Abstract

A synthetic estrogen 17α-ethinylestradiol (EE2) is the main active component of the hormonal contraceptive pills. The rise of consumption of hormonal contraceptives has increased the risk of the back negative effects of EE2 to aquatic organisms. EE2 belongs to the endocrine disruptive compounds known for mimicking natural hormones. A more detailed examination of the transformation of this compound *in vivo* and *in vitro* can contribute to a better understanding of its negative effects. This master thesis is therefore devoted to the study of the metabolism of EE2 in two selected model organisms.

The ligninolytic fungus *Pleurotus ostreatus* is the type of fungi with promising biodegradation ability to a lot of pollutants. These properties have led to numerous studies of the degradation potential of *P. ostreatus* towards EE2, with the possibility of removing this compound from the environment. EE2 has been degraded by the fungus *P. ostreatus in vivo* resulting in one hydroxylated metabolite, which estrogenic activity is in need for further study. *In vitro* studies were carried out with a microsomal fraction isolated from the mycelium of this fungus. The conversion of EE2 *in vitro* via CYPs dependent on NADPH has not been demonstrated, however using KHP as a cofactor, there was one metabolite of EE2 found, suggesting a possible peroxidase activity of microsomal enzymes.

The second approach uses laboratory rat as a model for EE2 metabolism in human body. The object of this part of thesis was to verify the involvement of CYP in the metabolism of EE2 and determine the specific isoforms using specific inducers/inhibitors of CYP and rat recombinant systems. Metabolites of EE2 were separated using high pressure-liquid chromatography and identified by mass spectrometry. Rat hepatic microsomes metabolized EE2 to two hydroxylated EE2 derivatives. Microsomes premedicated by inductors of CYP2B, 2C, 2E and 3A were efficient in the transformation of EE2. Studies with the inhibitors confirmed the role of CYP2E, 2B and 3A in the EE2 metabolism and rat recombinant CYPs showed on the role of CYP2A. Furthermore, a possible influence of EE2 on the metabolism of two natural hormones, progesterone and testosterone, was monitored. Results show that EE2 itself inhibits CYP3A and 2C activity, while its metabolites could modulate the activity of CYP2C. These obtained results are useful for understanding the transformation of EE2.

Keywords: 17α-ethinylestradiol, Cytochrome P450, Endocrine disruptor, *Pleurotus ostreatus*, Laboratory rat, Metabolism **(In Czech)**