Doktorant: Karol Jaroch

studia doktoranckie z zakresu nauk farmaceutycznych

Katedra Farmakodynamiki i Farmakologii Molekularnej

Promotor: dr hab. n. farm. Barbara Bojko

Tytuł rozprawy doktorskiej: Badania cytotoksyczności i metabolizmu kombretastatyny A4 oraz analizy metabolomiczne linii komórkowych z wykorzystaniem nowatorskich metod mikroekstrakcyjnych

Summary

Pre-clinical studies, including *in vitro* studies using cellular models and metabolism studies, play an important role in the study of new drug candidates. The results of these studies are used to estimate the patients' responses to a potential drug and are a key step limiting the entry of substances into the study using laboratory animals and subsequently clinical trials.

The search for modern analytical methods to obtain in-depth information is still ongoing, as opposed to standard cytotoxic research, concerning biochemical changes of cells after induction with new drug candidates. At the same time, the development of new extraction techniques that avoid the interference from components of the biological sample when used for mass spectrometers are an efficient and modern solution for the analysis of new drug candidates.

In the present study, Solid Phase Microextraction (SPME) was used in combination with high resolution mass spectrometry to analyze metabolomic changes in cell cultures under the action of the drug, and the course of metabolism reactions of this drug using microsomal enzymes.

The SPME in the form of microfibers was used to assess the effect of combretastatin A4 (CA4) on the non-small cell lung cancer cell line using untargeted metabolomics. Drug metabolism assessment was carried out with the use of multiple tools: *in silico* prediction, electrochemical reaction and rat liver microsomes (RLM) analyzed via protein precipitation (PP) and SPME.

The principal component analysis showed difference between cells exposed and unexposed to CA4. Employment of SPME for cell culture assessment was performed directly in 96-well plate where amount of sample is highly limited. The most important metabolomics changes were reflected in the levels of both intracellular and extracellular amino acids, low molecular mass acid and amides. The main path of CA4 metabolism is O-demethylation and aromatic hydroxylation. There was no differences found between PP and SPME analysis of RLM but more efficient sample clean-up and possibility of time course study with SPME makes the technology highly recommended for further metabolism analysis.

Due to no sample collection and non-depletive extraction SPME is a valid tool for *in vitro* metabolomic analysis of samples of limited volume. Thus, after conducting the desired number of measurements with SPME, it is possible to use conventional testing methods for cell lines. The possibility of producing SPME probes with different lengths of coverage with the extraction phase allows flexibility of application. The SPME specifics for *in vivo* testing in animals and humans, combined with *in vitro* tests give strong indications for the use of the described technology as a promising tool in *in vitro-in-vivo* extrapolation studies.