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Drug-metabolizing activity of human and rat liver, lung, kidney and intestine slices

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1. Organ-specific biotransformation was studied in human and rat liver, lung, kidney and small intestine slices and compared on a protein basis, using four model substances.

2. Deethylation of lidocaine was highest in liver slices from both man and rat, followed by the small intestine.

3. Metabolism of testosterone was highest in liver slices, but a different overall metabolic pattern was found between the different organs.

4. Lung, kidney and intestine slices prepared from human and rat organs showed mainly an unknown metabolite of 7-ethoxycoumarin identified as 4-ethoxy-2-hydroxyphenyl propionic acid (EPPA).

5. The maximal metabolism of 7-ethoxycoumarin in slices was equal with *in vivo* V_{max} in the rat.

6. Phase II metabolism of 7-hydroxycoumarin in kidney and intestinal slices was about 60% of the activity in liver slices.

7. In conclusion, organs other than the liver show a surprisingly high drug-metabolizing activity. Thus, the use of precision-cut slices of a combination of drug metabolizing organs in an *in vitro* test system from both animal and human origin is required for a proper systematic prediction of drug metabolism in man.

Introduction

The use of precision-cut liver slices from both animal and human origin is now a widely used model to study drug metabolism and toxicity (Bach *et al*. 1996, Olinga *et al*. 1998a, Lerche-Langrand and Toutain 2000). Lung and kidney slices have also been used for these purposes but not as extensively as liver slices (de Kanter *et al*. 2002). The use of slices prepared from the small intestine has been only very limited (Vickers *et al*. 1992, 1995).

Although the liver is generally considered to be the most important organ for the biotransformation of drugs, it is also recognized that the lung, kidney and intestine can make an important contribution to the fate of drugs in the body (Krishna and Klotz 1994). In addition, many examples of metabolism-induced toxicity in these organs have been described (Boyd *et al*. 1983). The role of the small intestine in metabolism of drugs is considered particularly substantial due to

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its high drug-metabolizing enzyme content (Krishna and Klotz 1994) and the first-pass phenomenon after oral drug administration.

When studying extrahepatic metabolism, whole cell systems are preferably used instead of microsomal preparations, because the latter does not contain all of the various drug-metabolizing enzymes (Pacifici *et al.* 1988). One example of such a whole cell model is that of precision-cut slices. It has the important advantage over the use of isolated cells that invasive cell isolation techniques, which are different for each organ and species, are not required. Moreover, in the use of precision-cut slices, preparation and incubation can be performed in essentially the same manner, which is important when comparing organ-specific metabolic capacity and species differences therein. Another advantage of the use of precision-cut slices is that the *in vivo* situation (including the presence of all cell types, cell–cell contacts and extracellular matrix) is more closely approached than in most other *in vitro* systems, such as isolated hepatocytes (liver), Clara or alveolar cells (lung), renal tubule fragments (kidney), or enterocytes (intestines).

Therefore, we have developed an *in vitro* test system using liver, lung, kidney and intestine slices to compare organ-specific metabolism and study differences between rat and man. Some preliminary data from this work on human lung slices and rat liver, lung and kidney slices have been described by de Kanter *et al.* (1999). The present paper expands our preliminary results on liver, kidney and intestine slices from both human and rat origin. To monitor the viability of these slices, ATP levels were measured during the 24 h of incubation and compared with *in vivo* values. Four representative test substrates were used to study xenobiotic phase I and II metabolism, i.e. testosterone (TST), lidocaine, 7-ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-HC), which are metabolized via different pathways. Slices were incubated with these test substrates at relatively high concentrations to be able to detect sufficient amounts of metabolites to study organ and species differences in metabolic pattern, and in metabolism capacity between rat and man.

Materials and methods

Materials

The following compounds were obtained from the sources indicated: lidocaine from Centrachemie (Etten-Leur, The Netherlands); 2α-, 7α-, 11α-, 19-, 16α- and 16β-hydroxytestosterone (TOH) from Steraloids (Newport, RI, USA); 7-EC from Fluka (Buchs, Germany); 2β-, 6α-, 6β-, 11β-, 14α- and 15α-TOH, TST, androstenedione, 7-methoxycoumarin, 7-HC, 7-hydroxycoumarin glucuronide (7-HC GLUC) and low melting agarose (type VII-A) from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands); University of Wisconsin organ preservation (UW) solution from DuPont Pharmaceuticals (Waukegan, IL, USA); Amphotericin B (Fungizone), penicillin-streptomycin solution and Williams' medium E (with Glutamax) from Gibco (Paisley, UK). Monoethylglycinexylidide (MEGX) was a kind gift of AstraZeneca (Södertälje, Sweden), and 7-hydroxycoumarin sulphate (7-HC SULF) was a kind gift from Mr P. Mutch, GlaxoWellcome (Herts, UK). All other chemicals were of analytical grade and were obtained from commercial sources.

Human organ tissue

Human liver tissue was obtained from redundant donor tissue after liver transplantation as part of a transplantation related research project. Human kidney, lung and intestine tissue was obtained from patients subjected to surgery for cancer in the organs concerned. Tumour free tissue considered as surgical waste was used. Human liver tissue was perfused with and stored in ice-cold UW (maximum 36 h) (Olinga *et al.* 1998b) while human lung, kidney and intestine (jejunum) material was stored in ice-cold Krebs–Henseleit buffer during transport from the hospital until arrival in the lab (maximum 2 h).

The research protocols were approved by the medical ethical committee of the Academic Hospital in Groningen, The Netherlands, and informed consent from the patients concerned was obtained.

Rat organ tissue

Male Wistar (HsdCpb:WU) rats (Harlan, Horst, The Netherlands) were housed in standard cages and had free access to food (standard chow, Hope Farms, Woerden, The Netherlands) and tap water. Rats (mean body weight 390 g) were anaesthetized by isofurane and N_2O/O_2, and the liver, lungs, kidneys and the small intestine were excized and placed in ice-cold Krebs–Henseleit, containing 10 mM Hepes and 25 mM glucose (pH 7.4). The intestines were flushed with ice-cold Krebs–Henseleit buffer to remove the contents, directly after excision, and all organs were weighed after removal of tissue (table 4). The trachea (rat) or the biggest bronchiole available (human) were cannulated and lungs instilled with 1.5% (w/v) low melting agarose solution containing 0.9% (w/v) NaCl at 37°C and allowed to gel in UW on ice. Small pieces of each organ were cut off before the organs were excized and snap-frozen to determine *in vivo* ATP levels as described below.

Preparation of slices

Tissue cylinders from liver, agarose-filled lung and kidney were prepared with a coring tool (i.d. 8 mm) (Alabama R&D, Munford, AL, USA) attached to a drilling machine with a variable rotation speed. The organ cylinders were stored in ice-cold UW until slicing (maximum 1 h).

Intestinal slices were punched out of the intestine with an 8 mm (human) or 3 mm (rat) biopsy core. After (human) or before (rat) coring the mucosa was carefully separated from the muscle layer and only the mucosa (upper 15 cm of the jejunum) was used for incubation. Precision-cut liver, lung and kidney slices were prepared using a Krumdieck tissue slicer (Alabama R&D) precooled and filled with oxygenated, ice-cold Krebs–Henseleit buffer. Kidney slices were derived mainly from cortex tissue. All slices were stored in ice-cold UW until incubation (time gap between coring and incubation: maximum 2 h).

Incubation of slices

Slices were incubated in 3.2 ml Williams' medium E, which was prewarmed and gassed with 95% O_2/5% CO_2 and supplemented with glucose (final concentration 25 mM) and for liver and lung slices gentamicin (50 $\mu g\,ml^{-1}$) was added. For kidney slices, penicillin (100 $U\,ml^{-1}$) plus streptomycin (100 $\mu g\,ml^{-1}$) was used, while for intestine slices gentamicin (50 $\mu g\,ml^{-1}$) and amphotericin B (2.5 $\mu g\,ml^{-1}$) was added, agents that are poorly metabolized and therefore considered not to interfere with biotransformation enzymes. Slices were individually incubated in six-well culture plates, which were placed in a plastic container, continuously gassed with humidified 95% O_2/5% CO_2, and shaken back and forth (90 times min^{-1}) in a cabinet at 37°C.

Viability of slices

The viability of the slices before and during incubation was determined by measurement of ATP content of individual slices. For this purpose, slices were placed in 1 ml 70% ethanol (v/v) containing 2 mM EDTA (pH 10.9) and snap-frozen in liquid nitrogen. After storage at −80°C and homogenization by sonication, ATP extracts were diluted 10 times with 0.1 M Tris HCl/2 mM EDTA solution (pH 7.8) buffer to lower the ethanol concentration. The ATP content was measured using ATP Bioluminescence Assay Kit CLS II from Roche (Mannheim, Germany) and a 96-well Lucy1 luminometer (Anthos, Durham, NC, USA) against an ATP-calibration curve.

Metabolic activity of slices

Metabolism of lidocaine (5 mM), TST (0.25 mM), 7-EC (0.5 mM) and 7-HC (0.5 mM) was studied by the addition of 32 µl stock-solution (to 3.2 ml Williams' medium E) in water (lidocaine), or methanol (TST, 7-EC, 7-HC). Metabolism was studied for 3 h of incubation, except in the case of rat liver slices incubated with TST, which was performed for 15 min to circumvent considerable substrate depletion. Medium samples (1 ml) of 7-EC incubations were acidified by directly adding 10 µl 2 M HCl after sampling, in order to prevent spontaneous formation of metabolites (if the pH of the incubation > 8.0). Medium incubated for 3 h with substrates, but without slices, served as controls and showed the absence of the particular metabolites (data not shown). Lidocaine, 7-EC and 7-HC medium samples were stored at −20°C until analysis. Preliminary experiments showed that no significant amounts of metabolites were retained in the slices (data not shown). After thawing, sodium azide was added to inhibit bacterial contamination (final concentration 0.1 $mg\,ml^{-1}$) and 50 µl of the centrifuged sample was analysed using HPLC as described above for the MEGX metabolite of lidocaine (Bargetzi *et al.* 1989) and for the metabolites of 7-EC (Walsh *et al.* 1995). For the metabolic biotransformation of TST, slices and incubation medium were harvested together and homogenized using sonication to extract the metabolites from the slice. Control experiments showed that metabolites of TST were significantly retained in

the slices (about 1.5 times the amount in medium, although metabolite profiles were similar) at the concentration used (data not shown). The homogenates were stored at −20°C. After thawing, 5 µl 11β-TOH, dissolved in methanol, was added as internal standard to 1 ml homogenate and 6 ml dichloromethane was then added. After removal of the water phase and protein interphase, the organic phase was evaporated and TST and its metabolites were dissolved in 130 µl 50% (v/v) methanol which was analysed using HPLC (van 't Klooster *et al.* 1993).

Protein content of slices

After incubation, five slices from each organ were taken and homogenized in their own incubation medium by sonication and diluted with 0.1 M NaOH. The protein content of the diluted homogenate was determined using the Bio-Rad protein assay dye reagent (Bio-Rad, Munich, Germany) against a BSA standard curve. Together with the wet weight of the slices, the percentage protein in the slices was determined (table 4). All metabolic activities and ATP levels are expressed as µg protein^{-1}.

Mass spectrometry

Samples were injected on to an Alltech Alltima C_{18} column (150 × 2.1 mm, 5 µm, Alltech Associates, Deerfield, IL, USA). Two series 200 micro-LC pumps (Perkin Elmer, Norwalk, CT, USA) delivered a gradient starting at 15% acetonitrile and 85% ammonium acetate (10 mM). One minute after the injection, the acetonitrile concentration was then linearly increased to 95% during 15 min. The system was operated at room temperature at 0.2 ml min^{-1}. The HPLC system was connected on-line with an API 3000 triple quadrupole mass spectrometer (PE-Sciex, Concord, Ontario, Canada) equipped with a TurboIonSpray interface. The conditions were set for negative ions, and full-scan spectra or product ion scan spectra were taken continuously. The MS was operated at such a low orifice voltage that 'up front' collision induced dissociation did not take place.

Results

Viability

To determine the relative viability of tissue slices in culture, ATP levels were assessed directly after slicing and after 1, 3 and 24 h of incubation (figure 1). ATP levels in both human and rat liver, lung and kidney slices showed an increase during 3 h of incubation and after 3 h a plateau level was reached, which was maintained during the incubation period (up to 24 h). However, the ATP level of intestine slices was lower than that in liver, lung and kidney slices, and did not increase during incubation.

To compare ATP results with *in vivo* levels, we determined the ATP content of small sections of organs taken from anaesthetized rats just before excizing the organs (table 1). Remarkably, ATP levels in rat liver, kidney and lung slices were higher (figure 1) than the *in vivo* levels (table 1). In contrast, ATP levels in intestine slices were the same as *in vivo* levels.

Metabolism studies

To determine the metabolic capacity of slices, incubations were performed with lidocaine, TST, 7-EC and 7-HC and the formation of metabolites was analysed. In this study, the *N*-deethylated metabolite, MEGX, of lidocaine was quantified. From table 2, it can be concluded that both rat and human liver slices have the highest capacity to form MEGX when compared with other organs. Rat liver shows a threefold higher formation rate of MEGX when compared with human slices.

As shown in table 3, a substantial organ-specific metabolic pattern of TST was observed for both rat and human slices. Human liver slices mainly formed 6β-TOH and androstenedione, whereas in human lung, kidney and intestine slices,

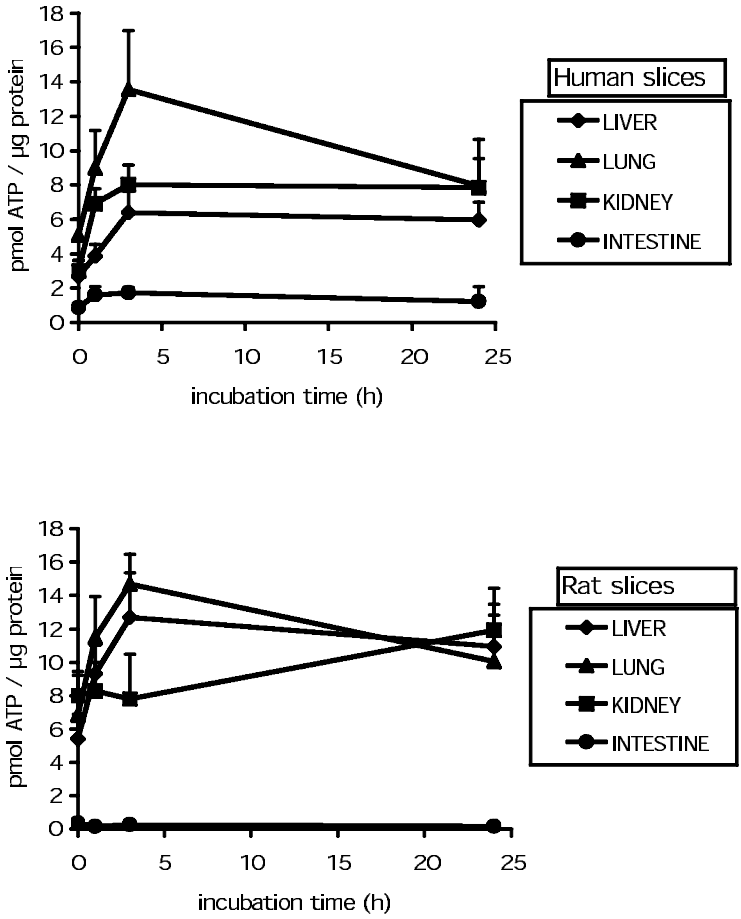

Figure 1. ATP levels in liver, lung, kidney and intestinal slices during 24 h of incubation (each time point representing the mean ± SEM of at least three organs, and three slices per organ).

Table 1. *In vivo* ATP levels in rat tissues.

Rat organ	ATP level[a]
Liver	1.81 ± 0.96
Lung	3.21 ± 0.15
Kidney	1.01 ± 0.41
Intestine	1.85 ± 0.88

[a] pmol ATP/µg protein
Data shown are the mean ± SEM from four organs.

mainly androstenedione was formed. Rat liver slices formed several metabolites, including 2α-TOH, which was not formed by human slices, and showed the highest activity of TST metabolism when compared with lung, kidney or intestine slices (15, 14 and 25% of rat liver slices, respectively). In addition, when regarding human organ slices, liver slices were most active but this was less pronounced than for rat organ slices (45, 63 and 61% of human liver slices, respectively).

Table 2. Metabolism of lidocaine in tissue slices.

MEGX formation[a]	*Human slices*	Rat slices
Liver	109 ± 16	363 ± 62
Lung	48 ± 24	25 ± 3
Kidney	6 ± 2	4 ± 3
Intestine	59 ± 30	71 ± 19

[a] pmol MEGX/3 h/μg protein.

Data are the means of at least four organs ± SEM, and three slices per organ.

Table 3a. Metabolism of testosterone in human tissue slices[a].

Metabolite	Human liver	Human lung	Human kidney	Human intestine
6α-TOH	0.8 ± 0.3	1.8 ± 1.6	n.d.	n.d.
6β-TOH	11.7 ± 1.7	3.9 ± 1.7	0.9 ± 0.2	0.8 ± 0.7
7α-TOH	1.3 ± 0.6	n.d.	n.d.	n.d.
15α-TOH	1.3 ± 0.8	n.d.	n.d.	0.4 ± 0.3
19-TOH	0.5 ± 0.2	n.d.	0.4 ± 0.2	n.d.
16α-TOH	0.9 ± 0.4	n.d.	0.5 ± 0.3	0.3 ± 0.3
16β-TOH	1.0 ± 0.5	0.2 ± 0.2	0.0 ± 0.0	n.d.
2α-TOH	0.1 ± 0.0	n.d.	0.2 ± 0.2	n.d.
2β-TOH	4.4 ± 1.5	n.d.	2.2 ± 2.2	0.2 ± 0.2
Androstenedione	40.1 ± 6.9	22.0 ± 13.4	34.6 ± 5.6	35.6 ± 15.6
Total	62.1 ± 12.8	27.9 ± 10.3	38.9 ± 13.0	37.4 ± 17.0

[a] pmol metabolite/3h/μg protein.

n.d. not detected.

Data are the means of at least four organs ± SEM, and three slices per organ.

Table 3b. Metabolism of testosterone in rat tissue slices[a].

Metabolite	Rat liver	Rat lung	Rat kidney	Rat intestine
6α-TOH	n.d.	2.2 ± 1.8	0.5 ± 0.5	n.d.
6β-TOH	15.7 ± 5.9	2.2 ± 1.7	0.7 ± 0.4	5.8 ± 1.7
7α-TOH	n.d.	1.0 ± 1.0	n.d.	n.d.
15α-TOH	0.4 ± 0.4	1.0 ± 1.0	n.d.	n.d.
19-TOH	9.2 ± 3.0	3.6 ± 1.3	0.6 ± 0.6	n.d.
16α-TOH	28.8 ± 8.4	1.5 ± 1.4	0.6 ± 0.4	1.5 ± 1.2
16β-TOH	1.5 ± 1.5	1.5 ± 1.5	0.9 ± 0.9	3.1 ± 2.4
2α-TOH	22.6 ± 7.3	1.1 ± 0.9	1.5 ± 0.2	n.d.
2β-TOH	n.d.	1.0 ± 1.0	n.d.	1.8 ± 1.1
Androstenedione	97.2 ± 24.6	11.3 ± 1.0	19.5 ± 2.2	31.4 ± 7.2
Total	175.3 ± 51.1	26.3 ± 12.5	24.4 ± 5.1	43.7 ± 13.6

[a] pmol metabolite/3 h/μg protein.

n.d. not detected.

Data are the means of at least four organs ± SEM, and three slices per organ.

Unlike liver slices that formed predominantly 7-HC and the conjugate 7-HC GLUC from 7-EC, lung, kidney and intestine slices from both man and rat formed mainly an, as yet, unidentified metabolite (figure 2). Minor amounts of this metabolite were also present in incubations without slice when the medium was not acidified (see above). Usually, the pH of WE increases towards 8.5 when the CO_2 atmosphere was not present. Incubations without slices or, with slices but

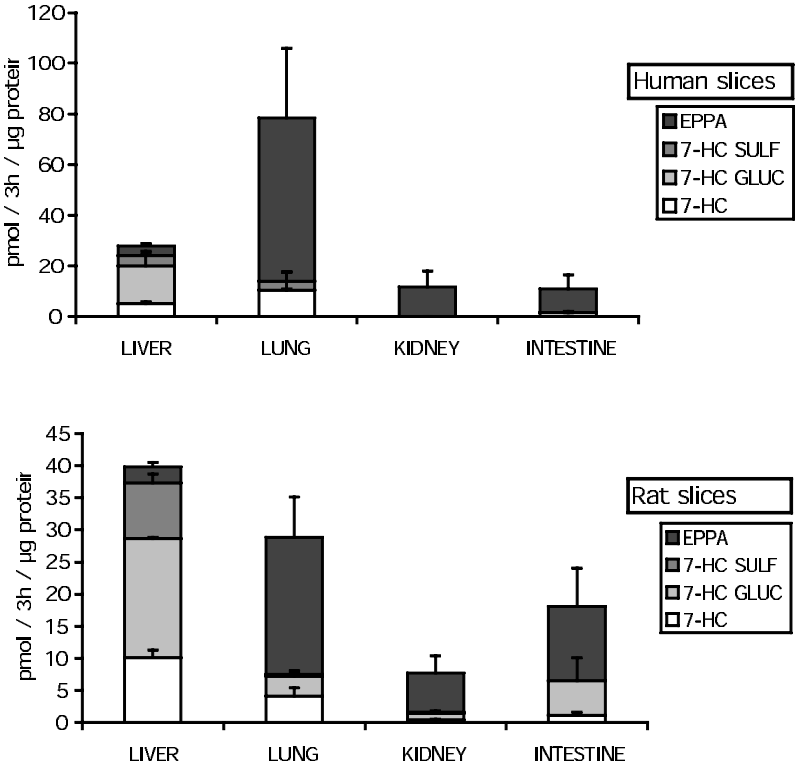

Figure 2. 7-EC (0.5 mM) metabolism during 3 h of incubation (data are means ± SEM of at least three organs, and three slices per organ).

performed at 4°C, showed no detectable formation of this metabolite when the medium was acidified after incubation.

To identify this metabolite, larger amounts of it were prepared by adding 5 µl 1M NaOH solution to 1 ml 0.5 mM solution of 7-EC in water (pH > 9). HPLC-MS analysis of this mixture showed mainly two molecular ions at m/z 207 $[M\text{-}H]^-$, with an empirical formula of $C_{11}H_{11}O_4$. A product ion scan of the $[M\text{-}H]^-$ ion at m/z 207 gave daughter ions at m/z 163, 133, 105 and 77, which are consistent with the loss of $[CO_2]$, $[C_2H_6]$, and twice [CO] from the parent ions. The structure of the metabolite was therefore presumed to be 4-ethoxy-2-hydroxyphenyl propionic acid (EPPA) (figure 3). This metabolite is in agreement with the finding that EPPA was not formed from 7-HC incubations (figure 4) and that similar additional HPLC peaks were formed when this experiment (increasing pH) was performed with 7-methoxycoumarin, analogous to 7-EC. The two peaks in the HPLC are thought to be the *cis* and *trans* forms of EPPA and its analogue formed from 7-methoxycoumarin, although this remains to be established.

To quantify the formation of EPPA we used a 7-EC standard curve, because the absorption coefficient at 320 nm appeared to be the same for EPPA and 7-EC, as is also the case for 7-HC and its conjugates (data not shown), which is in agreement with the preservation of the conjugated structure as depicted in figure 3.

Human and rat organ slices incubated with 7-HC at a concentration of 0.5 mm mainly formed 7-HC GLUC from 7-HC incubations, except human lung slices that produced only minor amounts of 7-HC SULF (figure 4). In contrast, rat lung

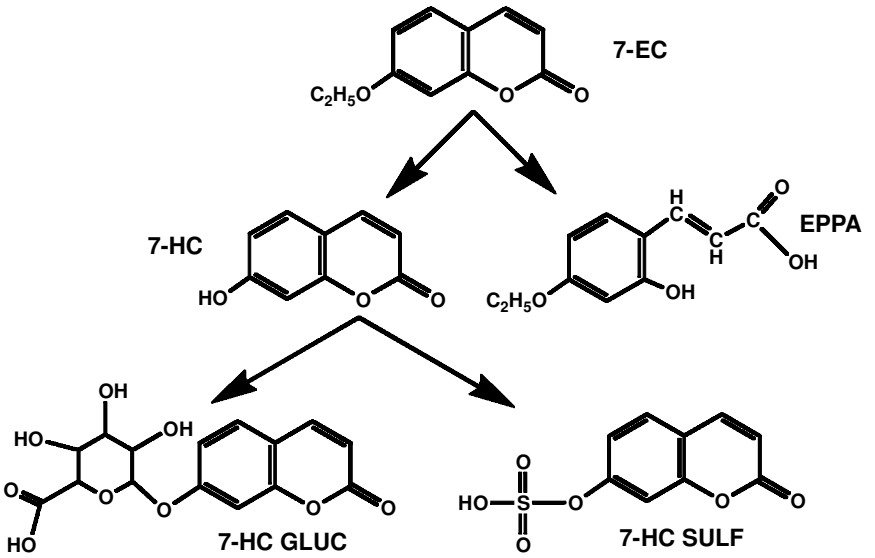

Figure 3. Metabolic pathway of 7-EC.

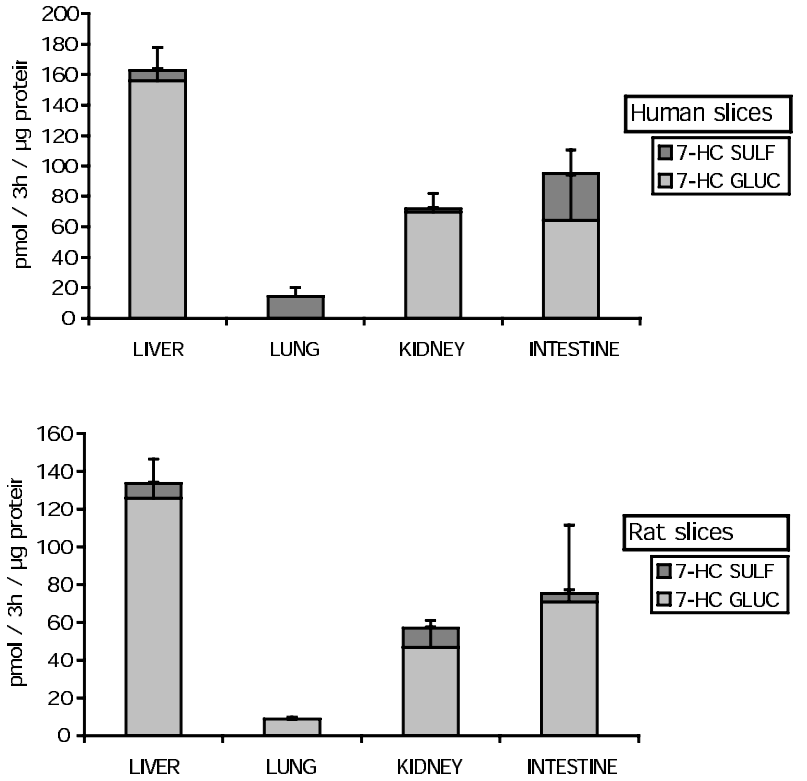

Figure 4. Phase II metabolism of 7-HC (0.5 mM), during 3 h of incubation (data are means ± SEM of at least four organs, and three slices per organ).

slices only produced some 7-HC GLUC. Both in man and rat, slices from kidney and intestine formed about equal quantities, of conjugate from 7-HC, that were about 60% of liver slices, when compared on a protein basis.

Discussion

The present study has shown for man and rat that the most important organs for drug metabolism (liver, lung, kidney, intestine) can be studied under similar conditions (preparation, handling, incubation) using tissue slices. Moreover, it has been shown that both phase I and II reactions can be studied simultaneously in tissue slices, being a major advantage above the use of microsomes. Metabolism in rat organs can also be studied simultaneously, using slices from the same animal. Tissue slices of human origin were made whenever tissue was available (normally one organ at a time). The period between explantation and slicing was much longer for liver (up to 36 h) than for the other human organs (maximum 2 h). It has been shown that this cold preservation time has no effects on ATP levels or metabolic capacity of liver slices (Olinga *et al*. 1998b).

We monitored tissue viability by measuring ATP levels in the slices. ATP concentrations in liver, lung and kidney slices increased during the first 3 h of incubation and were ultimately higher than the *in vivo* levels, as was shown for rat liver slices (Chapman *et al*. 1994), and then remained constant (figure 1 and table 1). However, it should be noted that it is very difficult to determine actual *in vivo* levels of ATP due to its rapid breakdown, even in small tissue samples (Faupel *et al*. 1972). As far as we know, the ATP content of human liver, lung, kidney or intestine slices has not been previously reported. However, another measure of viability, potassium content has been determined in human liver slices but it decreased during incubation (VandenBranden *et al*. 1998). Literature data on rat liver ATP levels (Wang *et al*. 1999), lung (Monteil *et al*. 1999) and kidney slices (Obatomi and Bach 2000) are in the same range as described herein. No data on ATP levels from rat intestine slices could be retrieved from literature. ATP levels from both rat and human intestine slices decreased slightly with time, unlike the slices from other organs. Therefore, it is concluded that for slices from liver, lung and kidney, viability is sufficient for our purposes. However, for intestine slices this might not be the case because ATP levels are quite low and did not increase with time of incubation (figure 1). Current research in our laboratory is ongoing to improve intestinal ATP levels and to study viability more extensively by examination of the morphology of the slices. Nevertheless, the results show that despite this relatively low viability, metabolic activity is remarkably high.

In the current study, the *N*-deethylated metabolite of lidocaine, MEGX, has been quantified. At the concentration used (5 mM), MEGX is by far the major metabolite both in rat liver (Hoke and Ravis 1991), rat lung and kidney (Tanaka *et al*. 1994) and man liver slices (Parker *et al*. 1996), although other metabolites have been reported at lower concentrations. It can be concluded from table 2 that liver slices have the highest capacity in both rat and man to metabolize lidocaine, although extrahepatic metabolism of lidocaine does takes place, which is in agreement with *in vivo* results in man (Sallie *et al*. 1992).

The current study analysed the phase I products of TST by HPLC. It was found that in human liver slices, 6β-TOH accounted for 19% of total metabolism. It was stated that 6β-TOH accounted for 75–80% of all metabolites in human liver

microsomes (Draper *et al.* 1998). This difference may be explained because only cytochrome P450-catalysed reactions were considered in the latter study using microsomes, in contrast to the circumstances in the present study, using human liver slices.

Apart from metabolism of TST towards TOHs by CYPs as discussed by de Kanter *et al.* (1999), TST can be metabolized by 17β-hydroxysteroid dehydrogenase, forming androstenedione (Farthing *et al.* 1981, 1982). Because we observed mainly androstenedione formation in lung, kidney and intestine slices (and only minor amounts of TOHs) (table 3), together with the finding that this enzyme is found in the small intestine (Farthing *et al.* 1981, 1982), it is possible that 17β-hydroxysteroid dehydrogenase is involved in the formation of androstenedione in the intestine, and possibly in lung and kidney too.

Many reports in the literature describe only three metabolites of 7-EC, namely 7-HC, 7-HC GLUC and 7-HC SULF. The main reason for this is that 7-HC and 7-HC conjugates (after enzymatic deconjugation) are often analysed by fluorescence (Rogiers *et al.* 1986). As shown in figure 3, these three metabolites are indeed the most abundant metabolites of 7-EC formed by liver slices from both rat and man. However, the main metabolite in lung, kidney and intestine slices appear to be EPPA, a metabolite that has not been described until now. Unidentified metabolites of 7-EC have been previously reported *in vivo* (Ritschel and Hardt 1983), in rat liver slices (Price *et al.* 1995, Walsh *et al.* 1995, Ball *et al.* 1996) and in rat microsomes (Jung *et al.* 1985). The latter study reported that 3-hydroxylation of 7-EC took place. By comparing the effect of deconjugation of rat liver slice metabolites, Ball *et al.* (1996) reported that out of 11 unidentified metabolites, three are glucuronic acid conjugates and four sulphate conjugates. A study on guinea pig and dog liver slices reported that ring opening of 7-EC can also take place, forming 4-ethoxy-2-hydroxyphenylacetic acid (EHPA) (Terada *et al.* 1996). However, EHPA differs from EPPA with respect to the length of the carboxyl moiety. In addition, EPPA appears to have the same absorption coefficient at 320 nm as 7-EC (and as 7-HC and its conjugates), unlike EHPA (Terada *et al.* 1996), most likely because of the difference between the structures.

We studied metabolism using relatively high substrate concentrations (0.25 mM for TST, 0.5 mM for 7-EC and 7-HC, 5 mM for lidocaine). In fact, these concentrations are probably sufficient to saturate the enzyme systems involved, implying that we actually measured V_{max} (capacity) rates. This opened the possibility to compare these V_{max} found *in vitro* with *in vivo* values. It has been reported in several studies that V_{max} and clearances were lower in slices when compared with isolated hepatocytes, especially when high clearance drugs such as 7-EC are considered (Ekins *et al.* 1995, Worboys *et al.* 1996a, b, 1997, Houston and Carlile 1997, Carlile *et al.* 1999). However, the rate of metabolism of lidocaine, which also results in a relatively high clearance, was similar for human liver slices and isolated hepatocytes (Olinga *et al.* 1993, 1998b). Moreover, even higher values have been reported for the metabolism of biphenyl using human and dog (but not rat) liver slices compared with isolated hepatocytes (Powis *et al.* 1989). Apart from species and substrate differences herein, we believe that it is difficult to compare slices and cells quantitatively. When normalizing to wet weight, adhering water may cause an underestimation of slices and this may also be the case for dry weight or protein content, because damaged cells on the cutting edges will contribute to

Table 4. *In vivo* scaling factors of rat slices.

Rat organ	Organ weight (g)	Percentage protein in slices[a]	Scaling factor (×1000)[b]
Liver	15.3	10.6	1631
Lung (agarose filled)	6.9	1.1	78
Kidney	2.9	9.3	268
Small intestine	7.2	12.0	865

[a] Determined through the wet weight and protein content of the slices.
[b] Calculated by multiplying the organ weight with the percentage slice protein.
Data are the means of 6 organs ± SEM (of each, organ five slices were determined).

the weight or protein content but are not metabolically active. In the case of isolated cells, damaged cells are simply removed by (Percoll) centrifugation.

An alternative approach to establish if metabolism rates in slices reflect *in vivo* values correctly is to compare the metabolic capacity of slices with *in vivo* values. The measured metabolic rates, which are expressed μg protein, can be scaled to the metabolic rates per μg organ using the protein percentage in slices and subsequently scaled to the total organ value by extrapolation to total organ weight. This scaling results in the scaling factors as indicated in table 4. In the present case, when the sum of the four measured metabolites of 7-EC from rat liver slices are taken from figure 2, and multiplied with the scaling factor from table 4, and corrected for rat weight, a $V_{max} = 0.42\,\mu mol\,min^{-1}\,SRW^{-1}$ (Standard Rat Weight of 250 g) for 7-EC metabolism in rat liver is derived. This value may largely underestimate the total rate of 7-EC metabolism because other organs than the liver contribute significantly (figure 2). When a similar calculation is made for lung, kidney and intestine, by summing all the metabolites from figure 2 of each organ, and multiplying by the scaling factors from table 4 for each organ, the following V_{max} are derived: lung, 0.01, kidney 0.01 and intestine 0.10 μmol $SRW^{-1}\,min^{-1}$.

The sum of V_{max} of liver, lung, kidney and intestine then gives a $V_{max} = 0.51\,\mu mol\,SRW^{-1}\,min^{-1}$, which correlates well for the reported V_{max} *in vivo*, namely 0.49 $mol\,SRW^{-1}\,min^{-1}$ (Carlile *et al*. 1998). Therefore, it is concluded that in the case of 7-EC metabolism, the use of slices reflects the *in vivo* metabolizing capacity correctly. Clearly, other organs than the liver contribute significantly to the total metabolism of the test compound.

Conclusions

It was reported (de Kanter *et al*. 1999) that using slices, it was possible to study xenobiotic metabolism, not only in liver, but also in lung and kidney from both rat and man, under similar experimental conditions. The present study has additionally examined intestinal slices and shown that, apart from the liver, lung and kidney, the intestine can also contribute considerably to the overall capacity of xenobiotic metabolism in the body of both rat and man. As shown, it is also possible to compare the results of slices (after up-scaling) to the *in vivo* situation.

This technology may lead to a more adequate choice of animal species used during drug development and can result in a considerable reduction in the use of experimental animals.

The use of a combination of liver, lung, kidney and intestine slices might be appropriate for most substrates because the most important organs involved in metabolism are included. Nevertheless, in a minority of cases significant drug metabolism takes also place in skin, nasal mucosa or other tissue and this aspect should not be neglected.

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