Available online at www.sciencedirect.com
SCIENCE DIRECT®
Toxicology 210 (2005) 37–44
TOXICOLOGY
www.elsevier.com/locate/toxicol

Cytotoxic effects in mammalian Vero cells exposed to pentachlorophenol

P. Fernández Freire, V. Labrador, J.M. Pérez Martín, M.J. Hazen *

Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

Received 9 December 2004; received in revised form 12 January 2005; accepted 12 January 2005
Available online 25 February 2005

Abstract

The effects of pentachlorophenol have been studied on diverse biological systems both in vivo and in vitro, however the cellular basis of the pronounced cytotoxicity of this organochlorine compound is poorly understood. In this work, morphological and biochemical analyses were carried out to identify the primary targets of pentachlorophenol toxicity in mammalian cells.

Our results show that pentachlorophenol is a very potent cytotoxic drug that displays an unusual and interesting mode of action in Vero cells. Although this compound is a powerful uncoupler of oxidative phosphorylation, we present the novel finding that lysosome destabilization is an early cytotoxic response that precedes the mitochondrial dysfunction. In addition, soon after exposure to moderate doses of pentachlorophenol, a significant number of cells initiate an apoptotic death process identified by the condensed and fragmented state of their nuclei.

These results demonstrate that there are multiple potential targets of PCP-induced toxicity in mammalian cells, and the need to develop further experimental studies for the risk assessment of this environmental pollutant.

Keywords: Cytotoxicity; Microscopy; Pentachlorophenol; Vero cells

1. Introduction

Pentachlorophenol (PCP) is a well-known organochlorine compound mainly used as a general herbicide, pesticide and wood preservative, as well as an insecticide and broad-spectrum biocide (ATSDR, 2001).

Even though the use of pentachlorophenol is severely restricted both in the USA and Europe, the US EPA has listed PCP as a priority pollutant since, due to its slow and incomplete biodegradation (Gupta et al., 2002; Chen et al., 2004; Hanna et al., 2004), it can be found in surface, groundwaters and in soils (Hattemer-Frey and Travis, 1989; Muir and Eduljee, 1999; ATSDR, 2001; Campbell et al., 2003; Hoekstra et al., 2003). Besides, recent studies indicate that even at low dose levels, PCP can exert synergistic effects when mixed with other aquatic pollutants such as cad-

* Corresponding author. Tel.: +34 91 497 8248; fax: +34 91 497 8344.
E-mail address: mariajose.hazen@uam.es (M.J. Hazen).

0300-483X/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2005.01.009

mium, copper, ammonium and polycyclic aromatic hydrocarbons (Zhu et al., 2001; Luckenbach et al., 2003).

The general population may be exposed to PCP primarily through the ingestion of water and food (Hattemer-Frey and Travis, 1989; Jorens and Schepens, 1993) but rather high doses are attained in occupational settings (Seiler, 1991). Thus, health effects of PCP among workers, as well as among the general population, are of great concern. Severe exposures to PCP may result in an acute and often fatal intoxication, both in humans and experimental animals, that implicate uncoupling of oxidative phosphorylation in mitochondria as the principal mechanism (Proudfoot, 2003).

The toxicological effects induced by lower doses of PCP have been studied on diverse biological systems both in vivo and in vitro. Results of different experiments have suggested that pentachlorophenol is a highly toxic compound to plants and aquatic organisms (Repetto et al., 2001; Nikkilä et al., 2003; Farah et al., 2004), a promoter of carcinogenesis in rodents (Chhabra et al., 1999), and an endocrine disruptor and probable carcinogen in humans (ATSDR, 2001; Proudfoot, 2003). However, the cellular basis for the adverse health effects associated with PCP exposure are poorly understood in mammals (IARC, 1991).

The present study is designed to define the effects and mechanisms of PCP action at the cellular level. Vero cells, a mammalian cell line established from the kidney of the African green monkey (*Cercopithecus aethiops*) that is recommended for screening chemical toxicity in vitro (ISO, 1999), were used to evaluate the cytotoxic effects of PCP. Furthermore, the cellular responses induced by PCP as a function of concentration and time of incubation were also evaluated by using fluorescence and transmission electron microscopy techniques.

2. Material and methods

2.1. Materials

Pentachlorophenol and neutral red were purchased from Merck (Darmstadt, Germany) while rhodamine 123 was obtained from Sigma Chemical Co. (St. Louis, USA). Acridine orange was purchased from BDH Chemicals (Poole, Dorset, UK), Hoechst 33258 from Riedel-de Haen (Seelze, Germany) and reagents for cell cultures were products of Bio-Whittaker (Verviers, Belgium).

2.2. Cell culture and treatments

Vero monkey cell line was maintained at 37 °C in 25 cm^2 flasks (Falcon, Becton Dickinson, USA) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% foetal calf serum, 100 U/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine. Exponentially growing Vero cells were seeded at a density of 10^5 cells/ml into 24-well tissue culture plates (Falcon), and cultured in complete medium for 24 h before adding the treatments. The monolayers were then washed once in phosphate-buffered saline (PBS), and the culture medium was replaced by new medium containing experimental solutions of pentachlorophenol ranging from 1 to 100 μM (0.26–26.63 μg/ml). Stock solutions of 10 mM PCP were prepared in ethanol and maintained in darkness at room temperature. The exposure solutions were prepared before use in the culture media and sterilized by filtration through a 0.22 μm Millipore® filter. Ethanol concentration in medium was 0.1% including the control groups.

2.3. Cell viability assays

Effects on cell viability were estimated for treated and untreated (control) cultures 24–72 h later by the neutral red uptake (NRU) method as described by Borenfreund and Puerner (1985). In brief, for the NRU test, medium was removed and after washing in PBS, a new medium containing neutral red (50 μg/ml) was added. Following an incubation period of 3 h, the medium was removed and intracellular neutral red was extracted by addition of 50% aqueous ethanol containing 1% acetic acid. Absorbances were measured at 540 nm on a Spectrafluor microplate reader (Tecan, Austria).

2.4. Morphological evaluation by fluorescence microscopy

Vero cells were grown on sterile coverslips and treated with pentachlorophenol at concentrations of 10, 40 and 80 μM for 3 and 24 h, then washed with PBS. Rhodamine 123 (Rh-123) and acridine orange (AO) were used as fluorescent probes for staining mitochon-