Asian Journal of Chemistry Vol. 20, No. 4 (2008), 2997-3001

Comparison of the Effects of Melatonin and Granulocyte-Colony Stimulating Factor in Cyclophosphamide Induced Cytopenic Rats

MUSTAFA BUYUKAVCI*, MEHMET EMIN BUYUKOKUROGLU† and MEHMET KOC‡
Division of Pediatric Oncology, Faculty of Medicine, Ataturk University
Lojmanlari, 50/2 Erzurum, Turkey
Fax: (90)(442)2361301; Tel: (90)(442)2361212(1059)
E-mail: buyukavci@hotmail.com; mbavci@atauni.edu.tr

Peripheral cytopenias including leukopenia, anemia and thrombocytopenia are important side effects of current chemotherapeutic regimens. Melatonin has been demonstrated to ameliorate some of these complications and the agent granulocyte-colony stimulating factor (G-CSF) has been widely used in treatment of chemotherapy-induced neutropenia. In this study, we compared the protective effects of melatonin and G-CSF in cyclophosphamide-induced cytopenic rats. White blood cell counts, hemoglobin levels and platelet counts were measured in six groups of animals: (i) control; (ii) cyclophosphamide (CPM) injected; (iii) melatonin injected; (iv) G-CSF injected; (v) CPM plus melatonin injected; (vi) CPM plus G-CSF injected. White blood cell counts, hemoglobin levels and platelet counts were significantly higher in cyclophosphamide plus melatonin treated rats than in cyclophosphamide alone treated animals. In the G-CSF plus cyclophosphamide treated group, only white blood cell counts were higher than the cyclophosphamide group. However, G-CSF raised the white blood cell count more than melatonin when administered following cyclophosphamide. Although melatonin has beneficial effects on the chemotherapy-induced anemia and thrombocytopenia, it is not as effective as G-CSF for preventing the leukopenia.

Key Words: Melatonin, G-CSF, Neutropenia, Rat.

INTRODUCTION

Melatonin, a major secretory product of the pineal gland, plays an important role in a number of physiological and pathophysiological processes, including circadian rhythm regulation, free radical scavenging, immunomodulation and apoptosis[1-3]. The association of melatonin with cancer has also been widely investigated. It was documented that melatonin possesses a wide range of activities in cancer growth and treatment[4-9].

†Department of Pharmacology, Faculty of Medicine, Ataturk University, Erzurum, Turkey.
‡Department of Radiation Oncology, Faculty of Medicine, Ataturk University, Erzurum, Turkey.

Melatonin augments the anticancer effect of chemotherapeutic drugs while decreasing some of the toxic side effects[10,11]. It has been shown to exert colony-stimulating activity *via* some cytokines such as interleukin-4 and to rescue myeloid precursor cells from apoptosis induced by chemotherapeutics[12,13]. It has also beneficial effects in the treatment of thrombocytopenia due to different causes including malignancies and reduces the frequency of chemotherapy-related thrombocytopenia in cancer patients[14-16].

Myelosuppression is a dose-limiting toxicity that prevents the administration of high doses of cytotoxic drugs especially in hematologic malignancies. Growth factors such as filgrastim, although very expensive, are widely used for amelioration of myelotoxicity resulting from prescribed toxic chemotherapeutic regimens. Either melatonin or granulocyte-colony stimulating factor (G-CSF), filgrastim, were previously shown to protect or stimulate the bone marrow in rats[17,18]. The aim of this study was to evaluate the protective effect of melatonin on chemotherapy-induced peripheral cytopenia in rats and to compare with the effect of G-CSF.

EXPERIMENTAL

48 Albino adult male rats (Sprague-Dawley strain) weighing 150-175 g were used and housed in an air-conditioned room (22 ± 1 ºC) with controlled 14 h light /10 h dark cycles. Rats were fed with standard laboratory chow and water, *ad libitum* and randomly divided into 6 equal groups (n = 8): 1) control; 2) cyclophosphamide (CPM) injected; 3) melatonin injected; 4) G-CSF injected; 5) CPM plus melatonin injected and 6) CPM plus G-CSF injected. CPM (Endoxan, Astra Medica Ag., Frankfurt, Germany) was administered as a single dose (75 mg/kg, intraperitoneally) on the first day of experiment. Melatonin (Sigma Chemical Co.) was dissolved in ethanol and then diluted with isotonic NaCl. It was injected daily (100 µg/dose, subcutaneously) for 8 consecutive days following the first day. G-CSF, filgrastim (Neupogen, F. Hoffmann-La Roche Ltd., Basel, Switzerland), was also injected (100 µg/kg, subcutaneously) in a same manner with melatonin. On tenth day of treatment, animals in all groups were anesthetized with tiopenthal sodium (50 mg/kg) and 2 mL blood samples were taken by cardiac puncture.

Hemoglobin levels, white blood cell (WBC) and platelet counts were determined with an automatic counter (Coulter Gen-S, Coulter Corporation, Miami, USA).

Statistical analyses were done using Student's-t test with the aid of the SPSS for Windows 10.0 package program.

RESULTS AND DISCUSSION

Hb levels, WBC and platelet counts were significantly reduced in CPM-treated rats in comparison with control animals. There was no statistically significant difference between the data of group 3 (Melatonin) and control. However, mean Hb levels and the WBC counts were higher in group 4 (G-CSF) with respect to control.

White blood cell counts, hemoglobin levels and platelet counts were significantly higher in cyclophosphamide plus melatonin treated rats than in cyclophosphamide alone treated animals. In the G-CSF plus cyclophosphamide treated group, only white blood cell counts were higher than the cyclophosphamide alone group. However, G-CSF raised the white blood cell count more than melatonin when administered following cyclophosphamide (Table-1).

TABLE-1
WHITE BLOOD CELL (WBC) COUNTS, HEMOGLOBIN (Hb) LEVELS AND PLATELET COUNTS (MEAN ± SE) IN CONTROL AND STUDY GROUPS

Groups	WBC (μL)	Hb (g/dL)	Platelet (10^3/μL)
Control	5300 ± 384	14.42 ± 0.30	1138 ± 71
CPM	2175 ± 77[b]	11.52 ± 0.37[b]	449 ± 57[b]
Melatonin	5775 ± 443	15.03 ± 0.08	1177 ± 32
G-CSF	6600 ± 408[a]	15.70 ± 0.37[a]	1122 ± 23
CPM + Melatonin	3712 ± 590[c]	13.43 ± 0.21[d]	680 ± 52[c]
CPM + G-CSF	7175 ± 630[d]	11.98 ± 0.35	392 ± 21

CPM: cyclophosphamide, G-CSF: granulocyte-colony stimulating factor
[a]$p < 0.05$, [b]$p < 0.001$ *vs.* group 1 (control);
[c]$p < 0.05$, [d]$p < 0.001$ *vs.* group 2 (CPM-alone treated).

Intraperitoneal administration of single dose CPM (50-75 mg/kg) induces prominent neutropenia[17-19]. We used this procedure and obtained marked bone marrow suppression represented by a significant decrease in WBC and platelet counts and Hb level.

Anwar *et al.*[18] reported that 10 d of intraperitoneally injected melatonin (100 μg/kg/dose) induced a significant increase in WBC and platelet counts. Nohynek *et al.*[17] reported that WBC count, but not RBC and platelets, were higher than control in rats receiving subcutaneous 100 μg/kg/dose of G-CSF for 4 d. In this study, daily subcutaneous injection of G-CSF alone produced a statistical significant rise in WBC count and Hb levels with respect to control group. However, melatonin injection did not lead to meaningful increase in those parameters. It means that G-CSF stimulated the production of WBC and erythrocyte but melatonin did not.

We found that melatonin has significant protective effect against to CPM induced cytopenia. Hb concentrations, WBC and platelet counts were significantly higher in CPM plus melatonin group than CPM only-treated rats. However, in CPM plus G-CSF group, only WBC count was higher than CPM group. These results were consistent with previous studies indicating a marked increase only in WBC of G-CSF treated rats as compared with CPM only-treated group[17]. Single-dose administration of G-CSF prior to chemotherapy protects WBC count in rats exposed to chemotherapeutics[20]. Anwar *et al.*[18] previously reported that concomitantly injected melatonin result in a significant increase in platelet counts but not leukocyte count in comparison with 10 d of aracytin treatment only. However, 10 d of daily melatonin treatment following aracytin therapy leads to an elevated leukocyte count. This may be associated with the myelosuppressive effect of aracytin which gradually disappears after discontinuation of therapy. In preliminary studies, we also observed the gradual rise of WBC in the fourth or fifth day after CPM injection.

The protective effect of melatonin on Hb levels and platelets may be the result of its ability as a scavenger for free radicals generated by cytotoxic drugs and its stimulatory effect on bone marrow cells[21-23]. G-CSF mobilizes not only granulocyte-macrophage but also erythroid progenitor cells[24]. In this study, however, G-CSF increased neither Hb levels nor platelet counts during the recovery phase of the WBC count following CPM administration. However, the WBC count was significantly higher in CPM plus G-CSF than CPM plus melatonin treated groups. Although it had no effect on thrombocytopenia and anemia, chemotherapy induced leukopenia was more protected by G-CSF than melatonin.

In conclusion, although both of melatonin and G-CSF reduced chemotherapy-induced reduction of WBC, this effect was more apparent when G-CSF was used. Melatonin, however, restored both the Hb concentrations and platelet counts in CPM-treated rats. These results indicated that although melatonin has beneficial effects on the chemotherapy-induced anemia and thrombocytopenia, it is not as effective as G-CSF for preventing the leukopenia.

REFERENCES

1. D.X. Tan, L.D. Chen, B. Poeggeler, L.C. Manchester and R.J. Reiter, *Endocr. J.*, **1**, 57 (1993).
2. A. Brzezinski, *N. Engl. J. Med.*, **336**, 186 (1997).
3. R.M. Sainz, J.C. Mayo, C. Rodriguez, D.X. Tan, S. Lopez-Burillo and R.J. Reiter, *Cell. Mol. Life Sci.*, **60**, 407 (2003).
4. J. Petranka, W. Baldwin, J. Biermann, S. Jayadev, J.C. Barrett and E. Murphy, *J. Pineal. Res.*, **26**, 129 (1999).
5. M.A. El-Missiry and A.F. Abd El-Aziz, *Cancer Lett.*, **151**, 119 (2000).

6. M.M. Marelli, P. Limonta, R. Maggi, M. Motta and R.M. Moretti, *Prostate*, **45**, 238 (2000).
7. R.M. Moretti, M.M. Marelli, R. Maggi, D. Dondi, M. Motta and P. Limonta, *Oncol. Rep.*, **7**, 347 (2000).
8. Vijayalaxmi, C.R. Jr. Thomas, R.J. Reiter and T.S. Herman, *J. Clin. Oncol.*, **20**, 2575 (2002).
9. M.M. Leon-Blanco, J.M. Guerrero, R.J. Reiter, J.R. Calvo and D. Pozo, *J. Pineal. Res.*, **35**, 204 (2003).
10. A. Panzer and M. Viljoen, *J. Pineal. Res.*, **22**, 184 (1997).
11. R.J. Reiter, D.X. Tan, R.M. Sainz, J.C. Mayo and S. Lopez-Burillo, *J. Pharm. Pharmacol.*, **54**, 1299 (2002).
12. G.J. Maestroni, A. Conti and P. Lissoni, *Cancer Res.*, **54**, 4740 (1994).
13. G.J. Maestroni, *Ann. N. Y. Acad. Sci.*, **840**, 411 (1998).
14. P. Lissoni, S. Barni, F. Brivio, F. Rossini, L. Fumagalli and G. Tancini, *J. Biol. Regul. Homeostat. Agents*, **9**, 52 (1995).
15. P. Lissoni, G. Tancini, S. Barni, F. Paolorossi, F. Rossini, P. Maffe and L. Di Bella, *Recenti. Prog. Med.*, **87**, 582 (1996).
16. P. Lissoni, S. Barni, M. Mandala, A. Ardizzoia, F. Paolorossi, M. Vaghi, R. Longarini, F. Malugani and G. Tancini, *Eur. J. Cancer*, **35**, 1688 (1999).
17. G.J. Nohynek, J.P. Plard, M.Y. Wells, A. Zerial and F. Roquet, *Cancer Chemother. Pharmacol.*, **39**, 259 (1997).
18. M.M. Anwar, H.A. Mahfouz and A.S. Sayed, *Comp. Biochem. Physiol.*, **119**, 493 (1998).
19. L. van der Laan, W.J. Oyen, E.C. Tan, A.A. Verhofstad, T. Hendriks and R.J. Goris, *J. Surg. Res.*, **82**, 346 (1999).
20. H. Ozan, F. Ozkalemkas, U. Ozan, K. Ozerkan, T. Bilgin and F. Kucukyildiz, *Eur. J. Gynaecol. Oncol.*, **22**, 463 (2001).
21. V. Rapozzi, S. Zorzet, M. Comelli, I. Mavelli, L. Perissin and T. Giraldi, *Life Sci.*, **63**, 1701 (1998).
22. G.J. Maestroni, *Adv. Exp. Med. Biol.*, **460**, 95 (1999).
23. L. Di Bella, C. Bruschi and L. Gualano, *Med. Sci. Monit.*, **8**, BR527 (2002).
24. W.P. Sheridan, C.G. Begley, C.A. Juttner, J. Szer, L.B. To, D. Maher, K.M. McGrath, G. Morstyn and R.M. Fox, *Lancet*, **339**, 640 (1992).

(*Received*: 20 July 2007; *Accepted*: 14 January 2008) AJC-6206