University of Groningen

Human and rat organ slices

de Kanter, Ruben

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2002

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
de Kanter, R. (2002). *Human and rat organ slices: a tool to study drug metabolism and toxicity*. s.n.

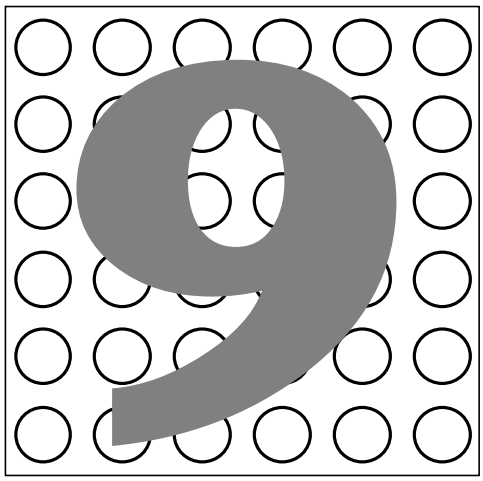
Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 12-10-2022



The use of human and rat slices from liver, lung, kidney and small intestine in toxicology

Ruben de Kanter
Marina de Jager
Annelies Draaisma
Dirk Meijer
Geny Groothuis

Groningen University Institute for Drug Exploration, Department of Pharmacokinetics and Drug Delivery, Groningen, Netherlands

Abstract

Precision-cut tissue slices have proven to be a useful in vitro system for biotransformation and toxicity studies. Since tissue slices can be readily prepared from a variety of tissues and species, they can easily be used for interspecies investigations and comparisons. Furthermore, tissue slices can also be readily and reproducibly prepared from human material. Thus human specific data can be obtained and comparisons (extrapolations) can be made between laboratory animals and humans.

The present study addresses the combined use of human and rat slices from liver, lung, kidney and intestine to test a number of model toxicants. Rat organ slices were exposed to paracetamol. Human liver, lung, kidney and intestinal slices were exposed to paracetamol (acetaminophen), paraquat, cephaloridine and flurbiprofen, respectively. We used the ATP content of the slices after 24 h of incubation as endpoint to determine viability after exposure.

After incubation with paracetamol, rat liver slices exhibited dose dependent toxicity whereas lung, kidney and intestine slices did not show the clearly decreased ATP content as observed in liver slices, at any of the used doses. In human organ slices, liver specific toxicity of paracetamol, lung specific toxicity of paraquat, kidney specific toxicity of cephaloridine and intestinal toxicity of flurbiprofen were confirmed. Unexpectedly however, flurbiprofen also induced major liver toxicity while paracetamol induced intestinal toxicity in human organ slices.

These results show that the combined use of precision-cut slices of several organs, from both man and rat, can be useful for in vitro toxicology studies and may at the same time reveal potential toxicities not readily observed in vivo.

Introduction

In early stages of drug development in pharmaceutical research, *in vitro* techniques provide useful tools for preliminary assessment of toxic potential of new compounds. *In vitro* techniques provide rapid results, require small numbers of animals, and, quite importantly, can also be applied on human tissue. Also investigations on mechanisms of toxicity are in principle possible using *in vitro* methods.

There are many approaches available for the study of toxicity *in vitro*, including cell lines, isolated cells and slices. The model of organ slices has regained popularity since the 1990s, both for studies on metabolism and toxicity.

Advantages of the use of slices can be summarized as follows: slices contain all relevant cell types and all transport proteins and biotransformation enzymes that are also present in the organ of origin and no proteolytic enzymes are needed to digest the tissue. Further, slices can be prepared from different species and

from several organs using essentially the same techniques of preparation, handling and incubation. In addition the technique is relatively simple, and no special facilities are needed for culturing, in contrast to the use of cell lines.
However, a disadvantage of the use of slices, compared with cell lines, is that slices can only be used for relatively short-term experiments (24-48 h), although for some purposes successful prolonged incubation has been claimed for 3-5 days [171].
Because many chemicals display organ-specific toxicity, slices from liver, lung and kidney have been used for toxicity studies in several occasions, as was reviewed earlier [63,171]. In most cases, slices from a single organ were used, in spite of the fact that the direct comparison between different organs is an attractive application of the use of organ slices. The use of slices from different organs in combination for toxicity studies has been described in only a few reports. Liver and kidney slices were used to compare the toxicity of fumosin B [217], benzo(a)pyrene [244], cyclosporin A [321] and atractyloside [223]. As far as the authors are aware of, lung or intestine slices have not been used before in combination with slices from other organs for comparative toxicological studies. A systematic comparison of the sensitivity of slices from the liver, lung, kidney and intestine to toxicants is still lacking.
Therefore we decided to investigate the usefulness of organ slices as an *in vitro* model to study organ-specific drug toxicity. Slices prepared from liver, lung, kidney and intestinal tissue were exposed to four model toxicants. The effect of paracetamol (APAP) was initially studied on rat organ slices and thereafter the effect of APAP and three additional compounds, paraquat (PQ), cephaloridine (CEP) and flurbiprofen (FLUR), was investigated in slices that were prepared from human organs.
We selected APAP, PQ, CEP and FLUR as toxicants for liver, lung, kidney and small intestine, respectively (see figure 1). These four compounds were chosen on basis of a supposed sensitivity of certain target organs for these toxic compounds in man. The liver toxicity of the analgesic and antipyretic agent APAP is supposed to result mainly from the formation of a reactive phase I metabolite in hepatocytes [28]. The herbicide PQ is claimed to be taken up selectively by the lungs and undergoes a single-electron cyclic reduction-oxidation with subsequent formation of harmful radicals in the lungs [120]. CEP, a beta-lactam antibiotic, is reported to accumulate in the tubular kidney cells, causing lipid peroxidation and inactivates renal tubular transporters [310]. The tissue damaging effect of FLUR in the intestine is supposed to be mediated by its inhibition of cyclo-oxygenases, although the precise mechanism of toxicity is not completely understood [27].
As an end-point for cellular toxicity, we selected the ATP content of the slices, as this has been proven to be a sensitive parameter for the viability of slices [101,230].

Materials and Methods

Materials. The following compounds were obtained from the sources indicated: APAP (acetaminophen), PQ (methyl viologen) and FLUR from ICN Biomedicals (Zoetermeer, the Netherlands); CEP, and low melting agarose (type VII-A) from Sigma-Aldrich Chemie (Zwijndrecht, the Netherlands); Viaspan (Belzer UW) organ preservation solution from Barr Laboratories (Pomona, NY, USA); amphotericin B (Fungizone), penicillin-streptomycin solution and Williams' medium E (with Glutamax) from Gibco (Breda, the Netherlands). All other chemicals were of analytical grade and were obtained from commercial sources.

Rat organ tissue. Animal handling was in accordance with the national laws on animal experimentation for the protection of vertebrate animals used for experimental and other scientific purposes. Male Wistar (HsdCpb:WU) rats, mean body weight 360 g (Harlan, Horst, the Netherlands) were housed in standard cages and had free access to food (RMH-chow, Hope Farms, Woerden, the Netherlands) and tap water. Rats were anaesthetised by isofurane and N_2O/O_2, and the liver, lungs, kidneys and intestines were excised and placed in ice-cold Krebs-Henseleit buffer containing 10 mM Hepes and 25 mM glucose, pH=7.4. The intestines were flushed thoroughly with ice-cold Krebs-Henseleit buffer to remove the contents. To prepare agarose filled intestinal slices, the intact intestines were first cut in 10 cm parts that were subsequently ligated on one side. These parts were then filled with 3% (w/v) low melting agarose solution in 0.9% (w/v) NaCl at 37°C and allowed to gel in ice-cold Krebs-Henseleit buffer. The agarose-filled intestines were cut in 1 cm parts and were embedded in the low melting agarose solution at 37°C using the Tissue Embedding Unit from Alabama R&D (Munford, AL, USA) so that agarose gel cylinders with a diameter of 16 mm were formed [67].

The lungs were filled with agarose solution as described earlier [64].

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. Tumor free tissue that was considered as surgical waste was used. Human liver tissue was perfused with, and stored in ice-cold UW (max. 36 hr) [228] 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 (max. 2 hr). The lungs were filled with agarose solution as described earlier [64]. The intestinal tissue was cut in strips of about 0.5 x 1 cm. These strips were then embedded into agarose as described for rat intestinal tissue [67].

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.

Preparation of slices. Tissue cylinders from liver, agarose-filled lung, kidney and agarose-filled and/or embedded small intestine 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. From these organ cylinders precision-cut slices (about 0.25 mm thickness, for lung slices 0.5 mm thickness) were prepared, using a Krumdieck tissue slicer (Alabama R&D, Munford, AL, USA), pre-cooled and filled with oxygenated, ice-cold Krebs Henseleit buffer. Kidney slices were derived mainly from cortex tissue. All slices were stored in ice-cold Belzer UW organ preservation solution until incubation. The time gap between coring and incubation was maximally 2 hrs.

Incubation of slices. Slices were incubated in 3.2 ml Williams medium E, pre-warmed at 37°C and gassed with 95% O_2/5% CO_2 and supplemented with glucose (final concentration 25 mM). For liver and lung slices gentamicin (50 µg/ml) was added. For kidney slices penicillin (100 U/ml) plus streptomycin (100 µg/ml) was used, whereas for intestine slices gentamicin (50 µg/ml) and amphotericin B (2.5 µg/ml) was added. Slices were individually incubated in 6-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) in a cabinet at 37°C. During incubation, the gel surrounding the slices, but not inside the ring of intestinal tissue, separated from the slices as observed visually.

Slices were pre-incubated for 3 h at 37°C. Previous experiments showed that the ATP content of organ slices is relatively low directly after preparation, but increases in the

first 1-3 h of incubation. Thereafter the ATP content of the slices remains stable for at least 24 h [61,88]. After this pre-incubation period, the slices were transferred to fresh medium and exposed to the compound of interest at the concentrations indicated in figure 1. After 24 h of additional incubation, the ATP content was determined. For administration of PQ a stock-solution in water was used, the other compounds were dissolved in medium.

Viability of slices. The viability of the slices after incubation was determined by measurement of ATP content. For this, individual 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 ten 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. All results are means from 3 slices per experiment and 3-7 individual organs were used. Data are given as mean ± SEM. Statistical significance of differences was calculated using two-tailed, two sample *t*-test, assuming equal variance.

Protein content of organs and slices. After incubation, slices were homogenized in their own incubation medium by sonication and stored at −20°C until analysis. After thawing, the homogenates were diluted with 0.1 M NaOH and the protein content was determined using the Bio-Rad protein assay dye reagent (Bio-Rad, Munich, Germany) against a bovine serum albumin standard curve. The ATP content of the slices was expressed per μg protein, and not per mg wet weight because in lung and intestinal slices agarose is present that contributes to the wet weight.

Results

Slices from human and rat organs were incubated for 24 hours to the toxicants as indicated in figure 1, after the pre-incubation without toxicant for 3 h.

	APAP (0-10 mM)	**PQ** (0-108 μM)	**CEP** (0-5 mM)	**FB** (0-10 mM)
liver	6	6	6	5
lung		3		
kidney			2	
intestine	2			2

Figure 1. The numbers of human organs that were used for slicing and exposure to toxicants are indicated. The shaded numbers indicate the combinations of supposed target organ with the corresponding compound.

Initially, rat liver, lung, kidney, small intestine and colon slices were incubated with different concentrations of APAP for 24 h after the pre-incubation period. The ATP content of these slices is depicted in figure 2.

As illustrated in figure 2, exposure of rat liver slices to 0.5 mM and 1 mM APAP had no significant effect on ATP content but APAP concentrations of 5 mM and 10 mM caused a concentration dependent decrease of the ATP content as measured after 24 h of incubation. In contrast, the ATP content of rat lung, kidney, small intestine and colon slices was not significantly influenced by the exposure to APAP during 24 h, in the concentration ranged studied (figure 2).

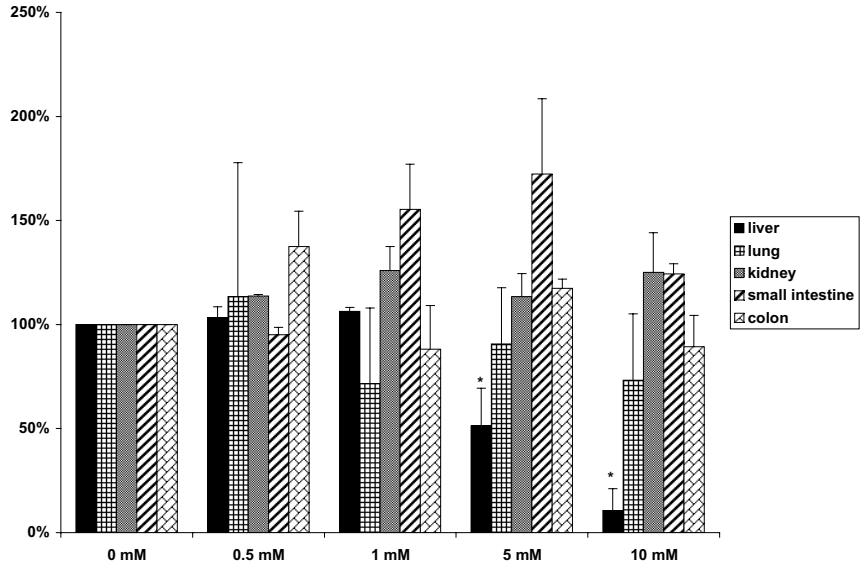


Figure 2. ATP content of rat organ slices exposed to 0 – 10 mM APAP for 24 h. The ATP content of slices that were not exposed to APAP is set as 100% for each experiment, and was 7.6 ± 1.2, 7.4 ± 4.8, 5.4 ± 1.1, 2.8 ± 0.6 and 4.3 ± 2.1 pmol /µg protein ± SEM, for liver, lung, kidney, small intestine and rat colon slices respectively (n=3-7).
* Significantly different ($p < 0.05$) vs. control slices.

When human liver and intestine slices were exposed to APAP (figure 3), it was found that the ATP content of human liver slices is similarly influenced by APAP exposure as that of rat liver slices. However, also human small intestine slices clearly showed APAP dependent loss of ATP content. This in contrast to rat intestinal slices, that even showed a slight increase of their ATP content.
Human lung slices, incubated with PQ, showed a concentration dependent loss of ATP as shown in figure 4. Also the ATP content of human liver slices was slightly (not significantly) affected, but only after exposure to 108 µM PQ.
In figure 5 the effect of exposure to CEP on human liver and kidney slices is shown. CEP did not lower the ATP content of human liver slices over the concentration range studied, but the ATP content of human kidney slices was lowered when exposed to 2 and 5 mM CEP.
The effect of FLUR on ATP content of human liver and intestinal slices is depicted in figure 6. As shown, human liver slices are more susceptible to FLUR, in terms of ATP loss, than human small intestinal slices. At exposure to 5 mM FLUR and higher, slices from both organs lost virtually all ATP after 24 h of incubation.

Sequential incubation of liver and kidney slices for toxicity studies
The influence of metabolites that are produced by liver slices on the viability of kidney slices was studied by means of sequential incubations with liver and kid-

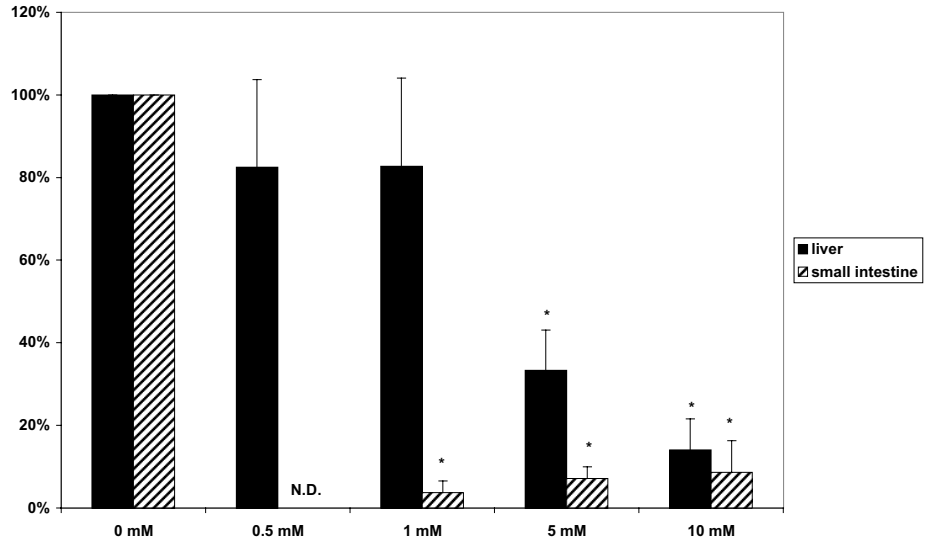


Figure 3. ATP content of human organ slices exposed to 0 – 10 mM APAP for 24 h. The ATP content of slices that were not exposed to APAP is set as 100% for each experiment, and was 8.7 ± 3.4 and 2.5 ± 1.2 pmol /µg protein ± SEM, for slices from human liver (n=6) and intestine (n=2), respectively.
* Significantly different ($p < 0.05$) vs. control slices.
N.D. Not determined.

ney slices. First, rat liver slices were incubated with varying concentrations of APAP for 24 h, and the incubation medium was stored at –20°C. Thereafter, in a separate experiment, kidney slices were incubated in this medium. The ATP contents of these slices were compared with rat kidney slices that were incubated in fresh medium with varying concentrations of APAP.
As is shown in figure 7, incubation of kidney slices with medium that originated from liver slice incubations with APAP caused a decrease of the ATP content dependent on the APAP concentrations used, which was most pronounced at 10 mM. However, the ATP content of kidney slices that were incubated in medium from liver slices that were incubated for 24 h without APAP, was also decreased when compared with control slices that were incubated in fresh medium (see figure 7; 0 mM bars).
During exposure to 10 mM APAP we observed a remarkable change in the macroscopic appearance of both rat and human liver slices, i.e. the color of the slices turned into black, as was clearly visible already after 3 h of exposure. The black reaction product dissolved in 70% ethanol, which was used as a fixative for the preparation of microscopic sections and as a consequence was not visible under the microscope.

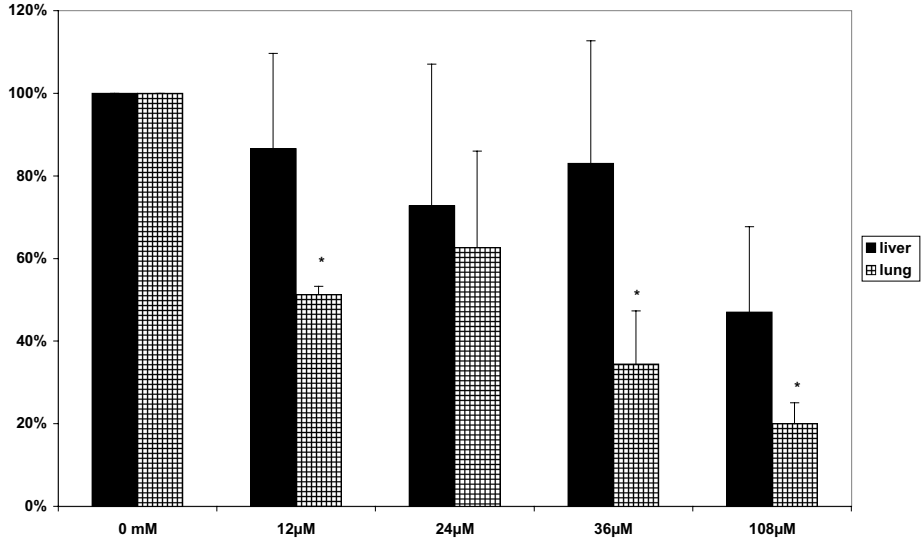


Figure 4. ATP content of human organ slices exposed to 0 – 108 µM PQ for 24 h. The ATP content of slices that were not exposed to PQ is set as 100% for each experiment, and was 8.7 ± 3.4 pmol /µg protein, for both slices from human liver (n=4) and lung (n=3). * Significantly different ($p < 0.05$) vs. control slices.

Discussion

In the current study, slices from several organs from both rat and man were used to confirm the organ-specific susceptibility to four well-studied model toxicants. In general, the data obtained from the applied combinations of organs and toxic compounds showed that tissue damage, measured as ATP content was observed in slices in an organ- and concentration dependent manner.

Using rat organ slices, liver-specific toxicity of APAP was convincingly demonstrated. Interestingly, it was found that human small intestine slices from two intestines were more sensitive for APAP induced ATP depletion than human liver slices (figure 3), but more research is necessary to investigate the significance of this finding.

Although APAP is usually described as hepatotoxic, renal toxicity *in vivo* has also been described [28]. Yet, this was not observed in kidney slices. This can be explained by the assumption that renal toxicity *in vivo* is at least partly due to metabolites formed in the liver, excreted into the bloodstream and activated in the kidney to a toxic metabolite [28]. This has been described for the APAP metabolite para-aminophenol [348] and for GSH conjugates of APAP [28]. The GSH conjugates can be converted to toxic metabolites via the γGT and β-lyase pathway [28] and para-aminophenol can undergo auto-oxidation resulting in renal damage [88]. To investigate whether this phenomenon could also be

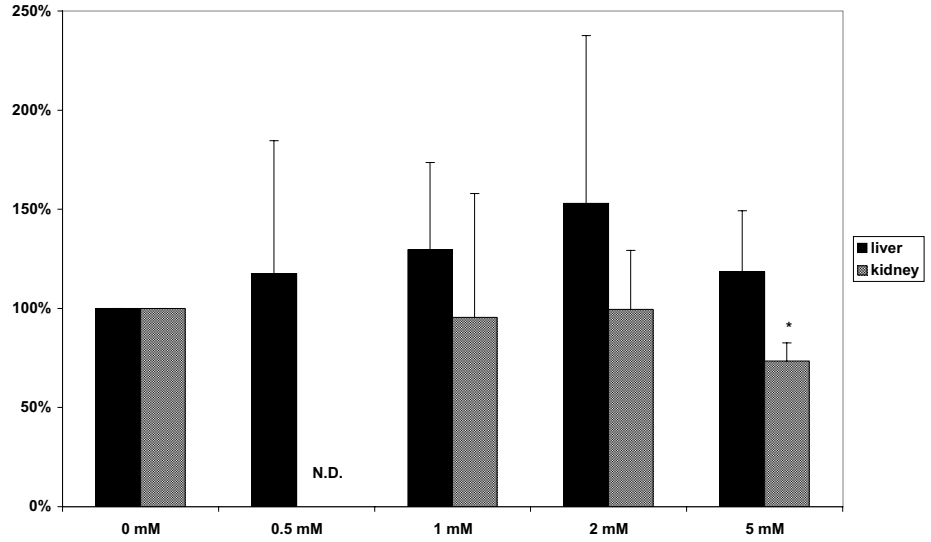


Figure 5. ATP content of human organ slices exposed to 0 – 5 mM CEP for 24 h. The ATP content of slices that were not exposed to CEP is set as 100%, and was 8.7 and 8.1 pmol /µg protein, for human liver and kidney slices, respectively.
* Significantly different ($p < 0.05$) vs. control slices.
N.D. Not determined.

detected in our *in vitro* system, we incubated kidney slices in medium that was previously used for incubation of liver slices with APAP.
Rat kidney slices that were incubated with medium that originated from incubations of liver slices in the presence of varying concentrations of APAP showed a decrease of ATP content, when compared with kidney slices that were incubated in fresh medium (figure 7). This is in agreement with the hypothesis that liver-derived metabolites can be responsible for toxicity in the kidney. However, also kidney slices that were incubated in medium originating from liver slices not exposed to APAP, appeared to have a lower ATP content when compared with kidney slices that were incubated in fresh medium. Apparently, liver slices deplete essential substrates and / or excrete substances in the medium that cause a decrease of the ATP content in kidney slices. In principle, this factor may act in concert with APAP metabolism to induce renal toxicity. Thus, more research is necessary to clarify the mechanisms underlying the reduced viability of kidney slices caused by the liver slice incubation media.
Human kidney slices showed a concentration dependent loss of ATP content after exposure of CEP. This is in agreement with described toxicity of this agent *in vivo*, a major reason for the fact that CEP is no longer used clinically [310].
The herbicide PQ is known to be highly toxic for the lung in mammals. This organ-specific toxicity was confirmed here using human lung slices. Of note, at high doses, also a loss of the ATP content of human liver slices was found (figure 4). This observation may be explained by the fact that the liver slices are exposed

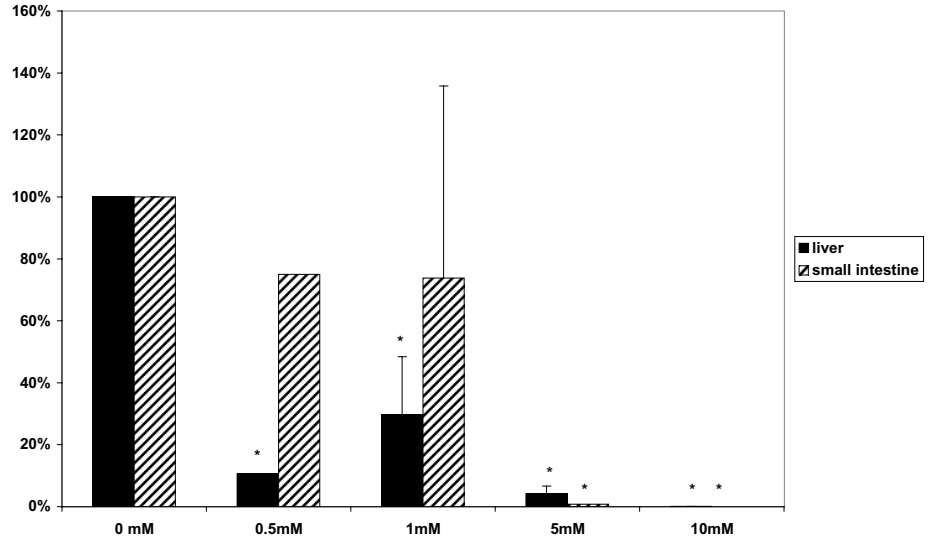


Figure 6. ATP content of human organ slices exposed to 0 – 5 mM FLUR for 24 h. The ATP content of slices that were not exposed to FLUR is set as 100%, and was 8.7 and 3.7 pmol /µg protein, for slices from human liver (n=3) and intestine (n=2), respectively. * Significantly different ($p < 0.05$) vs. control slices.

to a rather high concentration of 95% O_2, together with the finding that the toxicity of PQ is an oxygen driven process [132]. A similar toxicity has been described before, using isolated hepatocytes [270].

In clinical practice most side effects of FLUR are observed on the gastrointestinal level [27]. In contrast, we found that human liver slices from three donors were more sensitive to FLUR induced loss of ATP content, when compared with human small intestine slices of two organs (figure 6). Although we anticipated that the small intestine would be more susceptible, the present results are in agreement with the toxicity of FLUR observed in hepatocytes [41]. This phenomenon is supposed to be a result of the uncoupling of the mitochondrial oxidative phosphorylation in the liver by FLUR [197]. The observed specific intestinal toxicity of FLUR observed *in vivo* can be readily explained because after oral ingestion of FLUR much higher concentrations are reached in the lumen of the small intestine than in the liver.

The present study shows that it is well possible to study and compare responses to toxicants between different organs in certain species and between different species using slices. Yet, a limitation of the use of such *in vitro* studies is that it is difficult to extrapolate the findings to the intact organism in order to predict the potential toxic dose *in vivo* [29].

Another limitation of the use of toxicity studies performed *in vitro* in general is the risk of obtaining either false positive or negative results. False negative results can be caused by delayed toxicity that is only apparent after a longer peri-

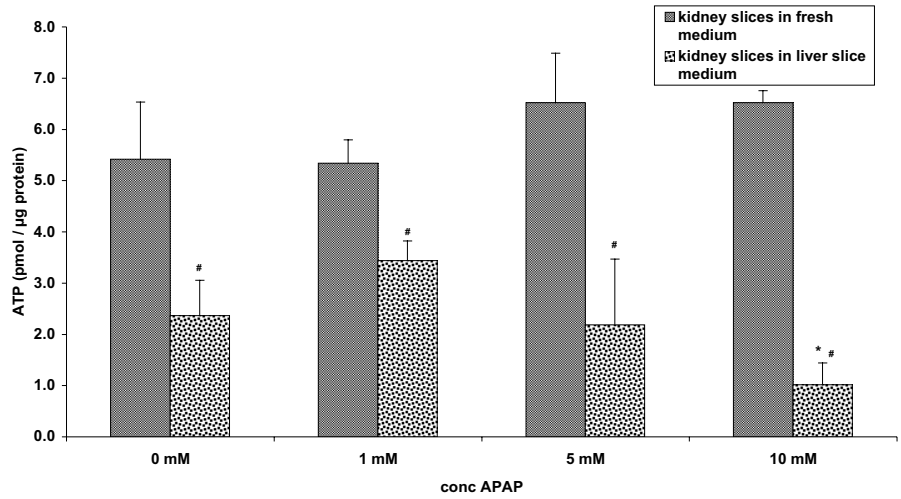


Figure 7. ATP content of rat kidney slices exposed to 0 – 10 mM APAP for 24 h. The slices were incubated in fresh medium, or in medium where rat liver slices have been incubated for 24 h, at the corresponding APAP concentrations (n=3).
* Significantly different ($p < 0.05$) vs. slices incubated in liver slice medium without APAP.
# Significantly different ($p < 0.05$) vs. corresponding slices incubated in fresh medium.

od of time than the incubation itself. Either early markers of toxicity or long-term experiments are needed to detect delayed toxicity *in vitro*.
Care should be taken that viability parameters are chosen that are sensitive enough to show toxic effects. The use of rather rough criteria such as dye exclusion and enzyme leakage can only provide clues after major cell damage. Other experimental factors, such as the composition of incubation media can also affect the sensitivity of the model system [95].
On the other hand, false positive results may also be obtained in *in vitro* studies. For example, the ATP content of slices, or cells, can be transiently lowered by energy demanding processes that are not related to toxic phenomena in the cell. It should be realized that ATP tissue content results from a dynamic equilibrium between ATP synthesis and ATP loss (either through consumption, cellular damage or uncoupling phenomena). For example, we found that ATP content of human liver slices is about 15% lower after incubation with 0.5 mM 7-ethoxycoumarin for 3 h (data not shown). As toxicity of 7-ethoxycoumarin has not been reported yet, a more likely explanation is that the ATP decrease is a result of the depletion of co-factors in these slices for the cytochrome P450 catalyzed reaction towards 7-hydroxycoumarin and the subsequent conjugation with glucuronide and sulphate. The synthesis of the cofactors NADPH, UDP-glucuronic acid (UDPGA) and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) are ATP demanding processes [88].

Therefore, it could be useful in the future to measure the ATP content, both directly after drug exposure and after an additional 1-3 h incubation without the compound of interest. ATP levels will remain low in case of cell damage, but may recover if only a high demand of energy-rich cofactors, for instance for the metabolism of the compound under study was the cause of the ATP decrease.
In conclusion, it is shown that slices from liver, lung, kidney, small intestine and colon, from both man and rat, can be used in an integrated manner for *in vitro* toxicity testing. ATP content of the slices is a convenient parameter to test potential cellular toxicity that is, if effects on ATP synthesis and consumption can be experimentally distinguished.