Original article:

Imatinib reduces proliferation of leukemic cells in vitro

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Abstract

Introduction: Philadelphia chromosome is a cytogenetic marker for chronic myeloid leukemia (CML). The main aims of this study were to assess the positive responses, side-effects and survival of CML patients treated with imatinib mesylate. ***Methods:*** All recently diagnosed CML patients who were treated with imatinib were recruited to this study. We investigated hematological and cytogenetic parameters by CBC, FISH and RT-PCR individually.

Results: Of the 10 cases, 7 (70%) were males and 3 (30%) were female. Four (40%) of the cases were analyzed retrospectively and 8 cases (80%) exhibited general exhaustion (75%), fever (80%), and splenomegaly (80%). Indications of bleeding and rashes were rarely seen at presentation. The majority of the patients had a generally low risk profile (70%), 30% had intermediate risk; with no subjects exhibiting high risk CML, 9 subjects (90%) were in remission. One patient (10%) had been in remission for 3 years, 4 (40%) had been in remission for 6 years, one was in remission after 7 years and 5 (50%) were in remission after 10 years. Most of the patients (90%) exhibited a deficient major molecular reaction, after 6 years of treatment and 42% of them had a deficient major molecular reaction after 10 years of treatment. No significant side effects associated with Imatinib treatment were reported by the patients. Imatinib treatment resulted in diminished expansion in CML CFU-GM cells. ***Conclusion:*** Imatinib mesylate is indicated for the treatment of Philadelphia chromosome-positive CP-CML with no significant adverse outcomes.

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Introduction

Philadelphia chromosome (Ph) results from the reciprocal translocation t(9;22) (q34;q11) of truncated chromosome 22 that is a hallmark of chronic myeloid leukemia (CML). This aberrant fusion gene encodes the break point cluster region-proto-oncogene tyrosine-protein kinase (BCR-ABL) oncogenic protein which leads to persistently enhanced tyrosine kinase activity with consequent cell proliferation, inhibition of differentiation and resistance to cell death[1-6]. Cytogenetic investigation of bone marrow samples has shown that 90–95% of

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CML patients are Ph chromosome positive[2]. CML disease develops as a clonal hemopoietic stem cell expansion, characterized by a chronic phase (CP), an accelerated phase followed by a blast crisis (BC) phase[3]. Each *Bcr-Abl* mRNA transcript is found in distinct phenotypes of CML and these are predictive of responses to therapy and overall clinical outcome[7]. Imatinib mesylate (STI-571, Gleevec) is a well-established first-generation ABL tyrosine kinase inhibitor (TKI) whose use has dramatically improved the prognosis of CML through decreased *Bcr-Abl* mRNA measured using real-time quantitative PCR (RQ-PCR) in CML patients. In contrast, hematological responders the platelet counts fall in response to Imatinib treatment with no cytogenetic change evident. This suggests that the relevant CML clone was Imatinib sensitive but that there was insufficient normal haemopoiesis to sustain cytogenetic reactions. This study was performed to examine the clinical presentation, hematological reaction, molecular reaction, survival and unfavorable effects of Imatinib treatment of CML patients at our hospital.

Material And Methods

Patients:

Ten patients (7 males, 3 females aged 22–71 years) were recruited to the study. Each was diagnosed to have a chronic myeloid leukemia (CP-CML) at our university hospital. This study was conducted between January 2015-Dec 2015 at the Leukemia Research Unit, PSAU, Al-Kharj, Saudi Arabia. Age and sex-matched control samples were acquired from bone marrow donated by volunteers giving cells for allogeneic transplantation or from volunteer blood donors. All patients were analyzed by symptoms, peripheral blood smear examination, bone marrow analysis with biopsies analyzed by quantitative RT-PCR. The oral dosage of Imatinib was 400 mg/day, administered between 2005 and 2015. At the mid-point of this period, peripheral blood smears, complete blood count (CBC) and clinical examination were performed at regular intervals with RT-PCR carried out at the last visit. Peripheral blood and bone marrow samples from patients with CP-CML were tested before and after Imatinib treatment for those subjects who were cytogenetic responders. The patients were considered as having a positive clinical reaction if they were free of manifestations and indications of CML.

Preparation of Mononuclear cells for Culture & CFU-GM culture and expansion:

Mononuclear cells (MNCs) were purified by density gradient centrifugation over Ficoll-Hypaque (1.077 g/ml Nyegaard, washed with HBSS (GibcoBRL) and, cells resuspended in the same medium and adjusted to a concentration of 5×10^6 in 5ml in MEM containing 15% FCS. We followed the same protocols previously published by Marley et al[5,8,9]. The susceptibility to Imatinib is expressed as the proportion between the AUC of the control samples and the AUC of the cells cultured following Imatinib treatment.

Statistics

Data were transferred to microsoft Excel spreadsheets and statistical analysis performed using Stat View SE+ package. Data distribution was analyzed by the Mann-Whitney U test and Wilcoxon Signed rank test. Significance was calculated by Spearman Rank test. The AUC was computed utilizing a Microsoft Excel spreadsheet.

Results

Nine (90%) out of twelve patients with Philadelphia-chromosome positive end stage CML were males and 25% were females (**Table-1**). The most common symptoms and clinical signs with which the patients presented were exhaustion 9 (90%), fever 8(80%), rash 4 (40%), and splenomegaly 8 (80%). The majority of the patients had a generally low risk profile (70%), 30% had intermediate risk; with no subjects exhibiting high risk CML; 9 subjects (90%) were in remission. WHO scoring framework. One patient had been in remission for 3 years, 4 (40%) had been in remission for 6 years, one was in remission after 7 years and (40%) were in remission after 10 years. One patient had a deficient major molecular reaction (MMR) to Imatinib treatment 12 years after initial diagnosis. Most of the patients (80%) exhibited MMR, after 6 years of treatment and 40% of them were in MMR after 10 years of treatment. Imatinib was well tolerated in these patients with no symptoms evident**.**

The morphology of the lymphocytes differed in size and shape from normal with some cells exhibiting a less mature phenotype. During the study period, none of the patients had increased numbers of lymphocytes in their bone marrow with a mean lymphocyte count of 3%. The mid-range of lymphocytes was 7%, however, there was a marked variability between patients. At study conclusion, the CML patients exhibited diminished B cell numbers in their bone marrow that contrasted with normal control values (10% of lymphocytes versus 29%), and no immature or maturating structures identified. Patients with a suboptimal reaction to Imatinib had diminished numbers of B cells in the bone marrow, though patients who responded well to Imatinib treatment

and with bone marrow lymphocytosis had normal or expanded numbers of B cells. In T cells, the CD4/CD8 proportion was typical and the extent of regulatory T cells (Tregs) in bone marrow was almost identical in various settings. The quantities of DC were equivalent to normal values in patients who responded well to Imatinib treatment.

We established responses to Imatinib treatment to establish the most those patients who did not exhibit a strong response to Imatinib (***data not shown***). We also observed a significant reduction in the AUC of those treated with Imatinib compared with untreated controls (p=0.002, n=10) **(Figure-1 and 2).** Strikingly, Imatinib reactions have no significant impact on NBM CFU-GM development compared with positive effects on CML CFU-GM formation.

In vitro testing demonstrated, clear differences between control and CML cell responses including cell multiplication, attachment, and reactions to Imatinib and IFN-α. The AUC in CML is higher than in control cells, and was significantly reduced by Imatinib or IFN-α although we observed, wide variations in both responses. It is well-established that Imatinib is a tyrosine kinase inhibitor that specifically hinders the tyrosine movement of ABL and BCR-ABL proteins and the development of CFU-GM colonies from CML patients.

Discussion

Chronic myeloid leukemia (CML) is a clonal myeloproliferative defect of the pluripotent immature stem cells with a rate of 1 per 100,000 in the west[10]. In contrast, the rate of occurrence in Saudi Arabia is not known. CML accounts for 6.2% of all leukemia with 4.6% and 6.7% due to CML in males and females respectively[1]. The median age of patients diagnosed with CML is 67 years-of-age in the west with slight male predominance[12]. In contrast, in this study the median age was 39 years with slightly higher rates in males although this is based on very small numbers. Prior to the introduction of Imatinib the usual chemotherapy regime for CML was dependent on Busulphan or hydroxyurea. However the side-effects were numerous with a median survival rate of under 3 years, hematological reduction of less than 80% with no cytogenetic remission[10]. Allergenic undifferentiated cell transplantation was the main remedial treatment for CML but it was challenging with a high death rate and unfavorable side-effects. Interferon Alpha was the treatment of choice before the development of tyrosine kinase inhibitors. The current treatment of choice for CML patients is Imatinib 400 mg daily[13]. The inhibition of BCR-ABL tyrosine kinase is a very successful treatment for Ph+ CML when compared with previously available treatments.

In the present study, we examined whether Imatinib treatment exhibits variations in 12 patients over a long time period. The major outcomes were that none of our patients reported any significant side-effects despite the fact that the length of treatment was from 3–10 years with 90% having been treated for more than 6years. These outcomes are in agreement with a number of local reports from Saudi Arabia[4-16].

A number of studies have demonstrated that following 6 years of treatment with Imatinib, 86–88% of patients with Ph+ CML remained in MMR, which was characterized as a 3-log decrease in the quantity of the Bcr-Abl transcript which has been established as an objective marker for a positive outcome in clinical studies[17-18].

Despite the fact that this study is based on small numbers, 83% of our patients remained in MMR following 6 years of treatment and 42% remained in MMR after10 years of treatment with few adverse effects reported or discontinuation of treatment with Imatinib. Our study is in agreement with studies which demonstrated a general survival rate in CML of 95.2% after 8 years[19-20]. We also explored whether the self-replication of CML CFU-GM could be decreased to the levels seen in normal bone marrow CFU-GMs following treatment with different concentrations of Imatinib. There was a significant reduction in CML CFU-GMs replication to the levels seen in normal bone marrow, with no effect on cell proliferation. These outcomes are in agreement with those reported by Gordon and colleagues[9]. These findings demonstrate that Imatinib inhibits progenitor cell multiplication, as shown by the AUC changes. CML related CFU-GM exhibit p210 expression, improved progenitor cell function and a positive reaction to remedial treatment in CML. The possibility that diverse downstream signaling pathways might be proportionately inactivated by treatment with Imatinib is supported by our preliminary discoveries on the impacts of pathway inhibitors in individual patients. Our information hastargeted the PI3-kinase pathway and we propose that downstream targets such as ERK and p70 might be differentially expressed in CML patients. These results support the use of orally administrated Imatinib as an effective treatment with few adverse effects compared with the alternative option of IFN-α treatment of CP-CML.

Conclusion

Imatinib mesylate is an effective, well-tolerated medication over a 10 year period of treatment and follow-up of patients with Ph+ CML.

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Table-1

Characteristics	value
Total number of patients	10
Male:Female	7:3
Range Age	22-71
Fever	8
Fatigue	6
Bleeding	1
Rash	4
Splenomegaly	8

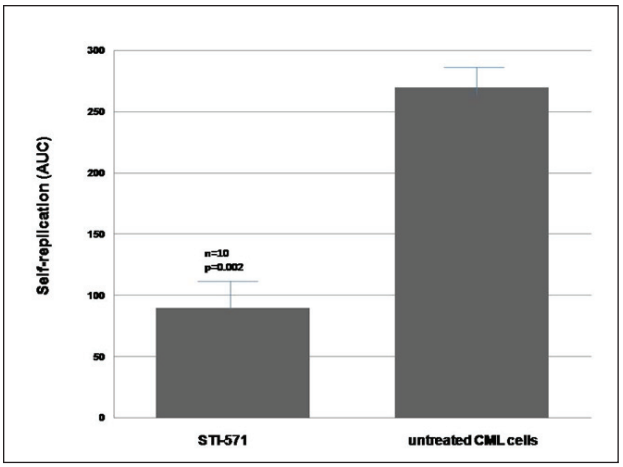

Figure-1: Self-renewal capacity (AUC) of CFU-GM from CML patients treated with STI-571. Self-renewal capacity (AUC) of CFU-GM from NBM controls treated with STI-571. There is no significant difference in the AUC of STI-571 compared to untreated controls (Wilcoxon Signed-rank test).

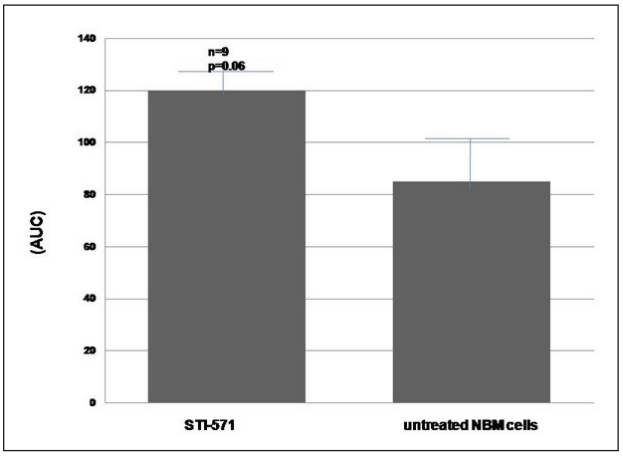

Figure-2: Self-renewal capacity (AUC) of CFU-GM from NBM controls treated with STI-571. There is no significant difference in the AUC of STI-571 compared to untreated controls (Wilcoxon Signed-rank test).

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