Induction, Expression and Maintenance of Cytochrome P450 Isoforms in Long-term Cultures of Primary Human Hepatocytes

Jing Yuan¹, Liegang Liu¹, Masashi Shimada¹, Aiguo Wang¹, Maren Ruhnke², Peter Heeckt¹, Andrea R. Müller*¹⁶, Natascha C. Nussler¹, Peter Neuhaus¹ and Andreas Nussler**¹³

¹Department General, Visceral and Transplantation Surgery, Charité Campus Virchow-Klinikum, Universitätsmedizin Berlin, Humboldt University of D-Berlin, ²Department of General and Thoracic Surgery, University Hospital D-Kiel, ³Fresenius-Proserve GmbH, D-Oberursel

Summary
Within the past decade, tremendous progress has been made in the isolation and culture of human hepatocytes for drug metabolism and toxicology, which could potentially reduce the number of animal experiments performed. However, human hepatocyte cultures are still not widely used for preclinical drug testing, partly due to inconsistent supply and quality of human tissue. Thus, the aim of this study was to evaluate primary cultured human hepatocytes from different patients over a study period of 14 days, by assays that characterise cell quality and function. We found urea production and albumin synthesis in all cell cultures over at least 7 days. Cytochrome P4501A2, CYP2D6, and CYP3A4 protein expression was demonstrated by Western Blot analysis and CYP1A112 and CYP3A4 induction by 3-methylcholanthrene, phenobarbital or rifampicin over 14 days. In addition, we saw that UDP-glucoronyltransferase activity was preserved in human hepatocytes over 2 weeks.

In conclusion, we could show that primary human hepatocytes isolated from discarded liver tissue can consistently be kept in culture over a long time period and are therefore well suited for preclinical drug testing.

Keywords: human hepatocytes, cytochrome P450, drug screening, toxicology

1 Introduction

The liver is the target organ for metabolism of endogenous and xenobiotic compounds. It performs central functions, which include biosynthesis, biotransformation, and biodegradation or detoxification. Xenobiotics, such as many drugs and environmental pollutants, are metabolised in the liver by either Phase I and/or Phase II enzymes (Berthou et al., 1989; Seddon et al., 1989). Cytochrome P450s are a group of primary enzymes responsible for the oxidative metabolism of xenobiotics, many of which are converted to more hydrophilic molecules in order to be more easily eliminated from the body. The cytochrome P (CYP) isoenzymes are a group of heme-containing enzymes embedded primarily in the lipid bilayer of the endoplasmic reticulum of hepatocytes. The enzymatic complex consists of two functional units, a cytochrome P450 monoxygenase (mixed-function oxygenase, MFO) and an NADPH-cytochrome P450 reductase (Nelson et al.,

Received 22 January 2004; received in final form and accepted for publication 11 February 2004
*present address: Clinic for General and Thoracic Surgery, University Hospital Kiel, D-Kiel
**present address: Fresenius-Biotech GmbH, D-Bad Homburg

ALTEX 21, Suppl. Linz 03/2004
The major human drug-metabolising CYPs belong to the families 1, 2, and 3. Among the numerous human isoenzymes identified, the major human CYP isoforms involved in drug metabolism are CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4.

Phase II enzymes are located in the cytoplasm and microsomes. Phase II enzymes primarily include glutathione-S-transferases (GSTs), UDP-glucuronyltransferases (UGTs), N-acetyltransferases (NATs), methyl-transferases and amino acid transferases. Phase II metabolism constitutes the conjugative phase of metabolism, in which a polar functional group on a drug is conjugated with a cofactor, usually inactivating it and so preparing it for excretion. Typical conjugation reactions include glucuronic acid, glutathione, sulphate, and glutaminic conjugations (Parkinson, 1996).

The liver consists mainly of hepatocytes. Primary cultured human hepatocytes retain uninterrupted metabolic pathways and tissue specific functions, and exhibit a more or less complete picture of the metabolic fate of xenobiotics in man, such as drug-drug interactions, drug toxicity, enzyme induction, and gene regulation (Ferrini et al., 1997; Li and Gorycki et al., 1999; LeCluyse, 2001; Yan and Caldwell, 2001; Yao et al., 2002). It is well known that discordance exists between animal and human data because of interspecies differences (Hod et al., 1982; Elshourbagy et al., 1986; Lin et al., 1995; Li and Lu et al., 1999; Grompe, 2001). Therefore, primary cultured hepatocytes are currently widely used as a valid and simplified model for metabolism and cytotoxicity studies of environmental pollutants and new drugs in humans (Berry et al., 1991a; Li and Lu et al., 1999; Gebhardt et al., 2003). However, cultured hepatocytes have certain limitations, such as the rapid loss of cytochrome P450 (CYP) and Phase II catalysing enzyme activities during the culture period (Skett, 1994). Several strategies have been employed to improve the quality of primary cultured hepatocytes (Silva et al., 1999), such as the cultivation of hepatocytes on different extracellular matrices using different culture systems (Kern et al., 1997), or the supplementation of media with hormones and growth factors (Blaauboer et al., 1994; Ferrini et al., 1997; Kern et al., 1997; Nussler et al., 2001; Pfaller et al., 2001). Additionally, it was reported that cocultivation with different cell types improves both quality and life span of hepatocytes. Recent reports demonstrated that cryopreservation of human hepatocytes can maintain P450 isoform activity and Phase II conjugation enzyme activity comparable to that of freshly isolated hepatocytes (Li and Gorycki et al., 1999; Li and Lu et al., 1999; Silva et al., 1999; Hengstler et al., 2000; Smrzova et al., 2001). This particular finding is of great value as it allows storage of unused human hepatocytes for later applications. However, if fresh human hepatocytes are available, it must be ensured that cell characteristics are maintained in culture for a certain time period for pharmacological and toxicological studies to be carried out.

At the Charité in Berlin we have regular access to discarded human liver tissue, either from surgical specimens or from organs not used for transplantation. So far these tissues are not routinely used to study important aspects of liver diseases and to perform preclinical drug testing. Increased use of human hepatocyte cultures could potentially reduce the number of animal experiments conducted in preclinical pharmacology and toxicology testing. At the same time, misleading data from animal experiments could be avoided. Some drugs even had to be withdrawn from the market because they posed a potential threat to patients despite inconspicuous preclinical animal data, constituting enormous financial consequences to drug manufacturers.

In order to demonstrate human hepatocyte cell culture functionality we evaluated isolations from different donors over a study period of 14 days. The hepatocyte cultures were characterised by studying cell quality by means of lactate dehydrogenase (LDH) and aspartate transaminase (AST) leakage, glucose and lactate metabolism, urea formation, albumin synthesis, expression of various CYP proteins, as well as cytochrome P450 enzyme and UDP-glucurononyltransferase activity.

2 Materials and methods

2.1 Isolation and culture of human hepatocytes

According to institutional guidelines, normal liver specimens were obtained from patients who underwent partial liver resections due to primary or secondary liver tumours. Approximately 30 g of tissue was used for the isolation of hepatocytes by a two-step collagenase perfusion technique, following the published standard procedure of our laboratory (Dorko et al., 1994). According to the designed experiments, harvested hepatocytes were seeded in monolayer culture at a density of 5×10⁶ cells/cm², either onto pre-coated 6-well plates or 90 mm Petri dishes (Becton Dickinson, Heidelberg, Germany) with 1% rat-tail collagen prepared as described (Berry et al., 1991b). Cells were left untreated for at least 12 h at 37°C in a humidified atmosphere of 95% O₂ and 5% CO₂. On the following day, medium was changed to remove floating cells and cell debris. Thereafter, the culture medium consisting of Williams’ medium E with L-Glutamine (Invitrogen, Karlsruhe, Germany), supplemented with 10% calf serum (PAA Laboratories GmbH, Austria), 100U/100μg/ml penicillin/streptomycin (Invitrogen, Karlsruhe, Germany), 1 μM insulin (Sigma, Taufkirchen, Germany), 1.4 μM hydrocortisone (Sigma, Taufkirchen, Germany), and 15 mM HEPES buffer (Invitrogen, Karlsruhe, Germany) was renewed every 24 h. Changes in cell morphology were examined daily under a phase contrast microscope.

2.2 Measurement of biochemical parameters and albumin synthesis

Supernatants of each culture were collected at the indicated time points and stored in aliquots at -80°C until further analysis. Lactate dehydrogenase (LDH) and aspartate transaminase (AST) were assayed using a commercial test kit from Sigma (Taufkirchen, Germany). Concentrations of urea, glucose and lactate were measured in the medium using commercial test kits from HITADO (Möhnesee Deleck, Germany), according to the manufacturer’s instructions.
2.3 7-ethoxyresorufin O-deethylase activity

The activity of 7-ethoxyresorufin O-deethylase (EROD) describes the rate of CYP1A-mediated deethylation of the substrate 7-ethoxyresorufin to form the product resorufin in human hepatocytes. Prior to the investigated study time points, cells were incubated with 2.5 μM 3-MC and 50 μM Omeprazol for 72 h. The culture medium was replaced daily during the induction period using fresh medium with or without the indicated inducers. To evaluate CYP1A1 activity, cell cultures were incubated with 5 μM ethoxyresorufin and 10 μM dicoumarol for 2 h at 37°C at the indicated time points. The fluorescent product 7-hydroxyresorufin, was determined using a Fluorescent Galaxy plate reader with 544 nm excitation and 590 nm emission wavelength, using a modified method described by Burke and Mayer (1974). The EROD activity was expressed in picomoles resorufin/min/mg protein.

2.4 7-ethoxycoumarin-7-O-deethylation activity

7-ethoxycoumarin-7-O-deethylation (ECOD) activity describes the rate of deethylation of the substrate 7-ethoxycoumarin to form the fluorescence product 7-hydroxycoumarin in human hepatocytes by several CYPs (Waxman and Chang, 1998). Prior to the investigated study time points, cells were exposed to 2 mM phenobarbital (PB), 2.5 μM 3-MC and 5 μM rifampicin (Rif) for 72 h. The culture medium was renewed daily in the presence or absence of the inducers. At the indicated time points, cell cultures were exposed to 25 μM ethoxycoumarin plus 1.5 mM salicylamide for 2 h at 37°C. The fluorescent product 7-hydroxycoumarin was determined using a Fluorescent Galaxy plate reader at 390 nm excitation and 460 nm emission wavelength. The ECOD activity was expressed in picomoles hydroxycoumarin/min/mg protein.

2.5 UDP-glucuronyl-transferase activity

UDPGT (uridine diphosphate glycosyltransferases) are a class of enzymes catalysing the process of conjugation of various endogenous and xenobiotic compounds. The 4-methylumbelliferone (4-MU, Sigma) glucuronidation assay was used to assess Phase II enzyme activity in human hepatocytes (Gomez-Lechon et al., 1997). At the indicated time points, cells were incubated with 1 ml 0.01 M PBS (pH 7.6) containing 100 μM 4-MU for 1 h at 37°C. The remaining 4-MU activity was measured using a Fluorescent Galaxy plate reader with 376 nm excitation and 460 nm emission wavelength. The corresponding cells were washed twice with ice-cold 0.01 M PBS (pH 7.6) and then scraped from the plates, centrifuged at 1700 x g for 5 min at 4°C, and re-suspended in 50 μl of 0.01 M PBS for the determination of total protein. The activity of produced conjugates was expressed as nanomoles of 4-MU conjugated (4-MU glucuronide) per minute per mg total protein.

2.6 Western Blot analysis for CYP450 protein expression

After cells were exposed to the different inducers or culture medium alone, cells were scraped off the culture plates and transferred to pre-cooled 1.5 ml Eppendorf tubes and pelleted by centrifugation at 240 x g for 5 min at 4°C. The pellets were washed twice with PBS and resuspended in approximately 50 μl of a solution containing 25 μg/ml aprotinin, 25 μg/ml Leupeptin, 25 μg/ml chymostatin, 50 μg/ml phenanthroline, 10μg/ml pepstatin and freshly added 100 μM PMSF. This suspension was subjected to 3 freeze/thawing cycles. After additional centrifugation at 20 000 x g at 4°C, the supernatant was stored at -80°C until further use.

Immunohistochemical analysis of primary cultured hepatocyte P450 isoforms was conducted as previously described (Nussler et al., 1994). Briefly, 25 μg of protein sample was separated on a 10% polyacrylamide gel containing 0.1% sodium dodecyl sulphate. Separated proteins were then transferred to nitrocellulose and exposed to antibodies directed against CYP1A2, CYP3A4 and β-actin. The immune complex was visualised using a polyclonal rabbit antibody purchased from Chemicon Int. Inc. (Temecula, USA). CYP2D6, a constitutively expressed CYP, was detected using a polyclonal rabbit antibody (a kind gift from the IKP Stuttgart, Germany). A horseradish-peroxidase-conjugated anti-mouse antibody (Amersham Freiburg, Germany) served as secondary antibody.

3 Results

3.1 Transaminases

The release of cytosolic enzymes, such as lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) was used for the evaluation of membrane integrity of primary human hepatocytes. In the present study, the levels of LDH, and AST were measured in monolayer cultures during the study period of 14 days. We observed a low but continuous LDH and AST membrane leakage in human hepatocytes over the whole study period (Tab. 1). However, the highest release of AST and LDH was seen on day 1, then rapidly decreased over the next 3 days and remained unchanged until day 14.

3.2 Glucose consumption and lactate formation

During the study period of 14 days, the glucose level in the supernatant medium was not influenced by culture time. However, levels were different in each tested
Tab. 1: Biochemical parameters in primary human hepatocyte cultures. Data are expressed as mean ± SD of at least 5 individual hepatocyte isolations.

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>LDH (U/Day/mg protein)</th>
<th>AST (U/Day/mg protein)</th>
<th>Glucose (μmol/Day/mg protein)</th>
<th>Lactate (μmol/Day/mg protein)</th>
<th>Urea (μmol/Day/mg protein)</th>
<th>Albumin (μmol/Day/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.29±3.84</td>
<td>2.16±2.80</td>
<td>93.3±45.38</td>
<td>9.56±4.56</td>
<td>26.04±4.63</td>
<td>72.21±37.02</td>
</tr>
<tr>
<td>3</td>
<td>0.12±3.00</td>
<td>0.46±7.70</td>
<td>69.42±40.31</td>
<td>10.00±5.41</td>
<td>23.12±4.63</td>
<td>57.03±39.84</td>
</tr>
<tr>
<td>5</td>
<td>0.35±1.35</td>
<td>0.32±1.35</td>
<td>79.45±45.61</td>
<td>14.91±13.38</td>
<td>24.30±9.19</td>
<td>45.56±18.24</td>
</tr>
<tr>
<td>7</td>
<td>0.60±2.43</td>
<td>0.44±2.43</td>
<td>82.02±53.45</td>
<td>21.55±13.53</td>
<td>12.33±2.24</td>
<td>34.01±16.70</td>
</tr>
<tr>
<td>9</td>
<td>0.22±1.36</td>
<td>0.34±3.02</td>
<td>89.89±56.67</td>
<td>20.06±10.65</td>
<td>15.32±12.53</td>
<td>29.78±32.70</td>
</tr>
<tr>
<td>11</td>
<td>0.57±3.43</td>
<td>0.22±1.01</td>
<td>84.07±44.00</td>
<td>25.04±14.52</td>
<td>10.45±3.24</td>
<td>25.92±18.85</td>
</tr>
<tr>
<td>13</td>
<td>0.21±1.78</td>
<td>0.06±5.00</td>
<td>81.10±63.48</td>
<td>30.19±12.36</td>
<td>10.53±3.18</td>
<td>19.66±26.62</td>
</tr>
<tr>
<td>15</td>
<td>0.46±2.01</td>
<td>0.11±2.05</td>
<td>67.48±29.80</td>
<td>34.03±12.50</td>
<td>7.34±0.82</td>
<td>8.10±5.31</td>
</tr>
</tbody>
</table>

3.3 Urea formation
The formation of urea takes place in the liver to eliminate toxic ammonium ions from the body. Thus, the production of urea by liver cells is a prognostic parameter for cell integrity and quality of the cell culture. The data in Table 1 show that human hepatocytes isolated from different donors synthesised urea at a high rate for 5 days. The capacity of urea formation was significantly reduced to approximately 50% on day 7. This second phase of reduced urea formation lasted another 7 days before it decreased to 25% of the initial value.

3.4 Albumin secretion
Albumin is a major plasma protein secreted by hepatocytes (Dufour et al., 2000). During the first 48 hours, while hepatocytes adapt to culture conditions, albumin secretion was unstable. Then, albumin secretion decreased with culture time. Albumin secretion in human hepatocytes isolated from various donors differed, which certainly depended on the initial hepatocyte quality after isolation and the patient's condition. However, our results indicate a relatively stable and continuous albumin secretion in hepatocyte cultures for at least seven days with a rapid decline thereafter.

3.5 CYP protein expression
As shown in Figure 1, CYP1A2 was expressed in human hepatocytes. We observed a faint band of CYP1A2 protein from the day of cell isolation to day 14 (lanes 1-4). When cells were exposed to 3-MC, we saw a strong induction on day 4 and only faint bands of CYP1A2 protein on days 10 and 14 (lanes 5-7).

CYP2D6 is also expressed in cultured human hepatocytes (Fig. 1). The strongest expression of CYP2D6 was seen on the day of cell isolation (lane 1), while the expression level decreased within 4 days by 50% (lane 2) and almost undetectable by days 10 and
14 (lanes 3-4). However, occasionally we could still see CYP2D6 protein expression up to 10 days (data not shown). When cells were exposed to various drugs we did not observe changes in the expression level (lanes 5-7). This result is consistent with the literature, which describes CYP2D6 to be constitutively expressed in the human liver (Michalets, 1998).

As seen in Figure 1, human hepatocytes always showed high CYP3A4 expression on the day of cell isolation (lane 1), whereas a continuous decrease was observed over the next 14 days. However, when cells were exposed to rifampicin for three days, we detected a consistent expression of CYP3A4 over 14 days (lanes 5-7).

3.6 EROD and ECOD enzyme activity
Human hepatocyte basal and induced CYP1A activity was measured in the presence or absence of 3-methylcholanthrene (3-MC) or Omeprazol (Om). The data clearly show that basal CYP1A activity was constant over 14 days with a slight decrease towards the end of the investigated time period. 3-MC and Omeprazol both showed sustained induction of EROD activity within 10 days and then reduced enzyme activity until the end of the study period (Fig. 2).

As shown in Figure 3, human hepatocytes showed a sustained induction of ECOD enzyme activity when cells were exposed to various inducers for 72 h. We found, that phenobarbital (PB) and rifampicin (Rif) led to a continuous increase in ECOD activity over 14 days while 3-MC only increased ECOD activity significantly until day 10.

3.7 CYP3A4-dependent metabolism of testosterone in human hepatocytes
It is well known that testosterone is metabolised in human hepatocytes into several metabolites, however the formation of 6β-hydroxytestosterone is clearly dependent on the activity of CYP3A4. As depicted in Figure 4, normal (untreated) human hepatocytes showed a rapid decline of 6β-hydroxytestosterone production within 4 days. However, pre-stimulation of human hepatocytes with 50 µM rifampicin over 3 days resulted in a strong formation of 6β-hydroxytestosterone over the whole study period, which is in agreement with our protein data demonstrated in Figure 1.

3.8 UDPGT enzyme activity
UDPGT enzyme activity in human hepatocytes showed a constant degree of glucuronidation of 4-methylumbelliferone in cells from different donors and did not change significantly over the study period of 14 days. In one experiment a high value of 33.9 nmol/min/mg protein
We observed that the level of glucose leakage in culture supernatants of human hepatocytes did not change during the entire study period. If cell cultures are not supplied with glucose, intracellular degradation of glycogen is dramatic. Since our cultures were not supplemented with glucose, it is likely that the constant glucose level was at least partly due to degradation of glycogen. In contrast, the continuous increase of lactate in supernatants of human hepatocytes suggests that the cells were deprived of oxygen particularly towards the end of the study period. Under these circumstances, oxygen deprived cells may change their metabolism from aerobic glycolysis to anaerobic glycolysis and transform pyruvate to lactate (Berry et al., 1991c). The reason for this oxygenation deficit in monolayer cultures has been described many times and may be improved by culturing cells in threedimensional culture systems or by increasing oxygen supply to the cells (Berry et al., 1991d).

The conversion of ammonia to urea is a vital sign of liver function and is therefore a good prognostic indicator of retained liver function (Watts et al., 1995). Our cultured human hepatocytes showed a good detoxification capacity over the first five to seven days in culture, which seems to be a dynamic process and correlates with other measured parameters. Protein degradation is a dynamic process, which can be influenced by
drugs and/or the metabolic status of the cell (Guggen-Guillouzo et al., 1983). Cultured hepatocytes are capable of secreting a large number of different proteins into the culture medium. In particular, the inhibition of protein synthesis has been suggested to be an early sign of cellular damage (Goethals et al., 1984). Albumin secretion, which is one of the major specific functions of hepatocytes in vivo, rapidly declined in monolayer cultures. Overall, this is not an unexpected finding, since studies of albumin metabolism have clearly shown that synthesis and secretion of albumin by isolated hepatocytes only takes a short time (Weigand and Otto, 1974). According to the applied culture conditions additional albumin storage and synthesis is only possible to a certain extent in monolayer cultures (Peters, 1977).

It is common knowledge that CYP450 proteins cannot be divided into "good" or "evil" in terms of health. However, increasing evidence suggests that some human liver P450 isoenzymes are induced by known noxics such as cigarette smoke, consumption of charcoal-grilled foods, or certain drugs (Tai et al., 1993; Parkinson, 1996). In the same line of evidence, it was observed that certain CYP450s are sometimes responsible for the generation of toxic, mutagenic, or carcinogenic metabolites from originally non-toxic substances (Shimada et al., 1989; Jover et al., 1993). However, some authors reported that rats pre-treated with certain CYP450 inducers are protected from the toxicity of many drugs (Selvay, 1971; Argus et al., 1978). Therefore, the evaluation of CYP450 function (protein expression and activity) depends on the characteristic of the xenobiatic or rather its metabolic pathway. However, the expression and activity of CYP450 proteins in hepatocyte cultures is a predictive parameter of whether or not a hepatocyte culture is useful to study various aspects of drug metabolism (Guillouzo et al., 1993; Li and Maurel et al., 1997; Kostrubsky et al., 1999; Gebhardt et al., 2003). Thus, the decrease of constitutive enzyme activity and/or the inductibility of CYPs in culture are good parameters to judge the quality of the cell culture. Changes of CYP activity are linked to enzymatic inhibition, a decrease in protein stability or to a decrease of mRNA synthesis (Rodriguez-Antona et al., 2002). Therefore, in this study, the effects of model inducers of cytochrome expression and enzymatic activity were investigated. First, we investigated the capacity of hepatocytes to express important CYP proteins. We found that human hepatocytes constitutively expressed CYP1A2, CYP2D6 and CYP3A4, which are all important drug metabolising enzymes (Parkinson, 1996). Our study confirmed that CYP1A2 and CYP3A4 expression is lost during culture time and can be induced by known standard inducers (Parkinson, 1996; Li and Maurel et al., 1997; Kostrubsky et al., 1999), while CYP2D6 cannot be induced and also loses its initial protein expression (Michalets, 1998). Thereafter, both EROD and ECOD activity were measured as markers of liver function, since both methods assess different families of CYP activity. While EROD metabolism assesses mainly CYP1A activity, ECOD turnover is due to a number of CYP proteins (Parkinson, 1996; Li and Maurel et al., 1997), of which CYP3A predominates (Wrighton and Stevens, 1992; Parkinson, 1996; Li and Maurel et al., 1997). Together these two CYP isoforms (CYP1A and CYP3A) comprise 60-90% of all CYP proteins in the liver (Parkinson, 1996). Thus, the measurement of these two activities in basal and induced states will provide an excellent estimate of the total drug metabolising capacity of the investigated cells. We saw modest but distinguished EROD activity in "normal" non-stimulated human hepatocytes, which was strongly induced by the typical CYP1A inducers 3-MC and Omeprazol.

As already mentioned, basal and induced ECOD enzyme activity covers various CYP families including CYP1A, CYP2A, CYP2B, and CYP3A. In this study we demonstrated that 3-MC, rifampicin and phenobarbital all increase ECOD activity in human hepatocytes approximately 20-fold, which is in agreement with several other studies (Parkinson, 1996; Li and Reith et al., 1997; Kostrubsky et al., 1999; Li, 2001). In terms of CYP3A4 activity we were able to demonstrate 68-hydroxysteroid formation in non-stimulated and stimulated hepatocytes over 14 days, although we observed a loss of constitutive CYP3A4 levels within a few days, confirming our observation that CYP3A4 protein expression was markedly reduced within 10 days.

In humans, a chemical substance is first functionalised by Phase I enzymes, and then conjugated by Phase II reactions, which facilitate the excretion of the resultant metabolite and usually inactivate the drug (Liska, 1998). This reaction is well recognised to be one of the major detoxifying mechanisms of toxic substrates in the body (Maruo and Sato, 2002). Therefore, it was important to study the major Phase II reactant UDP-glucuronosyltransferase to investigate whether hepatocytes retain this enzyme during the entire study period. In the present study, the low amount of free umbelliferone in human hepatocyte cultures during the study period indicates that coupling of Phase I and Phase II reactions was maintained.

In conclusion, the results presented in this study show that primary human hepatocytes in our culture system retained substantial integrity and maintained a wide range of differentiated hepatocyte functions, i.e. the rate of urea formation for ammonia removal, albumin synthesis, as well as Phase I and Phase II metabolism and detoxification. Thus, we believe that hepatocytes isolated from discarded liver tissue can easily be used for the study of liver diseases and for faster and safer preclinical drug testing. The latter is extremely important in order to reduce drug developmental costs and unreliable animal experiments. However, in order to achieve these goals, political and financial security must be provided for potential suppliers of human liver tissue. A possible alternative to the low supply of human liver tissue might be direct generation of liver cells from stem cells or stem cell-like cells in the near future.

References


Li, A. P. (2001). Screening for human

Correspondence to
Natascha C. Nussler
Department of General-, Visceral-, and Transplantation Surgery
Universitätsmedizin Berlin, Charité, Campus Virchow Klinikum
Augustenburger Platz 1
D-13353 Berlin
Germany
e-mail: andreas.nuessler@freenet.de