Involvement of ATP depletion and mitochondrial depolarization in the cytotoxicity of the algal toxins azaspiracids toward hepatocytes

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Azaspiracids (AZAs) are polyether marine toxins produced by dinoflagellates of the genera *Azadinium* and *Amphidoma* that can be accumulated in edible shellfish. Consumption of seafood contaminated by these compounds can induce a foodborne poisoning called Azaspiracid Shellfish Poisoning (AZP), characterized by severe gastrointestinal symptoms. Currently, more than 30 AZA analogues have been identified but only AZA-1, AZA-2 and AZA-3 are regulated in the European Union. *In vivo* studies on AZA-1 showed gastrointestinal effects as well as liver changes, visible as swollen organ, with fat droplets and vacuoles in hepatocytes but the mechanisms of action and the molecular targets of these toxins are still unknown. Hence, an *in vitro* study was carried out on immortalized human hepatic IHH cells to investigate the mechanisms of hepatotoxic effect of AZA-1, -2 and -3.

After short exposure time (24 h), AZAs induced a transient increase of mitochondrial activity in IHH cells (MTT assay) that was subsequently reduced in a concentration-dependent manner after longer (72 h) exposure [EC_{50}=1.2 x 10^{-11} M (95% confidence intervals, CI: 0.7-2.2 x 10^{-11} M), 7.0 x 10^{-11} M (95% CI: 3.3-14.6 x 10^{-11} M) and 3.8 x 10^{-11} M (95% CI: 2.0-7.0 x 10^{-11} M) for AZA-1, -2 and -3, respectively]. Accordingly, after 72 h exposure, AZAs induced a significant concentration-dependent mitochondrial depolarization: at the highest concentration (1.0 x 10^{-7} M) AZA-1, -2 and -3 induced 46, 41 and 45% depolarization, respectively, comparable to that induced by the positive control valynomicin (0.1 µg/ml, 42%). These effects seem to be dependent on AZAs ability to deplete intracellular ATP. Indeed, while AZAs did not significantly influence intracellular ATP after short exposure (24 h), they induced an almost complete ATP depletion after 72 h exposure. On the contrary, these effects appear to be not related to oxidative stress induced by AZAs.

Altogether, these results suggest that AZAs are able to induce a significant non-oxidative mitochondrial dysfunction in IHH cells, dependent upon ATP depletion. Further studies are in progress to elucidate the mechanisms at the basis of AZAs effects at the hepatic level.