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**Author:** Fredriksson, Lisa Emilia  
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ENGLISH SUMMARY

Adverse drug reactions, more commonly called side effects, are very problematic for both society and pharmaceutical industry. The costs are high in severe cases: for pharmaceutical companies due to the loss of intended income if a drug needs to be removed from the market; for society due to the extra healthcare that is required to treat the affected individuals. And, not to be forgotten, the burden to the patient in question.

Liver damage upon drug intake is the most common type of adverse drug reaction and reason for drug withdrawal. Drug-induced liver injury (DILI) may affect the liver to such an extent that the liver fails to function, which is potentially fatal. One of the major functions of the liver is drug clearance, meaning that it monitors the blood coming from the intestines for foreign substances, including drugs, takes these up and modifies them to species (metabolites) that can be removed via the urine or bile. Often this drug clearance process runs without trouble; however, sometimes drug metabolites are formed that actively damage the liver. Whether and why severe liver damage occurs seems to depend on the individual.

Sometimes DILI can be explained. For example, paracetamol is partially modified to form a harmful side product. Under normal circumstances this is detoxified by the liver’s own defense systems, but upon overdose the concentrations exceed the defense potential, resulting in acute liver failure. This process can be counteracted and by that avoided. However, the cause and mechanism of DILI by various other drugs is not (yet) known. Although infrequent, but severe, it is of major interest to prevent unexplainable DILI. Understanding how and why will therefore be very important. The knowledge could result in creating tests for drug safety that can be used by the pharmaceutical industry, to prevent the marketing of harmful drugs. My thesis dealt with this important problem: why does DILI occur and would it be possible to create tests to screen new drug-candidates for (liver) safety?

For some time it is known that not only the drug transformation or metabolism can damage the liver, but there is also a role for the immune system in DILI. Based on this knowledge, we tested in Chapter 2 the synergy hypothesis: simultaneous exposure to a drug that can cause DILI and a cytokine that promotes inflammation is more harmful to liver cells that are grown in a culture dish, than either of the two components alone. We tested this hypothesis for the model-drug diclofenac, the safe control drug naproxen and combined these with the cytokine TNFα: tumor necrosis factor α. Using this model we could confirm our hypothesis, TNFα indeed enhanced the cell-death response when combined diclofenac, but not by itself or when combined with naproxen. In this chapter we further show that this effect occurs because diclofenac sensitizes the liver cells to the dark side of TNFα, rather than the other way around. TNFα namely stimulates both pro-death as well as pro-survival signals in a cell. Under normal conditions, the balance between these opposites is favored towards survival. However, diclofenac shifts this balance by inhibiting the pro-survival signaling and thus promotes the cell-death response, resulting in enhanced liver cell damage.
The formation of drug metabolites induces stress in liver cells. In Chapter 3 we demonstrate the combination of three imaging methods to address the liver-injury potential of drugs. First, drug exposure may cause so called oxidative stress. Several drugs, including those that can cause unexplainable DILI, were tested in a imaging-based system to be able to detect this type of stress. This showed that many drugs, including diclofenac, cause oxidative stress. It is known that this might affect the survival-response to TNFα and therefore the activation of this response was monitored. The induction of oxidative stress by itself did not accurately predict the effect on the survival response. However, when this information was combined with the third assay, the cell-death assay to investigate the potential of TNFα to enhance the drug-induced liver cell death, we found that the drugs that both induced oxidative stress and inhibited the TNFα-induced survival signaling, caused more cell death. This demonstrated that a combination of readouts can be used to indicate the liver injury-inducing potential of drugs.

Since the induction of oxidative stress by drugs alone does not fully predict the enhancement of liver cell injury when combined with TNFα, other stress pathways must be involved. In Chapter 4 we took a gene expression approach to identify the types of signaling that are induced upon drug exposure. For the analysis we focused on two drugs whose toxicity is exceptionally enhanced when combined with TNFα: diclofenac and carbamazepine. Using signaling pathway analysis software we investigated the stress types that are induced by these drugs. This confirmed our earlier findings that these two drugs induce oxidative stress, but additionally indicated that they both affect genes controlling the protein synthesis machinery. Especially one gene, EIF4A1, was enhanced in expression under diclofenac and carbamazepine conditions, but not by drugs whose toxicity is not enhanced by TNFα. Interestingly, preventing this protein from being expressed reduced the amount of dying cells under these conditions. This shows its vital importance in the TNFα-enhanced drug-induced cell death response. Potentially, this gene could be used as a marker for the liver injury potential of drugs. Additionally, it might serve as a marker that can be used in an imaging setup, next to the oxidative stress reporter presented in chapter 3, to identify drugs that cause severe DILI.

In chapter 2 we reported that diclofenac leads to a shift in the survival response to TNFα to favor cell death. Diclofenac does this by inhibiting the activity of the TNFα-responsive transcription factor NF-κB, and thereby prevents the induction of pro-survival genes. In Chapter 5 we systematically investigated the role of individual proteins in the response dynamics of NF-κB. NF-κB needs to move in and out of the nucleus in a pre-determined manner in order to induce the appropriate genetic response. We identified several novel proteins as being important in controlling this movement, and were able to show that this had a direct impact on the cell death response under drug and TNFα conditions. This expansion of the knowledge about NF-κB regulation is not only important for understanding adverse drug reactions, but also in relation to cell growth and inflammation, which are responses important in areas such as cancer and chronic inflammatory diseases.
In summary, the work presented in this thesis provides a deeper understanding of how adverse drug reactions occur in the liver and emphasized on the role of inflammation in this process. The knowledge we gathered can be used to design and develop novel and better tests for toxicity assessment during drug development and I think that the work presented in chapter 5, the NF-κB screen, will prove useful to other fields of research, including cancer.