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Changes in the activity of the GPx-1 anti-oxidant selenoenzyme in mononuclear cells following imatinib treatment

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Abstract

Imatinib inhibits the ABL tyrosine kinase and is effective for the treatment of chronic myeloid leukemia (CML). ABL activates GPx-1, an enzyme associated with protection against oxidative DNA damage and disease. Enzyme activity was assessed in sample pairs consisting of mononuclear cells obtained from patients before and after imatinib therapy. Control sample sets obtained from patients not receiving imatinib showed little change in GPx activity over a several month interval. Five of 7 sample sets obtained from imatinib-receiving patients showed changes in GPx activity greater than 30%. One sample decreased 42% while 4 others increased 33% to 208%. Patients with the largest increase in activity were female and had the lowest baseline levels of GPx activity. Changes in GPx activity may influence the clinical outcome of patients being treated for CML.

Keywords

imatinib; glutathione peroxidase; CML

Introduction

CML therapy has been improved by approaches designed to specifically inhibit the BCR/ABL fusion protein using imatinib (mesylate) (Gleevec[®], CGP-57148, Novartis, Switzerland). Most patients with chronic phase CML achieve complete cytogenetic

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Authors' Contributions

ET performed the experiments and along with AD, PG, RM and MD, designed the studies, participated in the writing of the manuscript and data interpretation. In addition, PG and MD provided samples for analysis.

Conflicts of Interest

None of the authors expressed any potential conflicts of interest.

responses, with relatively mild side effects [1]. However, 20–25% exhibit primary or secondary resistance to imatinib, typically due to BCR-ABL point mutations or gene amplification [2]. Given this failure rate, strategies that predict or overcome resistance are warranted.

Reduced levels of the anti-oxidant enzyme glutathione peroxidase-1 (GPx-1) are associated with human disease, including asthma, heart disease, diabetes and cancer. GPx-1 was shown to be a substrate for ABL kinase activity with a consequential stimulation of enzyme activity that can be inhibited by treating the cells with imatinib [3]. Given the need to improve the clinical outcome of patients receiving imatinib and the role of GPx-1 in contributing to disease risk, we investigated whether there were changes in GPx activity in mononuclear cells obtained from patients before and after imatinib therapy.

Design and Methods

GPx activity was determined from archived mononuclear cells obtained from patients before and after undergoing imatinib therapy. Since the amount of protein available was insufficient to perform the assay multiple times, the reproducibility of the assay was established. Extracts prepared from the KU812 and MEG-01 human CML cell lines (ATCC) were freeze-thawed and assayed for GPx activity. To assess the variation in GPx activity expected to occur in samples from the same individuals over a period of time similar to that of patients treated with imatinib, mononuclear cells (MNC) were obtained from participants in a lycopene supplementation study (UIC IRB# 2005-0828, Peter Gann) and assayed for GPx activity. Samples were also obtained from patients prior to and after 3–6 months imatinib therapy as part of a study to predict clinical responsiveness (Oregon Health & Science University IRB # MOL-01018-L, B. Drucker). MNCs were isolated by Ficoll gradient centrifugation, frozen either as dry pellets or with maintaining viability, or made into protein lysates. Extracts were prepared and assayed for GPx activity as previously described [4].

Results and Discussion

Assay reliability

In order to determine the reproducibility of the GPx assay, extracts prepared from KU812 and MEG-01 cells were subjected to multiple freeze-thaw cycles and assayed on different days spanning a 4 month period. Four KU812 and 2 MEG-01 extracts were assayed (Figure 1A), where it was apparent that the variation from the mean did not exceed 20% for any of the tested extracts.

To determine the anticipated variation in GPx activity expected in MNCs from the same individuals over an approximately 6 month period, extracts were prepared from deidentified MNC cells from participants in a nutritional supplementation study not including drugs or substances that would alter GPx activity (unpublished observations). Five pairs of samples were studied (Figure 1B). The variation in measured activity for each of these sample sets was less than 20%, similar to the assay variation observed in the multiple retesting studies shown in Figure 1A.

Changes in GPx activity following imatinib therapy

GPx activity was assessed in paired samples obtained from the individuals before and after treatment with 400 mg/day imatinib and shown graphically in Figure 2. Patient information including gender, age and duration of treatment are included in Table 1. Of the 7 sample sets presented, 5 showed changes in enzyme activity that exceeded 20% and 2 did not. Of those 5, one showed a decrease in activity of 42% and 4 demonstrated increases ranging from 33%

to 208%. The sample set showing decreased GPx activity had the highest baseline level prior to treatment and the two demonstrating the greatest increases following treatment (166% and 208%) exhibited the lowest pre-treatment levels and were derived from the only 2 females in the patient population examined.

There are a number of limitations to this study. The amount of protein available for analysis permitted only a single assay per sample, although our assessments on assay variability indicated the data obtained is likely to be accurate. The total number of sample sets examined was also small (n=7). This, coupled with the large variation in the changes seen in the imatinib-treated patients, resulted in non-statistical significance ($p=0.18$) between treatment and control sets via a two-sample t-test with equal variance. However, five of 7 showed changes in excess of what was seen in the control analysis of untreated individuals. The sample size was also too small to make conclusions regarding any associations between changes in GPx activity and responsiveness to imatinib treatment as only one of the patients, number 4, had disease that did not improve with the drug.

In contrast to published data [3], we were unable to either detect tyrosine phosphorylation of GPx-1 nor an effect of mutating the likely ABL substrate [4] and exposure of the KU812 or MEG-01 human CML cell lines to 50 nM imatinib for 3 days did not result in a change in GPx enzyme activity (data not shown). Thus, there may be cell culture or cell type specific determinants of ABL phosphorylation of GPx-1. Nonetheless, most of the sample pairs examined showed a change in GPx-1 in MNC's following treatment. GPx may have a significant impact in influencing disease management and long term health of treated patients. As a major anti-oxidant enzyme, altered levels may influence the risk of DNA oxidations products leading to disease. In support of this, numerous reports have shown that a variant GPx-1 allele expected to exhibit reduced enzyme activity is associated with increased risk of a variety of diseases, including cancer [5]. Alternatively, enhanced GPx-1 activity may result in increased risk of diseases such as diabetes [6] or negatively effect therapeutic effectiveness by inhibiting apoptosis, as was first shown in T cells [7] and subsequently in a variety of cell types. These studies indicate the need for additional work to evaluate changes in GPx activity in patients undergoing treatment with ABL kinase inhibitors and to determine whether these changes impact the therapeutic outcome.

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Figure 1.
Reproducibility of the GPx enzyme assay.

A. Extracts were prepared from separate KU812 and MEG-01 cultures, and GPx activity assayed following 4 freeze-thaw cycles. Extracts were prepared from 4 individual cultures of KU812 cells (K1-4) and 2 individual cultures of MEG-01 cells (M1 and M2).

B. Extracts were prepared from MNC's derived from the same subjects 6–9 months apart and assayed for GPx activity. Individual patients are indicated by letters. GPx activity is presented as nmoles of NADPH oxidized/mg protein and the error bars indicate the S.D.

Figure 2.
GPx activity in MNC extracts derived from patients before and after imatinib therapy. Extracts were prepared from frozen and viable MNCs derived from patients prior to beginning imatinib treatment and after several months of receiving the drug (see Table 1 for details). GPx activity is presented as nmoles of NADPH oxidized/min/mg protein.

Table 1

Patient information and degree of change in GPx activity. Patients 1,2,5,6 and 7 achieved complete or near complete cytogenetic and/or molecular responses to imatinib therapy, patient 3's response was considered sub-optimal and patient 4's disease was considered resistant to imatinib therapy.

Patient	Gender	Age (yrs)	Duration of treatment (Mos.)	% Change
1	M	45	4	-42%
2	M	54	3	-
3	M	71	6	-
4	M	28	2	+75%
5	M	64	6	+33%
6	F	62	6.5	+208%
7	F	53	12	+166%