MONOCROTALINE INDUCES NEUROTOXICITY AND βIII-TUBULIN DESTABILISATION IN A MODEL OF CO-CULTURE NEURONS/GLIAL CELLS.

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Studies have shown cases of poisoning with plants from the genus *Crotalaria* (Leguminosae) mainly in animals with damages in central nervous system (CNS). It has been attributed to the pyrolizidine alkaloid monocrotaline (MCT) and its hepatic metabolites as dehydromonocrotaline (DHMC). Glial cells, particularly astrocytes are essential for biotransformation of xenobiotics in the CNS. Our previous studies demonstrated that MCT and DHMC induce changes on GFAP expression on astrocytes primary cultures. In this study we investigated the MCT effect on rat cortical glia/neurons primary co-cultures. Primary cultures were exposed to 10 or 100μM MCT and tested after 24 and 72h treatment. The MTT test and the measure of LDH activity on the culture medium revealed that, after 24h exposure, MCT was not cytotoxic to glia/neurons cells. However, the cell viability decreased in 10% and 20% after 72h treatment with 10 and 100μM MCT, respectively. The LDH test revealed significant increase at a rate of 12% and 23% after 72h treatment with 10 and 100μM MCT, respectively. Immunocytochemistry analyses revealed changes on βIII-tubulina polymerization in neuritis in co-culture, which was intensified after 72h exposure. Westernblot also showed a drastically decreased on βIII-tubulin expression, after 72h exposure to 10-100μM MCT. The Rosenfeld stain showed vacualizacion and megalocytosis in astrocytes. Moreover, we observed that MCT induced accumulation of nitrite in culture medium after 72h treatment, the stable form of nitric oxide, which indicates glial activation. These findings confirm cytoskeleton proteins as targets for MCT in CNS cells, and glial cells activation, and may be associated with neuronal damages observed on intoxicated animals.

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