Endothelial toxicity profiling of anti-cancer chemotherapy

Tomasz Wojcik, Ewa Szczesny, Katarzyna Majzner, Justyna Miszczyk, Malgorzata Lukawska, Irenea Oszczapowicz, Malgorzata Baranska, Wojciech Kwiatek, Stefan Chlopicki, Tomasz Wojcik, Ewa Szczesny, Katarzyna Majzner, Justyna Miszczyk, Malgorzata Lukawska, Irenea Oszczapowicz, Malgorzata Baranska, Wojciech Kwiatek, Stefan Chlopicki

Jagiellonian Centre for Experimental Therapeutics (JCET), Krakow, Poland; Faculty of Chemistry, Jagiellonian University, Jagiellonska 3, 30-068 Krakow, Poland; Department of Experimental Physics of Complex Systems, The H. Niewodniczański Institute of Nuclear Physics Polish Academy of Sciences, Krakow, Poland; Institute of Biotechnology and Antibiotics, 5 Starosinska, 02-516 Warsaw, Poland; Department of Experimental Pharmacology, Chair of Pharmacology, Jagiellonian University, Medical College, Grzegorzecka 16, Krakow, Poland.

Introduction
Cardiovascular toxicity is major reason for drug attrition during preclinical phases of drug development and could be due to vascular damage, induced by a direct endothelial damage, activation of coagulation fac tors or vascular inflammation. Since endothelial dysfunction constitutes an integrative response predictive for future cardiovascular events, we aimed to develop a comprehensive panel of endothelial cytotoxicity screening assays based on high-content screening (HCS) automated fluorescence microscopy, Raman microspectroscopy, chemiluminescence, and biochemical analysis. Using this approach we screened a group of classical and targeted chemotherapeutics.

Endothelial toxicity of anthracyclines and kinases inhibitors
Anthracyclines have been for several decades the most studied agents because of their known cardiovascular effects and relatively high incidence of heart failure. However, cancer patients are currently treated with newer chemotherapeutics such as tyrosine kinase inhibitors or cyclin-dependent kinases inhibitors that are also responsible of causing cardiovascular toxicities. The type of cardiovascular toxicity associated with these newer agents (type II) appears to be different that caused by anthracyclines (type I) [3]. Thus, we examined the effects of these newer agents on endothelial cells, using HCS fluorescence microscopy.

Effects of anthracyclines on nuclear area (chirarchical clustering)
Nuclear area of endothelial cells stained with Hoechst 33342 seems to be simple and accurate of nuclear accumulation of anthracyclines. In order to test if epimers of doxorubicin (EDOX) and daunorubicin (EDRN) accumulates in lower amounts than doxorubicin (DOX) and daunorubicin (DNR), cells were treated with these drugs for 24 hours, doxetaxel (DOCE) and 5-fluorouracil (5-FU) were used as negative controls in this assay. Obtained results were analysed using chierarchical clustering module of Spotfire software (Perkin Elmer).

Fosfolipidosis - neutral lipids (LipidTOX)
We demonstrated that anthracyclines (e.g. Doxorubicin, Daunorubicin) accumulate in the nucleus, causing nuclei swelling, genotoxicity as well as pro-inflammatory endthelium phenotype in nanomolar concentrations. Anthracycline epimers were less toxic (e.g. Epidoxorubicin, Epidaunorubicin). Nonspecific cycline-dependent kinase inhibitors (Dinaciclib, Flavopiridol), displayed high genotoxicity as well as pro-inflammatory endothelium phenotype in nanomolar concentrations. Anthracycline epimers were less toxic (e.g. Epidoxorubicin, Epidaunorubicin). Nonspecific cycline-dependent kinase inhibitors (Dinaciclib, Flavopiridol), displayed high genotoxicity as well as pro-inflammatory endothelium phenotype in nanomolar concentrations.

Conclusions
We demonstrated that anthracyclines (e.g. Doxorubicin, Daunorubicin) accumulate in the nucleus, causing nuclei swelling, genotoxicity as well as pro-inflammatory endthelium phenotype in nanomolar concentrations. Anthracycline epimers were less toxic (e.g. Epidoxorubicin, Epidaunorubicin). Nonspecific cycline-dependent kinase inhibitors (Dinaciclib, Flavopiridol), displayed high genotoxicity as well as pro-inflammatory endothelium phenotype in nanomolar concentrations. Anthracycline epimers were less toxic (e.g. Epidoxorubicin, Epidaunorubicin). Nonspecific cycline-dependent kinase inhibitors (Dinaciclib, Flavopiridol), displayed high genotoxicity as well as pro-inflammatory endothelium phenotype in nanomolar concentrations.

References:
[1] Laverty HG et al. (2011) How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? Br J Pharmacol 163(4); 675-693