INVESTIGATION OF THE MECHANISMS INVOLVED IN CYLINDROSPERMOPSIN TOXICITY: HEPATOCYTE CULTURE AND RETICULOCYTE LYSATE STUDIES.

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ABSTRACT

The aim of this study was to determine the extent to which protein synthesis inhibition, lowered glutathione (GSH) levels and toxin metabolism contribute to the toxicity of cylindrospermopsin. Both hepatocyte cultures and reticulocyte lysates were utilized as *in vitro* tools of investigation.

Cylindrospermopsin was purified from *Cylindrospermopsis raciborskii* extracts by high performance liquid chromatography (HPLC). The toxin (93% purity) was identified by UV absorbance maximum at 262 nm and mass spectral analysis of the M + H ion (416 *m/z*) by HPLC-MS/MS (HPLC coupled to tandem mass spectrometry).

Cylindrospermopsin (IC_{50} =120 nM) was three times more potent than cycloheximide (IC_{50} = 368 nM) for the inhibition of protein synthesis in reticulocyte lysates. Cylindrospermopsin was effective immediately upon addition to lysates, arresting the elongation stage of translation. The potency and nature of inhibition was reproduced in hepatocyte culture (IC_{50} ~ 200 nM) and could not be reversed, displaying behaviour similar to that of the irreversible inhibitor emetine.

In cultured hepatocytes 1-5 μM cylindrospermopsin caused significant cytotoxicity (52 – 82% cell death) after 18 hr incubation. GSH levels were extensively depleted by all toxic concentrations, to 14% and 6% of controls for 1 and 5 μM cylindrospermopsin respectively at 18 hr. Such GSH depletion preceded the loss of cell viability. Although the antioxidant capacity of the cells was compromised by the depletion of GSH, further investigation did not reveal a role for oxidative damage in the toxicity process. The lipid peroxidation product malondialdehyde (MDA) did not increase above controls in cylindrospermopsin treated cells and inhibition of glutathione reductase (GSSG-Rd) activity with 1,3-bis(chloroethyl)-1-nitrosourea (BCNU) did not alter the toxicity of cylindrospermopsin.

Protein synthesis inhibition was not correlated to cytotoxicity in hepatocytes. Furthermore, inhibition of cytochrome P450 (CYP450) activity with proadifen or

ketoconazole alleviated the toxicity of cylindrospermopsin, but not the effects on protein synthesis.

These findings imply that the inhibition of protein synthesis by direct action of the toxin cannot be considered a primary cause of hepatocyte cell death over an acute time frame. CYP450-derived metabolites may play a crucial role in cytotoxicity, and the toxicity process does not appear to involve oxidative damage.