

## 28.

**Modulation of BCL-2 gene expression in leukaemia cell line after treatment with a toxin isolated from *Bothrops* snake venom**

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Apoptosis and other molecular events involved in cancer development are important issues to be addressed. The knowledge of this complex network may lead to new approaches and better treatment for cancer patients and prevention for risk individuals. The modulation Bcl-2 expression has been described in a variety of human malignancies. Original data describes Bcl-2 expression in hematological cancers.

Semiquantitative RT-PCR and Realtime PCR were performed and Bcl-2 was in HL-60 cells. However, *Bothrops pirajai* venom presents a toxin denominated a phospholipase, piratoxin-I, which modulated the expression of Bcl-2 gene. When the line HL-60 cells were treated with 25 µg/mL of piratoxin-I we noticed an increase in the values of Ct, indicating low expression of the gene, compared to untreated culture where the values of Ct showed up high. In summary, our results indicate that there was a reduction in the expression of Bcl-2 when the cell line HL-60 was treated with piratoxin-I and we suggest that this toxin could be used as potential therapeutic compound for the treatment of cancer.

Financial support: FAPESP 06/51665-9 and 05/54855-0.

doi:10.1016/j.cbpa.2009.05.075

## 29.

**Cadmium, lead and metallothionein contents in tissues of the sea bream *Sparus aurata* from three different fish farming systems**

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Heavy metals in marine environment have a particular significance in ecotoxicology, since they are highly persistent and can be toxic in traces. Metals such as Pb and Cd are potentially toxic and pose a serial risk for human health when they enter the food chain. Fish are exposed to metals at different intensities through two major routes: metal ions dissolved in the water can be absorbed through the gills; metals bound to solid particles can be ingested and absorbed through the gut epithelium. For farmed fish, growing conditions (food and water chemistry) may determine metal composition of fish tissues as well as the response that these fish exhibit to metal toxicity. The distribution and bioaccumulation of dietary and waterborne cadmium and lead in tissues of sea bream *Sparus aurata* was studied in relation to three different fish farming systems. Metallothionein levels in fish tissues were also evaluated.

Results demonstrate that metal concentrations in various tissues significantly vary among fish culture systems. Different tissues show different capacities for accumulating heavy metals. The content of both cadmium and lead is not strictly correlated with that of metallothionein. Indeed, the marked accumulation of both metals in liver, as well as the high lead content found in gills and kidney, is not accompanied by a concomitant accumulation of MT in these tissues. No correlation is present between heavy metals and metallothionein content in muscle tissue: the high MT content in muscle is probably due to environmental stressors other than heavy metals. The results also demonstrate that cadmium accumulates mainly via dietary food, whereas lead accumulation is not of food origin. Finally, the low content of cadmium, lead and metallothioneins found in fish from the half-intensive farming

method demonstrates that, at least for *Sparus aurata*, this aquaculture practice guarantees the overall best quality products.

doi:10.1016/j.cbpa.2009.05.076

## 30.

**Toxicity of crude venom from the Scyphozoan *Pelagia noctiluca***

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One of the most relevant aspects of Cnidarian physiology is related to the biologically active compounds of nematocytes, organelles contained in specialized cells called nematocytes. Such compounds include proteins and secondary metabolites with toxic action whose mechanism is not completely understood so far. In general either an oxidative damage or pore formation on cell membrane target can be suggested to explain cell lysis after toxins treatment. Crude venom of the Scyphozoan *Pelagia noctiluca* collected in the Strait of Messina (Italy) is here being studied with the aim of comparing its toxic effect upon both red blood cells (RBCs) and nematocytes employed as a cell model and isolated from the Anthozoan *Aiptasia mutabilis*. Crude venom was obtained by sonication of 90 nematocytes/µl. As concerns the haemolytic assay 0.05% RBCs suspension was treated for 1 h with crude venom aliquots and then haemolysis was spectrophotometrically read at 414 nm. As concerns the cytolytic assay nematocytes isolated by 605 mM NaSCN from acontia of *Aiptasia m.* were treated with crude venom for 30 min. Thus treated cells were then checked under a light microscope and submitted to a typical cell volume regulation test under 35% hyposmotic shock. Our results show that, under an equal crude venom application, RBCs underwent lysis while nematocytes were anatomically intact as shown by Trypan blue test. Nevertheless treated nematocytes did not exhibit cell volume regulation capability. In the light of this, in the first case a pore-forming mechanism of crude venom may be hypothesized whereas in the second one an inhibitory effect on cell membrane transport mechanisms involved in RVD could occur. Further studies are needed to better ascertain these suggested actions.

doi:10.1016/j.cbpa.2009.05.077

## 31.

**Effects of cadmium on retinal development in lizard embryo: A molecular and morphological study**

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Eye malformations are commonly observed in vertebrate embryos after exposure to toxicants. Embryos of the lizard *Podarcis sicula* were used as model organism to investigate the effects of cadmium-contaminated soil (50 mg CdCl<sub>2</sub>/kg) on retinogenesis in a terrestrial oviparous vertebrate. At sampling (at regular time intervals from deposition to hatching), several embryos showed unilateral microphthalmia, often associated with brain and craniofacial alterations. Histological analysis showed hyperproliferation of the retina that folded invading the optic cup. These alterations usually occurred unilaterally, had an incidence of about 1/3 of treated embryos and appeared independent to the stage of development. Retinogenesis, however, was not affected and the hatching embryos showed a fully differentiated retina. The effect of cadmium was further studied using two early markers of retinal differentiation, Otx2 and Pax6, and Fzr, a cell-cycle regulator playing a crucial role in modulating cyclin destruction.

Northern blot analyses demonstrated a Cd-induced over-expression of Pax6 and Otx2, and a Fzr down-regulation. Because Fzr is required to arrest cells proliferation during embryogenesis, lzf deficiency could explain the increased proliferative index found in cadmium-exposed retina.

The cellular localization of the Otx2 and Pax6 mRNA in retinal cells was determined by *in situ* hybridization using homologous cDNA probes. Results demonstrated that the expression of these genes did not change in control and cadmium-treated eyes: in the early stages of retinogenesis, the two genes were expressed in all the cells with a perinuclear localization. In the differentiated retina, Otx2 remained in the cellular bodies of retinal cells forming the nuclear layers and the ganglion layer, Pax6 was expressed only in the cells of the inner nuclear layer and the ganglion layer. These data allow us to hypothesize that the increased expression of Pax6 and Otx2 observed by Northern blot analysis could be ascribed to the increased retinal cells number rather than to an effective Cd-induced gene over-expression.

doi:10.1016/j.cbpa.2009.05.078

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### 32.

#### Differential spatial expression of metal responsive transcription factor MTF-1 is related to functional organization of pituitary cells in *Cyprinus carpio*

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Transcription factors constitute central nodes in regulatory networks that orchestrate adaptation to environmental changes including exposition to heavy metals and other compounds that might affect the endocrine system. In carp we characterize hypothalamo-hypophyseal factors with the aim of identifying early response marker genes for evaluation of xenobiotic effects in the aquatic environment.

The metal transcription factor MTF-1 is necessary for homeostasis and response to heavy metal exposition. We prepared polyclonal antibodies against an oligopeptide designed from the derived amino acid sequence of *Cyprinus carpio*. Western blot analysis immunodetected a protein of about 60 kDa according to the derived molecular weight of MTF-1. The differential expression pattern of MTF-1 in pituitary gland showed strong immunoreaction in *proximal pars distalis* (somatotrophs, GH production), respect to *rostral pars distalis* (lactotrophs, PRL production) and *pars intermedia* (Somatolactin expression). In addition we characterize the metal response elements (MRE) in the promoter region of Pit1, the pituitary specific transcription factor regulating PRL, GH, TSH-beta subunit and SL cell line development and function, revealing stronger interaction with cell extracts from Zn treated respect to mock treated carp. This suggests specific effects on hypophyseal hormones mediating adaptation to environmental changes.

Acknowledgment: Chilean National Research Grant FONDECYT 1070724, 7070314 (G.K.); FONDECYT 1080571 (A.R.).

doi:10.1016/j.cbpa.2009.05.079

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