All-Trans-Retinoic Acid and Bleeding/Thrombosis

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All-trans-retinoic acid (ATRA) inhibits cell growth and proliferation by inducing cyto-differentiation and/or apoptosis in several cell types. These effects have become a therapeutic objective in human cancers. In addition to these functions, ATRA has been shown to affect cellular hemostatic properties [1]. Much understanding in this field has come from the clinical and experimental studies of human acute promyelocytic leukemia (APL), a distinct subtype of acute myelogenous leukemia (AML-M3), cytogenetically characterized by the balanced reciprocal translocation between chromosomes 15 and 17. In these cells, the fusion of the nuclear retinoic acid receptor (RAR α) gene on chromosome 17 with part of the PML gene on chromosome 15 results in the expression of a chimeric PML/RAR α protein, which is involved in the leukemogenesis and is the target for the myeloid differentiation effect induced by ATRA. The disease typically presents with a life-threatening hemorrhagic diathesis, which is worsened by cytotoxic chemotherapy. The bleeding disorder is particularly severe in the microgranular variant of APL (M3v), which is characterized by marked hyperleukocytosis. Before the introduction of ATRA for the management of APL patients, fatal hemorrhages due to the associated coagulopathy were a major cause of induction remission failure. In a large retrospective study, the overall remission rate was 62% and the prevalence of hemorrhagic deaths in induction 14% [2]. The use of ATRA for the remission induction therapy of

APL has raised the complete remission rate to greater than 90%, together with a rapid resolution of the coagulopathy, without causing bone marrow hypoplasia. ATRA promotes the terminal differentiation of leukemic promyelocytes, which is accompanied by prompt improvement of the coagulopathy typical of this disease [3]. Consistent with that, normalization in the plasma levels of hypercoagulation markers and downregulation of the two major blast cell-associated procoagulants (i.e. tissue factor, TF, and cancer procoagulant, CP) were observed [4]. A number of laboratory studies have subsequently confirmed the decrease or normalization of clotting and fibrinolytic variables during the first 1 or 2 weeks of therapy with ATRA. In spite of that, the impact of ATRA therapy on early hemorrhagic deaths and CR rate in APL remains uncertain compared to chemotherapy alone. In nonrandomized studies, APL patients administered ATRA showed 9% to 20% improvement of CR rate and 5% to 6% reduction of early hemorrhagic deaths, compared to historical controls receiving conventional chemotherapy [1, 5–8]. These preliminary findings have been more recently confirmed by randomized clinical trials. APL patients treated with different combinations of ATRA plus chemotherapy show a prevalence of early hemorrhagic deaths from 2.4% to 6.5% [9–13]. However, both nonrandomized and randomized clinical trials clearly show an improvement of event free and overall survival in patients receiving induction therapy

with ATRA. This raises the possibility that the effects of ATRA on the leukemic cell hemostatic properties influence the mechanisms of malignancy, independently from blood coagulation.

Mechanisms of ATRA Interaction with Malignant and Normal Cells

The beneficial effects of ATRA on the coagulopathy of APL are related to its capacity to interfere with the hemostatic properties of leukemic cells, which play an important role in the pathogenesis of this complication [1]. The principal hemostatic properties of the leukemic cells include the expression of procoagulant activities (tissue factor, TF, and cancer procoagulant, CP), fibrinolytic and proteolytic activities, and the secretion of inflammatory and angiogenic cytokines (interleukin-1 β , IL-1 β , tumor necrosis factor- α , TNF- α , and vascular endothelial cell growth factor, VEGF), which can affect the hemostatic properties of the vascular endothelium and leukocytes. ATRA can interfere with each of these properties, particularly it decreases the expression of both TF and CP, thus reducing the blast cell procoagulant activity; it increases both plasminogen activators and inhibitors, which result in unchanged or reduced fibrinolytic activity, it upregulates the expression of APL adhesion molecules, modifying the interaction of differentiating promyelocytic with the endothelium; and, finally, ATRA increases the production of cytokines, which can affect the hemostatic status of endothelium. The last two mechanisms, i.e. modifications in cell adhesion molecules and release of cytokines by APL cells during ATRA treatment, may have implications in the activation of the prothrombotic and proadhesive functions of endothelium.

Indeed, several studies demonstrate that ATRA affects the hemostatic balance of the endothelium. Particularly, ATRA induces the expression of endothelial cell thrombomodulin, the membrane receptor that binds and inactivates thrombin; it prevents the expression of TF, induced by standard and tumor-derived cytokines; and finally it induces the production of t-PA, favoring the profibrinolytic response at the vascular endothelium site.

ATRA and Thrombosis: Clinical Data

Intriguingly, thrombotic events have been reported in some series during ATRA therapy, including fatal myocardial infarction and cerebral thrombosis [10, 14, 15]. In another case, acute venous thrombosis and pulmonary

embolism were observed in a patient with APL who developed hyperleukocytosis after one week of treatment with ATRA [16]. The occurrence of thrombosis during induction treatment with ATRA combined with aprotinin and chemotherapy was described in a young patient who was homozygous for factor VQ 506 mutation [17]. Cases of acute renal failure, due to occlusion of renal vessels in a patient with APL treated with ATRA and tranexamic acid and with ATRA alone [18] have also been described. A patient with APL had fatal thromboembolism after receiving ATRA and tranexamic acid therapy [19].

Interestingly, Escudier et al. [20] noted that thrombotic events were more common in patients suspected of having ATRA syndrome. ATRA syndrome is the most severe side effect occurring in about a quarter of patients, usually between the second day and the third week of treatment. It is a protean syndrome of fever, respiratory distress, pulmonary infiltrates, pleuropericardial effusions, and edema, more common among patients who present with a high white blood cell count or develop rapid leukocytosis. If not promptly recognized and treated, the ATRA syndrome can lead to death from progressive hypoxemia and multiorgan failure.

Biological Mechanisms

Thromboembolic events were not characteristic features of APL before the ATRA era and the question arises on whether this drug, among its multiple effects on hemostasis, may also favor the onset of some potentially prothrombotic conditions. Particularly, modifications in the expression of cell adhesion molecules and the release of cytokines by APL cells during ATRA treatment have been associated with the occurrence of ATRA-related side effects. Attachment of leukemic cells to the vessel wall via adhesion molecules, with subsequent trans-endothelial migration represents one potential mechanism to explain the higher incidence of vascular complications in acute leukemia in association with high white blood cell counts. ATRA promotes the differential regulation of adhesion molecules on acute myeloid leukemia blasts, which may confer to blast cells new homotypic and heterotypic (blast/blast or blast/vascular cell) adhesion potential, facilitating cell migration and extravasation, and promoting localized coagulation. Our group demonstrated that ATRA treatment increased the adhesion of APL cells to the endothelium [21]. However, more recently we observed that, in the same functional assay, pretreatment of the EC with ATRA impaired adhesion of the APL cells to EC.

This anti-adhesive effect may be explained by the down-regulation of EC surface specific counter-receptors by ATRA [22]. Therefore, ATRA may act as a pro-adhesive stimulus on the leukemic cell, but may also exert some anti-adhesive effects on the endothelium.

Conclusion

The resolution of the coagulopathy makes for better management of patients and avoids the potentially catastrophic consequences of starting chemotherapy in the acute phase of the disease. Nevertheless, the clinical impact of ATRA on complete remission and early hemorrhagic deaths remains uncertain. An approximately 10% absolute increase of CR

and a variable reduction of hemorrhagic mortality in induction have been observed, but these limited improvements are difficult to confirm statistically in a rare disease such as APL. However, clearly ATRA improves disease-free survival and overall survival and there is a general consensus that the drug should be included in the management of APL. Although rare, thromboembolic events occurred under ATRA therapy. An answer may possibly lie in the very subtle balance between procoagulant and anticoagulant mechanisms in cancer. For instance, high concentrations of circulating cytokines in the presence of low levels of ATRA in the blood stream may definitely favor clotting activation and fibrin deposition at the vascular endothelium, whereas low cytokine levels with elevated ATRA in the blood clearly protect against fibrin deposition on the vessel wall.

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