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LP-55
Phylogeny and Toxin Profile of Two Cambodian Freshwater Pufferfish Species of the Genus *Pao*

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Pufferfish of the family Tetraodontidae contain potent neurotoxins, tetrodotoxin (TTX) and/or saxitoxins (STXs), but the toxin profile differs depending on the genus or species. Marine pufferfish of the genus *Takifugu* have TTX as their main toxin component [1–3], whereas freshwater pufferfish of the genus *Pao* generally have only STXs [4–6]. Some marine pufferfish, such as *Arothron* and *Canthigaster* species, possess TTX and STXs simultaneously [7–9]. Both TTX and STXs harbored by pufferfish are thought to be exogenous via the food chain [1], but the molecular mechanisms involved in the toxin accumulation remain unclear. On the other hand, the species classification of Cambodian freshwater pufferfish is incomplete and confusing, and scientific information on their toxicity and toxin profile is limited. In the present study, to accumulate information on the phylogeny and toxin profile of freshwater pufferfish, and to clarify the differences from marine pufferfish, thereby approaching to toxin accumulation mechanisms, we conducted simultaneous genetic-based phylogenetic and toxin analyses using freshwater pufferfish individuals collected from Phnom Penh and Kratie (designated PNH and KTI, respectively). Phylogenetic analysis of partial sequences of three mitochondrial genes (cytochrome b, 16S rRNA, and cytochrome c oxidase subunit I) determined for each fish revealed that PNH and KTI are different species in the genus *Pao* (designated *Pao* sp. A and *Pao* sp. B, respectively). A partial sequence of the nuclear tributyltin-binding protein type 2 (TBT-bp2) gene differentiated the species at the amino acid level. Instrumental analysis of the toxin profile revealed that both *Pao* sp. A and *Pao* sp. B possess STXs, comprising STX as the main component. In *Pao* sp. A, the toxin concentration in each tissue was extremely high, far exceeding the regulatory limit for STXs set by the Codex Committee. A strong positive correlation ($r = 0.862$, $p < 0.01$) was observed between the ovarian toxin concentration and gonadosomatic index (GSI), and the STXs amount in the ovary increased exponentially with increasing GSI. In *Takifugu* marine pufferfish, the TTX amount in the ovary increases linearly with increasing GSI [10], suggesting that the mechanism of toxin transfer to the ovary is slightly different between *Pao* freshwater pufferfish and *Takifugu* marine pufferfish. In *Pao* sp. B, in strong contrast to *Pao* sp. A, only the skin contained high toxin concentrations. The difference in the STX accumulation ability between the two species with different TBT-bp2 sequences suggests that TBT-bp2 is involved in STX accumulation in freshwater pufferfish.

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LP-56
Neutrophil: A key player in toxicity assessment of iron oxide nanoparticles

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The trend to use nanoparticles (NPs) as a new strategy for drug delivery is still increasing, mostly as some COVID-19 vaccines were developed as reliable nanoparticles delivery platforms to fight against the COVID-19 pandemic [1]. In this regard, the potential use of NPs as other candidates in the treatment of some medical treating conditions, including cancer, represent a very dynamic area of research. Among them, iron oxide nanoparticles (Fe_3O_4 NPs) which have the distinct property to be responsive to an external magnetic field and to be applied in thermotherapy are of great interests [2]. However, the lack of a comprehensive assessment regarding their interaction with polymorphonuclear cell neutrophils (PMNs), an essential component of the innate immune system, put them in a blurry state for advanced medical applications [3] [4].

Purpose of the study: To assess the interaction between PMNs and Fe_3O_4 NPs to determine at which extent these NPs could alter the normal biological functions of these key player cells in inflammation.

Methodology: PMNs were obtained from whole human blood of consent voluntary donors by using dextran sedimentation and Ficoll gradient techniques. The purity of cells was at least 95% after isolation. Cells were incubated with Fe_3O_4 NPs at concentration of 10 µg/ml or 100 µg/ml *in vitro* for different periods of times and several functions were studied. These NPs were endotoxin-free according to the classical *Limulus amebocyte lysate* (LAL) assay and as judged by absence of colonies when the NPs suspension was incubated in lysogeny broth agar plates for 72 h. Their size was approximately 10 nm as determined by transmission electron microscopy. PMN apoptosis was determined by cytology, exploiting the particularity of apoptotic PMNs to have a pyknotic nucleus. The production of reactive oxygen species was measured by spectrometry assay by using the H_2DCFDA dye. Cytokine production screening was assessed with a pool of supernatants from different blood donors using a cytokine array kit (36 cytokines/chemokines). The concentration of IL-1 β, TNF-α, IL-6 and IL-8 was then determined by specific ELISA kits with the supernatant of each donor. The phagocytosis function was assessed by using the opsonized-sheep red blood cells (SRBCs) assay. The capacity of PMNs to adhere onto a cell substratum was determined by using the human endothelial cell line EA.hy926 with a confluence greater than 85%. The chemotaxis function was evaluated by using Boyden chamber assay.

Results: Incubation ofPMNs with Fe_3O_4 NPs for 24 h demonstrates their capacity to inhibit spontaneous apoptosis of PMNs. Fe_3O_4 NPs did not induce reactive oxygen species production, which agrees with their non-cytotoxic and anti-apoptotic effect on PMNs. However, these NPs show a capacity to enhance phagocytosis for opsonized SRBCs and to promote the production of some pro-inflammatory cytokines (IL-1 β, TNF-α, IL-6, IL-8), and to enhance cell migration and adhesion.

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LP-57
T-2 mycotoxin and its impact on mitochondria – *in vitro* study

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Biological toxins are compounds produced by living organisms mainly for defence purposes. Trichothecenes are groups of chemically related mycotoxins produced by multiple filamentous fungal species such as *Fusarium*, *Trichoderma*, *Myrothecium*, *Trichothecium*, *Stachybotrys* and *Spicellum*. These compounds are linked to human and animal diseases by consuming contaminated cereals like wheat, barley, maize and oats. T-2 toxin is one of the main representatives of type-A trichothecenes and is considered as the most toxic compound. It is produced by different *Fusarium* species including *F. sporotrichioides*, *F. poae* and *F. acuminatum*. T-2 is commonly found in wheat, barley, oats but also is present in processed cereal products and animal feed. The cold climate and wet storage conditions increase the risk of T-2 contamination. T-2 poses a potential threat to humans and animals as a natural cereals contaminant and induces a wide range of toxic effects due to its strong toxicity. Observed acute toxicological effects are feed refusal, vomiting, hemorrhages, stomach necrosis, and dermatitis. At a cellular level, the major effect of T-2 is inhibition of protein synthesis, which leads to secondary DNA disruption and RNA synthesis. Compared to other trichothecenes, T-2 toxin has a toxic effect on the skin. Skin inflammation, skin fibroblast cells destruction, and skin damages similar to injuries caused by radiation are major topical effects of T-2 toxin. The aim of this *in vitro* study was to analyze the effect of T-2 mycotoxin on mitochondria physiology. The research material consisted of normal human foreskin fibroblast – Hs68cell line. Cells were treated with T-2 toxin in a concentration range of 0.001 to 10 μM for 24 h and 48 h.

To analyze the toxic effect of T-2 toxin on mitochondria and mitochondrial DNA (mtDNA), measurement of mitochondrial membrane potential (MMP) using JC-1 fluorescence probe. What is more, mtDNA damage using the semi-long run quantitative RT-PCR (SLR-qRT-PCR) were assessed and relative number of copies of human mtDNA by quantitative real-time PCR (qRT-PCR).

As a result of the study, decrease in the MMP of Hs68 cells was observed after exposure to the T-2 toxin what is probably related to the inhibition of the metabolic activity of cells. What is more, increase in mitochondrial DNA damage was observed, indicating a genotoxic effect of T-2 toxin on the mitochondrial genome. A significant concentration-dependent decrease in mtDNA copy number was also observed. The decrease in mtDNA copy number is due to the probable genetic effects of the T-2 toxin on the mitochondrial genome.

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LP-60
A multisystemic approach to investigate the role of polystyrene nanoparticles on neurodegeneration

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Objectives: Nano plastic particles (NPs) derived from the degradation of all sorts of disposable plastics can be found in the air, water, the food and in several day-to-day products. We are thus continuously confronted with these small particles and recent investigation could show their internalization into human cells thus raising the concern about their possible toxic effects for organismal health. The aim is to assess the effect of different NPs (non-modified and aminated polystyrene particles, PS/PS-NH_2) on different readouts primarily of relevance for the nervous system, through a multisystemic approach.

Material & Methods: The effect of NPs (50 nm) acute exposure was investigated *in vitro* (in undifferentiated and differentiated SH-SY5Y cells) by assessing cytotoxicity, neuronal like differentiation and secretion of Alzheimer's Disease related protein Aβ (β Amyloid). Moreover, the particle effect was evaluated upon chronic exposure *in vivo* (in *C. elegans*) looking at animals' development (4 days of exposure) and health-span (~20 days of exposure).

Results: Experiments in undifferentiated cells treated with PS and PS-NH_2 revealed a significant reduction in cell viability induced by PS-NH_2 (>5 μg/cm²) but not by PS. Moreover, Aβ ELISA assays indicated an increase in Aβ 1–42 secretion after exposure to PS-NH_2. Particle treatment in differentiated cells could show a degeneration of neurite outgrowth with very low concentrations of PS-NH_2 (2 μg/cm²). Worms' lifespan and movement was not altered when animals were treated with particles on solid agar plates, compared to liquid exposure, likely due to differences in particle availability and absorption. Instead, treatment with 50 μg/mL PS-NH_2 in liquid culture starting from embryo lead to a strong delay in animals' development and a sick phenotype, whereby treatment from young adult decreased the lifespan up to 20% compared to control. PS only slightly decreased lifespan at much higher (100 μg/mL) doses.

Conclusion: My results show aminated particles have toxic effects in both *in vitro* and *in vivo*. First investigations suggest a correlation of NPs exposure with neurodegeneration, by increasing Aβ secretion and impairment of neuronal outgrowths. Further work is planned to evaluate additional parameters of relevance for the nervous system *in vitro* as well as *in vivo* and to investigate the molecular mechanisms underlying their toxic effects.

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