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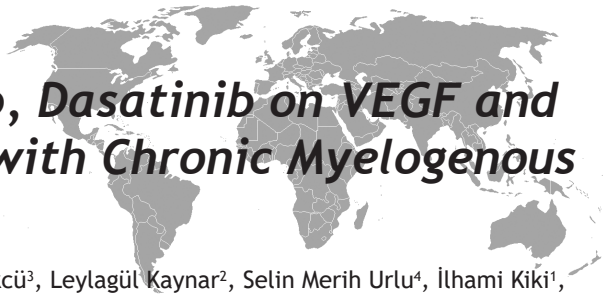

Effects of Imatinib, Nilotinib, Dasatinib on VEGF and VEGFR-1 Levels in Patients with Chronic Myelogenous Leukemia

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ABSTRACT

Objective: *Vascular endothelial growth factor (VEGF) is a protein that binding to VEGF receptors 1 (VEGFR-1) and accelerates angiogenesis. The relationship between angiogenesis and progression of tumors were observed in both solid and hematologic cancers. Monoclonal antibodies capable of inhibiting angiogenesis, tyrosine kinase inhibitors use for haematological cancer treatment. In this study; we investigated the effects of Imatinib mesylate (STI-571; Gleevec), Nilotinib (AMN107; Tasigna) and Dasatinib (BMS-354825; Sprycell) on serum levels of VEGF and VEGFR-1, in patients with chronic phase of chronic myeloid leukemia (CML).* ***Method:*** *Serum levels of VEGF and VEGFR-1 were measured in 65 patients with chronic phase of CML. Serum VEGF and VEGFR-1 levels were determined using quantitative sandwich enzyme immunoassay technique according to the manufacturers' instructions.* ***Results:*** *There were 33 (51%) male and 32 (49%) female patients in this study. 38 of 65 patients were using Imatinib, 15 Nilotinib, 12 Dasatinib. Mean serum VEGF and VEGFR-1 levels for the 65 patients with CML were 172.21±127.46 pg/mL and 199.62±122.22 pg/mL, respectively. In Dasatinib and Imatinib group, serum VEGF and VEGFR-1 levels were significantly higher than in control group (p= 0.008, p< 0.0001, and p< 0.0001, p< 0.0001). In Nilotinib group, serum VEGF levels were higher than control group, but; it was not statistically significant (p= 0.06) while . VEGFR-1 levels were significantly higher than those of controls (p< 0.0001).* ***Conclusion:*** *Imatinib, Nilotinib and Dasatinib were not superior to each other regarding to serum VEGF and VEGFR-1, but it may be said that Nilotinib may has slightly more effect on inhibition of anjiogenesis.*

Key words: *Angiogenesis, Tyrosin kinase inhibitors, VEGF, VEGFR-1*

Kronik Myeloid Lösemili Hastalarda İmatinib, Nilotinib, Dasatinib'in VEGF ve VEGFR-1 Düzeyleri Üzerine Etkileri

ÖZET

Amaç: *Vasküler endotelyal büyüme faktörü (VEGF), VEGF reseptörlerine bağlanarak angiogenezi hızlandıran bir proteindir. Anjiogenez ile tümörlerin progresyonu arasındaki ilişki hem solid hemde hematolojik kanserlerde saptanmıştır. Anjiogenez inhibisyonu yapan monoklonal antikorlardan tirozin kinaz inhibitörleri tedavide kullanılabilir. Bu çalışmada amaç kronik faz Kronik Myeloid lösemi (KML) hastalarında İmatinib mesylate (STI-571; Gleevec), Nilotinib (AMN107; Tasigna) and dasatinib'in (BMS-354825; Sprycell) serum VEGF ve VEGF reseptör 1 (VEGFR-1) düzeyine etkilerini araştırmaktır.* ***Yöntem:*** *65 kronik faz KML hastasında serum VEGF ve VEGFR-1 düzeyi ölçüldü. Serum VEGF and VEGFR-1 düzeyleri üretici firmanın önerisi doğrultusunda kantitatif sandviç enzim immunoassay yöntemi kullanılarak ölçüldü.* ***Bulgular:*** *Çalışmaya alınan hastaların 33'ü (% 51) erkek ve 32'si (% 49) kadındı. 65 hastadan 38'i İmatinib, 15'i Nilotinib ve 12'si Dasatinib kullanıyordu. Hastaların ortalama VEGF düzeyi 172.21±127.46 pg/mL iken VEGFR-1 düzeyi 199.62±122.22 pg/mL idi. Serum VEGF and VEGFR-1 düzeyleri imatinib ve dasatinib kullanan gruplarda kontrol grubundan anlamlı yüksekti (p< 0.0001, p<0.0001 ve p= 0.008, p< 0.0001). Nilotinib grubunda ise serum VEGF düzeyi kontrol grubundan yüksek ancak istatiksel olarak anlamlı değildi (p= 0.06). VEGFR-1 düzeyi ise kontrol grubundan istatiksel olarak anlamlı yüksekti (p< 0.0001).* ***Sonuç:*** *Serum VEGF ve VEGFR-1 düzeyi açısından İmatinib, Nilotinib ve Dasatinib birbirine üstün değildir. Ancak Nilotinibin anjiogenez inhibisyonuna biraz daha fazla etkili olduğunu söyleyebiliriz.*

Anahtar kelimeler: *Anjiogenez, tirozin kinaz inhibitörleri, VEGF, VEGFR-1*

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INTRODUCTION

Angiogenesis is a formation of new capillaries from blood vessels. It plays an important role in the progression and prognosis of solid tumors. Additionally, there is a relationship between angiogenesis and some hematologic malignancies (1). In other words, tumor angiogenesis plays a critical role in tumor growth and metastasis. Therefore, usage of agents suppressing of tumor angiogenesis like monoclonal antibodies, tyrosine-kinase inhibitors, transcription inhibitors and small-molecule inhibitors are one of the treatment modalities (2).

Some molecules released by both tumor and host cells, including endothelial cells, epithelial cells, mesothelial cells and leukocytes, induce angiogenesis. Among these, there are members of the fibroblast growth factor family, vascular endothelial cell growth factor, IL-8, epidermal growth factor, platelet derived endothelial cell growth factor, angiogenin, angiotropin, tumor necrosis factor and others (3). The most potent stimulating cytokines of angiogenesis are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (4-6). There are six secreted glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placenta growth factor (PlGF)-1, and PlGF-2 in VEGF family (7-9). It was found that expression of VEGF protein increased some hematological malignancies (10). For example, there was significantly more VEGF in fresh leukemic cells than cells from normal donors (11,12). Furthermore, in newly diagnosed patients with a range of FAB types of acute myeloblastic leukemia (AML), there is a direct relationship between increasing cellular VEGF content and shorter survival (13). Additionaly, bone marrow vascularization correlates with production of VEGF in patients with chronic myelogenous leukemia (CML) (14). The binding of VEGF-A to VEGF receptor 1 (VEGFR-1) induces endothelial cell migration (15).

CML characterized by the Philadelphia (Ph) chromosome, is a hematological stem cell disorder (16). Due to a reciprocal translocation between chromosome 9 and 22, the Ph chromosome encoding the chimeric Bcr–Abl oncoprotein with a constitutive tyrosine kinase activity is formed. Novel hybrid Bcr gene has enhanced tyrosine kinase activity. This Bcr-Abl tyrosine kinase is present in 95% of patients with CML and 10±15% of adults with acute lymphoblastic leukemia (ALL) (17).

CML can be effectively treated, during chronic phase (CP), with tyrosine kinase inhibitors (TKI) and they have been approved in clinical trials (18). In this manuscript, the effects of imatinib mesylate (STI-571; Gleevec), Nilotinib (AMN107; Tasigna) and Dasatinib (BMS-354825; Sprycell), TKIs on serum VEGF and VEGFR-1 were investigated.

MATERIAL AND METHODS

Serum levels concentrations of VEGF and its receptor VEGFR-1 were measured in serum samples of 65 patients with chronic phase CML at the Hematology Division of the Department of Internal Medicine of Ataturk University and Erciyes University Medical Schools. The study was approved by Medical School at Ethics Commitee. The patients took TKIs for at least six months. At the end of the sixth month, responses to TKIs treatment were assessed with standardized real quantitative polymerase chain reaction (PCR) and/or cytogenetics. It was found that patients were at complete cytogenetic remission (CCR) or had ≤%1 bcr-abl1 transcript level with PCR. Blood samples were obtained after an overnight fast and serum samples were stored at -800C. Serum VEGF and VEGFR-1 levels were determined commercially available kit (Adipo Bioscience Inc., USA.) using quantitative sandwich enzyme immunoassay technique according to the manufacturers' instructions. A monoclonal antibody specific for VEGF / VEGFR-1 has been pre-coated onto a microplate. Standarts and samples are pipetted into the wells and any VEGF / VEGFR-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for VEGF / VEGFR-1 is added to the wells. Following a wash to remove any unbound antibody, Streptavidin-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of VEGF / VEGFR-1 bound in the initial step. The color development is stopped and the intensity of the color is measured at 450 nm wavelength in microplate reader.

Statistical Analysis

Statistical analysis was performed using commercially available SPSS 11.0 software. Data were expressed as mean ± standard deviation. Comparison between groups was performed using the Mann-Whitney U test. $p< 0.05$ was considered statistically significant.

***Table 1**. Comparison of serum VEGF and VEGFR-1 levels in imatinib, nilotinib, dasatinib and control groups.*

	Control (n:20)	*Imatinib (n:38)*	*Nilotinib (n:15)*	*Dasatinib (n:12)*
VEGF (pg/ml)	*91.14±38.76*	*185.56±148.17**	*152.52±97.72*	*154.55±83.90***
VEGFR1 (pg/ml)	*91.82±41.57*	*205.71±147.24**	*175.23±37.69**	*210.83±105.36**

**;P < 0.0001 compared to controls, **;P = 0.008 compared to controls*

RESULTS

There were 33 (51%) male and 32 (49%) female patients. The mean age of the patients was 46.71 ± 14.92 years. Thirty eight of 65 patients were using Imatinib, 15 Nilotinib, 12 Dasatinib. Mean age of the patients who were used Imatinib was 46.53 ± 15.76 years and there were 16 male and 22 female patients in this group. There were 11 male and 5 female patients in Nilotinib group and their mean age was 48.87 ± 16.14 years. The mean age was 44.58 ± 10.83 years in Dasatinib group (6 male, 6 female). Accordingly, 20 healthy volunteers were included in the study as the controls (11 male, 9 female; mean age 47.30 ± 10.56 years). In this study, patients that had been used for at least six months TKIs, were taken. Response evaluation to TKIs treatment was performed with real quantitative polymerase chain reaction and/or cytogenetics. All patients had optimal response to TKIs treatment according to the European Leukemia Net expert panel. Mean serum VEGF and VEGFR-1 levels for the 65 patients with CML were 172.21 ± 127.46 pg/mL and 199.62 ± 122.22 pg/mL, respectively, they were 91.14 ± 38.76 pg/mL and 91.82 ± 41.57 pg/mL respectively, for the control group.

In Dasatinib group, serum VEGF (154.55 ± 83.90 pg/mL) and VEGFR-1 (210.83 ± 105.36 pg/mL) levels were higher than those of the controls ($p = 0.008$ and $p < 0.0001$, respectively). In Nilotinib group, serum VEGF levels (152.52 ± 97.72 pg/mL) were higher than those of the controls, but, it was not statistically significant ($p = 0.06$). VEGFR-1 levels (175.23 ± 37.69 pg/mL) were significantly higher than those of controls ($p < 0.0001$). In Imatinib group, serum VEGF (185.56 ± 148.17 pg/mL) and VEGFR-1 (205.71 ± 147.24 pg/mL) levels were higher than those of controls ($p < 0.0001$ for both) (Table I). Regarding serum VEGF and VEGFR-1 levels, the difference among Imatinib, Nilotinib and Dasatinib groups was not statistically significant ($p > 0.05$).

DISCUSSION

In the past two decades, inhibitors of angiogenesis have been developed for clinical use in various malignant diseases (19). The target of most angiogenesis inhibitors has been directed to the VEGF signaling pathway, such as the monoclonal antibody bevacizumab and two kinase inhibitors Sunitinib and Sorafenib. Also, small-molecule TKIs (sunitinib and sorafenib) effect VEGFR (20). Both drugs have shown beneficial in patients with renal cell cancer (21,22).

Imatinib specifically targets the TK activity of the oncogenic proteins encoded by Bcr-Abl-1 (23) and its discovery rapidly modified the treatment of CML and led to important changes in management (24) . Imatinib blocks platelet-derived growth factor receptor (25), and thus Imatinib modulates the PDGF-PDGFR signaling pathways in CML. But, over time, resistance to Imatinib may develop possibly depending on the excess of signaling pathways which are involved in angiogenesis. Thus other drugs, most of them also classifiable as TKIs, were developed (26). Two of them, Dasatinib and Nilotinib, have been commonly used for the treatment of patients with Imatinib-intolerant and Imatinib-resistant CML disease (27).

Previously, it was shown that VEGF secretion was increased in myeloid cells (28). Additionaly, VEGF plasma concentrations are significantly increased in chronic lymphocytic leukemia (29). Cellular VEGF protein levels in bone marrow samples increase (30) and this increase contributes serum VEGF levels. In the literature; plasma level of VEGF have also been shown to be significantly higher in CML patients compared with normal controls (11). Lundberg et al (14) reported the number of VEGF+bone marrow cells to be significantly higher in samples from CML patients than in normal controls and to correlate with bone marrow vascularity. In another study; the me-

dian VEGF value in CML samples was 1.6-fold higher than in normal control samples (30). Also mean serum levels of VEGF and VEGFR-1 for the 65 patients with CML were higher than those of the controls in our study.

In the study of Legros L et al. (31), it was demonstrated increase in VEGF production in patients with CML. After treatment with CCR for at least 6 months, plasma VEGF levels were lower when compared with VEGF concentration at diagnosis. Hence, they concluded that low plasma VEGF concentrations could be one of the characteristics of CCR. Serum levels of VEGF and VEGFR-1 were not statistically significant difference between Imatinib, Nilotinib and Dasatinib groups in our study. It may be associated with an optimal response to treatment of all patients.

Imatinib Mesylate is capable of reversing bone marow angiogenesis thereby decreasing the plasma levels of VEGF in CML patients. BCR-ABL stimulated angiogenesis by induced VEGF expresion can be supresed by treatment with TKIs such as Imatinib. Kvasnicka et al. (32) reported that first-line therapy with Imatinib induced a significant reduction of microvessels and that decreased bone marrow vascularity was associated with a CCR in most patients.

In our study, in Imatinib and Dasatinib groups, VEGF and VEGFR-1 levels were higher compared to control group. Because, at diagnosis, we did not measure VEGF and VEGFR-1 levels, comparison between values before and after treatment could not be done. This is our limitation for this study. We aim to measure baseline levels of VEGF and VEGFR-1 in future studies that associated with this study. In Nilotinib group, while VEGFR-1 levels were higher than in control group, VEGF levels were not statistically different from control levels. This may indicate that, Nilotinib may effectively decrease levels of VEGF in these patients. Additionaly, Nilotinib has antifibrotic effect in liver fibrosis model induced by carbon tetra chloride. This beneficial effect of Nilotinib is partly associated with suppressed expression of VEGF, and VEGFR (33).

Also, Sunitinib is a TKIs with antitumor and antiangiogenic activity that specifically inhibits VEGFR and PDGFR. In another study done in patients with metastatic breast cancer revealed it was found that Sunitinib therapy has been associated with decrease in plasma VEGFR, on the contrary, it increased VEGF levels (34).

Kantarjian H.M et al. (35) showed in last year, that with DASISION trial, patients with newly diagnosed chronic-phase CML were randomized to receive Dasatinib 100 mg or Imatinib 400 mg once daily. In this study, it revealed that Dasatinib useage was associated with significantly higher rates of complete cytogenetic response compared with Imatinib. In our study, all patients at CCR and Dasatinib was not superior to Imatinib in terms of reducing serum levels of VEGF and VEGFR-1. This result may be due to the small number of patients in Dasatinib group. Saglio G et al. (36) studied the effects of Nilotinib or Imatinib on CCyR in CML treatment and they showed that the rates of CCyR by 12 months were significantly higher for Nilotinib than for Imatinib. In our study really, in Nilotinib group, serum VEGF levels were closer to conrol group.

In conclusion, Imatinib, Nilotinib and Dasatinib were not superior to each other regarding to serum VEGF and VEGFR-1, but it may be said that Nilotinib may has slightly more effect.

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