Pharmacological studies on uptake, metabolism, and resistance to anti-cancer drugs: insights into the treatment of leukemia

Academic Thesis
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Leukemia is blood cancer that begins in the bone marrow and characterized by abnormal production of white blood cells. Due to advances in treatment, there has been a dramatic increase in survival rate for patients with different types of leukemia. Among cytotoxic agents for leukemia, anthracyclines and thiopurines represent two highly effective groups of such therapy. Anthracyclines are potent broad-spectrum cytotoxic drugs used for treatment of numerous cancers, including acute myelogenous leukemia (AML). However, side effects like dose-limiting bone marrow toxicity and the characteristic cumulative cardiotoxic effects, limit their clinical applications. These side effects are mainly caused by uptake of the drugs by normal cells, and the mechanisms behind their cellular uptake are not completely understood. Knowledge about uptake mechanisms could be beneficial to increase the selectivity of these drugs.

Thiopurines like 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) have been used extensively in the treatment of acute lymphoblastic leukaemia (ALL). Despite their wide-spread use, intrinsic and acquired resistance to thiopurines is a major problem. Understanding the mechanisms of resistance to these drugs should help optimize treatment and improve the survival rate.

In this thesis we investigated mechanisms and factors playing role in resistance to these two anti-leukemic groups by studying transport and intracellular metabolism by different enzymes:

In Study I we studied the uptake patterns of, daunorubicin (DNR), doxorubicin (DOX), epirubicin (EPI), idarubicin (IDA), and pirarubicin (PIRA) by human leukemic HL-60 cells in the presence of various inhibitors and investigated the possible involvement of specific carriers. Our data support the involvement of nucleoside transporters (NTs) in transmembrane transport of DNR, IDA, and PIRA. The results also showed a strong inhibitory effect of suramin on anthracycline uptake and cytotoxicity which requires further study. The significant reduction of cellular anthracycline uptake at low temperature strongly supports energy dependent carrier mechanisms.

In Study II we employed high-through-put characterization of genetic aberrations, including both expression microarrays and array-CGH, to elucidate the mechanisms underlying acquisition of resistance to thiopurines by human acute T-lymphocytic leukemia MOLT4 cells in an attempt to identify new markers and genes that may serve as valuable drug targets in the future. The downregulation of the two nucleoside transporters, CNT3 and ENT2 in both 6-MP-and 6-TG-resistant cells indicates that impairment of the transport of these agents contribute to drug resistance. In addition, elevated expression of the human terminal transferase enzyme, encoded by the DNTT gene, was found in both 6-TG- and 6-MP-resistant cells as compared to the wild-type cells. Specific inhibitors of this enzyme might be developed into a novel class of antitumor agents.

In Study III we evaluated three different methods for thiopurine methyltransferase (TPMT) phenotyping, including an HPLC-based assay modified and optimized in our lab, which is able to measure TPMT activity in RBC of patients and leukemic cell lines with requirement of only one million cells. We found significant relationships between the three methods and the distribution pattern of TPMT activity in RBC from 198 patients as determined by radiochemical, HPLC-UV, and HPLC-radiometric methods showed the classical trimodal distribution. Furthermore, the results indicated that the activity of TPMT enzyme is not changed in 6-MP-and 6-TG-resistant MOLT4 cells.

In Study IV we knocked down the expression of the TPMT enzyme in human MOLT4 leukemia cells employing specifically designed siRNA, in order to investigate the potential role of TPMT in the metabolism and thus, cytotoxicity of 6-MP and 6-TG. Our results indicate a 34% increase in sensitivity of MOLT4 cells to 1 µM 6-TG after treatment with TPMT-targeting siRNA, as compared to cells transfected with non-targeting siRNA, while sensitivity of the cells toward 6-MP was not affected significantly. We concluded that TPMT has a differential role in cytotoxicity of 6-MP and 6-TG, and probably inhibition of de novo purine synthesis (DNPS) by methylthioinosine monophosphate (meTIMP) makes a significant contribution to the cytotoxic action of 6-MP.