

Cryptolepine induced apoptosis in TNF α -stimulated A549 lung carcinoma cells through NF- κ B signalling pathway

Olumayokun Olajide¹, Colin Wright². ¹University of Huddersfield, Huddersfield, UK, ²University of Bradford, Bradford, UK

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- κ B 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- κ B signalling. In order to evaluate the effect on cell proliferation, cultured A549 cells were treated with cryptolepine (5-20 μ M) for 24 h, and number of viable cells determined using the MTT assay. Cultured cells pre-treated with cryptolepine (5-20 μ M) 30 min prior to stimulation with TNF α (1 nM) were evaluated for levels of caspase 3 using the Caspase-Glo[®] 3/7 Assay kit (Promega). The effects of cryptolepine on TNF α -induced I κ B phosphorylation, NF- κ Bp65 subunit nuclear translocation, and protein expressions of NF- κ B-regulated gene products of apoptosis (cyclin D1, survivin, XIAP, cIAP1, and Bcl-2) were investigated by treating cultured A549 cells with cryptolepine (5-20 μ M) 30 min before stimulation with TNF α (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 μ M of the compound, cell viability was reduced by $62.2 \pm 3.3\%$. Treatment with 10 and 20 μ M cryptolepine for 24 h was also found to cause significant ($p < 0.05$) induction of caspase-3. With 10 μ M, relative luminescence was 9038 ± 480.5 , and at 20 μ M, 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 μ M of cryptolepine inhibited TNF α -induced I κ B phosphorylation and NF- κ Bp65 nuclear translocation. Cells stimulated with TNF α (1 nM) showed elevated levels of Bcl-2, cyclin D1, surviving, XIAP and cIAP, which were reduced when pre-treated with cryptolepine (5-20 μ M). 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- κ B signalling.

References

Olajide OA et al Bioorg Med Chem 15:43, 2007

Olajide OA et al Eur J Med Chem 63:333, 2013

Olajide OA et al Evid Based Complement Alternat Med 2013:459723, 2013

Zhu H & Gooderham NJ, Toxicol Sci 91:132, 2006