in vivo *22*: 397-400 (2008)

A Study of Immunoendocrine Strategies with Pineal Indoles and Interleukin-2 to Prevent Radiotherapy-induced Lymphocytopenia in Cancer Patients

P. LISSONI[1], F. ROVELLI[1], F. BRIVIO[2], L. FUMAGALLI[2] and G. BRERA[3]

[1]*Division of Radiation Oncology and* [2]*Division of Surgery, San Gerardo Hospital, Monza, Milan;* [3]*Ambrosian University, Milan, Italy*

Abstract. *Background: Lymphocytopenia represents one of the most evident side-effects of radiotherapy (RT), particularly in the case of irradiation of pelvis, since it is the main location of bone-marrow proliferating cells in adults. Because of the fundamental role of lymphocytes in suppressing anticancer immunity, RT-induced lymphocytopenia could negatively influence the prognosis of cancer patients and the therapeutic efficacy of RT itself. In experimental conditions, the biological toxicity of irradiation appeared to be reduced by antioxidant agents, such as pineal hormones melatonin. A preliminary study was conducted to evaluate the influence of different immunobiological strategies with pineal indoles melatonin (MLT), 5 methoxytriptamine (5-MTT) or low-dose IL-2, the lymphocyte growth factor, on pelvic irradiation-induced lymphocytopenia in cancer patients suffering from rectal cancer or uterine cervix carcinoma. Patients and Methods: The study included 20 consecutive patients, who underwent pelvic irradiation for a total dose of 50.4 Gy. The patients were randomized to be concomitantly treated with MLT alone, with MLT plus 5-MTT or with s.c. low-dose IL-2 . Results: RT induced a significant decline in the mean number of lymphocytes while neither MLT alone, nor MLT plus 5-MTT were able to significantly reduce this decline. Conversely, IL-2 caused a statistically significant reduction of the RT-induced effect, so that the mean number of lymphocytes was significantly higher in patients concomitantly treated by IL-2 than in the other groups. Conclusion: This preliminary study showed that low-dose IL-2 was sufficient to reduce, even though not to completely abrogate, RT-induced lymphocytopenia. Further studies with different schedules and doses of IL-2 will be required to optimize the protective effect of IL-2 on irradiation-induced lymphocytopenia in humans.*

Correspondence to: Dr. Paolo Lissoni, Divisione di Radioterapia Oncologica, Ospedale S. Gerardo, 20052 Monza, Milano, Italy. Fax: +39 039 2332284, e-mail: p.lissoni@hsgerardo.org

Key Words: Interleukin-2, ionizing radiations, pineal hormones, radiotherapy.

Lymphocytopenia is one of the most unfavourable prognostic biological markers in cancer patients, since it has been proven to be associated with a poor prognosis in terms of both survival time (1, 2) and response to cancer chemotherapy (3). Lymphocytopenia may depend on cancer progression itself, or be induced by the various anticancer therapies. In fact, antitumor treatments, namely high-dose poly-chemotherapies (4) and radiotherapy (5), may determine an evident decline in lymphocyte count. Moreover, among the various radio-therapeutic strategies, pelvic irradiation represents the most lymphocytopenic radiotherapy (6), since in adults actively proliferating bone-marrow is particularly located in the pelvic region.

According to current knowledge, the main strategy to enhance lymphocyte number consists of the administration of the fundamental growth factor for lymphocytes, interleukin-2 (IL-2) (7). Unfortunately, no clinical study has been performed to evaluate the influence of IL-2 on radiotherapy-induced lymphocyte decline.

It should be noted that lymphocyte proliferation is not only regulated by the cytokine network, but it is also modulated by the endocrine system, in particular, it was shown that lymphocyte proliferation may be inhibited by cortisol (8) and amplified by the pineal neuro-hormone melatonin (MLT) (9), that appeared to stimulate the release of IL-2 by T helper lymphocytes, which may express MLT receptors (10). MLT, however, is not the only pineal neuro-hormone to stimulate lymphocyte proliferation, at least one other pineal indole, 5-methoxytryptamine (5-MTT), possibly exerting immuno-modulating effects (11).

Finally, other neuroactive substances, namely the endocannabinod agents such as anandamide (12), may have immunostimulating actions on several immune functions, including the anticancer immunity. Moreover, in addition to their well-documented immuno-stimulatory effects, both MLT (13) and 5-MTT (14) have appeared to play an evident antioxidant activity and to protect against irradiation toxicity on bone-marrow cell proliferation. In particular, high-dose

Table I. *Clinical characteristics of cancer patients undergoing pelvic irradiation.*

Characteristics	Controls	MLT	MLT + 5-MTT	IL-2
N40	8	6	6	
M/F	11/29	3/5	1/5	2/4
Median age(years)	57 (32-72)	56(38-71)	58 (42-68)	59 (39-73)
Tumor histiotype				
Cervical carcinoma	22	5	4	2
Rectal carcinoma	18	3	2	4

MLT=melatonin; 5-MTT=5-methoxytryptamine; IL-2= interleukin-2.

Table II. *Percent of patients with lymphocyte count greater than 1000/mm^3 at the end of pelvic irradiation (50.4 Gy).*

Patients +	Percent
Controls	1/40 (3%)
Uterine cervix	0/22
Rectal carcinoma	1/18 (6%)
MLT	0/8
MLT + 5-MTT	0/6
IL-2	5/6 (83%)*

*$p<0.001$ *vs*. Controls. MLT=melatonin; 5-MTT= 5-methoxytryptamine; IL-2= interleukin-2.

MLT was proven to prevent radiation-induced lymphocyte damage by enhancing DNA-repairing mechanisms (15).

Such neuroimmunobiological knowledge suggests several potential biological strategies to prevent the irradiation-induced decline in lymphocyte count and, in particular, to counteract the negative influence of RT on lymphocyte number and activity in cancer patients. The present preliminary study was performed to evaluate the influence of biological strategies with MLT, 5-MTT or low-dose IL-2 on lymphocyte number in cancer patients undergoing pelvic radiotherapy.

Patients and Methods

The study included 20 consecutive cancer patients, who underwent pelvic irradiation for rectal cancer or uterine cervix carcinoma. The clinical characteristics of thosepatients are reported in Table I.

They were randomized to receive MLT alone, MLT plus 5-MTT or *s.c.* low-dose IL-2. The eligibility criteria were: histologically-proven locally-limited uterine cervix carcinoma or rectal carcinoma, measurable lesions, no previous chemotherapy or radiotherapy and no concomitant treatments with steroid or other immuno-modulating agents. The experimental protocol was explained to each patient and written consent was obtained. The control group comprised 40 age- and sex-matched cancer patients affected by the same tumor histotypes.

In both groups of patients, radiotherapy consisted of 1.8 Gy daily fraction, for 5 days/week for 5 consecutive weeks, for a total dose of 50.4 Gy. MLT was given orally at 20 mg/day in the evening.

5-MTT was also given orally at 1 mg/day in the afternoon. Both pineal hormones were given every day until the end of RT, starting 7 days before the onset of RT, as an induction phase. IL-2 was injected *s.c.* at a dose of 3 MIU/ day for 5 consecutive days until the end of treatment, starting 7 days prior to RT, as an induction phase.

To evaluate the lymphocyte count, venous blood samples were collected in the morning after an overnight fast, before the onset of RT and at weekly intervals until the end of treatment. The absolute number of circulating lymphocytes was evaluated for each blood sample. Moreover, because of their importance in anticancer immunity (7), T-helper lymphocytes (CD4) were also measured by a flow cytometric assay and monoclonal antibodies supplied by Becton-Dickinson (Genova, Italy).

The data were reported as mean ± SE and statistically evaluated by the Student's *t*-test, the Chi-square test and the analysis of variance, as appropriate.

Results

The changes in total lymphocyte and CD4 cell mean numbers observed in the control group of patients with uterine cervix or rectal carcinomas are illustrated in Figure 1, while those found in control patients and in patients treated by the different immunobiological strategies for total lymphocytes and T-helper lymphocytes are illustrated in Figures 2 and 3, respectively.

As shown in Figure 1, the mean number of both total lymphocytes and $CD4^+$ observed on pelvic irradiation were significantly lower with respect to the pre-treatment values during the whole period of RT, both in patients with uterine cervix carcinoma and in those with rectal adenocarcinoma.

As reported in more detail in Table II, a lymphocyte count greater than 1000/mm^3 at the end of RT was observed in only 1/40 (3%) patients of the control group, in none of the MLT or MLT plus 5-MTT treated patients, and in 5/6 (83%) patients treated with low-dose IL-2.

The percent of patients with a lymphocyte count greater than 1000/mm^3 at the end of RT observed in the group concomitantly treated with IL-2 was significantly higher than that found in the other groups ($p<0.001$).

In addition, as shown in Table III, the mean percent of decline in total lymphocyte mean count observed in patients treated with IL-2 was significantly lower compared to other groups of cancer patients.

Moreover, as illustrated in Figure 2, the mean lymphocyte counts in patients concomitantly treated with low-dose IL-2 was significantly higher than in the other groups during the whole period of pelvic irradiation, even though they had significantly decreased with respect to the pre-treatment count.

Finally, as illustrated in Figure 3, a significant decrease in the mean number of $CD4^+$-cells during pelvic irradiation occurred in control patients, and in those treated with MLT or MLT plus 5-MTT. On the contrary, the $CD4^+$-cell mean number also decreased in patients concomitantly treated with low-dose IL-2, however without statistical significance with respect to the values found prior to radiotherapy. No

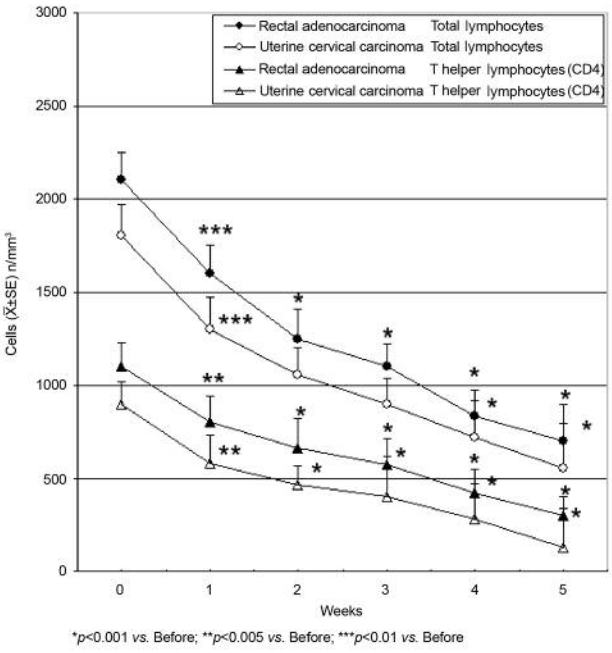

Figure 1. *Changes in the mean numbers of total lymphocytes and T helper lymphocytes (CD4) under pelvic irradiation in rectal cancer and uterine cervcal carcinoma patients treated with radiotherapy alone (50.4 Gy).*

important IL-2-related toxicity occurred, since the only side-effects were fever and asthenia during the first days of injection and, in particular, no IL-2-related thrombocytopenia or anemia occurred.

Discussion

Among the variety of biological strategies investigated in the present preliminary study attempting to prevent irradiation-induced lymphocytopenia, low-dose IL-2 immunotherapy proved to be the only approach able to reduce, even though not completely abrogate, RT-related declines in total lymphocyte count, as well as the CD4-subset, which plays a fundamental role in antitumor immunity by releasing IL-2 itself (7). No one biological strategy was effective in preventing RT-induced lymphocytopenia, including the administration of the pineal hormones MLT and 5-MTT. This failure is not surprising, since the pineal hormones were proven to prevent irradiation-induced lymphocyte damage in experimental conditions only at very high pharmacological doses (15), >30 times the pharmacological dosage utilized in the present study. Therefore, further studies with higher doses of MLT and 5-MTT will be required before excluding the potential efficacy of the pineal hormones in preventing RT-induced lymphocytopenia in humans, wich has been observed under experimental conditions in animals (15).

Table III. *mean decline in lymphocyte number in cancer patients treated with radiotherapy (RT) alone as controls, RT+ melatonin(MLT), RT + Mlt + 5-methoxytryptaniine (5-MTT) or RT + interleukin-2 (IL-2).*

Patients	Percent of decline (X±SE)
Controls	75±4%
Rectal cancer	76±6%
Cervical cancer	73±5%
MLT	69±6%
MLT+5-MTT	65±8%
IL-2	36±5% *

*$p<0.001$ *vs.* Controls; $p<0.025$ *vs.* MLT; $p<0.05$ *vs.* MLT + 5-MTT.

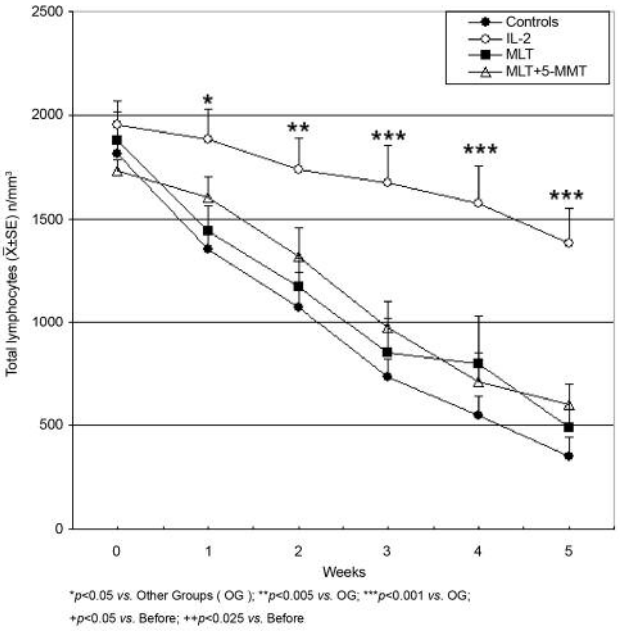

Figure 2. *Changes in the mean number of total lymphocytes in cancer patients treated with pelvic radiotherapy (RT) alone as a control group, or with RT plus melatonin (MLT), RT plus MLT plus 5-methoxytryptamine (5-MTT) or RT plus low-dose interleukin-2 (IL-2).*

Moreover, as previously demonstrated in patients with metastatic disease (16), the pineal hormones have been proven to enhance the antitumor efficacy of IL-2 and amplify IL-2-induced lymphocytosis. Therefore, they could be utilized in association with low-dose IL-2 to potentiate the IL-2-related prevention of lymphocyte decline determined by ionizing irradiation.

In the same way, the efficacy of IL-2 to prevent radiotherapy-induced lymphocytopenia is not surprising, since IL-2 is the physiological growth factor for lymphocytes (7). Moreover, in addition to the direct stimulatory effect of IL-2 on lymphocyte proliferation and differentiation, should be considered the potential role of tumor necrosis factor-alpha (TNF) released in response to IL-2 (17), since TNF has been proven to exert a radio-protective action on normal cells and a radio sensitizing

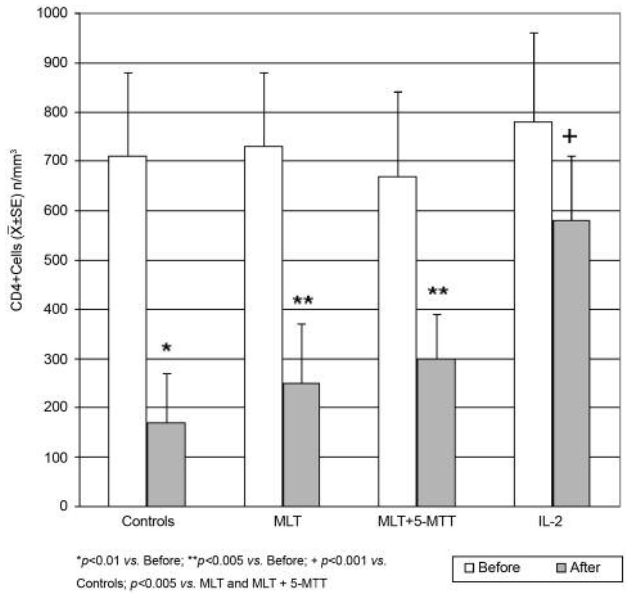

Figure 3. *T-helper lymphocyte (CD4) mean number observed before and after pelvic radiotherapy alone or in association with melatonin(MLT), MLT plus 5-methoxytryptamine (5-MTT) ,or interleukin-2 (IL-2).*

effect on cancer cells. Therefore, IL-2-prevention of irradiation-induced lymphocytopenia could be mediated, at least in part, by TNF produced in response to IL-2 itself.

Moreover, this study would suggest that the radio-protective action of IL-2 is particularly evident for T-helper lymphocytes, whose decline was less pronounced with respect to total lymphocytes.

As the lymphocyte count still decline under radiotherapy plus IL-2, lymphocyte subsets other than CD4 appear to be of less relevance in the generation of anticancer immunity in comparison to the fundamental role played by T-helper lymphocytes. The importance of $CD4^+$-cells in the prognosis of cancer patients was suggested by recent evidence of poor prognosis in advanced cancer patients with CD4 lymphocytopenia (18). Therefore, further studies in a greater number of patients, which analyze both the number and function of the different lymphocyte subpopulations, will be required to confirm and to better understood the protective action of IL-2 on irradiation-induced lymphocytopenia, which could have important clinical and military implications.

From a clinical oncological point of view, because of the well-documented negative prognostic significance of lymphocytopenia in cancer patients (1-3), the prevention of irradiation-induced lymphocyte decline by low-dose IL-2 could enhance the anticancer therapeutic effects of radiotherapy itself. On the other hand, it is known that dramatic lymphocyte damage is one of the main causes of ionizing radiation-induced death (15, 19). From a wide-view point , IL-2 could protect against lymphocyte damage during radio therapy for cancer treatment, but more generally from other irradiations, including that from atomic explosions, as an extreme example.

References

1 Riesco A: Five-years cancer cure:relation to total amount of peripheral lymphocytes and neutrophils. Cancer *25*: 135-140, 1970.

2 Maitoni M, Pirovano M, Nanni O *et al*: Biological indices predictive of survival in 519 Italian terminally ill cancer patients. J Pain Symptom Manage *13*: 1-9, 1997.

3 Lissoni P, Brivio F, Fumagalli L *et al*: Efficacy of cancer chemotherapy in relation to the pre-treatment number of lymphocytes in patients with metastatic solid tumors. Int J Biol Mark *19*: 135-140, 2004.

4 Blay JY, Chauvin F, Le Cesne A *et al*: Early lymphopenia after cytotoxic chemotherapy as a risk factor for febrile neutropenia. J Clin Oncol *14*: 639-643, 1996.

5 Miller GM, Kajioka EH, Andres ML and Gridley DS: Dose and timing of total-body irradiation mediate tumor progression and immunomodulation. Oncol Res *13*: 9-18, 2002.

6 Yamazaki H, Yoshioka Y, Inoue T *et al*: Changes in natural killer cell activity by external radiotherapy and/or brachytherapy. Oncol Rep *9*: 359-363, 2002.

7 Morgan DA, Ruscetti FW and Gallo RG: Selective *in vitro* growth of T lymphocytes from normal human bone marrow. Science *193*: 1007-1008, 1976.

8 Claman HN: Corticosteroids and the immune system. Adv Exp Med Biol *245*: 203-209, 1998.

9 Maestroni GJM: The immunoneuroendocrine role of melatonin. J Pineal Res *14*: 1-10, 1993.

10 Guerrero JM and Reiter RJ: Melatonin-immune system relationships. Curr Topics Med Chem *2*: 167-180, 2002.

11 Sze SF,Liu WK and Ng TB: Stimulation of murine splenocytes by melatonin and methoxytryptamine. J Neural Transm Gen Sect *94*: 115-126, 1993.

12 Grotenhermen F: Pharmacology of cannabinoids. Neuroendocrinol Lett *25*: 14-23, 2004.

13 Reiter R, Tang L, Garcia JJ and Munoz-Hoyos A: Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci *60*: 2255-2271, 1997.

14 Sacco S, Aquiiini L, Ghezzi P, Pinza M and Guglielmotti A: Mechanism of the inhibitory effect of melatonin on tumor necrosis factor production *in vivo* and *in vitro*. Eur J Pharmacol *343*: 249-255, 1998.

15 Vijayalaxmi, Thomas CR, Russel JR and Herman TS: Melatonin: from basic research to cancer treatment clinics. J Clin Oncol *20*: 2575-2601, 2002.

16 Conti A and Maestroni JGM: The clinical neuroimmunotherapeutic role of melatonin in oncology. J Pineal Res *19*: 103-110, 1995.

17 Beutier B and Cerami A: Cachectin: more than a tumor necrosis factor. N Engl J Med *316*: 379-385, 1987.

18 Borg C, Ray-Coquard I, Philip I *et al*: CD4 lymphopenia as a risk factor for febrile neutropenia and early death after cytotoxic chemotherapy in adult patients with cancer. Cancer *101*: 2675-2680, 2004.

19 Benotmane MA: Molecular aspects of individual radiosensitivity. J Biol Regul Homeost Agents *18*: 357-362, 2004.

Received January 9, 2008
Revised February 5, 2008
Accepted March 5, 2008