Abstract

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Title of Doctoral Thesis: **Interactions of cyclin-dependent kinase inhibitors with ABC drug transporters *in vitro* and *in situ***

In the present work, we focused on the study of pharmacokinetic interactions of cyclin-dependent kinase inhibitors (CDKi) with drug efflux transporters breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp). Using accumulation and transport methods with MDCKII-ABCG2 cells, we showed purvalanol A, olomoucine II, bohemine and roscovitine to inhibit BCRP. Employing accumulation method with MDCKII-ABCB1 cells, we further observed that purvalanol A potently inhibits P-gp and partial inhibition was recorded in the case of other studied CDKi (olomoucine II, roscovitine, flavopiridol, SNS-032). Transport method with the same cellular models was used for the study of substrate affinity of olomoucine II and purvalanol A when olomoucine II was determined to be a dual P-gp and BCRP substrate. Substrate affinity of purvalanol A toward any of the transporters tested was not observed. These findings were confirmed by the ATPase vesicular assay and using this experimental setup, we further showed that roscovitine is a P-gp substrate. Flavopiridol and SNS-032 seem to lack substrate affinity toward P-gp or to be P-gp substrates with a very low affinity. In the following part of this work we verified our *in vitro* results at organ level employing the method of dually perfused rat term placenta. We confirmed inhibition of the rat Bcrp by purvalanol A, olomoucine II, bohemine and roscovitine and substrate affinity of olomoucine II toward the rat P-gp as well as Bcrp. At the same time, we excluded the substrate affinity of purvalanol A toward both examined transporters. In the final part of the study, we tested our hypothesis that CDKi, which inhibit drug transporters, may increase the accumulation of concomitantly administered cytotoxic substrates and, at the same time, potentiate their effect by their own antiproliferative and cytotoxic activity. Combination index method of Chou-Talalay was employed for this purpose. Synergistic

or at least additive effects were observed in the combinations of olomoucine II or purvalanol A with mitoxantrone, an established BCRP substrate, in three cell lines which express considerable amounts of BCRP. Our hypothesis was further confirmed in the combinations of purvalanol A, roscovitine or olomoucine II with daunorubicin (P-gp substrate) when significantly higher synergistic effects of these combinations were observed in MDCKII-ABCB1 cells in comparison with parent cells. In the light of our results, it is possible to expect considerable impact of tested CDKi on the pharmacokinetic as well as pharmacodynamic behavior of concomitantly administered drugs, which are BCRP or P-gp substrates. This fact should be kept in mind in the case of possible introduction of CDKi into the clinical area. Our approach combining CDKi, which inhibit drug transporters, with the cytotoxic substrates of these transporters could, at least partially, help overcome the multidrug resistance which constitutes one of the main limitations in chemotherapy.