The Cytotoxicity of *p*-Chloro-*m*-xylenol in Primary Culture of Rat Hepatocytes

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INTRODUCTION

p-Chloro-*m*-xylenol (PCMX) is widely used as a general disinfectant and antiseptic agent in a number of products for both consumer and professional use (1). This antimicrobial agent is a phenol-derived compound that has frequently been substituted for other antiseptic agents, including chlorhexidine and hexachlorophene. At the present time, the Food and Drug Administration has not recognized PCMX as a safe and effective antimicrobial agent because of lack of toxicological evidence; however, it has been recommended for topical antifungal and germicidal applications in concentrations not to exceed 3.75% (w/v) (2). Since the use of PCMX is presently increasing in the United States, a broader knowledge about the toxicity of this phenolic compound merits investigation. Previous reports have suggested that PCMX does not produce significant systemic toxicity, and no target organ toxicity has been found except at high doses (3). In addition, some of the toxic effects seen in these studies have been attributed to the combination of the ingredients present in the antimicrobial products (3,4).

In previous studies we have demonstrated that primary cultures of postnatal rat hepatocytes is a suitable *in vitro* model for screening xenobiotics as potential hepatotoxicants (5,6). The purpose of this study was to evaluate the toxic effects of PCMX *in vitro* with hepatocytes as the target cells.

MATERIALS AND METHODS

Rat Hepatocyte Culture Procedure. The isolation and primary culture of parenchymal hepatocytes from Sprague–Dawley rats 8 to 10 days old were based on a method developed in our laboratory (7) and later modified (8).

Treatment of Hepatocyte Cell Cultures and Biochemical Assays. Chemicals and drugs were aseptically dissolved in culture medium containing no serum or antibiotics. *p*-Chloro-*m*-xylenol was dissolved first in dimethyl sulfoxide (DMSO) and diluted with culture medium to yield a final concentration of 1% (v/v). Control cultures were exposed to DMSO alone 1% (v/v) for comparison purposes. Cell cultures were treated with PCMX to yield final concentrations of 1×10^{-4}, and 5×10^{-4} *M* and were allowed to incubate for 2, 4, 8, and 12 hr. At set time periods, both media and cells were collected for biochemical assays. All experiments were performed in duplicate. The determinations of cell viability, cytosolic enzyme leakage [lactate dehydrogenase (LDH) and aspartate aminotransferase (AST)], lactate/pyruvate (L/P) ratios, and protein determinations were assayed utilizing procedures routinely used in our laboratory and have been described elsewhere (5–8).

Statistical Analysis. The statistical significance of differences in mean values of treated and control groups was evaluated by analysis of variance (ANOVA) and by a post hoc comparison test (Scheffe's methods of multiple contrast). $P < 0.05$ was chosen as the minimum level of significance.

RESULTS AND DISCUSSION

The findings from the present study demonstrate, for the first time in cultured cells, that PCMX is directly toxic to hepatocytes *in vitro*. Enzyme leakage correlated with cell viability and morphology in those cells treated with PCMX. For example, LDH (Fig. 1) and AST (data not shown) release was evident after 2 hr of exposure in cultured cells treated with PCMX at 5×10^{-4} *M*. The administration of PCMX at 5×10^{-4} *M* reduced cell viability to 32% at 2 hr of exposure and killed most of the cells within 12 hr. Cultured cells treated with 1×10^{-4} and 2×10^{-4} *M* did not release cytosolic enzymes into the medium or reduce cell viability at any time period, whereas 3.5×10^{-4} *M* over 4 hr was toxic to the cells (data not shown). In addition, the morphology of the hepatocytes treated with PCMX as revealed by phase-contrast microscopy showed a number of cytological changes such as size and shape deformation, jagged plasma membranes, vacuole formation, disruption of cell monolayer, and lysed cells (data not shown). These alterations induced by PCMX may indicate a loss of integrity of the plasma membrane structure.

PCMX had a profound influence on the metabolic functions of the hepatocytes. Changes in L/P ratios were observed as early as 2 hr with 5×10^{-4} *M* PCMX (Fig. 2). Further, there was a significant increase in L/P ratios after 8 hr in those cells treated with PCMX at 2×10^{-4} *M*. No metabolic changes were detected in cell cultures treated with the lowest concentration (1×10^{-4} *M*) compared to those of the control groups. Lactate and pyruvate measurements are commonly used to indicate the redox state of cells (12). Deficiencies in mitochondrial oxidative phosphorylation raise the $NADH/NAD^+$ ratio and hence cause an elevation of the intracellular L/P ratios (13). Pyruvate is produced in the cytoplasm by glycolysis and consumed in the mitochondrion by the tricarboxylic acid (TCA) cycle; further, it may increase when gluconeogenesis is inhibited (10). In addition, reduction of oxygen uptake and stimulation of glycolysis may be associated with an increase in lactate production (12,13). Therefore, the L/P changes seen in this study may be associated with the following: (i) an increase in glycolysis and/or decrease in gluconeogenesis, (ii) an inhibition of cellular respiration (oxidative phosphorylation), and (iii) a disruption of energy production by PCMX.

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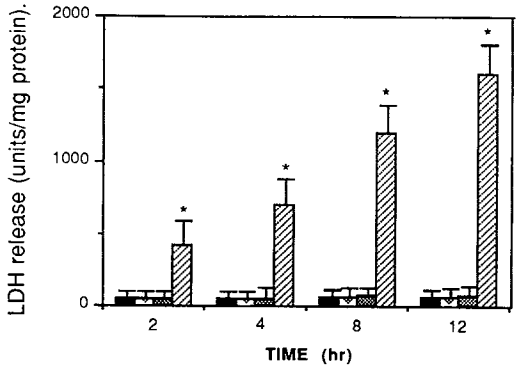

Fig. 1. Lactate dehydrogenase release in cultured hepatocytes treated with *p*-chloro-*m*-xylenol (PCMX) at 1×10^{-4} *M* (▨), 2×10^{-4} *M* (▩), and 5×10^{-4} *M* (▨) for 2, 4, 8, and 12 hr. The results are the means ± SE of four dishes. Asterisks indicate significant differences from controls (■) ($P < 0.05$). Assays in duplicate.

At the present time, little is known about the toxicity of PCMX in mammalian cells *in vitro*. PCMX may exert adverse effects in humans, including skin and eye irritation (Dettol; 4.8%, w/v) (3,4). If this chlorinated phenol is taken into the body in sufficient amounts, it may produce target organ toxicity. Plasma concentrations attained after oral or percutaneous administration in rats (Dettol, 25%, 4 ml/kg) reached an average peak of 38.8 μg/ml (in the 10^{-4} *M* range) or 7.8 μg/ml (in the 1×10^{-5} *M* range), respectively, in 30 min (9,10). In rats, the administration of high doses of PCMX (4.6–6.7 g/kg, in the 10^{-2} *M* range) may cause severe tissue damage (CNC, lungs, kidney, liver) and frequently death (3).

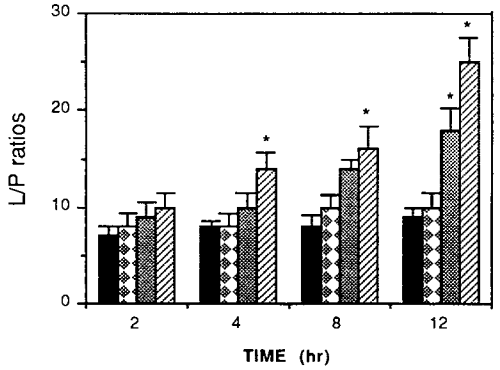

Fig. 2. Lactate/pyruvate (L/P) ratios measured in cultured hepatocytes after 2, 4, 8, and 12 hr of exposure to *p*-chloro-*m*-xylenol (PCMX) at 1×10^{-4} *M* (▨), 2×10^{-4} *M* (▩), and 5×10^{-4} *M* (▨). Each bar represents the mean ± SE ($n = 4$). Asterisks show significant increases in L/P ratios compared to controls (■) ($P < 0.05$). Assays in duplicate.

In humans, severe CNS and kidney injury and death have been reported after the ingestion of 150–300 ml (in the 10^{-1} *M* range) of Dettol (PCMX, 4.8%) (11). Selected concentrations of PCMX (ranging from 1×10^{-4} to 5×10^{-4} *M*) in this study were similar or close to *in vivo* situations reported in clinical and experimental cases.

In conclusion, the findings from this study indicate that PCMX was directly toxic to cultured hepatocytes. Further, these findings also suggest that the liver is an important target of toxicity by PCMX.

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REFERENCES

1. *British Pharmacopeia,* University Press, Cambridge, England, 1980, Vol. 1, p. 105.
2. Topical antifungal drug products for over-the-counter human use: Establishment of a monograph. *Fed. Reg.* 47:12533–12536 (1982).
3. W. C. Guess and M. K. Bruch. A review of available toxicity data on the topical antimicrobial, chloroxylenol. *J. Toxicol. Cut. Ocular Toxicol.* 5:233–262 (1986).
4. M. K. Bruch. The cutaneous absorption and systemic toxicity of antimicrobials. FDA concerns. In V. A. Drill and P. Lazar (eds.), *Cutaneous Toxicity,* Academic Press, New York, 1977, pp. 239–245.
5. D. Acosta, D. B. Mitchell, E. M. B. Sorensen, and J. V. Bruckner. The metabolism and toxicity of xenobiotics in a primary culture system of postnatal rat hepatocytes. In E. J. Rauckman and G. M. Padilla (eds.), *The Isolated Hepatocyte,* Academic Press, New York, 1987, pp. 189–214.
6. J. C. Davila, A. Lenherr, and D. Acosta. Protective effect of flavonoids on drug-induced hepatotoxicity *in vitro. Toxicology* 57:267–286 (1989).
7. D. Acosta, D. Anuforo, and R. V. Smith. Preparation of primary monolayer cultures of postnatal rat liver cells. *J. Tiss. Cult. Meth.* 6:35–37 (1980).
8. J. C. Davila, C. G. Reddy, P. J. Davis, and D. Acosta. Toxicity assessment of papaverine and papaverine-derived metabolites in primary cultures of rat hepatocytes. *In Vitro Cell. Dev. Biol.* 26:515–524 (1990).
9. B. Zondek and M. Finkelstein. Blood concentrations of p-chloro-xylenol in man following parenteral, percutaneous and rectal application. *Proc. Soc. Exp. Biol. Med.* 61:200–202 (1946).
10. B. Zondek. Fate of halogenated phenols in the organism. *Biomed. J.* 37:592–595 (1943).
11. D. Meek, D. M. Piercy, and R. Gabriel. Fatal self-poisoning with Dettol. *Post. Grad. Med. J.* 53:229 (1977).
12. A. L. Lehninger. *Biochemistry,* Worth, New York, 1977.
13. S. Seifer and S. Englard. Energy metabolism. In I. M. Arias, W. B. Jakoby, H. Popper, D. Schachter, and D. A. Shafritz (eds.), *The Liver: Biology and Pathobiology,* Raven Press, New York, 1988, pp. 279–315.