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## Human liver slices and isolated hepatocytes in drug disposition and transplantation research.

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

1996

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Olinga, P. (1996). *Human liver slices and isolated hepatocytes in drug disposition and transplantation research*. s.n.

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## Summary

The experiments described in this thesis were the result of structural collaboration between the Department of Pharmacokinetics and Drug Delivery (Faculty of Sciences) and the Division of Hepatobiliary Surgery and Liver Transplantation of the Department of Surgery in the Faculty of Medical Sciences.

The aim of the study was to investigate the utility of human liver slices and isolated hepatocytes in drug disposition and transplantation research. Therefore it was necessary to develop methods to measure drug transport and metabolism in these *in vitro* preparations of the liver. Furthermore, preservation methods of these preparations and the relation between results obtained *in vitro* with those found *in vivo* were investigated. In addition, the potency to predict liver graft function in the recipient using data obtained in *in vitro* experiments with human liver preparations was studied.

**Chapter 1** reviews the recent literature on the preparation and use of human liver slices and hepatocytes in drug metabolism and transport studies.

It is concluded that there is abundant information on drug transport and metabolism in rat liver whereas data from human liver are scarce. Therefore, there is a great need for the development of reliable liver preparations of human liver for *in vitro* research, for early pharmacokinetic screening and for the study of transport mechanisms in man.

The thesis contains sections dealing with evaluation, preservation of the *in vitro* preparations of the human and rat liver as well as the use of these preparations in transplantation related research and in studies on drug disposition.

## SECTION I

### Evaluation of *in vitro* preparations and incubation systems:

In **chapter 2**, five incubation systems for rat liver slices were evaluated for their ability to retain functionality and viability. After up to 1.5 hours of incubation we found no difference in the tested functions or viability of the rat liver slices in the different incubation systems. However, after 24 hours of incubation of the slices, the shaken Erlenmeyer flask, the Netwell insert in the 6 well culture plate and the 6 well shaker were superior to the 24 well incubation system and the Dynamic Organ Culture system. This indicates that for short-term culturing of liver slices the choice of the incubation system is of relatively little importance. Difference in functionality of the liver slices only become apparent after 24 hours of incubation.

Results obtained with human liver slices and hepatocytes may be influenced by the procurement of the liver tissue. Therefore, in **chapter 3** we investigated the influence of the procurement of the tissue on the function of isolated hepatocytes and liver slices. We studied isolated hepatocytes and liver slices from healthy human liver tissue obtained as material from either patients undergoing partial hepatectomy (PH-livers) or from donor tissue remaining after reduced size or split liver transplantation (Tx-livers). The hepatocytes from PH-livers and Tx-livers showed almost similar viability and functional capacities. Metabolism of model drugs in cells and slices from Tx-livers was found to be quantitatively comparable. However, liver slices from PH-livers showed a lower metabolic capacity compared to cells from the same tissue. This may indicate that only a part of the hepatocytes in the liver slice is participating in the metabolism of the compounds studied. In addition, this suggests that a selection of healthy cells takes place during isolation of the hepatocytes from PH-livers. From this study it can be concluded that hepatocytes from both Tx-livers and PH-livers can be used within one study without consideration of the procurement of the tissue. This is in contrast to human liver slices where the procurement of the tissue significantly influences the results obtained.

## SECTION II

### Preservation of isolated hepatocytes and liver slices:

The preservation of the *in vitro* liver preparations is of utmost importance for efficient use of the liver tissue.

In **chapter 4** we assessed whether the metabolic capacity of rat hepatocytes is retained after short-term (48 hours) storage. Preservation of rat hepatocytes in University of Wisconsin organ preservation solution (UW), results in a high recovery of viable cells with a well preserved metabolic energy state and therefore allows the use of these cells for metabolism studies up to 48 hours after isolation. This technology enables more efficient use of isolated cells and thereby reduces the number of experimental animals needed. In addition, short-term storage in UW was also investigated for human and monkey liver slices and hepatocytes. In **chapter 5** it is shown that *in vitro* preparations from monkey liver can be stored up to 18 hours with limited loss of metabolic capacity. Only one of the three tested biotransformation reactions, the phase I metabolism of 7-ethoxycoumarin, seems to be affected by UW storage. Furthermore, both isolated cells and slices of the Tx-liver and hepatocytes from PH-livers can be stored up to 18 hours without losing metabolic capacity. However, for the liver slices prepared from PH-livers cold storage is not recommended due to loss of metabolic function. Whether this is due to the warm ischemia occurring during the surgical procedure requires further investigation.

Long-term preservation can in principle be achieved with cryopreservation. In

application of human liver tissue in drug research, because it implies that more efficient use can be made of the scarce tissue and that cryopreserved human liver slices may be used at any desired time for studies concerning drug metabolism.

### SECTION III

#### Transplantation-related research:

In this section the predictive value of *in vitro* data (drug disposition and viability) of slices, hepatocytes and needle biopsies of human donor livers for graft function after transplantation was studied.

In **chapter 7**, the monoethylglycinexylidide (MEGX) formation rate from lidocaine in needle biopsies from donor livers was compared with the MEGX formation rate *in vivo* in the donor. In addition, the metabolic capacity of needle biopsies of donor livers was compared with graft function after transplantation. We found that MEGX formation rate in the biopsies *in vitro* properly reflect the metabolic function of the donor *in vivo*, and that liver needle biopsies may be a valuable tool to assess liver function before or after transplantation *in vivo*. However, the MEGX test alone clearly cannot be used as the golden standard to predict graft function after transplantation.

In **chapter 8**, the results obtained with human liver slices and isolated hepatocytes from livers used for reduced size or split liver transplantations were compared with the liver graft function in the recipient. The *in vitro* tests used in this study did not indicate those four livers, that after transplantation showed primary non-function of the grafts, indicating that these tests were not able to predict graft function. We conclude that either the function tests used are not sensitive enough to select non-viable donor livers or that other factors than liver viability influenced the graft function in the recipient in this specific patient group.

### SECTION IV

#### Drug transport:

To investigate the transport function of the human liver, we studied drug transport in human liver slices and hepatocytes.

In **chapter 9**, we investigated the possibilities to study drug transport in liver slices. The uptake of various compounds was investigated both with fluorescent microscopy and by measuring uptake of radioactive substrates. It was shown that human liver slices are a valuable tool to investigate the mechanisms and carrier specificity of transport of drugs, since carrier mediated uptake, stereoselective inhibition, adsorptive endocytosis and receptor mediated uptake of proteins can be demonstrated.

However, quantitatively, the uptake rate of compounds in the slice does not only reflect the uptake process in the cells involved, but is also influenced by the rate of penetration of the substrates into the slice. The time for substrates to penetrate into the whole slice amounts to minimally 5 minutes. The rate of penetration into the slice may therefore become rate limiting in the slice accumulation of the drug especially if the cellular uptake process for a particular substrate is very efficient.

In **chapter 10**, we investigated the large interindividual variation in uptake rate for taurocholic acid in human hepatocytes, which could only partly be explained by the variation in ATP concentration in these hepatocyte preparations. We found that taurocholic acid uptake rate in human hepatocytes shows age dependency. The uptake rate was significantly lower in the hepatocytes isolated from donors younger than 15 years than in adult donors. These results indicate that the low biliary secretion and high serum concentration of bile acids found in infants may be due to a smaller number of bile acid uptake carriers.

In **chapter 11**, we describe studies on the uptake of the organic cation rocuronium and the uncharged cardiac glycoside digoxin in human hepatocytes. Uptake in the human hepatocytes quantitatively resembled the uptake rates of these drugs in the human liver *in vivo*. Furthermore, when the data were compared with those obtained in rat hepatocytes clear species-differences in carrier specificity became apparent. This study supports the contention that extrapolation of drug transport data from rat to man is hazardous.

In **appendix A**, a method is described for the rapid analysis of the different adenosine nucleotides (ATP, ADP, AMP) and adenosine. This fast procedure was optimized for samples of hepatocytes and can be used with very low amounts of cells. The method allows rapid assessment of the energy charge of cells (being  $(ATP+1/2ADP)/(ATP+ADP+AMP)$ ). This ratio is maintained by the balance between energy-yielding and energy-consuming processes. The energy charge, as determined by the present method, is regarded as a sensitive indicator for the viability of isolated hepatocytes.

The experiments described in this thesis were performed within the framework of the Human Liver Group Groningen, in which the departments of Pharmacokinetics and Drug Delivery, Surgery, Paediatrics, Physiological Chemistry and Pathology collaborate. This combined expertise was shown to be essential for the optimal use of the scarcely available human liver tissue. This study was financially supported by the Alternatives to Animal Experiments Platform, Organon International BV and Solvay Duphar BV.