ORIGINAL ARTICLE

Imatinib mesylate affects tyrosine kinase activity in both leukemic and normal primary mononuclear blood cells

Kateřina Kuželová, Dana Grebeňová, Michaela Pluskalová, Iuri Marinov, Hana Klamová, Zbyněk Hrkal

Institute of Haematology and Blood Transfusion, U Nemocnice 1, 128 20 Praha 2, Czech Republic

Summary

Tyrosine kinase (TK) activity in primary mononuclear blood cells (MNBC) derived from chronic myelogenous leukemia (CML) patients in the chronic phase as well as from healthy donors was measured by a sensitive time-resolved fluorescence method using the Delfia[®] Tyrosine Kinase kit. The level of phosphotyrosine was assessed in parallel by flow-cytometry. The experimental protocol for Delfia[®] was optimized using a K562 cell line. A large part (20 to 50%) of the fluorescence signal from K562 cells was sensitive to Imatinib mesylate, an inhibitor of Bcr-Abl tyrosine kinase, which is currently the leading drug in CML treatment. In primary MNBC, the direct contribution of Bcr-Abl itself to the signal was low. However, a 48h treatment of MNBC with 5 μM Imatinib resulted in a significant reduction of the observed TK activity (mean TK activity value: 56% of control) paralleled by a decrease in the phosphotyrosine level in the CML group. Modification of TK activity by Imatinib was observed also in the donor group. Imatinib mesylate thus probably affects cell signalization even in Bcr-Abl negative cells.

Keywords: tyrosin-kinase activity – Imatinib – K562 – primary blood cells – leukemia

INTRODUCTION

Tyrosine phosphorylation is an important post-translational event occurring only to a limited extent in normal tissue. The level of phosphorylated tyrosine in many cellular proteins increases following various activation processes which are mediated through phosphotyrosine

✉ Kateřina Kuželová, Institute of Hematology and Blood Transfusion, U Nemocnice 1, 128 20 Prague 2, Czech Republic
☎ +420 221 977 285
🖷 +420 224 918 390
🖳 kuzel@uhkt.cz

kinases. In some cases, the aberrant expression of a tyrosine-specific kinase can be associated with an oncogenic transformation of the cell. Protein tyrosine kinases which act as receptors for a wide range of external signals are frequently deregulated in malignancies. In addition, many retroviral and cellular oncogenes encode tyrosine kinase variants that are constitutively active. Thus the constitutive tyrosine kinase (TK) activity of the chimeric fusion protein Bcr-Abl is the molecular cause of chronic myelogenous leukemia (CML), a disease which is characterized by the presence of the abnormal Philadelphia (Ph) chromosome in a clone of hematopoietic progenitor cells. The deregulated TK activity of Bcr-Abl maintains in permanent activity several signal transduction pathways and thereby enables the cell to escape from the usual regulation mechanisms of cell cycle progression and apoptosis

(Calabretta and Perrotti 2004, Steelman 2004, Manley et al. 2002). The most effective drug used so far to treat the chronic phase of CML, is Imatinib mesylate (Glivec®, formerly STI571), a specific inhibitor of Bcr-Abl TK activity (see Deininger et al. 2005 for a recent review). Studies on leukemic cell lines have shown that Imatinib treatment of Ph+ cells leads to rapid Bcr-Abl dephosphorylation and inactivation (Gambacorti-Passerini et al. 1997, Jacquel et al. 2003, Ferrao et al. 2003). As a result, the cell cycle progression is arrested and the cells frequently undergo apoptosis. Imatinib is also known to induce differentiation of Bcr-Abl positive cell lines into erythroid or megakaryocytic lineages (Jacquel et al. 2003, Fang et al. 2000, Kuželová et al. 2005, Kawano et al. 2004). The ability of Imatinib to suppress cell proliferation was equally well documented on primary mononuclear cells derived from CML patients (Deininger et al. 1997, Holtz et al. 2002). Moreover, the leukemic transformation seems to affect dendritic cells which are considered an important element in the induction of specific antitumor immune response (Dong et al. 2003). It has been suggested that the restoration of the dendritic cell function contributes to the beneficial effects of Imatinib-based therapy (Mohty et al. 2004).

While the processes leading to the positive clinical response to Imatinib are at least partly elucidated, little is known about the effects of the compound on nonmalignant hematopoietic cells. Imatinib potently inhibits not only the tyrosine kinase activity of Bcr-Abl, but also that of normal c-Abl kinase which undoubtly plays an important, although not yet clearly described physiological role (Shaul and Ben-Yehoyada, 2005). The activity of several other kinases, especially c-kit and PDGFR, is abolished by this drug with comparable efficiency (Manley et al. 2002, Deininger et al. 2005, Krystal 2004). The macrophage colony-stimulating factor receptor (c-fms) has recently been identified as an additional Imatinib target (Dewar et al. 2005). Imatinib was reported to be minimally toxic to normal counterparters of leukemic cells (Gambacorti-Passerini et al. 1997, Deininger 1997, Holtz 2002, Druker et al. 1996). However, recent works revealed that Imatinib inhibits the stimulated proliferation of both normal CD34^{+} progenitor cells (Bartolovic et al. 2004) and normal T lymphocytes (Cwynarski et al. 2004) *in vitro*. The *in vitro* development of the monocyte/macrophage lineage from normal bone marrow progenitors was also found to be impaired (Dewar et al. 2003). Furthermore, while Imatinib helps to restore the function of dendritic cells originating from CML progenitors, it seems to have an opposite effect on the development and function of dendritic cells generated from normal progenitor cells (Appel et al. 2004, Taieb et al. 2004).

Although long-term clinical experience with Imatinib is relatively limited, moderate hematological side effects are known to occur especially at higher Imatinib doses and at later stages of the disease (Talpaz et al. 2002, Sawyers et al. 2002, Kantarjian et al. 2004, Deininger 2005). An increase in the incidence of infections and other malignancies due to Imatinib treatment has also been reported (Mattiuzzi et al. 2003, Baskaynak et al. 2003).

In this work, we used a highly sensitive fluorescence method to measure the tyrosine kinase activity in primary mononuclear cells isolated from CML patients as well as from healthy donors. Our main aim was to determine the effects of Imatinib mesylate on the TK activity of primary cells and uncover possible differences between healthy and leukemia-affected cells.

MATERIAL AND METHODS

Chemicals
Imatinib mesylate was kindly provided by Novartis (Basel, Switzerland). It was dissolved in distilled sterile water at 10 mM stock and stored at −20 °C. A Delfia® Tyrosine Kinase kit (Wallac Oy, Turku, Finland) was purchased through PerkinElmer (Prague, Czech Republic). The antibody to Bcr was produced by Oncogene Res. Prod. (San Diego, CA). FITC-conjugated mouse monoclonal anti-phosphotyrosine antibody (clone PT-66) was obtained from Sigma (Prague, Czech Republic). It does not react with non-phosphorylated tyrosine or other phosphorylated amino acids, including serine and threonine.

Sample preparation
Blood samples were obtained with written informed consent from 17 patients in the early stage of chronic myelogenous leukemia (CML), the chronic phase, prior to any treatment. For 2 additional patients, the original diagnosis (CML) was not confirmed (Ph chromosome was not found by cytogenetic analysis and the bcr-abl transcript was not detected by multiplex RT-PCR). Control samples were prepared from buffy coats taken from 12 healthy volunteers. Peripheral blood mononuclear cells were isolated by standard density gradient centrifugation with Histopaque®-1077 (Sigma, Prague, Czech Republic). The samples derived from CML patients contained from 1 to 5% of blasts which were CD117 positive and usually CD34 positive (14 out of 17). The isolated mononuclear cells were resuspended in a RPMI-1640 medium supplemented with 10% fetal calf serum, 25 mM HEPES buffer, 100 U/ml penicillin, and 50 μg/ml streptomycin, and incubated for 24 h

at 37 °C. Imatinib mesylate (1 or 5 µM) was then added to appropriate samples and the cells were incubated for further 48 h.

K562 cell line
K562 cells were purchased from the European Collection of Animal Cell Cultures (Salisbury, UK). They were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (Sigma, Czech Republic), 100 U/ml penicillin, and 50 µg/ml streptomycin at 37 °C in 5% CO_2 humidified atmosphere. They were diluted to a density of 2×10^5 cells per ml three times a week.

Measurement of tyrosine kinase activity using Delfia® kit
Equal amounts (usually 5×10^6) of control and Imatinib-treated cells were washed once in PBS and lysed in 100 µl lysis buffer (50 mM TRIS, 5 mM EDTA, 300 mM NaCl, 1 % NP-40, supplemented prior to use by 1.5 mg/ml dithiothreitol, 1 mM phenylmethylsulphonyl-fluoride, 2 µg/ml aprotinin and 4 µg/ml leupeptin). The Phosphatase inhibitor cocktail II (5 µl, Sigma, Czech Republic) was immediately added to each sample.

The samples were incubated for 30 min on ice and centrifuged at 15 000 g for 10 min, at 4°C. The supernatant was directly used for TK activity assessment or rapidly frozen and analysed later. The activity of tyrosine kinases was measured using Delfia® Tyrosine Kinase kit following the manufacturer´s guidelines. The cell lysate (5 µl) was incubated at 37 °C in presence of biotinylated substrate (5 µl). The composition of the reaction buffer (30 µl) was as follows: 40 mM imidazole hydrochloride, 50 mM NaCl, pH 7.3, 1.5 mg/ml dithiothreitol, 1 mM phenylmethylsulphonyl-fluoride, 50 µg/ml aprotinin, 5 µg/ml leupeptin and Phosphatase inhibitor coctail II (10 µl per 1 ml of reaction buffer). The reaction mixture was supplemented by 10 µl of ATP/MgCl₂ solution (5 mM ATP and 50 mM $MgCl_2$ in 25 mM TRIS, 0.15 M NaCl, pH 7.2). After 1 h incubation, the reaction was stopped by adding 120 mM EDTA (10 µl), the mixture was diluted 10 times in Assay buffer and transferred into a streptavidin-coated 96-well microtitration plate.

The plate was slowly shaken at room temperature for 1 h, washed and incubated for 1 h with Europium-labelled anti-phosphotyrosine antibody. The unbound antibody was thoroughly washed out and Enhancement solution was added to induce the formation of highly fluorescent species. The time-resolved fluorescence signal from each well was then measured using a Fluostar Galaxy fluorescence reader (BMG Labtechnologies GmbH, Germany) equipped with 337 nm excitation filter and 615 nm emission filter. The time delay between the excitation and the signal detection was set to 400 µs and the integration time to 600 µs. The intensity of fluorescence of each sample is proportional to tyrosine kinase phosphorylation activity.

Bcr-abl expression level in primary cells
The presence of Bcr-Abl protein in cell lysates prepared for tyrosine kinase activity measurement was tested by means of Western-blotting. Western blots were prepared as described previously (Grebeňová et al. 2003) with some modifications. The adopted protocol allows for sensitive detection of Bcr-Abl protein (Guo et al. 1994). Proteins were transferred to nitrocellulose membrane by wet blotting at 90 V, at 4°C for 2 h. The antigen was detected using the enhanced chemiluminescence Western blotting detection system SuperSignal West Pico Chemiluminescent Substrate (Pierce, USA) according to the manufacturer's instructions and visualized by autoradiography on X-ray film (Hyperfilm™, Amersham Bioscinces, UK).

Assessment of phosphotyrosine level in cells
Cells (1×10^6) were washed once in PBS. A FIX&PERM cell permeabilization kit (An Der Grub, Kaumberg, Austria) was used to fix and permeabilize the samples following the standard manufacturer's protocol. The cells were labeled by FITC-conjugated anti-phosphotyrosine antibody and analyzed by flow-cytometry (Coulter Epics XL).

RESULTS

Tyrosine kinase activity in K562 cell line
Lysates from K562 cells (2×10^6) were prepared as described in the Material and Methods section. The assay for TK activity using Delfia® kit was carried out in the absence or presence of Imatinib mesylate which was added to the reaction buffer. The final concentration of this TK inhibitor in the reaction mixture was from 1 to 20 µM. Fig. 1 shows representative results of the time-resolved fluorescence measurement. The figure includes also two blank samples: cell lysate or biotinylated substrate were omitted from the reaction mixture (replaced by the same volume of the reaction buffer) in blank 1 and blank 2, respectively. The presence of Imatinib in the reaction mixture resulted in a decrease of TK activity by 20 to 50% (range of results from three independent experiments)

Bcr-Abl expression level in patient primary cells
The level of Bcr-Abl tyrosine kinase in the cell lysates intended for TK activity measurement was assayed by means of Western-blotting.

A considerable amount of Bcr-Abl protein could be detected in 1 out of 17 patient samples only (Fig. 2, lanes F, F+Imat). As expected, the band corresponding to Bcr-Abl was very intense in the sample obtained from K562 cells (Fig. 2).
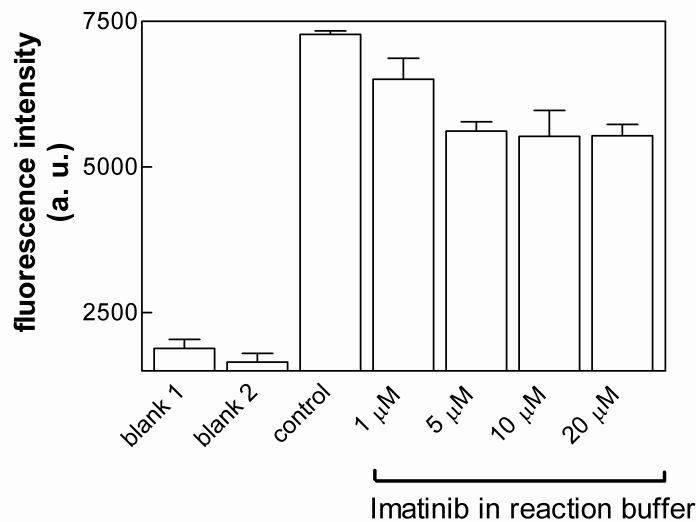

Fig.1: **Tyrosine kinase activity in K562 cells.** Cell lysate from K562 cells (2×10^6 in 100 µl lysis buffer) was assessed for TK activity using Delfia® Tyrosine Kinase kit. Imatinib mesylate was added to the reaction buffer (RB) at the indicated final concentrations. Blank 1: cell lysate was replaced by equal volume of RB. Blank 2: biotinylated substrate was replaced by an equal volume of RB. The assay was performed in duplicates. Representative example from three independent experiments.

Tyrosine kinase activity in patient and donor primary cells

The isolated mononuclear cells were incubated for 48 h in the absence or presence of Imatinib mesylate (5 µM, and usually also 1 µM), lysed and assayed for TK activity. An example of the measured fluorescence intensity is given in Fig. 3.
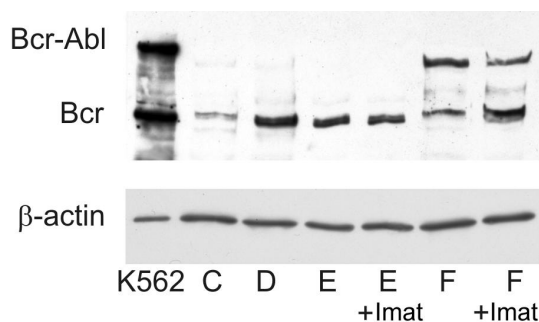

Fig. 2: **Bcr-Abl expression levels in K562 cell line and in primary blood cells.** Western-blot was labeled by anti-Bcr or anti-actin antibody, as indicated. Control samples (C, D, E, F) and samples treated with 5 µM Imatinib (E+Imat, F+Imat) were derived from four CML patients.

The fluorescence signal obtained from Imatinib-treated cells was lower than that from control cells in all 17 patient samples as well as in some samples (7 out of 12) derived from healthy donors. The attenuation of TK activity after 48 h incubation with 5 µM Imatinib is summarized in Fig. 4, which represents the relative values of TK activity in Imatinib-treated cells as per cent fractions of the corresponding controls.

The mean of TK activity in 5 µM Imatinib-treated samples was 56% of control in CML patients and 95% in donors (this difference was significant, P = 0.01 in t test). Interestingly, TK activity in samples obtained from 2 patients whose diagnosis was subsequently modified (not CML) was not reduced by Imatinib mesylate: the measured values in 5 µM Imatinib-treated cells were 104% and 170%. The effect of 1 µM Imatinib mesylate was tested in 13 out of 17 CML samples, the mean value of TK activity in treated cells was 71% of control (SD 28%).

In contrast to the results obtained with K562 cells, the addition of Imatinib mesylate to the reaction buffer had no detectable effect on the fluorescence signal detected in control patient and donor samples (cf Fig. 1 and example in Fig. 3).
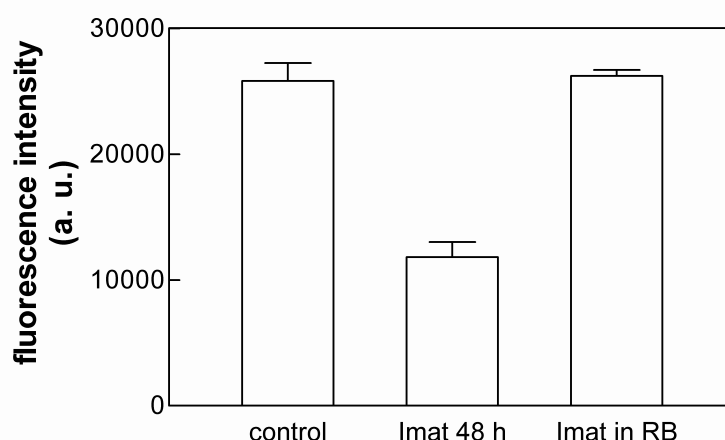
Fig. 3: **Example of TK activity in patient primary cells.** Lysates from control cells and cells treated for 48 h with 5 µM Imatinib were assessed for TK activity using Delfia® Tyrosine Kinase kit. Last column: lysate from control cells assessed in the presence of 5 µM Imatinib in the reaction buffer (RB). The assay was performed in triplicates.

Phosphotyrosine level in patient and donor primary cells

An assessment of the total amount of proteins phosphorylated on tyrosine using flow cytometry was introduced later in the course of the study. It was performed simultaneously with TK activity measurement on 6 patient and 6 donor samples.

Fig. 5 (panels A and B) shows the results of anti-phosphotyrosine labeling which are complementary to TK activity values presented in Fig. 3 (both sets of data were derived from the same sample). An example of the anti-phosphotyrosine staining of donor cells is also given in Fig. 5, panels C and D.
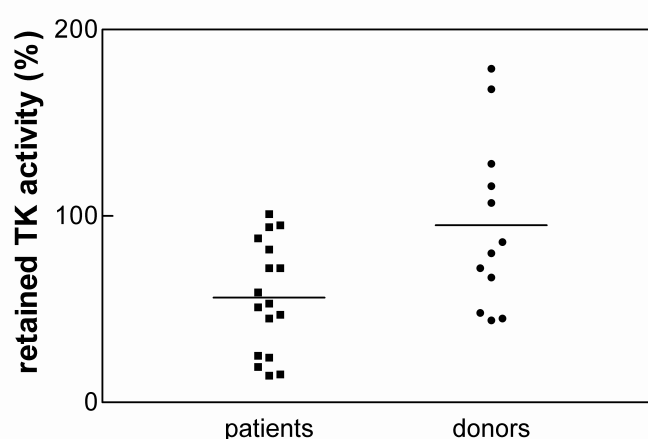

Fig. 4: **Reduction of TK activity by Imatinib treatment of primary cells.** Relative TK activity in patient and donor primary cells treated for 48 h by 5 µM Imatinib. Values are given as per cent fractions of TK activity in the appropriate controls.

In the majority of cases, the level of tyrosine-phosphorylated proteins was lower in Imatinib-treated samples compared to the controls. However, the difference was usually less pronounced in comparison with the extent of changes observed in TK activity measurement. The correlation between the shift of the mean fluorescence intensity obtained by flow cytometry and the suppression of

TK activity detected using Delfia® kit is summarized in Fig. 6.

Cell viability

The Trypan blue exclusion test was routinely performed in all primary mononuclear cell suspensions after incubation with Imatinib mesylate. No significant difference in necrotic cell

fractions was detected between controls and Imatinib-treated samples.

DISCUSSION

In a general way, the amount of proteins phosphorylated on tyrosine is related to the signaling activity of the cell. Presumably, the cells bearing the Philadelphia chromosome should exhibit an enhanced phosphotyrosine level due to the constitutive kinase activity of Bcr-Abl itself as well as to the Bcr-Abl-mediated permanent activation of multiple signaling pathways. Imatinib mesylate, which occupies the ATP-binding site of the chimeric kinase, induces a rapid Bcr-Abl inactivation which is followed by dephosphorylation of downstream signaling

kinases. In K562 cells, a cell line derived from a CML patient, the loss of Bcr-Abl phosphorylation occurs within a few minutes after the addition of Imatinib (Gambacorti-Passerini et al. 1997, Jacquel et al. 2003, Ferrao et al. 2003). Multiple reports have shown that the total amount of tyrosine-phosphorylated proteins present on Western-blots is dramatically reduced after 2 h incubation of K562 cells with 1 to 10 μM Imatinib (Mukai et al. 2003, Mahon et al. 2000, Weisberg and Griffin 2000). Phospho-specific immunoblots revealed that ERK 1/2 (Traina et al. 2003, Kawano et al. 2004), Akt (Fang et al. 2000) and STAT5 (Kotaki et al. 2003, Jacquel et al 2003) are among the most important downstream targets of Bcr-Abl. A large decrease in the total phosphotyrosine level after Imatinib treatment of K562 cells has also been documented by flow-cytometry (Mahon et al. 2003).
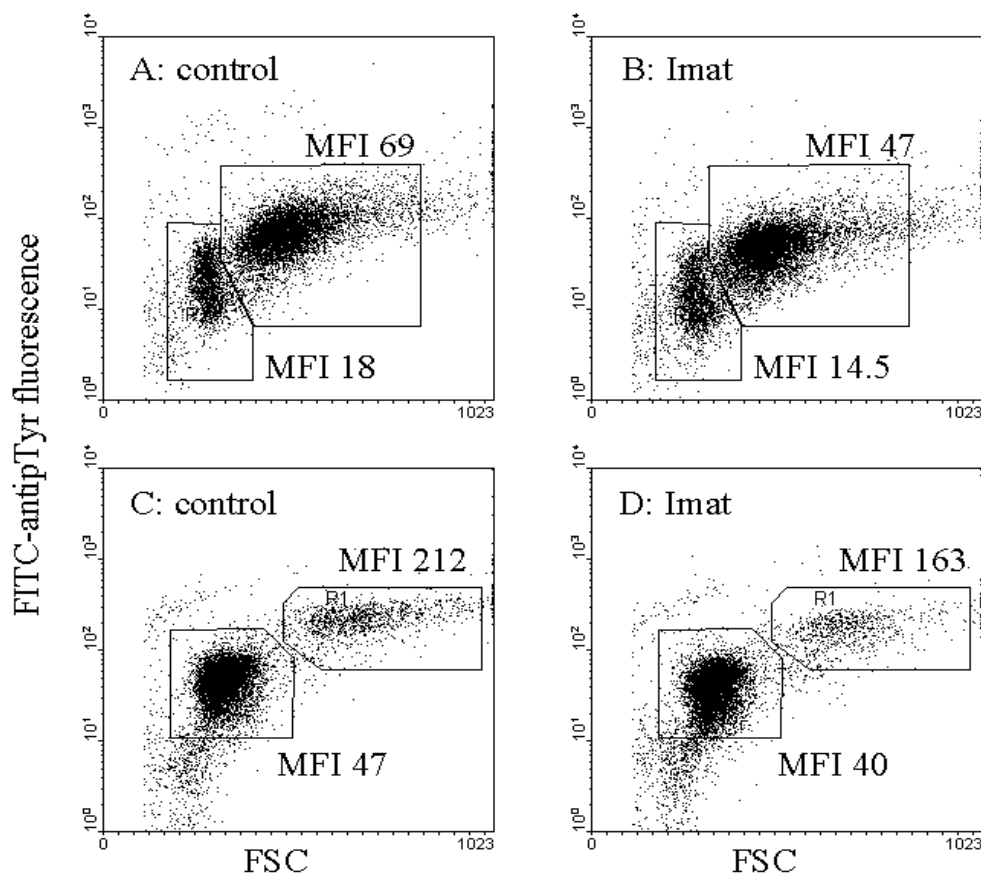
Fig. 5: **Examples of flow-cytometric analysis of total phosphotyrosine level.** Primary mononuclear cells derived from a CML patient (A, B; same patient as in Fig. 3) or from a donor (C, D) were incubated for 48 h with 5 μM Imatinib mesylate, labeled by FITC-antiphosphotyrosine antibody and analyzed by flow-cytometry. A, C: control cells, B, D: Imatinib-treated cells. MFI: mean fluorescence intensity of cells in the selected region (arbitrary units)

In this work, we present a direct measurement of tyrosine-specific kinase activity in cell lysates obtained both from K562 cells and from primary mononuclear blood cells (MNBC) isolated from CML patients as well as from healthy volunteers. For this purpose, we have optimized the parameters of a highly sensitive method based on the time-resolved fluorescence detection of the tyrosine-phosphorylated substrate. The long lasting fluorescence signal of europium chelates allows for a large reduction of the background fluorescence and, consequently, significant improvement of the signal/background ratio. The effects of Imatinib mesylate on the tyrosine kinase activity were studied in the concentration range which is achieved *in vivo* in the course of Imatinib-based therapy of CML (Deininger 2005, Le Coutre et al. 2004).

K562 cells carry about 20 copies of bcr-abl gene (Rodley et al. 1997, Wu et al. 1995) and express a high amount of Bcr-Abl protein (Fig. 2). In agreement with this fact, the overall tyrosine kinase activity detected in K562 cell lysates is partially sensitive to Imatinib mesylate (Fig. 1): 20 to 50% of the signal intensity is suppressed when Imatinib is added to the reaction mixture. The kinase activity of Bcr-Abl itself is thus likely to contribute to the observed signal.
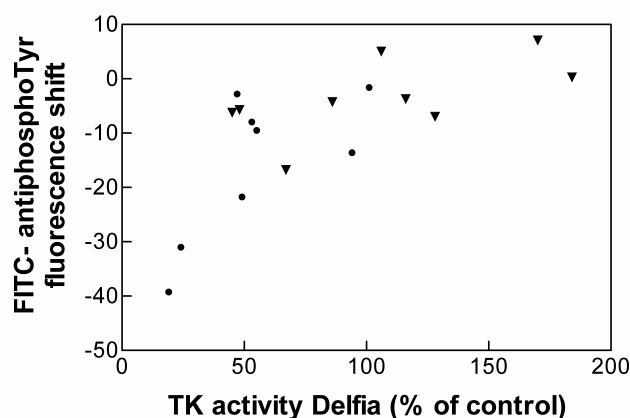

Fig. 6: **Correlation between TK activity and phosphotyrosine level.** Reduction of TK activity by 1 or 5 µM Imatinib assessed by Delfia® kit (see results presented in Fig. 4) vs shift of the mean fluorescence intensity in flow-cytometry dotplots (like that presented in Fig. 5) from the same samples. The figure includes all CML (circles) as well as donor (triangles) samples which were simultaneously tested for TK activity and phosphotyrosine level.

The mononuclear cell preparations from CML patients contained only 1 to 5 % of blasts which are likely to be bcr-abl positive. On the other hand, the majority of MNBC (about 60 to 80%) are T-lymphocytes which were reported to be bcr-abl negative in CML patients (Dong 2003, Takahashi et al. 1998). B-lymphocytes (about 5 to 15% of MNBC) form a mixed Ph$^-$/Ph$^+$ population (Takahashi et al. 1998). As all the patients included in this study were in the early stages of CML (chronic phase), the prevailing fraction of B-cells was probably also bcr-abl negative. The only cell type, besides the blasts, which is likely to be Philadelphia positive are the monocytes (Takahashi et al. 1998), which are usually reported to form about 10 to 30% of MNBC. As judged from flow-cytometry scattergrams, the fraction of monocytes present in our MNBC preparations was within this range. Taken together, we estimate that the cells bearing the bcr-abl fusion gene represented about 20 to 25% of MNBC derived from CML patients. A large population of cells in our CML samples was thus non-leukemic. Moreover, the expression rate of bcr-abl gene seems not to be high, as a considerable amount of Bcr-Abl protein was found only in 1 out of 17 patients. It is thus not surprising that the phosphorylation activity in samples derived from CML patients as well as from healthy donors was largely insensitive to Imatinib when it was added directly to the reaction mixture (Fig. 3, column labelled Imat in RB). However, an important effect was achieved by a 48 h incubation of cells with Imatinib prior to cell harvesting. In this case, the TK activity in Imatinib-treated samples was significantly weaker than in the controls (Fig. 4). This was true even for about a half of the samples derived from healthy donors. On the other hand, an enhancement of TK activity was observed in several other donor samples. As repeated tests performed on frozen cell lysates usually give well reproducible results, the variation of values obtained for healthy donors is probably not attributable to experimental errors. Thus, Imatinib seems to modify the phosphorylation activity even in normal cells.

An Imatinib effect is evident equally from the flow-cytometric analysis of the amount of proteins phosphorylated on tyrosine (Fig. 5) in individual cells. The decrease in the phosphotyrosine level detected by this method is less pronounced in comparison to the suppression of TK activity. This can be due to the fact that the total amount of phosphorylated proteins results from the equilibrium between TK activity and the opposing activity of tyrosine phosphatases which can also be affected by Imatinib. It is also likely that not all the kinases, which are active in intact cells, are extracted to the cell lysate. Moreover, the conditions (pH, type of substrate,...) used in our experiments may not be optimal for the kinase activity of all isolated kinases. However, although the shift in the mean fluorescence intensity (MFI) detected by flow-cytometry is less pronounced, it is in correlation with the suppression of TK activity induced by Imatinib (Fig. 6). It is noteworthy, that the shift of MFI is relatively homogeneous, i. e. it seems to apply not to a certain cell subpopulation but to all cells in the lymphocyte/blast as well as in the monocyte regions (Fig. 5). This finding supports the idea that Imatinib affects TK activity in all cells including those without bcr-abl expression.

In conclusion, we found that the TK activity of cells derived from CML patients was significantly lowered by a 48 h Imatinib treatment although the amount of Bcr-Abl protein in these cells was undetectable in all but one case under conditions used. This fact can be explained by the inactivation of tyrosine kinases which are downstream of Bcr-Abl. Although the TK activity of Bcr-Abl itself can be low with respect to the whole signal, the kinases network affected by this protein is extensive. However, the TK activity seems to be modified by Imatinib even in the samples derived from healthy volunteers and in non-transformed cells from CML patients. Our results are in agreement with previous reports describing Imatinib effects on normal cells. Likewise as other authors (Gambacorti-Passerini et al. 1997, Deininger et al. 1997, Holtz et al. 2002), we found no increased cell death due to Imatinib treatment. On the other hand, modification of TK activity indicates possible alterations in the cell signaling which can result into impaired proliferation and differentiation of progenitor cells (Bartolovic et al. 2004, Dewar et al. 2003) as well as of T-lymphocytes (Cwynarski 2004).

REFERENCES

Appel S., Boehmler A.M., Grünebach F. et al.: Imatinib mesylate affects the development and function of dendritic cells generated from CD34+ peripheral blood progenitor cells. Blood 103:538–544, 2004.

Bartolovic K., Balabanov S., Hartmann U. et al.: Inhibitory effect of imatinib on normal progenitor cells *in vitro*. Blood 103:523–529, 2004.

Baskaynak G., Kreuzer K.A., Schwarz M. et al.: Squamous cutaneous epithelial cell carcinoma in two CML patients with progressive disease under imatinib treatment. Eur. J. Haematol. 70:231–4, 2003.

Calabretta B., Perrotti D.: The biology of CML blast crisis. Blood 103:4010–4022, 2004.

Cwynarski K., Laylor R., Macchiarulo E. et al.: Imatinib inhibits the activation and proliferation of normal T lymphocytes *in vitro*. Leukemia 18:1332–1339, 2004.

Deininger M.W.N., Goldman J.M., Lydon N., Melo J.V.: The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. Blood 90:3691–3698, 1997.

Deininger M., Buchdunger E., Druker B.J.: The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 105:2640–2653, 2005.

Dewar A.L., Cambareri A.C., Zannettino A.C.W. et al.: Macrophage colony-stimulating factor receptor c-fms is a novel target of imatinib. Blood 105:3127–3132, 2005.

Dewar A.L., Domaschenz R.M., Doberty K.V. et al.: Imatinib inhibits the *in vitro* development of the monocyte/macrophage lineage from normal human bone marrow progenitors. Leukemia 17:1713–1721, 2003.

Dong R., Cwynarski K., Entwistle A. et al.: Dendritic cells from CML patients have altered actin organization, reduced antigen processing and impaired migration. Blood 101:3560–3567, 2003.

Druker B.J., Tamura S., Buchdunger E. et al.: Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med. 2: 561–566, 1996.

Fang G., Kim C.N., Perkins C.L. et al.: CGP57148B (STI-571) induces differentiation and apoptosis and sensitizes Bcr-Abl–positive human leukemia cells to apoptosis due to antileukemic drugs. Blood 96:2246–2253, 2000.

Ferrao P.T., Frost M.J., Siah S.P., Ashman L.K.: Overexpression of P-glycoprotein in K562 cells does not confer resistance to the growth inhibitory effects of imatinib (STI571) *in vitro*. Blood 102:4499–4503, 2003.

Gambacorti-Passerini C., Le Coutre P., Mologni L. et al.: Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. Blood Cells Mol. Dis. 23:380–394, 1997.

Grebeňová D., Kuželová K., Smetana K. et al.: Mitochondrial and endoplasmic reticulum stress-induced apoptotic pathways are activated by 5-aminolevulinic acid-based photodynamic therapy in HL60 leukemia cells. J. Photochem. Photobiol. B, Biol. 69:71–85, 2003.

Guo J.Q., Lian J.Y., Xian Y.M. et al.: BCR-ABL Protein expression in peripheral blood cells of chronic myelogenous leukemia patients undergoing therapy. Blood 83:3629–3637, 1994.

Holtz M.S., Slovak M.L., Zhang F. et al.: Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. Blood 99:3792–3800, 2002.

Jacquel A., Herrant M., Legros L. et al.: Imatinib induces mitochondria-dependent apoptosis of the Bcr-Abl positive K562 cell line and its differentiation towards the erythroid lineage. FASEB J. 17:2160–2162, 2003.

Kantarjian H., Talpaz M., O´Brien S. et al.: High-dose imatinib therapy in newly diagnosed Philadelphia chromosome-positive chronic phase chronic myeloid leukemia. Blood 103:2873–8, 2004.

Kawano T., Horiguchi-Yamada J., Saito S. et al.: Ectopic cyclin D1 expression blocks STI571-induced erythroid differentiation of K562 cells. Leuk. Res. 28:623–629, 2004.

Kotaki M., Motoji T., Takanashi M. et al.: Anti-proliferative effect of the abl tyrosine kinase inhibitor STI571 on the P-glycoprotein positive K562/ADM cell line. Cancer Lett. 199:61–68, 2003.

Krystal G.W.: Imatinib mesylate (STI571) for myeloid malignancies other than CML. Leuk. Res. 28S1: S53–S59, 2004.

Kuželová K., Grebeňová D., Marinov I., Hrkal Z.: Fast apoptosis and erythroid differentiation induced by Imatinib mesylate in JURL-MK1 cells. J. Cell. Biochem. 95:268–280, 2005.

Le Coutre P., Kreuzer K.A., Pursche S. et al.: Pharmacokinetics and cellular uptake of imatinib and its main metabolite CGP74588. Cancer Chemother. Pharmacol. 53:313–23, 2004.

Mahon F.X., Deininger M.W.N., Schultheis B. et al.: Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood 96:1070–1079, 2000.

Mahon F.X., Belloc F., Lagarde V. et al.: MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood 101:2368–2373, 2003.

Manley P.W., Cowan-Jacob S.W., Buchdunger E. et al.: Imatinib: a selective tyrosine kinase inhibitor. Eur. J. Cancer 38:S19–S27, 2002.

Mattiuzzi G.N., Cortes J.E., Talpaz M. et al.: Development of Varicella-Zoster virus infection in patients with chronic myelogenous leukemia treated with imatinib mesylate. Clin. Cancer Res. 9:976–80, 2003.

Mohty M., Jourdan E., Mami N.B. et al.: Imatinib and plasmacytoid dendritic cell function in patients with chronic myeloid leukemia. Blood 103:4666–4668, 2004.

Mukai M., Che X.F., Furukawa T. et al.: Reversal of the resistance to STI571 in human chronic myelogenous leukemia K562 cells. Cancer Sci. 94: 557–563, 2003.

Rodley P., McDonald M., Price B. et al.: Comparative genomic hybridization reveals previously undescribed amplifications and deletions in the chronic myeloid leukemia-derived K-562 cell line. Genes Chromosomes Cancer 19:36–42, 1997.

Sawyers C.L., Hochhaus A., Feldman E. et al.: Imatinib induces hematological and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 99:3530–9, 2002.

Shaul Y., Ben-Yehoyada M.: Role of c-Abl in the DNA damage stress response. Cell Res. 15:33–35, 2005.

Steelman L.S., Pohnert S.C., Shelton J.G. et al.: JAK/STAT, RaF/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia 18:189–218, 2004.

Taieb J., Maruyama K., Borg C. et al.: Imatinib mesylate impairs Flt3L-mediated dendritic cell expansion and antitumor effects *in vivo*. Blood 103:1966–1967, 2004.

Takahashi N., Miura I., Saitoh K., Miura A.B.: Lineage involvement of stem cells bearing the Philadelphia chromosome in chronic myeloid leukemia in the chronic phase as shown by a combination of fluorescence-activated cell sorting and fluorescence *in situ* hybridization. Blood 92:4758–4763, 1998.

Talpaz M., Silver R.T., Druker B.J. et al.: Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 99:1928–37, 2002.

Traina F., Carvalheira J.B.C., Saad M.J.A. et al.: BCR-ABL binds to IRS-1 and IRS-1 phosphorylation is inhibited by imatinib in K562 cells. FEBS Lett. 535:17–22, 2003.

Weisberg E., Griffin J.D.: Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 95:3498–3505, 2000.

Wu S.Q., Voelkerding K.V., Sabatini L. et al.: Extensive amplification of bcr/abl fusion genes clustered on three marker chromosomes in human leukemic cell line K-562. Leukemia 9: 858–862, 1995.