PROCEEDINGS ARTICLE

Inhibition of autophagy with clarithromycin: a new strategy to enhance sensitivity of CML stem cells to tyrosine kinase inhibitors

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Autophagy inhibition has been shown to sensitize tumor cells to cell death induced by tyrosine kinase inhibitors (TKIs). The remarkable responses obtained in seven patients with the combination of clarithromycin and TKIs support the hypothesis that the inhibition of autophagy may make chronic myeloid leukemia cells sensitive to killing by TKIs.

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Autophagy inhibition has been shown to sensitize tumor cells to cell death induced by tyrosine kinase inhibitors (TKI).^{1–4} The inhibition of autophagy may eliminate this survival mechanism by restoring 'sensitivity' of chronic myeloid leukemia (CML) stem cells to treatment with TKIs. Clarithromycin, similar to other macrolides, has been demonstrated to have *in vitro* activity in blocking autophagy.^{5–7}

Upon our Ethical Committee approval, seven patients (four patients with advanced CML and three high-risk patients at diagnosis) have been treated. Remarkable responses to the combination of TKI and clarithromycin have been observed.

A 43-year-old woman was diagnosed with high-risk Sokal CML in February 2000. She was previously treated with interferon alpha (IFN- α) and imatinib, nilotinib and dasatinib with persistence of 100% Ph⁺ metaphases. After 7 months of treatment with dasatinib, in March 2011, the bcr-abl/abl ratio increased to 6.1%. At that time, the patient had an infection (otitis/pharyngitis) sensitive to clarithromycin, which was added to dasatinib at a dose of 500 mg b.i.d. Cytogenetics performed on 20 April 2011 (d + 30) demonstrated Ph⁺ to 10% of 30 metaphases; together with a surprising reduction in the bcr-abl/abl transcript level to 0.5%. As of 25 June (d + 65), the value was 0.05%, and the patient continued to receive clarithromycin (500 mg/day) and dasatinib (100 mg/day). As of 16 July (d + 81), the disease was in complete cytogenetic response and the bcr-abl/abl transcript level was 0.09%. From 10 August, we stopped clarithromycin but on 22 August (d + 106), the transcript level increased to 7.07% and we restarted clarithromycin. On 19 September (d + 134), the transcript level reduced to 3.1%. Now she received clarithromycin on alternate days until 10 December.

A 53-year-old man was diagnosed with *de novo* lymphoid blast crisis CML in August 2010, with a WBC of $300 \times 10^9/l$; the bcr-abl/abl ratio was 95.2%. He was treated with chemotherapy plus imatinib (600 mg/day) beginning in September 2010. In November 2010, hematological control was lost and the bcr-abl/abl ratio was 22%. The patient was treated with dasatinib (70 mg b.i.d.), but no cytogenetic response was achieved. He underwent allogeneic transplant. Two months after transplant (May 2011), the disease progressed with 100% Ph⁺ cells and the bcr-abl/abl value had increased to 47%. He was restarted on dasatinib (100 mg/day) but

bcr-abl/abl transcript level increased in 4 weeks to 143%. We added clarithromycin to dasatinib on 2 June 2011. Three weeks later, the bcr-abl/abl value was reduced to 1.5% (d + 21). On 9 July (d + 39), we decided to stop clarithromycin and continued on dasatinib (100 mg/day) alone. Few weeks later, on 30 July (d + 60), WBC increased to $98 \times 10^3/l$ and the bcr-abl/abl transcript level increased to 40%. Bone marrow showed 100% lymphoid blasts. Clarithromycin was newly added to dasatinib on 1 August (d + 62), and on 17 August (d + 79) WBC decreased to $8 \times 10^3/l$. Despite this, the transcript level continued to increase to 80% and bosutinib treatment was started. The patient died of leukemia in September 2011.

A 68-year-old man was diagnosed with CML in October 1999. He was treated with chemotherapy, and autografting with Ph⁺ PBPC and IFN- α was performed.⁸ A complete cytogenetic response was achieved; in October 2000, the patient had a cytogenetic relapse. A second complete cytogenetic response was achieved in December 2001 after imatinib treatment (400 mg/day), which lasted for 6 years. In October 2006, WBC increased and 100% Ph⁺ marrow metaphases were found. He was treated with dasatinib (70 mg b.i.d.) with no cytogenetic response. In March 2011, the bcr-abl/abl ratio was 42.5%. Nilotinib (600 mg b.i.d.) was begun with no change in the bcr-abl/abl ratio after 2 months. On 8 June 2011, clarithromycin (500 mg b.i.d.) was added, and 3 weeks later (d + 21), the bcr-abl/abl ratio had decreased to 17%; on 6 July (d + 30), the value was 4% and 1 week later (13 July d + 37) it reached 0.0022% combined with complete cytogenetic response. We stopped clarithromycin treatment on 30 July, and a significant increase was verified on 24 August (bcr-abl/abl 17%). We reintroduced clarithromycin, and 1 month later (23 September), the transcript level reduced to 3.8%. Now the patient is receiving clarithromycin and nilotinib on alternate days.

A 70-year-old woman was diagnosed with CML in November 1998 and received low-dose cytarabine and IFN- α with only partial cytogenetic response. She was started on imatinib treatment (400 mg/day), but the karyotype did not change. Thereafter, she was started on nilotinib. At that time, chromosome analysis by FISH demonstrated 100% Ph⁺ marrow metaphases, and the bcr-abl/abl ratio was 26.5%. She continued nilotinib treatment until August 2010. At that time, WBC increased to $100 \times 10^9/l$. Dasatinib

(70 mg b.i.d.) was begun thereafter. Because blood count control was inadequate, homoarringtonine, hydroxyurea and Ara-C were added. In December 2010, the bcr-abl/abl ratio had increased to 140%, and E255V mutation was found. On 20 May 2011, clarithromycin (500 mg b.i.d.) was added. In 2 weeks (d + 14), WBC had decreased from 76×10^9 to $10 \times 10^9/l$, and 1 month later, the (d + 44) bcr-abl/abl ratio was 3%. For the evaluation done on 13 July, the (d + 53) bcr-abl/abl ratio was 0.096%. The patient continues to receive the combination of clarithromycin and dasatinib on alternate days.

Further, three patients with high-risk Sokal/Euro were treated with imatinib (one patient) and nilotinib (two patients). In all cases, clarithromycin was added to TKIs when WBC reached $\leq 15 \times 10^9/l$. A reduction of bcr-abl value from 178% (imatinib), 67% (nilotinib) and 110% (nilotinib) to 9%, 0.7% and 29% occurred at 16, 6 and 3 weeks, respectively. All patients were evaluated weekly for GOT/GPT/gGT, bilirubin, lipase, amilase and imatinibemia/nilotinibemia. Until now, biochemical modification has not occurred, but in all three patients an increase of imatinibemia/nilotinibemia > 1.000 ng/ml has been found; therefore, imatinib dose has been reduced to 300 mg daily and nilotinib to 400 mg b.i.d. daily. Clarithromycin treatment was stopped when the TKI plasma concentration increased more than 1.500 ng/ml. Soon after, conventional doses of imatinib (400 mg daily) and nilotinib at 600 mg b.i.d. daily were restarted.

In conclusion, the remarkable responses obtained in these seven patients support the hypothesis that inhibition of autophagy may make CML cells sensitive to killing by TKIs.^{1,2} These results cannot be explained by the inhibition of CYP3A3 alone, but probably the main underlying mechanism was reversal of the effects of autophagy. We checked the patients weekly, and we can conclude that this combination did not result in liver, renal or cardiac toxicity. Although prolonged response in advanced cases is unlikely, there is great potential for a combination of this type to increase the potential to cure CML if given early in the disease. To validate mechanistically our clinical observation, we will now start to examine the effects of clarithromycin on markers of autophagy

and on the induction of cell death in CML subsets from treated patients and in CML cells treated *in vitro*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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