Induction of Apoptosis by Salicylates in B-Cell Chronic Lymphocytic Leukemia

To the Editor:

We have read with great interest the report by Bellosillo et al demonstrating the induction of apoptosis in leukemic cells by aspirin and salicylate. This finding could have important implications for the understanding of the biology of chronic lymphocytic leukemia (CLL) as well as for the design on new therapeutic approaches for this disease. While the characterization of the phenomenon is clear, including the involvement of caspases in the effector phase, some points may deserve additional comments.

First, normal mononuclear cells (PBMC) were shown to be less sensitive than frozen CLL B cells to apoptosis induction by salicylates. We wonder whether the storage could have influenced the apoptosis resistance of the leukemic cells. In addition, if all experiments with leukemic cells were done at 5 × 10⁶ cells/mL, the high cell density could have influenced (at least partly) their susceptibility to apoptosis. Some additional data could have provided additional insight about the relevance and extent of the susceptibility reported by the authors, including the bcl-2 expression in the leukemic cells and the study of the effect of salicylate on cells from patients with lower cell counts.

A second point refers to aspirin and salicylate concentration required to induce apoptosis, which are quite high (IC₅₀ 5.9 ± 1.13 mM/L, and 6.96 ± 1.13 mM/L, respectively), as stated by Bellosillo et al. In the same reference they used (the Goodman and Gilman textbook of pharmacology), the lower value for toxic concentration of salicylate is 200 µg/mL, making quite improbable the use of these levels of aspirin or salicylate in the clinical setting. These concentrations are above those required for cyclooxygenase (COX) inhibition and the authors clearly demonstrate that COX-independent mechanism(s) must be involved, although the sample of other COX inhibitors used is too small to exclude all nonsteroidal anti-inflammatory drugs (NSAIDs) of sharing similar results. As stated below, some other targets of aspirin and salicyte, besides COX, are also affected by several NSAIDs.

Third, some other molecular targets of salicylates have been identified in the 100-year-long record of aspirin research, some of which may be involved in the triggering or regulation of apoptosis. For instance, nitric oxide is a mediator involved both in triggering or inhibition of apoptosis, depending on the cell and mechanisms under evaluation, although it is most frequently an inhibitor, through its effect on caspases (see ref 3 and references therein for recent review). Inducible nitric oxide synthase (iNOS) is inhibited by both aspirin and salicylate at concentration near the values reported by Bellosillo et al (IC₅₀, 3 mM/L and 20 mM/L, respectively). The effects are exerted at the level of translational/posttranslational modification and directly on the catalytic activity of iNOS.

A potentially significant target is the mitochondrial, which plays a central role in most models of apoptosis. Since the 1970s, aspirin has been known to uncouple the respiratory chain; it also inhibits enzymes such as NADH oxidase and cytochrome c oxidase. This effect associates with lipid peroxidation and damage, probably by apoptosis.

Recently, the mitochondrial protein cytochrome c has deserved substantial attention as a key component of the apoptosis machinery in several models. The release of cytochrome c from the mitochondria as a consequence of changes in their membranes has been shown to activate procaspase 9 and other caspases downstream. The most characterized change is the formation of a channel that includes several proteins of the bcl-2 family (bcl-Xs, bax) and participates in the regulation of Ca²⁺, pH, or electric properties of the matrix. Though controversial, this process is accompanied by an important change in the permeability of the mitochondrial membrane, referred to as permeability transition. Salicylate induces mitochondrial permeability transition. This effect has been involved in the pathophysiology of Reye’s syndrome induced by aspirin. Interestingly, several other NSAIDs also induce changes in the permeability of mitochondrial membranes.

In summary, the interesting report by Bellosillo et al deals with a very central point in cell and cancer biology, and the effect of salicylates they describe may have a significant impact on the way we consider these drugs in therapy. However, we suggest that the exploration of additional targets, in particular related to the effect of salicylates on mitochondria, deserves further attention.

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Response

We thank Drs Guerенко and Diez for their interest in our study and we appreciate the opportunity to respond to their thoughtful comments. For our experiments we used frozen normal peripheral blood mononuclear cells (PBMC). In this regard they raise the possibility that a "selection" effect may take place as a consequence of freezing. We have observed that a fraction of cells die during freezing (1% to 10% of B-CLL cells and 10% to 15% of PBMC), but we believe that this fraction is too low to explain the difference in sensitivity of PBMC and B-CLL cells to aspirin by a "selection" effect. Furthermore, B and T lymphocytes from normal donors and T lymphocytes from B-CLL patients are more resistant than B-CLL cells to aspirin-induced apoptosis, as determined by analysis of phosphatidylserine exposure by flow cytometry, where percentages are referred to the total number of cells analyzed. Moreover, cell density was 5 × 10⁶ cells/mL in cell viability assay performed by the MTT method, whereas for analysis of apoptosis cells were incubated at densities of 2 to 4 × 10⁶ cells/mL and sensitivities to aspirin similar to those obtained by the MTT assay were found for each patient. Therefore, we consider that cell density does not influence susceptibility to aspirin-induced apoptosis.

Regarding the mechanism of aspirin-induced apoptosis, aspirin did not modify Bcl-2 protein level (unpublished results, July 1997). As discussed in our study, inhibition of cyclooxygenase (COX) is not sufficient to induce apoptosis in B-CLL cells. We proposed several COX-independent mechanisms that may be involved in aspirin-induced apoptosis in B-CLL cells (inhibition of NFκB, inhibition of AP-1, activation of p38), but we agree that additional mechanisms should also be considered. In this respect, the mechanisms proposed by Drs Guerenco and Diez (induction of mitochondrial permeability transition and inhibition of nitric oxide synthase) deserve investigation. Interestingly, it has recently been described that B-CLL cells spontaneously express a functional inducible nitric oxide synthase, which has an anti-apoptotic role. Experiments are in progress to study the mechanisms involved in the apoptotic action of aspirin and salicylate in B-CLL cells.

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Burkitt’s Lymphoma: A Single Disease With Multiple Variants

We read with interest the insightful comments of Dr Wright, who is in a unique position to evaluate the historical evolution of the definition of Burkitt’s lymphoma (BL) and its variants. His observations emphasize the importance of precise disease definitions for biological and epidemiological studies. These principles were used by the Revised European-American Classification of Lymphoid Neoplasms (REAL), which proposed that disease entities should be defined by a constellation of morphological, biological, and clinical features.

The World Health Organization (WHO) classification will recognize several variants of BL, all of which are high-grade B-cell lymphomas that share deregulation of the c-myc, leading to the characteristic histological and clinical features of BL. In addition to endemic BL, sporadic BL, and AIDS-associated BL, as defined by Dr Wright, the WHO scheme will include an “atypical or pleomorphic” variant of BL. This subtype includes some cases that would have been diagnosed as “Burkitt-like” lymphomas in the REAL classification. These high-grade lymphomas show greater nuclear pleomorphism than classical BL, with occasional larger lymphoid cells resembling centroblasts. However, 100% of the cells are in cycle, and treatment recommendations are similar to those of classical BL. This variant is closely related to sporadic BL in its clinical and epidemiological features. Finally, the WHO classification recognizes that some BL may present with preferential involvement of the bone marrow and peripheral blood. Such cases were diagnosed as the L3 subtype of acute lymphocytic leukemia in the French-American-British Cooperative group classification.

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