

Original Article

In Vitro Effects of Imatinib on Glucose-6-phosphate Dehydrogenase and Glutathione Reductase

(glucose-6-phosphate dehydrogenase / glutathione reductase / imatinib mesylate / inhibition / kinetic properties)

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Abstract. Imatinib (Gleevec, STI571) is a drug used to treat certain types of cancer. Glucose-6-phosphate dehydrogenase and glutathione reductase are enzymes important for redox homeostasis and play key roles in many cellular processes. The purpose of the present work is to evaluate the *in vitro* effects of imatinib on sheep brain cortex glucose-6-phosphate dehydrogenase, and on bovine kidney cortex, bovine liver and yeast glutathione reductase. Kinetic studies on the inhibition of enzymes by imatinib have been investigated by using Lineweaver-Burk double reciprocal plot and values summarized with graphs by plotting the data using Lineweaver-Burk diagrams of $1/v$ against $1/[S]$ at each $[I]$. Imatinib inhibits glucose-6-phosphate dehydrogenase with an IC_{50} value of 0.7 mM. It inhibits bovine kidney cortex, liver and yeast glutathione reductase in a concentration-dependent manner with IC_{50} values of 0.8, 0.92, 1 mM, respectively. We have investigated the kinetic characteristics, inhibition types and constants (K_i). Inhibition of the glucose-6-phosphate dehydrogenase and glutathione reductase represents an attractive approach to the development of anticancer agents. This study shows the molecular effectiveness of the drug on purified enzymes of various sources. Understanding the kinetic mechanism of the drug and enzyme relationship may be a powerful approach to the fu-

ture drug studies concerning new cancer drugs, drug resistance and new aspects in cancer therapy.

Introduction

Imatinib is a small-molecule inhibitor of BCR-ABL tyrosine kinase activity. Chronic myeloid leukaemia cells contain a constitutively active BCR-ABL tyrosine kinase (Giles et al., 2008). Imatinib inhibits leukaemia cell glucose substrate utilization and growth (Boren et al., 2001). Resistance to multiple chemotherapeutic agents is a common clinical problem in the treatment of cancer (Giai et al., 1991). The resistance-related proteins (e.g., glutathione-dependent enzymes, metallothionein, O₆-alkylguanine-DNA-alkyltransferase, thymidylate synthase, dihydrofolate reductase, heat-shock proteins) can also be expressed in resistant tumours (Volm and Mattern, 1996; Volm, 1998). Modulation of cellular glutathione homeostasis can also have a profound effect on the sensitivity of cancer cells to chemotherapy. These effects are produced by several mechanisms that involve inactivation of toxic electrophiles by conjugation, modulation of cellular redox state, activation of drug transporter systems, and regulation of cell signalling and repair pathways (McLellan and Wolf, 1999).

In this study, we have clarified that imatinib inhibits the antioxidant enzymes such as glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GR). Glucose-6-phosphate dehydrogenase (G6PD, D-glucose-6-phosphate: NADP⁺ oxidoreductase, EC 1.1.1.49) is the first and rate-limiting enzyme of the pentose phosphate pathway and the principal intracellular source of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) (Leopold et al., 2007). Increased *G6PD* gene expression has been associated with increasing reduced form of glutathione levels (NADPH in turn maintains the level of glutathione) and resistance to oxidative stress (Wakao et al., 2008). The major function of G6PD is to supply ribose for incorporation into ribonucleic acids or coenzyme, particularly in proliferating cells (Baquer et al., 1988). There is a direct correlation between the activity of G6PD and the rate of cell growth (Tian et al., 1994).

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Abbreviations: 6-PGA – 6-phosphogluconate, G6PD – glucose-6-phosphate dehydrogenase, GSH – reduced glutathione, GSSH – oxidized glutathione, NADP⁺ – nicotinamide adenine dinucleotide phosphate, NADPH – nicotinamide adenine dinucleotide phosphate reduced form, U – unit of activity, V_m – velocity.

Glutathione reductase (GR, NADPH: oxidized glutathione oxidoreductase, EC 1.6.4.2) is a ubiquitous enzyme that catalyses the NADPH-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Glutathione is mainly involved in detoxification mechanisms: thiol transfer, destruction of free radicals, and metabolism of various exogenous and endogenous compounds (Rana et al., 2002). Inhibition of antioxidant components would be expected to increase reactive oxygen species and various electrophiles (Puglia and Powell, 1984; van Haaften et al., 2003). Renal excretion, hepatic metabolism and biliary excretion are the major pathways involved in the removal of endogenous and exogenous compounds, xenobiotics, and drugs including anticancer drugs. The toxic compounds are conjugated enzymatically or non-enzymatically with glutathione, glucuronic acid, or sulphate (Yamazaki et al., 1996).

Material and Methods

Materials

Sheep brain cortex, bovine kidney cortex and liver obtained from a local slaughterhouse were kept on ice and processed within 2–3 h of death.

G6P, NADP⁺, 6-phosphogluconate (6-PGA), NADPH reduced form, GSSG, tris(hydroxymethyl)aminomethane (Tris), DEAE Sepharose Fast Flow and Type VI Baker's yeast glutathione reductase were obtained from Sigma Chemical Co. (St. Louis, MO). 2',5'-ADP-Sepharose 4B was from Pharmacia Fine Chemicals (Uppsala, Sweden). BSA was from British Drug Houses Ltd. (London, United Kingdom).

Imatinib was provided by Novartis Pharma AG, Basel, Switzerland.

Assay of glucose-6-phosphate dehydrogenase

Enzyme activities were determined spectrophotometrically using an Ultrospec 2100 pro UV/visible spectrophotometer (GE Healthcare Life Sciences, Piscataway, NJ) by monitoring the NADPH production at 340 nm and at 37 °C ($\epsilon_{340} = 6.22 \text{ mM}^{-1}\text{cm}^{-1}$). The assay mixture contained 10 mM MgCl₂, 0.2 mM NADP⁺ and 0.6 mM G6P in 100 mM Tris/HCl buffer, pH 8.0 (Betke et al., 1967). Assays were carried out in duplicate and the activities were followed for 60 seconds. The reaction was linear during this time period.

One unit of activity (U) is the amount of enzyme required to reduce one μmol of NADP⁺ per min under the assay conditions. Specific activity was defined as units per mg of protein.

Assay of glutathione reductase

GR activity was determined according to a modified version of Stall's method (Acan and Tezcan, 1989). The incubation mixture contained 100 mM sodium phosphate buffer, pH 7.4; 1 mM GSSG; and 0.2 mM NADPH. The decrease in the absorbance of NADPH at 340 nm was monitored spectrophotometrically at 37 °C.

One U was defined as the amount of enzyme that catalyses the oxidation of one μmol of NADPH in 1 min under these conditions. Specific activity was defined as units per mg of protein.

Protein determination

Protein concentrations were determined as described by Bradford (1976) using bovine serum albumin as standard.

Purification of G6PD and GR

We have previously described a purification method for G6PD from bovine lens and sheep kidney cortex (Uluslu et al., 1999; Uluslu and Tandogan, 2006). We used the same method for the purification of G6PD from sheep brain cortex, which was similar to our previous purification protocols (Sengezer and Uluslu, 2007). Similarly, bovine kidney cortex and liver GR was purified by 2',5'-ADP Sepharose 4B affinity and DEAE Sepharose Fast Flow anion exchange chromatography (Tandogan and Uluslu, 2010).

Inhibition studies

Activities were measured by adding different concentrations of imatinib in the assay mixture given above for G6PD and GR measurement. Assays of G6PD and GR in the presence of imatinib were performed in the system without enzyme-inhibitor pre-incubation in which the reactions were initiated by adding the enzyme to a substrate-inhibitor mixture.

To investigate the *in vitro* effects of imatinib on the activity of G6PD, we primarily purified G6PD from the sheep brain cortex. Details of purification steps were explained in our previous study (Sengezer and Uluslu, 2007). In this study, we have investigated the *in vitro* effects of imatinib on GR from bovine kidney cortex, liver and yeast. We purified GR from bovine kidney cortex and liver and then analysed the effect of this drug on the purified enzyme. Baker's yeast GR was obtained from Sigma and the specific activity of the enzyme was 1.25 U/mg.

Kinetic studies on the inhibition of enzymes by imatinib were performed by using Lineweaver-Burk double reciprocal plot and values were summarized with graphs by plotting the data using Lineweaver-Burk diagrams of $1/v$ against $1/[S]$ at each $[I]$.

Statistical analysis of kinetic data

The data were analysed and the kinetic constants were calculated with the following equations (Segel, 1975) by means of a nonlinear curve-fitting program (Statistica StatSoft computer program):

Eq.1. Michaelis-Menten equation:

$$v = V_m * [S] / K_m + [S]$$

Eq.2. Non-competitive inhibition:

$$v = (V_m * S / (1 + I/K_i)) / (K_m + S)$$

Eq.3. Uncompetitive inhibition:

$$v = V_m * S / (1 + I/K_i) / (K_m / (1 + I/K_i) + S)$$

Eq.4. Pure competitive inhibition:

$$v = V_m * S / (K_s * (1 + I/K_i) + S)$$

v = Reaction rate

$[S]$ = Substrate concentration

V_m = Maximum rate

K_m = Michaelis-Menten constant (substrate concentration at half the maximal velocity (V_m))

Results

Inhibition of sheep brain cortex G6PD by imatinib

The activity of G6PD was measured by the increase in the different concentrations of imatinib (0.15–1 mM) and the results were summarized in Fig. 1. IC_{50} obtained value of imatinib was 0.7 mM.

Kinetics of imatinib inhibition of sheep brain cortex G6PD

Kinetic studies on the inhibition of bovine kidney cortex GR by imatinib were investigated by using Lineweaver-Burk double reciprocal plot (Figs. 2, 3): The data were analysed and the kinetic constants were calculated by means of a nonlinear curve-fitting program (Statistica StatSoft computer program) (Segel, 1975).

When G6P was the varied substrate, at constant and unsaturating $NADP^+$ concentration (0.1 mM), different fixed concentrations of imatinib (0.55–0.75 mM) were added into the assay mixture and initial velocities were measured. It was observed that imatinib acted as a non-competitive inhibitor with respect to G6P (Fig. 2, Table 1).

When $NADP^+$ was the varied substrate, at constant and unsaturating G6P concentration (0.4 mM), different fixed concentrations of imatinib (0.55–0.75 mM) were added into the assay mixture. It is shown that imatinib acted as a competitive inhibitor with respect to $NADP^+$ (Fig. 3, Table 1).

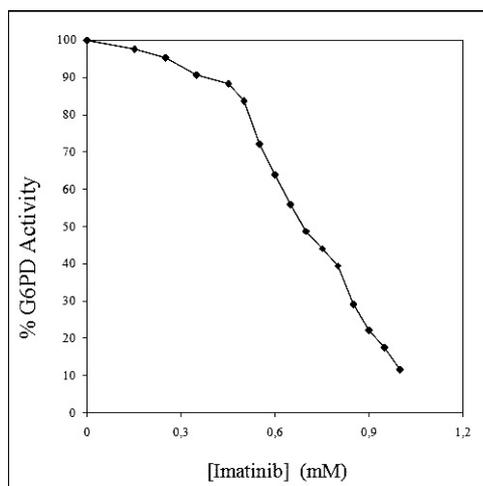


Fig. 1. Inhibition kinetics of sheep brain cortex G6PD by imatinib. $N = 3$, data in triplicate.

Inhibition of bovine kidney cortex GR by imatinib

Imatinib inhibited GR in a concentration-dependent manner (0.5–1 mM) and the obtained IC_{50} value of imatinib was 0.8 mM (Fig. 4).

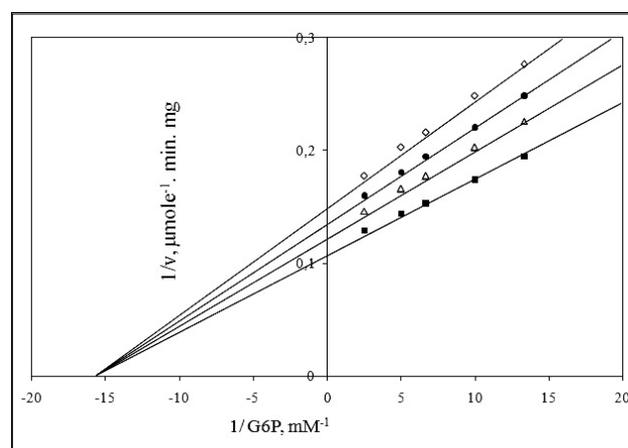


Fig. 2. Inhibition of sheep brain cortex G6PD by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against G6P as varied substrate and $NADP^+$ 0.1 mM constant concentration. $N = 3$, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.999, 0.025$; $R^2 = 0.999, 0.031$; $R^2 = 0.999, 0.034$; $R^2 = 0.998, 0.038$, respectively. The assays were carried out at 37 °C in 100 mM Tris/HCl buffer, pH 8.0 as described in Material and Methods. ■ 0.1 mM $NADP^+$ (no imatinib); △ 0.55 mM imatinib; ◇ 0.65 mM imatinib; □ 0.75 mM imatinib

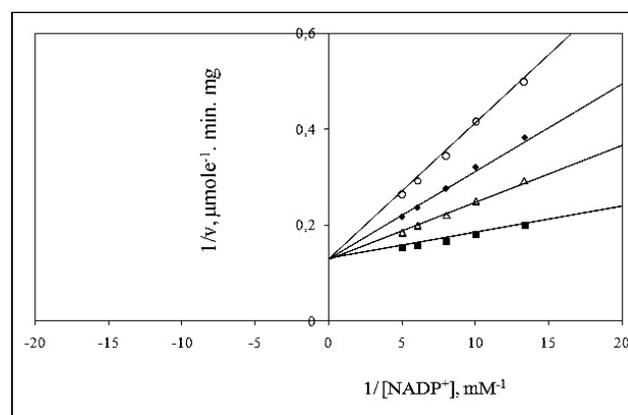


Fig. 3. Inhibition kinetics of sheep brain cortex G6PD by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against $NADP^+$ as varied substrate and G6P 0.4 mM constant concentration. $N = 3$, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.999, 0.018$; $R^2 = 0.999, 0.043$; $R^2 = 0.999, 0.067$; $R^2 = 0.998, 0.095$, respectively. The assays were carried out at 37 °C in 100 mM Tris/HCl buffer, pH 8.0 as described in Material and Methods. ■ 0.4 mM G6P (no imatinib); △ 0.55 mM imatinib; ◇ 0.65 mM imatinib; ○ 0.75 mM imatinib

Table 1. Mechanism of inhibition of sheep brain cortex glucose-6-phosphate dehydrogenase, bovine kidney cortex, bovine liver and yeast GR by imatinib, and K_i values

| | K_i (mM) | Type of inhibition |
|-------------------------|--------------------------------|--------------------|
| Sheep brain cortex G6PD | $K_{i_{G6P}}$ 2.320±0.210 mM | Non-competitive |
| | $K_{i_{NADP^+}}$ 0.311±0.002 | Competitive |
| Bovine kidney cortex GR | $K_{i_{GSSG}}$ 1.330±0.260 mM | Uncompetitive |
| | $K_{i_{NADPH}}$ 2.168±0.290 mM | Non-competitive |
| Bovine liver GR | $K_{i_{GSSG}}$ 1.221±0.200 mM | Uncompetitive |
| | $K_{i_{NADPH}}$ 2.249±0.343 | Non-competitive |
| Yeast GR | $K_{i_{GSSG}}$ 1.388±0.200 mM | Non-competitive |
| | $K_{i_{NADPH}}$ 0.762±0.007 | Uncompetitive |

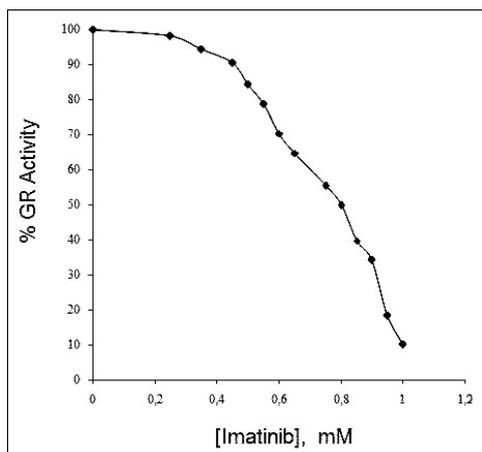


Fig. 4. Inhibition of bovine kidney cortex GR by imatinib. N = 3, data in triplicate.

Kinetics of imatinib inhibition of bovine kidney cortex GR

Kinetic studies on the inhibition of bovine kidney cortex GR by imatinib were performed. The data were analysed and the kinetic constants were calculated by means of a nonlinear curve-fitting program (Statistica StatSoft computer program) (Segel, 1975).

When GSSG was the varied substrate, at constant and unsaturating NADPH concentration (0.1 mM), different fixed concentrations of imatinib (0.6; 0.7; 0.8 mM) were added into the assay mixture and initial velocities were measured. Imatinib acted as an uncompetitive inhibitor with respect to GSSG (Fig. 5, Table 1)

When NADPH was the varied substrate, at constant and unsaturating GSSG concentration (0.7 mM), different fixed concentrations of imatinib (0.6–0.8 mM) were added into the assay mixture. It is shown that imatinib acted as a non-competitive inhibitor with respect to NADPH (Fig. 6, Table 1).

Inhibition of bovine liver GR by imatinib

It was found that this drug inhibits bovine liver GR in a concentration-dependent manner (0.5–1 mM) and the obtained IC_{50} value of imatinib was 0.92 mM (Fig. 7).

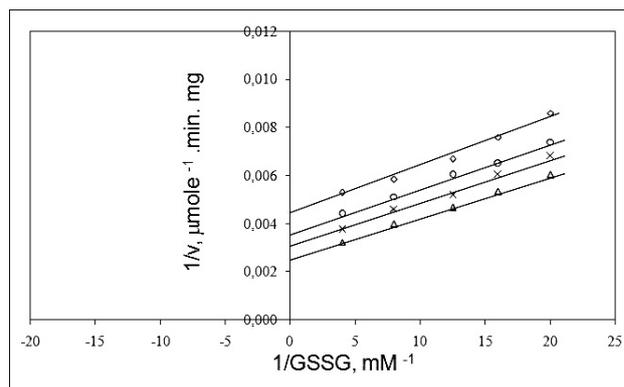


Fig. 5. Inhibition kinetics of bovine kidney cortex GR by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against GSSG as varied substrate and NADPH 0.1 mM constant concentration. N = 3, data in triplicate. Each point is the average of at least three determinations. Trend-line values and standard deviations are $R^2 = 0.999, 0.0009$; $R^2 = 0.993, 0.0010$; $R^2 = 0.996, 0.001$; $R^2 = 0.984, 0.001$, respectively. The assays were carried out at 37 °C in 100 mM sodium phosphate buffer, pH 7.4, as described in Material and Methods.

Δ 0.1 mM NADPH (no imatinib); \times 0.6 mM imatinib; \circ 0.7 mM imatinib; \diamond 0.8 mM imatinib

Kinetics of imatinib inhibition of bovine liver GR

Kinetic studies on the inhibition of bovine liver GR by imatinib were performed:

When GSSG was the varied substrate, at constant and unsaturating NADPH concentration (0.1 mM), different fixed concentrations of imatinib (0.55; 0.7; 0.75 mM) were added into the assay mixture and initial velocities were measured. It is shown that imatinib acted as an uncompetitive inhibitor with respect to GSSG (Fig. 8, Table 1).

When NADPH was the varied substrate, at constant and unsaturating GSSG concentration (0.7 mM), different fixed concentrations of imatinib (0.55; 0.7; 0.75 mM) were added into the assay mixture. It is shown that imatinib acted as a non-competitive inhibitor with respect to NADPH (Fig. 9, Table 1).

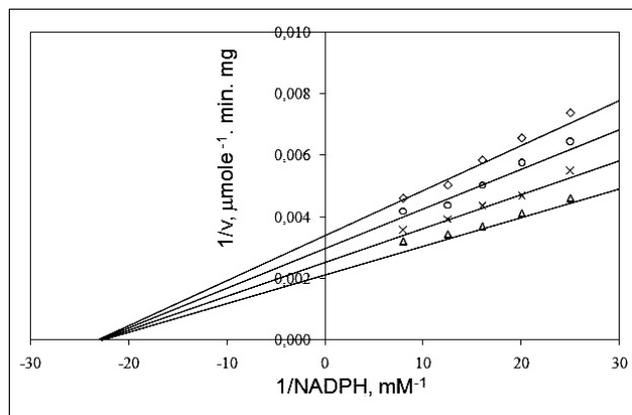


Fig. 6. Inhibition kinetics of bovine kidney cortex GR by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against NADPH as varied substrate and GSSG 0.7 mM constant concentration. N = 3, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.981, 0.0005$; $R^2 = 0.979, 0.0007$; $R^2 = 0.969, 0.0008$; $R^2 = 0.989, 0.001$, respectively. The assays were carried out at 37 °C in 100 mM sodium phosphate buffer, pH 7.4, as described in Material and Methods.

Δ 0.7 mM GSSG (no imatinib); x 0.6 mM imatinib; ○ 0.7 mM imatinib; ◇ 0.8 mM imatinib

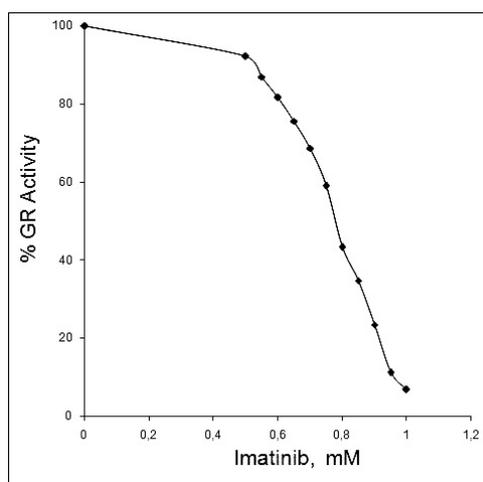


Fig. 7. Inhibition of bovine liver GR by imatinib. N = 3, data in triplicate.

Inhibition of yeast GR by imatinib

We have investigated the *in vitro* effects of imatinib on Baker's yeast glutathione reductase by using different (0.5–1.5 mM) concentrations of the inhibitor. The obtained IC_{50} value of imatinib was 1 mM (Fig. 10).

Kinetics of imatinib inhibition of yeast GR

When GSSG was the varied substrate, at constant and unsaturating NADPH concentration (0.1 mM), different fixed concentrations of imatinib (0.5; 0.75; 0.85 mM) were added into the assay mixture and initial velocities were measured. It is shown that imatinib acted as a non-

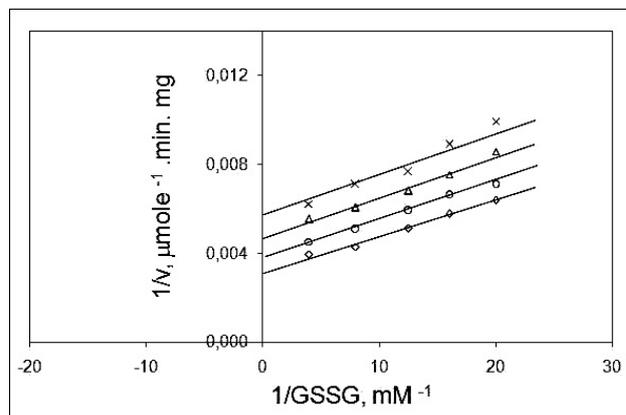


Fig. 8. Inhibition kinetics of bovine liver GR by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against GSSG as varied substrate and NADPH 0.1 mM constant concentration. N = 3, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.987, 0.0009$; $R^2 = 0.993, 0.0009$; $R^2 = 0.983, 0.001$; $R^2 = 0.975, 0.001$, respectively. The assays were carried out at 37 °C in 100 mM sodium phosphate buffer, pH 7.4, as described in Material and Methods.

◇ 0.1 mM NADPH (no imatinib); ○ 0.55 mM imatinib; Δ 0.7 mM imatinib; x 0.75 mM imatinib

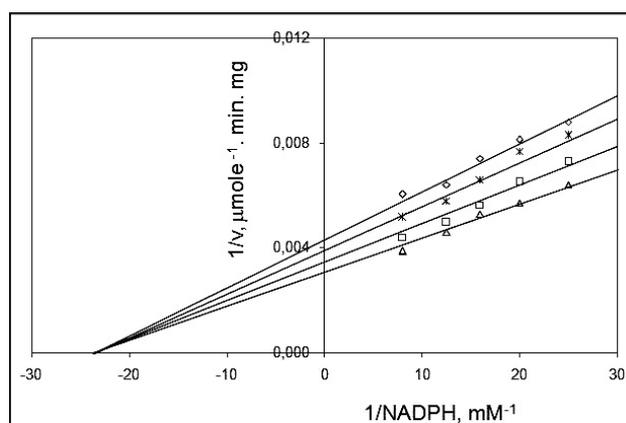


Fig. 9. Inhibition kinetics of bovine liver GR by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against NADPH as varied substrate and GSSG 0.7 mM constant concentration. N = 3, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.991, 0.0009$; $R^2 = 0.992, 0.001$; $R^2 = 0.982, 0.001$; $R^2 = 0.975, 0.001$, respectively. The assays were carried out at 37 °C in 100 mM sodium phosphate buffer, pH 7.4, as described in Material and Methods.

Δ 0.7 mM GSSG (no imatinib); □ 0.55 mM imatinib; x 0.7 mM imatinib; ◇ 0.75 mM imatinib

competitive inhibitor with respect to GSSG (Fig. 11, Table 1).

When NADPH was the varied substrate, at constant and unsaturating GSSG concentration (0.7 mM), different fixed concentrations of imatinib (0.5; 0.75;

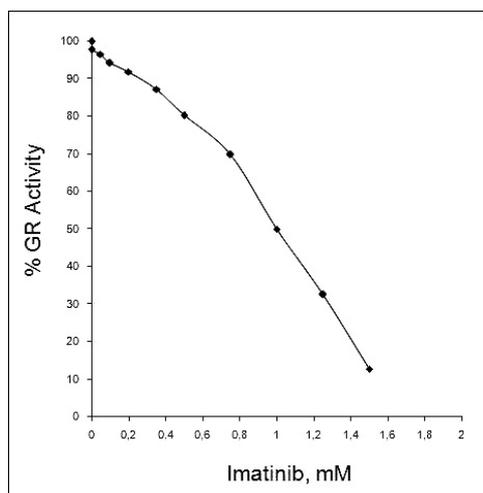


Fig. 10. Inhibition of yeast GR by imatinib. N = 3 Data in triplicate.

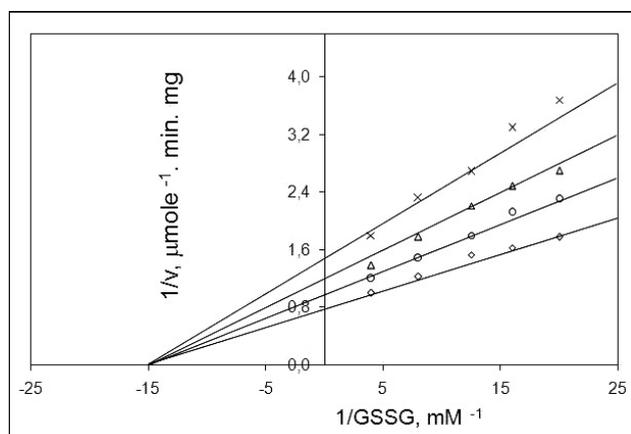


Fig. 11. Inhibition kinetics of yeast GR by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against GSSG as varied substrate and NADPH 0.1 mM constant concentration. N = 3, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.977, 0.280$; $R^2 = 0.993, 0.402$; $R^2 = 0.986, 0.477$; $R^2 = 0.990, 0.674$, respectively. The assays were carried out at 37 °C in 100 mM sodium phosphate buffer, pH 7.4, as described in Material and Methods.

◇ 0.1 mM NADPH (no imatinib); ○ 0.5 mM imatinib; Δ 0.75 mM imatinib; x 0.85 mM imatinib

0.85 mM) were added into the assay mixture. It is shown that imatinib acted as an uncompetitive inhibitor with respect to NADPH (Fig. 12, Table 1).

Discussion

Inhibition of glucose-6-phosphate dehydrogenase with imatinib mesylate

Recent studies have suggested that the G6PD enzyme has a critical role in proliferation of pre-neoplastic and

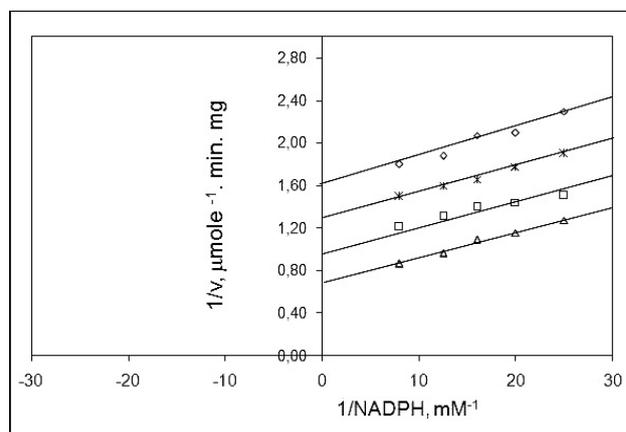


Fig. 12. Inhibition kinetics of yeast GR by imatinib. Lineweaver-Burk double reciprocal plot of initial velocity against NADPH as varied substrate and GSSG 0.7 mM constant concentration. N = 3, data in triplicate. Each point is the average of at least three determinations. Trendline values and standard deviations are $R^2 = 0.989, 0.143$; $R^2 = 0.968, 0.103$; $R^2 = 0.993, 0.139$; $R^2 = 0.959, 0.176$, respectively. The assays were carried out at 37 °C in 100 mM sodium phosphate buffer, pH 7.4, as described in Material and Methods.

Δ 0.7 mM GSSG (no imatinib); □ 0.5 mM imatinib; x 0.75 mM imatinib; ◇ 0.85 mM imatinib

neoplastic cells (Baba et al., 1989; Farquharson et al., 1993). Receptor tyrosine kinases are involved in oncogenesis, either by gene mutation or chromosome translocation, or simply by over-expression (Abella and Park, 2009). Imatinib mesylate is a competitive inhibitor of BCR-ABL through binding the ATP-binding site of tyrosine kinase (van Erp et al., 2007). On the other hand, increased G6PD activity is involved in tyrosine kinase synthesis (Boren et al., 2001). We have investigated the *in vitro* effects of imatinib on sheep brain cortex G6PD. We found that imatinib inhibited the sheep brain G6PD in a concentration-dependent manner. Inhibition of the enzyme was concentration-dependent with the IC_{50} value of 0.7 mM. However, *in vivo* exposure to imatinib inhibited the G6PD activity with an IC_{50} value of 6.8 μM. In response to STI571, proliferation of BCR-ABL-positive K562 cells showed a 57 % and 74 % decrease, respectively, whereas glucose label incorporation into RNA decreased by 13.4 % and 30.1 %, respectively (Boren et al., 2001).

Inhibition studies tell us that when G6P is the varied substrate, imatinib and G6P bind reversibly, randomly and independently to different sites. On the other hand, when $NADP^+$ is the varied substrate, imatinib inactivates G6PD competitively, as shown in Table 1 where imatinib and $NADP^+$ are mutually exclusive because of true competition for the same site (Segel, 1975).

Sheep G6PD (both isoforms) and mammalian G6PD proteins are reported to exist as two binding sites for $NADP^+$. The observed substitutions of human His with Arg in ovine $NADP^+$ binding site probably change the

electrostatic potential of the area, as His residue has a relatively weaker affinity for H^+ in comparison with Arg. Lysine is dispensable for substrate binding, while its substitution with other amino acids leads the substrate to react with another residue near the NADP binding site (Laliois et al., 2007). For this reason, it is possible to say that imatinib bound His residues, hereby inhibiting $NADP^+$ binding with the site. The G6P binds at the outer phosphate site. In the NADPH-G6P ternary complex, G6P is bound in a suitable orientation for proton transfer from its β -1-hydroxyl to the catalytic base His240 (Naylor et al., 2001). Thus, imatinib probably bound a histidine or cysteine residue on the surface of G6PD.

We have established by kinetic analysis that imatinib inhibited G6PD non-competitively with respect to G6P and competitively with respect to $NADP^+$. It is known that competitive inhibitors act on the catalytic site and non-competitive inhibitors act on the non-catalytic site of the enzyme. The inhibition potency and type of the inhibition is very important. All the chemotherapy drugs are very potent enzyme inhibitors.

Inhibition of glutathione reductase with imatinib mesylate

In this study we investigated whether imatinib could inhibit the GR from mammalian tissues and yeast. Yeasts are also among the most commonly used model organisms for genetics and cell biology. We found that imatinib inhibits the bovine kidney cortex, liver and yeast GR in a concentration-dependent manner. It was observed that inhibition of the enzyme was acting in a concentration-dependent manner with the IC_{50} values of 0.8; 0.92; 1 mM, respectively (Figs. 4, 7, 10). Imatinib is further an effective inhibitor for bovine kidney cortex and liver GR as compared with Baker's yeast GR. In our experiments we found that the yeast GR enzyme was inhibited at a higher imatinib concentration than the bovine enzymes. These results may indicate that IC_{50} concentration will be lower in human enzymes. As compared with bovine kidney cortex and liver GR, the inhibition type of imatinib was different from Baker's yeast GR. Here, we found that imatinib was also an inhibitor of Baker's yeast GR giving a non-competitive inhibition pattern when GSSG (Fig. 11) was the varied substrate and uncompetitive pattern when NADPH (Fig. 12) was the varied substrate. However, the kinetic studies of bovine kidney cortex and liver GR show that imatinib is an uncompetitive inhibitor with respect to GSSG and a non-competitive inhibitor with respect to NADPH (Figs. 5, 6, 8, 9 and Table 1). This is probably due to imatinib binding different amino acid residues or groups in the catalytic site of the enzyme. As it is well known, uncompetitive inhibitors are thought to bind the ES complex and not the enzyme, but non-competitive inhibition occurs when the inhibitor binds at a site away from the substrate binding site, causing a reduction in the catalytic rate (Segel, 1975). In consideration of the amino acid residue in the GSSG and NADPH binding

site of the catalytic site, cysteine, histidine, tyrosine residues were found to play an essential role in the catalytic mechanism of GR (Arscott et al., 2000). His-439 is thought to act in the catalytic mechanism as a proton donor/acceptor in the glutathione-binding pocket and Tyr-99 is a further possible proton donor in the GSH-binding pocket (Deonarain et al., 1989). Cys-58 and Cys-63 represent the enzyme's redox-active dithiol. Cys-90 with its location at the twofold axis forms a disulfide bridge with Cys-90 of the other peptide chain of the enzyme (Untucht-Grau et al., 1981). GR activity is thought to depend on the intactness of enzymes' SH-groups; the inhibitory effect of imatinib may be due to binding thiol groups in the enzyme.

Targeted drugs inhibit a specific target molecule directly involved in tumour progression in a more specific manner and represent a new perspective in the treatment of cancer (Petrelli and Giordano, 2008). Cancer cells also have the ability to become resistant to multiple different drugs by enzymatic deactivation (i.e., glutathione conjugation) and decreased permeability of drugs (Harris and Hochhauser, 1992). Inhibiting the GR will decrease the reduced form of glutathione and this may affect the drug resistance. High levels of glutathione in tumour patients can be used as the measure and prognostic criteria of drug resistance (Gorozhanskaya et al., 1998).

Imatinib has been used in the treatment of many types of cancers for many years. It is a well-designed anti-cancer drug. In this study we have shown the inhibition effect of this drug on two important enzymes from various sources to indicate the association of the drug and the enzyme kinetic mechanisms. It is known that irreversible inhibitors have many side effects. Therefore, understanding the drug and enzyme relationship may be a powerful approach to the future drug studies: new cancer drugs, drug resistance and new aspects in cancer therapy. It could also give us a new point of view on the drug studies.

In this study we investigated G6PD, which has a key role in detoxification, biosynthesis and cell proliferation. It is known that the drug metabolism and cell proliferation rate are extremely important factors in cancer therapy. Cancer cells have a typical different cellular metabolism. While most investigators accept that GSH has a role in drug resistance, the involvement of GSH-associated enzymes has been more contentious (Tew, 1994).

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