Institutionen för fysiologi och farmakologi

NOVEL HUMAN *IN VITRO* SYSTEMS FOR STUDIES OF DRUG INDUCED HEPATOTOXICITY

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras på engelska språket i Farmakologens föreläsningssal, Nanna Svartz väg 2, Karolinska Institutet, Solna,
fredagen den 26:e oktober, 2012, kl. 09.00.

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Stockholm 2012

ABSTRACT

Drug-induced liver injury (DILI) is a major human health concern, estimated to account for about half of all cases of acute liver failure in the general population. DILI also represents a significant problem in drug discovery, being one of the most common reasons for regulatory actions, including boxed warnings, restricted marketing and withdrawals of marketed drugs. Adverse drug effects in the liver are hard to detect at an early stage during drug development, much owing to the shortcomings of the currently available pre-clinical model systems. The work presented in this thesis aimed to refine and further develop more sensitive, human *in vitro* models and methods for better prediction of DILI and the underlying mechanisms.

Mono-culture of human primary hepatocytes is the closest *in vitro* model to the human liver, currently considered the golden standard in drug development. However, limitations, such as low availability of qualitative liver tissue and phenotypical instability of these cells in culture, require new sources of functional human hepatic cells. We show that high-density culture of the human hepatoma cell line Huh7 induces a spontaneous, hepatic, differentiation process, without the need for inducers as is usually the case. A particular increase of *CYP3A4* gene- and protein expression and catalytically activity is observed. Moreover, we found that the large increase in CYP3A4 expression seen in the confluent Huh7 cells is mediated by PXR nuclear translocation and increased PXR mediated transcriptional activity, most likely as a result of decreased CDK2 activity and cell cycle arrest. The high constitutive expression of CYP3A4 in the confluent Huh7 cells makes this cell system useful for studies of mechanisms for regulation of PXR and the *CYP3A4* gene.

The unique characteristics of stem cells make them an attractive large-scale source of hepatic cells for drug development and safety assessment. Using a novel stepwise differentiation protocol we have been able to differentiate human embryonic stem cells (hESC), via definitive endoderm and progenitor stages to hepatocyte-like cells which exhibiting many hepatocyte-specific features and functions, including CYP metabolic activities. A dynamic three-dimensional (3D) bioreactor system was shown to prolong and maintain the specific functions of primary hepatocytes, as well as facilitate the hepatic maturation of hESC into hepatocyte-like cells.

It has become increasingly evident that inflammatory event plays a significant role in many DILI events. Thus, *in vitro* systems containing a population of immune competent cells in combination with hepatic cells could be of great significance for studying mechanisms underlying DILI. A co-culture cell model consisting of hepatocytes and monocytes has been developed where the cells were separated by a semipermeable membrane. The hepatotoxic drug troglitazone caused a potentiated, and more rapid cytotoxic effect in cells treated in the co-culture as compared to the single cultures. Troglitazone treatment also resulted in an increased expression of several stress-related genes in the co-cultures compared to the single cultures. These results suggest a synergistic cytotoxic effect by soluble mediators released by the cells and underscores the importance of incorporating several hepatic cell types in order to generate more sensitive *in vitro* systems and better prediction of DILI.

ISBN 978-91-7457-869-0