ORIGINAL PAPERS

Adv Clin Exp Med 2006, **15**, 6, 979–982
ISSN 1230-025X

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Effect of Exogenous Melatonin on Rat Haematopoietic Cells Exposed to Cytosine Arabinoside and Etoposide

Wpływ egzogennej melatoniny na układ krwiotwórczy szczurów poddanych działaniu arabinozydu cytozyny i etopozydu

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Abstract

Background. There are reports that melatonin may protect the bone marrow cells from apoptosis induced by the chemotherapy especially by anthracyclines.
Objectives. The aim of the study was to determine whether exogenous melatonin can protect the bone marrow of rats treated with the antitumour drugs cytosine arabinoside (Ara-C) and etoposide (VP-16) widely used in haematology.
Material and Methods. The animals used were 60 male rats Buffalo, divided into six groups administered drugs as following: 1) VP-16 alone; 2) VP-16 + Melatonin; 3) Ara-C alone; 4) Ara-C + Melatonin; 5) Melatonin alone; 6) 0,9% NaCl (control). The animals were sacrificed and peripheral blood and bone marrow were evaluated.
Results Bone marrow hypoplasia was observed in all groups treated with cytotoxic drugs. The number of megakaryocytes and platelets was significantly higher in groups treated with melatonin. There was no statistical differences in granulocyte and erythroid cells.
Conclusions. Obtained data suggest that melatonin may protect megakariopoiesis from Ara-C and VP-16 toxicity (**Adv Clin Exp Med 2006, 15, 6, 979–982**).

Key words: melatonin, cytotoxic drugs, myelosuppression.

Streszczenie

Wprowadzenie. Istnieją doniesienia, że melatonina może chronić komórki szpiku kostnego przed apoptozą wywołaną niektórymi lekami cytostatycznymi, zwłaszcza antracyklinami.
Cel pracy. Ocena, czy egzogenna melatonina może mieć ochronne działanie na układ krwiotwórczy szczurów podczas stosowania w leczeniu nowotworów hematologicznych cytostatyków, takich jak arabinozyd cytozyny (Ara-C) i etopozyd (VP-16).
Materiał i metody. Badanie przeprowadzono na 60 samcach szczurów szczepu Buffalo podzielonych na sześć grup w zależności od stosowanych leków: 1) VP-16; 2) VP-16 + melatonina; 3) Ara-C; 4) Ara-C + melatonina; 5) melatonina; 6) 0,9% NaCl (grupa kontrolna). Następnie oceniano morfologię krwi obwodowej i szpiku kostnego.
Wyniki. Hipoplazję szpiku obserwowano we wszystkich grupach, w których stosowano cytostatyki. Liczba megakariocytów i płytek krwi była istotnie statystycznie wyższa w grupach, w których stosowano melatoninę. Podobnych różnic nie obserwowano w odniesieniu do linii granulocytarnej i erytroidalnej.
Wnioski. Uzyskane dane sugerują, że melatonina może wywierać działanie cytoprotekcyjne na układ płytkotwórczy podczas stosowania Ara-C i VP-16 (**Adv Clin Exp Med 2006, 15, 6, 979–982**).

Słowa kluczowe: melatonina, leki cytotoksyczne, mielosupresja.

Melatonin is an important part of the messenger/receptor network between the central nervous system and the immune system and participates in many physiological functions, such as sleep, circardian rhythm, mood, sexual maturation, reproduction, aging, immune response, and oncostatic actions. Besides its origin in pinealocytes, organs other than the pineal gland produce melatonin, including the retina, ovaries, testes, gut, and also thrombocytes, mononuclear blood cells, and bone marrow cells. Considering chemical structure, melatonin is an indolamine (N-acetyl-5-methoxytryptamine) synthesized via serotonin from tryptophan [1, 2].

Myelosuppression, cardiotoxicity, and nephrotoxicity are the most serious adverse effects of cancer chemotherapy which limit applicable dose intensity. Progenitor cells in bone marrow exhibit continuous proliferation and differentiation and are highly vulnerable to acute or chronic oxidative stress. There are several reports that melatonin may protect bone marrow, heart, and kidney cells from damage induced by cytotoxic drugs by its antioxidant properties and its enhancement of granulocyte-macrophage colony-stimulating factor (GM-CSF) production [3–6].

The protective effect of melatonin against the toxicity of adriamycin and bleomycin is well known [7, 8]. Antimetabolites (cytosine arabinoside, Ara-C) and inhibitors of topoisomerase (etoposide, VP-16) are antitumor drugs widely used in hematology. The aim of the study was to determine whether exogenous melatonin can protect the bone marrow of rats exposed to Ara-C and VP-16.

Material and Methods

The animals used were 60 male Buffalo rats 10–12 weeks of age (200–250 g body weight) obtained from the Department of Pathological Anatomy, Silesian Piasts Univeristy of Medicine in Wrocław. The animals were kept under a 12-h light-dark cycle at 21°C with free access to food and water. The study was approved by the Animal Care Ethics Committee of the Clinical Research of the Silesian Piasts Univeristy of Medicine in Wrocław and was performed according to the guidelines of the Polish Animal Care and Use Committee. The rats were randomly divided into six groups of 10 animals each according drug administration as follows: 1) VP-16 alone, 2) VP-16+melatonin (VP-16 + M), 3) Ara-C alone, 4) Ara-C+melatonin (Ara-C+M), 5) melatonin (M) alone, and 6) 0.9% NaCl (control).

Etoposide (Bristol Myers Squibb) was given in one dose of 80 mg/kg ip. Cytosine arabinoside (Merck) was given in one dose of 50 mg/kg ip. Melatonin (Sigma) was given at a dose of 10 mg/kg i.v. for six days after infusion of the cytotoxic drugs. On day 7 after infusion of the cytotoxic drugs, the animals were sacrificed and peripheral blood (granulocytes, erythrocytes, and platelets) and bone marrow were evaluated. Peripheral blood parameters were counted automatically using a Sysmex counter. Bone marrow smears were stained with May Grunwald Giemsa and examined by light microscopy. Statistical analysis was done using the Mann Whitney *U* test. The results were subjected to statistical analysis using STATISTICA 5.1 PL software (StatSoft, Kraków, Poland).

Results

Bone marrow hypoplasia was observed in all groups treated with cytotoxic drugs with and without melatonin (Fig. 1–4). Significantly higher megakaryocyte and platelet counts was observed

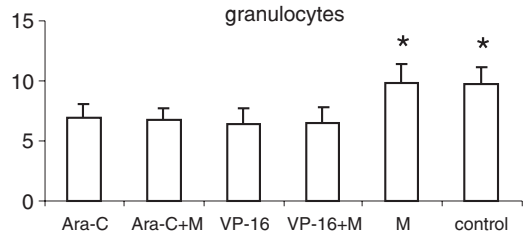

Fig. 1. Level (G/l) of peripheral blood granulocytes. Significant differences * $p < 0.001$ – control and M compared with Ara-C, Ara-C+M, VP-16, and VP-16+M

Ryc. 1. Stężenie (G/l) granulocytów krwi obwodowej. Istotne różnice * $p < 0.001$ między grupą kontrolną i otrzymującą M w porównaniu z grupami otrzymującymi Ara-C, Ara-C+M, VP-16 i VR-16+M

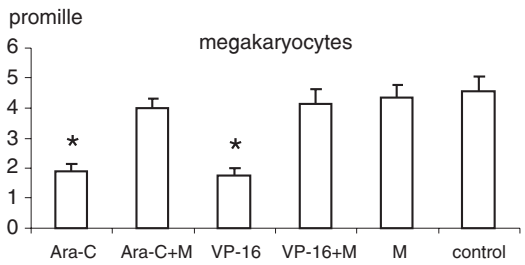

Fig. 2. Level [promille] of megakaryocytes in rat bone marrow. Significant differences * $p < 0.001$ – Ara-C and VP-16 compared with Ara-C+M, VP-16+M, control, and M

Ryc. 2. Stężenie (promile) megakariocytów w szpiku kostnym. Istotne różnice * $p < 0.001$ między grupami otrzymującymi Ara-C i VP-16 a otrzymującymi Ara-C+M, VP-16+M, kontrolną i otrzymującą M

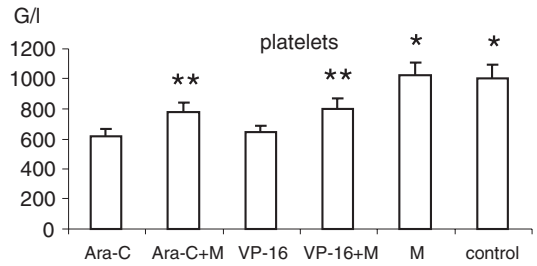

Fig. 3. Level (G/l) of peripheral blood platelets. Significant differences * $p < 0.001$ – control and M compared with Ara-C, Ara-C+M, VP-16, and VP-16+M; ** $p < 0.05$ – Ara-C+M and VP-16+M compared with Ara-C and VP-16

Ryc. 3. Stężenie (G/l) płytek krwi obwodowej. Istotne różnice * $p < 0.001$ między grupami: kontrolną i otrzymującą M w porównaniu z otrzymującą Ara-C, Ara-C+M, VP-16 i VP-16+M; istotne różnice ** $p < 0.05$ między grupami otrzymującymi Ara-C+M i VP-16 a Ara-C i VP-16

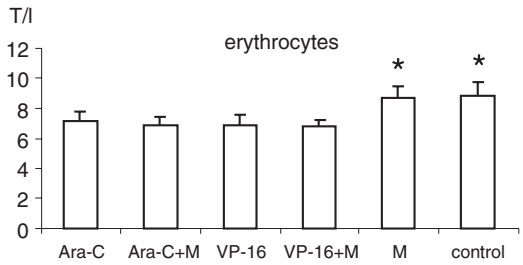

Fig. 4. Level (T/l) of peripheral blood erythrocytes. Significant differences * $p < 0.001$ – control and M compared with Ara-C, Ara-C+M, VP-16, and VP-16+M

Ryc. 4. Stężenie (T/l) erytrocytów krwi obwodowej. Istotne różnice * $p < 0.001$ między grupami: kontrolną i M a otrzymującą Ara-C, Ara-C+M, VP-16 i VP-16+M

in the Ara-C+M and VP-16+M groups compared with the groups treated without melatonin (Fig. 2–3). There were no statistical differences in the mean percentages of granulocytes and erythroid cells (Fig. 1 and 4).

Discussion

Chemotherapy still remains the most widely used tool in antitumor therapy. Melatonin appears to be a promising drug for combination with chemotherapy because of its antioxidant, immunoenhancing, and cytoprotective properties. It was reported that melatonin can decrease the toxicity of cytotoxic drugs without interfering with their anticancer action. In addition, melatonin is a substance without any severe adverse effects and therefore administration with chemotherapy does not increase its toxicity. [3–11]. Bone marrow is extremely vulnerable to endogenous and exogenous oxidative insults, such as environmental pollutants, ionizing irradiation, and cancer chemotherapeutic agents. It has been shown that melatonin is a potent scavenger of highly toxic oxygen-centered radicals, much more efficient than other antioxidants (e.g. mannitol, glutathione, vitamin E). This activity, not mediated by receptors, protects DNA from damage and reduces the risk of DNA mutations and consequent neoplasia. Recent reports confirm that melatonin protects the bone marrow of rats treated with myelotoxic drugs. However, the antioxidative activity of melatonin seems to need a higher level than normal and probably occurs only at pharmacological concentrations [6, 12].

Specific melatonin receptors have been described in rodent and human lymphocytes, granulocytes, thymocytes, splenocytes, and bone marrow stroma. Their activation in the bone marrow, with both physiological and pharmacological melatonin concentrations, results in enhanced release of interleukin (IL)-1, IL-2, IL-4, IL-6, tumor necrosis factor, interferon gamma, and opioid cytokines. These substances play significant roles in the correct proliferation, maturation, and stimulation of various immune effector cells, such as thymocytes, cytotoxic T lymphocytes, B lymphocytes, NK cells, and phagocytes [2, 6, 13, 14]. Melatonin enhances the synthesis of GM-CSF via IL-4 and in this way inhibits the apoptosis of myeloid progenitor cells induced by cytotoxic drugs. However, the cytoprotective effect of melatonin was noted mainly in granulocyte and macrophage lines. In contrast, melatonin does not protect more primitive, multipotent progenitor cells from the toxic effect of antitumor compounds, but rather decreases their number in the culture [7].

In this study, melatonin did not reduce the toxicity of Ara-C and VP-16 in granulocytes and erythroid cells of rat bone marrow, but seemed to have a significant protective effect on megakaryocytes. Reduced platelet count is a common complication of cancer. The etiology of thrombocytopenia includes chemotherapy-induced myelosuppression, bone marrow infiltration, and disseminated intravascular coagulation (DIC). It was reported that melatonin may not only prevent myelosuppresion during the chemotherapy, but may also contribute to its realization in metastatic cancer patients unable to tolerate the chemotherapeutic approach because of persistent thrombocytopenia [15]. There is also a report that melatonin plus a low dose of IL-2 not only neutralizes IL-2-induced thrombocytopenia, but also normalizes platelet counts in cancer patients with thrombocytopenia [16].

This study shows that the higher megakaryocyte and platelet counts in the groups treated with Ara-C and VP-16 plus melatonin may have clinical value in the therapeutic usage of this drug combination, but this needs further investigation.

These data show that melatonin does not reduce the toxicity of arabinoside cytosine and etoposide in granulocyte and erythroid lines of rat bone marrow. The higher megakaryocyte and platelet counts in the groups treated with melatonin need further investigation.

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Conflict of interest: None declared

Received: 3.01.2006
Revised: 8.11.2006
Accepted: 8.11.2006

Praca wpłynęła do Redakcji: 3.01.2006 r.
Po recenzji: 8.11.2006 r.
Zaakceptowano do druku: 8.11.2006 r.