

# Role of the toll-like receptor-adapter protein TIRAP in drug-induced liver toxicity

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## Abstract

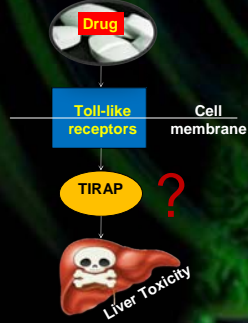
Drug-Induced Liver Injury (DILI) has become the rising health problem in the United States and the major reason for drugs removal from the market. DILI is known to be associated with inflammation. Inflammatory responses in liver are mediated by toll-like receptors and the intracellular adaptor proteins, including TIRAP. The goal of this research is to investigate the role of the toll-like receptor-adapter protein TIRAP in mediating the toxicity of drugs in the liver. We hypothesize that the toxicity of these drugs is mediated by TIRAP. In order to test this hypothesis, liver cells isolated from TIRAP wild-type mice and TIRAP knock-out mice were treated separately with Acetaminophen (APAP) or Chlorpromazine (CPZ) or Troglitazone (TGZ), followed by an enzyme assay to examine drug toxicity. The level of enzyme released upon cell lysis in TIRAP knock-out is lower than in TIRAP wild-type, which means the toxicity of drugs in the absence of TIRAP is reduced. The toll-like receptor-adapter protein TIRAP is likely to involve in drug-induced liver toxicity.

## Introduction

- Besides helping us to overcome diseases, some drugs have serious side effects that cannot be neglected. According to the FDA data in 2008, Drug Induced Liver Injury is the leading cause of acute liver failure in the United States and the main reason for drugs to be withdrawn from the market.
- The three drugs used for this study are Acetaminophen, Chlorpromazine and Troglitazone. Acetaminophen, which used for pain and fever reliever, has accounted for one-third of acute liver damage cases due to its toxicity of high doses. Chlorpromazine is an antipsychotic drug which is known to cause liver injury in certain individuals. Troglitazone had been approved for diabetes treatment by the FDA, then it was withdrawn from the market in the year 2000 for causing severe damage to liver.
- Studying about the mechanism of drug toxicity in liver tissues is necessary not only to protect the health of consumers but also to prevent the loss of pharmaceutical industry resulted from drugs withdrawal.

## Background

- The Toll-like receptor signaling pathway are involved in mediating inflammatory responses in the liver.
- Several studies have shown that inflammatory mediators are involved in drug-induced liver damage.
- TIRAP is one of the toll-like receptor adaptor proteins and a component of innate immune system. TIRAP is involved in mediating inflammatory responses; however, it is not known whether it is involved in mediating drug-induced hepatotoxicity.



## Hypothesis

The toll-like receptor adapter protein TIRAP is one of the contributing factors to the toxicity of drugs in liver.

## Materials and Methods

- Hepatocytes, or liver cells, were isolated from TIRAP knock-out (TIRAP<sup>-/-</sup>), or TIRAP wild-type mice (TIRAP<sup>+/+</sup>) and cultured into 96-well-culture-plates with concentration of 30,000 cells per well.
- After 24h, the cells were treated with Acetaminophen (0.1, 1, 5, 10, 15 mM or DMSO as control), or Chlorpromazine (5, 10, 15, 20, 40 μM or Saline for control), or Troglitazone (10, 20, 40, 80, 100 μM or DMSO for control)
- Prior to cells collection the next day, Triton X-100 was added into 2 out of 96 wells. Alanine aminotransferase assay (ALT) was performed to measure the concentration of the enzyme released by dead cells into the medium.
- Percent ALT values for the control samples treated with only DMSO or Saline were set to 100%. Percent ALT change due to drugs treatment were compared to the control values.

## Results

Figure 1: Acetaminophen toxicity in hepatocytes

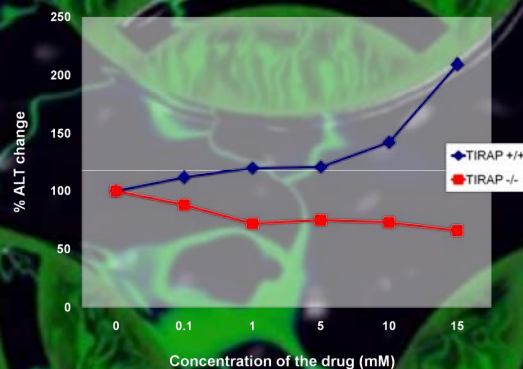
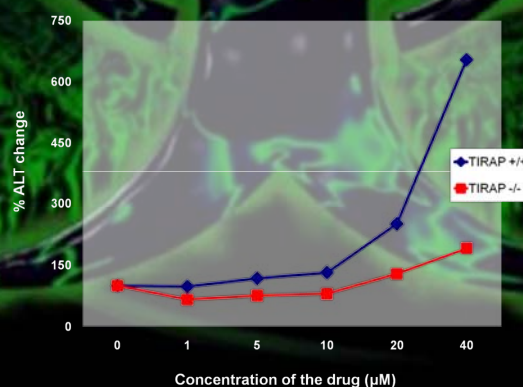
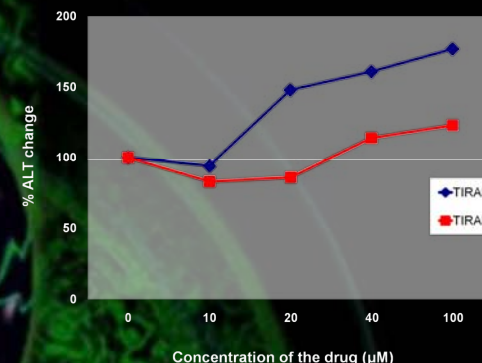


Figure 2: Chlopramazine toxicity in hepatocytes



## Results

Figure 3: Troglitazone toxicity in hepatocytes



## Discussion:

The toxicity of the drugs is measured by the concentration of ALT enzyme present in the medium environment. The more toxic the drugs are, the more cells break down and the more enzyme escapes from cells into the medium environment. The amount of ALT released into the medium is expressed as a percentage of total ALT inside and outside cells. The adding of Triton X-100 into two wells prior to collection served as positive control for maximum cell lysis and maximum enzyme released. The samples treated with DMSO or Saline (no drug) served as negative control and were set to 100%. Percent ALT change due to increasing concentration of drugs treatment were compared to this control value. In the experiments with three drugs, the percent ALT change upon treatment with drugs appeared to be lower in liver cells of TIRAP<sup>-/-</sup> than those of TIRAP<sup>+/+</sup>. That is, the toxicity of the drugs is reduced when the adaptor protein TIRAP is not present.

## Conclusion

- The research shows that the toll-like receptor adaptor protein TIRAP is involved in mediating the toxic responses in liver cells to selected drugs known to cause liver damage (APAP, CPZ and TGZ).
- The toll-like receptor adaptor protein TIRAP is likely to affect liver toxicity induced by additional hepatotoxic drugs.
- More experiments need to be conducted in order to determined the role of TIRAP in drug-induced liver toxicity. Future experiments should be performed not only in isolated liver cells but also in live animals and more hepatotoxic drugs need to be tested for accurate result.

## Acknowledgement

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