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Protective effect of polypeptide from *Chlamys farreri* on hairless mice damaged by ultraviolet A[1]

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KEY WORDS *Chlamys farreri*; ultraviolet rays; antioxidants; hairless mice; *bcl*-2 genes; nitric-oxide synthase; immunohistochemistry; topical administration

ABSTRACT

AIM: To study the protective effect of the polypeptide isolated from *Chlamys farreri* (PCF) on hairless mice skin damaged by ultraviolet A. **METHODS:** Enzymes and malondialdehyde (MDA) were determined by biochemical methods; the expressions of Bcl-2 protein and NOS protein were examined by immunohistochemical technique. The ultra-structure of the skin was observed through electronic microscope. **RESULTS:** PCF could enhance the activities of glutathione peroxidase (GSH-px), superoxide dismutase (SOD), and total anti-oxidative capacity (T-AOC). Also PCF could reduce the amount of MDA, increase the expression of Bcl-2 protein, and inhibit the expression of NOS protein. The ultra-structure of epidermis and fibroblasts remained normal in 20 % PCF groups; there were vacuoles in smooth endoplasm reticulum in epidermis of mice and the number of rough endoplasm reticulum in fibroblasts was decreased in model group. **CONCLUTION:** PCF had the protective effects on hairless mice skin damaged by ultraviolet A *via* its anti-oxidative mechanisms.

INTRODUCTION

Many reports have shown the oxidative damages of ultraviolet on skin as well as on cells[1]. Ultraviolet A (UVA) can cause more damages on the skin for its great penetrating capability[2]. UVA can penetrate the epidermis and the dermis and is the most intense light in ultraviolet.

Natural antioxidants can inhibit the oxidative injuries caused by UVA. They mainly come from plants or herbs and have been used in medicine and health care[3]. The reports mostly concerned about the oxidative damage models as nude mice or naked mice (immunodeficiency animal) and some kinds of cells. For instance, they are fibroblasts and horny cells, *etc*. We seldom see the reports on polypeptides as

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antioxidants, especially those from marine products and the reports on hairless mice (animal with normal immunity) as oxidative model[4,15]. In this study, we established the continuous oxidative damage model of hairless mice and then probed the protective effect of the polypeptides isolated from *Chlamys farreri*, on the mice skin.

MATERIALS AND METHODS

Drugs and reagents Polypeptides isolated from *Chlamys farreri* (PCF, M_r 800-1000), was purified and analyzed by HPLC, stored at 4 ℃, and isolated using biological engineering technique. Hairless mice: Kunming species, male, 18 g±2 g, bought from Beijing Medical University. Malondialdehyde (MDA) test kits and enzyme test kits including glutathione peroxidase (GSH-px), superoxide dismutase (SOD), and total anti-oxidative capacity (T-AOC): bought from Nanjing Jiancheng Bioengineering Institute, lot number: 20001102. Bcl-2 and NOS protein immunohistochemistry test kits: bought from Beijing Zhongshan Corporation, lot number: 20000102. Transmission electronic microscope: JEM-1200ES. Image analysis system: VIDAS-21, made in Japan. Radiometer and ultraviolet A light source: bought from Beijing Normal University and tissue homogenizer: Unicorn Medical Instrument Factory.

Establishment of oxidative model for hairless mice skin damaged by UVA Hairless mice (n=40) were randomly divided into five groups (8 per group). They were control group, model group, 5 % PCF group, 20 % PCF group, and 10 % vitamin C group. After routine breeding for about one week, saline, PCF, and vitamin C were smeared on the backs of the mice (1 mL per mouse a day) respectively[6]. One hour later, the mice were exposed to UVA (radiant intensity: 151.68 $J \cdot cm^{-2} \cdot d^{-1}$) except those of control group. Thirty days later (total radiant intensity: 4556.4 $J \cdot cm^{-2}$), the mice were put to death and the skin radiated by UVA was used to make the homogenate[7].

Observation of skin ultra-structure We used 5 % glutaral dehyde to fix the skin for 24 h and then dehydrated it with ethanol grade-by-grade, saturated the skin in embedding medium: epoxy resin-618. Then we cut it into very thin slices and stained them. Finally we observed the ultrastructure of the skin through transmission electronic microscope[8].

Immunohistochemistry detection of Bcl-2 protein and NOS protein First, we routinely prepared the skin tissue slices. Then the expression of Bcl-2 protein (the first antibody is rabbit-anti-mouse Bcl-2 IgG) and NOS protein (the first antibody is rabbit-anti-mouse NOS IgG) were examined by immunochemical technique following the kits directions and the results were processed by image processing system[9].

Effects of PCF on the antioxidative index of hairless mice skin damaged by UVA First, we prepared the homogenate of the skin by using cold PBS (pH 7.4, in ice bath) through tissue homogenizer. Second, we centrifuged the skin homogenate and get the supernatants for biochemical tests. After determining the amount of total protein in the supernatants, we detected the enzymes that include GSH-px, SOD as well as T-AOC and MDA by biochemistry method using the supernatants following the kits directions[10].

RESULTS

Bcl-2 immunohistochemical staining of epidermis in 20 % PCF group was deep compared with model group. NOS immunohistochemical staining of epidermis in 20 % PCF group was light compared with model group. Compared with model group, the expression of Bcl-2 protein in PCF groups rose obviously, while that of NOS in PCF groups decreased (P<0.05, Tab 1, Fig 1). PCF enhanced the enzyme activities and decreased

Tab 1. Effects of PCF on the expressions of Bcl-2 protein and NOS protein in hairless mice skin damaged by UVA. n=8 mice. Mean±SD. ^cP<0.01 *vs* model group.

Group	Bcl-2	NOS
Control	0.20±0.03	0.25±0.03
Model	0.14±0.06	0.34±0.07
5 % PCF	0.26±0.05^c	0.23±0.03^c
20 % PCF	0.31±0.08^c	0.22±0.03^c
10 % Vit C	0.22±0.03^c	0.24±0.04^c

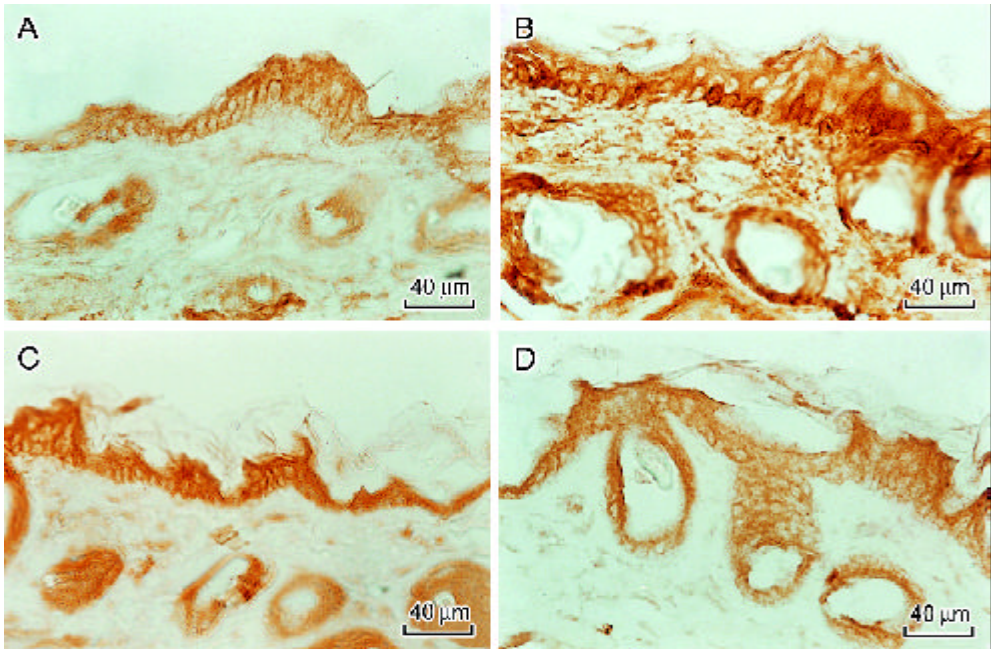

Fig 1. Immunohistochemical staining in epidermis of hairless mice. A) Bcl-2 in model group. B) Bcl-2 in 20 % PCF group. C) NOS in model group. D) NOS in 20 % PCF group. ×400.

the content of MDA in the skin of hairless mice (Tab 2).

Tab 2. Effects of PCF on the enzyme activities and MDA amount of skin homogenate of hairless mice damaged by UVA. *n*=8 mice. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* model group.

Group	GSH-px/U	SOD/ $\mu U \cdot L^{-1}$	T-AOC/ $U \cdot g^{-1}$	MDA/ $\mu mol \cdot L^{-1}$
Control	25.88±0.10	5.97±0.22	291±30	5496±1022
Model	26.09±0.11	5.1±0.3	194±40	13592±866
5 % PCF	26.46±0.13^{b}	6.1±0.6^{c}	323±42^{c}	8941±530^{c}
20 % PCF	26.61±0.12^{b}	6.4±0.5^{c}	382±63^{c}	7826±596^{c}
10 % Vit C	26.36±0.09^{c}	6.19±0.28^{c}	314±22^{c}	10746±539^{c}

After epidermis cells were exposed to UVA, we could see the damage with vacuoles in cytoplasm obviously. The ridges of mitochondria in epidermis cells in model group were broken and rough endoplasm reticulum expanded into vacuoles. The interspace between cells in epidermis of model group was broadened and there were some vacuoles in cytoplasm. The amounts of rough endoplasm reticulum, Golgi complex, and mitochondria of fibroblasts decreased dramatically and some nuclei became pycnosis (Fig 2A, B, C), which showed that the activity of the cell was weakened.

The constructions of epidermis and fibroblasts in dermis of 20 % PCF group were normal. The mitochondria and rough endoplasm reticula were distinct. The rough reticula were plentiful. The tonofilaments and intercellular bridges could be clearly observed in epidermis (Fig 2D, E).

The structure of epidermis and fibroblasts in dermis in 5 % PCF group were normal. The endoplasm tonofilaments were easily seen in cells but the mitochondria were obscure. The rough endoplasm reticula were plentiful in fibroblasts of dermis (Fig 2F, G).

DISCUSSION

From all the results, we could see that in model group UVA induced the deformation of the epidermis and dermis, inhibited the expression of Bcl-2 protein, and enhanced that of NOS. At the same time, UVA decreased the activities of antioxidative enzymes in the skin and increased the lipid peroxide. All this had made it clear that we had established the continuous UVA dam-

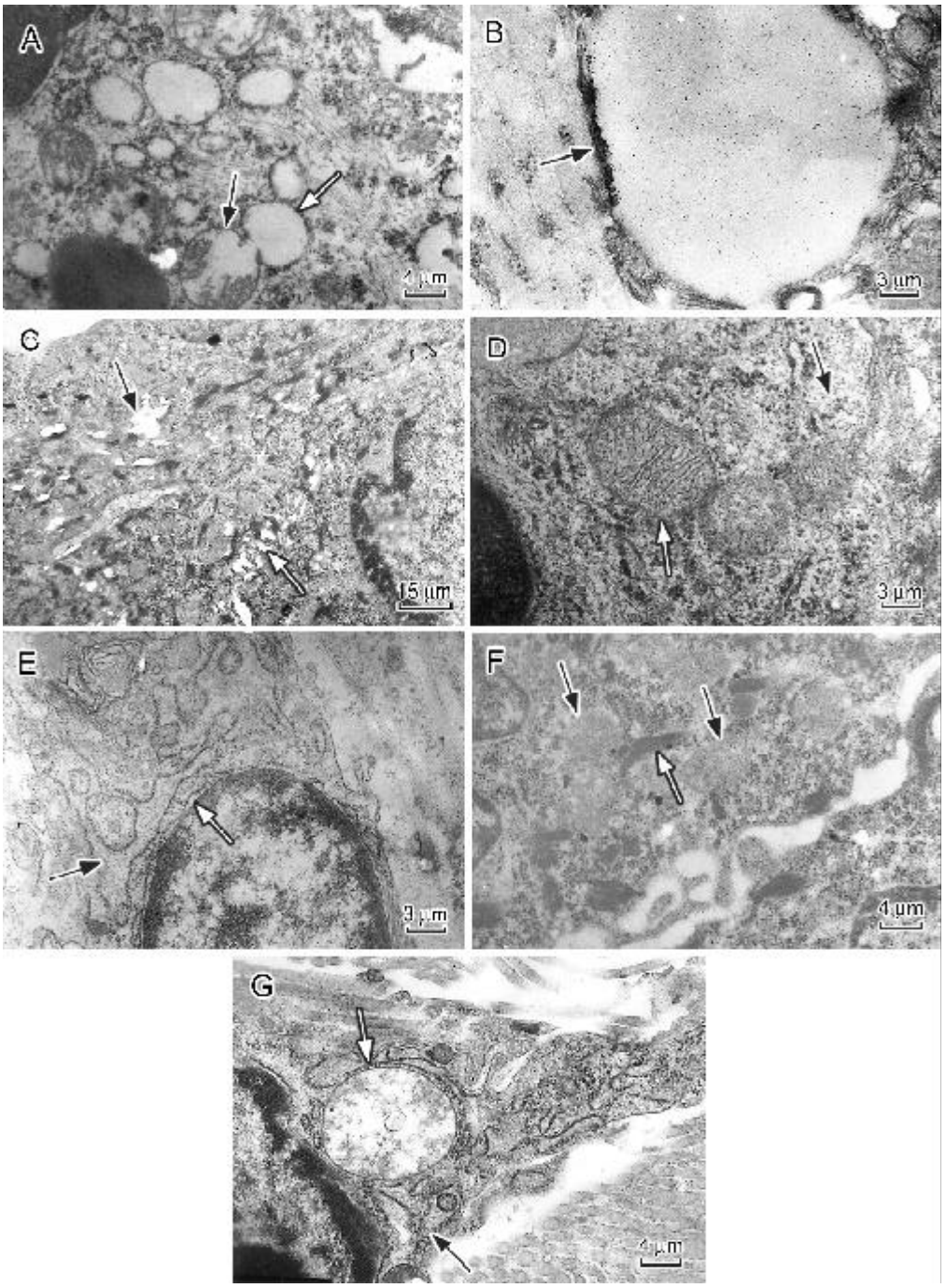

Fig 2. Ultrastructure in epidermis and dermis of hairless mice. A) Ridges of mitochondria and rough endoplasm in epidermis cells in model group. ×30 000. B) The interspace between cells and some vacuoles in cytoplasm in epidermis of model group. ×80 000. C) The big fibroblast of dermis of model group. ×40 000. D) Normal structure of epidermis in 20 % PCF group. ×40 000. E) Normal structure of fibroblasts in dermis of 20 % PCF group. ×40 000. F) Structure of epidermis in 5 % PCF group. ×30 000. G) Structure of fibroblasts in dermis of 5 % PCF group. ×30 000.

age model on mice successfully.

Electron microscope plays an important role in modern medical science. It is the most reliable method in observing the shape of cells. Under the electron microscope, we may study the character of the cell and see the small changes of the cell ultrastructure[11]. Our results showed that UVA could damage the epidermis as well as dermis of the mice. PCF protected the

mice from oxidative damage caused by UVA thus remained the ultrastructure of the cells in skin normal.

Fibroblasts in dermis can produce collagen fibers, elastic fibers, argyrophilic fiber, and dermis matrix, which have the function of maintaining the elasticity and the strength of the skin. When the fibroblasts in dermis are in dysfunction, the effect of skin will be in disturbance[12]. PCF could protect the fibroblasts in dermis thus maintained the skin function normal.

In 1988, Veux reported the function of Bcl-2 protein firstly. Bcl-2 is a kind of inhibitor of the cell apoptosis. It can prevent the apoptosis induced by free radicals and lipid peroxidation[13]. Bcl-2 has the antioxidative characteristics in cells through participating the reduction action and inhibiting the formation of active oxygen[14]. PCF could up-regulate the expression of Bcl-2 protein thus inhibit the lipid peroxidation induced by UVA.

Recently, many reports showed that NO· could induce the apoptosis of cells[15]. NO· combined with free radicals can perform nitroso which may causes the damage of DNA in cells. NO· itself also can injure the cells[16]. The expression of NOS protein indicates the amounts of NO· produced in cells. Our results showed that PCF inhibited the expression of NOS therefore prevented the damage caused by NO.

Several antioxidative enzymes including GSH-px, SOD, and CAT *etc* scavenge free radicals produced by ultraviolet[17]. The activities of the antioxidative enzymes reflect the potential anti-oxidative capability of the cell. UVA causes the cell of the skin to produce lipid peroxide[18]. The amounts of MDA reflect the level of lipid peroxidation *in vivo*[19]. Our results showed that PCF enhanced the activities of GSH-px and SOD and claimed that PCF was a potential antioxidant against ultraviolet damage. PCF can stimulate enzymes to remove free radicals therefore protect cells from the injury of radicals. Also PCF can enhance the activity of T-AOC that reflects the anti-oxidative capability of the skin. At the same time, PCF decrease the amounts of MDA which also means PCF reduces the lipid peroxidation of the cell and keeps the cells in skin out of the damage of the radicals.

In summary, on the background of successfully establishment of the continuous UVA damage model (irradiation intensity: 4556.4 J· cm^{-2}) of hairless mice, the results of this study indicated that PCF had the protective effects on hairless mice damaged by ultraviolet A. At the concentration from 5 % to 20 %, PCF obviously increased the activities of GSH-Px, T-AOC, and SOD, inhibited the lipid peroxidation, up-regulated the expression of Bcl-2 protein, and down-regulated that of NOS. Thus we observed the normal ultrastructures of epidermis and fibroblasts of mice in PCF group. PCF could protect not only the epidermis but also the dermis from the injury caused by UVA.

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扇贝多肽抗紫外线A对无毛小鼠皮肤的氧化损伤[1]

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关键词　扇贝；紫外线；抗氧化剂；无毛小鼠；*bcl*-2 基因；一氧化氮合酶；免疫组织化学；局部投药

目的：探究扇贝多肽(PCF) 抗紫外线 UVA 对无毛小鼠皮肤氧化损伤的作用．方法：昆明种无毛小鼠，随机分为双蒸水未照射组和模型组(双蒸水照射组、5 % PCF 组、20 % PCF 组、10 % 维生素 C 组)．酶法测定皮肤匀浆抗氧化酶(GSH-Px、T-AOC、SOD)活性和 MDA 的含量；免疫组织化学法测定皮肤 Bcl-2 和 NOS 蛋白表达；电镜观察皮肤组织超微结构．结果：PCF 能明显增加皮肤组织匀浆总抗氧化能力及 GSH-Px、SOD 活性，降低 MDA 含量．免疫组化结果表明，PCF 能上调 Bcl-2 蛋白的表达；抑制 NOS 蛋白的表达．超微结构显示 20 % PCF 组表皮细胞结构正常，成纤维细胞的细胞器结构正常；模型对照组表皮细胞损伤，胞质内可见空泡形成，真皮成纤维细胞内可见囊泡状扩张的滑面内质网，粗面内质网等细胞器减少，PCF组与模型对照组比较各项指标均有改善($P<0.05$)．结论：扇贝多肽具有抗紫外线 UVA 对无毛小鼠皮肤氧化损伤的作用．其机制与扇贝多肽上调 Bcl-2 蛋白表达，下调 NOS 蛋白的表达，提高抗氧化酶含量，抑制脂质过氧化有关．

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