To the editor:

The ABL kinase inhibitor STI571 does not affect survival of hematopoietic cells after ionizing radiation

The c-Abl gene encodes a widely expressed tyrosine kinase that is important for development, particularly of the nervous system. But the signaling pathways that employ c-Abl in normal cells are not well defined. Abl protein is located in both the nucleus and the cytoplasm, and recent studies suggest that a substantial component of Abl resides in the actin cytoskeleton and mediates signaling related to integrin activation. Other studies have linked Abl to the cellular response to DNA damage, including cell cycle arrest, DNA repair, and apoptosis. Abl tyrosine kinase is activated in response to ionizing radiation, and fibroblasts from Abl knockout mice have been reported to have reduced sensitivity to ionizing radiation. Importantly, STI571 inhibits Abl kinase activity at concentrations that are otherwise nontoxic to normal hematopoietic cells, and has shown impressive activity in early-phase clinical trials in patients with CML.

Because ionizing radiation activates Abl kinase and loss of the Abl gene has been associated with reduced sensitivity to ionizing radiation, it is possible that inhibition of Abl by STI571 would reduce sensitivity to ionizing radiation. To test this hypothesis, normal bone marrow cells from BALB/c mice exposed to 0, 0.1, or 1 μmol/L STI571 for 24 hours before being exposed to 0-6 Gy of gamma radiation, using a Gammacell 1000 (Atomic Energy of Canada, Mississauga, Canada). One μmol/L STI571 is sufficient to completely inhibit Bcr/Abl and c-Abl tyrosine kinase activity when assayed by the sensitive technique of immune complex kinase assay (Uemura, unpublished data, 1999). After irradiation, granulocyte/macrophage colony-forming cells (CFU-GMs) were measured. The methylcellulose medium contained STI571 at the same concentration as pretreatment (Figure 1A). The survival of CFU-GMs at each dose of radiation was the same with or without STI571.

The responses to genotoxic stresses are believed to be mediated by both p53-dependent and -independent pathways. The possibility that Abl kinase activity contributes to radiation sensitivity in cell lines in which p53 is mutated, therefore, considered. The p53 mutant hematopoietic cell line U937 was exposed to ionizing radiation (0-6 Gy), and the fraction of surviving cells was again measured by colony formation. Again, the addition of STI571 did not alter the surviving fraction or size of surviving colonies, after radiation (Figure 1B). Overall, our results suggest that, with the techniques used here, inhibition of c-Abl tyrosine kinase with STI571 at concentrations of up to 1 μmol/L does not affect radiation response where survival is the endpoint.

In a previous study, Dan et al treated U937 cells with etoposide and found that 10 μmol/L STI571 (previously known as CGP57148B) reduced apoptosis of U937 cells without interfering with JNK1/SAPK activation. 11 It was concluded that c-Abl acts downstream of caspases during the development of p53-independent apoptosis. Our study differs from that of Dan et al by the use of a lower dose of STI571. We found that 1 μmol/L STI571 was sufficient to essentially completely inhibit Bcr/Abl, Tel/Abl, or c-Abl kinase activity but, unlike 10 μmol/L STI571, was not associated with any nonspecific toxicity either on normal murine marrow cells or on U937 cells. It is also possible that Abl kinase is necessary for DNA repair responses to etoposide, but not to ionizing radiation.

Whereas c-Abl seems to be activated in response to ionizing radiation, the requirement for Abl tyrosine kinase activity to mediate cellular responses to DNA damage is less clear. For
example, while Abl−/− fibroblasts from mice have been reported to have increased survival after radiation, Abl−/− avian cells had a normal survival curve.7,12 Also, the role of Abl in DNA-damage-induced G1 arrest is controversial.5,6,12 In the present study, we found no evidence that pharmacologic kinase inhibition of c-Abl with STI571 altered radiation sensitivity in either murine hematopoietic cells or in a human p53 mutant cell line. However, some functions of c-Abl are kinase-independent,13,14 and it is possible that kinase activity is not required for some cellular responses to radiation. In any event, these results suggest that the effects of ionizing radiation in patients should not be altered significantly by STI571 treatment.

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References


To the editor:

Hyperprothrombinemia may result in acquired activated protein C resistance

Activated protein C (APC) resistance1 due to the factor V (FV) gene mutation G1691A (single-letter nucleotide codes)2 and the prothrombin gene mutation G20210A3 are the most common genetic disorders associated with venous thrombosis. APC resistance not due to the FV gene mutation G1691A,4,5 and the hyperprothrombinemia secondary to the prothrombin gene mutation G20210A,3 are also independent risk factors for venous thrombosis. Although it has been surmised that high levels of prothrombin in plasma may result in an increased rate of thrombin generation5 or increased thrombin potential,7 little is known on how hyperprothrombinemia leads to venous thrombosis. Recently, Smirnov and colleagues reported on an additional mechanism that might contribute to the pathogenetic role of hyperprothrombinemia.8 They showed that prothrombin may act as a modulator of the anticoagulant activity of APC both in a purified system and in plasma by inhibiting the ability of APC to inactivate FVas. As shown in the figure, there was an inverse relationship between n-APC-ratios and prothrombin activity (r = −0.32, P < .001). In particular, 7 of the 60 subjects (12%) with prothrombin levels higher than the 80th percentile (ie, 110 U/dL) had n-APC-ratios lower than the lower limit for APC resistance (ie, 0.76) established for this method at our laboratory. In contrast, only 2 of the 225 subjects (1%) with prothrombin levels lower than the 80th percentile had n-APC ratios lower than the lower limit (P < .0001, Fisher exact test). These results support the view that plasma prothrombin is a determinant of APC resistance and may explain the mechanism through which hyperprothrombinemia is associated with an increased risk for venous thrombosis.

Activated protein C (APC) resistance1 due to the factor V (FV) gene mutation G1691A (single-letter nucleotide codes)2 and the prothrombin gene mutation G20210A3 are the most common genetic disorders associated with venous thrombosis. APC resistance not due to the FV gene mutation G1691A,4,5 and the hyperprothrombinemia secondary to the prothrombin gene mutation G20210A,3 are also independent risk factors for venous thrombosis. Although it has been surmised that high levels of prothrombin in plasma may result in an increased rate of thrombin generation5 or increased thrombin potential,7 little is known on how hyperprothrombinemia leads to venous thrombosis. Recently, Smirnov and colleagues reported on an additional mechanism that might contribute to the pathogenetic role of hyperprothrombinemia.8 They showed that prothrombin may act as a modulator of the anticoagulant activity of APC both in a purified system and in plasma by inhibiting the ability of APC to inactivate FVas.

On the basis of this observation, one would predict that individuals with hyperprothrombinemia might also have acquired APC resistance as measured with the common aPTT-based test. To test this hypothesis, we selected from our files data on 285 individuals who served as controls in a previous study designed to assess the prevalence of the prothrombin gene mutation G20210A in our patient population.9 Individuals included in the analysis were those for whom APC resistance and prothrombin levels were available and those who were not carriers of the FV gene mutation G1691A. APC resistance was measured with the original aPTT-based test described by Dahlback et al1 as modified by Faiorini et al10; results were expressed as normalized APC ratios (n-APC-ratios) against a frozen pooled normal plasma. Prothrombin was measured using S-2238 (Chromogenix, Mölndal, Sweden) as substrate and Echis Carinatus (Sigma, St Louis, MO) as activator11; results were expressed as U/dL against a pooled normal plasma arbitrarily set at 100 U/dL.

As shown in the figure, there was an inverse relationship between n-APC-ratios and prothrombin activity (r = −0.32, P < .001). In particular, 7 of the 60 subjects (12%) with prothrombin levels higher than the 80th percentile (ie, 110 U/dL) had n-APC-ratios lower than the lower limit for APC resistance (ie, 0.76) established for this method at our laboratory. In contrast, only 2 of the 225 subjects (1%) with prothrombin levels lower than the 80th percentile had n-APC ratios lower than the lower limit (P < .0001, Fisher exact test). These results support the view that plasma prothrombin is a determinant of APC resistance and may explain the mechanism through which hyperprothrombinemia is associated with an increased risk for venous thrombosis.

Figure 1. Normalized activated protein C ratios as a function of prothrombin levels in subjects without the FV gene mutation G1691A. n-APC-ratios are inversely correlated to prothrombin levels (r = −0.32, P < .001).
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