Protective effect of resveratrol against oxidative damage of UVA irradiated HaCaT cells

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Abstract Objective To observe the photoprotective effect and possible mechanisms of resveratrol for ultraviolet A (UVA) irradiated HaCaT cells. Methods HaCaT cells under UVA irradiation with 5 J/cm² were interfered with 0.01 mmol/L and 0.1 mmol/L resveratrol. The testing objects were divided into a control and a UVA irradiation group and then we detected the proliferation capacity with methylthiazol tetrazolium (MTT) and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activity content of maleic dialdehyde (MDA) with hydroxylamine colorimetric, thiobarbituric acid (TBA) methods. The ultrastructure was observed under electron microscopy. Results Resveratrol could enhance the proliferation activity of SOD, GSH-Px activity of HaCaT cells under UVA irradiation and decrease the content of MDA in dose-dependent manner (P < 0.05). The electron microscope revealed that resveratrol could relieve the injury of HaCaT cells’ ultrastructure. Conclusion Resveratrol can relieve the inhibition to HaCaT cell proliferation injury of their ultrastructure and oxidation by UVA irradiation. The protection is dose-dependent. That resveratrol raises the oxidase activity and clears the oxyradical may account for these results.

Key words resveratrol; ultraviolet A; HaCaT cell; oxidative damage

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Skin aging is a complex physiological and pathological process which involves general dermal layers. In the past ultraviolet \textsuperscript{B} UVB\textsuperscript{4} radiation was considered the main cause of photoaging but UVA was thought harmless. Today more and more observations indicate that UVA is also the important cause of photoaging. This may be related to the production of excessive oxyradical in the skin and the degression of its antioxidative capacity under UVA irradiation. However there is little evidence about the mechanism of damage to the skin superficial layer by UVA and natural antioxidant which can prevent the damage. Resveratrol is a polyphenol compound which exists in the plants and has antioxidant activity\textsuperscript{11}\textsuperscript{14} anti-platelet aggregation\textsuperscript{12} anti-atherosclerosis\textsuperscript{13} anti-inflammatory activity\textsuperscript{14} growth inhibiting activity\textsuperscript{15} immunoregulation capacity\textsuperscript{16} et al. Therefore resveratrol is widely used in prevention and therapeutics for angiocardiopathy\textsuperscript{17} \textsuperscript{20}. The antioxidant activity of resveratrol in angiocardiopathy therapy is more powerful than those of vitamin C and E\textsuperscript{21}. But the literature about photoprotective effect of resveratrol has not been reported so far. In this study\textsuperscript{22} we discussed the protection mechanism to HaCaT cells through observing the proliferation activity\textsuperscript{23} ultrastructure and variation of antioxidant indexes\textsuperscript{24} then provide theoretical justification to the prevention and therapeutics for photoaging.

1 MATERIALS AND METHODS

1.1 Materials Resveratrol was obtained from Sikehua Company of Chengdu\textsuperscript{25} HaCaT cell line was present by the Second Xiangya Hospital of Central South University. MTT and dimethyl sulfoxide\textsuperscript{26} DM-SO\textsuperscript{27} were purchased from Sigma Company. MDA\textsuperscript{28} SOD and GSH-Px kits were purchased from Nangiqu Jiancheng. Biosteritron\textsuperscript{29} spectrum peak value is 290 nm \textsim 320 nm\textsuperscript{30} was from Sigma. Transmission electron microscope\textsuperscript{31} JEOI-1230\textsuperscript{32} was from Hitachi.

1.2 Test groups The samples in this study were divided into the following 4 groups\textsuperscript{33} normal control group\textsuperscript{34} without UVA irradiated and resveratrol treated\textsuperscript{35} UVA group\textsuperscript{36} only UVA irradiation\textsuperscript{37} UVA + 0.01 mmol/L resveratrol group and UVA + 0.1 mmol/L resveratrol group.

1.3 Cell culture The completed culture medium for HaCaT cells was Delbecco’s Modified Eagle’s Medium\textsuperscript{38} DMEM\textsuperscript{39} containing 10% fetal bovine serum\textsuperscript{40} 100 U/mL penicillin and 100 μg/mL streptomycin. HaCaT cells were expanded at 37 °C under 5% CO\textsubscript{2} and drawn the growth curve.

1.4 Testing the proliferation ability of HaCaT cells by MTT essay HaCaT cells of log phase were irradiated with 5 J/cm\textsuperscript{2} of UVA\textsuperscript{41} the culture medium was added drugs immediately after irradiation and then harvested after 24 h.

1.5 Detection of SOD\textsuperscript{42} GSH-Px and MDA HaCaT cells homogenate of each group were prepared. SOD\textsuperscript{43} GSH-Px and MDA were determined according to the manufacturer’s\textsuperscript{44} instructions.

1.6 Ultrastructure of HaCaT cells observed under electron microscope HaCaT cells of the normal control group\textsuperscript{45} the UVA group and the UVA + 0.1 mmol/L resveratrol group were transferred to culture dishes\textsuperscript{46} and rendered drugs immediately after irradiated with 5 J/cm\textsuperscript{2} of UVA\textsuperscript{47} then harvested after 24 h. Cells were fixed in phosphate buffer containing 2.5% glutaraldehyde\textsuperscript{48} then fixed in 2% osmium tetroxide\textsuperscript{49} dehydrated by acetone\textsuperscript{50} infiltrated in increasing concentrations of epon\textsuperscript{51} and embedded. Ultrathin sections were prepared\textsuperscript{52} then stained with uranyl acetate and lead citrate. Finally\textsuperscript{53} the HaCaT cells were examined and photographed by transmission electron microscopy.

1.7 Statistical Analysis SPSS13.0 was used for all statistical analysis. Data were denoted as means ± SD\textsuperscript{54} \bar{x} \pm s\textsuperscript{55} and evaluated by Kruskal Wallis H-test and one-factor analysis of variance. The significance level was set at P < 0.05.

2 RESULTS

2.1 Effect of resveratrol on proliferation capacity of UVA irradiated HaCaT cells OD value of UVA group was significantly decreased than that of the normal control group\textsuperscript{56} P < 0.05\textsuperscript{57}. Resveratrol increased OD value in UVA irradiated HaCaT cells in a dose dependent manner\textsuperscript{58} P < 0.05\textsuperscript{59} Table 1\textsuperscript{60}.

2.2 Effect of resveratrol on antioxidation ability
of UVA irradiated HaCaT cells Compared with the normal control group the activities of SOD GSH-Px of UVA group were significantly decreased and the concentration of MDA was increased $P < 0.05$. Resveratrol increased the activity of SOD GSH-Px and decreased the concentration of MDA in a dose-dependent manner $P < 0.05$. Table 2.

Table 1 Effect of resveratrol on proliferation ability of UVA irradiated HaCaT cell $x \pm s n = 6$

<table>
<thead>
<tr>
<th>Groups</th>
<th>OD value</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control group</td>
<td>0.889 ± 0.083</td>
</tr>
<tr>
<td>UVA group</td>
<td>0.542 ± 0.004</td>
</tr>
<tr>
<td>UVA + 0.01 mmol/L resveratrol group</td>
<td>0.753 ± 0.435</td>
</tr>
<tr>
<td>UVA + 0.1 mmol/L resveratrol group</td>
<td>0.892 ± 0.173</td>
</tr>
</tbody>
</table>

$*$ $P < 0.05$ vs normal control group $\triangle P < 0.05$ vs UVA group $#$ $P < 0.05$ vs UVA + 0.01 mmol/L resveratrol group

Table 2 Effect of resveratrol on antioxidation ability of UVA irradiated HaCaT cell $x \pm s n = 3$

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD U/mg</th>
<th>GSH-Px U/</th>
<th>MDA mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control group</td>
<td>579.263 ± 36.010</td>
<td>128.553 ± 3.325</td>
<td>25.930 ± 0.872</td>
</tr>
<tr>
<td>UVA group</td>
<td>84.343 ± 1.950</td>
<td>65.330 ± 5.541</td>
<td>74.735 ± 4.382</td>
</tr>
<tr>
<td>UVA + 0.01 mmol/L resveratrol group</td>
<td>440.503 ± 8.051</td>
<td>98.536 ± 15.035</td>
<td>36.542 ± 1.256</td>
</tr>
<tr>
<td>UVA + 0.1 mmol/L resveratrol group</td>
<td>576.100 ± 10.181</td>
<td>130.093 ± 14.616</td>
<td>27.437 ± 1.203</td>
</tr>
</tbody>
</table>

$*$ $P < 0.05$ vs normal control group $\triangle P < 0.05$ vs UVA group $#$ $P < 0.05$ vs UVA + 0.01 mmol/L resveratrol group

2.3 Ultrastructure change In the control group integrated cytomembrane small amounts of myelin-body affluent mitochondria and endoplasmic reticulum in endochylema appeared no edema in endochylema. Apoptosis and pyknosis were rarely seen Figure 1.

In the UVA group typical cellular apoptosis enriched and margined chromatin in nucleus appeared. Pyknosis and cell debris were commonly seen Figure 2.

In the UVA + 0.1 mmol/L resveratrol group vacuolization in endochylema and myelinbody were commonly seen. Extensive organelles were edema. Cellular shrinkage and pyknosis were commonly seen Figure 3.

Table 2

Fig. 1 Ultrastructure change of control group A Integrated cytomembrane no edema in endochylema $\times 5000$ B Small amounts of myelinbody and alveolar structure $\times 12000$ C Affluent mitochondria and tonofilament $\times 20000$

Fig. 2 Ultrastructure change of UVA group A Pyknosis and cell debris were commonly seen $\times 4000$ B Cellular apoptosis $\times 5000$ C Intracellular alveolar structure enriched and margined chromatin in nucleus $\times 8000$
3 DISCUSSION

Under normal condition the production and the elimination of the oxyradical in organism are relatively in balance. But certain pathological factors or ultraviolet can cause additional production of oxyradical. The theory of oxyradical is that the aging of skin is the consequence of obstruction between oxyradical production and elimination. The molecule of histiocyte in cutis is provoked after absorbing the light energy and can cause a series of photochemical reaction and produce chemically active oxyradical. Excessive oxyradical via oxygenation can cause injury of DNA cytomembrane and antioxidant in biomembrane e. g. Catalase CAT SOD GSH-Px etc. Besides the injury of histiocyte caused by ultraviolet leads to the peroxidation of lipids and multivalence unsaturated fatty acids in the biomembrane and the formation of lipid peroxidation LPO including MDA with generous consumption of abundance antioxidants. SOD is the scavenger of superoxide anion O2− which located in the cells and can turn O2− to O2 and H2O2. Active H2O2 is also harmful to human body and can be turned to H2O by GPH-Px or CAT and eliminated. GPH-Px can also oxidize LPO and produce alcohol with low toxicity. Han gave short term intensive exposure of ultraviolet to the cutis of mouse and found that the contents of MDA in cutis were significantly increased which indicated that the exposure of ultraviolet can accelerate the aging of skin and the oxyradical injury was the major mechanism of the skin aging.

Resveratrol is a non-flavone polyphenol exists in many species of plants and is considered as a phytoalexin. Researches demonstrate that resveratrol has the pharmaco-activity of anti-tumor cardiovascular disease therapy anti-mutation anti-oxidation anti-bacteria anti-inflammatory liver-protection apoptosis induction and estrogen-regulation. The injection of resveratrol glycoside which has the similar bioactivity as resveratrol can increase the activity of GSH-Px and SOD in cerebral cortex and hippocampus of cerebral ischemia mouse model reduce the oxyradical injury to the brain and decrease the LPO level in the brain of the cerebral ischemia mouse model especially the MDA level. Burkhardt et al. discovered that resveratrol can reduce the oxidative injury to thymocyte DNA caused by Cr3+ and H2O2 in vitro and pointed out that this kind of protection was related to the direct elimination of oxyradical by resveratrol. Hung et al. also found that resveratrol possessed strong function of oxyradical elimination especially in eliminating hydroxy radical to protect DNA from being damaged and the antioxidative effect of resveratrol was better than those of vitamin C and vitamin E. Moreover resveratrol can maintain glutathione in deoxidized form at high level by inhibit glutathione disulfide that is glutathione in oxidized form GSSG to decrease the production of oxyradical. In addition resveratrol can decrease the MDA level in blood and inhibit lipids peroxidation. In the experiment we cultured the HaCaT cells in vitro and used the UVA 5 J/cm2 which was close to sunshine that has reached the surface of the earth as the irradiation bump. We used this model to mimic the photaging of human epidermis after UVA irradiation and regarded the resveratrol as an intervention. Our findings suggest that the UVA radiation can decrease
the proliferation activity of HaCaT cells and damage cellular ultrastructure and significantly decreased the activities of SOD and GSH-Px and significantly increased the level of MDA. The result is identical to the records of domestically and abroad indicating that the UVA radiation can inhibit the proliferation of HaCaT cells and the elimination of active oxyradical. After the coaction of UVA and resveratrol the proliferation of cultured HaCaT cells was increased and the injury of cellular ultrastructure was partially recovered. the activities of SOD and GSH-Px were increased and the MDA level was decreased. These changes indicate that resveratrol protect the HaCaT cells through anti-UVA effect. In all we confer that resveratrol’s protection mechanism to keratinocytes against UVA may relate to its inhibition of UVA-induced increased apoptosis and its elimination of UVA-induced excessive reactive oxygen species ROS production in keratinocytes. In addition the findings of this research may also provide certain theoretical justification for the addition of appropriate concentration of resveratrol to skin care to antagonize UVA.

References


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