Nucleotide Requirements for the in Vitro Activation of the Apoptosis Protein-activating Factor-1-mediated Caspase Pathway*

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Adenine deoxynucleosides, such as 2-chlorodeoxyadenosine (2CdA) and fludarabine, induce apoptosis in quiescent lymphocytes, and are thus useful drugs for the treatment of indolent lymphoproliferative diseases. We previously demonstrated that the 5′-triphosphate metabolite of 2CdA (2CdATP), similar to dATP, can cooperate with cytochrome c and apoptosis protein-activating factor-1 (APAF-1) to trigger a caspase pathway in a HeLa cell-free system. We used a fluorometry-based assay of caspase activation to extend the analysis to several other clinically relevant adenine deoxynucleotides in B-chronic lymphocytic leukemia extracts. The nucleotide-induced caspase activation displayed typical Michaelis-Menten kinetics. As estimated by the $V_{max}/K_m$ ratios, the relative efficiencies of different nucleotides were Ara-ATP > 9-fluoro-9 β-D-arabinofuranosyladenine 5′-triphosphate > dATP > 2CdATP > 9-β-D-arabinofuranosylguanine 5′-triphosphate > dADP > ATP. In contrast to dADP, both ADP and its nonhydrolyzable α,β-methylenephosphonate analog were strong inhibitors of APAF-1-dependent caspase activation. The hierarchy of nucleotide activation was confirmed in a fully reconstituted system using recombinant APAF-1 and recombinant pro-caspase-9. These results suggest that the potency of adenine deoxynucleotides as co-factors for APAF-1-dependent caspase activation is due both to stimulation by the 5′-triphosphates and lack of inhibition by the 5′-diphosphates. The capacity of adenine deoxynucleotides to activate the apoptosis pathway may be an additional biochemical mechanism that plays a role in the chemotherapy of indolent lymphoproliferative diseases.

The purine nucleoside analogs 9-β-D-arabinofuranosyl-2-fluoradenine (fludarabine or F-Ara-A), 1 2-chlorodeoxyadenosine (cladribine or 2CdA), and 2′-deoxycoformycin are active to varying degrees in indolent lymphoproliferative diseases, including chronic lymphocytic leukemia (CLL), hairy cell leukemia, Waldenstrom’s macroglobulinemia, and low grade lymphomas (1, 2). They are unique among nucleoside antimetabolites by virtue of their ability to induce apoptosis in nonproliferating cells.

The regulation of cell death by apoptosis is thought to play a fundamental role in the natural evolution of malignancy and in the response of tumors to chemotherapy. CLL is an attractive model to investigate the regulation of apoptosis, independent of cell cycle progression, because of its slow population doubling time and the ready accessibility of malignant cells. Previous studies have documented abnormalities in the expression of certain Bcl-2 family proteins in CLL (3–6). This family of proteins plays a critical role in controlling cellular responses to apoptotic stimuli, including those induced by many chemotherapeutic drugs (7). Some of the actions of Bcl-2 are mediated by its ability to control the response of mitochondria to factors in the cytoplasm that induce the release of cytochrome c.

The molecular details of the activation of the final steps of the apoptotic pathway have been recently investigated (8, 9). The cascade is initiated by cytochrome c binding to the apoptosis-protein-activating factor APAF-1, which induces it to undergo a conformational change leading to the formation of APAF-1 multimers and to the recruitment of procaspase-9. The subsequent autocatalysis of procaspase-9 is followed by proteolytic activation of procaspase-3 and possibly procaspase-7. The entire multimeric complex has been defined as the functional apoptosome. Cytochrome c is normally sequestered inside mitochondria, between the inner and outer membranes of these organelles. However, it becomes released into the cytosol following exposure of cells to a variety of proapoptotic stimuli (10) (11–13). In addition to cytochrome c, the APAF-1-mediated in vitro activation of procaspase-9 and procaspase-3 requires dATP. Although ATP can supplant dATP, the deoxynucleotide is more active (12, 14). The peculiar preference of APAF-1 for dATP is unusual for energy-requiring processes not directly connected to DNA synthesis. In the present study, therefore, we designed experiments to compare the abilities of the purine deoxynucleoside analogs commonly used in the treatment of indolent lymphoproliferative diseases to activate the APAF-1-dependent apoptotic pathway and to define the kinetic parameters of the process.

*This work was supported in part by National Institutes of Health Grants GM23200 and CA81534. The costs of publication of this article were defrayed in part by the payment of page charges. This article must operate with cytochrome $c$ and apoptosis protein-activating factor-1 (APAF-1) to trigger a caspase pathway in a HeLa cell-free system. We used a fluorometry-based assay of caspase activation to extend the analysis to several other clinically relevant adenine deoxynucleotides in B-chronic lymphocytic leukemia extracts. The nucleotide-induced caspase activation displayed typical Michaelis-Menten kinetics. As estimated by the $V_{max}/K_m$ ratios, the relative efficiencies of different nucleotides were Ara-ATP > 9-fluoro-9 β-D-arabinofuranosyladenine 5′-triphosphate > dATP > 2CdATP > 9-β-D-arabinofuranosylguanine 5′-triphosphate > dADP > ATP. In contrast to dADP, both ADP and its nonhydrolyzable α,β-methylenephosphonate analog were strong inhibitors of APAF-1-dependent caspase activation. The hierarchy of nucleotide activation was confirmed in a fully reconstituted system using recombinant APAF-1 and recombinant pro-caspase-9. These results suggest that the potency of adenine deoxynucleotides as co-factors for APAF-1-dependent caspase activation is due both to stimulation by the 5′-triphosphates and lack of inhibition by the 5′-diphosphates. The capacity of adenine deoxynucleotides to activate the apoptosis pathway may be an additional biochemical mechanism that plays a role in the chemotherapy of indolent lymphoproliferative diseases.

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Ara-GTP, 9-β-D-arabinofuranosylguanine 5′-triphosphate; 8-Cl-ATP, 8-chloroadenosine 5′-triphosphate; ADPβP, β,γ-methyleneadenosine 5′-triphosphate; AMPγP, α,β-methyleneadenosine 5′-diphosphate; HEB, hypotonic extraction buffer; PIPES, 1,4-piperazinediethanesulfonic acid; AMC, Ac-DEVD-7-amino-4-trifluoromethylcoumarin; PAGE, polyacrylamide gel electrophoresis.
Nucleotides—Nucleosides were purchased from Sigma or Calbiochem. When nucleotides were unavailable commercially, they were synthesized by standard methods (15, 16) and checked for purity by TLC or high pressure liquid chromatography. The triphosphates studied included those of 9-β-D-arabinofuranosyladenine 5′-triphosphate (Ara-ATP), 2-fluoro-9-β-D-arabinofuranosyladenine 5′-triphosphate (F-Ara-ATP), 2-chloro-9-β-D-arabinofuranosyladenine 5′-triphosphate (CAFADTP), 2-chloro-2′-deoxyadenosine 5′-triphosphate (2CdATP), 9-β-D-arabinofuranosylguanine 5′-triphosphate (Ara-GTP), and 8-chloroadenosine 5′-triphosphate (8-Cl-ATP).

Cell Isolation—Heparinized peripheral blood samples from patients with CLL containing at least 80% malignant cells, were fractionated by Ficoll/Hypaque sedimentation. Nonadherent mononuclear cells were resuspended in complete medium (RPMI 1640 supplemented with 10% fetal bovine serum) at 1–2 × 10⁶ cells/ml.

Cell-free Extraction Preparation—CLL cells were isolated as described above. Cells were then washed at 4 °C and resuspended in a hypotonic extraction buffer (HEB; containing 50 mM PIPES, 50 mM KCl, 5 mM EGTA, 2 mM MgCl₂, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride). The cells were centrifuged at 1000 × g for 5 min to form a tight pellet, and the volume of the cell pellet was approximated. The supernatant was discarded, and HEB buffer was added to a volume between 0.5 and 1 times the pellet volume. The cells were allowed to swell for 20–30 min on ice and then lysed in a Dounce homogenizer with 100 strokes of a B-type pestle. The extent of lysis was monitored under the microscope by erythrosin B staining. The cell lysates were centrifuged at 100,000 × g for 60 min at 4 °C. The clarified supernatants were used immediately or stored in aliquots at −80 °C. The cytoplasmic fractions did not contain microscopically visible whole cells, nuclei, or mitochondria.

Cell-free Assays for Caspase Activity—CLL extracts were clarified by 0.2-μm filtration, and then 5–10 μl of extracts (100–200 μg of protein) were incubated in a 96-well plate with the indicated nucleotides, 2 μM of cytochrome c from bovine heart, and either 50 μM Ac-DEVD-7-amino-4-methylcoumarin (AMC) or 50 μM of Ac-LEHD-7-amino-4-trifluoromethylcoumarin (AMC) at 37 °C for 50–60 min in 50 μl with HEB buffer. The hydrolysis of the substrate was followed fluorometrically at 380 nm (excitation) and 460 nm (emission) for the caspase-3 substrate Ac-DEVD-AMC and at 400 nm and 505 nm for the caspase-9 substrate Ac-LEHD-AFC in a Cytofluor fluorescence plate reader (PerSeptive Biosystems, Framingham, MA). Base-line fluorescence values from reactions without nucleotides were subtracted from each data point. The specificity of the assay was validated using the caspase-3 inhibitor Ac-DEVD-CHO (aldehyde) at 1 μM. Ac-DEVD-AMC (sequence, N-acetyl-Asp-Glu-Val-Asp-AMC (17)), Ac-DEVD-CHO (18), and Ac-LEHD-AFC (sequence, N-acetyl-Leu-Glu-His-Asp-AFC (19)) were purchased from Calbiochem.

Immunoblotting—10 μl of CLL extracts were incubated at the indicated time points with the nucleotides and cytochrome c (2 μM) in a volume of 20 μl in HEB buffer at 37 °C. Proteins were resolved at 125 V on 14% gels and electrophoretically transferred to 0.2-μm polyvinylidene fluoride membranes (Millipore Corp., Bedford, MA) for 2 h at 125 V. Membranes were blocked overnight in 1X-tight blocking buffer (Tropix, Bedford, MA). Blots were then probed for 1 h with antibodies to procaspase-3 (Transduction Laboratories, Lexington, KY) or to caspase-9 (provided by S. Reed). The blots were developed with species-specific antiserum and visualized by alkaline phosphatase-based enhanced chemiluminescence (ECL; Tropix), according to the manufacturer’s instructions. The x-ray films were scanned, acquired in Adobe Photoshop, and analyzed with NIH Image software.

In Vitro Caspase-9 and Caspase-3 Assay—Cytosolic extracts were prepared as described above and stored at −80 °C. Human caspase-9 and caspase-3 expression plasmids were kindly provided by Dr. John Reed (Burnham Institute, La Jolla, CA). The respective procaspases were translated in vitro in the presence of [35S]methionine with a Promega (Madison, WI) TNT transcription/translation kit and purified from radioactive methionine and ATP through a desalting column (Bio-Rad).

Reconstituted System—The APAF-1/caspase-9 reconstituted system was recently described (20). Briefly, aliquots of 0.5 μl (0.2 μg) of His-tagged baculovirus-expressed recombinant procaspase-9 and 5 μl (0.8 μg) of baculovirus-expressed recombinant APAF-1 were incubated in the presence or absence of 10 μg/ml cytochrome c and the indicated nucleotides and 1 mM additional MgCl₂ at 30 °C for 1 h in a final volume of 20 μl of buffer A (20 mM Hepes-KOH, pH 7.5, 10 mM KCl, 1.5 mM MgCl₂, 1 mM NaEDTA, 1 mM NaEGTA, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) (20). After incubation, the samples were subjected to a 15% SDS-PAGE and transferred to a nitrocellulose filter, which was blotted with anticaspase-9 antibody. Procaspase-3 was translated and purified as described above. A 1-μl aliquot of in vitro translated procaspase-3 was incubated with the mixture of procaspase-9 activation reaction as described above. The samples were subjected to a 15% SDS-PAGE, and then the gel was transferred to a nitrocellulose filter, which was subsequently exposed to a phosphor imaging plate and visualized in a Fuji BAS-1500 phosphor imager.
of incubation (nmol/mg/h) using an AMC standard curve. AMC cleaved from the DEVD substrate per mg of CLL extract per 1 h incubation at 37 °C. Caspase-3-like activity was then expressed as nmol of HEB buffer, and fluorescence was then measured after a 30-min incubation. These results indicate that the intracellular ionic strength may play an important role in restraining APAF-1-mediated caspase activation. In the presence of 2 mM cytochrome c, the nucleotides (F-Ara-ATP (□), dADP (▲), dATP (●), CaFADATP (●), ATP (●), and ADP (□)) were added at the indicated concentrations in the presence of 2 mM cytochrome c in HEB buffer, and fluorescence was then measured after a 30-min incubation at 37 °C. Caspase-3-like activity was then expressed as nmol of AMC cleaved from the DEVD substrate per mg of CLL extract per 1 h of incubation (nmol/mg/h) using an AMC standard curve.

**Time Course of Nucleotide and Cytochrome c-Induced Caspase Activation**—In the presence of 2 mM cytochrome c, but without the exogenous addition of dATP, no activation of caspase-3 pathway in CLL extracts was detectable (Fig. 2). In contrast, 12.5 μM of dATP was sufficient to trigger the activation of the caspase after 15 min of incubation. The immunodepletion of caspase-9, APAF-1, or both, using specific polyclonal antibodies, completely abolished the nucleotide/cytochrome c-induced caspase activation (data not shown). These results indicate that caspase-9 and APAF-1 are critical for nucleotide/cytochrome c-initiated caspase-3 activation and cannot be substituted for by other caspases or CED-4 homologs present in the CLL extract.

In order to determine the possible role of intracellular nucleotides and low molecular weight compounds present in the CLL-cell-free extracts, we dialyzed the extracts and repeated the caspase assays in the presence of exogenous cytochrome c and various concentrations of nucleotides. Dialysis did not prevent cytochrome c and nucleotide-induced caspase activation. However, the preparation of CLL and HeLa cell-free extracts by manual homogenization led predictably to the release of small amounts of cytochrome c from mitochondria that could be visualized by immunoblotting (data not shown). In order to address the role of the released cytochrome c, immunodepletion experiments using anticytochrome c (native form) antibodies were carried out. The removal of the endogenous cytochrome c did not alter the observed nucleotide kinetics. In the immunodepleted extracts, exogenous cytochrome c was required to initiate procaspase-9 and procaspase-3 cleavage (results...
Nucleotide Regulation of APAF-1-mediated Caspase Pathway

TABLE II

Inhibition constants of nucleotides for cytochrome c- and dATP-induced caspase-3 activation in a CLL cell-free system (100 ng)

| Nucleotide          | $K_i$ (μM) |
|---------------------|------------|
| 2’,3’-dADP          | 89 ± 6     |
| ADP                 | 133 ± 5    |
| ATP                 | 163 ± 16   |
| AMPcP               | 1536 ± 250 |
| 3’-dATP (cordycepin)| 1685 ± 302 |
| ADPcP               | NA*       |
| dADP                | NA        |

*NA, no inhibitory activity.

Kinetic Parameters of Nucleotide Activators in CLL Extracts—A fluorometry-based assay was used to compare the effects of different nucleotides on APAF-1-dependent caspase activation in B-CLL cell extracts (Fig. 3). Caspase activation followed Michaelis-Menten kinetics, with a hyperbolic relationship between initial velocity and nucleotide concentrations. The ratio of $K_m$ to $V_{max}$ calculated by a nonlinear least-squares fit method, revealed the relative efficiencies of the different nucleotides (Table I). The most active compounds were as follows: Ara-ATP > F-Ara-ATP > CAFdATP > dATP > 2CdATP > Ara-GTP > dADP > ATP. ATP was a very weak activator, with a relative efficiency 25-fold smaller than dATP and 50-fold smaller than Ara-ATP. We also tested nucleotides and deoxynucleotides based on other bases (C, G, U, T, and I), but none activated caspases in CLL extracts, with the notable exception of Ara-GTP (Table I). These results confirmed the energetic requirement for APAF-1-mediated caspase activation and indicated that arabinofuranosyl nucleotides are more active than deoxynucleotides.

Kinetic Parameters of Nucleotide Inhibitors in CLL Extracts—In addition to studying the activation of APAF-1 by purine nucleoside 5’-triphosphate substrates, it also was important to determine if the 5’-diphosphates were product inhibitors. Both ADP and ATP dose-dependently inhibited dATP-induced caspase activation in CLL extracts (Fig. 4). The determination of the $K_i$ values (Table II) showed $K_i$ ($K_i$ = 133 μM) and ATP ($K_i$ = 163) to be nearly equipotent inhibitors. However, while a nonhydrolyzable α,β-methylene phosphonate analog of ADP (AMPcP) was also an inhibitor, a nonhydrolyzable analog of ATP (ADPcP) was ineffective (Fig. 4A). Hence, it is likely that the inhibitory action of ATP is due partly to its rapid conversion into ADP. It is noteworthy that dADP at concentrations up to 1 mM had no significant inhibitory effect on optimal dATP-induced caspase activation. The nonphysiological nucleotides 2’,3’-dADP and cordycepin (3’-dATP) showed an inhibitory capacity toward dATP-induced caspase activation.

Immunoblotting—Immunoblotting with anticaspase-3 and anticaspase-9 antibodies verified the time course of the nucleotide and cytochrome c-induced proteolytic activation of the procaspases in B-CLL extracts (Fig. 5). The results demonstrated that maximal procaspase-9 cleavage preceded maximal procaspase-3 proteolytic processing. Similarly, the depletion of caspase-9 was more rapid than that of caspase-3. After 20 min of incubation with 2 μM cytochrome c and 0.25 mM dATP (lane 2) both caspase-3 and caspase-9 appeared to be cleaved. After 40 min, no visible procaspase-9 was detectable in the extract. In contrast, 120 min of incubation were needed to fully deplete procaspase-3. The immunoblotting also confirmed the ATP/ADP inhibition. The addition of 0.5 mM ATP reduced the intensity of the dATP-activated procaspase-3 band (lane 7). The inhibitory effect of ATP/ADP on dATP-induced procaspase-9 activation was less visible, but the intensity of the lower band that corresponded to the activated caspase-9 was also reduced. The immunoblotting of caspase-9 showed two bands of cleaved products, which probably correspond to the p35 and p37 fragments resulting from cleavage at the processing sites D315 (p35) and D330 (p37). The p35 fragment, which is the result of the autocatalytic cleavage of procaspase-9, was more abundant in the immunoblot rather than the p37 fragment, which is thought to be the result of feedback cleavage of caspase-9 by caspase-3 (25).

Cleavage of in Vitro Translated Caspase-9 and Caspase-3—To support further the data obtained by immunoblotting, the proteolysis of in vitro translated procaspase-9 was studied (Fig. 6). The results confirmed the dose dependence of dATP-induced procaspase-9 degradation. In agreement with the fluorometric assays, F-Ara-ATP and Ara-ATP were more potent than dATP, while 2CdATP was slightly less effective. These data also confirmed the inhibitory properties of ADP, which almost completely inhibited dATP-induced procaspase-9 cleavage.

Nucleotide Effects in a Reconstituted System—To rule out any possible effects of contaminants on nucleotide-induced caspase activation, we utilized a recently described reconstituted system, based on highly purified cytochrome c, recombinant APAF-1, and recombinant procaspase-9 and -3 (20). The results were similar to those obtained with CLL extracts (Fig. 7). Visible procaspase-3 and procaspase-9 cleavage was observed with as little as 10 μM of F-Ara-ATP and dATP. The ranking of the tested nucleotides was the same as shown in Table I: F-Ara-ATP > CAFdATP > dATP > 2CdATP > ATP.

DISCUSSION

These results indicate that various nucleotides can have markedly different effects on APAF-1-mediated caspase activation. Although other reports have elucidated carefully the mechanism of activation of caspase-9 by APAF-1, the role of the nucleotides in the process has not been quantified (9, 20, 21, 25). It is important to address this issue because of the known ability of certain purine deoxynucleoside analogs to induce apoptosis in nondividing cells.

The purine analogues fludarabine or cladribine are active in CLL patients resistant to classical alkylating agents (2, 26). Other nucleosides that are capable of killing nondividing...
lymphoid cells include 2′-deoxyadenosine, 2′-β-α-arabinofuranosyladenine (27), 2-chloro-2′-β-α-arabinofuranosyl-2′-deoxyadenosine (28), and 2′-β-α-arabinofuranosylguanine (29). The cytotoxicity of these nucleoside analogs in lymphocytes depends mainly upon the selective and progressive accumulation of the 5′-triphosphate metabolites because of the high ratio of deoxycytidine kinase (EC 2.7.1.74) to cytosolic 5′-nucleotidase (EC 3.1.3.5) in lymphocytes, compared with other cell types (30, 31). The nucleoside 5′-triphosphates inhibit DNA polymerization and ligation and are incorporated into DNA (32). This leads to the progressive accumulation of DNA single strand breaks (33), followed by the activation of poly(ADP-ribose) polymerase, with resultant consumption of NAD and depletion of total adenine nucleotides.

Recently, we reported that 2CdATP was able to replace dATP in the activation of the procaspase-9/APAF-1 death pathway in HeLa cell extracts (14). In this paper, we demonstrate that 5′-triphosphate metabolites of the same nucleosides with

in vitro and in vivo cytotoxicity against indolent lymphoproliferative diseases are able to promote apoptosis activation both in CLL extracts and in a reconstituted “pure” system. The kinetics of nucleotide activation suggest that the apoptosis apoptotic pathway may play a relevant role in vivo. In fact, the concentrations of F-Ara-ATP in CLL lymphocytes of leukemic patients undergoing chemotherapy reach 30–60 μM (34), near or above the KM for the F-Ara-ATP-induced caspase cleavage (39 μM).

In the CLL extracts, the arabinofuranosyl-based adenine nucleotides were the most potent activators, although Ara-GTP also had some activity (Table I). Furthermore, dATP was 25 times more effective than ATP in promoting apoptosis activation. A 2′-fluoro substitution of Ara-ATP slightly reduced its potency as compared with Ara-ATP, whereas the 2′-arafluoro substitution of CAFdATP increased its caspase activating capacity compared with 2CdATP. In other experiments, 8-Cl-AATP had 2-fold greater efficiency than ATP, whereas 2′,3′-ddATP was devoid of caspase activating ability. Fig. 8 shows the structural features of nucleotides that appear to be optimal for APAF-1 activation of the apoptosome pathway: 1) a purine base (adenine > guanine), possibly with an 8-chloro substitution (R3), but without 2-halogenic substitutions (R2); 2) a ribofuranoside sugar with either no 2′-hydroxy group (R1) or a 2′-hydroxy or 2′-fluoro group in the “up” position and a 3′-hydroxy group in the “down” position; and 3) three 5′-hydrolyzable phosphate groups. Only a few proteins have been reported to show a preference for dATP as compared with ATP. These include DNA polymerases α, β, and γ as well as DNA primase (35, 36). Interestingly, 2-fluoro-2′-deoxyadenosine 5′-triphosphate was able to completely substitute for dATP using DNA polymerases α and γ, whereas the 2-chloro and 2-bromo analogs substituted poorly (35).

Recent evidence suggests that the intracellular levels of purine nucleotides may play an important role in the modulation of apoptotic and necrotic cell death signals (37). Adenine nucleotide depletion has been shown to induce apoptosis, which was prevented by the antiapoptotic protein Bcl-2 (38). In our system, ADP was a good inhibitor of APAF-1-dependent caspase activation, with a KM of 133 μM. Thus, earlier reports showing that ATP was an inefficient activator of the APAF-1-mediated caspase pathway are difficult to interpret due to its progressive conversion into ADP. In addition, our findings showed that a nonhydrolyzable analog of ADP (AMPCP) was a good inhibitor, while a nonhydrolyzable analog of ATP (ADP-P) was not. Normal lymphocytes and CLL cells have been reported to have average cell volumes of 200 and 160 femtoliters.
and ADP contents of 1127 and 873 pmol/10^7 cells, respectively (39–41). These values yield an estimated ADP concentration of about 400 μM, 3-fold higher than the K_i of ADP for apoptosis activation. Taken together, the data appear to indicate that ADP may work as a physiological intracellular inhibitor of the cytochrome c and APAF-1-mediated caspase pathway in both normal lymphocytes and CLL cells.

The correlation between the clinical relevance of the nucleosides and the capacities of their corresponding 5-triphosphate derivatives to activate the apoptosis pathway underscores the relevance of these effects in the chemotherapy of indolent lymphoproliferative diseases. However, the capacity of nucleotide analogs to activate directly the apoptosis pathway does not fully explain their diverse cytotoxicities toward CLL cells, both in vitro and in vivo. It is well known that 2CdA is more toxic than F-Ara-A when tested in purified CLL cells (42), and the in vivo dosage of F-Ara-A is approximately 5 times higher than 2CdA (43). In contrast, our results showed that F-Ara-ATP was more effective than 2CdATP in activating caspases.

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