Journal of Photochemistry and Photobiology B: Biology 130 (2014) 47–56

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Molecular mechanism of polypeptides from *Chlamys farreri* (PCF)'s anti-apoptotic effect in UVA-exposed HaCaT cells involves HSF1/HSP70, JNK, XO, iNOS and NO/ROS

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ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 4 November 2013
Accepted 5 November 2013
Available online 14 November 2013

Keywords:
Ultraviolet A-induced apoptosis
Heat shock transcription factor 1
Heat shock protein 70
c-Jun N-terminal kinases
Inducible nitric oxide synthase
Xanthine oxidase

ABSTRACT

This study investigated the molecular mechanisms of polypeptides from *Chlamys farreri* (PCF)'s anti-apoptotic effects in ultraviolet A-rays (UVA) exposed HaCaT cells. UVA-induced apoptosis in HaCaT cells was confirmed with Hoechst 33258 fluorescent staining; PCF treatment inhibited UVA-induced apoptosis in HaCaT cells, increased transcriptional activities of heat shock factor protein 1 (HSF1) and the expression of heat shock protein 70 (HSP70), whereas inhibited activation of c-Jun N-terminal kinases (JNK), expression of xanthine oxidese (XO), inducible nitric oxide synthase (iNOS) and release of nitric oxide (NO)/reactive oxygen species (ROS). Meanwhile, the HSF1 transcription inhibitor quercetin increased UVA-induced apoptosis, activation of JNK, expression of XO and iNOS and release of NO/ROS. Among the two NO release peaks we found in UVA exposed HaCaT cells, XO inhibitor oxypurinol was found to be able to inhibit NO release at 3 h post UVA exposure but not 18 h, while iNOS inhibitor S-methylisothiourea sulfate (SMT) was found to inhibit iNOS expression and NO release at 18 h but not 3 h. PCF's protection against UVA-induced apoptosis in HaCaT cells involves increased transcriptional activity of HSF1, increased expression of HSP70, and the subsequential inhibition of JNK pathway, XO and iNOS expression and ROS/NO release.

1. Introduction

UV irradiation is a common cause of skin damage [1]. The UV spectrum can be divided into three wavelength ranges, UVA, UVB and UVC [2]. While UVB is considered a carcinogen [3], UVA was also reported to cause cell damage, induce photoaging and lead to the formation of tumors in hairless mouse models [4,5]. The potential adverse health effects associated with UVA exposure, along with the facts that 90–99% of the solar radiation that reaches the surface of the earth is comprised of UVA [6] and that UVA penetrates deeper into the skin than UVB [7] guarantee the need for therapeutic agents that protect against UVA induced cell damage.

Polypeptides have multiple advantages comparing to traditional chemical-based drugs, such as better drug targeting and better cell penetration [8,9]. Considering their unique advantages and the quickly development, polypeptides is a promising group of potential new drugs. Polypeptides from *Chlamys farreri* (PCF) is a novel marine bioactive product developed by Yellow Sea Fisheries Research Institute Chinese Academy of Fishery Sciences. *C. farreri* (Chinese scallop) is common seafood found in East Asian sea, which was considered rich of nutrients [10]. This peptide isolated from *C. farreri* is a water-soluble octapeptide consists of Pro, Asn, Ser, Thr, Arg, Hyl, Cys and Gly. The molecular weight is 879 Da. Previous studies showed that PCF possessed anti-oxidant property and anti-apoptotic property, and the molecular mechanism involves p38MAPK, aSMase, c-Jun and COX-2 [11–13]. However, many still remain unknown for the complete molecular mechanism of PCF's anti-apoptotic effect.

A potential target of PCF is the HSF1/HSP70 pathway. Heat shock proteins (HSPs) are a group of highly conserved proteins, which have strong cytoprotective effects and behave as molecular chaperones [14,15]. Among many different HSP families, the HSP70 family appears to be the most evolutionarily conserved and widely distributed [16]. On heat stress, HSF1 undergoes phosphorylation, trimerlization and translocates to nuclear, then binds to the heat shock elements (HSE) in the promoter region of HSP70 gene, upregulating the latter's expression [17,18]. HSF1/HSP70 has been reported to play important roles in cancer and neurological diseases [19,20], but little attention had been paid to their roles in UV-induced damage. Moreover, HSF1/HSP70 pathway is known to inhibit JNK pathway, which contributes to its protective effects on mitochondrial ultrastructure and the promotion on mitochondrial

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http://dx.doi.org/10.1016/j.jphotobiol.2013.11.005

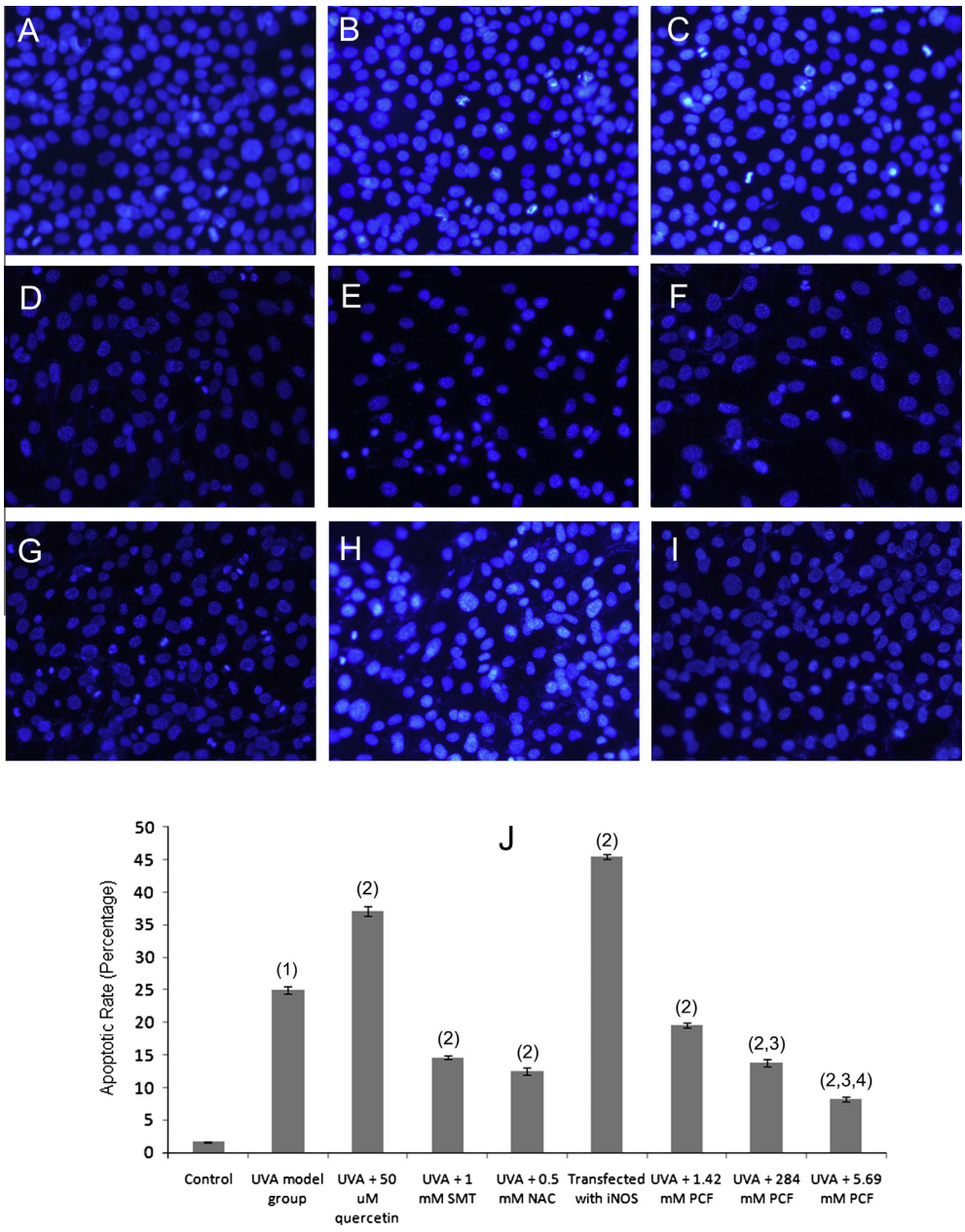

Fig. 1. Hoechst 33258 staining for apoptotic rates in HaCaT cells exposed to 8 J/cm^2 UVA with or without 50 μM quercetin, 1 mM SMT, 0.5 mM NAC, 1.42, 2.84 or 5.69 mM PCF (PCF1, PCF2 and PCF3, respectively), or HaCaT cells transfected with iNOS. HaCaT cells in logarithmic growth phase with cells fusion up to 80–85% were treated with the treatments described above for 2 h, then exposed to UVA for 3 h to reach total exposure of 8 J/cm^2. Cells were incubated for another 18 h with treatments in medium and were subjected to Hoechst 33258 staining for apoptotic rate. iNOS transfected cells were incubated in identical conditions without UVA or other treatments, and were subjected to Hoechst 33258 staining after 18 h incubation (For all groups, $N = 3$ from three independent experiments). A: Representative picture for control group. B: Representative picture for UVA model group. C: Representative picture for quercetin group. D: Representative picture for SMT group. E: Representative picture for NAC group. F: Representative picture for iNOS transfection group. G: Representative picture for 1.42 mM PCF group. H: Representative picture for 2.84 mM PCF group. I: Representative picture for 5.69 mM PCF group. Magnitude for all pictures was 400X. J: Quantification of the apoptotic rates for each group. (1) Statistically different from control group. (2) Statistically different from UVA model group. (3) Statistically different from PCF 1.42 mM group. (4) Statistically different from PCF 2.84 mM group. All error bars represent standard derivation.

membrane ion channel opening [5]. Because of their involvement in critical events in cell damage, we investigated HSF1/HSP70 and JNK as potential mechanisms of PCF's anti-apoptotic effect.

HSF1/HSP70 activation has multiple interactions with other cell signaling pathways, one important target is iNOS. iNOS is involved in the chronic inflammation and cell damage [21–23]. Multiple reports confirmed that HSF1 and HSP70 could inhibit the induction of iNOS [17,24], which seemed to be another contributor of HSF1/HSP70's cytoprotective effects, thus iNOS was also investigated as a potential mechanism of PCF's anti-apoptotic effects.

ROS generation plays important roles in cell apoptosis [25]. In our previous studies, PCF's protective effects were found to be associated with the suppression of ROS generation [12]. However, little was reported regarding to the role of NO, an important free radical and reactive nitrogen species (RNS) in cell apoptosis. Previously in our lab, we detected NO release in HaCaT cells following UV exposure and the peak at 18 h post UV exposure was associated with iNOS [26]. However, we also observed an early peak of NO which had not been elucidated. Recent studies have shown that XO could also catalyze nitrate and nitrite reduction to produce