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SELECTIVE INDUCTION OF APOPTOSIS IN MYELOID LEUKEMIC CELL LINES BY MONOACETONE GLUCOSE-3 BUTYRATE

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SUMMARY: Butyric acid is a potent cell growth inhibitor and differentiation inducer. Our previous studies have shown that MAG=3but, a monosaccharide ester of butyric acid, used at 1 mM, induces apoptosis in the HL-60 cell line. We report here that this drug can also induce apoptosis in the U-937 leukemic cell lines whereas the myeloblastic KG1 and the NB4 promyelocytic leukemic cell lines were refractory to induction of apoptosis. In order to determine what can trigger cells to undergo apoptosis, cell cycle analysis, induction of differentiation and p53, c-myc and Bcl-2 expression was studied. Apoptosis was correlated to an arrest of cell growth in the G1 phase of the cell cycle and to an induction of differentiation through the monocytic pathway in HL-60 and U-937 cells. Time course studies demonstrated DNA fragmentation after few hours incubation with the drug, while morphological signs appeared later (days 2 or 3).

Northern blot analysis and flow cytometric studies have shown that cell death induced by MAG=3but was not

Abbreviations: BUONa, butyric sodium salt; MAG=3but, Monoacetone Glucose-3 butyrate; NBT, nitroblue tetrazolium; PMA, phorbol 12-myristate 13-acetate.

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associated to an overexpression of c-myc and p53. However, in the HL-60 cells, BCL-2 protein expression was decreased after MAG=3but treatment, corroborating the apoptosis observed.

In subsequent years, butyric acid has been shown to act as a potent differentiation inducer (1, 2) and cell proliferation inhibitor in several tumor cell lines (3-5). However, butyric acid as the sodium salt has been administrated, in vivo, in only two clinical studies: one in which a partial remission in a child with acute myelogenous leukemia was reported (6) and a second in which nine patients with acute leukemia were treated but no responses were observed (7). Lack of clinical efficacy was mainly attributed to a rapid metabolism of butyric acid (8).

Previous studies have shown the pharmaco-therapeutic properties of butyric esters derived from natural polyhydroxylated compounds (9-14). These stable butyrate derivatives could improve the rapid turnover and the therapeutic action of butyric salts. One of the butyric esters, MAG=3but for 3-0-butanoyl-1,2-O-isopropylidène-α-D-glucofuranose, has been chosen for its high water solubility (9), absence of toxicity in rodents (10) anti-tumoral action in vitro and in vivo (11-13) and longer plasma half-life (14).

Recent data have demonstrated that apoptosis may be involved in cell death induced by chemotherapeutic agents such as taxol (15), cisplatin (16, 17), cytarabine (18), topoisomerase I or II inhibitors such as camptothecin, etoposide and tenoposide (16, 19-21). Cells undergoing apoptosis are characterized by several morphological and biochemical events such as cellular shrinkage, chromatin condensation (22, 23) and DNA cleavage by a calcium-magnesium sensitive endonuclease in fragments multiples of 180-200 base pairs, producing a characteristic "ladder" on agarose gel electrophoresis (24). Apoptosis has now been reported to play an important role in embryogenesis (25), carcinogenesis (26), hormone-dependent atrophy of tissues (27) and regulation of the immune system (28) and cell survival promoted by hematopoietic colony-stimulating factors (29). Indeed, these factors such as erythropoietin (EPO) (30, 31), IL-3 (29, 32), granulocyte-macrophage colony stimulating factor (GM-CSF) (29) and granulocyte colony-stimulating factor (G-CSF) (29) have all been

shown to suppress apoptosis in cells that depend on them for survival.

Furthermore, several groups have shown that apoptotic cell death can be a c-MYC, BCL-2 and p53 dependent phenomenom. Indeed, it has been found that an overexpression of c-MYC induces apoptosis (33, 34), but it has also been reported that apoptosis can be detected without any overexpression of c-MYC (35) through a BCL-2 independent pathway (36). Other studies have demonstrated that p53 is required for DNA damage-induced cell death but not neccessarly for all forms of apoptosis (37, 38).

Our previous studies have shown that MAG=3but induces apoptosis in the HL-60 cell line (39). In the present paper, we studied the effect of MAG=3but on three other leukemic cell lines characterized by their different level of blockage in the hematopoietic lineage, KG1, U-937 and NB4.

We show that myeloid leukemic cells that differ in their competence to induction of differentiation by MAG=3but also differ in their sensitivity to induction of apoptosis. Furthermore, MAG=3but-induced apoptosis in HL-60 and U-937 cells is a phenomenom which is c-myc and p53 independent but Bcl-2 dependent.

METHODS

Cells

The HL-60 and the U-937 cell lines were provided by Dr T. Breitman. The NB4 cell line (40) was a gift from Dr M. Lanotte, INSERM U 301, Hôpital Saint-Louis. The KG1 cell line was pursached from the ATCC.
Cells were seeded at an initial concentration of $2x10^5$/ml in RPMI 1640 medium containing 15% (v/v) fetal calf serum (FCS), 100 IU/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine. Cells were maintained at 37°C in an atmosphere of 95% air, 5% CO_2. MAG=3but (ARTAC, France) was added at the beginning of the culture. Stock solution was stored at -20°C.

Cell viability and differentiation assessment

Cells were counted on a Malassez hemocytometer and were assessed for viability by trypan blue exclusion test. To quantify differentiation, the nitroblue tetrazolium (NBT) reduction test was performed using standard methodology with phorbol myristate acetate (PMA), and the percentage of cells containing intracellular

black formazan deposits determined as previously described (41). Cytospin slide preparations of untreated and MAG=3but-treated cells were stained with May-Grünwald-Giemsa stain (Laboratoire central d'hématologie, Hôpital Saint-Louis, France) for evaluation of nuclear morphology and cellular differentiation. Counts were performed under light microscopy on a minimum of 200 cells.

DNA extraction and electrophoresis

At the end of the culture, cells ($10x10^6$/ml) were washed twice in PBS at 4°C. The pellets were lysed with 2 ml of lysis buffer (50 mM Tris-HCl pH 8/ 20 mM EDTA pH 8/ 2% SDS), incubated at 37°C for 18 hours and chilled on ice for 10 minutes. To precipitate proteins, 0.8 ml of a satured NaCl solution was added and tubes were left on ice for 5 minutes. Precipitates were centrifuged at 3000 rpm for 1 hour to separate intact from fragmented chromatin. RNase A was then added to supernatants at a final concentration of 20 µg/ml and incubation at 37°C was continued for 15 minutes. DNA was then precipitated by adding two volumes of 100% ethanol. Prior to electrophoresis, loading buffer (containing 0.25% bromophenol blue, 0.25% xylene cyanol, 15% ficoll) was added to each sample (40 µg DNA) at a 1:5 (v/v) ratio. DNA was electrophoresed on a 1.25% agarose gel overnight. Electrophoresis was carried out in 2 mM EDTA pH 8/ 89 mM Tris/ 89 mM boric acid (TBE). A Hind III- digest of λ-DNA and Hae III- digest of Φ-DNA was used as molecular size standard. After the completion of electrophoresis, DNA in the gel was visualized by soaking the gel for 1 hour in TBE buffer containing 1 µg/ml ethidium bromide and destained briefly in distilled water. The stained gel was viewed by transillumination with UV light (302 nm) and photographed.

RNA extraction and Northern blot analysis

Cells were washed twice in PBS and total cellular RNA was purified by extraction with guanidine isothiocyanate and cesium chloride gradient centrifugation (42). Purified total RNA (30 µg) were analysed by electrophoretic separation in 1% agarose-formaldehyde gel, run overnight in MOPS buffer, and transferred to nitrocellulose filters (Hybond^{TM} - C Extra, Amersham). Filters were hybridized to p53 (1.9 kb XbaI/XbaI fragment) (a gift from Dr M. Oren and Dr D. Givol, The Weizmann Institute of Science, Rehovot, Israel), bcl-2 (1.5 kb HindIII/Eco RI fragment) (a gift from Dr M. L Cleary, Stanford University School of Medecine, California, USA) c-myc (1.5 kb SacI/SacI fragment) (a gift from Dr D. Stéhelin, Institut Pasteur, Lille) probes, labelled with [^{32}P]dCTP by the random priming method (43) (Kit Multiprime DNA Labelling System, Amersham). Prehybridation for 3 hours and hybridation for 20 hours were carried out at 42°C in 50% (v/v) formamide, 10%

dextran sulfate, 5X SSC, 0.1% (w/v) SDS, 1X Denhardt's solution, and 100 μg/ml denatured salmon sperm DNA. Hybridized blots were washed four times at room temperature in 2X SSC containing 0.2% (w/v) SDS, and then four times for 20 min in 0.2X SSC and 0.2% SDS at 60 °C and exposed to autoradiographic identification. The filters were reprobed with a fragment of the GAPDH gene (44) to quantitate the amount of RNA in each lane.

Antibodies

The anti-BCL-2 antibody BCL-2/124, an IgG1 isotype, directed against the peptide 41-54 of the BCL-2 protein was a gift from Professor D. Y. Mason (Oxford, United Kingdom) and was used undiluted (45).
The anti-p53 antibody, an IgG2a isotype directed against the N-terminal region of the protein, was generously provided by Professor T. Soussi, INSERM U 301, Hôpital Saint-Louis, Paris. It was used at a 1 μg/ml concentration.
The anti-c-MYC antibody (Cambridge Research Biochemicals), an IgG2b isotype directed against the Ala-Pro-Ser-Glu-Asp-Ile-Trp-Lys-Lys-Phe-Glu-Leu-Cys myc sequence was used at a 1:400 dilution.
IgG1, IgG2a, IgG2b irrelevant control antibodies (used at a 1:40 dilution) and FITC-goat anti-mouse antibody (used at a 1:200 dilution) were purchased from Coulter, France.

Flow cytometric assay

Cells ($1x10^6$) were washed twice in PBS fixed and permeabilized by addition of 1 ml of a 1% paraformaldehyde solution containing 20 μg/ml lysolecithin and incubated for 2 minutes at room temperature. The cells were then centrifuged for 5 minutes at 1500 r.p.m. at 4°C and kept for 10 minutes on ice in 2 ml of ice-cold (-20°C) absolute methanol. After another centrifugation, cells were resuspended in 1 ml of 1% Triton X-100 and placed on ice for 5 minutes. Cells were then resuspended in a PBS solution containing 0.5% BSA and incubated for 20 minutes at 4°C with primary antibodies. Cells were then washed and incubated with goat anti-mouse-FITC antibody at the same conditions as before. After another wash, cells were stained by a solution of propidium iodide (50 μg/ml) and RNAse A (50 μg/ml) for cell cycle analysis and apoptotic cells detection. Flow cytometric studies were performed on an EPICS profile II flow cytometer (Coulter) and percentage of cells in each phase of the cell cycle was analysed by the Multicycle software (Coulter). Relative BCL-2, p53, and C-MYC levels was also determined by calculation of a corrected mean fluorescence intensity [difference in fluorescence intensity of Immunoglobulin control and respective antibody].

RESULTS

Cell growth inhibition in MAG=3but-treated cells is associated with a growth arrest in the G1-phase of the cell cycle

In standard liquid suspension culture conditions, the HL-60, U-937, NB4 and KG1 human myeloid leukemic cell lines presented a specific exponential cell growth. HL-60 and U-937 cells reached a doubling time of 1.5 to 2 days whereas NB4 and KG1 cells were less proliferative with a doubling time of 3 to 4 days. The exponential growth of HL-60, U-937, NB4 and KG1 cells was inhibited by the presence of 1 mM MAG=3but concentration and was significantly reduced in HL-60 and U-937 cell lines (Fig. 1a, b, c, d). This inhibition appeared on the second day of incubation and reached the highest level on the 6th day for HL-60 with a percentage of 75% inhibition and on the 5th day for U-937 with 60% inhibition while it did not exceed 30% in NB4 or KG1 cells. In the presence of lower MAG=3but concentrations (0.1 to 0.75 mM) no effect on cell growth

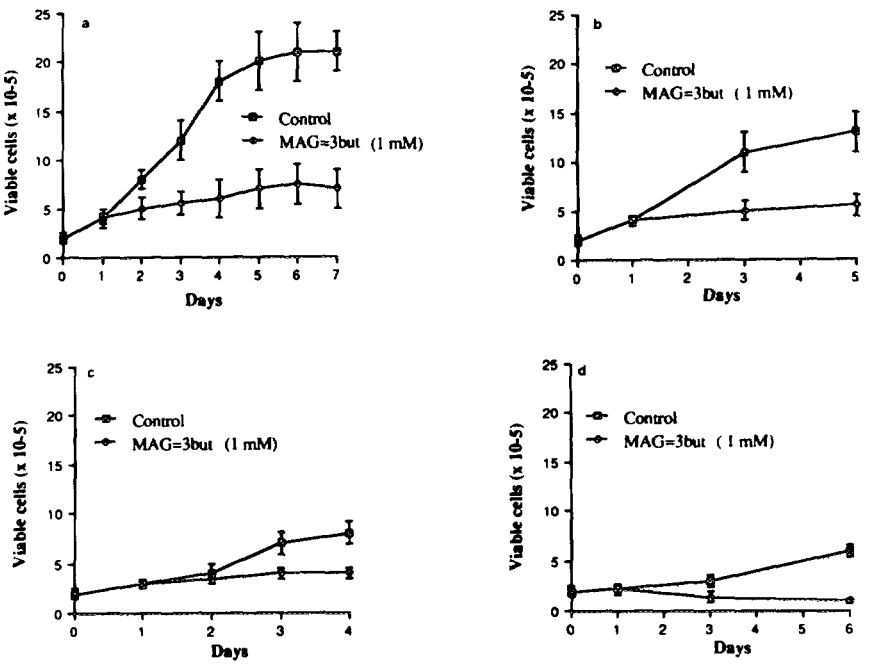

Fig. 1. Time course studies of HL-60, U-937, NB4 and KG1 cell viability. Cells were incubated in medium alone (-o-), or with 1 mM MAG=3but (-•-). Results are expressed as the mean (± s.e.m) of three experiments.

was observed while incubation with higher concentrations (≥ 3 mM) resulted in a rapid loss of cell viability (data not shown). Cell cycle analysis of MAG=3but-treated cells revealed a progressive decrease of the percentage of cells in the S through G1 phase. After 3 days of culture with MAG=3but, the percentage of cells in the S phase of cell cycle was 10% for HL-60 and U-937 cells and 17% for NB4 cells (Fig. 2b, d, f), corresponding to a 4-fold decrease compared to

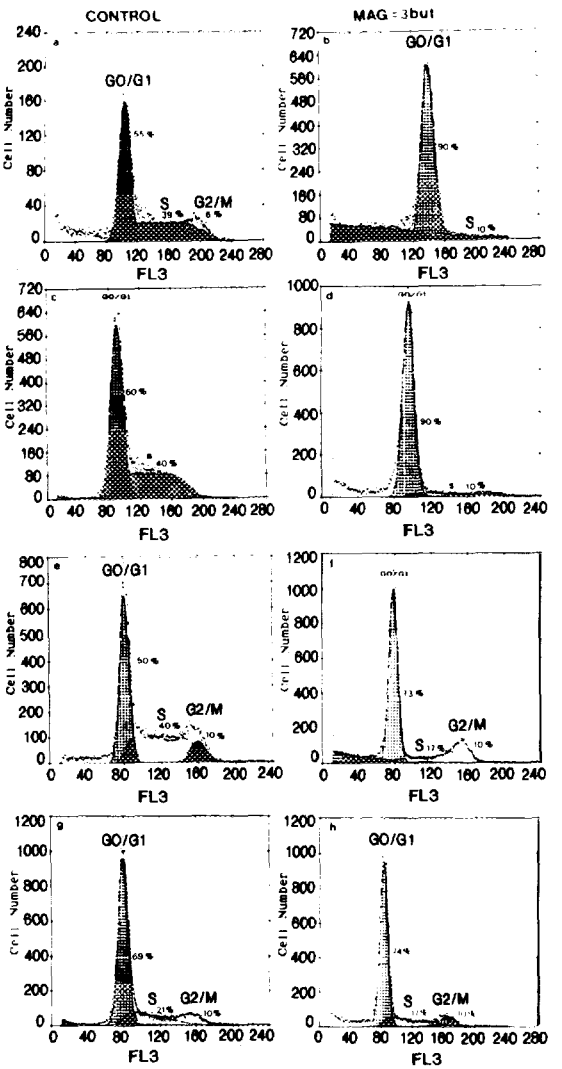

Fig. 2. Flow cytometric analysis of cell cycle distribution in control HL-60 (a), U-937 (c), NB4 (e) and KG1 (g) or treated with 1 mM MAG=3but for 3 days. HL-60 MAG=3but (b), U-937 MAG=3but (d), NB4 MAG=3but (f), KG1 MAG=3but (h).

controls (Fig. 2a, c, e). However, in KG1 cells we did not observe a significant decrease of the S phase with a percentage of 17% in treated cells and 21% in controls (Fig 2g, h). At this time, after MAG=3but treatment, the percentage of cells in the G1 phase was 90% for HL-60 and U-937, 73% for NB4 cells and 74% for KG1 cells vs respectively 55% for HL-60 cells, 60% in U-937 and NB4 cells and 69% in KG1 cells. Thus MAG=3but-induced inhibition of cell growth was correlated to an arrest in the G1 phase of the cell cycle; HL-60 and U-937 cells being more sensitive than NB4 and KG1 cells.

MAG=3but induces apoptosis in myeloid leukemic cell lines

HL-60 and U-937 cells grown under the influence of 1 mM MAG=3but harboured features of cells with highly condensed chromatin and fragmented nuclei (Fig 3a). These features were characteristic of apoptotic cells as already described extensively (23, 24). MAG=3but treatment did not produce any morphological features of apoptosis in the NB4 or KG1 cells, at any time of incubation. Because the process of apoptosis is associated with DNA degradation in many models, agarose gel electrophoresis of DNA extracted from treated and untreated cells was performed. Exposure of HL-60 and U-937 cells to 1 mM MAG=3but for only 4 hours resulted in the characteristic ladder of DNA fragments of approximatively 180 base pair multiples of DNA (Fig. 3b, lanes 2, 4). Such a ladder of DNA fragments was not observed when DNA was extracted from MAG=3but-treated NB4 or KG1 cells (Fig. 3b, lane 6, 8) even for periods up to 6 days.

Kinetics of MAG=3but-induced apoptosis: light microscopy and flow cytometric analysis

The induction of apoptosis in HL-60 and U-937 cells following exposure to MAG=3but was time-dependent. By light microscopy, apoptotic cells appeared from day 2 of incubation for U-937 cells and day 3 for HL60 cells. A maximum of 15 ± 2% for U-937 and 25 ± 5% for HL-60 was reached at day 6 (Fig. 4a, b). Kinetic studies of flow cytometric cell cycle analysis of propidium iodide-stained cells treated with 1 mM MAG=3but revealed the appearance of a distinct cell cycle region below the GO/G1 region. This extra "sub-G1" peak displaying reduced fluorescence of the DNA is due to a reduction of

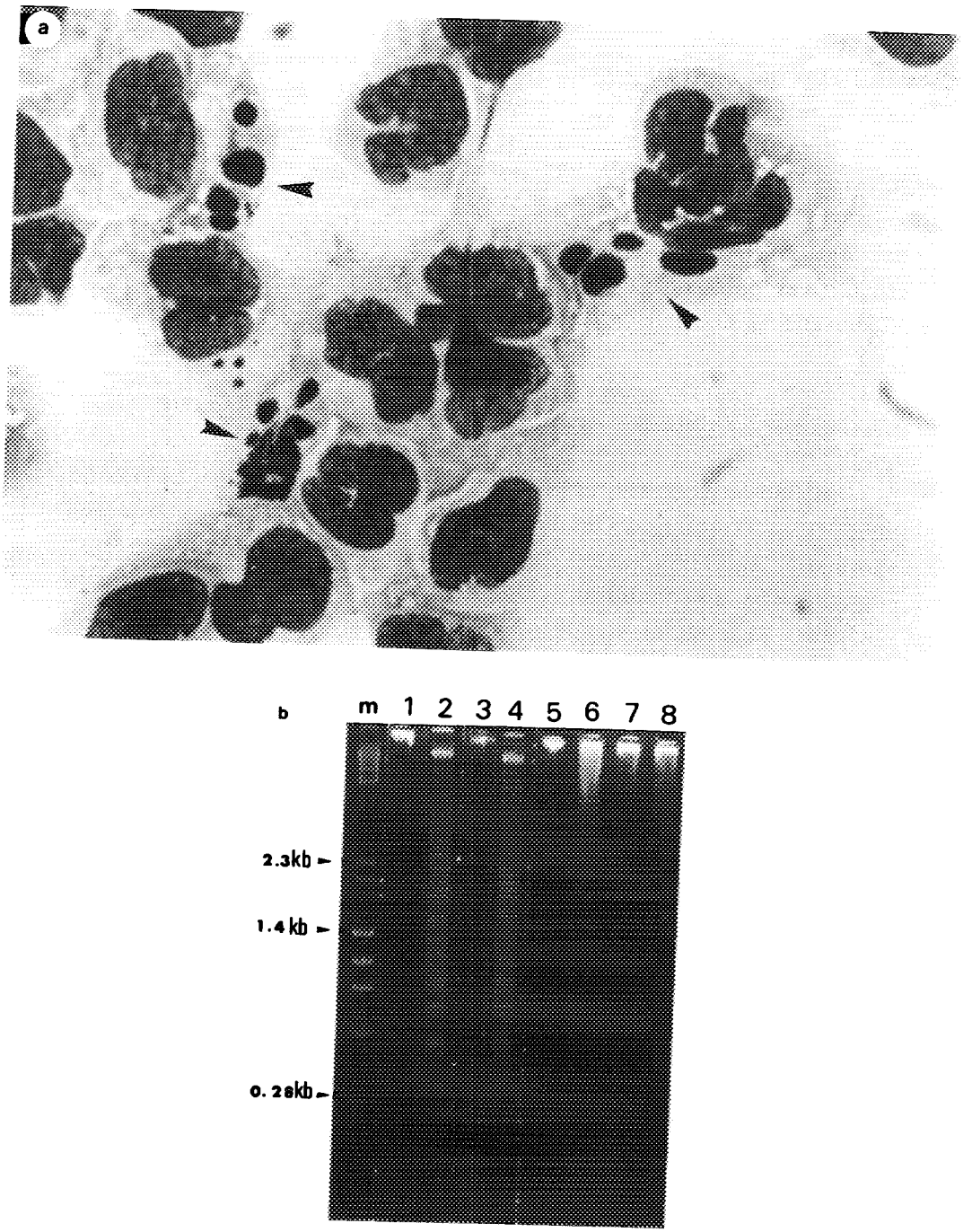

Fig. 3. a, Morphological appearance of HL-60 cells treated for 6 days with 1 mM MAG=3but. Note the morphology of apoptotic cells with highly condensed chromatin and apoptotic bodies (arrows). b, Agarose gel electrophoresis of HL-60 (1,2), U-937 (3,4), NB4 (5,6) and KG1 (7,8) DNA after 4 hours of culture.
m: DNA size markers.
(1, 3, 5, 7): control cells.
(2, 4, 6, 8): cells treated with 1 mM MAG=3but.

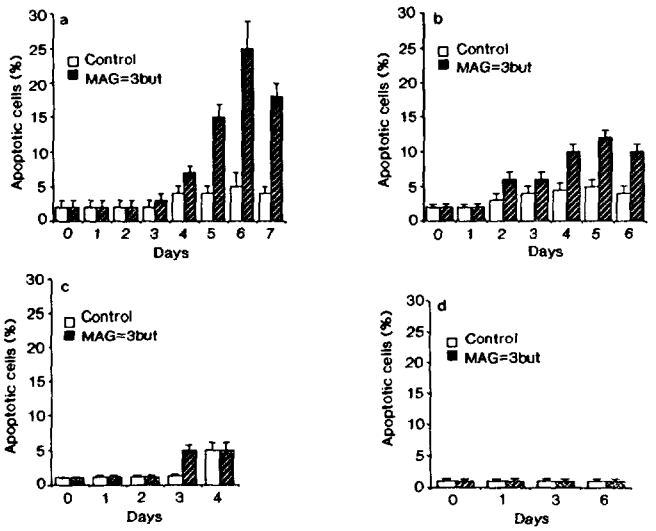

Fig. 4. Percentage of apoptotic cells in HL-60 (a), U-937 (b), NB4 (c) and KG1 (d). Apoptosis was assessed morphologically for up to 7 days in cells incubated in the presence or absence of 1 mM MAG=3but. The values represent the average of three separate determinations (± s.e.m).

cell volume and nuclear condensation characteristics of apoptotic cells (46). At day 6, the percentage of apoptotic cells was 38.8% for HL-60 and 16% for U-937 (Fig. 5b, d) whereas it was respectively 8% and 6% in controls (Fig. 5a, c). Apoptotic cells were also selected by the computer from a cytogram of the light scatter against propidium iodide fluorescence (Fig. 5B). The apoptotic cells have a slight decrease of the foward light scatter compared to viable or necrotic cells. After 3 days of culture in presence of MAG=3but, HL-60 cells exhibited 12% of apoptotic cells, 22% of necrotic cells and 61% of viable cells vs respectively 1%, 7% and 91% in controls. It is important to note that no signs of apoptosis, assessed either by cytospin slide preparations (Fig. 4 c, d) or by flow cytometry was detectable in NB4 and KG1 cells at different time intervals of culture.

Expression of bcl-2, c-myc and p53 mRNA and proteins in the myeloid leukemic cells after MAG=3but treatment

c-MYC protein was present in 60-80% of the cell population in all cell lines (Fig. 6) with a mean fluorescence intensity (FI ≥ 7

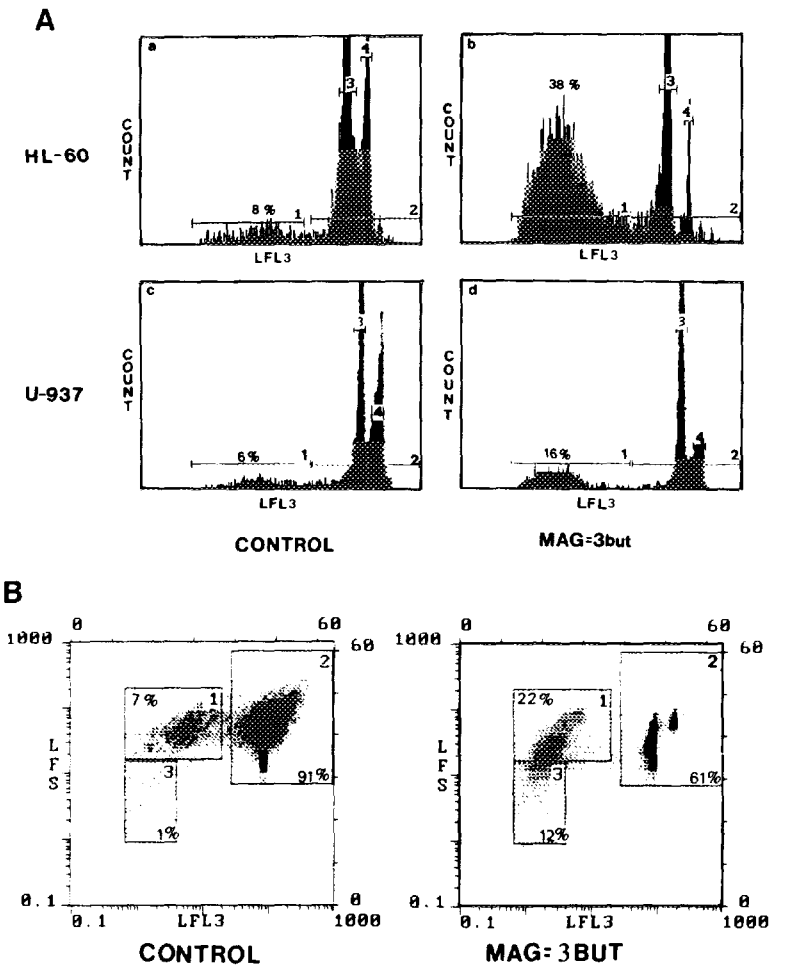

Fig. 5. A, Flow cytometric studies of propidium iodide-stained HL-60 and U-937 control cells (a, b) or treated for 6 days with 1 mM MAG=3but (c, d). Apoptotic cells can be recognized by their disminished stainability with propidium iodide and appear as a "sub-G1" peak. B, light scattering properties of control or MAG=3but-treated HL-60 cells for 3 days.
1) Necrotic cells 2) Viable cells 3) Apoptotic cells.

units) (data not shown). BCL-2 and p53 proteins were differently expressed. BCL-2 was not detected in U-937 and KG1 cells, was present in only 20% of HL-60 cells, however 75% of NB4 cells harboured the protein (Fig. 6). The corresponding p53, c-myc, Bcl-2 genes were also differently expressed in these cell lines (Fig. 7), closely correlated to the presence of the proteins. p53 gene was detected only in the NB4 cell line. A lack of expression of p53 is observed in the HL-60 cell line due to structural alterations of the

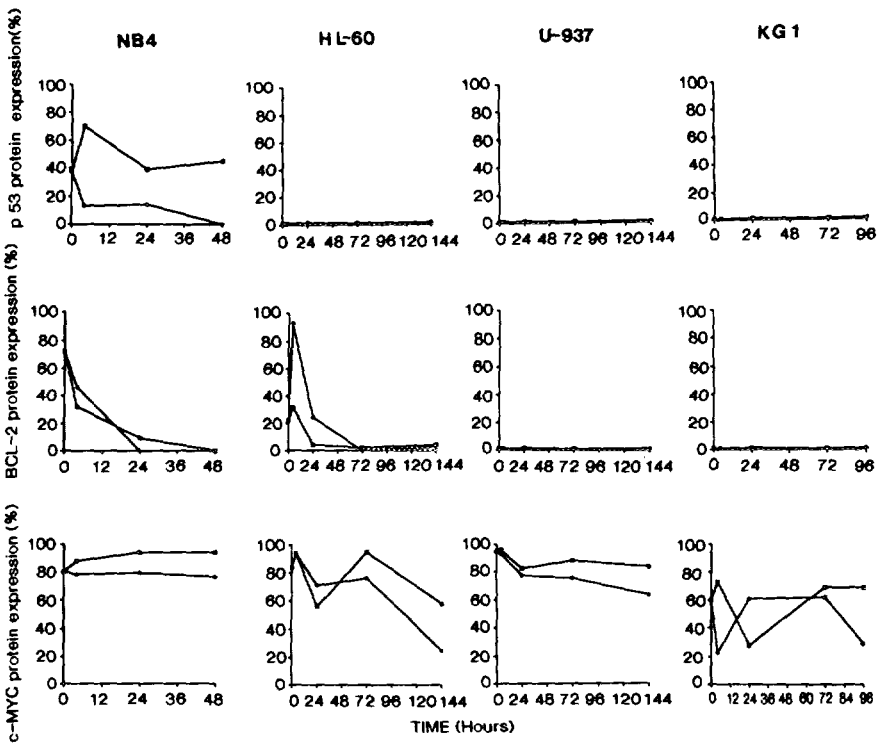

Fig. 6. Time course studies of cellular expression of c-MYC, BCL-2 and p53 evaluated by flow cytometry in HL-60, U-937, NB4 and KG1 control cells or treated with 1 mM MAG=3but for time up to 7 days. One representative experiment out of three. (▬) control cells (◆) MAG=3but-treated cells.

p53 gene region (47). Incubation with MAG=3but resulted in the downregulation of c-myc gene (Fig. 7) and reduced protein expression in HL-60, U-937, NB4 and KG1 cells. BCL-2 is also regulated in HL-60 and NB4 cells by MAG=3but, reaching undetectable levels in the HL-60 cell lines after 72 hours incubation with MAG=3but (Fig. 7), with a significant reduction of the mean fluorescence intensity (FI < 2 units vs 5 units in controls).

Differentiation of myeloid leukemic cell lines by MAG=3but

HL-60 and U-937 cells grown under the influence of 1 mM MAG=3but exhibited morphological and functional (NBT test) changes characteristic of more mature cells in the monocytic differentiation. No signs of differentiation were seen in presence of

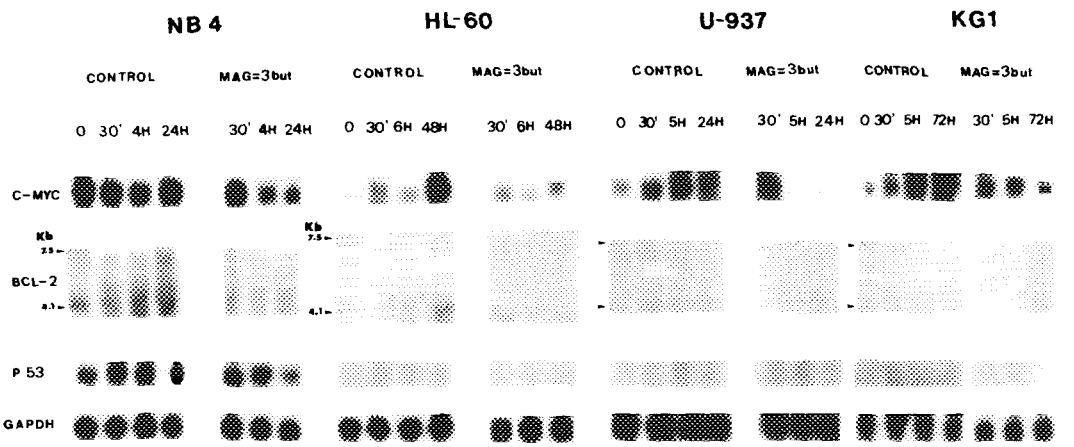

Fig. 7. Northern blot analysis of bcl-2, c-myc and p53 gene expression in HL-60, U-937, NB4 and KG1 cells after exposure for different time intervals with 1 mM MAG=3but. Total RNA (30 µg) purified from each cell culture was probed successively for p53, c-myc, bcl-2 and GAPDH.

the drug in NB4 and KG1 cells. The maximum of differentiated cells, assessed by the NBT test, was obtained at day 6 for HL-60 and U-937 cells and day 4 for NB4 and KG1 cells with respectively a percentage of 60 ± 5%, 50 ± 2%, 7 ± 2% and 1 ± 0.5% (Table 1).

DISCUSSION

The results presented herein show that MAG=3but, a monosaccharide ester of BuONa, used at 1 mM concentration inhibits KG1, HL-60, U-937 and NB4 myeloid cell growth. Whereas arrest in G1 phase of cell cycle was observed in all cell lines, induction of apoptosis was noted in only HL-60 and U-937 cells. Apoptosis, assessed morphologically by cytospin slide preparations, flow cytometry and biochemically by DNA fragmentation was shown to be dose and time dependent. Indeed, incubation of HL-60 and U-937 cells with higher concentrations of MAG=3but (≥ 3 mM) resulted in a suppression of DNA fragmentation while increased cytotoxicity was observed. Time course studies demonstrated that DNA fragmentation was detected 4 hours after incubation with the drug, while morphological signs appeared from day 1 by flow cytometry and day 2 or 3 by cytospin slide preparation. Maximal percentage of cells with apoptotic features never exceeded 30% at any given time due to a rapid elimination of these cells.

Table 1: Differentiation assay by NBT test after 6 days for HL-60 and U-937 cells and 4 days of culture for NB4 and KG1 cells in presence or absence of 1 mM MAG=3but

		Differentiated cells (%)	Viable cells (%)
HL-60	Control	7 ± 2%	80 ± 1%
	MAG=3but (1 mM)	60 ± 5%	60 ± 2%
U-937	Control	5 ± 2%	80 ± 2%
	MAG=3but (1 mM)	50 ± 2%	50 ± 2%
NB4	Control	7 ± 2%	75 ± 2%
	MAG=3but (1 mM)	7 ± 2%	60 ± 5%
KG1	Control	1 ± 0.5%	87 ± 1%
	MAG=3but (1mM)	1 ± 0.5%	70 ± 2%

A variety of anti-cancer drugs including taxol (15), 2-Chloro-2'-deoxyadenosine and 9-β-D-Arabinosyl-2-fluoroadenine (48) have been shown to induce apoptosis at appropiate concentration in human myeloid leukemia cells and chronic lymphocytic leukemia. The susceptibility of leukemic and other cancer cells to induction of apoptotic cell death during chemotherapy is a major and novel determinant in the therapy of cancers.

The data presented shows that one drug may not induce apoptosis in all myeloid leukemic clones, stressing the necessity to determine which parameters are involved in the susceptibility of malignant cells to undergo apoptosis.

It is known in various cell systems that induction of apoptosis can be triggered by regulated Bcl-2, c-myc or p53 expression (49-58). The induction of apoptosis in hematopoietic cells can be inhibited by transfection of regulated Bcl-2 gene (26, 49-51) or by addition of cytokines (29, 52, 53). In addition, the endogenous level of Bcl-2 expression is highly suggestive of resistance to undergo apoptosis (54, 55). c-myc expression, under conditions of growth restriction, facilitates apoptosis in 32D myeloid cells (56) and Rat-1 fibroblasts (57) as well as p53 deregulated expression (52, 58).

In this study, we noted that endogenous levels and/or regulation of c-myc or p53 expression in myeloid leukemic cells did not change their susceptibility to undergo apoptosis in the presence of MAG=3but. c-myc gene expression was rapidly down regulated by MAG=3but irrespective of whether or not apoptosis was induced.

In fact, c-myc was more certainly related to be an early gene modulation of growth arrest, as already reported (59) and p53 downregulation to an arrest of the cells in the G1 phase of the cell cycle (60). On the contrary, the endogenous level of Bcl-2 in the leukemic cell lines studied, appeared as a good indication of a cell's susceptibility to undergo apoptosis. Compared to NB4 cells which expressed high levels of the Bcl-2 protein, U-937 and HL-60 cells either did not or weakly expressed the protein. So, high levels of BCL-2 protein could explain the resistance of NB4 cells to undergo apoptosis with MAG=3but. Presence of Bcl-2 is linked to cell survival (49-51), and high levels of Bcl-2, genetically engineered, blocks apoptosis and endogenous cleavage of DNA. Interestingly, levels of regulated Bcl-2 but not c-myc was linked to apoptosis in mouse myeloid leukemic cells (61).

Another explanation to understand the difference in leukemic cell susceptibility to undergo apoptosis may be their proliferative characteristics. Decision to undergo apoptosis is a cell cycle dependent event (62).We have noted, that in our culture conditions, the NB4 cell line and KG1 cell clone had a very low doubling time adding to their resistance to undergo apoptosis.

It is striking that apoptosis was observed in only the two cell lines which were triggered to terminal differented cells in the presence of MAG=3but. Bone marrow aspirates show that Bcl-2 expression is down regulated as differentiation occurs: myeloid precursors cells are positive whereas neutrophils and reticulocytes are negative (54). Mature neutrophils and monocytes are known to age within hours and spontaneously undergo apoptosis (63). Induction of leukemic cell differentiation towards neutrophils or monocytes, such as observed in this study by MAG=3but, may also trigger the apoptotic process, as a consequence of the differentiation process.

Thus, the resultant of different parameters such as the anti-apoptotic signals like the level of BCL-2 protein, cell cycle characteristics and differentiation induction in myeloid leukemic cells may allow the as yet undefined molecular signals to induce apoptosis by MAG=3but. Further studies to explore these molecular signals for MAG=3but-induced apoptosis may reveal potential targets of this drug.

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