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Potential protection of green tea polyphenols against ultraviolet irradiation-induced injury on rat cortical neurons

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ABSTRACT

The present study was performed to investigate the possible protective effects of green tea polyphenols against ultraviolet (UV)-C light irradiation-induced cell death in the cultured rat cortical neurons. We found that UV-C light irradiation induced marked cell death tested by 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and TdT-mediated biotin-dUTP nicked-end labeling (TUNEL) assay. Protective effects of green tea polyphenols on UV-C light irradiation-induced apoptosis in cortical neurons were demonstrated by testing the content of Bax, which is involved in cell death. The expression of active Bax in cultured rat cortical neurons was inhibited significantly by green tea polyphenols compared to UV irradiation group tested by the immunoprecipitation assay and Western blot assay. However, there were no significant changes in the contents of total Bax after treatment with green tea polyphenols in UV-C light-irradiated rat cortical neurons. Our results demonstrated that the green tea polyphenols inhibited the active Bax expression, suggesting a neuroprotective effect of green tea polyphenols against the UV-C light irradiation-induced injury on cortical neurons.

Green tea is a popular beverage and is used widely in the world. It is known that green tea has many beneficial effects to our body, such as antimutagenic, antiproliferative and anticarcinogenic properties, as well as neuroprotective activity in degenerative disorder models [1,3,8,10,12,15]. Polyphenols are the main compounds of green tea and there are a class of polyphenolic flavonoids known as catechins (the most abundant component) comprise (−)-epigallocatechin-3-gallate (EGCG), and (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC) respectively [26]. EGCG is thought to be the most pharmacologically active of the catechins. Previous studies have demonstrated that green tea polyphenols improved the memory impairment and reduced hippocampal neuronal damage induced by ischemia [11,19].

Ultraviolet (UV) light, a potent mutagenic and genotoxic agent, is divided into three parts by wavelength: UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (200–280 nm) [7,11]. UV induced apoptosis in many cell types [4,16], and Bax is the proapoptotic factor in the signal pathway [13,24]. Previous study demonstrated that UV induced increases in the synthesis of p21 and Bax, both of them can promote apoptosis [5]. Green tea polyphenols have protective effect on the hippocampus neurons treated by amyloid β [2]. The present study was performed to explore the possible protective effects of green tea polyphenols on UV-C light irradiation-induced injury on cortical neurons.

Primary cultures of rat cortical neurons prepared from 1-day-old newborn rats as described previously [17,18,21]. Rat cortical neurons were treated with 0.25% trypsin at 37 °C for 20 min, and triturated with a pasteur pipette, dispersed cells were diluted to a concentration of 5×10^5 cells/ml on poly-L-lysineprecoated 6-well plates or 35-mm dishes. Cultures were maintained in modified Eagles' medium, 10% new born bovine serum, 5% D(+)-glucose, 50 IU/ml penicillin and 0.05 mg/ml streptomycin at 37 °C with 95% air and 5% CO_2. On the second or third day, 5 μg/ml cytosine arabinoside were added to the medium to suppress the proliferation of the glia cells. The medium was renewed every 3 days during the culturing period. Uptake and transport experiments were carried out after 6–7 days in culture.

To study the influence of UV-C light irradiation on the cortical neurons, three groups of cultured rat cortical neurons were exposed to 20 W/m^2 (80 J/m^2) UV-C light (260 nm) for 4 s, 20 s or 1 min. The UV-C light irradiation was 2 cm above the dishes or 96 wells plate. The medium was added, the control group is without UV irradiation, and the cells were incubated for 4 h at 37 °C. Active Bax is detectable after 4 h.

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The cell viabilities after UV-C light irradiation were tested by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide) assay according to previous methods [22].

The green tea polyphenols (98% purity, a gift from Dr. Bao-Lu) contain of 50% EGCG, 22% ECG, 18% EGC, and 10% EC, analyzed by high-pressure liquid chromatography [9].

Data from MTT and Western blot analysis were expressed as the mean ± S.E.M. of percentage values of the control level. The difference between two groups are analyzed by Student's *t*-test (nonparametric) and $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ are compared with control group.

As shown in Fig. 1A, there were significant cell death in the groups received UV-C light irradiation for 4 s ($n = 12$; $t = 1.40$, $p < 0.01$), 20 s ($n = 12$; $t = 8.00$, $p < 0.001$) and 1 min ($n = 12$; $t = 7.18$, $p < 0.001$) tested by MTT assay compared to the control group.

To detect the neuronal apoptosis induced by UV-C light irradiation, the cultured cortical neurons received UV-C light irradiation for 20 s and the neuronal viability were determined by TdT-mediated biotin-dUTP nicked-end labeling (TUNEL) assay, and DAPI were used to stain nuclei of the cultured cortical cells, the results are shown in Fig. 1B(a and d). In the control group, 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) stained nuclei of the cultured cortical cells were shown in Fig. 1B(a), and there was a few TUNEL-positive cells observed in Fig. 1B(b). In the UV-C light irradiation group, DAPI stained nuclei of the cultured cortical cells were also shown in Fig. 1B(d). The TUNEL-positive cell number increased after UV-C light irradiation as shown in Fig. 1B(e), and TUNEL-positive cells exhibited typical nuclear condensation and fragmentation. The whole DAPI nuclei and TUNEL-positive cells were merged and shown in Fig. 1B(c and f). Cell death rate in the UV-C light irradiation group increased to 51.92% compared to the control group (4.2%) by TUNEL assay.

The protective effects of green tea polyphenols against UV-C light irradiation-induced apoptosis were further determined in cortical neurons. The cultured cortical neurons were exposed to UV-C light irradiation for 20 s, then were treated by green tea polyphenols at concentration of 10 pM, 1 nM, 100 nM, 1 μM or 10 μM. As shown in Fig. 1C, UV-C light irradiation induced marked cell death, and the cell viability increased significantly after treatment with 10 μM of green tea polyphenols compared to the control group ($n = 5$; $t = 2.67$, $p < 0.01$) tested by MTT assay, while there were no marked changes in the cell viability after treated by 10 pM ($n = 5$; $t = 2.69$, $p = 0.51$), 1 nM ($n = 5$; $t = 8.50$, $p = 0.06$), 100 nM ($n = 5$; $t = 2.07$, $p = 0.07$), 1 μM ($n = 5$; $t = 0.59$, $p = 0.07$) of green tea polyphenols. The results are shown in Fig. 1C.

Bax is a cytoplasmic protein, which is considered as to promote cell death. Our above results demonstrated that green tea polyphenols are able to protect the cultured rat cortical neurons against the UV-C light irradiation-induced injury. Was there any influence of green tea polyphenols on the expression of Bax in the cultured rat cortical neurons after UV-C light irradiation? The contents of active Bax and total Bax were detected by immunoprecipitation and Western blot assay according to previous methods [20].

Cells were washed in PBS and lysed in lysis buffer (Beyotime, China) with 1% phenylmethanesulfonyl fluoride (PMSF). Cell lysates containing 1 mg of protein (as determined by the BCA protein assay kit) were brought to a final volume of 100 μl, mixed with five micrograms of monoclonal anti-Bax antibody (6A7) and 10 μl of Protein A-Sepharose Fast Flow was added to the PBS buffer. The sample mixtures were rotated at 4 °C overnight. The samples were then centrifuged at 3000 × *g* for 1 min, and immunoprecipitates were eluted in 5× SDS sample buffer. Samples were fractionated on a 12% SDS-PAGE gel and electrophoretically transferred onto PVDF membranes. The membranes were blocked for 2 h with 5% non-fat milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) at

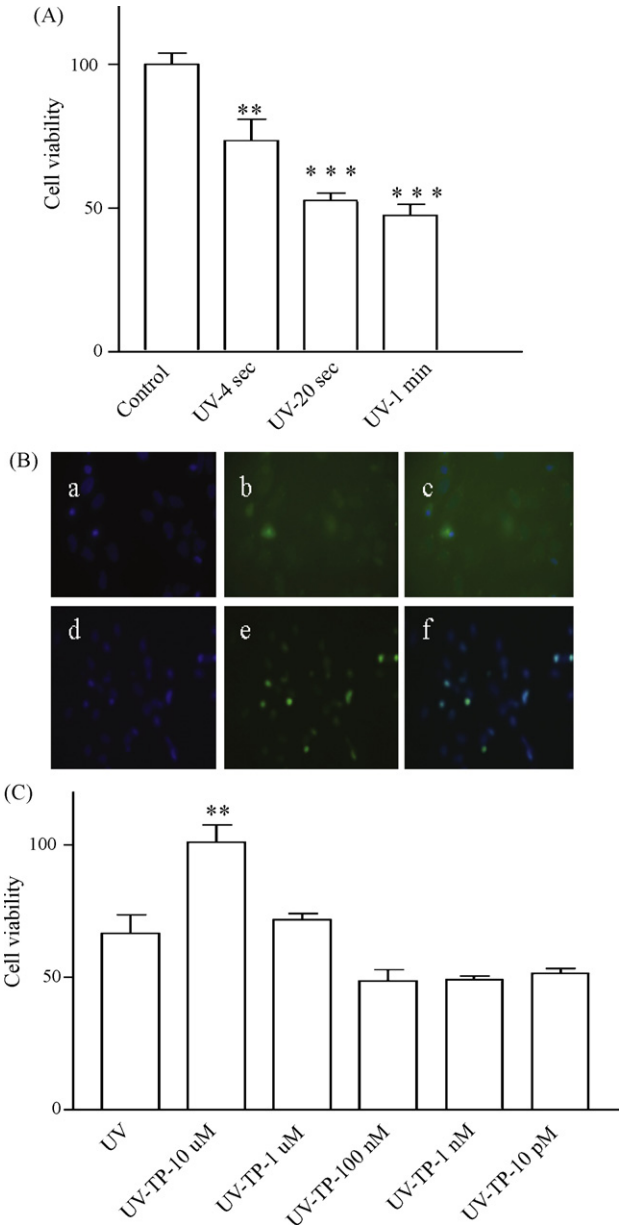

Fig. 1. Effects of green tea polyphenols on UV-irradiation on UV-irradiation-treated cultured rat cortical neurons. Cell viability were shown in A at different time (4 s, 20 s, and 1 min) of UV-C light irradiation on cultured rat cortical neurons. The results from TUNEL assay were shown in B. DAPI visualized nuclei (blue) in the control group B(a), and TUNEL-positive cells (green) in the control group shown in B(b). B(c) is merged with blue and green in the control group. B(d–f) shown the results from TUNEL assay in the UV-C light irradiation group. And DAPI visualized nuclei (blue) shown in B (d). TUNEL-positive cells (green) shown in B(e). Merged pictures with green and blue shown in B(f). Protective effects of green tea polyphenols at different concentrations on UV-C light irradiated cortical neurons were tested by MTT assay, cell viability were shown in C. $^{**}p < 0.01$ and $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

37 °C, and then incubated with a polyclonal goat anti-Bax antibody (N20, 1:1000) overnight at 4 °C. After washing 4 × 10 min in TBST, the membranes were incubated with an HRP-conjugated anti-goat antibody (1:3000) for 2 h. After four washes with TBST, the protein was visualized with the ECL detection system.

The content of active Bax increased significantly in cultured cortical neurons after treatment with UV-C light irradiation compared to control group ($n = 5$; $t = 7.82$, $p < 0.001$) determined by immuno-