SUMMARY

Glutathione is the most abundant intracellular non-protein thiol. This tripeptide, which occurs as the reduced (GSH) and the oxidized (GSSG) form, belongs among the most potent antioxidants. The aim of present work was to introduce and optimize a glutathione assay that, in comparison to mostly used HPLC methods, would possess low cost and short time of the measurement. We chose the spectrofluorimetric method based on detection of a reaction product between GSH and o-phthaldialdehyde. After optimization, we gained following analytical parameters: GSH (calibration: R^2=1, 0-500 µmol/l; intra-assay: CV%<<5%), GSSG (calibration: R^2=0.99, 0-200 µmol/l; intra-assay: CV%<5%). We tested the levels of both GSH and GSSG by optimized method and by HPLC/FL method. We found very strong correlation, r = 0.99. We conclude, we established optimized glutathione assay which possessed high specificity, sensitivity, accuracy and, in additon, is comparable to HPLC method.

Another aim of our work was to evaluate the acetaminophen (AAP) toxic influence *in vitro*. The cause of AAP toxicity is found in metabolic activation to NAPQI (N-acetyl-p-benzoquinone imine). This compound reacts with GSH to form AAP-GSH and thus it results in GSH depletion. In AAP overdose, the necrosis of hepatocytes appears and it may lead to acute liver failure. Despite a number of studies, the mechanism of AAP injury remains still unknown in part.

We used rat hepatocytes incubated with AAP (1-20 mmol/l) during 1-24 h time period. Using WST-1, we found time dependent decrease in cell viability in all AAP concentrations. These results were confirmed by LDH test in cells treated with the highest AAP doses. Except of other assays, we tested also the changes of glutathione reductase (GR) activity. Interestingly, we proved the dose dependent decrease of GR activity in all AAP treated cells already after 3 h of treatment. Consequently, we studied the mechanism of GR inhibition and we discovered a significant influence of an AAP metabolite that was considered to be harmless till now. We prepared and tested the influence of this compound and we proved strong inhibition of hepatocyte GR, i.e. by 97% compared to control. Our results provide absolutely new advices into AAP toxicity and lead to new consequences in description of AAP toxic causation.