RETENTION OF STRUCTURAL AND FUNCTIONAL POLARITY IN CULTURED SKATE (*RAJA ERINACEA*) HEPATOCYTES

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Isolated liver parenchymal cells in culture have proven to be invaluable for addressing a multitude of biochemical, physiological, and toxicological questions (Alpini et al., Hepatology 20:494-514, 1994). Hepatocytes have been isolated from many species, including marine and freshwater fish (Baski and Frazier, Aquatic Toxicol. 16:229-256, 1990). Cultured hepatocytes offer a number of advantages over in vivo or isolated perfused liver models, including elimination of systemic effects, better control of environmental conditions, reduced variability between experiments, simultaneous and repeated sampling in a single experiment, and more efficient use of animals, reagents, and time. Short-term cultured hepatocytes closely mimic the functions of cells in the intact liver and provide a useful model for studying acute effects of drugs and xenobiotics, whereas primary monolayer cultures allow for measurement of long-term effects (days to weeks). However, one drawback of most hepatocyte culture models is that the cells begin to dedifferentiate and lose transport polarity immediately after isolation. In contrast, our previous experience with short-term cultures of hepatocytes isolated from the little skate indicates that these cells largely retain hepatobiliary polarity for at least 8 h (Miller et al., Am. J. Physiol. 270:G887-G896, 1996; Smith et al., J. Exp. Zool. 241:291-296, 1987; Henson et al., J. Exp. Zool. 271:273-284, 1995). When compared with mammalian hepatocytes, skate hepatocytes are easier to isolate and maintain in short-term culture, and they exhibit high viability, stability, and maintenance of normal hepatocyte functions (Smith et al., J. Exp. Zool. 241:291-296, 1987; Henson et al., J. Exp. Zool. 271:273-284, 1995). Skate hepatocytes have been used to evaluate mechanisms of hepatic metabolism, detoxification, membrane transport, and cell volume regulation (e.g., Ballatori et al., Toxicol. Appl. Pharmacol. 95:279-291, 1988; Am. J. Physiol. 267:G285-G291, 1994; Mol. Pharmacol. 48:472-476, 1995; Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992).

The goal of the present study was to develop conditions for long-term culture of skate hepatocytes that allow for the retention of membrane polarity. Skate hepatocytes were isolated by a mild collagenase perfusion technique and incubated at 12°C in serum-free elasmobranch Ringer solution. Pilot experiments compared some enriched culture media, and in particular a modified DMEM/F12 medium, to a simple elasmobranch Ringer solution (both media supplemented with 0.1 μM dexamethasone and penicillin/streptomycin, 100 U: 0.1 mg/ml). Surprisingly, only small differences in the overall preservation of metabolic and structural integrity were found. However, the enriched medium had one major shortcoming, it was highly conducive to the growth of bacteria and other parasites that were occasionally present in the livers of these wild animals and that were inadvertently co-isolated with the hepatocytes. In

contrast, these organisms were unable to thrive in elasmobranch Ringer solution containing penicillin/streptomycin. Thus, all subsequent studies were carried out using elasmobranch Ringer as the culture medium.

Isolated skate hepatocytes contained numerous lipid-filled vesicles, which presumably provide their major source of metabolic energy, and most of cells were present as clusters of 3-12 hepatocytes surrounding a bile canaliculus, rather than as single cells. The number of single cells in the preparation could be increased by lengthening the time of collagenase perfusion. These cells and cell clusters readily attached to untreated plastic culture dishes, and formed a loose, network-type three dimensional structure when dexamethasone was present in the medium. However, attachment to the culture dishes was only moderately strong, particularly for the larger hepatocyte clusters, given that only a small fraction of the total surface area of these clusters was in contact with the surface of the dish. In contrast to plastic dishes, attachment to glass cover slips was weak, although it was markedly improved by coating the cover slips with poly-L-lysine, or with type I or type IV collagen. The effects of the collagen or poly-L-lysine coatings was transient, however, as the cells gradually detached from treated glass over 2-4 days in culture.

Trypan blue and propidium iodide exclusion were measured on days 0-7 of culture, and were found to be >98%. During this time interval the cells maintained high intracellular concentrations of K^+, ATP and reduced glutathione (GSH), and high ratios of ATP/ADP and GSH/GSSG. Cultured skate hepatocyte clusters also maintained a relatively normal morphology and a polarized distribution of apical and basolateral membrane domains. Polarity was confirmed both morphologically, using antibodies raised against the skate canalicular bile salt transporter, Bsep, and antibodies to the canalicular multispecific organic anion transporter, Mrp2, and functionally, by monitoring secretion of the fluorescent organic anions NBD-taurocholate, a Bsep substrate, and fluorescein-methotrexate, an Mrp2 substrate, into the bile canalicular spaces. Quantitative RT-PCR analysis revealed that mRNA levels of the hepatic transporters Oatp, Bsep, and Mrp2 declined only slowly over the 7-day period to levels of 70, 35 and 40% of initial values, respectively. Examination of microtubules, actin filaments and cytokeratin filaments by immunofluorescence microscopy revealed that the cytoskeleton maintained a largely polarized distribution during the culture period.

These studies indicate that in contrast to mammalian hepatocytes, isolated skate hepatocyte clusters retain polarity of structure and function in culture, and should provide an excellent system for investigating long-term effects of drugs and xenobiotics on hepatobiliary functions. (Supported by National Institute of Health Grants ES03828, ES01247, DK34989, DK25636, DK48823, NSF DBI 9820400, and by the Burroughs Wellcome Foundation).