9 SUMMARY

Liver is the main target organ for toxic effect of various compounds. This is due to a number of reasons, but it is generally related to drug clearance and metabolism. Hepatotoxins may react with the basic cellular constituents and induce almost all types of lesion of the liver. Isolated hepatocytes are a suitable model system for toxicity testing of xenobiotics.

Mitochondria and mitochondrial energy providing system serve as a common target for hepatotoxic drugs. Experiments on isolated mitochondria are thus also useful model system for evaluation of the toxic effect of various hepatotoxins. Oxidative stress is one of the most important mechanisms through which hepatotoxins induce cell death. Effective protection of cellular damage induced by oxidants requires more information about reactions involved in this process. Tert-butylhydroperoxide (tBHP) has been widely used as a model compound to mimic the effect of oxidative stress in various cell types. This organic hydroperoxide is metabolized in cells into free radicals which cause cellular injury. S-adenosyl-L-methionine (SAMe) is involved in transmethylation and transsulfuration reactions including synthesis of glutathione. Acute toxicity studies in animals have shown that SAMe is capable to prevent damage induced by various hepatotoxins. The aim of this study was to characterize toxic injury of isolated rat hepatocytes and mitochondria induced by tBHP and to evaluate a possible protective effect of SAMe against oxidative damage of hepatocytes.

Hepatocytes were isolated from male Wistar rats by two-step collagenase perfusion. A portion of cells was used for measurement of oxygen consumption (Oxygraph Oroboros-2k, Aut) and for evaluation of mitochondrial membrane potential (MMP) by tetraphenylphosphonium (TPP^+) selective electrode. Remaining hepatocytes were cultivated in suspension or on collagen coated Petri dishes. Changes of morphological features were observed using phase contrast microscopy (Olympus CK 40). The rate of hepatocytes injury was evaluated by lactate dehydrogenase (LDH) activity in the culture medium. The level of lipid peroxidation was determined from malondialdehyde production. Mitochondrial membrane potential was evaluated by measuring the accumulation of Rhodamine 123 (Rho123) and from uptake of fluorescence probe JC-1. Redox state of cells was assessed by GSH and GSSG content. Mitochondria were isolated from rat liver by differential centrifugation. Mitochondrial ROS production was monitored by fluorescence emission of CM-H_2DCFDA (FDA) and mitochondrial swelling was measured spectrophotometrically by monitoring of the decrease in absorbance at 520 nm.

Tert-butylhydroperoxide increases ROS production and lipoperoxidation which precedes LDH leakage, and decreases activity of respiratory Complex I and Complex II, MMP and GSH/GSSG. These changes are in proportional relation to the tBHP concentration and to the time of incubation. Respiratory Complex I activity is significantly more sensitive to the peroxidative action of tBHP than the activity of Complex II. We also found that the mechanism of the tBHP effect on mitochondrial membrane potential depends on the respiratory substrates and we can suppose at least two different mechanisms. The first is inhibition of respiratory Complex I and the second one is opening of mitochondrial permeability transition pore. We found that SAMe presents protective effect against toxic injury of rat hepatocytes induced by tBHP. Since the protective effect of SAMe against GSH depletion was not confirmed, it seems that this effect is ascribed more to transmethylation reactions than to transsulfurations.