Effect of Melatonin on the Cytotoxicity of Chemotherapeutic Drugs in Human Leukemia Cells

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Abstract. Background/Aim: Limited data are available on the effect of melatonin (MLT) on the cytotoxicity of chemotherapeutic drugs in tumor cells. In this study, we aimed to evaluate the effect of MLT on the cytotoxicity of different chemotherapeutic agents in leukemia cells in vitro. Materials and Methods: The experiments were carried out using human leukemia cell lines, Jurkat, MOLT-4, Daudi, HL-60, CMK, and K562, and two patient samples. Leukemia cells were incubated with cytarabine, daunorubicin, and etoposide with or without 10\(^{-5}\) M and 10\(^{-3}\) M concentrations of MLT. Cytotoxicity was measured by detecting apoptosis using flow cytometry. Results: Overall, co-incubation with melatonin did not alter the cytotoxicity of chemotherapeutic drugs in cell lines and patient samples except one. In a patient sample with acute myeloid leukemia, etoposide treatment in combination either concentrations of MLT resulted in increased elimination of the leukemia cells. Conclusion: Melatonin does not interfere with the cytotoxic effect of cytarabine, daunorubicin and etoposide in leukemia cells.

Melatonin (MLT), a neurohormone produced by the pineal gland, is known as a natural oncostatic agent. Although its mechanism of action is not fully understood, MLT has been demonstrated to exert oncostatic effects on tumor cells including breast, prostate, ovarian, skin and liver cancers, both in vivo and in vitro (1, 2). At physiological concentrations, MLT seems to inhibit cell proliferation, while at pharmacological concentrations it has cytotoxic activity on cancer cells (3, 4). However, in studies carried out using different leukemia cell lines, a spectrum of effects from no effect to inhibition of proliferation, induction of apoptosis and enhanced cytotoxicity were observed depending on the concentration of MLT used (5-10).

MLT reaches high concentrations in bone marrow (11) and protects the bone marrow and lymphoid tissues from the damaging effects of cytotoxic drugs and stimulates the bone marrow suppressed by chemotherapeutics in rats (12). In humans, concomitant administration of MLT and conventional chemotherapeutic drugs or interleukin-2 prolongs the survival time in untreatable advanced hematologic malignancies and metastatic solid tumors, and substantially reduces the frequency of thrombocytopenia, neurotoxicity, cardiotoxicity, stomatitis and asthenia (2, 13, 14).

We have previously reported that 0.1 M concentration of MLT induces the production of reactive oxygen species and has a moderate cytotoxic effect in CMK, Jurkat and MOLT-4 cells but not in HL-60, K562 and Daudi cells in vitro (8). However, the mechanisms of MLT on the cytotoxicity of chemotherapeutic drugs are not currently well understood, despite encouraging results for MLT administration in hematological malignancies. A recent study showed that MLT does not attenuate doxorubicin cytotoxicity in mammary adenocarcinoma, colon carcinoma or mouse leukemia cell lines and it intensifies cytotoxicity of doxorubicin in human keratinocytes, non-small cell lung cancer (A-549) and laryngeal cancer cell lines (Hep-2) (6, 15). In this study, we investigated the effect of MLT on the cytotoxicity of commonly used anti-leukemia drugs in human leukemia cells.

Materials and Methods

Cell cultures. Human T lymphoblastic leukemia cell lines (Jurkat and MOLT-4), human B lymphoblastic leukemia cell line (Daudi), and human myeloid leukemia cell lines (HL-60 and K562) were obtained from the American Type Culture Collection (Manassas, VA, USA), and CMK (human myeloid leukemia cell line) was...
obtained from DSMZ (Braunschweig, Germany). Cell viability was determined by trypan blue exclusion test prior to treatment, where a viability of >90% was required to proceed. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco BRL, Gaithersburg MD, USA), 2 mM glutamine, 50 mM MEM non-essential amino acids, 1 mM sodium pyruvate and 10 mM BME and maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air. All cells were plated at 5x10^5 per 1.88 cm^2 well in 24-well plates. Bone marrow samples on two patients, one with acute myeloid leukemia (AML) and the other with T-lineage acute lymphoblastic leukemia (T-ALL) were included in the study as part of an ongoing study approved by the Human Investigational Committee at our institution.

Melatonin. MLT, N-acetyl-5-methoxytryptamine, obtained from Sigma Chemical Co. (St. Louis, MO, USA), was dissolved in 100% ethanol and prepared as a 0.5 M stock solution, then diluted with medium for obtaining required concentrations.

In vitro treatment. Leukemia cell lines (HL-60, CMK, K562, Daudi, Jurkat, MOLT-4) and two patient samples were incubated with cytarabine (Ara-C) (10 μM), daunorubicin (DNR) (0.5 μM), and etoposide (ETO) (20 μM) with or without 10^-5 M and 10^-3 M concentrations of MLT in 1 ml RPMI-containing wells. All chemotherapeutics were added 15 minutes after MLT treatment. Cytotoxicity was assessed after overnight incubation. All experiments were repeated at least three times. The doses of chemotherapeutics and MLT were selected based on earlier studies (8, 16, 17).

Cytotoxicity assessment. At the end of the incubation period, cells were harvested from plates and were stained with annexinV-FITC (AnnV)/propidium iodide (PI) as described previously (18, 19). Briefly, 100 μl of cell suspension were transferred to clean 12x75 tubes containing 100 μl 1× binding buffer. Then 10 μl of AnnV-FITC/PI working solution was added and the cell suspension was incubated in the dark for 15 min. At the end of incubation, 0.4 ml 1× binding buffer and 20 μl of flow count fluorospheres (Coulter Corp., Miami, FL, USA) were added to each tube prior to analysis on a Coulter XL Epics Flow Cytometer (Coulter Corp., Miami, FL, USA). Absolute counts of viable cells were obtained by quantifying the number of AnnV-negative/PI-negative events representing viable cells in FL1/FL3 and using Coulter’s calibration software to adjust numbers to absolute counts by monitoring bead acquisition in FL2.

Cytotoxicity was calculated using the following formula:

Cytotoxicity (%)= ((fluorosphere-adjusted counts of viable cells in the control sample)−(fluorosphere-adjusted counts of viable cells in treated sample))/ (fluorosphere-adjusted counts of viable cells in the control sample) ×100

Fluorosphere-adjusted counts of AnnV-negative/PI-negative events (i.e., the viable cell population) were used in the calculations to account for growth inhibition and different forms of cell death that might have occurred. Thus, the results reflect growth inhibition and total cell loss occurring during the entire incubation period rather than the rate of cell apoptosis/death at the time of sampling.

Statistical analysis. Statistical analyses were performed using SPSS 10.0 for Windows (Chicago, IL, USA). All values were expressed as mean±standard deviation. The cytotoxicities provided by chemotherapeutics alone and chemotherapeutics with either concentration of MLT (10^-3 M and 10^-5 M) were compared using one way analysis of variance (ANOVA) followed by Tukey test.

Results

Cytarabine, ETO and DNR treatment resulted in varying degrees of cytotoxicity to all leukemia cells. MOLT-4 was the most affected cell line by all drugs. The cells from the patient with AML (Patient 2) were the most resistant to all drugs except Ara-C (Table I). Since the direct cytotoxicity of MLT was evaluated in a previous study by our group (8), it was not re-evaluated in this study.

Effect of MLT on Ara-C cytotoxicity. Cytarabine mostly affected the T lymphoblastic leukemia cell lines, Jurkat and MOLT-4. However, it had minimal toxicity on the cells from the patient with T-ALL (Patient 1). In addition, it appeared that Ara-C with either concentration of MLT had a stronger cytotoxic effect on the cells from this patient; however, it was not statistically significant. Cytarabine cytotoxicity on cells from the other patient was also less than that of the cell lines. MLT enhanced Ara-C cytotoxicity without statistical significance in both patient samples. The results were similar in the Ara-C-treated myeloid (HL-60, CMK, K562) and B lymphoblastic leukemia (Daudi) cells with or without melatonin.

Effect of MLT on DNR cytotoxicity. Daudi cells and cells from Patient 2 were the least affected by DNR. Melatonin, especially at 10^-3 M, slightly increased the cytotoxicity in cells from Patient 2 but not in Daudi cells. The highest rates of cytotoxicity were observed in Jurkat, MOLT-4 and HL-60 cell lines. Although some concentrations of MLT increased the DNR toxicity particularly in myeloid leukemia cell lines and cells from Patient 1, the difference was statistically insignificant.

Effect of MLT on ETO cytotoxicity. Etoposide cytotoxicity was more prominent in MOLT-4 and HL-60 than others. Etoposide was the most effective drug on myeloid leukemia cell lines (HL-60, CMK and K562). However, ETO had almost no effect on cells from Patient 2. Etoposide with either concentration of MLT killed the leukemia cells more than ETO-alone in this group (p<0.05). This was the only statistically significant effect of MLT in this study. MLT exerted no effect on ETO cytotoxicity in cell lines and cells from Patient 1.

As a result, MLT neither reduced nor enhanced the cytotoxicity of chemotherapeutic drugs in the studied cell lines significantly; however, it increased the cytotoxicity of ETO in leukemia cells from Patient 2 with AML.
Discussion

There are several studies indicating the beneficial effects of MLT in patients with cancer (1, 2, 13, 14). These observations may be attributed to various actions of MLT. MLT may have protective activities against the adverse effects of chemotherapeutic agents, or may exert additive cytotoxic effects on tumor cells. However, it is not clear if MLT attenuates the effectiveness of antineoplastic drugs on cancer cells. This study investigated the effect of melatonin on the cytotoxicity of Ara-C, ETO and DNR in leukemia cells in vitro, which are the commonly used agents in treatment of childhood leukemia.

Fas-induced cell death was shown to be enhanced by MLT in a human leukemia cell line (Jurkat) via its pro-oxidant activity (20). MLT, at concentrations greater than 10^{-3} M, reduces cell number to 50% of the control after seven days of incubation in human erythroleukemia cell line (K562) (7). Panzer et al. (5) reported that melatonin concentrations of 10^{-5} M to 10^{-3} M have no effect on the growth and cell cycle of human lymphoblastoid cell line (TK6). The cytotoxic effect of 10^{-3} M MLT in CMK, Jurkat and MOLT-4 cells, which was associated with significant reactive oxygen species generation, was previously reported (8). All these studies demonstrate that the effect of MLT on different leukemia cells is related to its concentration.

Although MLT itself has shown some cytotoxic effects on tumor cells in earlier studies, the data presented in this study showed that MLT did not enhance the cytotoxicity of chemotherapeutics in leukemia cells in vitro. None of the drugs used, Ara-C, ETO and DNR, exerted enhanced cytotoxicity when co-incubated with 10^{-5} M and 10^{-3} M concentrations of MLT in Jurkat, MOLT-4, HL-60, Daudi, K562 and CMK cells.

Anthracyclines are effective and widely used chemotherapeutic agents in the treatment of childhood leukemias. One of the dose-limiting side-effects of anthracyclines is cardiotoxicity. Dexrazoxan (ICRF-187) is a well-known and clinically available agent for protection of the heart against anthracycline-induced cardiotoxicity (21). However, there is preliminary in vitro evidence that ICRF-187 antagonizes the antitumor activity of DNR probably due to inhibition of topoisomerase II activity, and potentiates the hematotoxicity of doxorubicin (16, 22, 23). It appears that MLT exerts its protection without demonstrating any side-effects. The beneficial effect of concomitant administration of MLT with chemotherapeutic drugs in patients with hematologic malignancies has been previously demonstrated in many studies (1, 13), and this may be attributable to its protective action on normal tissues rather than its cytotoxic effects on cancer cells. MLT protects peripheral blood mononuclear cells against antiproliferative effects of chemotherapeutic agents in vitro and activates monocytes and induces their cytotoxic properties (24, 25). Oxidative damage induced by doxorubicin in brain, heart, and kidney tissues can be reduced by melatonin (26). MLT significantly attenuates doxorubicin-induced cardiac dysfunction, ultrastructural alterations, and apoptosis in mouse hearts without interfering with its antitumor effect (27). It does not prevent doxorubicin cytotoxicity on mammary adenocarcinoma, colon carcinoma or mouse leukemia cell lines; however, intensifies cytotoxicity of doxorubicin in human keratinocytes, non-small cell lung cancer and laryngeal cancer cell lines as emphasized earlier (6, 15). In this study, MLT did not attenuate the daunorubicin cytotoxicity in human leukemia cell lines or patient cells. Daunorubicin effect was not enhanced by 10^{-5} M or 10^{-3} M

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Table I. Mean cytotoxicity (%) induced by different chemotherapeutics with or without melatonin in different leukemia cells.

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<tr>
<th></th>
<th>HL-60</th>
<th>CMK</th>
<th>K-562</th>
<th>Daudi</th>
<th>Jurkat</th>
<th>MOLT-4</th>
<th>Patient 1 (T-ALL)</th>
<th>Patient 2 (AML)</th>
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<tr>
<td>Ara-C</td>
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<td>Control (alone)</td>
<td>32.0±9.8</td>
<td>41.5±14.8</td>
<td>22.0±4.2</td>
<td>26.0±14.1</td>
<td>47.0±9.8</td>
<td>86.0±4.2</td>
<td>7.0±5.6</td>
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<td>with Mel 10^{-5}</td>
<td>36.00±14.1</td>
<td>39.00±0.0</td>
<td>25.50±13.43</td>
<td>27.50±14.84</td>
<td>36.50±21.92</td>
<td>86.50±4.94</td>
<td>14.00±8.48</td>
<td>28.00±7.07</td>
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<td>with Mel 10^{-3}</td>
<td>25.00±11.3</td>
<td>38.00±8.4</td>
<td>24.50±4.94</td>
<td>31.00±7.07</td>
<td>37.50±14.84</td>
<td>70.00±7.07</td>
<td>27.50±16.26</td>
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<td>Control (alone)</td>
<td>56.50±0.7</td>
<td>25.00±2.8</td>
<td>14.00±11.31</td>
<td>11.50±13.43</td>
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<td>with Mel 10^{-5}</td>
<td>61.50±3.5</td>
<td>37.50±6.3</td>
<td>23.00±9.89</td>
<td>11.50±13.43</td>
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<td>50.50±0.7</td>
<td>39.00±8.4</td>
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<td>48.50±12.02</td>
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<td>Control (alone)</td>
<td>82.50±6.3</td>
<td>63.50±3.5</td>
<td>58.00±7.07</td>
<td>38.50±10.60</td>
<td>68.50±19.09</td>
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<tr>
<td>with Mel 10^{-5}</td>
<td>80.50±3.5</td>
<td>66.00±1.4</td>
<td>71.00±16.97</td>
<td>25.50±10.60</td>
<td>68.50±20.50</td>
<td>98.00±0.00</td>
<td>46.50±0.70</td>
<td>14.50±0.70*</td>
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<tr>
<td>with Mel 10^{-3}</td>
<td>74.00±4.2</td>
<td>62.00±11.3</td>
<td>54.00±5.65</td>
<td>33.00±9.89</td>
<td>63.00±12.72</td>
<td>98.00±0.00</td>
<td>36.50±10.60</td>
<td>5.00±2.82*</td>
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*p<0.05 Melatonin vs. control groups. Death percentages are expressed as mean±standard deviation. Mel: melatonin; Ara-C: cytarabine; DNR: daunorubicin; ETO: etoposide.
concentrations of MLT. However, MLT enhanced the cytotoxicity of ETO only in leukemia cells from a patient with AML. It seems that the effect of MLT is cell type dependent. Although the highest rates of cytotoxicity were observed with ETO treatment in myeloid leukemia cell lines, co-incubation with MLT had no effect.

In conclusion, concomitant treatment of using MLT with drugs does not interfere with the cytotoxicity of commonly used chemotherapeutic agents such as Ara-C, ETO and DNR in leukemia cells.

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