Abstract

Vandetanib, lenvatinib and cabozantinib are inhibitors of receptor tyrosine kinases approved to treat locally advanced or metastatic thyroid gland, kidney and liver cancers. These multi-kinase inhibitors, inhibit phosphorylation of tyrosine moieties of protein, thus modulate cell signalization in cancer cells. Metabolites of vandetanib, lenvatinib and cabozantinib were detected *in vitro* as well as *in vivo* in blood and urine. Cytochromes P450 and flavin monooxygenases were identified as primary enzymes participating in metabolism of these drugs. Literature lacks information regarding pharmacological efficacy of vandetanib, lenvatinib and cabozantinib metabolites.

The aim of this diploma thesis was the investigation of pharmacological efficacy of *N*-oxides of vandetanib, lenvatinib and cabozantinib. The viability measurement under normoxic and hypoxic conditions was employed to determined their efficacy. The expression of enzymes of the first phase of xenobiotics metabolism (CYP 450 1A1, 1B1, 3A4 a CYP 450 oxidoreductase) and receptor tyrosine kinases RET and VEGFR2, as well as mechanism of changes in their expression were investigated using western blotting and flow cytometry. High performance liquid chromatography was utilised to investigate possible metabolism of tyrosine kinase inhibitors and their *N*-oxides.

Tyrosine kinase inhibitors exhibit increased toxicity under hypoxic conditions in comparison to normoxic conditions. Metabolites were highly significantly less effective than their parental molecules, irrespectively of oxygen content. Tyrosine kinase inhibitors decreased expression of VEGFR2 but not RET. Hypoxia decreased VEGFR2 expression and proved synergy with decrease caused by lenvatinib. Inductors of autophagy decreased VEGFR2 in the same manner as lenvatinib. In contrary autophagy inhibitors prevented the decrease of VEGFR2 by lenvatinib. Results indicate possibility that autophagy is responsible for decreased VEGFR2 expression. Metabolites of tyrosine kinase inhibitors were not detected in cultivation medias and expression of biotransformation enzymes was not altered.

Overall results indicate lower pharmacological efficacy of tyrosine kinase inhibitor metabolites. Based on results showed in this thesis tyrosine kinase inhibitors may increase autophagy thus decrease expression of VEGFR2.

Key words: tyrosine kinase inhibitors, pharmacological efficacy, metabolites VEGFR2, RET, autophagy, hypoxia [IN CZECH]