Arsenic Trioxide, a Novel Mitochondriotoxic Anticancer Agent?

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During the last few years, it has become increasingly clear that mitochondria play a major rate-limiting role in apoptosis *(1–3).* In general terms, the apoptotic process can be subdivided into three phases: the initiation phase, the decision/effector phase, and the degradation phase. During the heterogeneous initiation phase, which is essentially premitochondrial, specific pro-apoptotic signal transduction pathways or nonspecific damage pathways are activated. These pathways converge on the mitochondria during the decision/effector phase, where they trigger progressive permeabilization of mitochondrial membranes, mostly as a result of the action of the permeability transition pore complex (PTPC). Thus, the mitochondrion (or to be more precise, the PTPC, which interacts with the Bcl-2/Bax complex) "decides" the cell's fate and determines the point of no return of the process *(4,5).* The morphologic and biochemical features of apoptosis become manifest during the postmitochondrial degradation phase, in which soluble intermembrane proteins (SIMPs) released from mitochondria play an active role: AIF (apoptosis-inducing factor) translocates to the nucleus, where it induces large-scale DNA fragmentation *(6);* cytochrome c triggers the activation of pro-caspase-9 *(7);* and procaspases 2, 3, and 9 intervene in a cascade of proteolytic destruction *(8,9).* Inhibition of SIMPs does not prevent cell death as such, although it may cause a shift from apoptotic to nonapoptotic cell death *(2).*

The goal of chemotherapy is to kill tumor cells, mostly by inducing apoptosis. What then is the practical implication of PTPC and SIMPs? Some gross alterations in mitochondrial function, such as a dissipation of the mitochondrial inner transmembrane potential ($\Delta\Psi_m$) or the release of SIMPs (via the outer membrane), are near-to-general features of apoptosis *(1–3).* The mere detection of mitochondrial changes thus does not distinguish between two fundamentally different possibilities, namely, that a chemotherapeutic agent acts directly on mitochondria or rather that it compromises mitochondrial function in an indirect fashion, by activating extramitochondrial pro-apoptotic signal transduction or damage pathways. To discriminate between these possibilities, it is necessary to perform experiments in which the chemotherapeutic agent is added to purified mitochondria *in vitro* and local effects (activation of the PTPC and release of SIMPs) are studied. One particularly interesting possibility is to combine different components of the cell (e.g., nuclei, cytosols, and mitochondria) *in vitro* to study the minimum requirements for caspase activation or induction of nuclear apoptosis induced by anticancer agents. With this approach, it has become clear that a number of experimental drugs act primarily on mitochondria to induce apoptosis. This applies to lonidamine (an agent used in phase II studies of breast cancer therapy) *(10),* betulinic acid (an agent that selectively kills neuroectodermal cells) *(11,12),* thiol-cross-linking agents such as diamide *(13),* and ligands of the peripheral benzodiazepine receptor *(14)* including photo-activable porphyrin derivatives *(15).* In contrast, classical chemotherapeutic agents such as etoposide, doxorubicin, or cisplatin have no direct mitochondrial effects *(11).* Such agents act by inducing p53, by activating ceramide, by stimulating the CD95/CD95L pathway, and/or by inducing major shifts in redox potentials. Each of these alterations then can affect mitochondria in an indirect fashion, p53 by affecting the redox balance (*see below*) *(16,17),* ceramide by formation of ganglioside GD3 and/or influencing the function of BAD (which interacts with Bcl-2) *(18),* CD95 by apical caspases (which act on mitochondria) *(19),* and redox imbalances by effects on redox-sensitive sites within the PTPC *(20).* The level at which a chemotherapeutic agent acts is likely to have some practical impact because, on theoretical grounds, agents that directly affect mitochondria should bypass any resistance due to interruption of the indirect death-inducing pathways (e.g., mutations of p53 and loss of CD95). Whether this is the case for arsenic trioxide awaits further confirmation.

Arsenic trioxide has recently become a therapeutic agent of choice for the treatment of acute promyelocytic leukemia (APL). The report by Zhu et al. *(21)* in this issue of the Journal shows that arsenic trioxide induces (as do most other chemotherapeutic agents) an early $\Delta\Psi_m$ collapse, thus extending the observation that arsenite (the trivalent inorganic salt formed by arsenic trioxide) causes the release of cytochrome c from mitochondria to the cytosol *(22).* Cell-free systems of apoptosis reveal that arsenite requires mitochondria to induce nuclear apoptosis *in vitro (23).* Moreover, arsenite acts on isolated mitochondria to induce the opening of PTPC, presumably by acting as a thiol-oxidizing agent *(20).* In accordance with this idea, intracellular glutathione levels determine the dose of arsenite required to kill cells *(21,24).* Arsenite also acts on purified PTPC reconstituted in liposomes *in vitro (23).* In such a system, recombinant Bcl-2 prevents the opening of PTPC, which is in agreement with the fact that transfection-enforced overexpression of Bcl-2 protects cells against the pro-apoptotic effect of arsenite *(23).*

If these data suggest that arsenic trioxide can induce apoptosis via a direct mitochondrial effect leading to caspase activation, they do not rule out that this pleiotropic agent may have additional effects that also contribute to induction of cell death.

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Indeed, arsenic may interfere with a staggering variety of cellular processes, e.g., cell cycle progression, DNA repair, ubiquitination, tubulin polymerization, nitric oxide synthesis, and oncogene expression or activation. In view of this large number of potential cellular targets, issues such as dose, cell type, or cellular environment become critical. From a therapeutic perspective, differences in arsenic sensitivity should exist between tumor cells and normal cells. Some reports have highlighted clear differences in arsenic sensitivity of some cell lines compared with others. For example, human T-cell lymphotropic virus type I-infected cells, myeloma cells, and transformed lymphocytes are exquisitely sensitive to arsenic *(25,26)*. However, results observed in cell lines should be interpreted with caution. Indeed, cellular levels of glutathione and selenium (both of which potentially titrate intracellular arsenic) are major determinants of arsenic-triggered cell death *(21,24,27)*. The redox state of cell lines in culture is likely to differ from that of cells *in vivo*. In that sense, very little is known about the consequences of *in vivo* exposure to micromolar arsenic concentrations, with the notable exception of APL, in which arsenic trioxide induces dramatic clinical remissions. Although nonleukemic cells show some toxicity, the selective disappearance of leukemic cells *in vivo* implies their greater sensitivity to the drug.

The mechanism by which eradication of the leukemic clones is achieved *in vivo* is still unclear. *In vitro* studies in cell lines initially suggested the mechanism to be apoptosis, while lower doses were later found to induce terminal differentiation *(27–30)*. In contrast to the observations in established APL cell lines, primary APL blasts are relatively resistant to rapid apoptosis induction by arsenite when cultured *ex vivo*. In patients or animal models, apoptosis and differentiation seem to coexist in the few cases analyzed, but their respective contribution to clinical remissions is elusive *(30,31)*. At the molecular level, arsenic was reported to target the leukemia-specific PML/RARα (i.e., promyelocytic leukemia protein/retinoic acid receptor α) fusion protein for degradation *(21,30,32,33)*. Since the PML/RARα fusion protein may antagonize both differentiation and apoptosis, its degradation could trigger these two events, yet, in some settings, PML/RARα degradation is observed in the absence of apoptosis *(27)*. Arsenic has also been reported to alter the intranuclear localization of PML protein, to enhance its pro-apoptotic properties, and finally to induce its degradation *(32,34,35)*. However, mouse cells lacking the PML (or PML/RARα) genes were shown to undergo normal cell death in response to arsenic *(36)*, a finding conflicting with the results of other reports *(35,37)*. Finally, prolonged exposure to arsenic can induce the degradation of RARα *(27)*, which points to a possible effect of this drug on the retinoic acid (RA) response. In support of this possibility, arsenic sensitizes some APL cells to RA-triggered differentiation *(27)*. Moreover, arsenic and RA cooperate to induce tumor regression and prolong survival in an animal model of APL *(31)*. This observation suggests that, especially when used at relatively low doses (in the low μ*M* range), arsenite may have effects that can be dissociated from its acute mitochondriotoxicity.

Most anticancer agents have been clinically used before their modes of action have been understood, and arsenic is no exception to this rule. The future will tell to which extent progress in fundamental understanding will invert this order and boost rational drug design. Irrespective of the molecular details, it appears that arsenic constitutes a welcome addition to the clinician's armamentarium for the chemotherapy of leukemia.

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