P 22.02

Phenolic derivatives with potential anticancer properties – a structure-activity relationship study

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Recently, there has been a growing interest in the understanding of the mechanisms underlying the biochemical role of phenolic compounds, namely as inhibitors of deleterious oxidative processes (e.g. in cancer and inflammation [1]). This biological activity is ascribed to be strongly dependent on the conformational characteristics of the compounds [2]. Thus, the understanding of these structure-activity relationships (SAR's) may, hopefully, contribute to the wider goal of developing new and more effective therapeutic agents. In the present work, several phenolic agents – caffeic and gallic acid esters, as well as β-nitrostyrenes – were studied: (i) their structural behaviour was determined by vibrational spectroscopy coupled to theoretical methods; (ii) their cytotoxic activity was evaluated for distinct human cancer and healthy cell lines, the results thus obtained being interpreted in the light of the previously performed conformational analysis. A significant growth-inhibition effect was assessed for propyl trans-3-(3,4-dihydro-xyphenyl)-2-propenoate, and propyl-3,4,5-trihydroxybenzoate (an official anti-oxidant used as a food additive), at 50 μM concentration. Furthermore, toxicity of both esters towards noncancer cells was verified to be significant only for higher concentrations (above 75 μM) (Fig. 1). The number of OH ring substitutive groups was found to be a determinant factor of the cytotoxic effect of such phenolic compounds. This type of structure-activity study may, hopefully, contribute, in a near future, to the understanding of the mechanisms of carcinogenesis, and thus to the development of new chemopreventive and chemotherapeutic strategies.

References

1 Sergediene, E. et al. FEBS Lett. (1999) **462** 392.
2 Marques, M.P.M. et al. J. Med. Chem. (2003) **46** 5395.

N. Milhazes acknowledges FCT for a PhD fellowship (PRAXIS XXI/BD/18520/98).

P 22.03

The cytoprotective effect of Bunte salt derivative (Sat3) in mammalian cell

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Prior studies with Sat3 [2-(butylamino)-1-phenylethane-1-thiosulfuric acid] (Moreira, L.S., Piló-Veloso, D., Nelson, D.L., Química Nova, 2000; 23(4): 447) showed that this substance exhibits schistosomicide and trypanosomicide activities. In this study, we evaluated the cytotoxic and mutagenic potentials for the acid. Sat3 was not toxic in the concentrations assayed (10^{-12} to 10^{-4} mol/L) on four normal cell lines by the tetrazolium salt reduction assay (MTT). The compound did not exhibit mutagenic activity by the Ames test. Moreover, a reduction in the spontaneous reversion of *Salmonella typhimurium* his$^-$ T100 line, was induced by Sat3 (143.3 ± 2.4 vs. 3.0 ± 1.2 with Sat3 10^{-7} mol/L, $P < 0.05$, ANOVA). The micronuclei test confirmed these results, as a significant reduction of the spontaneous and mitomycin (MMC)-induced micronucleation was observed on CHO cells (0.062 ± 0.006 - control; 0.051 ± 0.002 - Sat3 10^{-7} mol/L; 0.132 ± 0.002 - MMC 100 ng/mL and 0.083 ± 0.003 MN/CB - Sat3 10^{-7} mol/L followed by MMC, $P < 0.05$, ANOVA). However, Sat3 protection did not prevent the lethal effects provoked by anticancer drugs and γ-radiation. We conclude that Sat3 although unable to prevent cell death by anticancer therapy, may act as a protective drug against the mutagenic effect induced by that treatment. Supported by CNPq and FAPEMIG.

P 22.04

Prenatal carbon monoxide exposure impairs myelination in rat offspring

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Experiments have been carried out in order to investigate possible consequences of prenatal carbon monoxide (CO) exposure on myelination and sphingomyelin homeostasis in the sciatic nerve of rat offspring. The influence of gestational CO exposure on motor activity has been also explored. Pregnant rats were exposed to 0 or 150 p.p.m. of CO mixed with air from day 0–20 of pregnancy as previously described (Carratù et al., Life Sci. 2000; 67: 1759–1772). Male pups were sacrificed on postnatal days 3, 8, 18, 40 and 90. Sciatic nerves were removed and processed for (i) computer-assisted morphometric analysis; (ii) determination of sphinganine and sphingosine levels by the HPLC-OPA reagent fluorescence method; and (iii) evaluation of motor activity in Macrolon cages by infrared monitoring. Prenatal exposure to CO (150 p.p.m.) produced a significant reduction in myelin sheath thickness of sciatic nerve fibres in 40- and 90-day-old rats with respect to controls ($P < 0.01$, Dunnett's *t*-test), whereas the developmental pattern of axonal diameters was not affected. In utero exposure to CO (150 p.p.m.) significantly increased sphingosine (SO) levels in sciatic nerve from 90-day-old rats with respect to controls ($P < 0.05$, overall one-way ANOVA), whereas sphinganine (SA) concentrations were not altered ($F = 0.69$, d.f. = 1/10, n.s.). Finally, prenatal CO (150 p.p.m.) exposure did not significantly impair motor activity ($F = 0.25$, d.f. = 1/10, n.s., overall one-way ANOVA). The present findings show that prenatal exposure to a CO level resulting in maternal blood HbCO concentrations equivalent to those found in human cigarette smokers produces, in male rat offspring, subtle alterations in myelin sheath thickness and sphingomyelin homeostasis which are not associated with functional deficits.

P 22.05

3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') activates microglia in rat brain by a process not dependent on hyperthermia

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This study evaluated the effect of a neurotoxic dose of MDMA on glial cell activation and the relationship of activation with the acute hyperthermic response induced by the drug. Male Dark Agouti rats (175–200 g) received MDMA (12.5 mg/kg, i.p.) or saline at an ambient room temperature (Ta) of 21 or 4 °C. Rectal temperature was monitored before and up to 12 h after MDMA. Animals were sacrificed between 1 h and 7 days after treatment. The density of peripheral benzodiazepine (PBZ) receptors, overexpressed during microglial activation (Pappata et al., Neurology 2000; 55: 1052–1054), and glial fibrillary acidic protein (GFAP) levels, a marker of astrogliosis (O'Callaghan et al., Brain Res. 1990; 521: 73–80), were determined in hypothalamus and cortex by [^{3}H]-PK11195 binding assays and ELISA, respectively. OX-42 (which labels microglial CD11b surface antigen; Tikka et al., J. Neurosci. 2001; 21: 2580–2588) and GFAP immunoreactive cells were visualized by histochemical staining of anterior hypothalamus sections. At 21 °C, MDMA induced a progressive increase in [^{3}H]-PK11195 binding to the hypothalamus and cortex between 3 and 48 h, peaking at 24 h. There was also increased immunoreactivity for OX-42 in both areas 3 and 24 h after MDMA, the staining being intensified at 24 h. These effects were not modified when MDMA was given at low Ta, although the hyperthermia was abolished. No changes were observed in GFAP in cortex at any of the time points. These results show that MDMA increases the number of PBZ receptors and immunoreactive cells for OX-42, indicating that MDMA induces a short-lasting activation of microglia in the rat brain. The fact that these two parameters are not significantly reduced when rats are kept at low Ta indicates that it might be a process independent of the changes in body temperature induced by MDMA.

P 22.06

Thiolic antioxidants protect against cisplatin-induced Ca^{2+}-dependent mitochondria dysfunction: relevance for toxicity mechanisms

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The antitumour drug cisplatin (CisPt) is used for the treatment of cancer of a wide range of tissues. Mitochondria dysfunction, because of ROS formation, seems to play an important role in cisplatin-induced hepatotoxicity and nephrotoxicity. Recently, the cytotoxicity of cisplatin has been related with the induction of apoptosis. However, the mechanisms of CisPt effects are not yet understood. It has been recognized that the stimulation of the mitochondrial permeability transition (MPT), which is triggered by ROS, is the major signalling pathway for the apoptosis. Therefore, since CisPt stimulates the apoptosis and MPT is linked to the activation of apoptotic cascade, we studied some oxidative events related with the induction of MPT, attempting to clarify the underlying mechanisms on CisPt-induced apoptosis. When pre-incubated with CisPt, rat liver mitochondria show a Ca^{2+}-dependent depolarization of DY, Ca^{2+} release and mitochondrial swelling. Moreover, CisPt induces oxidation of membrane protein sulfhydryl groups, matrix glutathione and pyridine nucleotides that are involved in the MPT induction. Such effects are prevented by cyclosporin A, a potent and specific inhibitor of the MPT. Furthermore, CisPt at high concentrations uncouples the mitochondrial respiration and inhibits the ATP synthesis. Antioxidants and thiol reducing agents DTT, GSH, N-acetylcysteine and cysteine also protect mitochondria from CisPt-induced effects. Our data indicate that this drug increases the sensitivity of mitochondria to the Ca^{2+}-dependent induction of MPT by a mechanism involving the ROS formation and that MPT is responsible for the stimulation of apoptosis and may induce cell death by depletion of ATP. Moreover, this work points towards antioxidants and thiol protecting compounds as promising therapeutic adjuvants in the prevention of CisPt cytotoxicity.

P 22.07

Heat pretreatment increases MDMA-induced heat shock protein 70 expression in the rat cortex but does not protect against 5-HT neurodegeneration.

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Heat shock protein 70 (Hsp70) is produced in response to diverse stimuli and is believed to provide neuroprotection (Yenari et al., Mol. Med. Today 1999; 5: 525–531). The aim of this study was to determine if prior exposure to heat could potentiate the induction of Hsp70 induced by MDMA in rats and prevent the long-term 5-HT neurodegeneration induced by the drug (Colado et al., Br. J. Pharmacol. 1997; 121: 823–833). The rectal temperature of adult male Dark Agouti rats (175–200 g) was increased 1.5 °C above normal baseline values by exposing them to a high ambient temperature with a heating blanket and maintaining this hyperthermia for 1 h. MDMA (12.5 mg/kg, i.p.) was administered 24 h later at an ambient temperature of 21 °C and the rectal temperature monitored for 6 h. Animals were killed either 3 h or 7 days after MDMA administration and the expression of Hsp70 (Western blot analysis) or the density of 5-HT uptake sites ([^{3}H]-paroxetine binding) determined in the cortex. MDMA produced a long-lasting (at least 6 h) hyperthermic response, compared with saline-treated animals (controls), which was not altered by heat pretreatment. Three hours after its administration, MDMA induced a marked increase in expression of Hsp70 (800% of controls), which was further increased by prior heat exposure (1800% of controls). Heat pretreatment alone had no effect on Hsp70 levels compared with controls. Seven days after treatment, MDMA-treated rats had a reduced density of 5-HT uptake sites (35%) indicating neurodegeneration, which was not modified by prior heat exposure. These findings indicate that, although prior heat exposure increases the expression of Hsp70 induced by MDMA, this overexpression of Hsp70 does not confer protection against the neurodegenerative effects of the drug.