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Polydatin, A Natural Precursor of Resveratrol, Induces β-Defensin Production and Reduces Inflammatory Response

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Abstract—It is well known that human keratinocytes produce the anti-microbial peptide β-defensin 2. Its production is enhanced by pathogenic microorganisms or other environmental stressors. In this study, we evaluated the effect of resveratrol, a polyphenol found in several dietary source as grape seed, and its natural precursor, polydatin on heat-stressed human keratinocytes. By reverse transcription-polymerase chain reaction and enzyme-linked immunoadsorbent assay, we demonstrated that resveratrol used in combination with polydatin was able to modulate interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha gene expression. In addition, our data show that resveratrol and polydatin increased the heat shock protein (Hsp)70B′ gene expression, a Hsp that plays an important role in the cytoprotection and repair of cells and tissues. Worthy of note, polydatin used alone or in combination with resveratrol, increased the release of human β-defensin 2. These results highlighted the ability of polydatin and resveratrol to reinforce cytoprotective response in stress conditions and suggest their use in cosmetic or pharmaceutical preparations.

KEY WORDS: resveratrol; polydatin; HaCat.

INTRODUCTION

The human epidermis provides a first defence barrier against environmental stressor. Keratinocytes, which are the major constituent of the epidermis, not only make up the physical barrier against microbial pathogens, but also play an active role in innate immune responses [1]. Recognition of pathogens by innate immune cells is mediated by pattern-recognition receptors that recognise conserved pathogen-associated molecular patterns. One major group of pattern-recognition receptors is the toll-like receptors (TLRs) which transduct signals leading to the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thus driving the induction of several pro-inflammatory cytokines, chemokines [2, 3] and anti-microbial peptides and the up-regulation of adhesion and costimulatory molecules involved in innate and acquired immune responses [4].

Anti-microbial peptides produced by keratinocytes, among which the β-defensin family, contribute to the host's defence mechanism against bacterial, fungal and viral infections in all species from insects to man [5]. They represent a first line of defence system that is present constitutively but may be enhanced in the case of inflamed or injured skin, events that alter the skin protective functions. Moreover, cytokines released in the skin strongly affect the expression of heat shock proteins (Hsp), highly conserved proteins that have a

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protective function against subsequent cellular injury [6]. This protein is constitutively expressed in specific skin cells, such as epidermal keratinocytes, providing a natural barrier against potential environmental stressful attacks. The Hsp70B′, another member of Hsp family, is strictly stress inducible and absent in unstressed cells.

Resveratrol (3,4′,5,-trihydroxy-trans-stilbene) (Fig. 1) is a naturally occurring polyphenol and is particularly found in grape skin, nuts, pomegranate and *Poligonum cuspidatum*. It has been hypothesised that resveratrol contributes to the ability of polyphenols-rich Mediterranean diet to reduce the incidence of age-related diseases such as coronary heart disease, cancer and dementia [7, 8]. In support of this hypothesis, resveratrol displays a broad variety of beneficial effects including cardioprotective, neuroprotective, anti-microbial and chemopreventive properties. Resveratrol has been shown to down-regulate vasoactive peptides such as endothelins, to inhibit oxidised low-density lipoprotein and cyclooxygenase, to inhibit the clearance and neurotoxicity of beta-amyloid, to modulate apoptotic signalling pathways and to activate sirtuin and AMP-activated protein kinase which are believed to be involved in the caloric restriction-longevity effect [9]. Recently, it was found that in the grape juices and in *P. cuspidatum*, the average concentration of piceid (resveratrol-3-*O*-β-mono-D-glucoside, polydatin), the glycoside form of resveratrol, is seven times that of resveratrol [10, 11] and this is probably the most abundant form of resveratrol in nature [12]. Piceid has the glucoside group bonded in position C-3 substitutes of a hydroxyl group (Fig. 1). This substitution gives rise to conformational changes of the molecule, resulting in changes in the biological properties. Piceid is more resistant to enzymatic oxidation than resveratrol is soluble in water, and unlike resveratrol which penetrates the cell passively, it enters the cell via an active mechanism using glucose carriers [10]. A number of studies hypothesise that piceid may have biomedical properties similar to those abovementioned for resveratrol: anti-carcinogenic effects and inhibition of platelet aggregation and LDL oxidation [13]. Moreover, a recent study performed on human peripheral blood mononuclear cells has demonstrated the capability of these polyphenolic compounds to decrease interleukin (IL)-17 production. [14].

In this study, we investigated the effects of resveratrol and polydatin on heat-stressed human keratinocytes (HaCat). Here, we demonstrate that resveratrol and polydatin reduce inflammatory response of thermally stressed HaCat and reinforce cytoprotective response through the human β-defensin 2 (HBD-2) and Hsp70B′ induction.

MATERIALS AND METHODS

Chemicals

Natural resveratrol and polydatin were extracted and kindly supplied by Fondazione Edmund Mach, Istituto Agrario di San Michele all'Adige (ISMA), Italy. The purity of both compounds tested by HPLC-MS, UV and MNR was higher than 99 % (method patented and described in EP2087894A1) [15, 16]. Resveratrol and polydatin were dissolved in ethanol at 100 mM stock solution. All stock solutions were stored at −80 °C and diluted in culture medium just prior to use.

MTT Cell Proliferation Assay

About 3×10^3 HaCat was seeded in 96-well plates in culture medium in a final volume of 100 μL Dulbecco's modified Eagle's medium (DMEM), at 37 °C and 5 % CO_2. After 24 h, the cells were treated with different concentrations of resveratrol, polydatin and resveratrol and polydatin combination (5.5, 11, 22, 44 and 88 μM). After treatment for 24 h, the medium was discarded and 30 μL of 0.5 mg/mL 3-(4,5-dimethylthiazol-

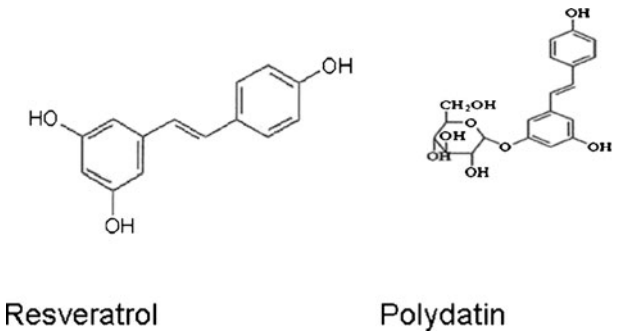

Fig. 1. Chemical structure of resveratrol and polydatin.

2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO) was added to each well followed by incubation for 3 h at 37 °C. MTT (Sigma-Aldrich) is a tetrazolium salt that can be cleaved by active mitochondria of viable cells to form a purple formazan product which can be measured colorimetrically. The purple formazan formed was then solubilised by 150 μL DMSO/well. The absorbance was recorded at 560 nm using an enzyme-linked immunoadsorbent assay (ELISA) plate reader (Bio-Rad Laboratories, Hercules, CA).

Cell Culture and Treatments

HaCat cell lines (HaCat cell line spontaneously immortalised, non-tumorigenic) were cultured in DMEM supplemented with 10 % fetal bovine serum (Gibco Laboratories, Grand Island, NY), penicillin (50 U/mL), streptomycin (50 mg/L) and 2 mM L-glutamine. For the treatments, 3×10^5 cells were plated in 6-well plates (35 mm diameter) with 2 mL complete medium and pre-incubated for 24 h at 37 °C with resveratrol (44 μM), polydatin (44 μM) and resveratrol and polydatin combination. At the end of the incubation period, cells were heat stressed for 40 min at 42 °C.

Reverse Transcription-Polymerase Chain Reaction Analysis

Total RNA, isolated by High Pure RNA Isolation Kit (Roche Diagnostics, Milan, Italy) from 1×10^6 HaCat was transcribed by reverse transcriptase (Expand Reverse Transcriptase, Roche Diagnostics) at 42 °C for 45 min according to the manufacturer's instructions. Two microlitres of complementary DNA were amplified in a reaction mixture containing 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 200 μM dNTP and 2.5 units of *Taq* DNA polymerase (Roche Diagnostics) in a final volume of 50 μL. For the co-amplification of TLR-2, TLR-4, IL-8, IL-6, tumor necrosis factor-alpha (TNF-α), HBD-2 and Hsp70B′, the PCR was carried out in the presence of 0.5 μM sense and anti-sense TLR-2, TLR-4, IL-8, IL-6, TNF-α, HBD-2 and Hsp70B′ primers and 0.05 μM sense and anti-sense glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β-actin primers. Conditions and size of products are reported in Table 1. The reaction took place in a DNA thermal cycler (Mastercycler gradient, Eppendorf, Milan, Italy). β-actin reverse transcription-polymerase chain reaction (RT-PCR) was performed on mRNAs to confirm that mRNAs were suitable for RT-PCR analysis. The PCR products were analysed by electrophoresis on 1.8 % agarose (Eppendorf, Milan, Italy) gel in Tris–Borate-EDTA buffer (Fluka, BioChemiKa, Switzerland). Densitometric analysis of ethidium-bromide-stained agarose gel was carried out by NIH image V1.6 software. The ratio between the yield of each amplified product and that of the co-amplified internal control allowed a relative estimate of mRNA levels in the sample analysed. The internal control (GAPDH or β-actin) was a housekeeping gene whose PCR product did not overlap with the studied gene.

ELISA Assay for HBD-2

Semi-confluent HaCat were treated or not with resveratrol, polydatin and resveratrol/polydatin for 24 h. Cell supernatants were recovered by centrifugation and assayed for the HBD-2 detection by an ELISA (Phoenix Pharmaceuticals, Inc). The results were expressed in picogrammes per millilitre of culture medium.

Protein Extraction and Western Blotting Analysis

About 4×10^5 HaCat were treated or not with resveratrol, polydatin and resveratrol/polydatin for 24 h. The cells were scraped with 1 mL phosphate-buffered saline (PBS), and the cell pellet was homogenised with 300 μL ice-cold buffer (50 mM HEPES at pH 7.5, 150 mM NaCl, 1 % glycerol, 1 % Triton, 1.5 mM $MgCl_2$ and 5 mM EGTA) supplemented with 20 mM sodium pyrophosphate, 40 μg/mL aprotinin, 4 mM PMSF, 10 mM sodium orthovanadate and 25 mM NaF. Total extracts were cleared by centrifugation for 30 min at 4 °C at 10,000 rpm and assayed for the protein content by Bradford's method. Fifty microgrammes of protein from each cell lysates were separated by a 10 % SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes; the filters were stained with 10 % Ponceau S solution for 2 min to verify equal loading and transfer efficiency. Blots were blocked overnight with 5 % non-fat dry milk, then incubated with anti-human TLR-2 (H-175) and anti-human TLR-4 (H-80) rabbit polyclonal antibodies (10 μg/mL; Santa Cruz Biotechnology), anti-IL-6 rabbit polyclonal antibody (Santa Cruz, CA), anti-IL-8 rabbit polyclonal antibody (Chemicon, Temecula, CA), anti-Hsp70B′ rabbit polyclonal antibody (Stressgen, Biotechonologies) and anti-TNF-α mouse polyclonal antibody (Santa Cruz), 1 μg/mL in TBS (150 mM NaCl, 20 mM Tris–HCl at pH 8) for 2 h at room temperature. After washing with 0.1 % Tween-20 PBS, the filter was incubated with 1:2,500 peroxidase-

Table 1. Human Primers Sense and Anti-Sense Sequences and Expected PCR Products (bp)

Gene	Sense and anti-sense sequences	Conditions	bp
IL-6	5′-ATg AAC TCC TTC ACA AgC gC-3′	30 cycles at 95 °C for 30 s	628
	5′-gAA gAg CCC TCA ggC Tgg ACT g-3′	55 °C for 71 s and 72 °C for 142 s	
IL-8	5′-ATg ACT TCC AAg CTg gCC gTg-3′	30 cycles at 94 °C for 60 s	297
	5′-TgA ATT CTC AgC CCT CAA AAA CTT CTC	55 °C for 60 s and 72 °C for 60 s	
TNF-α	5′-gAg CAC TgA AAg CAT gAT CCg-3′	33 cycles at 94 °C for 60 s	682
	5′-AAA gTA gAC CTg CCC AgA CTC gg-3′	60 °C for 60 s and 72 °C for 60 s	
HBD-2	5′-CCA gCC ATC AgC CAT gAg ggT-3′	33 cycles at 94 °C for 60 s	200
	5′-ggA gCC CTT TCT gAA TCC gCA-3′	63 °C for 60 s and 72 °C for 60 s	
TLR-2	5′-gCC AAA gTC TTg ATT gAT Tgg-3′	32 cycles at 95 °C for 45 s	347
	5′ TTg AAg TTC TCC AgC TCC Tg-3′	54 °C for 45 s and 72 °C for 60 s	
TLR-4	5′-gAA ATg gAg gCA CCC CTT C-3′	32 cycles at 95 °C for 45 s	628
	5′-Tgg ATA CgT TTC CTT ATA Ag-3′	54 °C for 45 s and 72 °C for 60 s	
HSP-70	5′-ctc caG cat ccG aca aGa Agc-3′	33 cycles at 94 °C for 30 s	234
	5′-acG GtG ttG Tgg ggg TTC Agg-3′	63 °C for 30 s and 72 °C for 30 s	

conjugated anti-rabbit immunoglobulins (Santa Cruz) and 1:2,500 anti-mouse immunoglobulin (Santa Cruz) for 1 h at 22 °C. It was extensively washed and finally analysed using the Enhanced Chemiluminescence System (Amersham, Little Chalfont, UK). Protein loading was checked by reprobing the membranes with α-tubulin, in order to show that protein levels were not changed.

Statistical Analysis

All the experiments were conducted with three molecules (resveratrol, 44 μM, polydatin, 44 μM and association of resveratrol and polydatin, 44 μM) with at least three replicates for each group. All the experiments were performed also on stressed cells. The *P* value was generally evaluated between 0.01 and 0.03, confirming the statistical significance of results ($P<0.05$). The three experimental groups were compared with control group using a one-way analysis of variance. Post hoc group comparison was conducted using the Student-Newman–Keuls test.

RESULTS

Resveratrol and Polydatin Did Not Affect HaCat Viability

To evaluate the effects of resveratrol and polydatin on HaCat cells viability, MTT assay was performed and HaCat cells were treated with different concentrations (5.5, 11, 22, 44 and 88 μM). As shown in Fig. 2, resveratrol and polydatin did not affect HaCat cells viability at all the concentrations used. In our experimental model, we used heat-stressed HaCat with consequent activation of inflammatory cytokines. The best concentration at which resveratrol and polydatin induced the anti-inflammatory response was 44 μM (data not shown).

Resveratrol and Polydatin Associations Up-regulates TLR-2 Gene Expression in Heat-Stressed HaCat Cells

To assess if resveratrol and polydatin might modulate the expression of TLRs, the gene expression of TLR-2 and TLR-4 was examined in HaCat cells, heat stressed or not, by RT-PCR. The treatment with 44 μM resveratrol and polydatin for 24 h, used alone or in combination, did not modulate TLRs gene expression in HaCat cells (Fig. 3a, b). When the cells were heat stressed, a significant induction of TLR-2 mRNA expression was demonstrated (Fig. 3a) while TLR-4 was not modulated (Fig. 3b). Interestingly, when the cells were pre-treated with the combination of resveratrol or polydatin and heat stressed, a stronger expression of TLR-2 was observed while when separately used the molecules were not able to induce TLR-2 expression respect to heat-stressed control. Western blot analysis was conducted to confirm the data obtained (Fig. 3c).

Resveratrol and Polydatin Reduce Proinflammatory Cytokine IL-6, IL-8 and TNF-α

To evaluate the ability of resveratrol and polydatin to modulate the proinflammatory response, activated by the heat stress, the gene expression of the proinflammatory cytokines IL-6, IL-8 and TNF-α was analysed.

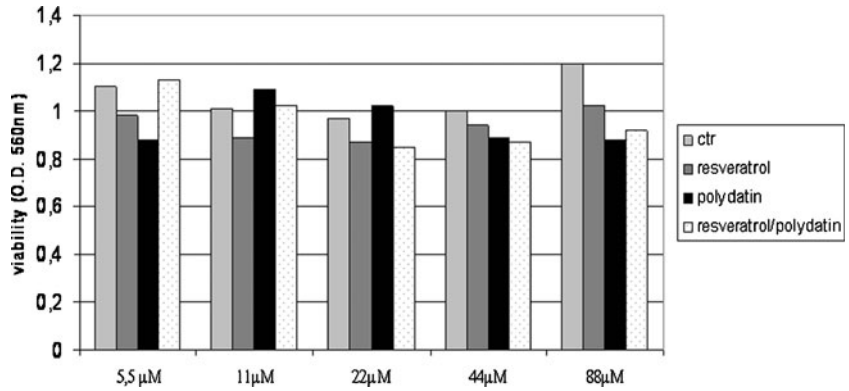

Fig. 2. Effect of resveratrol and polydatin on HaCat cell proliferation.

HaCat cells did not express these cytokines, and resveratrol and polydatin, alone or in combination, did not induce their expression (Fig. 4). As shown in Fig. 4a–c, heat-stressed HaCat cells displayed high level of TNF-α, IL-6 and IL-8 gene expression. When HaCat cells were pre-treated with 44 µM resveratrol and polydatin for 24 h, alone or in combination, and successively heat stressed, the gene expression of TNF-α, IL-6 and IL-8 was down-regulated (Fig. 4a–c). In particular, the combination of both molecules was able to strongly reduce the expression of the SU-cited cytokines, with the best effect on IL-6 whose gene expression was completely abrogated. These results were reinforced by protein assessment (Fig. 4d).

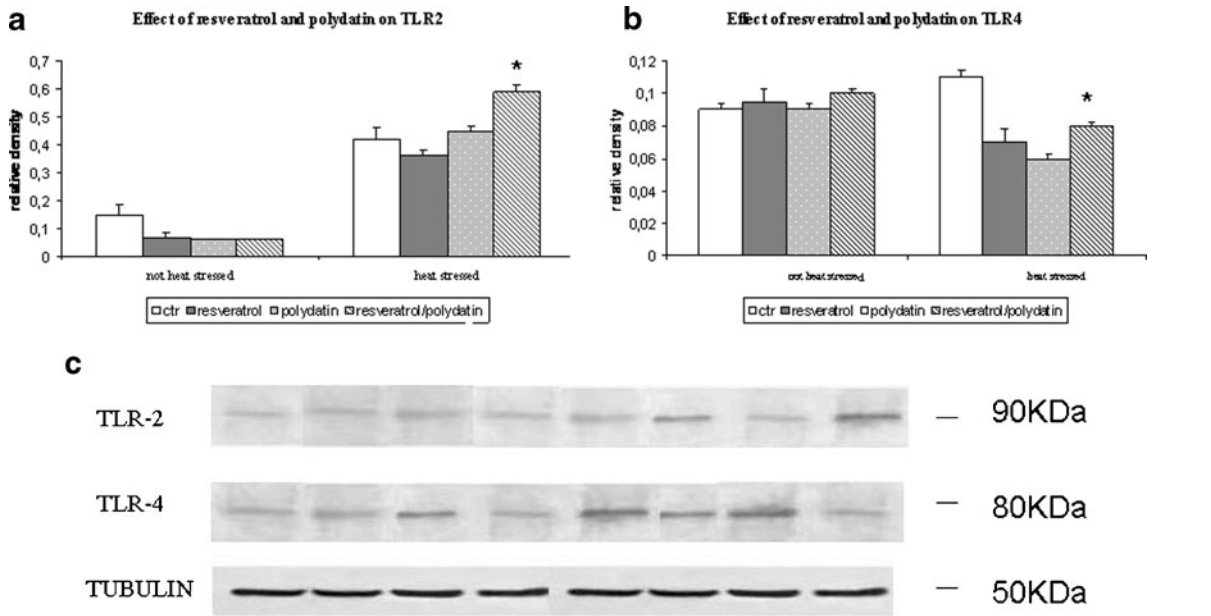

Fig. 3. Effect of resveratrol and polydatin on TLR-2 and TLR-4 mRNA expression and Western blot analysis in heat-stressed HaCat cells. RT-PCR analysis using specific primers for TLR-2 (**a**) and TLR-4 (**b**). *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 µM of resveratrol; *3*, HaCat cells treated with 44 µM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. **c** Western blot analysis with an anti-TLR-2 and anti-TLR-4, respectively. *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 µM of resveratrol; *3*, HaCat cells treated with 44 µM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. The data shown are representative of three different experiments±SD. *$P<$ 0.05, significantly different compared with control.

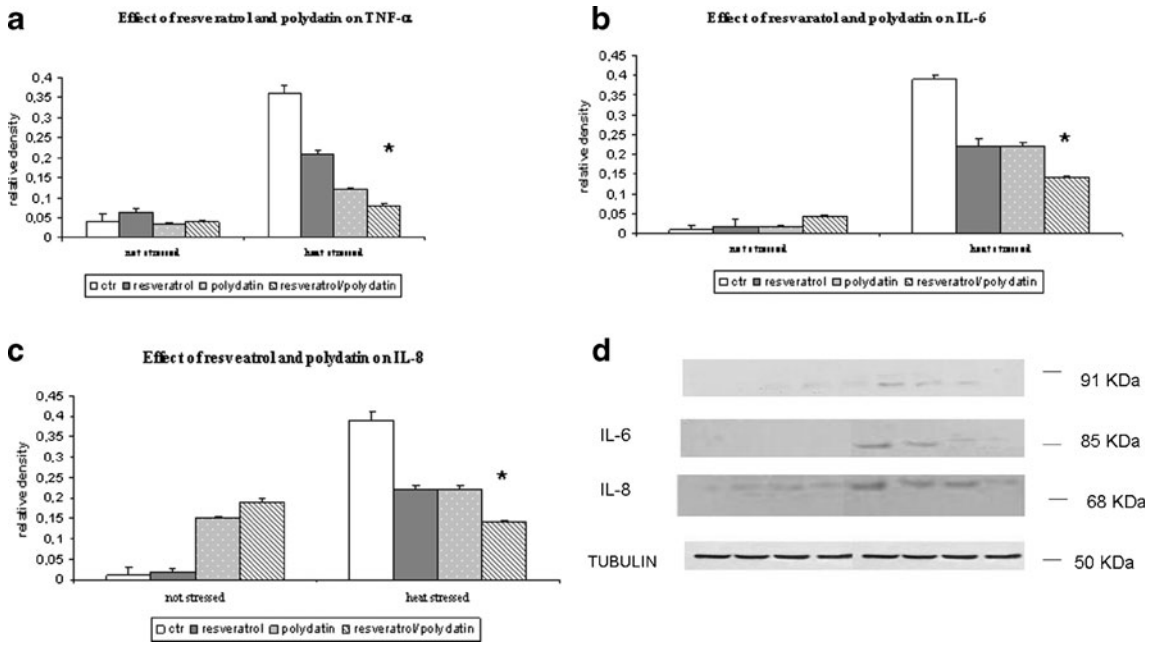

Fig. 4. Effect of resveratrol and polydatin on TNF-α, IL-6 and IL-8 mRNA expression and Western blot analysis in heat-stressed HaCat cells. RT-PCR analysis using specific primers for TNF-α (**a**), IL-6 (**b**) and IL-8 (**c**). *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 μM of resveratrol; *3*, HaCat cells treated with 44 μM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. **d** Western blot analysis with an anti-TNF-α, anti-IL-6 and anti-IL-8, respectively. *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 μM of resveratrol; *3*, HaCat cells treated with 44 μM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7* heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. The data shown are representative of three different experiments±SD. **P*<0.05, significantly different compared with control.

Resveratrol and Polydatin Combination Induces HBD-2 Release in Heat-Stressed HaCat Cells

To demonstrate if resveratrol and polydatin were able to induce the release of HBD-2, HaCat cells were pre-treated with the molecules, alone or in combination for 24 h, and successively heat stressed. As shown in Fig. 5a, heat stress did not induce the HBD-2 expression, while polydatin alone or in combination with resveratrol induced the HBD-2 expression in heat-stressed HaCat. In contrast, resveratrol was unable to induce the same effects. We also tested the release of HBD-2 in the medium of heat-stressed cells. As demonstrated by ELISA assay (Fig. 5b), the association of resveratrol and polydatin induced a strong release of HBD-2 (191 ng/mL) with respect to the heat-stressed control (9 ng/mL).

Resveratrol/Polydatin Association Up-regulates Hsp70B′ Gene Expression

To analyse the resveratrol and/or polydatin cyto-protective effects, HaCat cells were incubated for 24 h with resveratrol, polydatin and their combination and, successively, heat stressed. As shown in Fig. 6a, heat stress induced the Hsp70B′ gene expression in untreated cells, with respect to the not stressed cells. When the cells were pre-incubated with resveratrol and polydatin combination, and successively heat stressed, a stronger increase of Hsp70B′gene expression was observed. These data have been confirmed by Western blot analysis (Fig. 6b).

DISCUSSION

Anti-microbial peptides, which are synthesised in the skin at the sites of microbial entry, provide a soluble barrier that acts as an impediment to infection. In the case of infection or injury, anti-microbial peptide expression in the skin is up-regulated due to increased synthesis by keratinocytes and deposition from degranulation of recruited neutrophils. HBD-2 is virtually absent in normal skin and its expression in HaCat

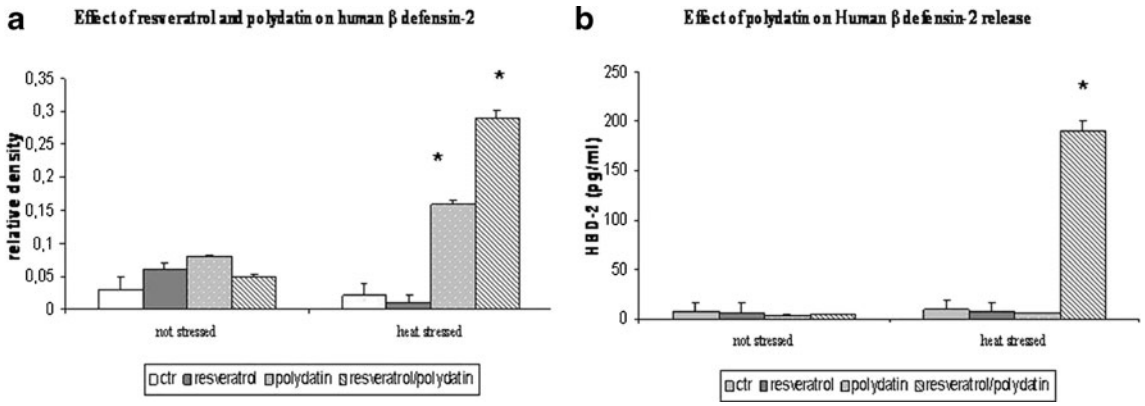

Fig. 5. Effect of resveratrol and polydatin on human β defensin-2 (*HBD-2*) mRNA expression and release in heat-stressed HaCat cells. RT-PCR using specific primers for h-BD2 (**a**). *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 μM of resveratrol; *3*, HaCat cells treated with 44 μM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. **b** Release of HDB-2 in the HaCat culture medium. ELISA assay. *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 μM of resveratrol; *3*, HaCat cells treated with 44 μM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. The data shown are representative of three different experiments±SD. **P*<0.05, significantly different compared with control.

requires stimulation by cutaneous pathogens or other environmental stressor [17]. In recent years, it has also been demonstrated that defensins exert additional biological activities, apparently unrelated to their anti-

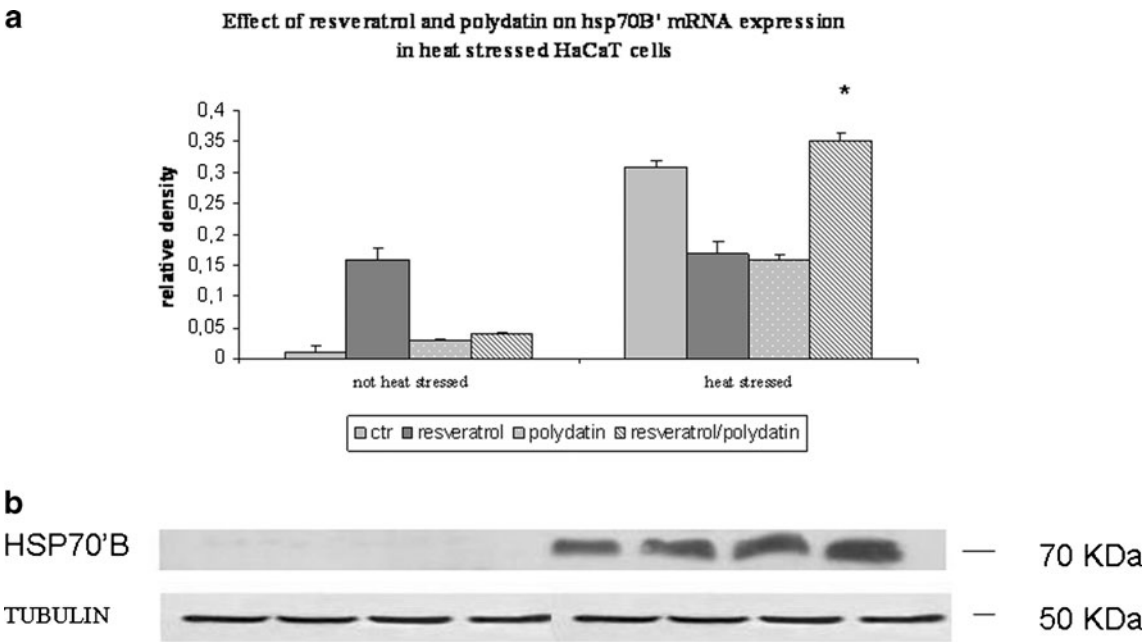

Fig. 6. Effect of resveratrol and polydatin on Hsp70B′ mRNA expression and Western blot analysis in heat-stressed HaCat cells. **a** RT-PCR analysis using specific primers for Hsp70B′. *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 μM of resveratrol; *3*, HaCat cells treated with 44 μM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. **b** Western blot analysis with an anti-Hsp70B′. *Lanes 1*, untreated HaCat cells (control); *2*, HaCat cells treated with 44 μM of resveratrol; *3*, HaCat cells treated with 44 μM of polydatin; *4*, HaCat cells treated with resveratrol and polydatin association; *5*, untreated heat-stressed HaCat; *6*, heat-stressed HaCat treated with resveratrol; *7*, heat-stressed HaCat treated with polydatin; and *8*, heat-stressed HaCat treated with resveratrol and polydatin association. The data shown are representative of three different experiments±SD. **P*<0.05, significantly different compared with control.

microbial action [18]. Several studies have indicated that HBDs are potential modulators of inflammation due to their effects on polymorphonuclear (PMN) apoptosis. Nagaoka *et al*. (2008) have demonstrated that HDBs can potentially suppress PMN apoptosis and prolong their lifespan, thereby contributing to defence against external insults [19]. In this study, we evaluated the ability of resveratrol and its piceid, the polydatin, to induce the release and gene expression of HBD-2 in heat-stressed HaCat. Our results showed that polydatin induces HBD-2 expression in heat-stressed keratinocytes, and its effects were strongly enhanced by the combined use of polydatin with resveratrol. In addition, TLR-2 gene expression was also increased by resveratrol and polydatin treatment, whereas the expression of TLR-4 was not modulated.

Considerable evidences have demonstrated a close association between β-defensins and TLRs because several epithelial cell types, including keratinocytes, react to external stimuli by inducing TLR-2 or TLR-4 and up-regulating HBD-2 [20, 21]. Heat stress may produce cellular alteration associated mainly to protein damage. To restore cellular homeostasis, some mechanism of cellular defence might be activated by inducing the expression of various genes and Hsp [22]. Hsp have an important role in cytoprotection and repair of cells against stress and trauma [23]. As expected in our experimental model, Hsp70B′ was induced after heat stress. When HaCat cells were treated with resveratrol and polydatin, and successively heat stressed, a marked over-expression of Hsp70B′ was observed, compared with the heat-stressed control. Moreover, the treatment with resveratrol and polydatin, alone or in combination, strongly reduce the proinflammatory response of heat-stressed HaCat cells. Our results are in accordance with recent literature demonstrating that resveratrol blocks the TNF-α-induced activation of NF-κB, a transcriptor factor strongly associated with inflammatory diseases. This might suggest a possible mechanism of action of resveratrol [24]. In addition, our results illustrate that resveratrol activity is potentiated by its natural piceid, the polydatin, a naturally occurring agent that might provide protection from skin damages.

The results reported here highlight the ability of polydatin, and its association with resveratrol, to enhance the production of the anti-microbial peptide HBD-2 and to reduce the expression of proinflammatory cytokines. The association also prevents cell damage by maintaining an elevated level of Hsp70B′.

Skin cells are also equipped with an elaborate system of anti-oxidant substances and enzymes, which maintains the balance between oxidative stress and anti-oxidant defence and keeps the cells away from oxidative stress damage [25]. The major oxidative stress is sunlight with its ultraviolet radiation A (UVA) [26]. These radiations enhance skin damage to cellular proteins, lipids and saccharides. In recent years, it has been reported that resveratrol prevented oxidative stress caused by UVA irradiation [27, 28]. Liu *et al*. reported that resveratrol protects HaCat from UVA-induced oxidative stress damage by down-regulating Keap1 expression. [29]. However, in our system the action mechanism of resveratrol and polydatin is still not fully understood. Further study is needed to determine the molecular mechanism of resveratrol and polydatin on our experimental model.

Our results might pave the way for a rational use of resveratrol and polydatin association in dermocosmetics or pharmacological preparations. These molecules used in combination might thus activate the cytoprotective response and protect the skin from damage caused by pathogenic microorganisms or other environmental stressors.

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