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ORIGINAL ARTICLE

A new neuroimmunotherapeutic strategy of subcutaneous low-dose interleukin-2 plus the long-acting opioid antagonist naltrexone in metastatic cancer patients progressing on interleukin-2 alone

Paolo Lissoni[1], Fabio Malugani[1], Veronica Bordin[1], Ario Conti[2], George Maestroni[2] & Gabriele Tancini[1]

1. Division of Radiation Oncology, S. Gerardo Hospital, Monza, Milan, Italy.
2. Institute of Pathology, Locarno, Switzerland.

Correspondence to: Dr. Paolo Lissoni
Divisione di Radioterapia Oncologica
Ospedale S. Gerardo dei Tintori
20052 Monza (Milano), ITALY.
FAX: +39 2332284

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Abstract

OBJECTIVES: Recent advances in knowledge of Psychoneuroimmunology have shown that several neuroactive substances, including neurohormones and neuropeptides, may exert immunomodulatory effects. However, despite the great variety of potential neuroimmune interactions, at present we may recognize two major neuroendocrine systems exerting a physiological neuroimmunomodulatory function, consisting of the pineal gland and the brain opioid system, provided by immunostimulatory and immunosuppressive effects, respectively. Recent in human studies have demonstrated the possibility to amplify the biological activity of IL-2, the major anticancer cytokine, by pineal indoles.

MATERIALS & METHODS: The present study was carried out to draw some preliminary in human results on the possible immunomodulatory effects of the inhibition of the brain opioid activity by a long-acting opioid antagonist, naltrexone (NTX). The study was performed in 10 metastatic renal cell cancer patients, who had progressed on a previous immunotherapeutic cycle with IL-2 alone. Patients were treated with the same doses of IL-2 (6 million lU/day subcutaneously for 6 days/week for 4 weeks) plus an oral administration of NTX at a dose of 100 mg every 2 days.

RESULTS: The clinical response consisted of a partial response in 1 and a stable disease in 5 patients, whereas the other 4 patients progressed. Therefore, the percent of non-progressive disease was 6/10 (60%). Moreover, mean lymphocyte increase achieved during IL-2 plus NTX was significantly higher ($P<0.05$) than that obtained during the previous treatment with IL-2 alone.

CONCLUSIONS: This study shows that a blockade of the brain opioid system, which plays a physiological immunosuppressive role, may improve the anticancer effects of IL-2 in humans.

Introduction

Recent advances in knowledge of neuroimmunomodulation (NIM) have shown that most neurohormones and neuropeptides may play immunomodulatory effects on lymphocyte and macrophage functions by acting on specific neurohormonal and neuropeptidergic receptors expressed by the immune cells [1,2]. Unfortunately, most immunomodulating effects played by neuropeptides and neurohormones are extremely varied, depending on dose, way of injection, experimental conditions and psychoneuroendocrine status of the investigated subjects. Therefore, it is still difficult to translate *in vivo* the great variety of immunomodulatory effects described *in vitro*. Then, the definition of the physiological significance of NIM is still at the beginning. However, despite the controversial experimental data, at present it has been already possible to demonstrate that the endogenous opioid system, particularly through the mu-opioid receptors, plays a major immunosuppressive role, namely on cellular immunity, including the antitumor immune response [3]. Even though the mechanisms responsible for the immunosuppressive role of the opioid agents are still unclear, it has been shown that the opioids may induce immunosuppression namely by inhibiting T helper-1 (TH1) lymphocyte functions and stimulating T helper-2 (TH2) lymphocyte activity [3]. Since the anticancer immunity is a TH1 dependent phenomenon, whereas the TH2 activity inhibits antitumor immune response [4, 5], the inhibition of TH1 functions and activation of TH2 lymphocytes by the opioid agonists may allow a suppression of immune reactions against cancer growth. TH1 lymphocytes stimulate the anticancer immunity namely through the release IL-2, which is the main anticancer cytokine, whereas TH2 lymphocytes immunosuppress the antitumor immunity mainly through the secretion of IL-10, which exerts immunosuppressive effects by inhibiting secretion and activity of IL-2 itself [4, 5]. The altered TH1/TH2 ratio, consisting of TH1 hypofunction and TH2 hyperfunction, would represent one of the most important cancer-related immune dysfunctions which is responsable for most immune cell anomalies described in advanced cancer patients [6], including the decrease in the circulating number of NK, LAK, dendritic cells (DC) and lymphocytes. This statement is justified by the fact that a diminished TH1 function determines a decreased release of IL-2, which is the main growth factor for the immune cells involved in tumor cell destruction [7]. Therefore, the normalization of TH1/TH2 ratio would constitute the main endpoint of the recent cancer immunotherapies with cytokines. At least from a theoretical point of view. The normalization of TH1/TH2 ratio may be obtained by a stimulation of TH1 functions and/or by an inihibition of the TH2 hyperfunction. Several neuroendocrine agents, such as the indole hormones released from the pineal gland, have been proven to activate TH1 functions [8]. In particular, melatonin (MLT), which represents the most investigated pineal hormone, has appeared to promote IL-2 production from TH1 lymphocytes by acting on specific melatoninergic receptors expressed by TH1 cells [9,10]. Because of the physiopathological importance of neuroimmune interactions [1], the concomitant administration of immunomodulating neurohormones and neuropeptides could enhance the therapeutic efficacy of the anticancer cytokines by replacing physiological links between neuroendocrine and immune systems, which are often altered in the advanced neoplastic disease [11,12]. Therefore, the concomitant administration of anticancer cytokines, such as IL-2, plus immunostimulating neurohormones could deserve important therapeutic implications, by representing a new possible strategy in the treatment of human neoplasms, the so-called neuroimmunotherapy (NIT) of cancer. In particular, it has been shown that the concomitant administration of pharmacological doses of MLT may enhance the biological and anticancer therapeutic effects of low-dose IL-2 with respect to IL-2 alone in humans [13]. At present, however, the administration of IL-2 plus MLT or other pineal indoles would constitute the only clinically investigated example of NIT in the treatment of cancer [13,14]. Another potential NIT approach could consist of a concomitant administration of anticancer cytokines plus neuroactive substances capable of inhibiting the action of immunosuppressive neuroendocrine areas, such as the brain opioid system. In other words, according to NIM knowledge, an improvement in immune functions may be achieved by either activating immunostimulatory neuroendocrine areas, such as the pineal gland [8–10], or blocking the action of immunosuppressive neuroendocrine structures, such as the opioid system [3], in an attempt to enhance TH1-functions and to reduce TH2-activities respectively. The block of the type mu-opioid system may be obtained through the administration of opioid antagonists, such as naltrexone (NTX) [15]. Therefore, administration of long-acting mu-opioid antagonist NTX could enhance immune functions, including anticancer immunity, namely by inhibiting TH2 activity. In fact, at least in experimental conditions, NTX has been shown to reduce IL-10 release and to enhance that of IL-2, with a following amplification of anticancer immunity. On this basis, a study was planned in an attempt to analyze the influence of a concomitant NTX administration on the immunological and therapeutic effects of IL-2 in advanced cancer patients.

Materials and methods

The study included 10 consecutive metastatic renal cell cancer patients (M/F: 6/4; median age: 59 years, range 49–66), who progressed in response to a previous immunotherapeutic cycle with IL-2 alone at the same doses. The neuroimmunotherapeutic cycle with IL-2 plus NTX was, as follows: IL-2 at a dose of 3 mil-

lion IU twice/day subcutaneously for 6 days/week for 4 consecutive weeks plus a concomitant admistration of NTX at a dose of 100 mg orally every two days until the end of IL-2 injection. Eligibility criteria were, as follows: histologically proven metastatic renal cell carcinoma, measurable lesions, progression after a previous immunotherapeutic cycle with IL-2 alone at the same doses, no concomitant chronic therapy with steroids because of their immunosuppressive action and no concomitant chronic therapy with morphine or other opioid substances for cancer-related pain because of the opioid antagonist action of NTX itself. The experimental protocol was explained to each patient, and written consent was obtained.

The immunobiological results obtained during IL-2 plus NTX were compared to those observed in the same patients during IL-2 alone by evaluating the absolute number of total lymphocyte, being the lymphocytosis the most important biological favourable prognostic marker of the efficacy of IL-2 cancer immunotherapy [16]. Moreover, the clinical response was assessed according to WHO criteria. The results were statistically analyzed by the chi square test, the Student's *t-test* and the analysis of variance, as appropriate.

Results

An increase in lymphocyte number greater than 30% with respect to that obtained during IL-2 alone occurred during IL-2 plus NTX in 6/10 (60%) patients. The changes in mean lymphocyte number, as assessed at weekly intervals, observed during IL-2 alone or IL-2 plus NTX are illustrated in Fig.1. Mean lymphocyte number observed after 7, 14 and 21 days of IL-2 injection was significantly higher during IL-2 plus NTX than during IL-2 alone. Moreover, as illustrated in Fig.2, mean maximum lymphocyte increase occurring during IL-2 plus NTX was statistically significant higher than that achieved during IL-2 alone ($P<0.05$). As far as the clinical response to IL-2 plus NTX is concerned, a partial response (PR) was achieved in one patient with nodes as dominant metastasis sites. A stable disease (SD) occurred in five other patients, whereas the remaining four patients had a progressive disease. Therefore, the percent of non-progressive disease obtained during IL-2 plus NTX was 6/10 (60%) patients. Finally, the percent of patients with lymphocyte increase greater than 30% was significantly higher in patients who showed no progressive disease (PR + SD) than in those who progressed on treatment (5/6 vs 1/4,$P < 0.05$).

The treatment was substantially well tolerated in all patients, and in particular, despite the potential hepatotoxicity of NTX, no statistically significant increase in transaminases levels occurred during concomitant administration of NTX with respect to the values observed during IL-2 alone.

Discussion

This preliminary study shows that concomitant administration of long-acting opioid antagonist NTX may improve IL-2-induced lymphocytosis and reestablish a control of neoplastic growth in metastatic cancer patients who progressed on IL-2 alone, by suggesting that a blockade of the opioid system by NTX may *in vivo* enhance immunobiological and anticancer properties of IL-2 immunotherapy. At present, the only published example of the application of the psychoneuroendocrine knowledge to the clinical treatment of human diseases characterized by immune dysfunctions, such as cancer, is represented by the association of IL-2 plus pineal indoles namely MLT [11,12], as a

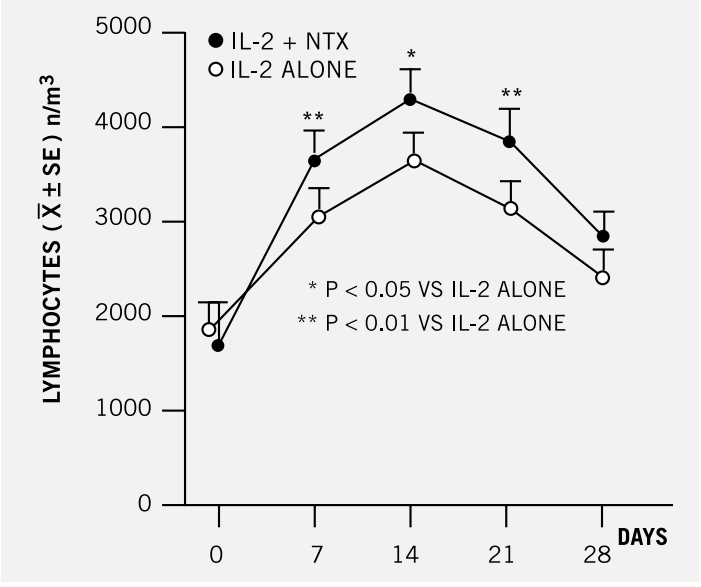

Figure 1: Changes in lymphocyte mean number during IL-2 alone or IL-2 plus naltrexone (NTX) in metastatic cancer patients.

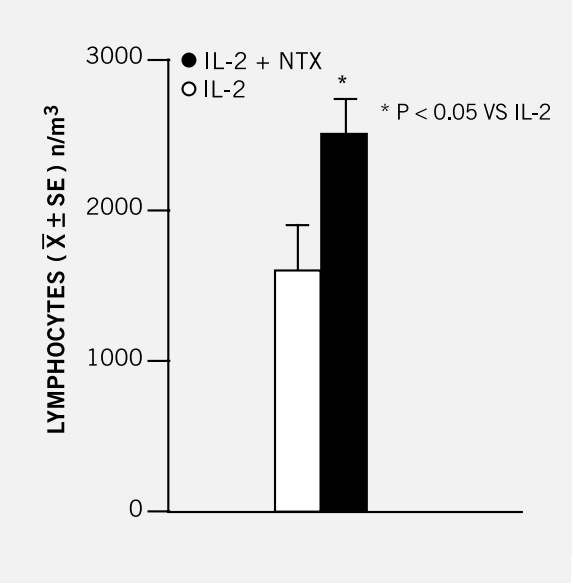

Figure 2: Mean increase in lymphocyte number occurring in metastatic cancer patients during IL-2 or IL-2 plus naltrexone (NTX).

NIT of cancer. Therefore, this preliminary study would show a new, other potentially effective neuroimmunotherapeutic strategy, consisting of the administration of an anticancer cytokine, such as IL-2, plus a neuroactive substance capable of blocking the opioid system and removing its immunosuppressive action on IL-2-induced anticancer immune reaction, such as NTX. Then, according to knowledge available until now, an improvement of the efficacy of the anticancer immunotherapy by a psychoneuroendocrine manipulation may be achieved through two different ways:

– consisting of the activation of immunostimulatory neuroendocrine areas, such as the pineal gland by the exogenous administration of pineal indoles,
– and of the inhibition of immunosuppressive neuroendocrine areas, such as the brain opioid system by the administration of long-acting opioid antagonists blocking the mu-opioid receptor.

Further studies, by evaluating several immune parameters, will be required to better define neuroimmunomodulating effects of NTX in humans. Moreover, further clinical studies will be needed to establish whether the concomitant administration of pineal indoles and opioid antagonists plus IL-2 may enhance the anticancer efficacy of IL-2 with respect the single agents.

In conclusion, this study would suggest that the block of the opioid tone by long-acting opioid antagonist NTX may amplify IL-2-induced lymphocyte increase and reestablish a control of the neoplastic proliferation in metastatic cancer patients progressing on IL-2 alone, by confirming in humans the immunosuppressive role of the opioid system on the anticancer immunity.

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