Metabolism and Toxicological Studies in Immortalised Rat Hepatocyte Cell Lines

C MacDonald(1), F Reid(1), K Anderson(2), L Yin(2), E Hill(1), H Kelly(1) and M H Grant(2), (1)Department of Biological Sciences, University of Paisley, Paisley PA1 2BE, UK, (2)Bioengineering Unit, University of Strathclyde, Glasgow G4 0NW, UK.

Abstract

We have investigated the potential use of immortalised rat hepatocyte lines as an alternative to primary rat hepatocytes for drug metabolism and screening. Primary hepatocytes are widely used in the pharmaceutical industry for in vitro studies because established hepatoma cell lines no longer express the relevant drug-metabolising enzyme systems. Immortalised hepatocyte lines have been isolated by transfection of primary cells with SV40 early region DNA. These lines have been investigated for the retention of drug-metabolising enzymes such as glutathione-S-transferases, cytochrome P450s and bilirubin UDPGT. We have shown that immortalised lines retain a drug-metabolising capability but that these enzymes demonstrate the instability of expression with time in culture which is also a feature of primary cells.

Introduction

Primary hepatocytes are widely used in the pharmaceutical industry for in vitro drug metabolism studies. However, there are problems associated with the use of primary cells, because drug metabolism is not maintained in a stable fashion. They cannot be replaced by established hepatoma cell lines because the existing lines no longer express the relevant drug-metabolising enzyme systems. Immortalised hepatocyte cell lines have been isolated by transfecting primary cells with SV40 early region DNA (Yin et al, 1996; MacDonald and Willett 1996), but these lines behave like primary cells and instability is still a problem. Levels of drug metabolising enzymes can be induced in primary cells, eg by compounds such as benzanthracene and phenobarbitone (Jauregui et al, 1991) and we have investigated this approach for the immortalised cell lines. We have also modified the culture conditions for the cells and shown here and elsewhere (Goncalves et al, this volume) that this is another approach to increase the activity of the drug metabolising enzymes.
Procedure

Materials and Methods

Cell culture. Hepatocytes were transfected with SV40 early region DNA by either calcium phosphate precipitation (SV40RH1, P9 and C2.1.2) or electroporation (LQC3 and LQC6) and cultured as previously described (MacDonald et al, 1994; Willett and MacDonald, 1996).

Drug metabolism. Enzyme activities were measured in cell homogenates harvested with a ‘rubber policeman’ and prepared in 0.1M sodium phosphate buffer pH 7.6 as described previously (Grant et al, 1988). GST activities were measured towards the general substrate 1-chloro-2,4-dinitrobenzene (CDNB; 50μM) and towards ethacrynic acid (EA; 0.2mM). The activities were measured in 0.1mM sodium phosphate buffer, pH 7.6, in the presence of 1mM reduced glutathione (GSH) as described by Habig and Jakoby (1981). Bilirubin UDPGT activity was measured in the presence of 0.4mM bilirubin, 4mM UDP-glucuronic acid and 10mM MgCl₂ using the diazotisation procedure described by Dutton (1980). Total cell protein was quantified by the method of Lowry and coworkers (1951) using bovine serum albumin as standard. Cytochrome P450 dependent mixed function oxidase (MFO) activities were measured using 5μM ethoxyresorufin as substrate in the presence of 0.3mM NADPH. Reactions were carried out for 2 hours at 37°C under yellow light and the fluorescence of the product, resorufin, detected at 580nm excitation and 600nm emission as described previously (Grant et al, 1988). Testosterone metabolism was used as a marker for the activity of several cytochrome P450 isoforms. Freshly isolated or primary cells were incubated for 20 minutes with 100μM testosterone and cell lines for 24 hours then the metabolic profiles were analysed by HPLC (Wortelboer et al, 1990).

Results and Discussion

As has been previously reported (MacDonald et al, 1994) the immortalised lines retain many of the characteristics of primary hepatocytes. They express bilirubin UDP glucuronosyltransferase (UDPGT) at levels which are comparable with those of fresh hepatocytes. They do not show γ-glutamyl transferase (γGT) activity (a marker found in pre-neoplastic hepatic tissue, but not in normal cells) and have a high ratio of total glutathione-S-transferase (GST) activity measured by CDNB conjugation compared to that detected by metabolism of EA. Initially this latter activity was thought to be due to the presence of the pi isoform of GST but Western blotting suggests that other isoforms capable of metabolising EA may also be present (data not shown). In addition, the immortalised cell lines retain the ability to synthesise GSH from L-methionine via the cystathionine pathway. This ability is lost during primary culture of hepatocytes and is also absent from de-differentiated hepatoma cell lines. Although these enzyme activities are present in the immortalised lines, levels of expression are not stable: for example, the ratio of CDNB to EA GST activities and GSH resynthesis decline as the cells are passaged. In contrast, however, γGT has not been detected even after continued culture.