ABSTRACT

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Title of Thesis: Analytical and bioanalytical evaluation of novel potential drugs from the group of iron chelators

High performance liquid chromatography (HPLC) gained its unprecedented position among bioanalytical techniques due to its effectivity and versatility. If tuned properly, it can separate complex mixtures and can be used for qualitative and quantitative analysis simultaneously, usually within a short time. Reliability of the HPLC methods is proven by their validation performed according to accepted guidelines.

Iron chelation is considered to be an effective concept of treatment of various pathologies – from iron overload disease to cancer or neurodegenerative disorders. To develop new chelators for these indications it is important to investigate their structure-activity relationship, the fate of the drug in organism and its relationship to biological effects and toxicity etc. Modern analytical methods are essential tools for these studies.

The first part of this work presents validated LC-UV and LC-MS methods for assay of aroylhydrazone iron chelators and related pro-drugs in biological materials. In the study, the structure-activity relationship in the group of the aroylhydrazones was examined. We proved that alkyl substitution of hydrogen in the vicinity of the hydrazone moiety is key in enhancing their chemical stability due to steric hindrance of the hydrazone bond.

Additionally, utilising developed analytical methods, we proved in further experiments that: 1) an oxidation-activated prodrug BSIH is more stable *in vitro* and has better pharmacokinetic properties *in vivo* than its active form SIH, 2) BSIH is less toxic and protects cells from hydrogen peroxide induced damage, 3) BSIH decomposes to SIH but also to salicylaldehyde which augments the protective effect of the drug.

The next study was focused on the prochelator BHAPI, based on aroylhydrazone with improved stability in plasma (compared to SIH) – HAPI. The study proved that the prochelator is equimolarly activated to HAPI and both BHAPI and its active form reduce

the toxicity induced by catecholamines and their oxidative products. These results show the concept of boronated prodrugs to be a promising approach for development of new effective chelating therapeutics.

The second part of this work deals with systematic assessment of metabolism and pharmacokinetics of the cardioprotective drug dexrazoxane (DEX) and its metabolite (ADR-925). A validated LC-MS method was utilised in this study to analyse samples from both *in vitro* and *in vivo* experiments. Recorded data were processed in population study. Our results show that the experimental model utilising rabbits for cardioprotection studies provides high similarity in pharmacokinetics to clinical models. This work challenges the hypothesis that the cardioprotective effect of DEX is caused by its iron chelating metabolite – ADR-925, which should hamper Fenton chemistry in the heart. In addition, the current data corroborate the modern theory that parent compound DEX may protect the heart from anthracycline induced cardiotoxicity via inhibition of topoisomerase 2β.

This new hypothesis is also augmented by the last paper included in this dissertation. It is focused on a DEX analogue JR-311 (where one methyl group was replaced by an oxo-group). New LC-MS method was developed to investigate stability, metabolism and penetration to cardiomyocytes of JR-311. The study revealed that JR-311 is quickly hydrolysed to an ADR-925-like compound – which leads to a complete loss of its protective effect. This finding pointed at the need for significant modification of design of *in vitro* experiments. The modified *in vitro* study showed that JR-311 interacts with TOP2B as well as it protects the heart cells from anthracycline induced damage. Study showed that the presented structural modification does not hamper the activity of JR-311, but future development should be focused on more stable DEX analogues.