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Research Article

Vinorelbine Increases the Progression to Apoptosis in Hl-60 Acute Promyelocytic Leukemia Cell Line

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ABSTRACT

BACKGROUND

Vinorelbine is a semi-synthetic vinca alkaloid that has been employed both as a single agent and in combination, and has shown significant antitumor activity. Dexamethasone which acts on glucocorticoid and meperidine which acts on mu opioid receptors have pro-apoptotic and anti-proliferative effects. We investigated the effects of dexamethasone and meperidine on the cytotoxicity of vinorelbine on HL-60 human acute promyelocytic leukemia cell line.

METHODS:

The cytotoxic effects of vinorelbine alone and in combination with dexamethasone or meperidine were examined on the HL-60 cell line by performing plating efficacy, BrdU, caspase 3, bcl-2 immunocytochemistry and flow cytometry.

RESULTS

We determined that vinorelbine inhibited cell proliferation and decreased the rate of synthase phase cells. Furthermore, by immunocytochemistry and flow cytometry analyzes we found that vinorelbine increased the rate of apoptotic cells via decreasing bcl-2 and increasing caspase-3 activation. No additive effects were observed when dexamethasone was combined with vinorelbine. However, meperidine increased the apoptotic effect of vinorelbine.

CONCLUSION

Vinorelbine promotes apoptosis in HL-60 cells and may be helpful in the treatment of acute promyelocytic leukemia patients. Moreover using meperidine with vinorelbine in the treatment may be beneficial.

Keywords: Dexamethasone; HL-60; Leukemia; Meperidine; Vinorelbine

INTRODUCTION

Acute myeloid leukemia (AML) is a clinically and molecularly heterogeneous disease. Cytogenetic and/or molecular studies are used to assign 30% to 40% of AML cases carrying specific genetic lesions to different prognostic subgroups in order to monitor minimal residual disease and to select patients who could benefit from targeted therapies [1]. However, they cannot be applied to 40% to 50% of patients with AML who at conventional cytogenetics exhibit a normal karyotype [1, 2].

Vinorelbine is a semi-synthetic vinca alkaloid known to exert its antitumor activity by interfering with the polymerization of tubulin. It has shown a broad spectrum of activity in some advanced carcinomas of lung, breast and ovary [3]. It is a semi-synthetic vinca alkaloid that binds to tubulin and inhibits mitotic microtubule polymerization and stops cell cycle at G2 / M transition [3, 4]. Vinorelbine has significant antineoplastic activity against various types of tumours such as lymphoma, breast and lung cancer [5, 6].

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Glucocorticoids remain the cornerstone in the treatment for a number of haematological malignancies, including leukemia, lymphoma and myeloma. Extensive literature suggests that the efficacy of glucocorticoids stems from their ability to mediate apoptosis . Dexamethasone is a glucocorticoid class drug used in the treatment of edema because it reduces vascular permeability and also has anti-inflammatory and immunosuppressive effects. Glucocorticoids were introduced in oncological practice, as is evident, solely on the basis of pro-apoptotic effects in lymphoid cells and on their effectiveness in treating tumour related edema, inflammation, pain and electrolyte imbalance, stimulating appetite, and most importantly, preventing nausea and emesis caused by cancer therapy . It is used to prevent nausea and vomiting associated with chemotherapy . Glucocorticoids have pro-apoptotic and anti-proliferative effect in lymphoid cells in cell culture.7

Meperidine is a synthetic opioid used as pain reliever . There is data indicating that opioid species increase apoptosis . Opioids bind to opioid receptors and show their effectiveness. HL-60 cell line has been shown to contain mu opioid receptors from opioid receptors [14]. While endogenic opioid peptides induce apoptosis through Bcl-2 in HL-60 cells, it has also been shown that codeine and codeinone cause apoptotic cell death via caspase 3 in HL-60 cell line.

There is no study stating the effects of dexamethasone and meperidine drugs which acts on glucocorticoid and mu opioid receptors and have pro-apoptotic and anti-proliferative effects, on the cytotoxicity of vinorelbine that is a microtubule polymerization inhibitor, on AML.

We investigated the effects of these drugs on AML cell line (HL-60) in separate and combined use by plating efficacy, immunocytochemistry and flow cytometry. With this study, the effectiveness of these substances and their combinations on HL-60 cell line will be enlightened, contributing to the treatment of patients with AML in the clinic.

MATERIALS AND METHODS

CELL CULTURE AND DRUGS

HL-60 cells established from the American Type Culture Collection (ATCC) (ECACC no: 85011431) were maintained in Dulbecco's modified Eagle's Medium (DMEM, containing 100U/ml penicillin and 100 µg/ml streptomycin) (Biological Industries, Haemek, Israel) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Biological Industries, Haemek, Israel). The flasks were kept in an incubator with a humidified atmosphere of 5% CO2 at 37 °C. We used early passages (4-7th) of HL-60 cells after obtaining the cell line. Vinorelbine (Navelbine, Pierre Fabre Medicament), Dexamethasone (Onadron İ.E.Ulagay) and Meperidine (Aldolan, Liba Laboratories) were dissolved in bidistilled water and added into cell culture in equal volumes.

PLATING EFFICACY

Cells prepared in 2 ml of DMEM were plated into a 24-well plate in 5 x 104/ml concentration with 100% vitality. All drugs were added in equal volumes of 100µl, and after 24, 48 and 72 hour, cells were counted. 0,1; 1; 10; 100µM concentrations of vinorelbine, dexametasone and meperidine were added in equal volumes of 100µl, and after 24, 48 and 72 hour, cells were counted. ID50 (Inhibition Dose %50) of vinorelbine and dexamethasone is determined as 10 µM, and ID50 of meperidine is determined as 100 µM. ID50 doses were used for all assays.

For plating efficacy, cells prepared in 2 ml of DMEM were plated into a 24-well plate in 5 x 104/ml concentration with 100% vitality. All drugs were added in equal volumes of 100µl, and after 24, 48 and 72 hour, cells were counted. 10µM concentrations of vinorelbine and dexametasone and 100µM concentration of meperidine were added in equal volumes of 100µl, and after 24, 48 and 72 hour, cells were counted.

BROMODEOXYURIDINE-LABELLING INDEX (BRDU-LI)

BrdU (5-bromo-2-deoxyuridine (2µM, 1:200 dilution)) was added to the cells' medium with subsequent incubation for an additional 1 hour. Thereafter cells were gently removed and washed in p*hosphate*-buffered saline (PBS) (pH 7.4). Cells were dropped onto the slides and were fixed in 70% ethanol and then washed in PBS for 10 minutes. Cells were incubated in a solution of 3% H2O2 for 10 minutes, then washed with PBS and incubated with primary mouse anti-BrdU antibody (1:250 dilution, LabVision, UK). Cells were incubated with biotinylated IgG followed by streptavidin- peroxidase conjugate (LabVision, UK). The slides were then washed and incubated with the DAB chromogen substrate system and counterstained with Mayer's haematoxylin (Lab Vision TA-125-MH). The immunostained slides were observed under light microscopy at magnifications of x 40. BrdU-labelled cells were observed by the same person. BrdU-positive cell types were determined by observing dark brown nuclear staining. Unlabelled nuclei with only blue haematoxylin staining and pale brownish nuclei were considered to be negative. Ten slides and at last 3000 cells were evaluated for each group.

CASPASE-3 IMMUNOSTAINING

Cells were dropped onto the slides and were fixed in 70% ethanol and then washed in phosphate-buffered saline (PBS) for 10 minutes. Cells were incubated in a solution of 3% H2O2 for 10 minutes, then washed with PBS and incubated with polyclonal rabbit anti-caspase 3 antibody (Lab Vision RB-1197-P) for 2 hours at room temperature on humidified environment. Cells were incubated with biotinylated IgG followed by streptavidin- peroxidase conjugate (LabVision, UK). The slides were then washed and incubated with the AEC chromogen substrate system and counterstained with Mayer's haematoxylin (Lab Vision TA-125-MH). The immunostained slides were observed under light microscopy at magnifications of x 40 and were observed by the same person. Caspase 3-positive cell types

were determined by observing dark brown AEC nuclear staining. Unlabelled nuclei with only blue haematoxylin staining and pale brownish nuclei were considered to be negative. Ten slides were observed for each group.

BCL-2 IMMUNOSTAINING

Cells were dropped onto the slides and were fixed in 70% ethanol and then washed in p*hosphate*-buffered saline (PBS) for 10 minutes. Cells were incubated in a solution of 3% H2O2 for 10 minutes, then washed with PBS and incubated with polyclonal rabbit anti-bcl-2 antibody (Santa Cruz sc-7382) for 2 hours at room temperature on humidified environment. Cells were incubated with biotinylated IgG followed by streptavidin-peroxidase conjugate (LabVision, UK). The slides were then washed and incubated with the AEC chromogen substrate system and counterstained with Mayer's haematoxylin (Lab Vision TA-125-MH). The immunostained slides were observed under light microscopy at magnifications of x 40 and were observed by the same person. Bcl-2-positive cell types were determined by observing dark brown AEC nuclear staining. Unlabelled nuclei with only blue haematoxylin staining and pale brownish nuclei were considered to be negative. Ten slides were observed for each group.

FLOW CYTOMETRY

The apoptotic index was evaluated by using flow cytometric Annexin-V-fluorescein isothiocyanate/ propidium iodide (Annexin-VFITC/PI) staining. Following the instruction manual of the kit (BD Pharmingen, San Diego, CA, USA), briefly, cells were washed twice with PBS and resuspended by binding buffer containing 0.01 M HEPES, 0.14 mM NaCl, and 2.5 mM CaCl2 . A cell suspension (1×105 cells in 100 µL) in binding buffer was incubated with 5 µL of FITC-labelled Annexin V (BD Pharmingen) dye and PI for 15 min in the dark at room temperature. After incubation, the PI fluorescence and Annexin V were measured simultaneously in a BD FACS Calibur and analysed with the instrument's operating software (CellQuest: BD Pharmingen). Data acquisition and analysis were undertaken with CellQuest and WinMDI programs.

STATISTICAL ANALYSIS

The results were statistically analysed using the independent Student's t-test. Data were represented as means ± standard error mean (SEM) and at least in triplicate. Results were considered significant with p<0.05.

RESULTS

PLATING EFFICACY

Figure1: Cell proliferation results of Hl-60 cells at 24, 48 and 72 hours. Control group exerted exponentially grown. Vinorelbine and vinorelbine combinations with meperidine or dexamethasone inhibited proliferation at all times. *(p<0.05), *(p<0.01)

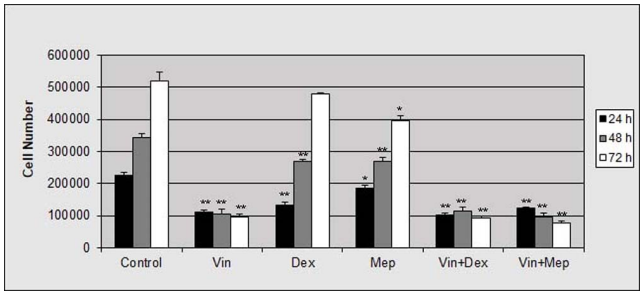


Figure 1: clearly shows that vinorelbine (10 µM) decreased cell proliferation in comparison to control group at all-time points (p<0.01). Dexamethasone (10 µM) decreased cell number at 24th and 48th hours (p<0.01 and p<0.05). Meperidine (100 µM) significantly decreased cell number at 24th, 48th and 72th hours compared to control (p<0.05, p<0.01, p<0.05).

The exposition of vinorelbine and dexamethasone combination inhibited cell proliferation at 24th, 48th and 72th hours (p<0.01). But this result was similar to vinorelbine alone group and dexamethasone statistically did not show additional effect on decreasing cell number (p>0.05). This result was same in vinorelbine+meperidine combination group as well (p>0.05). Vinorelbine+meperidine combination exhibited proliferation inhibition along 72 hour according to control group (p<0.01). Meperidine enhanced vinorelbine cytotoxicity more than vinorelbine alone group but this result was not found statistically significant (p>0.05). These results suggest that vinorelbine is effective on cell proliferation inhibition and vinorelbine combination with dexamethasone or meperidine does not make difference on its cytotoxicty effect.

BRDU-LABELLING INDEX (BRDU-LI)

The labelling index of control group was determined as 59.32%, 49.53%, 37.95% at 24th, 48th, 72th hours, respectively (figure 2, 3A).

Figure 2: BrdU-Labelling index *(p<0.05), **(p<0.01))

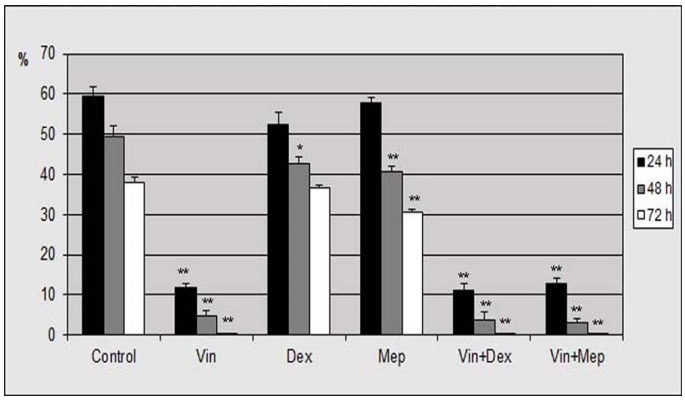
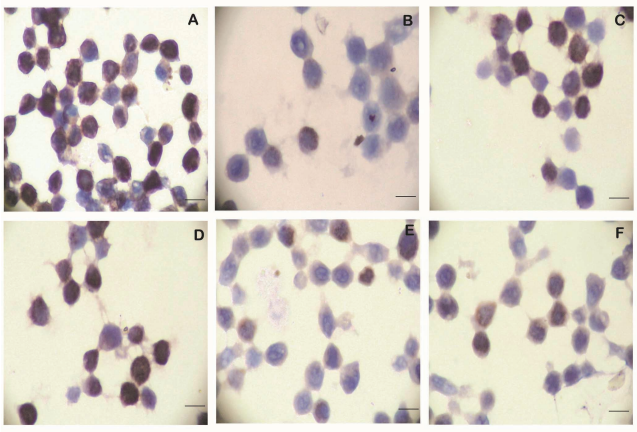


Figure 3: HL-60 cells stained with BrdU immunocytochemistry at 24h. (A) Control, (B) Vinorelbine, (C) Dexamethasone, (D) Meperidine, (E) Vinorelbine+dexamethasone, (F) Vinorelbine +meperidine, (Bar: 25µ))



Vinorelbine reduced the labelling of the cells as 11.68% at 24th hour, which was statistically significant according to the control ($p<0.01$) (figure 2, 3B). The labbelling index of cells in vinorelbine group was 4.88% at 48th hour and decreased to 0.15% at 72th hour. These results were also significant according to the control group ($p<0.01$). Labelling indexes of dexamethasone were 52.43%, 42.67%, and 36.46% at 24th, 48th and 72th hours, respectively (figure 3C). Decreasing labelling of the cells was significant at 48th hour ($p<0.05$). Meperidine exposured cells represented 57.72%, 42.67%, 30.51% labelling at 24th, 48th and 72th hours, respectively ($p>0.05$), ($p<0.01$), ($p<0.01$) (figure 3D). Combination of vinorelbine and dexamethasone exhibited low labelling indexes 11.27%, 3.89%, 0.15% at 24th, 48th and 72th hours, respectively ($p<0.01$) (figure 3E). While the results compared with dexamethasone alone group, these results were found significant ($p<0.01$). It seems that decreasing of S phase cell numbers was because of vinorelbine's effect. Vinorelbine and meperidine combination exhibited low labelling indexes 12.76%, 3.1%, 0.15% at 24th, 48th and 72th hours, respectively ($p<0.01$) (figure 3F). While the results compared with meperidine alone group, these results were found significant ($p<0.01$). Meperidine did not show additive effect on vinorelbine ($p<0.01$). These results improves that vinorelbine alone, meperidine alone and vinorelbine combination with dexamethasone or meperidine inhibited cell proliferation along 72 hours.

CASPASE-3 LABELLING INDEX

Caspase-3 labelling indexes of control group were determined 0.95%, 1.44% and 1.1% at 24h, 48h and 72h, respectively (figure 4, 5A)

Figure 4: Caspase-3-Labelling index *($p<0.05$), **($p<0.01$))

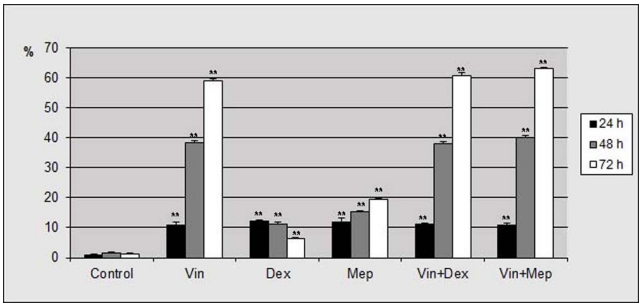
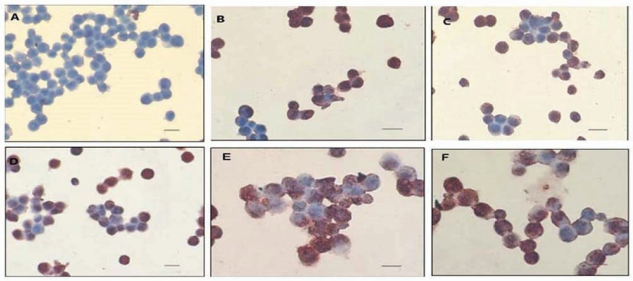


Figure 5: HL-60 cells stained with caspase 3 immunocytochemistry at 24h. (A) Control, (B) Vinorelbine, (C) Dexamethasone, (D) Meperidine, (E) Vinorelbine +dexamethasone, (F) Vinorelbine+meperidine, (Bar: 25µ))



After using vinorelbine, labelling indexes were increased to 10.77%, 38.51% and 59.02% at 24h, 48h and 72h ($p<0.01$) (figure 5B). Dexamethasone exposured group had 12.1% LI at 24h, 11.08% LI at 48h and 6.3% LI at 72h ($p<0.01$) (figure 5C). Meperidine group cells showed increasing caspase-3 LI by the time; 11.98%, 15.27% and 19.3%, at 24h, 48h and 72h, respectively ($p<0.01$) (figure 5D). When vinorelbine combined with dexamethasone, caspase-3 LI was determined 11.24% at 24h, 38.05% at 48h and 60.6% at 72h ($p<0.01$) (figure 5E). These results were significant according to control at all times and according to dexamethasone at 48h and 72h ($p<0.01$). In vinorelbine+meperidine group, caspase-3 LI were 11.48% at 24h, 40.2% at 48h and 63% at 72h and they all were statistically significant according to control ($p<0.01$) (figure 5F). When it was compared to meperidine alone group, caspase-3-LI was not significant at 24h ($p>0.05$) but was significant at 48h and 72h ($p<0.01$).

BCL-2 LABELLING INDEX

Bcl-2 labelling indexes of control group were determined 69.4% at 24h, 49.5% at 48h and 38.77% at 72h (figure 6, 7A).

Figure 6: Bcl-2-Labelling index *($p<0.05$), *($p<0.01$)

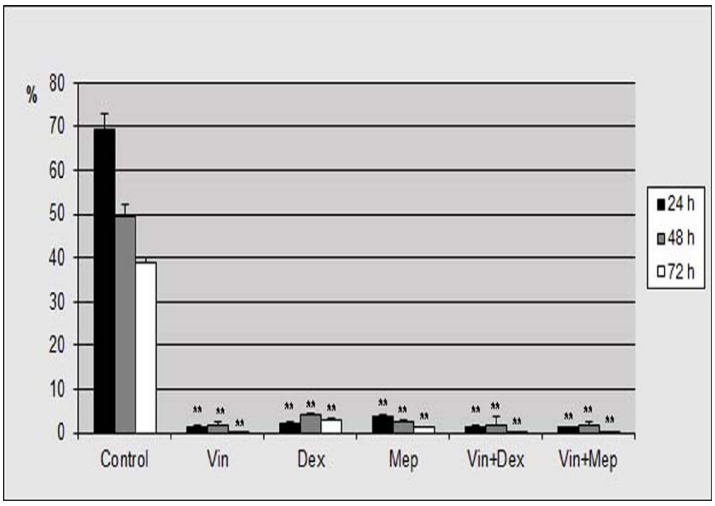
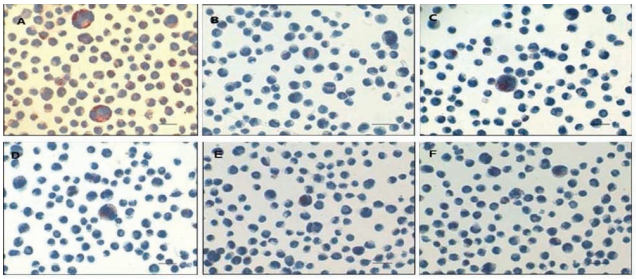


Figure 7: HL-60 cells stained with bcl-2 immunocytochemistry at 24h. (A) Control, (B) Vinorelbine, (C) Dexamethasone, (D) Meperidine, (E) Vinorelbine+dexamethasone, (F) Vinorelbine +meperidine, (Bar: 25µ))

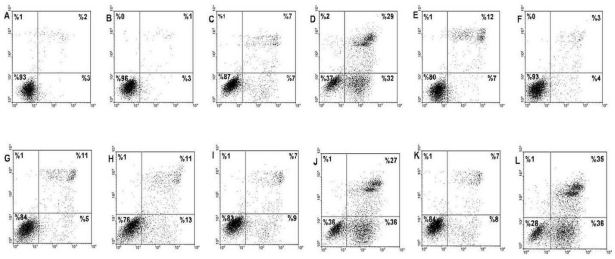


When vinorelbine was exposured, bcl-2 labelling index decreased according to control group, 1.36% at 24h, 1.8% at 48h and 0.15% at 72h ($p<0.01$) (figure 7B). Dexamethasone decreased bcl-2 LI rates 2.32% at 24h, 4.16% at 48h and 2.92% at 72h statistically significant ($p<0.01$) (figure 7C). Meperidine also decreased bcl-2 LI rates 3.75% at 24h, 2.41% at 48h and 1.24% at 72h statistically significant ($p<0.01$) (figure 7D). Vinorelbine and dexamethasone combination exhibited low levels of bcl-2 staining. Dexamethasone did not effect bcl-2 inhibition of vinorelbine. (1.46% at 24h, 1.93% at 48h and 0.15% at 72h) ($p<0.01$) (figure 7E). Vinorelbine and meperidine combination decreased bcl-2 LI 1.27% at 24h, 1.86% at 48h and 0.15% at 72h ($p<0.01$) (figure 7F). Meperidine did not have an effect on vinorelbine inhibition. It was statistically significant when compared to meperidine alone group at 24h, 48h, and 72h ($p<0.01$).

FLOW CYTOMETRY

The rate of apoptosis was determined as 5% at the 24th hour in the control group and 4% at the 72th hour (figure 8A, 8B).

Figure 8: Flow cytometric analyses (A) Control 24th hour, (B) Control 72th hour, (C) Vinorelbine 24th hour, (D) Vinorelbine 72th hour, (E) Dexamethasone 24th hour, (F) Dexamethasone 72th hour, (G) Meperidine 24th hour, (H) Meperidine 72th hour, (I) Vinorelbine+dexamethasone 24th hour, (J) Vinorelbine +dexamethasone 72th hour, (K) Vinorelbine+meperidine 24th hour, (L) Vinorelbine+meperidine 72th hour)



In the vinorelbine group, the rate increased from 14% at 24 hours to 61% at 72 hours (figure 8C, 8D) and was significant compared to control ($p<0.01$). When dexamethasone was administered, the rate of apoptosis decreased from 19% at 24 hours to 7% at 72 hours (figure 8E, 8F) ($p<0.01$). While meperidine caused apoptosis at 16% at 24 hours, it increased this rate to 24% at 72 hours (figure 8G, 8H) ($p<0.01$). In the vinorelbine+dexamethasone group, the rate of apoptosis increased from 16% at 24 hours to 63% at 72 hours (figure 8I, 8J) ($p<0.01$). Dexamethasone did not make any difference between single exposure and combination with vinorelbine. When vinorelbine was administered in combination with meperidine, the rate of apoptosis increased 15% at 24 hours and 71% at 72 hours (figure 8K, 8L) ($p<0.01$). Meperidine had increased Vinorelbine's apoptotic index when it was combined with vinorelbine at 72h ($p<0.01$).

DISCUSSION

It was reported earlier that vinorelbine had antitumoral effect on C6 glioma cell lines in vitro . But the effects of vinorelbine on leukemia still has not been detected clearly. It was reported that vinorelbine has dose-dependent cytotoxic activity on leukemia cell lines and inhibits cell growth of myeloma cells . Uckun et al. reported that vinorelbine causes apoptotic death in freshly obtained primary leukaemia cells from 53 patients with haematological malignancies four patients with AML . It was proved with flow cytometric analyses that vinorelbine caused apoptosis on fresh cultured primer leukemia cells which were derived from acute lymphoblastic leukemia, cronic lymphocytic leukemia and acute myeloid leukemia patients. Vinorelbine induced apoptosis was determined on myeloma cell lines by flow cytometry, too . The Bcl-2 protein is encoded by the B-cell lymphoma/leukemia-2 gene and is a member of a family of well conserved regulatory proteins involved in the regulation of apoptosis . Over-expression of bcl-2 inhibits apoptosis . Increased bcl-2 level was releated to leukemia's poor prognosis and showed two effects. Glucocorticoids promote apoptosis through induction of expression of proapoptotic Bcl-2 proteins such as Bad in thymocytes or through a decrease in the expression of prosurvival proteins like Bcl-2 and Bcl-xL in leukemic cells [23] and other cell types . The suppression of prosurvival Bcl-2 members by glucocorticoids and abrogation of glucocorticoid-induced apoptosis by overexpression of Bcl-2 suggest a causal relation between glucocorticoid-induced apoptosis and Bcl-2 proteins via a mechanism that may require generation of reactive oxygen species or altered mitochondrial transmembrane transport . High expression of bcl-2 was associated with a low complete remission rate after intensive chemotherapy and with a significantly shorter survival. In multivariate analysis, the percentage of bcl-2+ cells (or the blast survival in culture), age, and the percentage of CD34+ cells were independently associated with poor survival Vinorelbine induced apoptosis via caspase-3 activation on cells which was derivated from patients with leukemia . It was proved with flow cytometric analyses that Vinorelbin caused apoptosis on fresh cultured primer leukemia cells which were derived from acute lymphoblastic leukemia (ALL), cronic lymphocytic leukemia (KLL) and acute myeloid leukemia (AML) patients [28]. Our findings are consistent with earlier reports. According to bcl-2, caspase 3 immunocytochemistry and flow cytometry analyses, we determined that vinorelbine caused apoptosis by decreasing bcl-2 levels and increasing caspase 3 activation in HL-60 cells.

Glucocorticoids (GCs) are frequently used as cotreatment with anticancer drugs. Because they may have potent proapoptotic properties and they reduce side effects of chemotherapy and radiotherapy such as nausea, hyperemesis, and acute toxicity on

normal tissue. It was detected that glucocorticoids have pro-apoptotic and anti-proliferative effects on lymphoid cells in cell culture [7, 29, 30] and have cytotoxic effects in some cell types, such as lymphocytes, myeloma cells and lymphoma cells [31]. In addition, Ovali et al. found that dexamethasone reduced cell numbers in leukemia cells derived from patients with AML .
In our study, dexamethasone significantly reduced the number of cells, too. Various parameters were measured in the leukocyte population isolated from the blood of 26 patients with acute leukemia and it was emphasized that dexamethasone-induced cell death may be related to the number of cells in S phase [33]. Similarly, we detected that the number of cells in the S-phase was decreased in the dexamethasone group.

Opioid drugs are widely used in pain control in cancer patients at various stages of neoplastic growth and progression. It was found that endomorphine 1 (EM1) and endomorphine 2 (EM2), which are endogenic μ-opioid receptor agonists, have antiproliferative effects on the generation of human cytotoxic T lymphocytes (CTL) against HTLV-I induced T-cell leukemia cells (MT-2 line) . Morphinone, the oxidative metabolite of morphine, does not activate caspase-8 and caspase-9 in HL-60 and HSC-2 (oral squamous cell cancer) cell lines, but activates caspase-3 dose-dependently. Based on this, it was suggested that morphinone induces non-apoptotic cell death in HL-60 cells . In another study with HL-60 cells was found that codeinone, the oxidative metabolite of codeine, induces internucleosomal DNA fragmentation. In addition, it was shown that codeinone does not activate caspase-8 and caspase-9 but activates caspase-3 dose-dependently . Endomorphins, an endogenic μ-opioid receptor agonist decreased the level of bcl-2 in HL-60 cells and increased the expression of Bax, Fas and FasL. As a result, endomorphins induce apoptosis by activating Bcl-2-Bax and Fas-FasL in HL-60 cells . Codeinone led to increase in apoptotic cells in HL-60 cells . When morphine was applied to HL-60 and A549 (lung cancer) cell lines, early apoptotic markers were produced, and the increase in necrotic cells in MCF-7 (breast cancer) cells was induced . However, there exist no study which investigated whether vinorelbine efficacy could be augmented with opioids in HL-60 AML cells. In the present study, meperidine inhibited cell proliferation at all hours and decreased S-phase cell number at 48th and 72th hours. By flow cytometric analysis, it was shown that codeinone led to increase in apoptotic cells in HL-60 cells . Similarly, meperidine increased the number of apoptotic cells and the apoptotic effect of vinorelbine compared to control.

BDMV (Bortezomib, Dexamethasone, Mitoxantrone, and Vinorelbine) is an active reinduction regimen for children with relapsed ALL who cannot receive asparaginase. Further prospective clinical trials are warranted to evaluate the safety and efficacy of BDMV . GVDex (Gemcitabine, Vinorelbine, and Dexamethasone) is a low-toxicity, cost-effective, highly effective out-patient salvage regimen with high response rate in relapsed/refractory Hodgkin's lymphoma . Although vinorelbine is added to combined therapies of leukemia patients in the clinic, there is no study investigating the effects of using vinorelbine in combination with meperidine and dexamethasone on HL-60 AML cells in vitro.

CONCLUSION

In summary, our results showed that vinorelbine may increase the progression to apoptosis in HL-60 cells and may be useful in the treatment of patients with AML. The combination of vinorelbine with meperidine will be more beneficial in the treatment than dexamethasone.

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