Mitochondrial Effects of the Marine Algal Toxins Azaspiracids: Role of the Ionic Imbalance in Human Hepatocytes

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Azaspiracids (AZAs) are polyether marine toxins produced by dinoflagellates of the genus *Azadinium* that currently include more than 30 analogues. These compounds can contaminate edible shellfish inducing foodborne poisonings in humans, called Azaspiracid Shellfish Poisoning (AZP) and characterized by severe gastrointestinal symptoms. Among these compounds, only AZA-1, AZA-2 and AZA-3 are regulated in the European Union, being the most significant for the occurrence and toxicity. Besides gastrointestinal effects, *in vitro* studies on AZA1 showed liver changes, visible as swollen organ, with fat droplets and vacuoles in hepatocytes but the the mechanisms of action and the molecular targets of these toxins are still undefined. Hence, an *in vitro* study was carried out on the immortalized human hepatic cell line IHH, as a predictive model to assess AZA-1, -2 and-3 effects in hepatocytes.

AZAs reduced mitochondrial activity (MTT assay) in a concentration-dependent manner only after 72h exposure, with EC_{50} values of 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. Interestingly, the effect was almost undetectable after 48h, whereas a significant concentration-dependent increase of mitochondrial activity was observed at 24h. This effect seems to be independent on a proliferative stimulus since no increase of cell density (Sulphorodamine B, SRB, assay) was observed after 24h exposure. By contrast, the increased mitochondrial activity seems to be related to a metabolic stimulation at the mitochondrial level. Indeed, 5 μM rotenone and 1 mM tenoyl trifluoroacetone (TTFA), inhibitors of mitochondrial electron transport chain complex I and II, respectively, significantly inhibited the effect observed at 24h by the MTT assay. These results suggest a functional activation of these complexes by AZAs. To further investigate this effect, experiments were carried out in specific ion-free media, observing that AZAs effect on mitochondrial activity was significantly reverted in Na^+-free, Cl^--free and mainly K^+-free media, but not in a Ca^{2+}-free medium. Hence, cells exposure to specific inhibitors and/or activators of K^+ transporters led to hypothesize that K_{ATP} channels, hERG channels and Na^+/K^+ ATPase opening can be involved in AZAs mechanism of mitochondrial damage. On the whole, these results gain new insight on AZAs mechanism of action at the hepatic level.