CYTOCHROME P450 ACTIVITY IN PRIMARY CELL CULTURES OF SKATE HEPATOCYTES

Maria Runnegar[1], Ned Ballatori[2], Greg Connolly[2], Isabel Heine[3] and James L. Boyer[4]

[1]Center for the Study of Liver Diseases, School of Medicine, University of Southern California, Los Angeles, CA 90033

[2]Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, NY 14642

[3]Riverside High School, Durham, NC 27704

[4]Department of Medicine and Liver Center, Yale University School of Medicine, New Haven, CT 06510

A long-term primary culture model of skate hepatocytes has been established to study the long-term effects of drugs, toxins and environmental pollutants. A number of biochemical and morphological parameters have already been determined on these cultures (Ballatori et al., Bull. MDIBL 37:85-86, 1998). The aim of this study was to determine how cytochrome P450 activity is altered with time in culture.

The cytochrome P450 family consists of hemoproteins that catalyze the monooxygenation of a wide range of lipophilic xenobiotics and endogenous compounds such as steroids and carcinogens. These enzymes are quantitatively the most important for drug metabolism, and are particularly abundant in the liver, the site of metabolism of most xenobiotics. A striking property of these enzymes is their multispecificity, which enables them to attack most lipophilic compounds. The enzymes are inducible by environmental chemicals such as polycyclic hydrocarbons, drugs such as phenobarbital, and steroids such as pregnenolone-16a-carbonitrile, and are regulated by various endogenous factors such as hormones, age, sex, and nutritional conditions. In mammalian hepatocyte culture models, cytochrome P450 activity decreases rapidly with time in culture, unless elaborate culture conditions are established (Grant et al., FEBS Letters 190: 99-103, 1985). The goal of the present study was to evaluate the cytochrome P450 metabolic capacity of skate hepatocytes in culture.

The isolation and culture conditions for skate hepatocytes have been previously described (Ballatori et al., Bull. MDIBL 37:85-86, 1998). Extracts from cell cultures or livers were prepared in 0.1 M phosphate buffer pH 7.4 containing 20% glycerol and 0.15 M KCl as in Sadar et al. (J. Biol. Chem. 271:17635-17643, 1996). After centrifugation (10 min, 12,000 g) aliquots of these extracts were used to assay P450 activity. The P450 assay conditions were essentially as in Burke et al., (Biochem. Pharmacol. 34: 3337-3345, 1985). Briefly, 7-ethoxyresorufin*O*-deethylase (EROD, P4501A1, *CYP1A1*) activity of cell extracts was determined by measuring the release of resorufin from ethoxyresorufin. The reaction was in 0.1 M K_2HPO_4/NaH_2PO_4 at pH 7.6, with 250 μM of NADPH and 5 μM ethoxyresorufin substrate. The reaction was followed fluorimetrically at an excitation wavelength of 530 nm and emission wavelength of 585 nm with a Perkin Elmer 650 fluorescence detector. Activity was quantitated by the addition of a standard solution of resorufin to the reaction. Results are given as picomoles of resorufin released/min/mg protein (room temperature).

The cytochrome P450 activity of freshly isolated skate hepatocytes (3.61±0.43 pmoles of resorufin /min/mg protein), was of the same order of magnitude as the activity measured in liver extracts (1.54±0.20 pmoles of resorufin /min/mg protein). The quantitative difference between liver and isolated hepatocytes most likely reflects changes in activity that occurs during the isolation, washing and plating of hepatocytes. The fact that nonparenchymal cells

are also present in the liver but not in the isolated hepatocytes may also account in part for the difference in activity. For isolated hepatocytes P450 activity decreased with time in culture (Figure 1). At 3 days, P450 activity had decreased to 30±3 % of freshly isolated skate hepatocytes, whereas at 6 days it had decreased to 16±4 % of control.

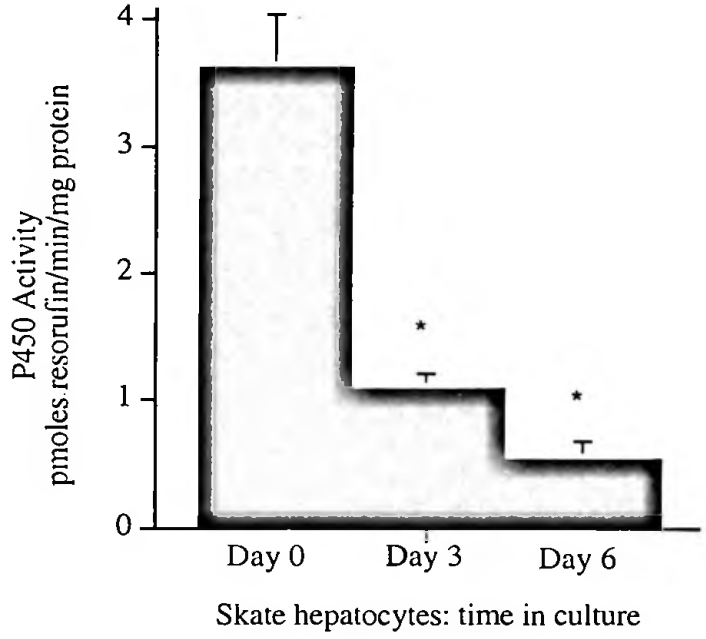

Figure 1. Changes in P450 activity (pmoles of resorufin /min/mg of protein) of skate hepatocytes with time in culture. N (cell isolations) = 5, results are mean±SEM, * $p \leq 0.05$ from controls.

Unlike mammalian hepatocytes in culture, skate hepatocytes have been shown to retain polarity in the distribution of apical and basolateral membrane domains (Ballatori et al., Bull. MDIBL 37:85-86, 1998). In contrast, the decrease in P450 activity of skate hepatocytes with time in culture is similar to that seen in rat hepatocytes (Grant et al., FEBS Letters 190: 99-103, 1985). It remains to be established how the activity of other components of the drug metabolism complex such as cytochrome b5, NADH-cytochrome b5- and NADPH-cytochrome c-reductases are maintained in these long-term hepatocyte cultures.
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