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ScienceDirect
Leukemia Research 32 (2008) 980–983
Leukemia Research
www.elsevier.com/locate/leukres

Brief communication

Comparison of imatinib, dasatinib, nilotinib and INNO-406 in imatinib-resistant cell lines

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Received 26 June 2007; received in revised form 2 November 2007; accepted 2 November 2007
Available online 8 January 2008

Abstract

We compared the growth-inhibitory effects and inhibition profile of the SRC family kinases (SFKs) of imatinib, dasatinib, nilotinib and INNO-406. Dasatinib exhibited the strongest potency against BCR–ABL with little selectivity over SFKs. Nilotinib exhibited a weaker affinity than the other inhibitors, but was highly specific for ABL and may be useful for the treatment of P-glycoprotein overexpressing leukemic cells. INNO-406 had an intermediate affinity for BCR–ABL between that of dasatinib and nilotinib, and inhibited only SFKs LCK and LYN among SFKs. Both nilotinib and INNO-406 were potent inhibitors of the dasatinib-resistant T315A, F317L and F317V BCR–ABL mutations.

Keywords: Imatinib; Dasatinib; Nilotinib; INNO-406; SRC family kinases

1. Introduction

The use of imatinib mesylate, an ABL tyrosine kinase inhibitor, has led to a dramatic change in the management of chronic myeloid leukemia (CML); however, several patients, especially those in the advanced phase of the disease, develop resistance to imatinib therapy. Several second-generation ABL tyrosine kinase (TK) inhibitors, including dasatinib [1], nilotinib [2] and INNO-406 (formerly NS-187) [3] have been developed to override the imatinib-resistance mechanisms that are caused by BCR–ABL overexpression and/or point mutations within the ABL kinase domain. All of the second-generation ABL TK inhibitors exhibit a stronger affinity to ABL than imatinib and are effective against most of the mutated forms of ABL; however, the relative affinity to ABL, the effects on mutated ABL and the inhibitory profile for other TKs differs amongst these agents.

A comparative analysis of dasatinib and nilotinib has been previously described; however, data comparing these drugs to INNO-406 has not been reported. We therefore compared the growth-inhibitory effects and inhibition profile for SFKs of imatinib, dasatinib, nilotinib and INNO-406 in imatinib-sensitive and -resistant CML cell lines.

2. Materials and methods

2.1. Reagents and leukemic cells

INNO-406 and imatinib were synthesized and purified at Nippon Shinyaku (Kyoto, Japan). Dasatinib and nilotinib were obtained from Bristol-Myers Squibb (New York, USA) and Novartis Pharma (Basel, Switzerland), respec-

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doi:10.1016/j.leukres.2007.11.008

tively. K562, BV173, KU812, MEG01, KT-1 and MYL were derived from a CML patient. HL60 was established from an acute myeloid leukemia (AML) patient. K562-IMR and MYL-R were established by single-cell culture after the exposure of each parental line to stepwise increments of imatinib. P-glycoprotein (P-gp)-overexpressing K562/D1-9, Ba/F_3 cell lines expressing BCR–ABL/wild-type (wt), M244V, G250E, Q252H, Y253F, T315I, T315A, F317L, F317V, M351T or H396P [3] and fetal liver cells (FLC) transfected with wt *bcr–abl* (FLC/wt) or wt *bcr–abl* and p53 deleted (FLC/wt/p53$^{-/-}$) [4] were established previously. The parental Ba/F_3 cell line was not transfected with *bcr–abl* and was maintained in 10% WEHI-conditioned medium as a source of IL-3.

2.2. Cell proliferation assays

Each cell line was plated in 96-well plates in triplicate at 1×10^4 cells/well. Cells were incubated with serial dilutions of each agent for 3 days. Cell proliferation was measured using the MTT assay with the SF reagent (Nacalai Tesque, Kyoto, Japan), and IC_{50} values were calculated using CalcuSyn software (Biosoft, Cambridge, UK).

2.3. Inhibitory effects against SFKs

The inhibitory effects of dasatinib and INNO-406 against eight SFKs (SRC, LCK, LYN, FGR, BLK, FYN, YES, HCK) were tested using the QuickScout® Select Panel (Carna Biosciences, Kobe, Japan).

3. Results and discussion

We initially compared the growth-inhibitory effects of the ABL TK inhibitors against six CML cell lines and HL60 (Table 1). None of the agents suppressed the growth of HL60 or the parental Ba/F3 cell line at concentrations less than 2000 nM. Dasatinib, nilotinib and INNO-406 exhibited 31.1–276.5 (mean 98.6), 4.7–17.9 (mean 9.85) and 6.0–44.3 (mean 16.4) times more potency, respectively, against the CML cell lines when compared to imatinib. Imatinib, nilotinib and INNO-406 are all 2-phenylaminopyrimidine derivatives; however, INNO-406 demonstrated 2.5 times more activity than nilotinib against the BV173 and KU812 cell lines. There was no significant difference on the growth-inhibitory effects of these two inhibitors on the other four CML cell lines. INNO-406 exhibited a stronger efficacy than imatinib and nilotinib; this could be explained by the trifluoromethyl group at the phenyl ring that is adjacent to the methylpiperazine group of INNO-406 effecting phosphorylated BCR–ABL [3].

Next, we compared the effect of the inhibitors on three imatinib-resistant cell lines: K562/D1-9 (P-gp overexpressing), K562-IMR (BCR–ABL overexpressing) and MYL-R (BCR–ABL and LYN overexpressing). In these cell lines, dasatinib exhibited more potency than nilotinib or INNO-406. INNO-406 was 3.1 times more effective than nilotinib against MYL-R, suggesting a possible effect of INNO-406 on LYN. Nilotinib exhibited more potency against K562/D1-9 than INNO-406, probably because nilotinib is unlikely to be a P-gp substrate [5] whereas INNO-406 is [6]. The IC_{50} val-

Table 1
Imatinib, dasatinib, nilotinib and INNO-406 IC_{50} values (nM) for cellular proliferation

	Imatinib	Dasatinib	Nilotinib	INNO-406
K562	414.8	1.5	24.3	24.7
BV173	873	10.9	48.7	19.7
KU812	202.5	3.5	29.7	11.8
KT-1	269.8	4.2	42.9	39.3
MEG01	196.2	6.3	42	27.3
MYL	529.6	6.5	84.5	87.7
HL60	>2000.0	>2000.0	>2000.0	>2000.0
K562/D1-9	>2000.0	91	309.7	970.3
K562-IMR	>2000.0	63.8	1550	879.3
MYL-R1	1575.8	6.4	993.6	324.1
Ba/F3 (parental)	>2000.0	>2000.0	>2000.0	>2000.0
Ba/F3/wt BCR–ABL	1712	87.6	642.3	152.9
Ba/F3/M244V	>2000.0	93.1	891.4	874.2
Ba/F3/G250E	>2000.0	27.2	866	852
Ba/F3/Q252H	>2000.0	62.3	895.5	888.1
Ba/F3/Y253F	>2000.0	16.8	975.3	490.8
Ba/F3/T315I	>2000.0	>2000.0	>2000.0	>2000.0
Ba/F3/T315A	>2000.0	>2000.0	949.2	422.5
Ba/F3/F317L	>2000.0	>2000.0	929.8	293.5
Ba/F3/F317V	1053.7	>2000.0	286.9	284
Ba/F3/M351T	>2000.0	88	580.4	582.9
Ba/F3/H396P	>2000.0	8.9	986.9	280.1
FLC/wt	309.7	3.8	55.6	44
FLC/wt/p53$^{-/-}$	464.5	18.5	170.8	104.8

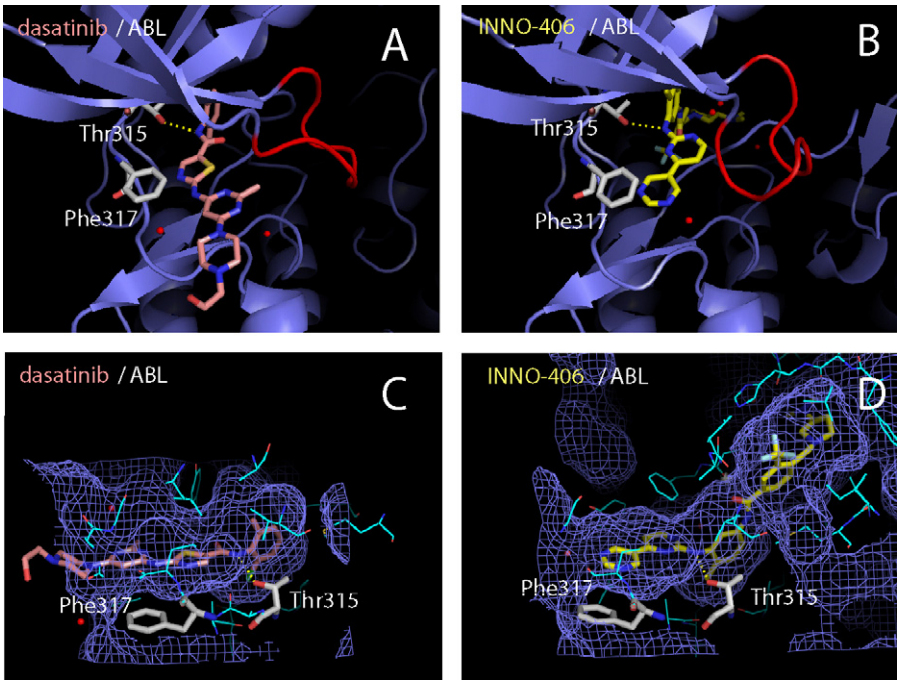

Fig. 1. X-ray crystal structures of the dasatinib/ABL (A and C) and INNO-406/ABL (B and D) complexes. (A) P-loop does not interact with dasatinib directly, (B) closely located P-loop tightly grips INNO-406, (C) Thr315 and Phe317 contribute largely to the binding and (D) Thr315 and Phe317 do not contribute as much for dasatinib.

ues for the ABL TK inhibitors against FLC/wt/p53$^{-/-}$ cells were 1.5-4.9 times higher than those of the parental FLC/wt cells, suggesting that the effects of the ABL TK inhibitors partially depend on the p53 pathway.

None of the ABL TK inhibitors reduced the growth of Ba/F_3/T315I cells. In cell proliferation assays using Ba/F3 cells harboring a series of BCR–ABL mutants, dasatinib exhibited at least six-times more potency than nilotinib and INNO-406 for all of the mutations studied, with the exception of T315A, F317L and F317V (Table 1). Data from a mutagenesis assay predicted that dasatinib would be insensitive to T315A, F317L and F317V [7]; indeed, T315A, F317L and F317V mutations were recently detected in dasatinib-resistant CML patients. Both nilotinib and INNO-406 inhibited T315A, F317L and F317V, with the latter compound being more potent against T315A and F317L. Nilotinib and INNO-406 might therefore be effective for the treatment of patients expressing these three dasatinib-resistant mutant forms of BCR–ABL.

In order to determine why dasatinib was ineffective against the mutated ABL at amino acid 315 and 317, the X-ray crystal structures of the dasatinib/ABL (PDB ID: 2GQG) and INNO-406/inactive conformation of ABL (PDB ID: 2E2B) complexes were closely explored. While the P-loop of ABL (colored in red) tightly grips INNO-406 (Fig. 1B), it is remotely located from dasatinib and does not directly interact with this inhibitor (Fig. 1A). The T315A, F317L and F317A mutations cause decreased steric and hydrogen-bonding interactions. Accordingly, the P-loop is likely to compensate for these decreased interactions in the case of INNO-406 but not for dasatinib (Fig. 1C and D).

Next we compared the inhibitory effect of dasatinib and INNO-406 against SFKs. Nilotinib, like imatinib, has been reported not to inhibit any SFKs [8]. As previously reported [1], dasatinib inhibited all eight SFKs at very low concentrations, while INNO-406 inhibited only LCK and LYN, which may be involved in imatinib-resistance (Table 2). SFKs have diverse but important roles in vivo and little is known what happens when all of the SFKs are strongly suppressed for prolonged periods of time. The relative specificity of INNO-406 for SFKs may therefore lead to a more favorable side effect profile in patients.

In conclusion, dasatinib exhibited the strongest affinity for ABL and the least specificity for SFKs. Nilotinib showed weaker affinity for SFKs compared to the other compounds, but was highly specific for ABL and may be useful for the treatment of P-glycoprotein overexpressing leukemic cells. INNO-406 had an intermediate affinity for BCR–ABL

Table 2
Effects of dasatinib and INNO-406 against SRC family kinases

	Dasatinib	INNO-406
SRC	2.7	4300.0
LCK	1.0	120.0
LYN	1.7	230.0
FGR	7.1	860.0
BLK	3.5	1700.0
FYN	3.1	2800.0
YES	8.9	3600.0
HCK	7.1	7700.0

between that of dasatinib and nilotinib, and also inhibited the SFKs LCK and LYN. Both nilotinib and INNO-406 were potent inhibitors of the dasatinib-resistant T315A, F317L and F317V BCR–ABL mutations. These findings should be useful for treating imatinib-resistance patients with second generation ABL TK inhibitors.

Acknowledgments

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, Technology of Japan, the Kobayashi Foundation of Innovative Cancer Chemotherapy, Japan Leukaemia Research Fund and Research Grant of the Princess Takamatsu Cancer research Fund and Kobayashi Foundation for Innovative Cancer Chemotherapy. KT-1, MYL(-R), K562/D1-9 and K562-IMR were kindly provided by Prof. Yasukawa, Dr. Tanaka, Dr. Urasaki and Dr. Yamauchi, respectively.

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