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Hepatocytes cultured in alginate microspheres: an optimized technique to study enzyme induction

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Abstract

An important application of hepatocyte cultures is identification of drugs acting as inducers of biotransformation enzymes that alter metabolic clearance of other therapeutic agents. In the present study we optimized an in vitro system with hepatocytes cultured in alginate microspheres that allow studies of enzyme induction with excellent sensitivity. Induction factors obtained with standard inducers, such as 3-methylcholanthrene or phenobarbital, were higher compared to those with conventional hepatocyte co-cultures on collagen coated dishes. This is illustrated by activities of 7-ethoxyresorufin-*O*-deethylase (EROD) after incubation with 5 μM 3-methylcholanthrene (3-MC), a standard inducer for cytochrome P4501A1 and 1A2. Mean activities for solvent controls and 3-MC exposed cells were 2.99 and 449 pmol/min/mg protein (induction factor: 150) for hepatocytes cultured in microspheres compared to 2.72 and 80.6 pmol/min/mg (induction factor: 29.6) for hepatocytes on collagen coated dishes. To compare these in vitro data to the in vivo situation male Sprague Dawley rats, the same strain that was used also for the in vitro studies, were exposed to 3-MC in vivo using a protocol that guarantees maximal induction. Activities were 29.2 and 1656 pmol/min/mg in liver homogenate of solvent and 3-MC treated animals (induction factor: 56.7). Thus, the absolute activities of 3-MC exposed hepatocytes in microspheres are lower compared to the in vivo situation. However, the induction factor in vitro was even higher compared to the in vivo situation (150-fold versus 56.7-fold). A similar scenario was observed using phenobarbital (0.75 mM) for induction of CYP2B and 3A isoenzymes: induction factors for testosterone hydroxylation in position 16β were 127.5- and 50.4-fold for hepatocytes in microspheres and conventionally cultured hepatocytes, respectively.

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The new in vitro system with hepatocytes embedded in solid alginate microspheres offers several technical advantages: (i) the solid alginate microspheres can be liquefied within 60 s, allowing a fast and complete harvest of hepatocytes; (ii) alginate capsules are stable allowing transport and mechanical stress; (iii) high numbers of hepatocytes can be encapsulated in short periods; (iv) defined cell numbers between 600 hepatocytes, the approximate number of cells in one capsule, and 18×10^6 hepatocytes, the number of hepatocytes in 6 ml alginate, can be transferred to a culture dish or flask. Thus, encapsulated hepatocytes allow a flexible organization of experiments with respect to cell number. In conclusion, we optimized a technique for encapsulation of hepatocytes in alginate microspheres that allows identification of enzyme induction with an improved sensitivity compared to existing systems.

Keywords: Primary hepatocytes; Alginate beads; In vitro systems; Enzyme induction; Phenobarbital; 3-Methylcholanthrene; Testosterone hydroxylation

1. Introduction

The use of primary hepatocytes is now well established in pharmacology and toxicology research (Gebhardt et al., 2003; Li et al., 1999; Ringel et al., 2002; Hengstler et al., 2000a, 2000b, 2003; Karim et al., 2000; Beerheide et al., 2002; Tanner et al., 2003; Carmo et al., 2004; Arand et al., 2003). An important application of hepatocyte cultures is identification of drugs acting as inducers or inhibitors of biotransformation enzymes that alter metabolic clearance of other therapeutic agents (Ringel et al., 2002; Hengstler et al., 2000a, 2000b). Well-known examples represent induction of cytochrome P450 3A4 (CYP3A4) by rifampicin, carbamazepine or phenytoin leading to loss of protection in women taking oral contraceptives or to lowered serum concentrations of the immunosuppressant cyclosporin, causing serious clinical complications such as organ rejection after transplantation.

It is generally accepted that several differentiated functions of hepatocytes are better maintained in three-dimensional than in two-dimensional culture systems (Gebhardt et al., 2003). Frequently applied three-dimensional in vitro systems are collagen sandwiches where hepatocytes are cultured between two layers of collagen, Matrigel cultures and hepatocytes microencapsulated in alginate. Although sandwich or Matrigel cultures of freshly isolated hepatocytes are well accepted for studies of enzyme induction, two problems have been observed, namely difficulties in transport and cryopreservation. Transport of hepatocyte in vitro systems may be necessary, since isolation and cultivation of hepatocytes and the final experiment are often performed in different laboratories, especially if hepatocytes from man or from not readily available animal species are in question. In our experience the collagen layers of sandwich cultures can easily be damaged during transport. In addition cryopreservation of sandwich or Matrigel cultures did not reproducibly lead to acceptable drug metabolism data. In contrast, there is no transport problem with hepatocytes microencapsulated in alginate, since the alginate capsules are extremely stable. Another advantage of encapsulated hepatocytes is the possibility of cryopreservation, since a good maintenance of drug metabolizing activities has been reported (Mahler et al., 2003; Canaple et al., 2001; Rialland et al., 2000; Guyomard et al., 1996). For instance protein neosynthesis, urea production, P-450 content, EROD and PROD activities were almost unaltered in cryopreserved and thawed hepatocytes (Guyomard et al., 1996). Encapsulated hepatocytes can be easily harvested, since citric acid liquefies alginate within seconds.

The major field of research where encapsulated hepatocytes have been applied is treatment of liver failure. Promising results have been obtained with encapsulated hepatocytes either after intraperitoneal or intrasplenic transplantation into rats (Liu and Chang, 2003; Aoki et al., 2002; Hamazaki et al., 2002; Cai et al., 2002) or as a component extracorporeal bioartificial livers (Sun et al., 2003; Desille et al., 2002). However, in vitro systems with encapsulated hepatocytes have not yet been optimized for studies of enzyme induction. Indeed, we initially obtained only a weak enzyme induction with standard inducers, when published encapsulation protocols for transplantation purposes were applied. In the present study we optimized an in vitro system with microencapsulated hepatocytes. We present a protocol that allows studies of enzyme induction with