 0.5 %, 1 %, and 2 % (w/v) respectively. Incubated 10 min later, the cells were exposed to UVA (radiant intensity: 3650 $\mu J \cdot cm^{-2}$) for 2 h except those in control group. Then we detected the proliferation of the cells using MTT method at wavelength of 490 nm by microplate reader (Anthos Labtec Instruments GmbH)[5].

Effects of PCF on apoptosis and death rates of Hela cells damaged by UVA (detected using Annexin V method) Cells in logarithmic growth phase were adjusted to $5 \times 10^8 L^{-1}$, then put into 6-well plate, triplicate well a group with 10 mL as final volume per well. After exposed to UVA (radiant intensity: 3650 $\mu J \cdot cm^{-2}$) for 2 h, the cells were taken out and washed twice by cold PBS buffer. Then the cells were centrifuged at 67 $\times g$ for 5 min, and adjusted to a concentration of $1 \times 10^9 L^{-1}$. At this time, we took out 100 μL of cells suspension (without fixation) and added 5 μL fluorescent dye to the suspensions respectively. After mixing the fluid, we kept the cells in dark for 15 min. Then we added 1×binding buffer into 400 μL cell suspension and determined the apoptosis and death rates by flow cytometry (Coulter Co Ltd, USA)[6].

Effect of PCF on the concentration of $[Ca^{2+}]_i$ in Hela cells damaged by UVA Hela cells were randomly divided into six groups including control group, model group, 0.5 % PCF group, 1 % PCF group, 2 % PCF group, and 1 % vitamin C group. Cells in logarithmic growth phase were set to $5 \times 10^8 L^{-1}$, then put into 6-well plate, triplicate for a group with 10 mL as final volume per well. After incubated for 24 h, PCF and vitamin C were added to culture media respectively in a concentration as previously described. After 2 h, the cells were exposed to UVA (radiant intensity: 3650 $\mu J \cdot cm^{-2}$) for 2 h except those in control group. Two hours later, the cells were washed by cold PBS buffer and

then prepared into a concentration of $1 \times 10^9 L^{-1}$. Fluorescent dye (Fluo-3 AM) 100 μL were added to 100 μL cell suspension respectively. After stained in 37 °C water bath for 30 min, the cells were washed by cold PBS buffer thrice. Then fluorescent signal intensity was detected by flow cytometry and the average value was calculated[7].

Effects of PCF on the anti-oxidative index of Hela cells damaged by UVA The cells were randomly divided into six groups including control group, model group, 0.5 % PCF group, 1 % PCF group, 2 % PCF group and 1 % vitamin C group. Cells in logarithmic growth phase were set to $5 \times 10^8 L^{-1}$, then put into 6-well plate, triplicate for a group with 10 mL as final volume per well. After incubated for 24 h, PCF and vitamin C were added to culture media respectively in a concentration as previously described. After 2 h, the cells were exposed to UVA (radiant intensity: 3650 $\mu J \cdot cm^{-2}$) for 2 h except those in control group. Two hours later, the cells were centrifuged and the supernatant was collected respectively, then enzymes including GSH-px, SOD, and CAT were detected by chromatometry following the kits directions[8,9].

Data analysis The data were expressed as mean±SD and treated with one-way ANOVA.

RESULTS

Cell growth was inhibited after ultraviolet A radiation. But PCF (0.5 %-2 %) pretreatment stimulated Hela cells proliferation in a concentration-dependent manner ($P<0.01$, Tab 1).

Compared with model group, the concentrations of $[Ca^{2+}]_i$ in PCF groups rose obviously, while the apoptosis and death rates decreased in PCF groups in concentration-dependent manner ($P<0.05$, Tab 1, Fig 1, 2).

PCF enhanced the enzyme activities in Hela cells. The results of GSH-px and SOD correlated with the PCF concentration ($P<0.05$, Tab 2).

DISCUSSION

All the results in model group indicated that UVA could induce Hela cells to apoptosis as well as death and inhibit the proliferation of the cells. Furthermore UVA can suppress the activities of anti-oxidative enzymes in cells[10]. So we successfully established the oxidative damage model of Hela cells in this study.

Our results showed that PCF could protect the

Tab 1. Effects of PCF on the proliferation, the concentration of $[Ca^{2+}]_i$, apoptosis and death rates of Hela cells damaged by UVA. *n*=8 wells of Hela cells. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* model group.
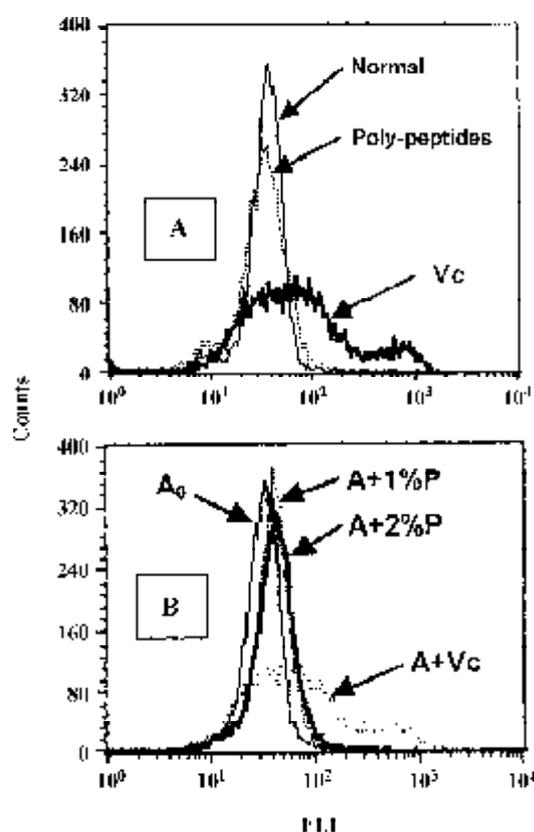
Group	*OD* value	$[Ca^{2+}]_i$	Apoptosis rate/%	Death rate/%
Control	0.81±0.04	35.813±0.011	0.547±0.024	0.963±0.013
Model	0.410±0.013	30.5±0.6	3.352±0.021	3.447±0.022
0.5 % PCF	0.598±0.01[c]	38.12±0.09[c]	3.16±0.03[b]	3.407±0.012[b]
1 % PCF	0.847±0.016[c]	38.23±0.03[c]	3.05±0.05[b]	3.35±0.03[b]
2 % PCF	0.951±0.014[c]	43.22±0.04[c]	2.033±0.012[c]	1.752±0.012[c]
1 % VitC		75.26±0.09	1.584±0.019	1.418±0.009

Tab 2. Effects of PCF on the enzyme activites of Hela cells damaged by UVA. *n*=8 holes of Hela cells. Mean±SD. [b]*P*<0.05, [c]*P*<0.01 *vs* model group.

Group	GSH-px/U	SOD/$\mu U \cdot L^{-1}$	CAT/$U \cdot g^{-1}$
Control	4.9±0.3	50.3±0.4	0.03±0.003
Model	4.8±1.1	3.8±1.3	0.006±0.004
0.5 % PCF	66.4±2.4[c]	8.5±1.7[c]	0.008±0.003
1 % PCF	66.4±2.4[c]	18±3[c]	0.043±0.017[b]
2 % PCF	97±3[c]	21.5±2.1[c]	0.04±0.03[b]
1 % vitC	4.3±0.6	5.8±0.4	0.033±0.005

Fig 1. Fluorescent signal intensities of Hela cells. A) Normal fluorescent signal intensities of Hela cells. B) Fluorescent signal intensities of Hela cells damaged by UVA, with the obviously higher intensities in 2 % PCF group.

cells from UVA injury. Cells apoptosis is a multi-factor related process including gene promotion and mutation. The radiation produced by ultraviolet can cause cells to apoptosis. When cells apoptosis takes place, the structures of plasma membrane will change. These changes can be detected by FCM in very early stage of cells apoptosis[11]. The method we used in this study is Annexin V, a kind of phospholipid conjugated protein method. When cells apoptosis occurs, phosphatidyl serine (PS) turns over from intramembrane to lateral, a process occurring before the other changes, and combines with Annexin V. Our study showed that PCF inhibited apoptosis and death rates of Hela cells induced by UVA in a concentration-dependent manner.

Some investigations claimed that ultraviolet could make the concentration of $[Ca^{2+}]_i$ higher[12]. But others showed that following the apoptosis and death of cells, the concentration of $[Ca^{2+}]_i$ might not increase[13]. $[Ca^{2+}]_i$ as a second messenger in cells play an important role in cells growth and metabolism. Our results made it clear that concentration of $[Ca^{2+}]_i$ in PCF groups was elevated. The single concentration of $[Ca^{2+}]_i$ as an in-

dex in determining cells apoptosis is not precise. The determining factor in cells apoptosis is the unbalance in $[Ca^{2+}]_i$ contents.

Several antioxidative enzymes including GSH-px, SOD, CAT, *etc* scavenged free radicals produced by ultraviolet. The amounts of MDA reflect the level of lipid peroxidation *in vivo*[14]. Our results showed that PCF enhanced the activities of GSH-px, SOD, and CAT
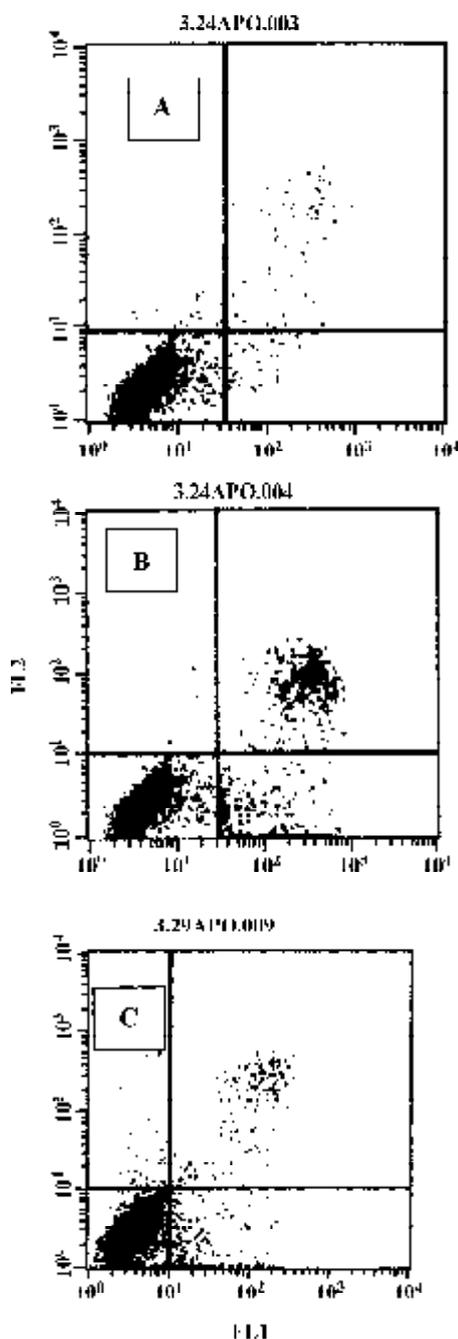
Fig 2. Apoptosis and death rates of Hela cells (the right upper area shows the death rate and the right down area indicates the apoptosis rate). A) Normal apoptosis and death rates of Hela cells. B) Apoptosis and death rates of Hela cells damaged by UVA. C) Apoptosis and death rates of Hela cells in 2 % PCF group.

in concentration-dependent manner and claimed that PCF was a potential antioxidant against ultraviolet damage. PCF could stimulate enzymes to remove free radicals, therefore protected cells against the injury of radicals. At the same time, PCF decreased the amounts

of MDA which also meant PCF reduced the lipid peroxidation of the cells and kept the cells out of the damage of the radicals.

In summary, on the background of successfully establishment of the unitary UVA damage model (irradiation intensity: 3650 $\mu J \cdot cm^{-2}$) of Hela cells, the results of this study indicated that PCF had the protective effect on Hela cells damaged by UVA in the concentration range of 0.5 %-2 %. PCF obviously increased the proliferation of Hela cells and decreased the apoptosis and death rates. Its was related to its abilities of enhancing the activities of GSH-Px, CAT, and SOD, inhibiting lipid peroxidation, and increasing the concentration of $[Ca^{2+}]_i$ in Hela cells.

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