Anti-inflammatory and anti-proliferation by AB-E4 on HSC-3 human oral cancer cell line

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ABSTRACT

Ajuga bracteosa **inhibited cell proliferation and induced apoptosis in oral cancer HSC-3 cell lines. In this study, we isolated active compounds AB-E4 from *Ajuga bracteosa*, and investigated the effects of AB-E4 on the cytotoxicity, induction of apoptosis, and putative pathways of its actions in human oral cancer cell. We are therefore interested in whether AB-E4 is capable of causing abnormal apoptosis processes, and whether this condition can be rectified through AB-E4 herb treatment. We used different AB-E4 concentrations. The MTT reduction assay was employed to quantify the differences in cell activity and viability. DNA ladder formation on agarose electrophoresis was also performed. The NFkB, IKK, IKBα, P65 and p50 expression level was monitored using immunoblotting techniques. AB-E4 exhibited inhibitory activities in the IKK and p50 assays, whereas those of AB-E4 showed inhibitory activities against NFkB. Taken together, drastic morphological changes, reduced cell viability and the presence of inhibit NFκB pathway all indicated that AB-E4 is capable of inducing anti-inflammation and anti-proliferation in HSC-3 cell lines.**

MATERIALS AND METHODS

1. Cell viability

Cells (2 × 10^5) were cultivated in 96-well for 1 day to become nearly confluent. Then cells were cultivated with samples in the presence of 100 ng/mL LPS (lipopolysaccharide) for 24 h. After that, the cells were washed twice with DPBS and incubated with 100 μL of 0.5 mg/mL MTS for 2 h at 37 °C testing for cell viability. After 30-min incubation, absorbance at 570 nm was read using a microplate reader.

2. Measurement of nitric oxide/nitrite

The cells were incubated with samples in the presence of LPS (100 ng/mL) at 37 °C for 24 h. And then cells were dispensed into 96-well plates, and 100 μL of each supernatant was mixed with the same volume of Griess reagent and incubated at room temperature for 10 min, the concentration of nitrite was measured form absorbance at 540 nm

3. Western blot analysis

The RAW264.7 macrophage cells (1.0 × 10^7) were washed with ice-cold phosphate-buffered saline and suspended in 0.2 ml hypotonic lyses containing 0.5% Nonidet P-40 and microcentrifuged at 12,000 × *g* for 1 min. The cytoplasmic and nuclear protein extracts (40 and 20 μg, respectively) were separated using 12% SDS–PAGE and transferred to a nitrocellulose membrane. Various primary antibodies in TBS. Thereafter, the blot was washed, exposed to horseradish peroxidase-conjugated secondary antibodies for 1 h, and then developed through enhanced chemiluminescence. PCNA and α-tubulin were used as internal controls in nuclear and cytoplasmic experiments, respectively.

RESULTS

In LPS-stimulated RAW264.7 cells, AB-E4 inhibited the production of NO and/or TNF-α and also blocked the LPS-induced expression of NO synthase (Fig. 1). AB-E4 inhibited the activation of NF-κB induced by LPS, associated with the abrogation of IκBα degradation, with a subsequent decrease in nuclear p65 and p50 protein levels (Fig. 2). AB-E4 also shown anti-proliferative effect on human oral cancer HSC-3 cell (Fig. 3 & 4).

CONCLUSION

In the present study, we found that AB-E4 had anti-inflammation and anti-proliferative effect on human oral cancer HSC-3 cell. AB-E4 shown time and dose-dependent anti-inflammatory activity, and that this protection is probably due to the suppression of macrophage activation.

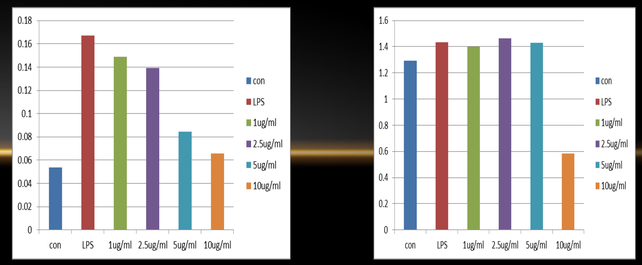

Fig. 1 AB-E4 treatment decreased the NO levels

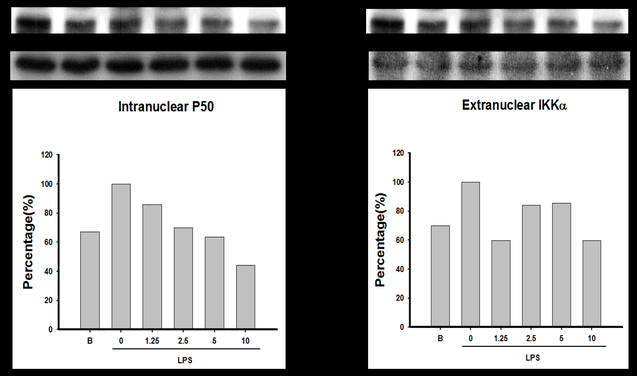

Fig. 2 Effect of AB-E4 on LPS-induced NFκB nuclear translocation in RAW264.7 cells. The ratios of immuno-intensity between the p65, p50 and the loading control PCNA were calculated.

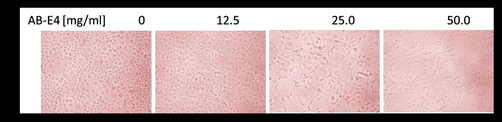

Fig. 3 Effects of AB-E4 on Morphology in HSC-3 cells.

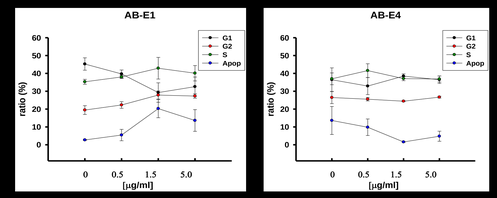

Fig. 4 Effects of AB-E4 on cell cycle in HSC-3 cells.

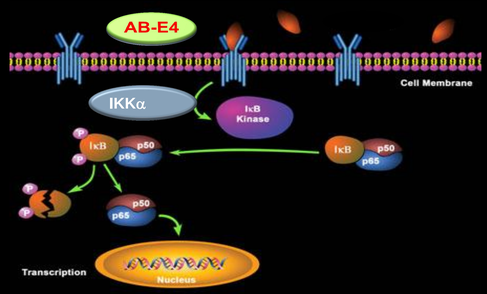

Fig. 5 AB-E4 that lead to NF-κB activation.