THE ROLE OF CALCIUM IN PARACETAMOL (ACETAMINOPHEN) CYTOTOXICITY IN PC12 CELLS TRANSFECTED WITH CYP4502E1

A. HOLOWNIA 1*, J.-F. MENEZ 2 AND J.J. BRASZKO 1
1Medical Academy of Bialystok, Ludwik Zamenhof Children's Hospital, Clinical Pharmacology Unit, J. Waszyngtona 17, 15-274 Bialystok, Poland; 2Laboratoire de Biochimie-Nutrition, Faculté de Médecine, 22 Avenue Camille Desmoulins, 29285 Brest, France
*Correspondence

ABSTRACT
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Paracetamol-induced toxicity is mainly due to the accumulation of its CYP450-mediated N-hydroxylation product – N-acetylimidoquinone. We examined cell viability, proliferation rates and intracellular calcium in PC12 cells and in a PC12 cell line transfected with cytochrome P4502E1 exposed to paracetamol. This drug had a concentration-related effect on cell survival and a LD50 which was significantly different between both cell types. A 48% decrease of PC12 cells was found following application of 5 mmol/L paracetamol for 48 h. A total 73% decrease in cell numbers was found in cells metabolizing the drug. Culture protein levels were diminished in a similar manner. Paracetamol increased intracellular calcium (by 66%) only in CYP4502E1-transfected cells. The protective role of EGTA and verapamil modulating calcium homeostasis was more evident in CYP4502E1-transfected cells. These results suggest that biotransformation of paracetamol by CYP2E1 increases its cytotoxicity and that a calcium imbalance may have a key role in the initiation of cell injury.

Keywords: paracetamol, acetaminophen, cytochrome P4502E1, intracellular Ca2+, PC12 cells

INTRODUCTION
Paracetamol (acetaminophen; N-acetyl-p-aminophenol) has been increasingly used as an antipyretic since the finding that aspirin causes Reye's syndrome. Paracetamol is metabolized mainly by microsomal enzymes. It was demonstrated that cytochrome P450(CYP1A2/CYP2E1)-mediated N-hydroxylation results in the formation of N-acetyl-benzo-quinoneimine, a highly reactive intermediate which may play a role in the drug effect [1,2]. We have shown that the cytotoxicity of paracetamol in PC12 cells transfected with CYP4502E1 (DB#7 cells) is related to its metabolism [3,4]. This relationship was also found in cultured hepatocytes transfected with CYP4502E1 [5]. It is known that perturbations of intracellular calcium homeostasis are involved in chemically-induced cell death [6–8] and that calcium antagonists protect against paracetamol-induced DNA fragmentation and cell death both in mice [9] and in cultured mouse hepatocytes [10]. As most biochemical aspects of paracetamol toxicity relative to its metabolism are not clear, it appeared of interest to examine the effect of
the drug on cell viability and cellular calcium levels using a PC12 cell line transfected with cytochrome P4502E1.

MATERIALS AND METHODS

PC12 cells and a previously developed [4] PC12 cell line expressing a transfected human liver cytochrome P4502E1 gene (DB-7 cells) were maintained in Falcon flasks in Dulbecco's modified Eagle's medium containing 5% heat-inactivated horse serum and 5% fetal bovine serum (both from Gibco, Grand Island, NY, USA) supplemented with 100 U/ml of penicillin and 100 mg/ml of streptomycin. Transfected cells were supplemented with 500 mg/ml of a neomycin analogue – sulphate G418 (Cergy-Pontoise, France).

The metabolic activity of the transfected cell line was tested using the chlozoxazone hydroxylation test with HPLC detection of the reaction product as previously described [4].

Synchronization of cell cultures

Cells were detached with a solution containing 0.2 g/L of EDTA, 154 mmol/L NaCl, 20 mmol/L potassium phosphate pH 7.2 and 0.1% glucose supplemented with 0.5 g/L of porcine trypsin, placed in Petri dishes (5 cm diameter) and grown to confluence. The cells were then trypsinized and replated in 25-ml Falcon Flasks at low densities. Culture media were supplemented 24 h thereafter with different doses of sterile acetaminophen (Sigma Chemical Co., St Louis, MO, USA) in saline and kept for 48 h.

LD<sub>50</sub> determination

Paracetamol concentrations lethal to 50% of cell populations (LD<sub>50</sub>) were determined in PC12 and DB-7 cells using an AlmarBlue kit (Interchim, Asnieres, France). Cells were grown (100,000 cells per well) in Multiwell Tissue Culture Plates (Falcon, New Jersey, USA), and were treated with 1.25 mmol/L, 2.5 mmol/L, 5 mmol/L, 7.5 mmol/L and 10 mmol/L paracetamol for 48 h. At the end of the treatment period, Almar Blue was added to the culture media and cells were incubated for an additional 3 h. Following this, the optical densities of metabolized and non-metabolized compounds were determined using a Uvikon 860 Spectrophotometer (Kontron).

Intracellular calcium

Intracellular calcium levels were determined in cultured control–saline cells, in cells treated with 5 mmol/L paracetamol for 48 h and, additionally, in paracetamol-treated cells when the levels of calcium ions in the culture media were diminished by 50% of