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Organ Slices as An *In Vitro* Test System for Drug Metabolism in Human Liver, Lung and Kidney

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Abstract—Metabolism of xenobiotics occurs mainly in the liver, but in addition, the lungs and kidneys may contribute considerably. The choice of the animal species during drug development as a predictive model for the human condition is often inadequate due to large interspecies differences. Therefore, a universal method for the preparation and incubation of human and animal liver, lung and kidney tissue is being developed for drug metabolism and toxicity testing using precision-cut organ slices. Human tissue was obtained from surgical waste material. Slices were made from rat and human liver, kidney and agar-filled (1.5%, w/v) lung tissue using a Krumdieck tissue slicer and incubated in six-well plates. The morphology and the ATP content show that viability is maintained during 3 hours of incubation. These organ slices show a variety of phase I (hydroxylation, oxidation and *O*- and *N*-deethylation) and phase II (glucuronidation and sulfation) metabolic routes using lidocaine, testosterone, 7-ethoxycoumarin and 7-hydroxycoumarin as substrates. The metabolic patterns and rates were found to be different for the various organs and species studied. The use of human tissue slices will enable us to collect more human-specific data on drug metabolism and toxicity. This may lead to a more adequate choice of animal species used during drug development and will result in a considerable reduction in the use of experimental animals.

Keywords: slices; liver; lung; kidney; human tissue; drug metabolism; species differences.

Abbreviations: 7-EC = 7-ethoxycoumarin, 7-HC = 7-hydroxycoumarin; 7hc-GLUC = 7-hydroxycoumarin glucuronide, 7hc-SULF = 7-hydroxycoumarin sulfate; CYP = cytochromes *P*-450, MEGX = monoethylglycinexylidide; UW = University of Wisconsin organ preservation solution.

INTRODUCTION

Metabolism of many xenobiotics, including drugs and toxic compounds, occurs mainly in the liver. Therefore, until now, *in vitro* studies on metabolism of xenobiotics were usually performed using liver preparations, such as microsomes, isolated hepatocytes, slices or isolated perfused livers. It becomes, however, increasingly evident that other organs, such as lung, kidney and intestine can also contribute considerably to the metabolism of xenobiotics. This may not only be important for the clearance of drugs from the body, but may also result in organ-selective toxicity. During drug development, animal experiments are obligatory to study metabolism and toxicity before administration to humans is allowed. Usually the rat and the dog are selected in the first instance. Also, most of the fundamental research on drug metabolism and research is performed in the rat. From the presently collected research data, however, it becomes increasingly clear that the differences in metabolic profiles and toxic reactions between species can be quite large. These differences include not only the *kind* of metabolites that are formed, but also concern the contribution of the various organs in the body in the process of clearance and toxicity. These species differences appear to be almost specific for the compound under study, and despite extensive research, structure–metabolic profile relations are difficult to establish. In particular, extrapolation of results obtained in animal experiments to the human situation is precarious.

Therefore, it is of the utmost importance to develop an *in vitro* test system with human and animal tissue to predict organ-specific drug metabolism and toxicity in man in an early phase of drug development, and to establish possible species differences therein. The development of the technique of preparation (Krumdieck *et al.*, 1980) and use of organ slices from liver (Smith *et al.*, 1985), kidney (Ruegg *et al.*, 1987) and lung (Stefaniak *et al.*, 1988) caused

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an increasing use of animal and human liver slices in studies on drug metabolism and toxicity. This technique is generally accepted as a good model to obtain human-specific data (Bach *et al.*, 1996; Olinga *et al.*, 1998a; Parrish *et al.*, 1995; Vickers, 1994). Slices are relatively easy to prepare and they remain viable for at least 24 hr. Recently, even maintenance of viability and drug metabolic capacity of liver slices up to 3–4 days was reported, enabling studies on induction and toxicity (Gokhale *et al.*, 1997; Price *et al.*, 1998). Moreover, human and animal liver slices can be cryopreserved with maintenance of their biotransformation capacity for at least 3 hr after thawing (de Kanter and Koster, 1995; de Kanter *et al.*, 1998).

Kidney slices have also been used extensively for toxicity and uptake studies, (for a review, see McGuinness *et al.*, 1993); however, human kidney tissue is used less frequently for drug metabolism studies (Connors *et al.*, 1996; Vickers, 1994). Kidney slices can be prepared from cortex and medulla selectively (Ruegg *et al.*, 1987), enabling the study of regioselective drug metabolism, which is important for the prediction of drug–drug interactions. Also, lung slices have been used successfully in metabolic and toxicity studies (Fisher *et al.*, 1994; Vickers *et al.*, 1997). The preparation of lung slices necessitates the infusion of low melting agar to give the tissue enough solidity to enable slicing (Stefaniak *et al.*, 1988).

In this paper we describe initial experiments aimed to develop a test system with human and rat liver, lung and kidney slices to identify species and organ specific metabolism. We prepared slices from human tissue that was obtained as surgical waste resected from tissue obtained from cancer patients during surgery. The viability of these slices during incubation is determined by ATP content and morphology. These parameters are generally considered as being the most sensitive viability parameters (Blackmore *et al.*, 1997; Gokhale *et al.*, 1997; Vickers *et al.*, 1997). Moreover, we assessed the metabolic rate and pattern of four model substrates: lidocaine, testosterone, 7-ethoxycoumarin (7-EC) and 7-hydroxycoumarin (7-HC). These substrates are selected because they have been used before to characterize metabolic capacity of human liver slices since they show a variety of phase I (hydroxylation, oxidation, *N*- and *O*-deethylation) and phase II (sulfation and glucuronidation) metabolic routes, and because a large number of cytochromes *P*-450 (CYP) isoenzymes is involved (see Discussion). This report describes some preliminary results on drug metabolism and viability, using rat and human liver, kidney and lung slices.

MATERIALS AND METHODS

Materials

The following compounds were obtained from the sources indicated: lidocaine from Centrachemie (Etten-Leur, The Netherlands); 7-EC and testosterone from Fluka (Buchs, Switzerland); 6β-, 16α-, 11β-hydroxytestosterone, androstenedione, and 7-HC from Sigma (St Louis, MO, USA); University of Wisconsin organ preservation solution (UW) from Du Pont Critical Care (Waukegan, IL, USA); Williams' medium E (with Glutamax-I) from Gibco BRL (Paisley, UK). Monoethylglycinexylidide (MEGX) was a kind gift of ASTRA (Södertälje, Sweden) and 7-hydroxycoumarin glucuronide (7hc-GLUC) and 7-hydroxycoumarin sulfate (7hc-SULF) were kind gifts from Mr P. Mutch, Glaxo Wellcome Research and Development (Herts, UK). All other chemicals were of analytical grade and were obtained from commercial sources.

Organs

Human lung and kidney tissue was obtained from patients subjected to surgery for carcinoma. All lung donors were male (aged 56–82 yr). Kidney donors were all female (aged 49–63 yr). Tumour-free tissue that was considered as surgical waste was used. Human lung and kidney material was stored in ice-cold Krebs–Henseleit buffer (Olinga *et al.*, 1998b) until the start of the slicing procedure (total cold storage period: max 2 hr). Human liver tissue was obtained from redundant donor tissue after split liver transplantation that was perfused with ice-cold UW, as described previously (Olinga *et al.*, 1998b). Liver donors were both male and female (aged 7–69 yr). The research protocols were approved by the medical ethical committee and consent from the patients was obtained.

Animals

Male Wistar rats were housed in standard cages and had free access to food and tap water. Rats were anaesthetised by halothane, NO_2/O_2, and the liver, kidneys and lungs were excised and placed in UW on ice.

Preparation of slices

The lung trachea (rat) or the biggest bronchiole available (human) was cannulated and lungs were instilled with 1.5% (w/v) low melting agarose solution containing 0.9% (w/v) NaCl at 37°C and allowed to gel on ice. Tissue cylinders from liver, kidney and lung were prepared with an 8 mm diameter motor-driven coring tool as described before for liver (Smith *et al.*, 1985) and kidney (Ruegg *et al.*, 1987). From the cylinders, precision-cut organ slices were prepared as described (Smith *et al.*, 1985). Kidney slices were prepared perpendicular to the cortical–papillary axis to distinct cortex and medulla derived slices (Ruegg *et al.*, 1987). The wet

weight of liver and kidney slices was 15–20 mg (thickness 270–360 μm), lung slices were 20–25 mg (thickness 360–450 μm). Slices were stored in ice-cold UW until incubation.

Incubation

Slices were individually submerged in 3.2 ml Williams' medium E in a well of a six-well culture plate, which was placed in a plastic container and gassed with humidified 95% O_2/5% CO_2, and shaken back and forth (90 times/min) in a cabinet at 37°C. Metabolism of lidocaine (5 mM), testosterone (0.25 mM), 7-EC (0.05 mM) and 7-HC (0.5 mM) was studied during 3 hr (lidocaine incubations with liver slices: 1 hr). Incubation, sampling, handling and analysis was performed as described earlier (de Kanter *et al*., 1998).

Viability

Slice ATP content was determined in homogenates [containing 70% methanol (v/v) and 2 mM EDTA], using ATP Bioluminescence Assay Kit CLS II from Boehringer Mannheim. Slices were fixed in 10% formalin for histopathological analysis. Paraffin sections (5 μm) were stained by haematoxylin and eosin staining.

RESULTS

Viability

Rat and human kidney cortex paraffin-embedded sections of slices incubated for 3 hr contained glomeruli, proximal and distal tubuli, whereas medulla slices lacked glomeruli and mainly collecting tubes and tubules of the loop of Henle were visible (not shown). There were no signs of damage in slices incubated for 3 hr compared with slices directly after slicing. Sections of lung slices exhibited open alveoli and small bronchioli, and no damage was seen after 3 hr of incubation of both rat and human slices (not shown).

ATP levels in lung slices were assessed directly after slicing and after 1, 3 (lung, 2–4), 15 and 24 hr (lung, 6) of incubation. ATP levels in lung slices showed a two- to fivefold increase during 3 hr of incubation (Fig. 1). After 3 hr, a plateau level is reached that was maintained during the incubation period (until 24 hr) in slices from human lung 6. After 3 hr, the ATP content of human kidney and liver slices increased in the same way to a plateau of 88 ± 28 pmol ATP/μg protein (six human kidneys) and 42 ± 18 pmol ATP/μg protein (two human livers).

Metabolism

Human lung and liver and rat lung, liver and kidney medulla slices metabolized lidocaine (5 mM) to an *N*-deethylated metabolite, monoethylglycinexylidide (MEGX). The data are shown in Fig. 2. No MEGX formation was found using rat kidney cortex slices.

Metabolites in rat lung and kidney cortex and medulla formed after 3 hr of incubation with 0.25 mM testosterone are given in Fig. 3(A), together with the levels of three of the metabolites found in liver slices. In rat liver slices also 2α-, 6α-, 7α-, 15α-, 16β- and 19-hydroxytestosterone were formed (not shown). In rat lung and kidney, mainly androstenedione was formed in addition to small amounts of 16α-hydroxytestosterone. The data from human lung (n = 1) and liver (n = 3) are given in Fig. 3(B). In the samples of the human lung, two extra peaks appeared in the HPLC chromatogram, which could not be identified with the standards used routinely. Apart from these two metabolites, human lung slices mainly formed androstenedione and small amounts of 16α-hydroxytestosterone, but no 6β-hydroxytestosterone, which is one of the main metabolites found in human liver. Preliminary data from slices from human kidney showed that the same metabolites were found as in human lung slices.

In Fig. 4, the data are given for the formation rate of 7-HC, 7hc-GLUC and 7hc-SULF after incubation for 3 hr with 0.05 mM 7-EC. Rat and human kidney and rat lung slices showed no detectable metabolic activity of 7-EC. In Fig. 5, the formation rate of 7hc-GLUC and 7hc-SULF after incubation

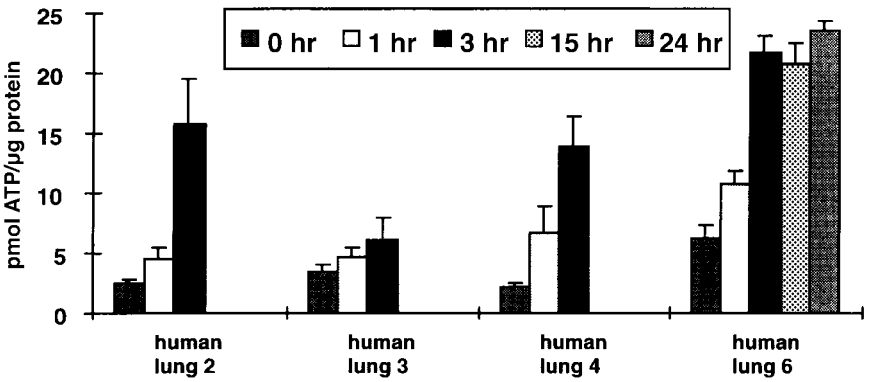

Fig. 1. ATP levels in lung slices of individual lungs during incubation (means of three slices $\pm$ SEM).

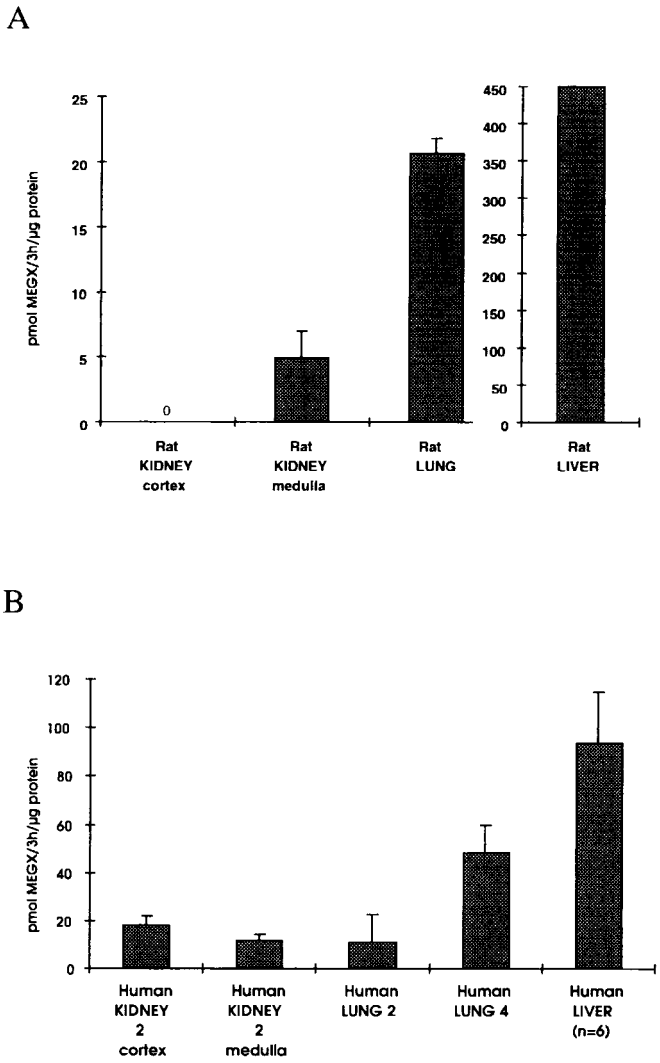

Fig. 2. Lidocaine (5 mM) metabolism during 3 hr of incubation towards MEGX is shown for both rat (n = 3, panel A) and human (panel B) organ slices (means of three slices ± SEM).

with 0.5 mM 7-HC. Human lung 6 and 7 showed no metabolic activity when incubated with 0.5 mM 7-HC.

DISCUSSION

The results of this study show that organ slices can be prepared successfully from the non-malignant parts of human surgical waste tissue of human lung and kidney cancer patients as was shown previously for liver (Olinga *et al.*, 1998b). They maintain good viability during incubation and can successfully be used for drug metabolism studies, since they perform a variety of phase I reactions, such as the *N*-deethylation of lidocaine, hydroxylation and oxidation of testosterone, and the *O*-deethylation of 7-EC. Moreover, all organs show phase II conjugation reactions: the sulfation and/or glucuronidation of 7-HC.

The data on morphology indicate that the slices remain viable for at least 3 hr of incubation. Lung slices showed increase in ATP levels up to 3 hr and maintenance of ATP levels for 24 hr (Fig. 1). This pattern of an initial recovery period followed by a constant plateau level of ATP was found earlier in lung (Vickers *et al.*, 1997), liver (Smith *et al.*, 1985) and kidney slices (Stubenitsky *et al.*, 1995), and is readily explained by resynthesis of ATP, lost during the cold storage period that is inevitable between excision of the organs and slicing. In a previous study we compared the viability of human liver slices obtained from surgical waste from cancer patients with those from donor tissue (Olinga *et al.*, 1998b). We concluded that slices prepared from liver tissue after partial hepatectomy have somewhat decreased metabolic capacity, probably due to the warm ischaemia during surgery, but are still useful to study drug metabolism patterns. In addition, our data on morphology and ATP content

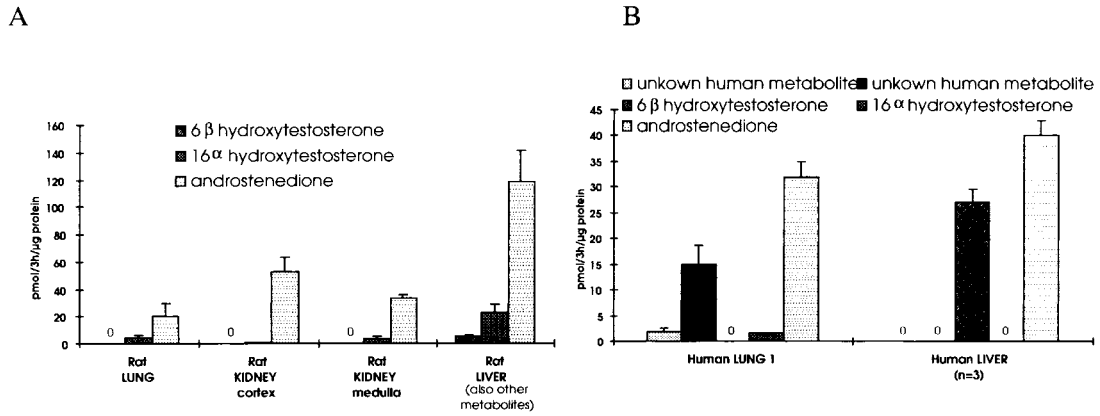

Fig. 3. Testosterone (0.25 mM) metabolism during 3 hr incubation towards hydroxytestosterone and androstenedione is shown for both rat (n = 1, panel A) and human (panel B) organ slices (means of three slices ± SEM).

in lung and kidney slices do not show severe impairment of viability.

With respect to drug metabolism, the data clearly show that the three different organs differ in their relative activities of the various biotransformation reactions, and in addition that clear interspecies differences are present.

Lidocaine metabolism in human lung amounts to about 50% of liver values when expressed per mg protein, whereas in rat lung the activity is only 5%

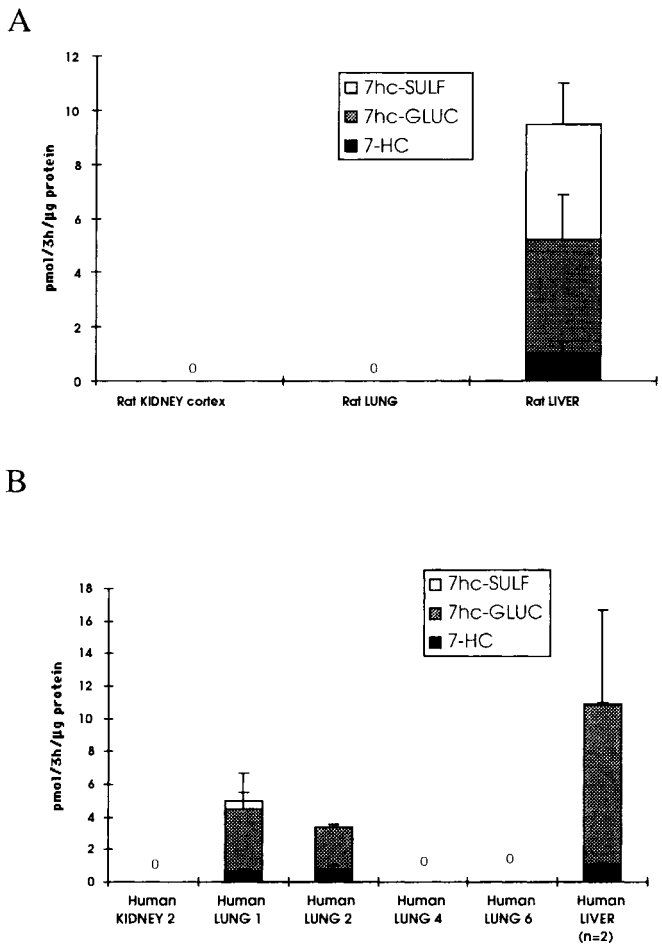

Fig. 4. 7-EC (0.05 mM) metabolism during 3 hr incubation towards 7-HC, 7hc-GLUC and 7hc-SULF is shown for both rat (n = 1, panel A) and human (panel B) organ slices (means of three slices ± SEM).

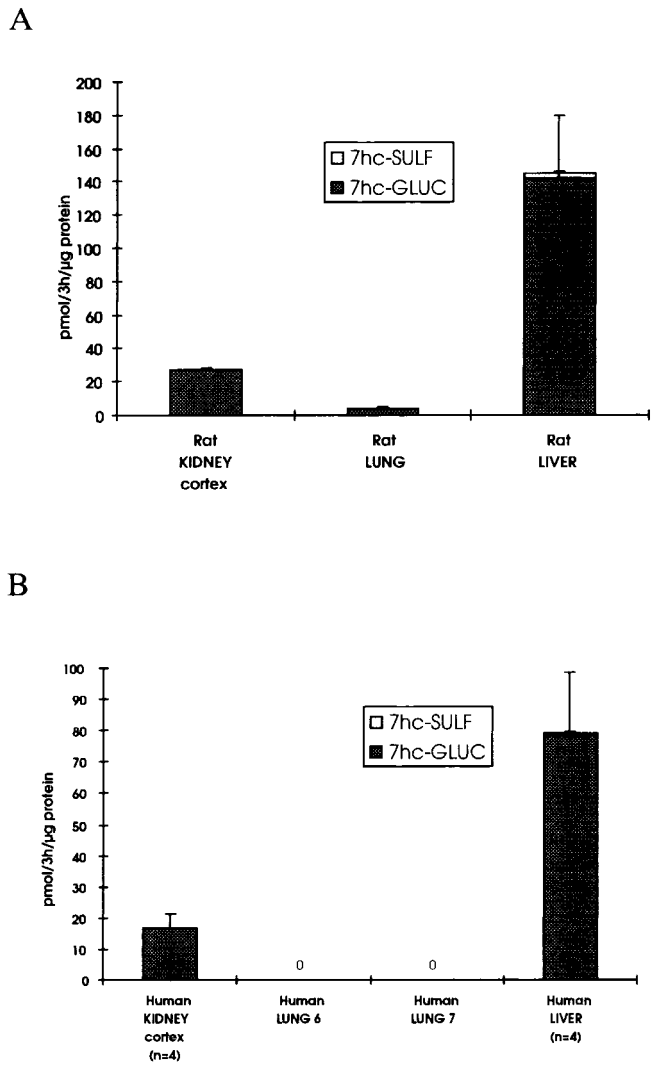

Fig. 5. 7-HC (0.5 mM) phase II metabolism during 3 hr incubation towards 7hc-GLUC and 7hc-SULF is shown for both rat (n = 1, panel A) and human (panel B) organ slices (means of three slices ± SEM).

of liver values. On a protein basis human lung slices have a twofold higher metabolic rate compared with rat lung slices, whereas human liver has only 20% of rat liver activity. CYP3A4 is responsible for lidocaine metabolism into MEGX in human liver (Sotaniemi *et al.*, 1995), and possibly also in human lung because CYP3A4 is found to be present in human lungs (Anttila *et al.*, 1997). In rat liver, MEGX is formed by CYP2C11 and CYP2B1, and possibly by CYP3A2 and CYP1A2 (Imaoka *et al.*, 1990), while in rat lung and kidney CYP2B1 is involved (Tanaka *et al.*, 1994). Surprisingly, rat renal cortex slices did not form detectable amounts of MEGX, whereas significant amounts of MEGX were formed in medulla slices. Apparently the isoenzyme responsible is not present in the cortex. Renal cortical slices from the same rats did show testosterone and 7–HC metabolism, indicating that the lack of MEGX formation is not due to lack of viability.

Striking differences both quantitatively and qualitatively were found between human and rat organs with respect to testosterone metabolism. In human lung slices, two unidentified metabolites were detected that were not found in human liver slices nor in any of the rat organs. Human liver slices mainly form 6β-hydroxytestosterone and androstenedione, and minor amounts of 2β-hydroxytestosterone whereas rat liver slices form relatively little 6β-hydroxytestosterone but produce several other metabolites, among others androstenedione and 16α-hydroxytestosterone. In human liver, the known CYP isoforms involved in testosterone metabolism are CYP3A3/4/5 (formation of 2β- and 6β-hydroxytestosterone) and CYP2C9/19 (androstenedione formation) (Waxman *et al.*, 1991;

Yamazaki and Shimada, 1997), while CYP4B1, which is present in human lung but not in human liver, is found to be able to catalyse 6β-hydroxytestosterone formation (Waxman *et al.*, 1991). However, we did not find 6β-hydroxytestosterone in human lung slice incubations.

In rat liver, many CYP isoenzymes are involved in testosterone metabolism, among them are the following CYP isoenzymes (formed hydroxytestosterones in parentheses): 1A1 (6β), 1A2 (6β), 2A1 (7α), 2A2 (6β, 7α), 2A4/5 (7α), 2B1 (6β, 16α, 16β, androstenedione), 2B2 (16α, androstenedione), 2C7 (2α, 16α, 7α), 2C11 (2α, 16α, androstenedione), 2C13 (6β), 3A1 (6β, 15β), 3A2 (6β), 3A4 (15β) (Gokhale *et al.*, 1997; Yamazaki and Shimada, 1997), and references therein.

The main CYP isoforms in rat lung are CYP2B1 and CYP4B1 (Hoet *et al.*, 1997). Probably CYP2B1 is involved in the formation of the main metabolite, androstenedione, in rat and human lung, as it is in liver. This might be the case in kidney too, where the same metabolic pattern is observed as in lung. Quantitatively similar amounts of testosterone metabolites were found in human liver and lung slices, whereas in rat lung slices metabolism of testosterone is only 20% of that in liver. The formation rate of the main metabolite, androstenedione, in human liver is 30% of that in rat liver. The contribution of kidney cortex is somewhat higher than of the medulla, the lung slices exhibit the lowest activity.

When slices are incubated with 0.05 mM 7-EC, integrated phase I and phase II metabolism takes place: *O*-deethylation to 7-HC, followed by glucuronidation and sulfation of the newly formed hydroxylgroup. The total phase I metabolic rate (7-HC + 7hc-GLUC + 7hc-SULF) in human liver is about 50% of that in rat. In contrast, some human lungs show detectable 7-EC metabolic activity while rat lung slices do not. This is in accordance with data from human lung microsomes (Shimada *et al.*, 1996) but not with a report about rat lung slices, which did show metabolic activity of 7-EC (Price *et al.*, 1995). This might be explained by rat strain differences; Price *et al.* used Sprague–Dawley rats, whereas Wistar rats were used in the present study. Wheeler and Guenthner (1991) found that total CYP per mg microsomal protein in rat lung is 20 times higher than in human lung. This suggests that the specific isoenzymes involved (1A1/2 and 2E1; Shimada *et al.*, 1996) in *N*-deethylation of 7-EC is present in higher amounts in human than in rat lung. Rat liver was the only organ that formed appreciable amounts of sulfate conjugate. Rat lung and kidney cortex showed no detectable metabolism of 7-EC. In the human lungs that we tested, total phase I metabolism was present in two individuals, but absent in two other lungs. From the ATP data (Fig. 1) and the presence of MEGX formation in slices from lung 4, one can conclude that this was probably not due to a low viability but to interindividual variation.

When slices were incubated with 0.5 mM 7-HC, mainly glucuronides were formed, which can be explained by the much higher 7-HC concentration than during incubation with 7-EC and the much higher V_{max} for glucuronidation than for sulfation (Mulder *et al.*, 1984). Again, human liver slices show 50% lower activity than rat liver slices, and rat lung slices did not show any activity.

Taking all the data together, one can conclude that the contribution of the three organs in man and in rat shows a rather unpredictable pattern. The relative contribution of the three organs within one species is different per substrate and parallelism is hardly found between rat and human organs. Although the data are far from complete, one could only speculate that for the substrates tested, rat liver is more active than human liver, but that the reverse is true for lung: human lung is more active than rat lung. This supports the necessity of using human organ slices in order to predict human metabolism *in vivo*.

In conclusion, slices are a powerful tool to study human and organ specific metabolism *in vitro*. To determine the contribution of the three organs to the total body clearance, data on the intrinsic clearances per organ should be calculated using not only data on V_{max} and K_m of the various metabolic routes and the blood flow through the organs, but also on the net uptake rate of the substrates into the cells.

The use of human organ slices during drug development could result in a safer first administration to man and in a reduction in the number of experimental animals used.

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REFERENCES

Anttila S., Hukkanen J., Hakkola J., Stjernvall T., Beaune P., Edwards R. J., Boobis A. R., Pelkonen O. and Raunio H. (1997) Expression and localization of CYP3A4 and CYP3A5 in human lung. *American Journal of Respiratory Cell and Molecular Biology* **16,** 242–249.

Bach P. H., Vickers A. E. M., Fisher R., Baumann A., Brittebo E., Carlile D. J., Koster H. J., Lake B. G., Salmon F., Sawyer T. W. and Skibinski G. (1996) The use of tissue slices for pharmacotoxicology studies. The report and recommendations of ECVAM workshop 20. *ATLA* **24,** 893–923.

Blackmore M., Richardson J. C., Rhodes S. A., Patterson L., Spencer A. J. and Gray T. J. B. (1997) Rat renal cortical slices: maintenance of viability and use in *in vitro* nephrotoxicity testing. *Toxicology in Vitro* **11,** 723.

Connors M. S., Larrauri A., Dannecker R., Nufer R., Brendel K. and Vickers A. E. M. (1996) Biotransformation of a somatostatin analogue in precision-cut liver and kidney slices from rat, dog and man. *Xenobiotica* **26,** 133–141.

de Kanter R. and Koster H. J. (1995) Cryopreservation of rat and monkey liver slices. *ATLA* **23,** 653–665.

de Kanter R., Olinga P., Hof I., de Jager M., Verwillegen W. A., Slooff M. J. H., Koster H. J., Meijer D. K. F. and Groothuis G. M. M. (1998) A rapid and simple method for cryopreservation of human liver slices. *Xenobiotica* **28,** 225–234.

Fisher R. L., Smith M. S., Hasal S. J., Hasal K. S., Gandolfi A. J. and Brendel K. (1994) The use of human lung slices in toxicology. *Human and Experimental Toxicology* **13,** 466–471.

Gokhale M. S., Bunton T. E., Zurlo J. and Yager J. D. (1997) Cytochrome P450 isoenzyme activities in cultured rat and mouse liver slices. *Xenobiotica* **27,** 341–355.

Hoet P. H. M., Demedts M. and Nemery B. (1997) In vitro modulation of the P450 activities of hamster and human lung slices. *Cell Biology and Toxicology* **13,** 185–192.

Imaoka S., Enomoto K., Oda Y., Asada A., Fujimori M., Shimada T., Fujita S., Guengerich F. P. and Funae Y. (1990) Lidocaine metabolism by human cytochrome P-450s purified from hepatic microsomes: comparison of those with rat hepatic cytochrome P-450 s. *Journal of Pharmacology and Experimental Therapeutics* **255,** 1385–1391.

Krumdieck C. L., Dos Santon J.E. and Ho K-J. (1980) A new instrument for the rapid preparation of tissue slices. *Analytical Biochemistry* **104,** 118–123.

McGuinness S., Gandolfi A. J. and Brendel K. (1993) Use of renal slices and renal tubule suspensions for in vitro toxicity studies. *In Vitro Toxicology* **6,** 1–24.

Mulder G. J., Dawson J. R. and Pang K. S. (1984) Competition between sulphation and glucuronidation in the rat in vivo: enzyme kinetics and pharmacokinetics of conjugation. *Biochemical Society Transactions* **12,** 17–20.

Olinga P., Meijer D. K. F., Slooff M. J. H. and Groothuis G. M. M. (1998a) Liver slices in *in vitro* pharmacotoxicology with special reference to the use of human liver tissue. *Toxicology in Vitro* **12,** 77–100.

Olinga P., Merema M., Hof I. H., de Jong K. P., Slooff M. J. H., Meijer D. K. F. and Groothuis G. M. M. (1998b) Effect of human liver source on the functionality of isolated hepatocytes and liver slices. *Drug Metabolism and Disposition* **26,** 5–11.

Parrish A. R., Gandolfi A. J. and Brendel K. (1995) Precision-cut tissue slices: applications in pharmacology and toxicology. *Life Sciences* **57,** 1887–1901.

Price R. J., Ball S. E., Renwick A. B., Barton P. T., Beamand J. A. and Lake B. G. (1998) Use of precision-cut rat liver slices for studies of xenobiotic metabolism and toxicity: comparison of the Krumdieck and Brendel tissue slicers. *Xenobiotica* **28,** 361–371.

Price R. J., Renwick A. B., Beamand J. A., Esclangon F., Wield P. T., Walters D. G. and Lake B. G. (1995) Comparison of the metabolism of 7-ethoxycoumarin and coumarin in precision-cut rat liver and lung slices. *Food and Chemical Toxicology* **33,** 233–237.

Ruegg C. E., Gandolfi A. J., Nagle R. B., Krumdieck C. L. and Brendel K. (1987) Preparation of positional renal slices for study of cell-specific toxicity. *Journal of Pharmacological Methods* **17,** 111–123.

Shimada T., Yamazaki H., Mimura M., Wakamiya N., Ueng Y. F., Guengerich F. P. and Inui Y. (1996) Characterization of microsomal cytochrome P450 enzymes involved in the oxidation of xenobiotic chemicals in human fetal liver and adult lungs. *Drug Metabolism and Disposition* **24,** 515–522.

Smith P. F., Gandolfi A. J., Krumdieck C. L., Putnan C. W., Zukowski C. F., 3d, Davis W. M. and Brendel K. (1985) Dynamic organ culture of precision liver slices for in vitro toxicology. *Life Sciences* **36,** 1367–1375.

Stefaniak M. S., Gandolfi A. J. and Brendel K. (1988) Adult rat lung slices in dynamic organ culture: a new tool in pharmacology. *Proceedings of the Western Pharmacology Society* **31,** 149–151.

Stubenitsky B. M., Ametani M., Danielwicz R., Southard J. H. and Belzer F. O. (1995) Regeneration of ATP in kidney slices after warm ischemia and hypothermic preservation. *Transplant International* **8,** 293–297.

Tanaka K., Oda Y., Asada A., Fujimori M. and Funae Y. (1994) Metabolism of lidocaine by rat pulmonary cytochrome P450. *Biochemical Pharmacology* **47,** 1061–1066.

Vickers A. E., Jimenez R. M., Spaans M. C., Pflimlin V., Fisher R. L. and Brendel K. (1997) Human and rat lung biotransformation of cyclosporin A and its derivatives using slices and bronchial epithelial cells. *Drug Metabolism and Disposition* **25,** 873–880.

Vickers A. E. M. (1994) Use of human organ slices to evaluate the biotransformation and drug-induced side-effects of pharmaceuticals. *Cell Biology and Toxicology* **10,** 407–414.

Waxman D. J., Lapenson D. P., Aoyama T., Gelboin H. V., Gonzalez F. J. and Korzekwa K. (1991) Steroid hormone hydroxylase specificities of eleven cDNA-expressed human cytochrome P450s. *Archives of Biochemistry and Biophysics* **290,** 160–166.

Wheeler C. W. and Guenthner T. M. (1991) Cytochrome P-450-dependent metabolism of xenobiotics in human lung. *Journal of Biochemical Toxicology* **6,** 163–169.

Yamazaki H. and Shimada T. (1997) Progesterone and testosterone hydroxylation by cytochromes P450 2C19, 2C9, and 3A4 in human liver microsomes. *Archives of Biochemistry and Biophysics* **346,** 161–169.