Arsenic and Apoptosis in the Treatment of Acute Promyelocytic Leukemia

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Remarkable progress has been made in identifying the oncogenic fusion proteins that are produced as a result of specific chromosomal translocations in the human acute leukemias (1). Because these proteins are expressed only by cells within the leukemic clones, they hold great promise as targets for new drugs that will specifically destroy leukemic cells while leaving normal cells unharmed. Both all-trans-retinoic acid (t-RA) and arsenic trioxide (As$_2$O$_3$) have shown this type of activity in patients with acute promyelocytic leukemia (APL) whose blast cells harbor the t(15;17) chromosomal translocation and express the PML/RAR$_a$ fusion protein. Each of these agents was identified empirically in innovative clinical trials conducted by investigators at the Shanghai and Harbin Institutes of Hematology in China (2,3). A plausible biochemical basis for the selective action of t-RA became apparent once the PML/RAR$_a$ fusion gene was cloned, since the aberrant protein contains the retinoic binding domain of retinoic acid receptor (RAR) (4-8). Subsequent work showed that t-RA can induce differentiation of leukemic promyelocytes (2,9-11), and clinical trials combining t-RA with standard cytotoxic drugs have led to improved survival in patients with APL (12-16).

More recently, As$_2$O$_3$ was shown to be active against APL that was resistant to t-RA and other forms of chemotherapy (3); i.e., among 15 patients with this disease, 14 achieved complete remission after treatment with arsenic (17). Few clues to the specific activity of As$_2$O$_3$ in APL can be gleaned from an analysis of the structure of the PML/RAR$_a$ protein. However, studies by Shao et al. (18), published in this issue of the Journal, together with recent reports from Z. Chen’s laboratory (17,19,20,25), afford important insights into the mechanism of action of this new agent.

Differences in the points of attack of t-RA and As$_2$O$_3$ in leukemic promyelocytes expressing the PML/RAR$_a$ fusion protein are shown in Fig. 1. Both agents induce degradation of PML/RAR$_a$, with As$_2$O$_3$ apparently targeting the aminoterminal sequences of PML rather than RAR$_a$, the binding partner of t-RA. Wild-type PML protein is also degraded in As$_2$O$_3$-treated cells, whereas with t-RA therapy, this novel nuclear protein assembles into normal macromolecular organelles called PML oncogenic domains, or PODs. This difference does not appear to account for the lethal effects of As$_2$O$_3$, however, as leukemic myeloid cells lacking PML/RAR$_a$ also show down-regulation (i.e., loss) of wild-type PML protein when treated with As$_2$O$_3$, yet they continue to grow and survive as before (19,20). Wild-type RAR proteins, including RAR$_a$ (18), are not affected by As$_2$O$_3$ in contrast to their avid interaction with t-RA. The net effect of these changes is either differentiation to end-stage granulocytes lacking proliferative potential (in the case of t-RA) or rapid induction of apoptosis, as demonstrated by the presence of DNA “ladders” typical of programmed cell death (17-20) (in the case of As$_2$O$_3$ therapy).

The observation that As$_2$O$_3$ can induce apoptosis without differentiation in both t-RA-sensitive and t-RA-resistant promyelocytes (18) raises intriguing questions about the molecular basis of cell death due to arsenic exposure. For example, does degradation of PML/RAR$_a$ trigger an event within a defined apoptotic pathway or is it merely part of a spectrum of arsenic-induced cellular changes that collectively activate the apoptotic machinery?

Several studies (21-23) have shown that leukemic cell lines conditionally expressing PML/RAR$_a$ depend on the antiapoptotic effects of this fusion protein for survival in the absence of growth factors. Thus, PML/RAR$_a$ may inhibit a conserved growth factor-dependent apoptotic pathway, analogous to the one subverted by the E2A/HLF fusion protein in leukemic lymphoblasts (24). In this context, the survival and differentiation of promyelocytes following t-RA-induced loss of PML/RAR$_a$ could reflect the influence of pharmacologic concentrations of the retinoid interacting with wild-type RAR proteins. Conversely, rapid apoptosis would be the expected outcome of PML/RAR$_a$ degradation by As$_2$O$_3$, in the absence of compensatory activation of a survival/differentiation pathway.

Because the apoptotic effects of As$_2$O$_3$ are equally pronounced in cells sensitive or resistant to t-RA, both in vitro and in vivo (3,17,18,25), this agent appears to add a new pathway of cell kill to those available with t-RA or conventional antileukemia drugs. Biochemical studies (18,19,25) predict that t-RA and As$_2$O$_3$ would not produce enhanced activity when administered together, but rather should be given sequentially, either by themselves or in combination with standard therapy for APL, to achieve optimal clinical responses. Resistance to t-RA typically develops in almost all patients after a few months of therapy (26,27). That interval may well be the best time to introduce As$_2$O$_3$, possibly in combination with daunorubicin or other genotoxic agents. Because of its apparent lack of effects on normal bone marrow cells at clinically effective concentrations (17), As$_2$O$_3$ is not likely to increase the dose-limiting hematopoietic toxicity of drugs commonly used to treat APL.

Identification and characterization of arsenic trioxide as an effective antileukemia agent in APL cells with the PML/RAR$_a$ fusion protein extends hope that other drugs will be found whose primary targets are the translocation-induced fusion proteins of acute lymphocytic leukemia and acute myelogenous leukemia (1). The availability of several different genetically tractable model organisms may help to streamline the discovery of drugs with a selective killing capacity (28).

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Fig. 1. Effects of all-trans-retinoic acid and arsenic trioxide on PML/RARα and other nuclear proteins in the blast cells of acute promyelocytic leukemia (APL). Retinoic acid interaction with PML/RARα leads to rapid degradation of this fusion protein and assembly of the wild-type PML protein into normal nuclear structures called PODs (PML oncogenic domains). In addition, retinoic acid binding to wild-type retinoic acid receptors (RARs) in the cell nucleus may contribute to blast cell differentiation. By contrast, arsenic trioxide treatment induces even more rapid degradation of PML/RARα and eliminates wild-type PML protein as well, apparently through effects mediated by PML amino acids that are included in the fusion product. Wild-type RARs are not degraded, apparently through effects mediated by PML that sometimes accompanies programmed cell death induced by the arsenic compound. These observations suggest that all-trans-retinoic acid and arsenic trioxide act through quite different biochemical pathways. Indeed, transformed promyelocytes resistant to retinoic acid appear to be as sensitive to the effects of arsenic trioxide as previously untreated cells.

References

(23) Rogaia D, Grignani F, Grignani F, Nicoletti I, Pelicci PG. The acute pro-
When and to What End Do Pathologists Agree?

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We are certainly pleased with the central message of the study by Wells et al. (1) that appears in this issue of the Journal which we take to be that pathologists are generally in agreement regarding diagnoses of benign and malignant breast disease. It was also a pleasure to find that so many pathologists were willing to take part in a cooperative exercise regarding diagnostic agreement. However, several points of clarification regarding the study and its conclusions are needed. These discussion points relate to special considerations of diagnostic implications for clinical management (2) and the performance of clinical trials. Indeed, a major use of this study may be to pinpoint those situations in which pathologists are likely to disagree so that we may target these elements for study.

The practical question presented with every medical diagnosis is the diversity of the decision tree, and, fortunately, for most breast biopsies, that is straightforward, because most of the lesions are benign as was the case in this exercise conducted by Wells et al. (1). This dominance of benign diagnoses was particularly true in the premammographic era when large, well-developed lesions (e.g., fibroadenomas, cysts, cancers, etc.) made up the majority of the specimens submitted to a surgical pathologist (3).

It is certainly refreshing to focus on agreement, not disagreement. The agreement presented in the study by Wells et al. (1) relates to diagnoses of “cancer-yes” versus “cancer-no,” a simple dichotomy. However, Wells et al. (1) document the difficulty in making an assignment of invasive or in situ status for some biopsy specimens, and the authors state that “there are situations when anything less than perfect agreement may be clinically unacceptable.” The category of atypia was accorded generic status by Wells et al., yet they did not reference the body of literature on the subject, which includes studies of histologic criteria linked to epidemiologically validated outcomes (4–7). Indeed, the paper by Schnitt et al. (5) is incorrectly cited as a study that documents diagnostic disagreement. In fact, that study was designed to target particularly difficult lesions of atypical hyperplasia, benign hyperplasias, and minimal carcinomas in situ, and the results indicated that there was greater than 90% agreement when the several pathologists involved used agreed-upon criteria. Epidemiologists are now documenting different disease patterns for different patterns of atypical hyperplasia using these same criteria (7), which have also been adopted in large part by the breast cancer screening program in the U. K. Use of these criteria for the recognition and separation of atypical hyperplasia from minimal examples of ductal carcinoma in situ (DCIS) appears to foster interobserver agreement (5,6,8,9).

Another way to look at disagreements has to do with biologic and clinical relevance. Since Wells et al. provide very little in the way of specifics regarding the type of specimen and the extent of the lesions for each of the different categories, we can only speculate about the levels of disagreement (and the reasons for them). Although at face value we might think that any disagreement between benign and malignant could not be tolerated in clinical practice, in actuality, atypical ductal hyperplasia and non comedo DCIS straddle the benign/malignant fence. For small examples of noncomedo DCIS, the biologic potential is probably more similar to atypical ductal hyperplasia than to lesions toward the other end of the spectrum of malignant potential (i.e., possibly comedo DCIS and certainly lesions in the malignant invasive category). We also might think that it should be very straightforward to separate noninvasive from invasive carcinoma, but consider the example of high-grade (comedo) DCIS with areas suggestive of microinvasion—such lesions are often greatly distorted by sclerosis, making the diagnosis of microinvasion quite difficult. In the vast majority of cases, microinvasive carcinoma behaves no differently from DCIS (10,11), suggesting that the clinical behavior may dictate the seriousness of a disagreement and reduce its importance in this special case.

This consensus on benign versus malignant diagnoses was obtained without recourse to discussions of criteria or special circumstances, such as the small core biopsies in which less than

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