EFFECTS OF CYLINDROSPERMOPSIN ON FUNCTIONS OF HUMAN GRANULOSA CELLS AND SPERMATOZOA

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Abstract

The aim of this study was to investigate the effects of Cylindrospermopsin (CYN) on human reproductive cells. CYN is a potent alkaloid cyanotoxin that inhibits protein synthesis (PSI) and causes cytochrome P450 metabolism-dependent cytotoxicity and genotoxicity in mice and mouse cells *in vitro*. In the last two decades, evidence has suggested that CYN can have deleterious health effects in humans and animals; however, further research into CYN toxicity is essential. Currently, little is known about the effects of CYN on human reproductive cells, although preliminary data suggest that low levels of CYN may adversely affect metabolic pathways in granulosa cells and cause oxidative damage to spermatozoa membranes. Both have the potential to affect cell viability and functionality.

In vitro cultures of primary-derived granulosa cells (GC) and spermatozoa provide good reproductive models to investigate a compound's toxicity. Both are readily obtained from patients seeking assisted reproductive technology (ART). In this study, GC were isolated from women undergoing ART and cultured in defined medium. After an initial 24-h adherence period, GC were then exposed to 0 – 5 µM CYN for 24, 48 or 72 h. After each time point, cell viability was measured and conditioned medium was collected for quantification of secreted steroid hormones by radioimmunoassay. The presence of cytoplasmic cholesterol lipid substrates required for steroidogenesis, and 3β-hydroxysteroid dehydrogenase (3βHSD) enzyme activity (which enables GC to produce progesterone from pregnenolone cholesterol substrate) were also assessed.

Exposure of GC to CYN concentrations of ≥ 1 µM for 48 h was cytotoxic. Decreased levels of cytoplasmic cholesterol lipid, 3β HSD activity and steroid hormone production 72 h after addition of ≥ 1 µM CYN was likely due to this loss of cell viability.

CYN toxicity is mediated indirectly via its CYP metabolites, but also directly by inhibition of protein synthesis (PSI). Even partial PSI may decrease GC steroid hormone production in cells, at doses without measurable loss of viability. To test this, *de novo* protein synthesis in GC exposed to CYN was measured using [^{3}H]-Leucine. The hypothesis was confirmed with observations of dose-dependent decreases in

progesterone production, and protein synthesis, after 6 h exposure to CYN concentrations up to 5 µM.

Mature, human spermatozoa lack CYP activity and do not rely on *de novo* protein synthesis, the two main mechanisms by which CYN confers its toxicity. However, spermatozoa cell membranes are densely packed with lipids, which are rapidly oxidised by reactive oxygen species when antioxidant defence mechanisms have been depleted. Since CYN has been shown to reduce antioxidant defence mechanisms, lipid peroxidation in spermatozoa is a potential secondary toxicity that could still affect fertility. The effects of CYN on both fresh and cryopreserved human spermatozoa were investigated through the use of four different viability assays: the MTT, MTS, Eosin Y exclusion, and ATP assays. Neither the MTT nor MTS assay proved capable of measuring spermatozoa viability in control experiments under the conditions used in this study. CYN concentrations up to 3 µM were not cytotoxic to cryopreserved spermatozoa after 72 h as determined by Eosin Y exclusion. There were significant decreases in ATP levels in fresh spermatozoa, but only after 24 h exposure to 30 µM CYN.

The current guideline for safe levels of CYN in drinking water is below 1 $\mu g.L^{-1}$. Short-term exposure of mature human spermatozoa and human granulosa cells to such levels of CYN is unlikely to affect the viability or functionality of the cells. However, the effects of long-term exposure to CYN need to be further investigated. The ability of CYN to decrease steroid hormone production and protein synthesis in GC may indicate a potential to disrupt normal reproductive cell functionality and development.