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Highlight report: towards the replacement of in vivo repeated dose systemic toxicity testing

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Recently, the European Union, together with the European cosmetics industry represented by Colipa, initiated the "SEURAT-1" program to develop in vitro and in silico test systems with the goal to eventually replace in vivo repeated dose systemic toxicity testing. The results and concepts will be published in a series of six annual books, the first of which was recently released (Schwarz and Gocht 2011).

Repeated dose systemic toxicity testing has been a major topic frequently addressed in our journal (Bruchajzer et al. 2010; Heo et al. 2010; Pestka 2010; Tasaki et al. 2009; Kumar and Gill 2009). Of note, Rupp et al. (2010) have recently applied a computational QSAR tool to predict chronic oral LOAEL. In their study, they predicted LOAELS for 807 industrial chemicals and compared the predicted values to the experimental LOAELS. Due to the exclusion criteria of the QSAR tool, prediction was not possible for 460 compounds. Of the remaining 347 chemicals, 34–62% were predicted within a range of 0.2- and fivefold compared to the experimentally obtained LOAELS. Their results illustrate the ambiguity of in silico chronic LOAEL prediction and the need for more refined prediction tools (Rupp et al. 2010).

The relevance of repeated dose testing is illustrated by numerous recently published studies, e.g., the role of chronic 4-n-nonylphenol or methylmercury exposure on aortic vasoconstriction and hypertension (Hsieh et al. 2009; Grotto et al. 2009), the relevance of chronic oxidative stress in alcohol-induced liver injury (Cederbaum et al. 2009), immunotoxicity by chronic exposure to perfluorooctanesulfonate (Dong et al. 2009), and renal dysfunction as a consequence of chronic exposure to depleted uranium (Zhu et al. 2009). It is also well established that induction or inhibition of detoxifying enzymes (Gebhardt et al. 2003; Hengstler et al. 2000; Hewitt et al. 2007) or protective factors (Ilowski et al. 2010, 2011) plays an important role in repeated dose toxicity. Moreover, much progress has been achieved in physiologically based toxicokinetic modelling to predict relevant concentrations for in vitro testing (Mielke et al. 2011). However, relatively little is known about the differences in mechanisms relevant for repeated dose and acute toxicity. Is repeated dose toxicity simply a consequence of the prolonged activity of the same toxic pathways that cause acute toxicity? An example of repeated dose hepatotoxicity can be used to illustrate that this is not necessarily the case:

Liver fibrosis

Single administration of high doses of CCl_4 causes cell death to a fraction of hepatocytes located at the centre of the liver lobules, because only the central hepatocytes express the metabolically activating CYP2E1 (Hoehme et al. 2010; Bauer et al. 2009). In less than 10 days, the dead cell area completely regenerates. The regenerated liver is functionally and morphologically undistinguishable from control livers. However, if lower doses of CCl_4 are administered over 6 weeks (two injections per week), liver fibrosis is observed. Interestingly, fibrosis occurs in the periphery of the liver lobules, and not at the centre where cell killing of hepatocytes takes place. This scenario is

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most probably explained by the release of cytokines by stressed or dying hepatocytes. These cytokines activate liver stellate cells to form myofibroblasts that secrete large amounts of extracellular matrix, finally leading to fibrosis and destruction of the liver architecture. Since (for unknown reasons) stellate cells and myofibroblasts prefer to localize to the periportal region, the pattern of CCl_4-induced cell death (in the centre of liver lobules) does not correspond to the pattern of fibrosis (in the periphery of liver lobules). Although CCl_4 is not clinically relevant, it represents the best studied model compound that is prototypical for other compounds causing pericentral liver damage, and for which metabolic activation by CYP2E1 is relevant. Clinically relevant examples include ethanol or paracetamol. In addition, liver fibrosis after bile duct ligation, mdr2 knockout (which leads to bile salt toxicity), viral hepatitis or alcohol abuse (Hengstler et al. 2009) all seem to be caused by a secondary mechanism where toxicity to hepatocytes activates stellate cells.

Idiosyncratic drug-induced liver injury (DILI)

One mechanism leading to DILI is that drugs are activated to reactive intermediates in hepatocytes and form protein adducts that may function as haptens. It may take several weeks to months and repeated doses over a longer period until immune cells have proliferated and matured sufficiently in order to induce a clinically evident DILI. Recently, evidence has been presented that even complex mechanisms requiring repeated doses and interaction of several cell types may be predicted by relatively simple in vitro systems: considering both, protein binding of compounds to liver proteins and the daily dose of drugs allows a good differentiation between DILI inducing and negative compounds (Usui et al. 2009; Nakayama et al. 2009). Using this approach, the positive DILI compounds acetaminophen, alpidem, bromfenac, carbamazepine, diclofenac, flutamide, imipramine, nefazodone, tacrine, ticlopidine, tienilic acid, and troglitazone could be differentiated from negative compounds acetylsalicylic acid, caffeine, dexamethasone, losartan, ibuprofen, paroxetine, pioglitazone, rosiglitazone, sertraline, theophylline, venlafaxine, and zolpidem (Usui et al. 2009).

Steatosis and steatohepatitis

Several drugs can induce steatosis, the accumulation of lipid droplets in hepatocytes. Examples are amiodarone that inhibits beta-oxidation, tamoxifen that increases triacylglycerol biosynthesis or fialuridine that compromises mitochondrial functions (review: Amacher 2011a, b). Also the activation of Kupffer cells, the tissue macrophages of the liver, may contribute to steatosis, since Kupffer cells secrete cytokines such as IL-1beta that suppress PPARalpha expression a control factor of several genes involved in transport and oxidation of free fatty acids (Amacher 2011a, b). Already the induction of a fully established steatosis may require repeated doses. Drug-induced steatosis may progress to steatohepatitis, a serious condition of fibrosis or cirrhosis which may lead to liver failure. The "two hit theory" tries to explain pathogenesis of steatohepatitis (review: Amacher 2011a, b). The first hit is induced by repeated doses of a drug leading to steatosis. Next a "second hit" causes progression of usually asymptomatic steatosis to clinically evident steatohepatitis. Although the exact nature of the "second hit" is not fully understood, it is clear that steatosis, e.g., induced and maintained by repeated doses of drugs, clearly enhances the risk of steatohepatitis.

The above examples may be sufficient to illustrate that one compound may cause both acute and repeated dose toxicity by different pathways. However, research on the mechanisms of repeated dose toxicity is still at an early stage but promises an unusually dynamic and successful field of research. The book series by Schwarz and Gocht (which can be down loaded from the following website: http://www.seurat-1.eu/pages/library/seurat-1-annual-report.php) will be interesting for anyone interested in repeated dose systemic toxicity testing.

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