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RESEARCH ARTICLE

Synergistic effect of imatinib mesylate and fludarabine combination on Philadelphia chromosome-positive chronic myeloid leukemia cell lines

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ABSTRACT

Fludarabine-containing combinations have additive cell killing against leukemic blasts in vitro. It has also been shown that imatinib mesylate combined with fludarabine or cladribine had an additive effect on CML CFU-GM cells. In this regard, we aimed to investigate the effect of fludarabine-imatinib mesylate combination against CML blastic phase cell lines K562 and Meg-01. XTT test was performed for proliferation and inhibition assay. According to obtained data, five different effective concentrations of each drug in 25 different combinations were tested. Results of the combination studies were analyzed with isobologram. At IC_{20}, imatinib mesylate and fludarabine combination showed synergism and strong synergism in K562 and Meg-01 cells, respectively. At IC_{50} and IC_{75}, combination indexes (CI) indicated strong synergism and synergism. Based on our results, the fludarabine- based chemotherapy regimens can be used for those patients with CML blastic phase in combination with imatinib mesylate.

Key Words: Fludarabine, Imatinib mesylate, CML, K562, Meg-01

ÖZET

İmatinib mesilat ve fludarabin kombinasyonunun Filadelfiya kromozomu pozitif kronik myeloid lösemi hücre serileri üzerindeki sinerjistik etkisi

In vitro koşullarda fludarabin içeren kombinasyonların lösemik blastlar üzerinde ve fludarabin ya da kladribinin imatinib mesilat ile kombinasyonlarının KML CFU-GM hücrelerine karşı aditif etkinliğe sahip oldukları gösterilmiştir. Bu gözlemlere dayanarak biz de fludarabin ve imatinib mesilat kombinasyonunun KML blastik faz hücre serileri K562 ve Meg-01 üzerine olan etkinliğini araştırmayı planladık. Proliferasyon ve inhibisyon göstergesi olarak XTT testini kullandık. Elde edilen verilere göre, her ilacın beş farklı etkin konsantrasyonu 25 farklı kombinasyon halinde test edildi. Kombinasyon çalışmalarının sonuçları izobologram ile analiz edildi. İmatinib mesilat ve fludarabinin kombinasyonunun K562 ve Meg-01 hücre serileri için IC_{20} kombinasyon indeksi değerleri sırası ile sinerjistik ve güçlü sinerjistik, IC_{50} ve IC_{75} değerlerinde de güçlü sinerjistik ve sinerjistik etkinliği göstermekte idi. Bu sonuçlar KML blastik fazdaki hastalar için imatinib mesilat ile kombine edilen fludarabin bazlı rejimlerin kullanılabileceğini göstermektedir.

Anahtar Sözcükler: Fludarabin, imatinib mesilat, KML, K562, Meg-01

INTRODUCTION

Imatinib mesylate is a specific inhibitor of bcr-abl tyrosine kinases [1]. It also inhibits the growth of Philadelphia-positive (Ph^+) cell lines in vitro [1-3]. However, the apoptosis of Ph^+ cells by imatinib mesylate may be incomplete, in that combination of this agent with other antileukemic agents seems to be an important target to obtain complete elimination of the disease. The purine analogue fludarabine when combined with other antileukemic agents has promising activity against acute leukemias [4], and is given for immune suppression as a part of the allogeneic bone marrow transplantation procedure. It has been shown that fludarabine-containing combinations have additive cell killing against leukemic blasts in vitro [5,6], and further that imatinib mesylate used together with both fludarabine and cladribine had an additive effect on chronic myelogenous leukemia (CML) CFU-GM cells [7].

In order to further improve its effectiveness against different Ph^+ cell lines, many combinations of imatinib mesylate with different antileukemic drugs including hydroxyurea, interferon alpha or cytarabine have been investigated. The highly varied experimental conditions and analytical methods for evaluating the effects of the drug combinations have yielded different findings; nevertheless, the vast majority of studies imply that combination of imatinib mesylate with other antileukemic agents against Ph^+ cell lines produces better antileukemic activity than monotherapy [8-10]. These findings led us to think that the combination of imatinib mesylate and fludarabine might have a good antileukemic activity against Ph^+ leukemic blasts. Hence, we planned to study the effect of this combination against Ph^+ CML blastic phase cell lines K562 and Meg-01.

MATERIALS and METHODS

Cell lines

K-562 [European Collection of Cell Cultures (ECACC)] and Meg-01 (ECACC), the Ph + human CML blastic cell lines, were maintained in plastic tissue culture flasks containing RPMI 1640 (Biological Industries, Israel) with L-glutamine

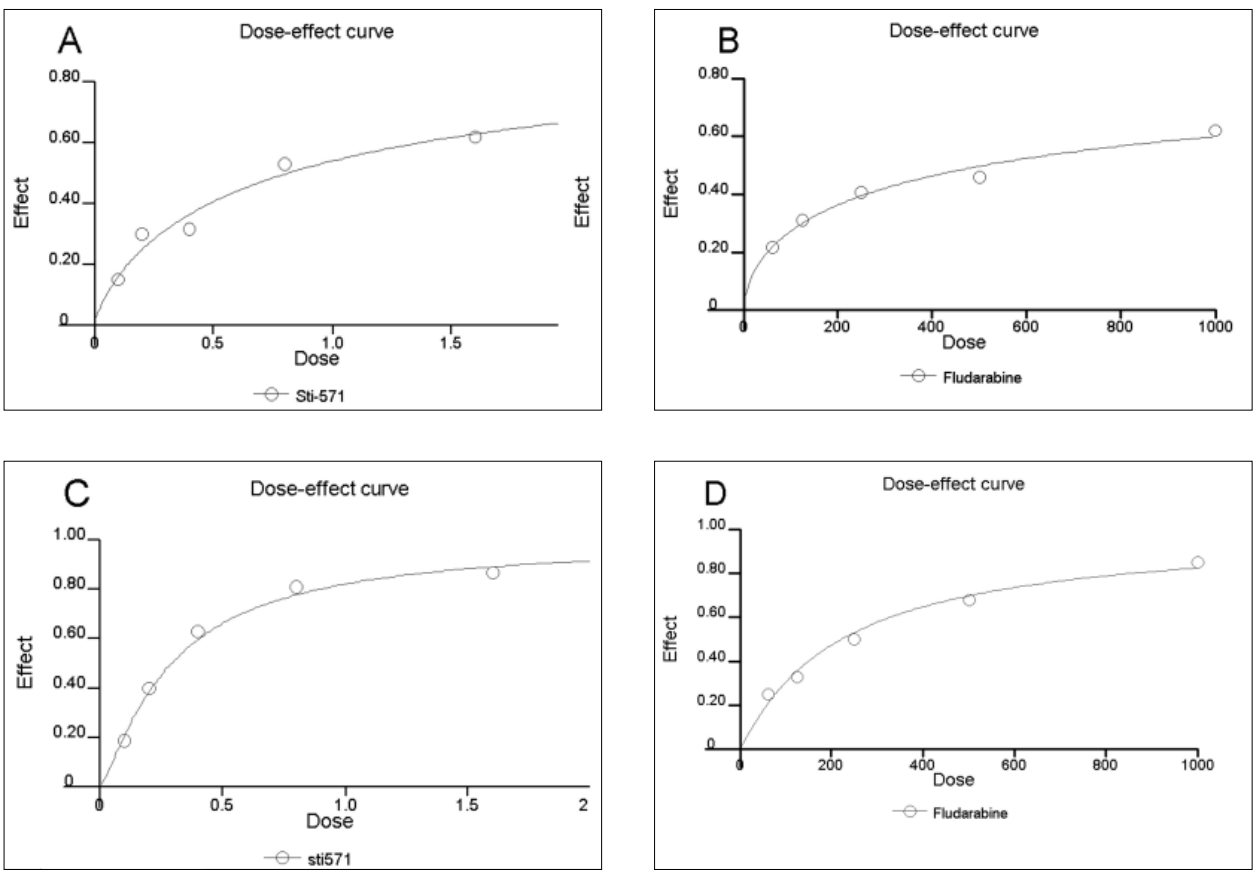
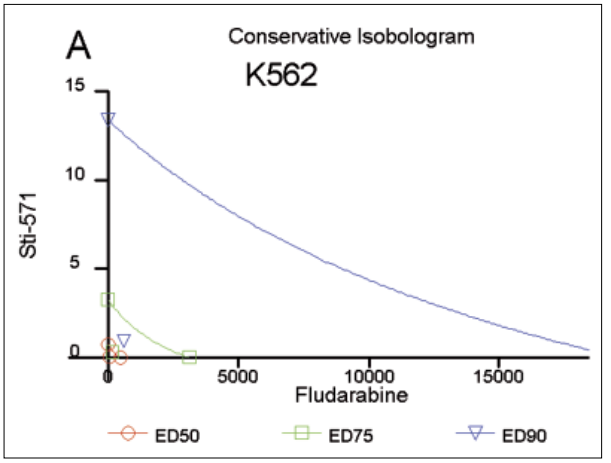


Figure 1. Dose-effect curves of cell lines; **A:** STI571 for K562, **B:** Fludarabine for K562 and **C:** STI571 for Meg-01, **D:** Fludarabine for Meg-01.



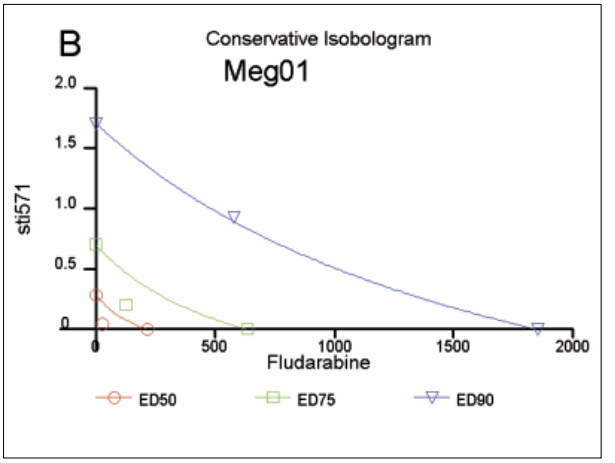


Figure 2. Isobologram analyses of K562 **(A)** and Meg-01 **(B)**.

Table 1. Combination indexes of imatinib mesylate and fludarabine combinations for cell lines

	IC20	*IC50*	*IC75*
K562	0.58 (synergism)	0.28 (strong synergism)	0.17 (strong synergism)
Meg-01	0.15 (strong synergism)	0.30 (synergism)	0.55 (synergism)

medium supplemented with 10% heat-inactivated fetal calf serum (FCS) (Biological Industries, Israel) and penicillin-streptomycin-amphotericin-B (Biological Industries) in a humidified atmosphere of 95% air 5% CO_2 at 37^{0}C.

Drugs

Imatinib mesylate was kindly provided by Novartis (Basel, Switzerland). According to the manufacturer's instructions, 10 µM stock solution of imatinib mesylate was prepared and stored at -20^{0}C and diluted with RPMI 1640 medium before use.

Fludarabine was obtained from Schering AG (Germany). 100 µM stock solution of the agent was prepared and stored. Appropriate drug concentrations were made by dilution with fresh medium (RPMI 1640 medium with 10% heat-inactivated FCS) immediately before each experiment.

Cytotoxicity assay

XTT (Roche Diagnostica, Germany) assay was performed for proliferation and inhibition assay as described previously [11]. Cells from each line were harvested from the medium and resuspended to a final concentration of 5 x 10^3 cells per well and exposed to escalating doses of imatinib mesylate and fludarabine independently. 96-well plates were then incubated at 37^{0}C in 5% CO_2. After a 72 hour incubation period, XTT solution was added to wells and incubated again for four hours. The spectrophotometric absorbances of the samples were measured with a microplate (ELISA) reader. All combinations were assayed in triplicate. The wavelength to measure absorbance of the formazan product was between 450 and 500 nm. The reference wavelength was 650 nm.

Regarding the proliferation-inhibition values, we obtained dose-response curves for each cell line with each agent and then calculated 50% inhibition of proliferation (IC_{50}) values. According to obtained data, five different effective concentrations of each drug in 25 different combinations were tested. Results of the combination studies were analyzed with isobologram [12], and combination index (CI) was calculated by the CI=d1/D1 + d2/D2 formulation. CI values were defined as follows: smaller than 1 synergism, equal to 1 additive, and over 1 antagonism.

Data analysis

Isobologram analyses and graphics were carried out using CalcuSyn for Windows 1.1 software program.

RESULTS

Calculated IC_{50} values of imatinib mesylate for K562 and Meg01 were 0.8 and 0.29 µM, and

IC_{50} values of fludarabine for K562 and Meg-01 were 504 and 219 μM, respectively (Figure 1). According to obtained data from proliferation-inhibition tests, five different effective concentrations of each drug (for imatinib mesylate 0.1, 0.2, 0.4, 0.8 and 1.6 μM and for fludarabine 62.5, 125, 250, 500 and 1000 μM) in 25 different combinations were tested.

At IC_{20}, imatinib mesylate and fludarabine combination showed synergism (CI=0.58) and strong synergism (CI=0.15) in K562 and Meg01 cells, respectively. At IC_{50}, CIs were 0.28 (strong synergism) and 0.30 (synergism), and at IC_{75}, CIs were 0.17 (strong synergism) and 0.55 (synergism) (Table 1 and Figure 2).

DISCUSSION

CML in the chronic phase can be controlled by anticancer agents such as interferon alpha, hydroxyurea, cytarabine, and busulfan, but survival is extremely short after the onset of blastic crisis. Leukemia cells in blastic phase are extremely resistant to antileukemic agents. Allogeneic bone marrow transplantation is the best chance to cure CML, and more than 50% of patients will be cured. However, transplantation is only available for less than 30% of patients. It is obvious that development of novel therapies is required to decrease morbidity and mortality of CML.

Imatinib mesylate, a specific inhibitor of bcr-abl tyrosine kinases when used as a single agent, demonstrates significant activity in patients with CML. Imatinib mesylate also inhibits the growth of Ph^+ cell lines in vitro [1-3]. However, the apoptosis of Ph^+ cells by imatinib mesylate may be incomplete. In order to further improve its effectiveness against different Ph^+ cell lines, many combinations of imatinib mesylate with different antileukemic drugs including hydroxyurea, interferon alpha or cytarabine have been investigated. Even though the highly varied experimental conditions and analytical methods for evaluating the effects of the drug combinations have yielded different findings, the vast majority of the studies imply that addition of standard agents used for the treatment of various stages of CML to imatinib mesylate produces better antileukemic activity than monotherapy [8-10].

An experiment by Kano *et al.* [8] was conducted using four human Ph^+ leukemia cell lines: KU812, K-562, and TCC-S from patients with CML myeloblastic crisis, and TCC-Y from a patient with pre-B-cell acute lymphoblastic leukemia (ALL). In this study, viable cell growth was determined by MTT reduction assay, and the cytotoxic interactions of imatinib mesylate with other agents at the point of IC_{80} were evaluated by isobologram. They showed that when combined with imatinib mesylate, recombinant interferon alpha had synergistic activity against TCC-S cell line, and 4-hydroperoxy-cyclophosphamide had synergistic activity against all Ph^+ four cell lines, and that except for methotrexate, which had antagonistic activity, other agents including hydroxyurea, cytarabine, homoharringtonine, doxorubicin, etoposide and vincristine exhibited additive properties against four Ph^+ cell lines. Barteneva et al. [9] reported that imatinib mesylate pretreatment enhanced cytotoxic activity of interferon alpha in K562 cells. Thiesing and coworkers [10] studied the activity of combinations of imatinib mesylate with antileukemic agents including interferon, hydroxyurea, daunorubicin and Ara-C on MO7e, a human megakaryoblastic cell line, MO7p210, a derivative of MO7e engineered to express Bcr-Abl, and K562 cell line, and showed that there was no change in the inhibition of proliferation of MO7e cells when imatinib mesylate was added to interferon, hydroxyurea, daunorubicin and Ara-C compared with each of the antileukemic agents alone. When imatinib mesylate was combined with interferon, MO7p210 cells were highly sensitive to imatinib mesylate. However, when imatinib mesylate was combined with daunorubicin or Ara-C, the IC_{60} for these agents dropped to concentrations lower or equal to those of the MO7e parental cell line. There was no change in the IC_{60} when imatinib mesylate was combined with hydroxyurea. As with the MO7p210 cells, the combinations of imatinib mesylate plus interferon or daunorubicin produced additive antileukemic effects on K562 cells. Imatinib mesylate plus Ara-C produced a synergistic effect.

In view of these studies, we aimed to investigate the effect of combination of imatinib mesylate and fludarabine against Ph^+ K562 and Meg01 cell lines in vitro, and we have shown that imatinib mesylate significantly augmented the sensitivity of the Ph^+ cell lines against fludarabine.

Compared with the results of the combination studies by Kano et al., Barteneva et al. and Thiesing et al., which were mainly additive, combination of imatinib mesylate and fludarabine resulted in synergistic to strong synergistic ac-

tivity on K562 and Meg01 cell lines. The exact mechanism of this combined cytotoxic activity of these two different drugs remains to be elucidated.

Although the pharmacokinetic interaction and the toxic effects of the drug combinations can not be measured in vitro and the differences between in vitro and clinical systems would influence the cytotoxic interaction of imatinib mesylate and other agents, the fludarabine-based chemotherapy regimens, which are fairly toxic, were used in AMLs quite successfully [13,14], and can be used for those patients with CML blastic phase.

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