MRS Ability to Predict Resistance Development to Imatinib Mesylate

J. Miljus[1], J. V. Melo[2], N. Anderson[1], L. Boros[3], S. G. Eckhardt[4], N. S. Serkova[1]

[1]Anesthesiology, University of Colorado Health, Denver, CO, United States, [2]Hematology, Imperial College, London, United Kingdom, [3]Pediatrics, UCLA, Los Angeles, CA, United States, [4]Medical Oncology, University of Colorado Health, Denver, CO, United States

Introduction. Chronic myelogenous leukemia (CML) is generated by a t(9;22) translocation that gives rise to the Philadelphia chromosome. This contains the fused *BCR-ABL* oncogene that encodes a tyrosine kinase fusion protein. Imatinib mesylate (Gleevec™) functions through competitive inhibition at the ATP-binding site of the Bcr-Abl enzyme, which leads to the inhibition of tyrosine phosphorylation of proteins involved in Bcr-Abl oncogenic signaling. The major concern regarding imatinib treatment is, to date, the development of drug resistance in CML patients. Although the correlation between the molecular mechanisms and imatinib efficacy in clinical trials is well established, there is no precise information about the changes in cell metabolism in CML cells during exposure to the drug. However, it has been suggested [1] that the control of glucose-substrate flux is an important mechanism of the antiproliferative action of imatinib since *BCR-ABL* positive cells express high-affinity GLUT-1 glucose transporter and have increased glucose uptake. Unlike solid-tumor patients, CML patients cannot undergo clinical PET evaluations on glucose metabolism. The goal of the present MRS study was to establish the metabolic signature of human CML cells with different sensitivity to imatinib. This MRS based protocol could be later applied for clinical metabolic monitoring of leukocytes, isolated from peripheral blood of CML patients, for early prediction of imatinib resistance development.

Materials and Methods. Two paired imatinib-sensitive and resistant clones of two CML cell lines, K562 and LAMA84, were used to evaluate metabolic effects of imatinib resistance. LAMA84-r cells are resistant due to Bcr-Abl and P-glycoprotein overexpression; the mechanism of resistance in K562-r cells is still unknown (may seems to be independent of Bcr-Abl). Resistant clones were grown in imatinb-containing medium (K562-r: 5μM; LAMA84-r: 1μM). Sensitive cells were incubated with a broad imatinib concentration range (0.1 to 10 μM) for up to 96 h. [1-^{13}C] glucose was added to all cells for the last 4h. Cells were subsequently extracted using 12% PCA. The extracts were analyzed by ^{1}H-, ^{13}C- and ^{31}P-MRS using a Bruker 500-DRX spectrometer. External TMSP and MDP standards were used for metabolite quantification in ^{1}H- and ^{31}P-MRS, respectively. For a pilot study on isolated leukocytes, 20 mL whole blood was collected from a healthy subject and leukocytes were isolated using Ficoll gradient centrifugation. Isolated leukocytes (9×10^{3} cells) were subsequently incubated in 10 mL of AIM-V medium containing 5 mmol/L [1-^{13}C] labeled glucose for 4 h. Due to the low cell count, leukocyte extracts were analyzed using a novel Bruker 1-mm microprobe (instead of the standard 5-mm probe). All incubation media were collected prior to the extraction and analyzed by ^{1}H- and ^{13}C-MRS for glucose uptake and lactate export.

Results. Imatinib decreased glucose uptake in the *BCR-ABL*-positive cells in a dose- and time-dependent fashion by switching from glycolysis to Krebs cycle metabolism and improving the cell energy state. Already after 24 h of incubation, sensitive cells, treated with the therapeutically relevant concentration of 1 μM imatinib, showed slightly increased mitochondrial TCA cycle (C4-glutamate signal increased to 137% and 114% vs. untreated K562-s and LAMA84-s, Table 1). Imatinib sensitive cells have highly decreased glucose uptake as well as decreased lactate export compared to untreated cells (Fig. 1 and Table 1). In resistant K562-r cells mitochondrial activity was significantly lower (C4-glutamate decreased to 47%, $p<0.001$, Table 1) with decreased citrate ($p<0.05$) and pyruvate ($p<0.05$) concentrations and decreased NTP/NDP-ratios (45%, vs. K562-s $p<0.05$). The *de novo* ^{13}C-lactate export into the media was highly increased in K562-r (168%, $p<0.05$, all compared to K562-s), with a subsequent increase in glucose uptake vs. that of sensitive cells (17.2 mmol/L/g compared to 13.6 mmol/L/g, $p<0.05$, Fig. 1). LAMA84-r showed the same metabolic pattern; however, the changes in this cell line were less pronounced. A significant decrease in phosphodiester concentrations, markers for membrane degradation, provides another metabolic end-point for imatinib resistance [21% of sensitive cells, $p<0.005$ (K562-r) and 55%, $p<0.05$ (LAMA84-r)]. On the other hand, phosphocholine, a marker for membrane biosynthesis, was increased to 293% in LAMA84-r vs. LAMA84-s ($p<0.001$). The important metabolic end-points, which were established in K562-r and LAMA84-r cells (glucose metabolism, energy state, and phospholipid synthesis/catabolism), can be also assessed in human leukocytes from a small blood sample volume (Table 1).

Discussion. Despite their different mechanisms of imatinib resistance, K562-r and LAMA84-r cells showed a metabolic profile of the resistant phenotype with similar changes compared to their sensitive counterparts. Mitochondrial metabolism and energy state were decreased significantly in K562-r ($p<0.05$), while non-oxidative metabolism and glucose uptake were increased. These changes were similar but less pronounced in LAMA84-r. We therefore conclude that the failure of imatinib in inhibiting cytosolic glycolysis and improving mitochondrial metabolism, as well as maintaining high phosphocholine and low phopshodiester concentrations, could serve as a metabolic signature of imatinib resistance. Furthermore, the use of a Bruker 1mm microprobe opens a more convenient way to measure and quantify samples with a very small volume/weight/cell count. MRS-based quantitative analysis of small volumes of blood can be used as a clinical assay for early detection of treatment failure in CML patients, for whom no clinical metabolic PET protocols exist.

	Sensitive untreated	Sensitive +imatinib	Resistant	Leukocytes
[NTP/NDP]	3.85	6.47	3.17	4.77
C1-Glucose	n.d.	n.d.	n.d.	0.95
C3-Lactate	2.45	1.41	2.33	0.17
C4-Glutamate	1.05	1.42	0.68	0.23
[TCA/Glycolysis]	0.43	1.01	0.29	2.71
[Glc/Lactate]extrac	0.73	2.68	0.50	8.77
[PME/PDE]	1.46	1.21	9.85	1.33

Table 1: Absolute concentrations of selected metabolites [μmol/g] in human CML (n=3) and isolated leukocytes (n=1) after incubation with 5mM 1-^{13}C-Glucose for 4 h. The leukocytes spectra have been obtained with less then 10^{4} cells using a 1mm probe

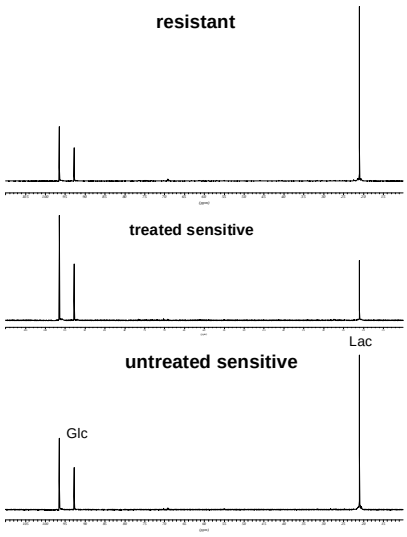

Figure 1: 13C-MRS on incubation media

References:
[1] Boros et al. N Engl J Med **347:** 67-68, 2002