

WP7.3: Improving in vitro/in vivo correlations – Hepatotoxicity

The main goal of WP7.3 has been to develop novel assays for the evaluation of compounds that are liver toxic.

Markers for hepatotoxicity

To identify a set of characteristic markers that can be used in high throughput screening of acute liver toxicity, 21 ACuteTox reference chemicals have been tested in metabolic competent cells (rat hepatocytes), non-competent hepatic cells (HepG2) and non hepatic cells (3T3 fibroblasts), using the MTT assay as a cytotoxic endpoint. Also, the following biochemical functions were examined in the cells: cellular ATP levels, formation of reactive oxygen species (ROS), cellular protein content and mitochondrial membrane potential. The results were compared with the MTT assay and suggest that neither of the assays, when measured at early time points (5 hr of exposure) or late times (24 hr of exposure) allowed a better discrimination of the chemicals than the one obtained with the MTT assay.

The rat hepatocytes, HepG2 and 3T3 cell cultures with MTT assay as end-point measurements have been selected for the additional testing of further 41 reference chemicals in collaboration with WP6. In addition to the chemicals selected by the Consortium, also more known hepatotoxic compounds will be tested.

Evaluation of cholestatic chemicals

The envisaged strategies for evaluation of cholestatic chemicals (i.e. compounds impairing hepatocyte bile acids and bilirubin transport) have included the development of a cell bank expressing hepatocellular bile salt and xenobiotic transporters, as well as fluorescent bile salt derivatives. The cell bank consists of five CHO cell lines stably transfected with the major forms of human liver organic anion transporters, as well as four CHO cell lines stably transfected with rat liver organic anion transporters. These transport proteins are involved in the uptake of endogenous substances, for example bile acids and xenobiotics (drugs and toxins), into hepatocytes. Each of the established cell line expresses single hepatocellular transporters and was used to characterize the transport properties of the developed fluorescent bile acid derivatives (see below). In addition, the cell lines can be suitable for testing the effects of chemicals on individual human and rat organic anion transporters, and whether a transporter is involved in the uptake of a given xenobiotics in human (or rat) liver.

The new fluorescent analogues of bile acids (i.e. fluorescent conjugates) were developed by linking different fluorophores to different positions in the bile-acid moiety. The fluorescent bile salt derivatives were then investigated for their uptake and transport across the cell membrane, as well as inhibition of uptake by drugs known to block active bile transport. The cellular *in vitro* model used to examine the behavior of the bile analogues and the possible interaction between chemicals and bile acids were the cell lines described above, as well as freshly isolated rat hepatocytes kept in suspension, which express a satisfactory level of anion and bile-acid transporters.

For the liver export system, two recombinant baculoviruses expressing rat and human ABC-transporters, namely the bile salt export pump Bsep/BSEP, are available. Inhibition of BSEP (Bile Salt Export Pump by drugs and xenobiotics constitutes an important pathophysiological mechanism of acquired cholestasis.

A protocol outlining a test system to identify/alert for substances which are potentially hepatotoxic due to their capability to impair hepatic transport is to be expected.