Inducibility of ethoxyresorufin deethylase and UDP-glucuronosyltransferase activities in two human hepatocarcinoma cell lines KYN-2 and Mz-Hep-1

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Abstract

Two human hepatoma cell lines, KYN-2 and Mz-Hep-1 were characterized in terms of glucuronidation capacity and inducibility of cytochrome P4501A1/1A2 and several UDP-glucuronosyltransferases (UGTs). Cytochrome P4501A1/1A2 activity was measured using 7-ethoxyresorufin and that of UGTs with 16 different substrates. The effects of dimethyl sulfoxide (DMSO), β-naphthoflavone, α-naphthoflavone, and rifampicin on these drug-metabolizing enzyme activities were studied. DMSO treatment increased in a dose-dependent manner the ethoxyresorufin O-deethylase (EROD) activity in KYN-2 cells, while an opposite effect was observed in Mz-Hep-1 cells. In KYN-2 cells, EROD was more responsive toward β-naphthoflavone treatment in combination with DMSO. This activity was enhanced in Mz-Hep-1 cells more than 83 times by β-naphthoflavone. The enhancement of EROD activity by DMSO and β-naphthoflavone treatments of KYN-2 cells was abolished by α-naphthoflavone treatment. In Mz-Hep-1, only the inducing effect of β-naphthoflavone was abolished by α-naphthoflavone treatment. Rifampicin treatment of KYN-2 cells reversed both the DMSO and β-naphthoflavone effects on the EROD activity. Glucuronidation of steroids, bile acids, fatty acids and drugs was effective in KYN-2 and Mz-Hep-1 cells. Both 1-naphthol glucuronidation and the level of UGT1*6 protein detected by immunoblot and supporting this activity were lowered by DMSO treatment and increased by β-naphthoflavone treatment in KYN-2 cells. In Mz-Hep-1 cells, DMSO and β-naphthoflavone had no effect on 1-naphthol glucuronidation activity. DMSO, β-naphthoflavone and rifampicin also affected the glucuronidation of various substrates supported by different UGT isoforms. These results indicate that KYN-2 and Mz-Hep-1 cells can be used as new in vitro models for the studies of drug metabolism and the regulation of the corresponding enzymes.

Abbreviations: DMSO, dimethyl sulfoxide; EROD, ethoxyresorufin O-deethylase; NF, naphthoflavone; P450, cytochrome P450 (EC 1.14.14.1); RIF, rifampicin; UGT, UDP-glucuronosyltransferase (EC 2.4.1.17).
Introduction

The activity of drug-metabolizing enzymes is mainly associated with the liver. Isolated and cultured hepatocytes are powerful tools for studying the metabolism of drugs and xenobiotics, and performing regulation studies of the enzymes involved in that process. However, for ethical reasons, cultures of human hepatocytes are becoming less and less available and human hepatoma cell lines therefore remain an efficient model for studying the activity and regulation of drug-metabolizing enzymes. Different hepatoma cell lines have been established and characterized to date, but few have a differentiated phenotype. KYN-2 is a hepatocarcinoma cell line, established from a surgical sample of a 52-year-old Japanese male patient (Yano et al., 1988). The hepatocarcinoma cell line Mz-Hep-1 was established from a hepatitis B marker negative 64-year-old Caucasian female (Dippold et al., 1985). These two cell lines secrete plasma proteins of the hepatocyte phenotype such as albumin, transferrin or ferritin. Mz-Hep-1 cells are \( \alpha \)-fetoprotein negative (Dippold et al., 1985). The secretion of \( \alpha \)-fetoprotein and of the carcinoembryonic antigen by KYN-2 cells depends upon culture conditions (Yano et al., 1988). KYN-2 and Mz-Hep-1 cells have not been characterized in terms of drug-metabolizing enzyme activity and inducibility.

In hepatocytes, oxidation and conjugation reactions are catalyzed by multienzymatic systems, which mainly include the cytochrome P450-linked monooxygenase complexes (P450) and UDP-glucuronosyltransferases (UGT) (Mulder et al., 1990; Gonzalez, 1993). These enzymes belong to multigenic families of proteins (Burchell et al., 1991; Nelson et al., 1993). The induction of human P450s, in particular P4501A, is well established in vivo and in vitro (Gonzalez, 1993), whereas little is known about the inducibility of human UGTs and the molecular mechanisms of their regulation.

In this work, the glucuronidation capacities of KYN-2 and Mz-Hep-1 cells using 16 different substrates was investigated. The effects of several inducer molecules, such as dimethyl sulfoxide, naphthoflavone and rifampicin, on 7-ethoxyresorufin O-deethylase and on different UGT isoforms were studied.

Materials and methods

Materials

\( \beta \)-NF, \( \alpha \)-NF, RIF, UDP-glucuronic acid (sodium salt), 1-naphthol, and \([1-^{14}C]\)naphthol (7.1 mCi/mmol) were from Sigma (St Quentin Fallavier, France). Dulbecco’s MEM was purchased from Gibco-BRL (Cergy Pontoise, France). UDP-[U-\(^{14}C\)]glucuronic acid (282.2 mCi/mmol) was from Dupont de Nemours (NEN Division). DMSO was from Merck (Nogent-sur-Marne, France). Culture materials were from Becton Dickinson (Pont de Claix, France). All other chemicals were analytical grade.

Cell culture and treatments

Hepatoma cells KYN-2 (Yano et al., 1988) and Mz-Hep-1 (Dippold et al., 1985) were generously given by Dr M. Kojiro (First Department of Pathology, Kurume University, School of Medicine, Kurume-Shi, Japan) and by Dr W.G. Dippold (Department of Internal Medicine, Pathology, Genetic and Microbiology, Johannes Gutenberg-Universität, Mainz, Germany), respectively. These cell lines were grown in Dulbecco’s MEM supplemented as described previously (Abid et al., 1994). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO\(_2\). Before treatment, cells were trypsinized and seeded at \( 1 \times 10^6 \) cells per 90-mm Petri dish. After 24 h, the solvent (DMSO) and the inducers, \( \beta \)-NF, \( \alpha \)-NF, RIF, were added to the medium and