Cryptolepine induced apoptosis in TNFα-stimulated A549 lung carcinoma cells through NF-κB signalling pathway

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Cryptolepine, the major alkaloid of the west African shrub Cryptolepis sanguinolenta, has been shown to induce cell cycle arrest and apoptosis in A549 cells (Zhu and Godderham, 2006). We have also reported the inhibitory effects of this compound on NF-κB in various cell types (Olajide et al., 2007; 2013a; 2013b). In this study, we have investigated whether the apoptosis-inducing action of the compound is mediated through NF-κB signalling. In order to evaluate the effect on cell proliferation, cultured A549 cells were treated with cryptolepine (5-20 µM) for 24 h, and number of viable cells determined using the MTT assay. Cultured cells pre-treated with cryptolepine (5-20 µM) 30 min prior to stimulation with TNFα (1 nM) were evaluated for levels of caspase 3 using the Caspase-Glo® 3/7 Assay kit (Promega). The effects of cryptolepine on TNFα-induced IκB phosphorylation, NF-κBp65 subunit nuclear translocation, and protein expressions of NF-κB-regulated gene products of apoptosis (cyclin D1, survivin, XIAP, cIAP1, and Bcl-2) were investigated by treating cultured A549 cells with cryptolepine (5-20 µM) 30 min before stimulation with TNFα (1 nM), followed by In Cell western analysis. Results showed that cryptolepine produced dose-dependent and significant (p<0.05) reduction in A549 cell proliferation after 24 h of treatment. At 20 µM of the compound, cell viability was reduced by 62.2±3.3%. Treatment with 10 and 20 µM cryptolepine for 24 h was also found to cause significant (p<0.05) induction of caspase-3. With 10 µM, relative luminescence was 9038±480.5, and at 20 µM, relative luminescence was 9776±266.4, compared with relative luminescence of 1151±74.5 recorded in control cells. Protein analyses revealed that 10 and 20 µM of cryptolepine inhibited TNFα-induced IκB phosphorylation and NF-κBp65 nuclear translocation. Cells stimulated with TNFα (1 nM) showed elevated levels of Bcl-2, cyclin D1, surviving, XIAP and cIAP, which were reduced when pre-treated with cryptolepine (5-20 µM). Our results showed that cryptolepine downregulated the expression of anti-apoptosis proteins. We have also demonstrated that cryptolepine induces apoptosis in A549 lung carcinoma cells by interfering with NF-κB signalling.

References


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