

Neuroimmunotherapy of untreatable metastatic solid tumors with subcutaneous low-dose interleukin-2, melatonin and naltrexone: Modulation of interleukin-2-induced antitumor immunity by blocking the opioid system

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Abstract

OBJECTIVES: The preliminary applications of the psychoneuroimmunological knowledges to the treatment of human diseases have confirmed the possibility to amplify IL-2-dependent anticancer immunity by the pineal hormone melatonin (MLT) or by opioid antagonist, such as naltrexone (NTX), which act by activating TH1 lymphocytes or suppressing TH2 lymphocytes, respectively. At present, however, there are no data about the immunobiological effects of a concomitant administration of both MLT and NTX on IL-2-induced anticancer immunity. This preliminary study was carried out to evaluate whether the association of NTX may further enhance the lymphocytosis induced by the neuroimmunotherapy with IL-2 plus MLT. **MATERIALS & METHODS:** The study included 14 consecutive untreatable metastatic solid tumor patients. According to a cross-over randomized study, the patients were treated during two consecutive immunotherapeutic cycles at 21-day intervals with IL-2 plus MLT alone or with IL-2 plus MLT plus NTX. IL-2 was injected subcutaneously at 3 MIU/day for 6 days/week for 4 weeks, MLT was given orally at 20 mg/day in the evening every day, and NTX was given orally at 100 mg in the morning every next day. For the immune evaluation, venous blood samples were drawn before the onset of treatment and at weekly intervals. **RESULTS:** Lymphocyte mean number significantly increased after both IL-2 plus MLT and IL-2 plus MLT plus NTX. However, the concomitant administration of NTX induced a significantly higher increase in lymphocyte mean number with respect to that achieved with IL-2 plus MLT alone. In contrast, the increase in eosinophil mean number was significantly higher on IL-2 plus MLT alone. **CONCLUSIONS:** This preliminary study shows that the association of NTX further amplifies the lymphocytosis obtained by IL-2 plus MLT. Since the lymphocytosis represents the most important favourable prognostic variable predicting the anticancer efficacy of IL-2 immunotherapy, it is probable that a cancer neuroimmunotherapy with IL-2 plus both MLT and NTX to activate TH1 and suppress TH2 cells respectively, may deserve more promising results in the treatment of human neoplasms according to the psychoneuroimmunological knowledge.

Introduction

After several years of experimental and clinical studies, at present it is known that the immune system may mediate both activation and suppression of the immunological response against cancer growth [1–4]. The activation of the anticancer immunity would be namely mediated by T helper-1 (TH1) lymphocytes through the release of interleukin-2 (IL-2) [1,2], whereas its suppression is depending on the activation of T helper-2 (TH2) lymphocytes, which release interleukin-10 (IL-10), that has been proven to block IL-2 secretion and IL-2-induced generation of cytotoxic lymphocytes [3,4]. The lymphocytes have appeared to express receptors for several neurohormones and neuropeptides [5,6], and this evidence would explain the influence of the psychoemotional status on the immune system, including the anticancer immunity [5,7]. Within the neuroendocrine system, the pineal hormone melatonin (MLT) would represent one of the major neuroactive substances responsible for TH1 activation [7–10] with a consequent stimulation of the anti-cancer immunity [1], whereas TH2 activation would be mainly under an opioid stimulatory control [11], particularly mediated by the μ -opioid receptor. Therefore, the opioid stimulation would suppress the anticancer immunity by activating TH2 lymphocytes [11]. In fact, in experimental conditions, the long-acting opioid antagonist naltrexone (NTX) has been proven to induce immunostimulation by determining an enhanced IL-2 production and a diminished IL-10 secretion [11]. On the other side, the concomitant administration of MLT has appeared to amplify the biological effects of IL-2 in cancer patients [12]. Finally, preliminary studies in cancer patients would suggest that the administration of NTX may also enhance the immunobiological effects of IL-2 [13]. At present, however, there are no data about the influence on IL-2 activities of a concomitant administration of both MLT and NTX in an attempt to activate TH1 and to suppress TH2 lymphocytes, respectively. Therefore, at present it is still unclear whether the concomitant activation of TH1 cells by MLT and the suppression of TH2 cells by NTX may further amplify IL-2-induced anticancer immunobiological functions with respect to each single strategy with MLT alone or NTX alone. The present study was performed to analyze the immunobiological effects of IL-2 plus MLT with or without a concomitant NTX administration in patients with metastatic solid neoplasms.

Materials and methods

The study included 14 consecutive untreatable metastatic solid tumor patients (M/F:10/4; median age 61 years, range 49–76). Eligibility criteria were as follows: histologically proven metastatic solid neoplasms, measurable lesions, progression on previous standard anticancer therapies and no availability of further conventional effective treatments, and no double tumor. Patients under chronic therapy with morphine or other opioid agents were excluded from the study. The exper-

imental protocol was explained to each patient, and written consent was obtained. Tumor histotypes were, as follows:

renal cell carcinoma: 4; colorectal cancer: 3; gastric cancer: 2; malignant thymoma: 2; pancreatic cancer: 1; undifferentiated neuroendocrine tumor: 1; unknown primary tumor: 1.

Finally, dominant metastasis sites were, as follows: soft tissues: 1; bone: 1; lung: 4; liver: 4; lung + liver: 2; peritoneum: 1; brain: 1.

Patients underwent a cross-over randomized study, consisting of 2 consecutive neuroimmunotherapeutic cycles at 21-day intervals with low-dose IL-2 plus MLT with or without a concomitant NTX administration. Each cycle consisted of IL-2 at 3 million IU/day subcutaneously in the evening for 6 days/week for 4 consecutive weeks plus MLT at 20 mg/day orally during the dark phase of the day every day without interruption. NTX, which was given during only one of the 2 planned cycles, was administered orally at 100 mg in the morning every next day. Patients were evaluated for their clinical response according to WHO criteria. Moreover, they were biologically analyzed by considering the results obtained during IL-2 plus MLT alone with respect to those achieved during IL-2 plus MLT plus NTX, and the biological evaluation consisted of the analysis of changes in the absolute number of lymphocytes and eosinophils, which represent the 2 main biological prognostic variables predicting the efficacy of IL-2 cancer immunotherapy [14]. The results were statistically analyzed by the *chi-square test*, the *Student's t test* and the analysis of variance, as appropriate.

Results

The subcutaneous injection of IL-2 was well tolerated in all patients. On the contrary nausea, vomiting and anorexia requiring a 50 dose reduction occurred after NTX administration in 4/14 (29%) patients, who continued their NTX therapy at a dose of 50 mg every next day, whereas no important increase in transaminase levels was observed on NTX administration.

Changes in lymphocyte and eosinophil mean numbers observed during IL-2 plus MLT with or without NTX are illustrated in *Figs 1* and *2*, respectively. The concomitant administration of NTX induced a statistically significant increase in lymphocyte mean number with respect to that achieved during IL-2 plus MLT only, with a peak after 2 weeks of IL-2 injection. On the contrary, the increase in eosinophil mean number was significantly higher during IL-2 plus MLT alone than during the concomitant NTX administration.

As far as the clinical response is concerned, the radiological examinations, including CT scan, made after the 2 cycles of IL-2 immunotherapy, documented a partial response (PR) in 3/14 (21%) patients. The first patient had a malignant thymoma with lung metastases, the second patient showed lung and brain metastases due to renal cell carcinoma, and the third patient who had a PR was suffering from liver metastases due to pancreatic adenocarcinoma. A stable disease

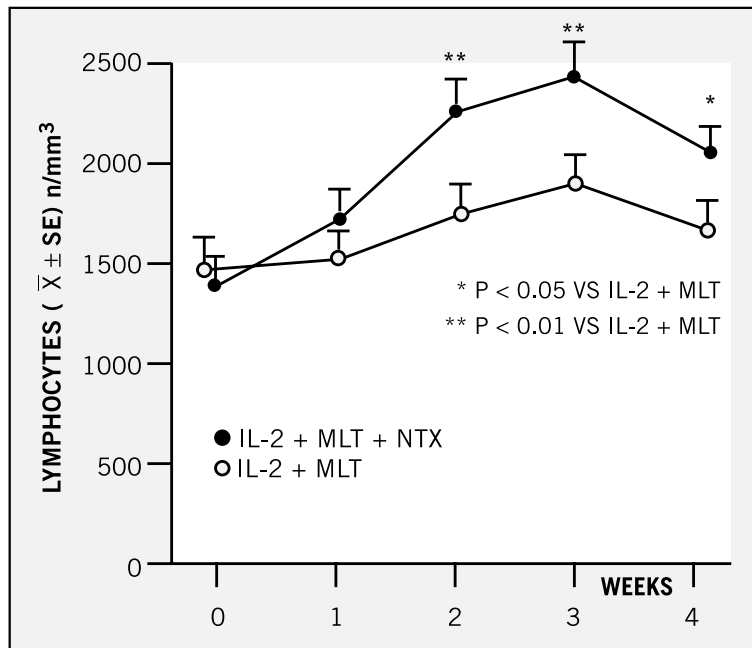


Figure 1: Changes in lymphocytes mean number during immunotherapy with IL-2 plus melatonin (MLT) and naltrexone (NTX) or during IL-2 plus MLT alone.

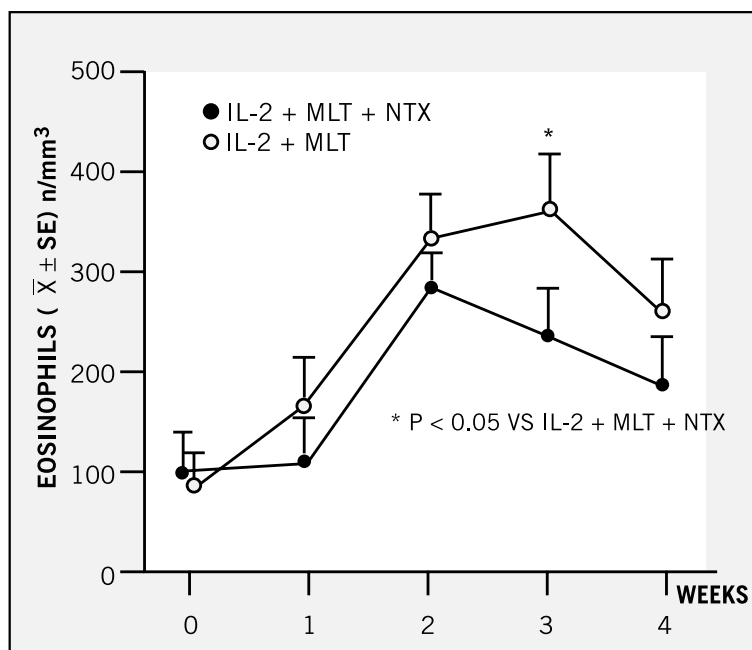


Figure 2: Changes in eosinophil mean number during immunotherapy with IL-2 plus melatonin (MLT) and naltrexone (NTX) or during IL-2 plus MLT alone.

(SD) was achieved in other 8/14 (57%) patients, whose tumor histotype was, as follows: renal cell carcinoma: 3; colorectal cancer: 2; malignant thymoma: 1; unknown primary tumor: 1; gastric carcinoma: 1. The remaining 3/14 (21%) patients had a progressive disease (PD). Therefore, a control of the neoplastic growth (PR+SD) was achieved in 11/14 (79%) patients, with a median duration of response of 9 months (range 4–11 months). After the radiological evaluation, non-progressing patients (PR+SD) continued their therapy with MLT alone at 20 mg/day without interruption, in association with IL-2 for 6 days every month as a maintenance treatment. As far as the biological response

is concerned in relation to the clinical response, an increase in lymphocyte number greater than 1000/mm³ occurred in 9/11 patients with PR or SD and in none of the patients with PD. This difference was statistically significant (P<0.01).

Discussion

This preliminary study, carried out to analyze the possibility to modulate the immunobiological effects of the neuroimmunotherapy with low-dose IL-2 plus MLT by the use of opioid antagonists, seems to suggest that the concomitant block of the opioid system by MLT

may further amplify the already documented modulatory action of the pineal indole MLT on IL-2 induced stimulation of lymphocyte proliferation. In any case, it has to be remarked that in this study we have used high-dose NTX, corresponding to the dosage commonly employed to completely block the effects of exogenous opioids [15], and the aim of the administration of an opioid antagonist was to block the activation of TH2 lymphocytes, which is responsible for cancer-related immunosuppression by releasing IL-10 [11]. However, it has to be taken into consideration that the inhibitory effect of the opioid antagonist NTX on IL-10 secretion would not be the only possible action responsible for the potential immunomodulating antitumor activity of NTX itself. In fact, other researchers have demonstrated the anticancer efficacy of low-dose NTX [16–18]. The antitumor effect of low-dose NTX would be mediated by an increase in metenkephalin and β -endorphin blood concentrations, which may inhibit cancer cell growth at least through three different antitumor mechanisms, consisting of stimulation of NK and T cytotoxic lymphocyte functions by β -endorphin [19], possible direct oncostatic effect of metenkephalin and an increased opioid receptor expression on cancer cell surface, which would make cancer cells more sensitive to the action of β -endorphin, that may induce apoptosis in cancer cells [16–18]. Moreover, it has to be further remarked that the studies of low-dose NTX have used an administration during the dark period of the day, whereas in the present study an administration during the light phase of the day was utilized. Our biological end-point was to achieve an inhibition of TH2 activation by NTX, in an attempt to reduce IL-10 secretion, with a following potential increase in the efficacy of IL-2-induced lymphocytosis [1–3], and this effect has appeared to be achieved on the basis of the results of this study, even though further clinical investigations in a greater number of patients will be required to confirm these data. In addition to a more pronounced increase in lymphocyte number during a concomitant administration of NTX, another biological evidence which could confirm the possibility to block TH2 cell activation is represented by the lower increase in eosinophil number during IL-2 plus MLT plus NTX than during IL-2 plus MLT alone, since IL-2-induced eosinophilia is determined by interleukin-5 (IL-5) produced by TH2 lymphocytes [2,7,14]. In any case, successive clinical investigations by measuring IL-10 and IL-5 blood concentrations will be required to clearly confirm the possibility to block TH2 cell activation by the long-acting opioid antagonist NTX. Because of the potential cytotoxic properties of eosinophils [2, 14], their increase following TH2 activation could constitute a favourable biological event, which, however, would be vanifed by the concomitant enhanced production of IL-10 by the activated TH2 lymphocytes. In fact, it is known that the evidence of eosinophilia during IL-2 immunotherapy may play a relevant positive prognostic significance when it is associated with a concomitant increase in lymphocyte number [2, 4, 14].

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