Original Article

Effects of Imatinib mesylate (Glivec) on some circulating immunocytes in a group of Nigerians with Chronic Myeloid Leukaemia: a preliminary study

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ABSTRACT

AIM Glivec is a selective inhibitor of the bcr/abl tyrosine kinase, deregulated expression of which is involved in the pathogenesis of chronic myeloid leukaemia (CML). Reports of tuberculosis and lymphopenia in CML patients on Glivec who are not exposed to mycobacterial infection and are HIV negative have suggested the possibility of immunosuppressive properties for Glivec; and hence the need to investigate its effects in Nigerian CML patients currently on the drug.

METHODS Baseline complete blood count (CBC) and CD4+ T lymphocyte count of confirmed Ph+ CML patients were done pre-Glivec therapy and while on the drug. CBC was done using manual method, while absolute lymphocyte count (ALC) was calculated from the total WBC and the differential count for lymphocytes. CD4+ lymphocyte counts were done using Cyflow automatic cell counter. Glivec, at a dose of 400mg, was used for between 12 and 68 weeks before a repeat CBC and circulating CD4+ lymphocyte count were done.

RESULTS Ten patients with a mean (±SD) age of 41.90 ± 8.034 years were investigated. The mean CD4+ lymphocyte count while on imatinib therapy (841.80 ± 373.57/µL) was significantly lower ($p = 0.003$) than the pre-therapy mean (1774.50 ± 1044.0/µL). There was also a significant reduction ($p = 0.009$) in the mean absolute lymphocyte count (ALC) from 20071.20 ± 17267.99/µL pre-therapy to 2696.60 ± 1587.69/µL post therapy. The reduction in circulating CD4+ lymphocytes correlated ($r = +0.67$, $p = 0.35$) with reduction in ALC.

CONCLUSION Glivec appears to cause significant lymphopaenia. The clinical significance of this finding requires further study.

Keywords: Imatinib; Leukemia, Myelogenous, Chronic, BCR-ABL Positive; immunosuppression; Nigeria; Glivec; Lymphocyte Count, CD4.

INTRODUCTION

T helper cells, also known as CD4+ T lymphocytes, play an important role in cell- mediated immunity. They are involved in activating and directing other cells of the immune system, including B cell antibody class switching, activation and growth of cytotoxic T lymphocytes, and in maximizing bactericidal activity of phagocytes. Imatinib mesylate (Glivec, STI571) is a selective inhibitor of the bcr/abl tyrosine kinase. Deregulated expression of bcr/abl gene is involved in the pathogenesis of chronic myeloid leukaemia (CML). Imatinib is a relatively new first line drug in the treatment of CML. However, recent observations in its use have shown it to inhibit T cell proliferation by arresting the cells in Go/G1 phase without affecting the viability of the cells.[1] The immunosuppressive properties have been confirmed in animal models [2] in which the feeding of mice with imatinib mesylate caused impaired induction of a protective anti-tumour immunity in these animals. Recently, Senn et al (2005) [3] reported a case of peritoneal tuberculosis and global lymphopenia in a CML patient on Glivec who was not exposed to mycobacterial infection and was HIV negative, while Santachiara et al (2008) [4] reported the development of hypogammaglobulinemia in CML and gastrointestinal gist tumour patients treated with imatinib. Although imatinib has been shown to be immunosuppressive in Caucasian studies, its use is new in Nigerian patients and hence the need to investigate its effects in these patients, as race and ethnicity can influence the mechanism of action and effects of drugs [5–9].

METHODS

Between January 2005 and December 2009, 10 consenting consecutive Philadelphia chromosome positive (Ph+) chronic myeloid leukaemia (CML) patients seen at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife were prospectively investigated after obtaining approval from the Ethics and Research Committee of the hospital.

Baseline complete blood count (including PCV, WBC and differentials, and platelet) and circulating CD4+ lymphocyte levels were assessed before being commenced on Glivec and 6 months after being on the drug. Complete blood counts were done using a manual method as described by Lewis and colleagues [10], while the absolute lymphocyte count was calculated from the total WBC count and the differential count for lymphocytes. CD4+ lymphocyte counts were done using Cyflow automatic cell counter (Partec, Germany). All blood samples were taken between 9.00 am and 12.00 Noon. Data is presented as means (± SD). SPSS for Windows version 16 was used for computing all statistical calculations.

RESULTS

The patients were 8 males and 2 females with a mean age of 41.90 ± 8.034 years. Glivec, at a dose of 400 mg, was used for between 12 and 68 weeks before a repeat complete blood count and circulating CD4+ lymphocyte counts were done. The mean CD4+ lymphocyte count pre-Glivec therapy was 1774.50 ± 1044.0/µL compared to 841.80 ± 373.57/µL during Glivec therapy, showing a significant reduction ($p = 0.003$), (Table 1). There was also a significant reduction ($p = 0.009$) in the mean absolute lymphocyte count from 20071.20 ± 17267.99/µL pre-therapy to 2696.60 ± 1587.69/ µL post therapy. Expectedly, there were significant reductions in total white count (WBC) during Glivec therapy. The mean granulocyte count during therapy (3325.40 ± 2987.69) was significantly lower ($p = 0.022$) than the pre-therapy count (142000 ± 158348.94). These values are presented in Table 2. Except for patients 2, 6, and 8, all patients had reduced post-therapy immunocyte levels.

As expected, there was a significant improvement in haematocrit level during therapy. Reductions in platelet count during Glivec were not significant. The improvement in the patients' haematocrit level correlated positively ($r = 0.93$, $p = 0.001$) with the reductions in WBC count. Reductions in absolute lymphocyte count and circulating CD4+ lymphocyte levels also correlated significantly with reductions in WBC count ($r = 0.81$, $p = 0.004$; $r = 0.72$, $p = 0.018$, respectively). Similarly, reductions in circulating

Table 1. Mean (± SD) of Laboratory parameters before and after Imatinib Mesylate therapy

Mean ± SD	Baseline	After imatinib	t-value	p-value	95% C I
PCV	30.1 ± 4.067	37.60 ± 4.949	3.78	0.002	3.24 - 11.76
WBC (/µL)	449000 ± 910600	6016 ± 4045	3.28	0.004	62257.06 -283710.94
ALC (/µL)	20071.20±17267.99	2696.60±1587.69	3.17	0.005	5882.68 -28924.12
CD4+ (/µL)	1774.60 ± 1044	841.80 ± 373.57	7.90	0.001	684.62 -1180.99
Platelet (/µL)	274000 ± 305400	212000 ± 1587.69	0.58	0.567	161396 -285396.21

CI, Confidence Interval; PCV, packed cell volume; WBC, white blood cell; ALC, absolute lymphocyte count.

Table 2. CD4+ lymphocytes, absolute lymphocyte count and absolute neutrophil counts before and after Imatinib Mesylate therapy

	CD4+ Lymphocytes (/µL)		Absolute Lymphocyte Count (/µL)		Absolute Neutrophil Count (/µL)	
Patient	**Baseline**	**After imatinib**	**Baseline**	**After imatinib**	**Baseline**	**After imatinib**
1	1,008	515	14,500	660	14,500	2,700
2	1,063	536	21,900	980	19,710	3,280
3	3,269	1,254	12,000	4,758	288,000	3,042
4	542	628	1,922	3,245	1,478	2,655
5	2,944	1,603	44,775	4,278	452,725	2,622
6	1,162	524	1242	1512	1458	3888
7	1,941	631	30,613	2,320	247,687	1,680
8	649	600	2,277	2,442	1,023	1,258
9	2,027	1,050	22,121	1,656	178,979	744
10	3,141	1,028	49,362	5,115	210,438	11,385

CD4+ lymphocyte counts correlated (r = 0.67, p = 0.35) with reductions in absolute lymphocyte counts. The circulating CD4+ lymphocyte level correlated positively with the granulocyte level (r = 0.877, p = 0.001) but negatively with the haematocrit value (r = - 0.758, p = 0.011). No correlation was found between length of Glivec therapy and reduction in circulating CD4+ levels, absolute lymphocyte counts, total white cell counts or platelet counts.

DISCUSSION AND CONCLUSION

This study showed an increase in circulating CD4+ T lymphocyte and absolute lymphocyte counts in chronic myeloid leukaemia (CML) patients before treatment, but a significant reduction after a variable length of time on Glivec therapy. The increase in lymphocyte count pre-therapy has been suggested to be due to an immunological response to neoplastic cell proliferation [11] in line with the concept of immune surveillance which proposes

that lymphocytes recognise and destroy developing cancer cells. The CD4+ T lymphocytes are central to the function of the immune system as they control the adaptive immunity against pathogens and cancer by activating other effector immunocytes. The importance of a reduction of CD4+ T lymphocytes can be observed in HIV infection in which there are often serious opportunistic infections when there is significant reduction in CD4+ T cells.

Several workers have reported severe microbial infections in chronic myeloid leukaemia (CML) subjects using imatinib. Senn et al [3] reported a case of reactivation of pulmonary tuberculosis in a CML patient on Glivec who had significant reduction in circulating CD4+ T lymphocytes, while Speletas et al [12] reported pneumonia caused by Candida organisms in CML patients receiving imatinib. Herpes zoster infection in a gastrointestinal stromal tumour (GIST) patient receiving imatinib has also been reported, though with normal circulating CD4+ T lymphocytes. [13] In our study, the mean absolute number of circulating CD4+ lymphocytes was within normal limits, and none of the patients developed any infection as at the time of investigation. It can however be argued that the length of time of follow up might have been too short for the patients to develop severe enough lymphopaenia to predispose them to infection. Other cellular elements may also mitigate the effects of lymphopaenia. Polymorphonuclear leukocytes are efficient phagocytes with intracellular killing capacity [14, 15]. Only one of the patients had granulocyte count of less than 1000 cells per microlitre at the point of second sampling, showing that the high granulocyte level in these patients could also be responsible for the reduction in their susceptibility to infections at the point of observation. Additionally, there was no significant reduction in platelet count. Platelets could mitigate the effects of lymphopaenia. Studies have shown that in addition to their role in coagulation, platelets have receptors which enable them to contribute to molecular and cellular host defences. [16-18] This suggests that CML patients on imatinib may benefit from close monitoring for infections if they have thrombocytopaenia in addition to when they have granulocytopaenia.

Glivec appears to cause significant lymphopaenia. The clinical significance of this finding requires further study.

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FOOTNOTES

Conflicts of interest: The authors declare no competing conflicts of interest

REFERENCES

1. Dietz AB, Souan L, Knutson GJ, Bulur PA, Litzow MR, Vuk-Pavlovic S. Imatinib mesylate inhibits T cell proliferation in vitro and delayed-type hypersensitivity in vivo. Blood. 2004;104:1094- 9.
2. Taieb J, Maruyama K, Borg C, Terme M, Zitvogel L. Imatinib mesylate impairs Flt3L-mediated dendritic cell expansion and antitumour effects in vivo. Blood. 2004;103:1966-7.
3. Senn L, Telenti A, Kovacsovics T, Meylan P. Peritoneal tuberculosis as a possible complication of Imatinib therapy for chronic myelogenous leukaemia. European Society of Clinical Microbiology and Infectious Diseases. 15th European Congress of Clinical Microbiology and Infectious Diseases. Copenhagen/Denmark. Abstract No.1135_128. April 2-5, 2005.
4. Santachiara R, Maffei R, Martinelli S et al. Development of hypogammaglobulinemia in patients treated with Imatinib for chronic myeloid leukaemia or gastrointestinal stromal tumor. Haematologica. 2008;93:1252-5.
5. Munoz C, Hilgenberg C. Ethnopharmacology. Am J. Nurs. 2005;105:48-9.
6. El Rouby S, Mestres CA, LaDuca FM, Zucker ML. Racial and Ethnic differences in Warfarin response. J. Heart Valve Dis. 2004;13:15-21
7. Johnson JA. Predictability of the effects of race or ethnicity on pharmacokinetics of drugs. Int J. Clin Pharmacol Ther. 2000; 38: 53-60
8. Johnson JA. Influence of race or ethnicity on pharmacokinetics of drugs. J. Pharm Sci. 1997; 86:1328-33.
9. Jamerson K, DeQuattro V. The impact of ethnicity on response to antihypertensive therapy. Am J Med. 1996;101:22S-32S.
10. Lewis SM, Bain BJ, Bates I.

Practical Haematology 9th Ed. Churchill Livingstone Edinburgh. 2001; p633
11. Dowding C, Th'ng KH, Goldman JM, Galton DA. Increased T lymphocyte numbers in chronic granulocytic leukaemia before treatment. Experimental Haematology. 1984;12:811- 5.
12. Speletas M, Vyzantiadis TA, Kalala F et al. Pneumonia caused by Candida krusei and Candida glabrata in a patient with chronic myeloid leukaemia receiving Imatinib mesylate treatment. Med Mycol. 2008;46:259-63.
13. Durosinmi MA, Ogbe PO, Salawu L, Oyekunle AA. Herpes zoster complicating Imatinib mesylate for gastrointestinal stromal tumour. Singapore Med J. 2007; 8: e16-e18
14. Soehnlein O, Kenne E, Rotzius P, Eriksson EE, Lindborn L. Clinical and Experimental Immunology. 2007;151:139- 45.
15. Soehnlein O, Kai-Larsen Y, Frithiof R et al. The Journal of Clinical Investigation. 2008;118(10):3491-3502.
16. Yeaman MR. Bacterial-Platelet interaction: virulence meets host defence. Future Microbiol. 2010;5:471-506.
17. Yeaman MR, Bayer AS. Antimicrobial peptides from platelets. Drug Resist Updates. 1999;2:116- 26.
18. Yeaman MR. Platelets in defense against bacterial pathogens. Cell Mol Life Sci. 2010;67:525-44.