Induction of CYP3A and associated terfenadine N-dealkylation in rat hepatocytes cocultured with 3T3 cells

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Abstract

Long-term culture of hepatocytes has been challenged by the loss of differentiated functions. In particular, there is a rapid decline in cytochrome P450 (CYP). In this study, we cocultured rat hepatocytes with 3T3 fibroblasts for 10 days, and examined hepatocyte viability, morphology, and expression of CYP3A. Terfenadine was incubated with the cultures, and its biotransformation was quantitatively analyzed by HPLC. Terfenadine is metabolized by two major pathways: C-hydroxylation to an alcohol metabolite which is further oxidized to a carboxylic acid, and N-dealkylation to azacyclonol. In rat liver, only the N-dealkylation pathway appears to be mediated by CYP3A since anti-rat CYP3A antibody inhibited azacyclonol but not alcohol metabolite formation in incubations of terfenadine with liver microsomes. Freshly isolated rat hepatocytes were seeded on top of confluent 3T3 cells. Cultures were maintained in Williams' E medium supplemented with 10% fetal bovine serum and either 0.1 µmol/L or 5 µmol/L dexamethasone. In pure hepatocyte cultures, viability, as determined by lactate dehydrogenase (LDH) activity, decreased steadily to less than 30% of initial levels by day 10. In cocultures, LDH activity remained high and was 70% of initial levels on day 10. The half-life of terfenadine disappearance was optimally maintained in cocultures treated with 5 µmol/L dexamethasone, and was associated with the increased formation of azacyclonol. On day 5, nearly 50% of added 5 µmol/L terfenadine was converted to azacyclonol within 6 h, whereas the conversion was only 4% on day 1. Western and RNA-slot blot analyses confirmed that treatment with 5 µmol/L dexamethasone induced CYP3A mRNA expression and CYP3A protein expression. This coculture system could offer a useful approach in the study of drugs and xenobiotics metabolized by CYP3A.

Abbreviations: BSA, bovine serum albumin; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; LDH, lactate dehydrogenase; PCN, pregnenolone-16α-carbonitrile; SDS, sodium dodecyl sulfate; SSC, saline sodium citrate.

Introduction

Cultured hepatocytes are a useful tool for studies of xenobiotic metabolism and toxicity (McQueen and Williams, 1987). However, their use has been restricted primarily to short-term culture following cell isolation. It would be desirable to develop cultures of long-
er duration which could be used in repeated exposure experiments, thereby better mimicking \textit{in vivo} exposures. Long-term culture of hepatocytes has been challenged by the loss of differentiated functions (Croci and Williams, 1985). In particular, there is a rapid decline in cytochromes P450 (CYP), which catalyze the oxidative metabolism of a wide variety of endogenous and xenobiotic compounds. For example, rat hepatocytes cultured for 24 h lose 50–80% of their initial CYP content (Paine, 1990). Numerous attempts have been made to maintain CYP in cultured hepatocytes, including modifications of the culture medium, such as addition of nicotinamide, metyrapone, or dimethyl sulfoxide (DMSO), replacement of cystine/cysteine with δ-aminolevulinic acid (Paine, 1990; Villa et al., 1991), incubation at 30°C instead of 37°C (Padgham et al., 1992), use of a substratum of reconstituted basement membrane (matrigel) (Schuetz et al., 1988) and, perhaps most promisingly, coculture with other cell types (Guguen-Guillouzo et al., 1983). Most coculture studies have used rat epithelial cells derived from primitive biliary ducts to prolong survival and differentiated functions of human or rodent hepatocytes (Guillouzo et al., 1990; Niemann et al., 1991; Perrot et al., 1991; Akrawi et al., 1993). Established murine cell lines, of fibroblastic origin, such as C3H/10T½ and 3T3, offer important advantages for routine coculture because of their immortality, rapid growth, and stable phenotype. In previous studies, cocultures of rat hepatocytes with 3T3 or C3H/10T½ cells showed improved maintenance of hepatocyte morphology and total CYP content, and expression of some CYP-associated activities (Langenbach et al., 1979; Kuri-Harcuch and Mendoza-Figueroa, 1989; Donato et al., 1990; Utesch et al., 1991).

One of the most important cytochrome CYP families is CYP3A. The enzymes belonging to the CYP3A family are responsible for the metabolism of numerous clinically and toxicologically important substrates. Four CYP3A proteins have been isolated from human liver: CYP3A4 appears to be the major form, and is related to rat liver CYP3A proteins (Nebert et al., 1991). Levels of CYP3A or CYP3A4 in human liver and CYP3A1 in rat liver are inducible by glucocorticoids, macrolide antibiotics and phenobarbital (Watkins et al., 1985; Gonzalez et al., 1986).

In this study, we investigated the effect of coculture of rat hepatocytes with 3T3 cells, over a ten-day period, on hepatocyte viability, morphology, and expression of CYP3A, as determined by RNA and Western blotting, and CYP-associated activity. We used the antihistamine terfenadine as a substrate for CYP3A. Terfenadine is biotransformed by two oxidative pathways, reported to involve CYP3A4 in human liver (Yun et al., 1993): C-hydroxylation to an alcohol metabolite which is further oxidized, via a postulated aldehyde intermediate, to a carboxylic acid, and N-dealkylation to azacyclonol (Figure 1). We also examined the ability of the glucocorticoid dexamethasone to induce CYP3A in the coculture system.

\section*{Materials and methods}

\subsection*{Materials}

Analytical reference standards of azacyclonol, and the alcohol and carboxylic acid metabolites of terfenadine were provided by Marion Merrell Dow Research Institute (Cincinnati, OH, USA). Polyclonal antibodies raised in rabbits to purified rat CYP3A1/2, used in immunoinhibition experiments, were a generous gift from Dr Stelvio Bandiera, University of British Columbia. Polyclonal antibody to rat CYP3A used in Western blots, PCN-induced positive control rat microsomes, biotinylated secondary antibodies, streptavidin–horse-radish peroxidase conjugate, BSA, enhanced