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Mimosine induces apoptosis in the HL60 human tumor cell line

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Mimosine, a plant amino acid not found in proteins, has been widely used as a synchronizing agent, blocking the progression of cell cycle on the G1/S phase border. The mechanism by which this block is achieved is still unclear. We report that in HL60 cells the synchronization is related to an increase in apoptosis. Another human tumor cell line, K562, is insensitive to both phenomena thereby demonstrating that apoptosis observed in HL60 is line-specific. We hypothesize that the mimosine-induced apoptosis and alteration of the cell cycle is due to the inhibition of hypusine generation.

Keywords: Apoptosis; cell cycle synchronization; HL60; hypusine; K562; mimosine.

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Introduction

Cell cycle progression appears to be regulated by sequential activation and subsequent inactivation of a growing family of serine/threonine protein kinases, the cyclin-dependent protein kinases (cdk).[1] Many physiological substrates of cdks have been identified, including histone H1, lamins, nucleolin, and microtubules. During the cell cycle, the cyclins appear and disappear at specific regulatory points. In mammalian cells, progression through G1 seems to be controlled by the C-, D-, and E-type cyclins, whereas progression through the rest of the cell cycle seems to be controlled by the A- and B-type cyclins. Another important factor which controls cell cycle progression is the intracellular level of iron, which is required for DNA synthesis.[2,3] The main cellular requirement for iron occurs late in the G1 and S phases, and is due to the increased activity of the ribonucleotide reductase.[4] Therefore, by modifying the expression of cyclins or the intracellular iron levels, it is possible to modify cell cycle progression. Mimosine {α-amino-β-(3-hydroxy-4-oxo-1,4-dihydropyridine-1-yl)propanoic acid} is a naturally occurring amino acid found in the seeds and foliage of the legume genera *Mimosa* and *Leucaena*. The compound was shown to cause inhibition of hair growth and loss of hair in mice,[5] to act as a defleecing agent in sheep,[6,7] and to induce fetal resorption in rats.[8] Mimosine was also shown to block the cell cycle earlier than aphidicolin,[9] an inhibitor of DNA-polymerase activity.

Furthermore, mimosine inhibits various mammalian enzymes *in vitro*, such as tyrosinase,[10] dopamine β-hydroxylase,[10] deoxyhypusyl hydroxylase (DOHH)[11] and, H1 kinase.[12]

Recent studies[13] demonstrated that cyclin D levels are decreased after treatment with mimosine in MDA-MB-453 human breast cancer cells and that this agent also inhibits cyclin-E-associated kinase activity.[14] Moreover, mimosine is able to chelate transition metal ions such as Fe^{2+} and binds to a 50-kDa protein identified as serine hydroxymethyltransferase (SHMT), an enzyme involved in the penultimate step of thymidine biosynthesis.[15] It is not yet clear if the mimosine-induced cell cycle block is due to the alteration of one or more of these metabolic pathways or if an upstream event directly modified by mimosine exists. One of the most investigated pathways altered by mimosine is the activity of DOHH. This enzyme allows the formation of the amino acid hypusine in the eukaryotic initiation factor 5A (eIF-5A).[16] Hypusine is present in eIF-5A only, is essential for its activity

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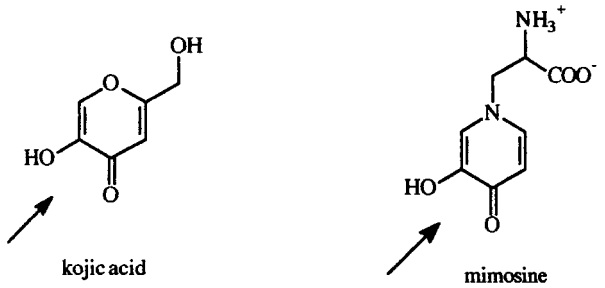
Figure 1. Chemical structures of mimosine and kojic acid. Arrows indicate metal-chelating domains.



in vitro and has been well preserved throughout the evolution of living organisms. There is strong evidence that hypusine and eIF-5A are vital for eukaryotic cell proliferation; in fact, inactivation of both of the eIF-5A genes is lethal in yeast.[17] Evidence was provided that the transition from the G1 to the S phase of the eukaryotic cell cycle is a hypusine-sensitive event and it was proposed that eIF-5A is required to commit cells to initiate DNA synthesis.[18] Additionally, inhibitors of either deoxyhypusine synthase or deoxyhypusine hydroxylase exert strong anti-proliferative effects in mammalian cells, including many human cancer cell lines.[15] More recently, the DOHH inhibitors (mimosine and deferiprone, an iron chelating agent) were shown to have antiretroviral effects and to promote apoptosis of the retrovirus-infected human T lymphocytes.[19] The reported induction of apoptosis in infected T lymphocytes prompted us to study the effects of mimosine on the progression of the cell cycle in two human cancer cell lines, HL60 and K562. Mimosine is a well-known DOHH inhibitor[17] and its molecule contains a metal-chelating domain[20] (Figure 1, arrow) that seems to play a role in its binding to the enzyme. On the basis of the known anti-DOHH activity of L-mimosine we hypothesized that the ensuing reduced levels of hypusine and of eIF-5A would result in apoptosis.[21,22] In order to distinguish the effect of mimosine as DOHH inhibitor from its effect as an iron chelator[20] we used kojic acid, which has a metal-chelating domain[23] identical to that of mimosine but is devoid of DOHH inhibitory activity.[11] The K562 cell line is an "apoptosis-resistant" tumor cell line due to its *bcr-abl* genetic rearrangement that allows most cells to escape apoptosis.[24] No data regarding the effects of mimosine on K562 cells are available in the literature but assuming that this line would remain unaffected by any mimosine-induced apoptosis we identified it as a negative control for the study of this compound.

Flow cytometry, light and electron microscopy were utilized to quantify and characterize apoptotic cells.

Materials and methods

Chemicals

L-mimosine was purchased from Aldrich. D,L-mimosine was prepared by racemization of L-mimosine according to the literature.[25] Kojic acid (2-hydroxymethyl-5-hydroxy-γ-pyrone) and propidium iodide were purchased from Sigma.

Cell cultures

HL60 human promyelocytic leukemia cells and K562 human chronic myeloid leukemia cells were routinely cultured in RPMI 1640 medium buffered with sodium bicarbonate and supplemented with 10% fetal calf serum, 1000 U/1000 μg/ml penicillin/streptomycin, 100 mM pyruvic acid and 200 mM glutamine. All reagents were purchased from Seromed (Munich, Germany). Standard culture conditions were used.

Cell treatment

Both cell lines (1×10^6 cells/ml) were incubated for 18 h with different concentrations of mimosine (40–400 μM) dissolved in RPMI 1640 in 25 ml culture flasks at 37°C in an atmosphere of 5% CO_2 saturated with water. The duration of the treatment was optimized in the 12 to 72 h range by preliminary experiments at the highest mimosine concentration (400 μM). In various experiments 400 μM kojic acid was added to both cell lines with or without mimosine (200 or 400 μM). Furthermore, dose-response experiments were performed using either L- (all experiments) or D,L-mimosine to check the stereospecificity of the interaction.

The cell cycle and apoptosis-related DNA degradation

Cell cycle analysis was performed using propidium iodide (PI) staining of DNA.[26] Briefly, 0.5×10^6 cells were fixed in 70% cold ethanol for 30 min at 4 °C, washed twice with phosphate buffered saline (PBS, pH 7.4) and centrifuged at 2000 rpm for 10 min. Next the cells were incubated at 37°C in citrate buffer (0.05 M Na_2HPO_4, 25 mM sodium citrate, 0.1% Triton X-100, pH 7.8), stained with 100 mg/ml PI (Sigma) for 30 min (for dye stabilization) in the presence of RN-ase (1 mg/ml, Sigma) and finally examined with a cytometer. Apoptosis was confirmed by the appearance of a sub-diploid peak in the cell cycle diagram, that is indicative of DNA fragmentation.[27]

Flow cytometry

All the experiments were performed using a FACScan cytometer (Becton-Dickinson) equipped with 15 mW air-cooled argon ion laser operating at 488 nM. PI red fluorescence was measured through a long pass 620-nM filter. Red fluorescence was amplified in both a linear and a log mode in order to identify cell cycle phases and the apoptotic sub-diploid peak, respectively. A minimum of 10,000 events per sample were collected at a low flow rate setting (12 ml/min) to improve the coefficient of variation on the DNA histograms. DNA data analysis was performed with Cell-fit software version 2.1 (Becton-Dickinson).

Light and electron microscopy

HL60 and K562 control, mimosine- and kojic acid-treated cells were sedimented at 1800 rpm for 10 min.

Cell pellets were fixed immediately with 2.5% glutaraldehyde in 0.1 PBS, post-fixed with OsO_4 1% in the same buffer, dehydrated with alcohol and embedded in araldite.[26] Semi-thin sections, stained with 1% toluidine blue in water solution at 40°C, were observed with a Vanox light microscope (Olympus).

Thin sections, collected on nickel grids and conventionally stained with uranyl acetate and lead citrate, were observed with a Philips CM10 electron microscope, at 80 KV.

Statistical analysis

Statistical evaluation of the results was performed by using the Student's t test and the χ^2 test. Probability values equal to or lesser than 0.05 were considered significant.

Results

Flow cytometry

Exposure of HL60 cells to 400 μM mimosine alone for 18 h blocked the cell cycle on the G1/S border (Figure 2), although residual S and G2/M phases were still present (Figure 3). Together with the well-described synchronizing effect, mimosine also induced massive apoptosis in HL60 cells, as shown by the sub-diploid peak present in the cell cycle histogram (Figure 2). At the same time, the level of necrosis present in the treated samples was qualitatively and quantitatively comparable to that observed in the control experiments. Kojic acid (400 μM, 18 h) did not induce apoptosis or synchronization (Figure 2), but rather only a small, statistically not significant, decrease in the cells in the G2/M phase (Figure 3). Experiments were also performed (400 μM kojic acid plus 200 or 400 μM mimosine) in order to observe a kojic acid-induced potentiation of action with the mimosine effects. In these experiments, the effect of mimosine was unaltered (data not shown). This synchronising/apoptotic effect was dose-dependent (Figure 4), reaching a maximum at 200–400 μM.

The slightly enhanced apoptotic effects of L- vs. D,L-mimosine and the inactivity of kojic acid can be explained on the basis of the known stereochemical requirements of the interaction of mimosine with DOHH (*vide infra*). Mimosine behaved as a putative antagonist of DOHH whereas kojic acid was inactive (Figure 4) as we expected on the basis of its lack of inhibitory activity towards DOHH.

Figure 2. Fluorescence histogram representing the HL60 cell cycle. The apoptotic subpopulation is identified by the presence of a sub-diploid peak. *x* axis = FL3 propidium iodide (PI) fluorescence; *y* axis = number cells/channel.

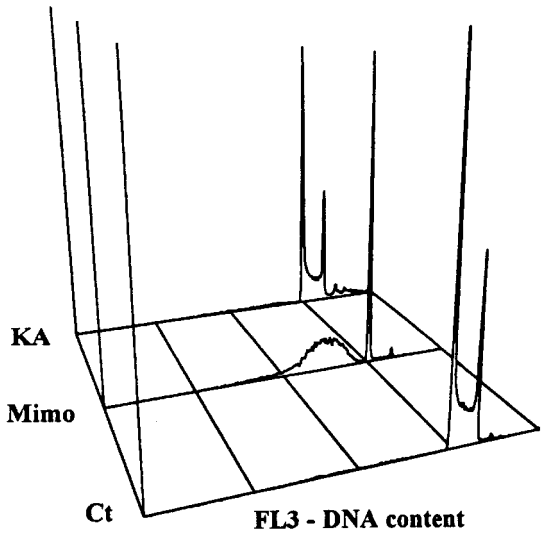


Figure 3. Variation in cell cycle phases induced by mimosine (400 μM) and kojic acid (400 μM) in HL60 cells (n = 6).

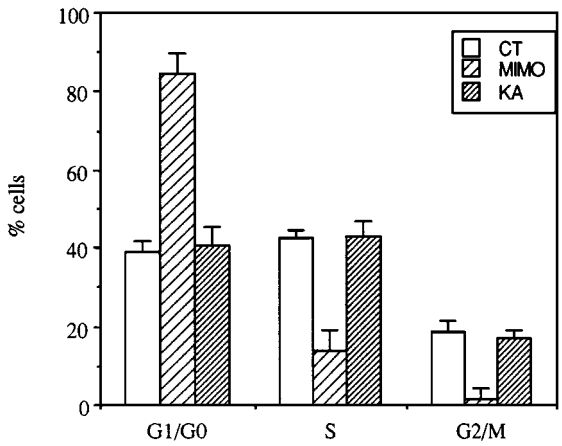


Figure 4. Dose-dependent induction of apoptosis by L- and D,L-mimosine in HL60 ($n = 3$).

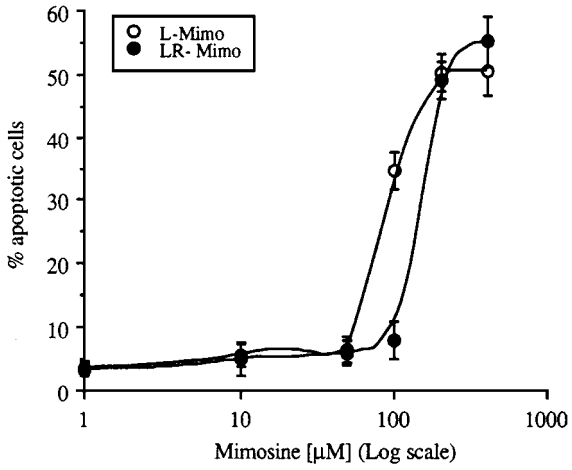
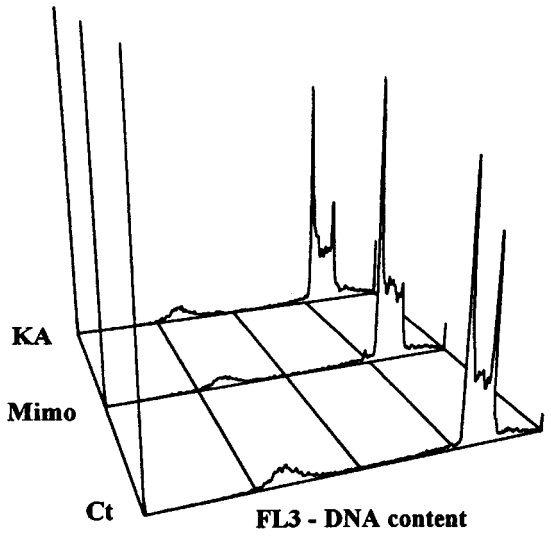


Figure 5. Fluorescence histogram representing the K562 cell cycle. x axis = FL3 propidium iodide (PI) fluorescence; y axis = number cells/channel.



Mimosine (400 μM) seems to delay the K562 cell cycle, reducing the G2/M phase, although no synchronizing effect in the G1 phase was observed even after 72 h incubation (data not shown).The addition of kojic acid (400 μM) to K562 cells caused a decrease in the G2/M phase (Figure 5)

Light microscopy

Semi-thin sections provided an overview of the morphology of both mimosine- and kojic acid-treated cells and the controls (Figure 6).

In mimosine-treated samples (Figure 6b), numerous apoptotic cells appeared alongside cells with normal morphology. These cells mostly showed advanced apoptotic features, such as strong cytoplasmic condensation and micronuclei. In kojic acid-treated cells (Figure 6c) no apoptosis was detected and the cell morphology was similar to that of the controls. A number of mitotic cells were also detected in the controls (Figure 6a).

Electron microscopy

Ultrastructural investigation revealed the characteristics of both treated and control cells. As shown in Figure 7a, the initial chromatin margination, typical of early apoptosis in mimosine-treated cells, appeared in the presence of a still good preservation of the cytoplasmic organelles.

More advanced apoptotic patterns are those of Figure 7b, which shows a cell with many electron-dense micronuclei surrounded by a double envelope the outher membrane of which is outlined by rybosomes.[28]

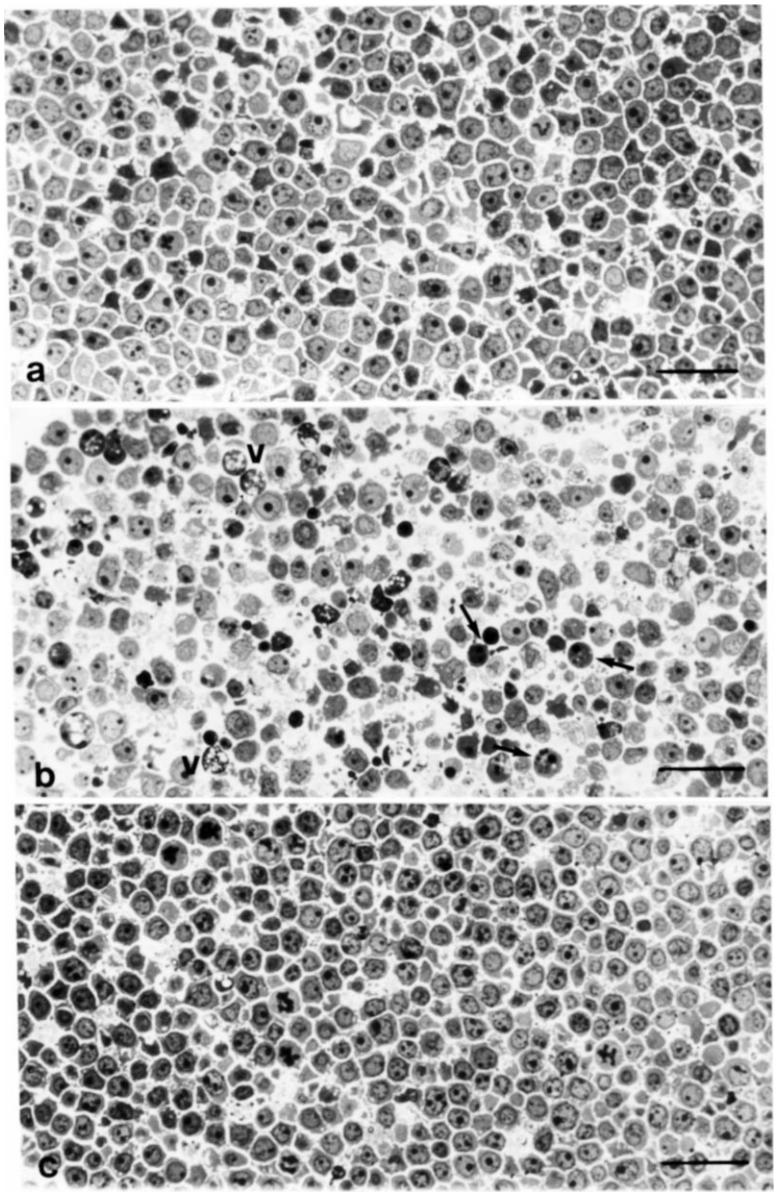
To better investigate the effects of mimosine on the HL60 cells, we also examined the residual non-apoptotic cells. A characteristic vacuolization of the cytoplasm, in the presence of a good preservation of the plasma membrane and of the organelles (Figure 7c) was a remarkable feature of these cultures.

Furthermore, high amounts of glycogen stored within the cytoplasm were present as electron-dense single-particle aggregates (Figure 7d). Treatment with kojic acid did not seem to affect the cells which were apparently identical to the controls (Figure 8a and b).

Discussion

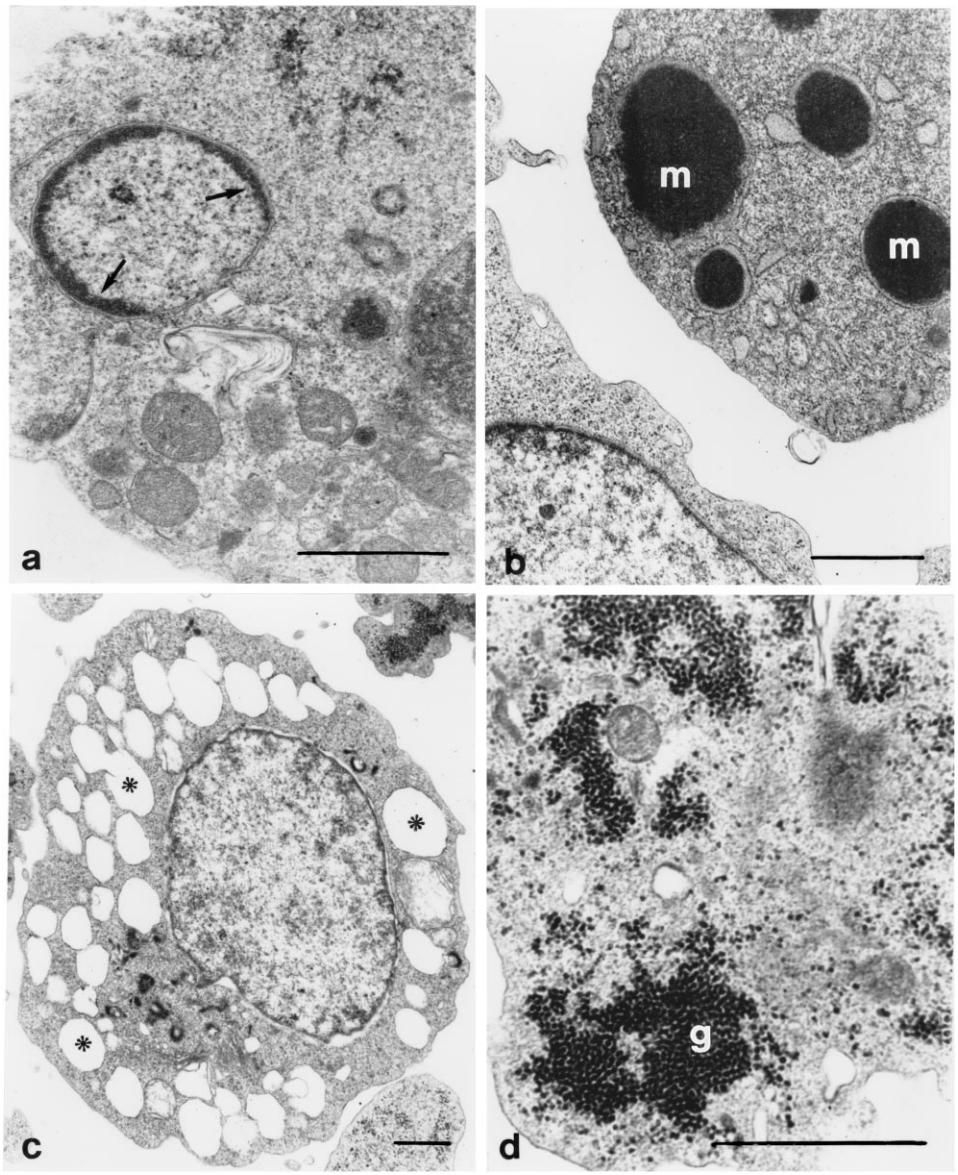
Chemicals that affect DNA synthesis are potentially useful for the development of anticancer drugs. Mimosine is considered a reversible synchronizing agent for various tumor cell lines, human fibroblasts and PHA-activated lymphocytes,[29,30] in that it blocks the cell cycle on the G1/S border. Its action seems to precede the block mediated by aphidicolin, but in any event it acts after DNA synthesis begins.[31] The biochemical pathway leading to this block is still unknown, but it has been hypothesized that mimosine modifies the nucleotide pool levels through binding to SHMT [32] thus modifying one or more essential replication proteins. DOHH, an enzyme regulating the formation of hypusine in eukaryotic initiation factor 5A (eIF-5A)[33] is an additional target for mimosine; eIF-5A is highly preserved and the site of deoxyhypusine hydroxylation has been maintained throughout phylogeny, from the archae bacteria to man. This site may thus be a vital genetic element for cell proliferation.[34] Furthermore, it has been observed that inhibitors of *in vitro* deoxyhypusine hydroxylation block the cell cycle in CHO and LAZ463 cells.[17] All these compounds bind to DOHH and chelate iron at the same time. In this study we used kojic acid, which bears an iron-chelating domain identical to that

Figure 6. Light microscopy of semithin sections of control (**a**), mimosine- (**b**) and kojic acid-treated (**c**) HL60 cells. A high rate of apoptosis appears after incubation with mimosine (**b**, ‡). Among apoptotic cells, most in the final stages, numerous vacuolized cells (**b**, v) can also be detected. Cells incubated under control (**a**) conditions and with kojic acid (**c**) showed comparable normal morphology; bar = 50 μm.



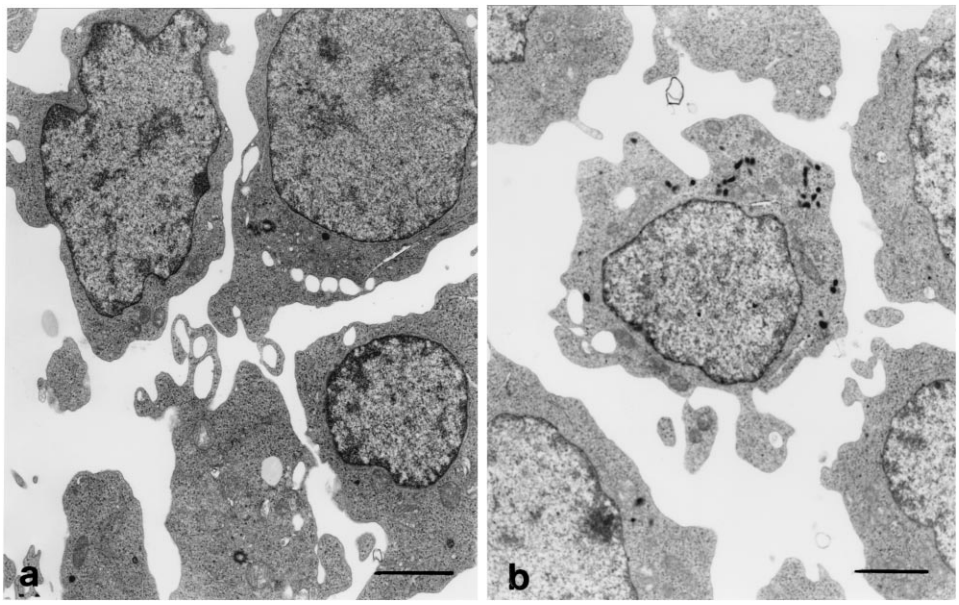
of mimosine but is ineffective as DOHH inhibitor,[11,35] to discriminate between the chelating effect and inhibition of DOHH. Kojic acid has only a slight effect on the HL60 and K562 cell cycle and G2/M phase and it does not show an additive or synergistic effect with mimosine. In fact, mimosine synchronized HL60 cells mainly in the G1 phase, whereas it was ineffective towards the K562 cells. The cause of the different sensitivity shown by the two cell lines is a matter of speculation; it could be due to a different uptake of mimosine or to a different expression of eIF-5A isoforms. In fact, it is possible that also in K562 cells different eIF-5A isoforms exist, as it was observed in HeLa cells.[36] In the HeLa cell line, the two minor isoforms observed possess a free lysine side chain in the place of hypusine, and therefore are unaffected by inhibition of DOHH; moreover, these isoforms were localized in the cytoplasm only. It is important to note that the role of eIF-5A is still unclear, although it was formerly thought to be involved in the formation of the first peptide bond in protein synthesis. It has also been suggested that eIF-5A can play a role in mRNA export from the nucleus.[19,37] We observed that mimosine not only induces synchronization

Figure 7. Transmission electron microscopy of mimosine-treated HL60 cells. Initial chromatin margination (**a**, ‡) and micronuclei (**b**, m) characterize the apoptotic cells. Widespread vacuolization (**c**, ∗) and glycogen aggregates (**d**, g) are consistent features of identically treated, non-apoptotic cells; bar = 1 μm.



of the cell cycle in HL60 cells but also causes apoptosis, as shown by the DNA degradation observed by using flow cytometry (Figure 2) and by the typical apoptotic morphological pattern observed with light and electron microscopy (Figures 6, 7 and 8). Cytotoxicity has been reported to be induced by high doses (10^{-1} M) of mimosine in cultured carp hepatocytes[38,39] and it has been also reported that mimosine is a potent clastogen in hamster fibroblasts,[40] however no toxic effects have been observed in synchronization experiments.[29] In our study, we found both synchronization and apoptosis in HL60 cells when using mimosine doses and exposure times that have been reported to block DOHH activity *in vitro* and to synchronize cells on the G1/S boundary. We observed that the two phenomena are dose-dependent and that at higher doses (200–400 μM) they are not affected by the chirality of mimosine (Figure 3). The slight difference between L- and D,L-mimosine is not surprising in view of the steric requirements of the active site of DOHH,[35] of the defleecing effects and of the toxicity of mimosine and related compounds.[7,41,42] The independence from chiral effects is in line with the reported apoptotic activity of mimosine and of deferiprone, which is an achiral molecule, on retrovirus-infected ACH-2 cells.[19] These data and the inactivity of kojic acid suggest that mimosine induces apoptosis possibly via inhibition of DOHH.

Figure 8. Control (**a**) and kojic acid-treated (**b**) HL60 cells, show a well-preserved, comparable ultrastructural morphology; bar = 2 μ.



Morphological analysis was carried out to characterize mimosine-induced apoptosis. Unusual nuclear changes had already been described after exposure of various cell lines to mimosine,[38,39] but its role as inducer of apoptosis had not yet been reported. Our study demonstrates that, in HL60 tumor cells, apoptosis progression is closely comparable to that described in the same model, under different experimental condition,[43] and for other cell lines.[44] Nuclear chromatin marginates and clusters at the nuclear periphery, while the plasma membrane and organelles are well preserved. Finally, a number of uniformly electron-dense micronuclei are formed and features of secondary necrosis progressively appear.

An unusual behavior was observed in non-apoptotic mimosine-treated cells. Even in the presence of a good preservation of the nucleus and plasma membrane, numerous membrane-bound, apparently empty vacuoles appeared. This feature, absent in the control and kojic acid-treated cells, must be related to the treatment with mimosine and is presumably an early event. This phenomenon has also been described in cells committed to apoptosis. In this particular model it is probably correlated with initial apoptotic changes preceding nuclear alteration.

In addition, large masses of single-particle glycogen appeared in the mimosine-treated non-apoptotic cells and are absent in control and kojic acid-treated specimens. Glycogen is a recognized energy source and these macromolecules frequently accumulate in the cytoplasm of some cell types. In our case, its unusual storage in mimosine-treated cells could be due to the blocking of the cell cycle or to a number of other cellular metabolic activities.

We speculate that the apoptosis induced by mimosine in HL60 cells could be due to two different consequences of the inhibition of DOHH. The lack of hypusine formation causes a reduction in the activity of eIF-5A that seems to be involved in the translation of messenger RNA. The alteration of this flow of information could slow the synthesis of DNAs in HL60[45] and cause apoptosis. At the same time, the lack of hypusine formation also leads to the inhibition of the transcription of mRNAs for methionine adenosyl-transferase and cytochrome-c oxidase subunit I, both of which are critical enzymes for cell proliferation.[46] Other hypusine-dependent mRNAs are those for cyclin A and cdc2, the levels of which are reduced by mimosine.[12] Therefore, mimosine could induce alterations in DNA synthesis in actively proliferating cells, inhibiting the expression of key proteins through the alteration of DOHH activity. This alteration in the cell cycle could activate the apoptotic machinery in cells of lymphoid origin, *i.e.* genetically committed to promptly activate an apoptotic response. In fact although mimosine-treated cells accumulate in the G1 phase as shown in Figure 4, it is also possible to note that in the DNA histogram obtained from the same cells, the number of cells in G1 is the same as in the control cells. This discrepancy is due to the fact that the percentage of cells in G1 was calculated for the total number of "live cells", *i.e.* cells in the normal cell cycle, without counting cells in the hypo-diploid peak. Thus, it is possible that mimosine induces apoptosis in cells actively replicating or at the end of the cell cycle.

The ability of mimosine to induce apoptosis could help to explain the physiological role of DOHH and eIF-5A. It is conceivable that a modulation of eIF-5A activity

through DOHH or other enzymes involved in hypusine synthesis could occur during normal development or carcinogenesis.

Our model could help to increase our understanding of the establishment and propagation of cancer as well as its control; moreover, chemical optimization of the mimosine structure could yield more potent analogs potentially useful as leads for new anticancer agents.

Acknowledgments

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References

1. Norbury C, Nurse P. Animal cell cycle and their control. *Annu Rev Biochem* 1992; **1**: 441–470.
2. Hayashi I, Sato GH. Replacement of serum by hormones permits growth of cells in a defined medium. *Nature* 1976; **259**: 132–134.
3. Cherington PV, Smith BL, Pardee AB. Loss of epidermal growth factor requirement and malignant transformation. *Proc Natl Acad Sci USA* 1979; **76**: 3937–3941.
4. Eriksson S, Graslund A, Skog S, Thelander L, Tribukait B. Cell cycle-dependent regulation of mammalian ribonucleotide reductase. The S phase-correlated increase in subunit M2 is regulated by de novo protein synthesis. *J Biol Chem* 1984; **259**: 11695–11700.
5. Crounse RG, Maxwell JD, Blank H. Inhibition of growth of hair by mimosine. *Nature* 1962; **194**: 694–695.
6. Reis PJ, Tunks DA, Hegarty MP. Effects of mimosine, a potential chemical defleecing agent, on wool growth and the skin of sheep. *Aust J Biol Sci* 1975; **28**: 495–501.
7. Ward KA, Harris RLN. Inhibition of wool follicle DNA synthesis by mimosine and related 4(1H)-pyridones. *Aust J Biol Sci* 1986; **29**: 189–196.
8. Dewreede S, Wayman O. Effect of Mimosine on the rat fetus. *Teratology* 1970; **3**: 21–27.
9. Lalande M. A reversible arrest point in the late G1 phase of the mammalian cell cycle. *Exp Cell Res* 1990; **186**: 332–339.
10. Hashiguchi H, Takahashi H. Inhibition of two copper-containing enzymes, tyrosinase and dopamine beta-hydroxylase, by L-mimosine. *Mol Pharmacol* 1977; **13**: 362–367.
11. Abbruzzese A, Hanauske-Abel HM, Park M-H, Henke S, Folk JE. The active site of deoxyhypusyl hydroxylase: use of catechol peptides and their component chelator and peptide moieties as molecular probes. *Biochim Biophys Acta* 1991; **1077**: 159–166.
12. Feldman ST, Schonthal A. Negative regulation of histone H1 kinase expression by mimosine, a plant amino acid. *Cancer Res* 1994; **54**: 494–498.
13. Kulp KS, Green SL, Vullet PR. Iron deprivation inhibits cyclin-dependent kinase activity and decreases cyclin D/C/D K4 protein levels in asynchronous MDA-MB-453 human breast cancer cells. *Exp Cell Res* 1996; **229**: 60–68.
14. Alpan RS, Pardee AB. $p21^{WAF1/CIP1/SD11}$ is elevated through a p53-independent pathway by mimosine. *Cell Growth & Diff* 1996; **7**: 893–901.
15. Linn HB, Falchetto R, Mosca PJ, Shabanowitz J, Hunt DF, Hamlin JL. Mimosine targets serine hydroxymethyltransferase. *Biol Chem* 1996; **271**: 2548–2556.
16. Abbruzzese A, Park MH, Folk JE. Deoxyhypusine hydroxylase from rat testis. Partial purification and characterization. *J Biol Chem* 1986; **261**: 3085–3089.
17. Park MH, Joe YA, Kang KR, Lee YB, EC Wolff. The polyamine-derived aminoacid hypusine: its post-translational formation in eIF-5A and its role in cell proliferation. *Amino Acids* 1996; **10**: 109–121.
18. Hanauske-Abel HM, Park MH, Hanauske AR, Popowicz AM, Lalande M, Folk JE. Inhibition of the G1-S transition of the cell cycle by inhibitors of deoxyhypusine hydroxylation. *Biochim Biophys Acta* 1994; **1221**: 115–124.
19. Andrus L, Szabo P, Grady RW, *et al.* Antiretroviral effects of DOHH inhibitors: a hypusine-dependent host cell mechanism for replication of human immunodeficiency virus type 1 (HIV-1). *Biochem Pharmacol* 1998; **55**: 1807–1818.
20. Adams R, Johnson JL. Leucenol. VI. A total synthesis. *J Am Chem Soc* 1949; **7**: 705–708.
21. Wiley JW, Tyson JNH Jr, Steller JS. The Configuration of Complex Kojates Formed with Some Transition Elements as Determined by Magnetic Susceptibility Measurements. *J Am Chem Soc* 1942; **64**: 963–964.
22. Reno' F, Burattini S, Rossi S, *et al.* Phospholipid rearrangement of apoptotic membrane does not depend on nuclear activity. *Histochem Cell Biol* 1998; **110**: 467–476.
23. Darzynkiewicz Z, Bruno S, Del Bino G, *et al.* Features of apoptotic cells measured by flow cytometry. *Cytometry* 1992; **13**: 795–808.
24. Kretchmar-Nguyen S, Craig A, Raymond KN. Transferrin: the role of conformational changes in iron removal by chelators. *J Am Chem Soc* 1993; **115**: 6758–6764.
25. Tome ME, Fiser SM, Payne CM, Gerner EW. Excess putrescine accumulation inhibits the formation of modified eukaryotic initiation factor 5A (eIF-5A) and induces apoptosis. *Biochem J* 1997; **328**: 847–854.
26. Tome ME, Gerner EW. Cellular eukaryotic initiation factor 5A content as a mediator of polyamine effects on growth and apoptosis. *Biol Signals* 1997; **6**: 150–156.
27. Amarante-Mendes GP, Naekyung KC, Liu L, *et al.* Bcr-Abl exerts its antiapoptotic effect against diverse apoptotic stimuli through blockage of mitochondrial release of cytochrome C and activation of caspase-3. *Blood* 1998; **91**: 1700–1705.
28. Columbaro M, Gobbi P, Renó F, *et al.* A multiple technical approach to the study of apoptotic cell micronuclei. *Scanning* 1998; **20**: 541–548.
29. Lalande M. Synchronization of primary human fibroblast and lymphocytes with mimosine. *Meth Cell Sci* 1996; **18**: 135–142.
30. Hoffman BD, Hanauske-Abel HM, Flint A, Lalande M. A new class of reversible cell cycle inhibitors. *Cytometry* 1991; **12**: 26–32.
31. Hughes TA, Cook PR. Mimosine arrests the cell cycle after cells enter S-phase. *Exp Cell Res* 1996; **222**: 275–280.
32. Kalejta RF, Hamlin JL. The dual effect of mimosine on DNA replication. *Exp Cell Res* 1997; **231**: 173–183.
33. Park MH, Wolff EC, Folk JE. Hypusine: its post-translational formation in eukaryotic initiation factor 5A and its potential role in cellular regulation. *BioFactors* 1993; **4**: 95–104.
34. Schnier J, Schwelberger HG, Smith-McBride Z, Kang HA, Hershey JW. Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast Saccharomyces cerevisiae. *Mol Cell Biol* 1991; **11**: 3105–3114.

35. McCaffrey TA, Pomerantz KB, Sanborn T, *et al.* Specific Inhibition of eIF-5A and collagen hydroxylation by a single agent. *J Clin Invest* 1995; **95**: 446–455.
36. Klier H, Csonga R, Joao HC, *et al.* Isolation and structural characterization of different isoforms of the hypusine-containing protein eIF-5A from HeLa cells. *Biochemistry* 1995; **34**: 14693–14702.
37. Ruhl M, Himmelspach M, Bahr GM, *et al.* Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating transactivation. *J Cell Biol* 1993; **123**: 1309–1320.
38. Vogt G, Bohm R, Segner H. Mimosine, a naturally occurring drug interfering primarily with the cell nucleus. *J Submicrosc Cytol Pathol* 1993; **25**: 247–256 .
39. Vogt G, Bohm R, Segner H. Mimosine-induced cell death and related chromatin changes. *J Submicrosc Cytol Pathol* 1994; **26**: 319–330.
40. Jha AN, Hande PM, Mullenders LH, Natarajan AT. Mimosine is a potent clastogen in primary and transformed hamster fibroblasts but not in primary or transformed human lymphocytes. *Mutagenesis* 1995; **10**: 385–391.
41. Takahara S, Takahshi H. An amine inducing alopecia in mice. *J Biochem* 1972; **72**: 195–197.
42. Hegarty MP, Lee CP, Christie GS, De Munk FG, Court RD. Comparative toxicities of mimosine and some chemically related compounds to mouse bone marrow cells in liquid culture. *Aust J Biol Sci* 1978; **31**: 115–121.
43. Stuppia L, Gobbi P, Zamai L, Palka G, Vitale M, Falcieri E. Morphometric and functional study of apoptotic cell chromatin. *Cell Death and Differentiation* 1996; **3**: 397–405.
44. Mariani AR, Columbaro M, Zauli G, *et al*. Lineage-related susceptibility of human haemopoietic cell lines to apoptosis. *Anatomical Record* 1999; **254**: 1–6.
45. Martin SJ, Lennon SV, Bonham AM, Cotter TG. Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA or protein synthesis. *J Immunol* 1990; **145**: 1859–1867.
46. Hanauske-Abel HM, Slowinska B, Zagulska S, *et al*. Detection of a sub-set of polysomal mRNAs associated with modulation of hypusine formation at the G1-S boundary. Proposal of a role for eIF-5A in onset of DNA replication. *FEBS Lett* 1995; **366**: 92–98.