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PI.37

Effects of ammonium metavanadate on liver cythocrome P450 enzymes of young Wistar male rats

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Vanadium (V) is a heavy metal used in several human activities as component of insecticides and dyes and in harden steel process which is evolved in several toxicological effects. The ammonium metavanadate a pentavalent salt of vanadium is described in literature as very toxic compound which molecular effects are poorly studied. Consequently, the aim of this work was to evaluate the in vivo effects of different doses of NH4VO3 on liver cytochrome P450 system of young Wistar male rats. Three groups of five animals were intraperitonealy injected with vehicle (control) and in doses of ammonium metavanadate of 50 and 100 µmol/kg (treated groups), during six days.The animals were sacrificed by decapitation and the livers were immediately removed. After tissue homogenising the microsomes were prepared by differential centrifugation and used for determination of cythocromes b5 and P450 concentration as well as NADPH reductase, etoxicoumarin O-deethylase (ECOD) and lauric acid ω-hydroxylase (LAω-OH) activities. The experiments showed that NH4VO3 lead to a significantly increase ($P<0,01$) of cythochrome b5 and P450 concentration, as well as the ECOD and NADPH reductase enzymatic activities. Moreover, we observed that ammonium metavanadate only increased significantly ($P<0,01$) the lauric acid ω-hydroxylase activity in the group treated with 100 µmol/kg, the higher dose tested in this work. Our results suggest that ammonium metavanadate, in the doses studied, could induce the liver biotransformation capacity and eicosanoids biosynthesis pathway of young Wistar male rats which are mediated by cytochrome P450 enzymes, particularly the members of CYP1A and CYP4A families.

PI.38

Mitochondrial dysfunction as a major determinant in bile acids induced cell death.

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Accumulation of potentially toxic bile acids, such as chenodeoxycholic acid (CDCA), due to impairment of bile flow, results in irreversible liver damage. Administration of ursodeoxycholic acid (UDCA), although questionable, is the currently accepted method for treating cholestasis. The goal of this investigation was to determine whether CDCA-induced apoptosis in cultured human HepG2 cells was prevented by UDCA or its taurine derivative, tauroursodeoxycholic acid (TUDC), and to characterize the involvement of mitochondria in the process. CDCA-induced apoptosis, as determined by Hoechst 33342-stained nuclei, was evident upon coincubation with TUDC. Exposure to UDCA plus CDCA caused membrane permeability. Caspase-9-like activity, PARP cleavage and DNA fragmentation were detected in CDCA-exposed cells and in cells coincubated with TUDC, but not UDCA. CDCA caused a decrease in mitochondrial membrane potential and depletion of ATP, both of which were potentiated by UDCA but not TUDC. The results suggest that UDCA potentiates CDCA cytotoxicity, probably at the level of induction of the mitochondrial permeability transition (MPT). Consequently, as suggested by the lack of the main hallmarks of the apoptotic pathway, in the presence of UDCA, CDCA-induced apoptosis is not properly executed but degenerates into necrosis. A.P.Rolo is supported by FCT (SFRH/BPD/14615/2003). This work was partially financed by a Portuguese Research Council-FCT grant (Ref: POCTI/CBO/42486/2001).

PI.39

Effect of glutamate excitotoxicity on glutamatergic and GABAergic markers
in cultured hippocampal neurons: neuroprotection by BDNF

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Glutamate is responsible for massive neuronal cell death during ischemia and in brain trauma, being the hippocampus particularly vulnerable. In this work we studied the effect of glutamatergic and GABAergic markers in cultured hippocampal neurons. Stimulation with glutamate under conditions where cell death occurs by apoptosis caused a time-dependent decrease in the protein levels of the vesicular glutamate transporters, VGLUT1 and VGLUT2, although with different kinetics. The $t_{1/2}$ for the effect of VGLUT1 and VGLUT2 was 2.2h and 4.3h. The maximal effect was observed 8h after the insult, when VGLUT1 and VGLUT2 protein levels where reduced to about 50% of the control. The protein levels of GAD-65 (Glutamic Acid Decarboxylase) and GAD-67, the enzymes responsible for GABA production from glutamate, also decreased in a time-dependent manner after excitotoxicity stimulation with glutamate, with $t_{1/2}$ of 2.8h and 4.1h. These results show that the two isoforms of GAD are differentially degraded during the apoptotic process. Pre-incubation of the cells with Z-VAD-FMK, a broad range caspase inhibitor, prevented partially glutamate induced loss of VGLUTs and GADs, indicating that this effect occurs downstream of caspase activation. The neurotrophin BDNF, which was previously shown to protect hippocampal neurons from glutamate toxicity, also reduced to some extent the loss of glutamatergic and GABAergic markers, suggesting that it is able to preserve the function of both types of neurons. The protection of GABAergic neurons by BDNF was further confirmed by immunocitochemistry experiments, where we found that pre-incubation with the neurotrophin decreased glutamate-induced apoptotic death of GABBAergic positive neurons.
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PI.40

Characterization of the antioxidative system on *Castanea sativa* Mill. in association with the ectomycorrhizal fungi *Pisolithus tinctorius* and Amanita muscaria during the early stages of contact.

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Mycorrhizae are symbiotic associations between soil borne fungi and the root system of higher plants having a beneficial role on plant growth. The fungus receives carbon from plant, while the plant increased nutrient uptake mediated via the fungus. In agroforestry systems, most of the mycorrhizae belong to ectomycorrhizal (ECM) group being the mechanisms controlling its development poorly defined. During arbuscular mycorrhizal (AM) establishment, some evidences suggest that a temporal and spatial activation of different defence mechanisms by plants are activated. The present work pretends to assess the influence of ECM inoculation on the activity of antioxidant enzymes from roots of *Castanea sativa* Mill., during the early stages of contact.
The experimental work was carried out in an "in vitro" system, established between two symbiotic associations: *Castanea sativa* Mill. / *Pisolithus tinctorius* and *Castanea sativa* Mill. / *Amanita muscaria*. In these systems, plants were harvested at different times of fungi contact. The levels of H_2O_2 and the activity of oxidative stress enzymes, namely catalase (EC 1.11.1.6), superoxide dismutase (EC 1.15.1.1) and ascorbate peroxidase (EC 1.11.1.11), were determined in roots.
The results suggest that in the early stages of plant-ECM fungi interaction the oxidative metabolism could be involved, in a similar way as described for plant-AM interactions, where the plants produce enzymatic as well as non-enzymatic defence responses. Preliminary results will be presented and discussed in order to understand the effect of ectomycorrhizal fungi contact on oxidative stress enzyme activities of the host.**Keywords**: Ectomycorrhizal, oxidative stress, *Pisolithus tinctorius*, *Amanita muscaria*, *Castanea sativa* Mill..