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Modulation of Immune and Anti-Tumor Effects of Cancer Immunotherapy with Anti-Pd-1 Monoclonal Antibodies by the Pineal Hormone Melatonin: Preliminary Clinical Results

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Abstract

The recent cancer immunotherapy with inhibitors of the expression of PD-1 and its ligands represents one of the most promising strategies in cancer cure. Then, the main question is how to enhance its therapeutic efficacy, which could potentially achieved by its association with chemotherapy, Il-2 immunotherapy, and anti-angiogenic strategies. By taking into consideration that the immune system is physiologically under a neuroendocrine regulation, another possible strategy to enhance the efficacy of cancer immunotherapy could consist of a neuro immune approach, namely by the pineal hormone melatonin (MLT), which at present constitutes the most investigated endogenous neuroendocrine molecule provided by immune stimulatory anticancer properties. On these bases, a study was planned to evaluate the effects of a concomitant administration of high-dose MLT in metastatic cancer patients treated by the anti-PD-1 monoclonal antibody Nivolumab (NIVO). The study included 14 patients, and the results were compared to those observed in a control group of 50 patients. No small cell lung cancer and melanoma were the most frequent tumor histo types. MLT was given orally at 100 mg/day during the dark period of the day, and NIVO was injected i.e. at 3 mg/kg b.w. at 15-day intervals. The percentage of both objective tumor regressions and disease control (DC) were higher in patients concomitantly treated by MLT, even though the only difference in DC was statistically significant. This evidence was associated with a significantly higher increase in lymphocyte-to-monocyte ratio (LMR) in MLT group, by suggesting that MLT may be successfully associated with anti-PD-1 monoclonal antibodies to pilot the immune response in an antitumor way by stimulating lymphocyte proliferation and inhibiting macrophage-induced inflammatory status, which suppresses the antitumor immunity.

Keywords

Anti-PD-1 monoclonal antibodies; Cancer immunotherapy; Checkpoint inhibitors; Melatonin; Neuro immune modulation.

Introduction

The recent advances in the area of the Psycho-neuro-endocrine-immunology (PNEI) have demonstrated that the immune responses, including the anticancer immunity, are physiologically under a complex neuroendocrine regulation, which constitutes the biochemical mediation of the psycho spiritual life [1-3]. Then, the immune responses do not depend only on the activation of immune cells themselves, but also on their neuroendocrine control. Despite the complexity of the neuro immune regulation, it is possible to affirm that the immune responses are namely inhibited by catecholamine's [4] and brain opioid system [5], whereas they are stimulated by the pineal gland [6] through its connection with brain cannabinoid system [7,8]. In fact, the pineal hormone melatonin (MLT), the most investigated pineal hormone [6], has been proven to stimulate the immune system, namely the anticancer immunity [9]. In more detail, today it is known that the anticancer immunity is mainly mediated by TH1-lymphocytes through the release of IL-2 [10], cytotoxic T lymphocytes, and NK cells after their evolution into LAK cells induced by IL-2 itself [10], whereas it is namely suppressed by macrophage-mediated inflammatory response, and by regulatory T lymphocytes (T reg) through the release of TGF-beta [11], which constitutes the main endogenous immunosuppressive agent. The immunosuppressive action of T reg lymphocytes is related to cell surface expression of specific molecules, including CTLA-4 and PD-1, the so-called immune checkpoints, whose inhibition by monoclonal antibodies has appeared to control tumor growth [12]. Unfortunately, most clinical studies with anti-checkpoint monoclonal antibodies, including anti-PD-1, anti-PD-L1, and anti-PD-L2, have been limited to the only evaluation of the control of tumor growth, without taking into consideration its relation with changes in the immune biological response. In any case, preliminary clinical results would suggest that the efficacy of checkpoint inhibitors is associated with an increase in lymphocyte count [13], as well as the previous cancer immunotherapy with IL-2 [10], and with a decline in monocyte count. This evidence is not surprising, since the antitumor immunity is essentially mediated by lymphocytes and suppressed by the monocyte-macrophage system, as confirmed by the negative prognostic significance of lymphocytopenia in cancer patients [14], as well as by an increase lymphocyte count, and in the values of lymphocyte-to-monocyte ratio (LMR) [15]. Then, LMR may be considered as the most simple and less expensive laboratory biomarker to monitor the immune status in cancer patients [16]. Moreover, more recent studies have demonstrated that MLT, in addition to its well documented immunomodulatory effects namely due to TH1 cell stimulation by acting on specific MLT lymphocyte receptors [9], has been proven to inhibit PD-1 expression, as well as that of its ligands PD-L1 and PD-L2 [17,18]. MLT has also appeared to inhibit indole amine 2,3 dioxygenase-1 (IDO-1) expression, which in contrast

may stimulate PD-1, PD-L1, and PD-L2 expression [18]. On these bases, a preliminary clinical study was planned, in an attempt to evaluate the effects on tumor growth and antitumor immunity of the PD-1 inhibitor Nivolumab (NIVO), a fully human IgG4 anti-PD-1 monoclonal antibody (MAB) with dual blockade of both PD-L1 and PD-L2 [19,20], in association with high-dose MLT in metastatic cancer patients, by comparing the results to those observed in a historical control group of patients treated by the only PD-1 inhibitor.

Methods and Materials

The study included 14 consecutive metastatic cancer patients, who were treated with the anti-PD-1 MAB NIVO plus high-dose MLT. The experimental protocol was explained to each patient, and written consent was obtained. The results were compared to those obtained in a historical control group of 50 patients with comparable tumor histotype and extension, who had received NIVO alone. The characteristics of patients and controls are reported in Table 1.

Table 1: Clinical characteristics of metastatic cancer patients treated with high-dose melatonin (MLT) plus the anti-PD-1 monoclonal antibody Nivolumab (n=14), or the only Nivolumab (n=50), as a control group.

CHARACTERISTICS	MLT plus NIVOLUMAB	NIVOLUMAB
M/F	4-Oct	36/14
MEDIAN AGE (years)	56 (42-74)	58 (38-71)
TUMOR HISTOLOGY		
-Non small cell lung cancer	6	33
- Squamous cell carcinoma	4	22
- Adenocarcinoma	2	11
- Melanoma	7	16
-Biliary tract cancer	1	1
TUMOR METASTASES		
- Lung	6	27
- Liver	4	13
- Liver plus Lung	2	7
- Brain	2	3

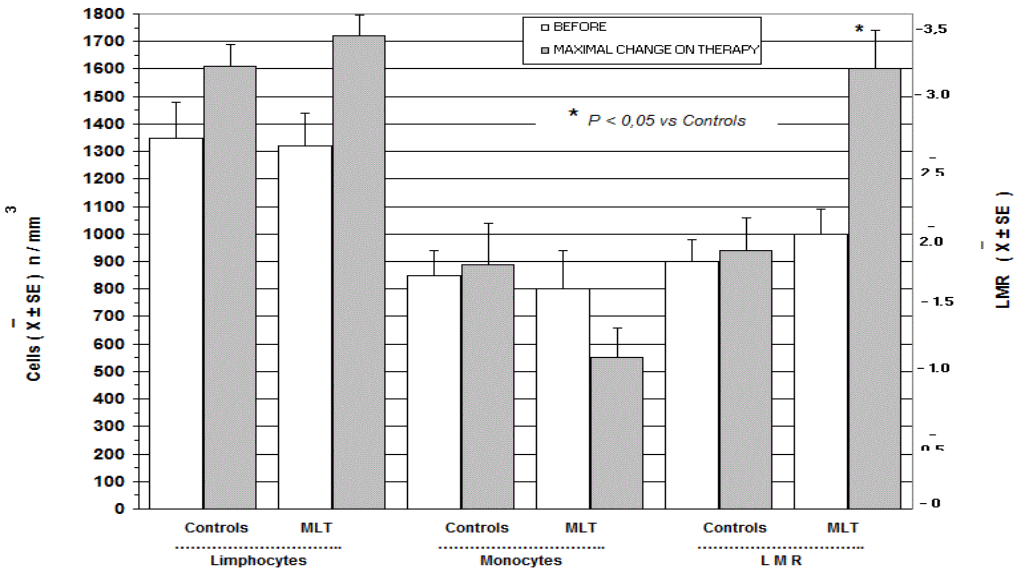
Eligibility criteria were, as follows: histologically proven metastatic solid neoplasm, measurable lesions, no double tumor, no previous therapy with other anti-PD-1, PD-L1, and PD-L2 inhibitors, and no previous history of autoimmunity. NIVO was injected intravenously at a dose of 3 mg/kg b.w. at 15-day intervals. Patients were evaluated by CT scan, NMR, and PET before the onset of therapy and after four cycles of treatment. The clinical response was assessed by WHO criteria. MLT was given orally at 100 mg/day during the dark period of the day, corresponding to its light/dark rhythm [6], by starting 7 days prior to NIVO injection to prepare, and positively modulate the immune status of cancer patients. The immune response on treatment was investigated by determining LMR before the onset of therapy, and at 15-day

intervals, by considering the maximal variation on therapy. Data were statistically evaluated by the chi-square test, the Student's t test, and the analysis of variance, as appropriate.

Results

A complete response (CR) was achieved in 1/14 (7%) patients treated with NIVO plus MLT, and in only 1/50 (2%) patients treated by NIVO alone, as a control group. Both patients were suffering from melanoma. A partial response (PR) was obtained in other 4/14 (29%) patients treated with NIVO plus MLT (non-small cell lung cancer (NSCLC): 2; melanoma:1; biliary tract cancer:1), and in 8/50 (16%) control patients (NSCLC: 6; melanoma: 2). Then, the percentage of objective tumor regressions (CR+PR) obtained in patients treated with NIVO plus MLT was higher than that found in those, who received the only NIVO, even though the difference was not statistically significant. A stable disease (SD) was found in 7/14 (50%) patients treated with NIVO plus MLT, and in 19/50 (38%) control patients. Then, the percentage of disease control (DC) (CR+PR+SD) obtained in patients treated with NIVO plus MLT was statistically significantly higher than in those, who received the only NIVO (12/14 (86%) *vs* 27/50 (54%), P<0.05). On the contrary, the percentage of progressive disease (PD) observed in patients treated with NIVO plus MLT was significantly lower than that found in controls (2/14 (14%) *vs* 23/50 (46%), P<0.05). The treatment was substantially well tolerated in both groups of patients, and those concomitantly treated by MLT referred an apparently less as then as controls. Finally, as far as immune changes occurring on treatment are concerned, lymphocyte and monocyte mean numbers, and LMR mean values observed in both groups of patients before and on therapy are illustrated in Figure 1.

Figure 1: Lymphocyte, monocyte and lymphocyte-to-monocyte ratio (LMR) mean values in metastatic cancer patients treated by the anti-PD-1 monoclonal antibody Nivolumab alone as a Control group (n=50), or Nivolumab plus Melatonin (MLT).



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As shown, lymphocyte mean count found on treatment in patients who concomitantly received MLT was higher than in controls, without, however, statistical differences. On the contrary, monocyte mean number seen on treatment in patients concomitantly treated with MLT was lower than in controls, even though the difference did not reach the statistical significance. Then, LMR mean values found on treatment in patients treated with NIVO plus MLT was significantly higher ($P<0.05$) with respect to those occurring in patients, who received the only NIVO.

Discussion

This preliminary phase-2 study, by showing an apparently greater efficacy of patients concomitantly treated with high-dose MLT and NIVO in terms of both tumor regression and DC, would suggest that the pineal hormone MLT may be favorably associated with anti-checkpoint inhibitors to enhance their antitumor efficacy. According to these preliminary results, MLT would exert its immunomodulatory effects on the action of PD-1, PD-L1 and PD-L2 expression inhibitors by stimulating lymphocyte response and inhibiting the monocyte-macrophage function, since monocyte count is correlated to tumor macrophage infiltration and macrophage-mediated chronic inflammation [21], which suppresses the antitumor immunity by promoting T reg cell production and activation [15]. Moreover, MLT could amplify the efficacy of the anticancer immunotherapy with PD-1 and PD-1 ligand expression inhibitors by stimulating the TH1-related secretion of IL-2, which has appeared in experimental conditions to enhance the antitumor efficacy of immunotherapy with anti-PD-L1 inhibitors [22]. Then, the results of this study justify further randomized studies with anti-PD-1 or its ligands MABs alone or in association with high-dose MLT in the future immunotherapies of cancer, in an attempt to associate to the inhibition of immune checkpoint expression a stimulation of lymphocyte proliferation and activation, as well as an inhibition of macrophage-mediated immunosuppression of the anticancer immunity.

Conclusion

This study, by showing an apparent enhanced anticancer efficacy of anti-PD1 through the concomitant administration of the pineal neuroimmunomodulating hormone MLT, would suggest the possibility to improve the antitumor immune activity of anti-PD1 inhibitors not only by chemotherapy, other MABs, or cytokines, but also by acting on the neuroendocrine control of the immune responses, including the antitumor immunity, which is namely stimulated by pineal gland and brain cannabinoid system, and inhibited by corticosteroids, catecholamines, and brain opioid system, mainly acting on mu-opioid receptor.

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