SUMMARY

Liver damage has a variety of causes. Due to the role of liver in the metabolism of xenobiotics, liver damage is often caused by toxic substances. Despite the variety of causes, the mechanisms of damage may be common. Most often, oxidative stress is induced in the cell or important organelles are directly damaged. This leads to cell death, the initiation of an inflammatory response and the development of fibrosis. A major problem in Western civilization is Non Alcoholic Fatty Liver Disease (NAFLD), in which fat is stored in liver tissue. Steatotic hepatocytes may be more sensitive to damage compared to non-fatty ones.

The study of hepatotoxicity and possible hepatoprotection requires suitable models for *in vitro* experiments. The first part of this work is therefore dedicated to the introduction of a model background using hepatocytes isolated from rat liver. **First, we introduced a method of culturing hepatocytes in a sandwich and evaluated its benefit over cultivation in a *monolayer***, even for experiments lasting 48 hours, which are frequently used in our laboratory. Significant differences were recorded from day 4 of culture, when the sandwich model provides better preservation of morphological and functional parameters of hepatocytes. However, for our purposes of studying the mechanisms of hepatotoxicity and hepatoprotective effects on cultures up to 48 hours old, a *monolayer* culture methodology should suffice.

We also introduced an *in vitro* model of steatosis induced by the addition of oleic acid (OA), palmitic acid (PA) and their mixtures to the culture medium. In primary culture of rat hepatocytes **in *monolayer***, each of the two fatty acids used can induce a significant increase in triacylglycerol (TAG) content, which, however, is accompanied by a significant cytotoxic (lipotoxic) effect in the case of PA. The use of a mixture of OA and PA is accompanied by the development of steatosis associated with lower cytotoxicity and better preserved functional capacity of hepatocytes. When choosing a model, it is necessary to take into account its use and the situation to be simulated. It is appropriate to use a model with OA alone, or OA in combination with PA to induce simple steatosis while maintaining a high degree of cell viability A model using PA can be chosen to induce steatosis accompanied by apoptosis and cytotoxicity.

In the second part of this work, we verified, using a model of hepatocytes cultured in *monolayer*, whether incubation of hepatocytes with epigallocatechin gallate (EGCG)

shows a protective effect against damage by model hepatotoxin D-galactosamine (D-GalN). EGCG belongs to plant polyphenols, an important source of which is green tea. Many of its effects are described, due to which it has the potential to have a hepatoprotective and antisteatotic effect. Only preventive incubation with EGCG showed a protective effect, while co-administration of EGCG with D-GalN failed. Reduction of lipid peroxidation from the lipid bilayer may have contributed to the protection of the plasmatic membrane. Although a positive effect of EGCG on GSH production cannot be ruled out, the oxidative stress induced in our work probably exceeded the ability of antioxidant mechanisms to reverse GSH deficiency in hepatocytes. A promising strategy for a possible enhancement of the hepatoprotective effect of EGCG could be to prolong the preventive incubation.

In the next step, we evaluated the susceptibility of steatotic hepatocytes to D-GalN damage in a currently established *in vitro* model of steatosis. Our results confirm a higher sensitivity, the degree of sensitivity depends on the presence of PA in the steatogenic medium. It can be speculated that oxidative stress and mitochondrial dysfunction, which PA significantly supports, play a role. The protection of OA against the cytotoxic effects of PA probably did not apply in this case, but the mechanism needs to be further investigated. We have verified that our established model of steatosis induced *in vitro* can be used to study hepatotoxicity.

Using an established *in vitro* model of steatosis, we also tested the potential protective effect of EGCG on the development of steatosis and lipotoxicity. EGCG reduced the lipotoxic effect. A decrease in oxidative stress was observed even in non-steatotic hepatocytes. However, the effect of EGCG on the fat content of hepatocytes alone is not clear in our work. A decrease of TAG was observed only in the development of OA-induced steatosis, when PA was present in a mixture with OA or alone, no decrease was observed. In this regard, studies are needed to provide further information on the effect of EGCG on the regulation of lipid metabolism with respect to the fatty acids used. Furthermore, we observed a proapoptotic effect of EGCG not only on steatotic hepatocytes, but surprisingly also on non-fatty hepatocytes. These findings also require further research.