Medroxyprogesterone acetate reduces the production of cytokines and serotonin involved in anorexia/cachexia and emesis by peripheral blood mononuclear cells of cancer patients.

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The aim of the present study was to evaluate whether MPA at pharmacological doses in vitro is able to influence the production and/or release of IL-1β, IL-2, IL-6, TNFα and 5-HT by peripheral blood mononuclear cells (PBMC) of advanced stage cancer patients stimulated by PHA. Furthermore, in order to confirm its immunosuppressive effect, we have also studied the effect of MPA on the in vitro proliferative response of PBMC to PHA, anti-CD3 monoclonal antibody (mAb) and recombinant IL-2 (rIL-2) and on the expression of membrane-bound IL-2 receptor (IL-2R) subunits, α (CD25) and β (CD122) chain or both, by PHA- or anti-CD3 mAb-stimulated PBMC of the same cancer patients. Peripheral Blood Mononuclear Cells Separation PBMC from freshly drawn heparinized peripheral blood samples of 10 cancer patients in advanced stage of disease (6 squamous cell head and neck cancer, 2 colon adenocarcinomas, 1 non small-cell lung cancer and 1 ovarian carcinoma) were separated as previously described [1]. Mitogens and Drug To stimulate PBMC proliferation we used PHA-M, anti-CD3 mAb and rIL-2 (all from Boehringer-Mannheim, Mannheim, Germany). PHA, diluted in RPMI 1640 medium, was used at the concentration of 5 μg/mL. Anti-CD3 mAb, diluted in PBS ( Gibco) was used at the concentration of 5 μg/mL. Recombinant IL-2 was used at the concentration of 200 IU/mL. MPA (Pharmacia, Milan, Italy) was reconstituted in DMSO (Sigma, St. Louis, MO, USA) at the concentration of 100 mg/mL and diluted in RPMI 1640 medium to the concentrations of 0.1, 0.2 and 0.4 μg/mL, respectively. Proliferative Response of PBMC to PHA, anti-CD3 mAb or rIL-2 ± MPA at different concentrations PBMC proliferative response was evaluated as previously described [1]. Fifty μL of MPA were added in the appropriate wells at the aforementioned concentrations. Control wells contained 1x10⁵ cells in 125 μL of complete medium plus 125 μL of RPMI 1640 containing the different mitogens. The results were expressed as cpm. Assay for IL-1β, IL-2, IL-6, TNFα and 5-HT Production in Cultures of PBMC Stimulated with PHA ± MPA 0.2 μg/mL. The levels of IL-1β, IL-2, IL-6, TNFα and 5-HT were assessed in culture supernatants from PBMC stimulated with PHA in the presence or absence of MPA 0.2 μg/mL. The cytokine production was evaluated with a sandwich ELISA test, as previously reported [1]. The results were expressed in pg/mL as mean ± standard error of mean (M±SE). Serotonin was evaluated with ELISA test (Immunotech SA) based on the competition between 5-HT in the previously acetylated sample and 5-HT–enzyme conjugated, used as a tracer, for binding to the mAb coated onto the microtiter plate. Acetylated samples and standards plus 5-HT-acetylcholinesterase were added to the wells, where they compete for a limited number of mAb-binding sites. Enzymatic activity was measured by adding a chromogenic substrate and the absorbance of the resulting coloured solution was measured with a spectrophotometer. The concentrations were determined by extrapolating them from the standard curve plotted correlating standard concentrations with their corresponding absorbance values.

The range of the assay was from 1.8 to 1000 nM, intra-assay variation was 9% and inter-assay variation was 8%. The results were expressed in nM as M±SE. Detection of Membrane-Bound IL-2 Receptor, α (CD25) and β (CD122) Subunits, on PBMC Stimulated with PHA or anti-CD3 mAb ± MPA 0.2 μg/mL. PBMC cultures were stimulated with PHA or anti-CD3 mAb and supplemented with MPA 0.2 μg/mL in the appropriate wells for 72 hours. The detection of membrane-bound IL-2 receptor, α (CD25) and β (CD122) subunits was performed as previously described [2]. The results were evaluated as percentage of cells expressing CD25 or CD122 or both. Statistical Analysis All results were expressed as M±SE. The statistical analysis of the results was performed by using Student’s two tailed t test. Peripheral Blood Mononuclear Cells Proliferative Response ± MPA The proliferative response of PBMC to PHA, anti-CD3 mAb or rIL-2 was studied in 10 advanced stage cancer patients. The addition of MPA induced significant changes of proliferative response to PHA and anti-CD3 mAb, but did not induce changes in the response to rIL-2. The reduction of the proliferative response to PHA and anti-CD3 mAb was dose-dependent so that a greater reduction was observed with highest MPA dose used (0.4 μg/mL), whereas the proliferative response to rIL-2 was not affected by MPA addition. Production of IL-1β, IL-2, IL-6, TNFα and 5-HT in Cultures of PBMC Stimulated with PHA ± MPA The production of IL-1β, IL-6, TNFα and 5-HT in culture supernatants from PHA-stimulated PBMC of 10 advanced stage cancer patients was assayed. A comparison was made with the production of the same cytokines and 5-HT by PHA-stimulated PBMC of 20 age-sex-matched normal healthy subjects. The levels were significantly higher in cancer patients than in controls. The addition into cultures of MPA 0.2 μg/mL was able to significantly reduce the production of IL-1β, IL-6, TNFα and 5-HT but not IL-2. Detection of Membrane-Bound IL-2 Receptor, α (CD25) and β (CD122) Subunits, on PBMC Stimulated with PHA or anti-CD3 mAb ± MPA. The percentage of cells expressing membrane-bound IL-2 receptor, α (CD25) or β (CD122) subunits or both, on PBMC from 10 advanced stage cancer patients after stimulation with PHA or anti-CD3 mAb was not influenced by the addition of MPA 0.2 μg/mL.

In conclusion, our study provides further evidence to previously reported data by us and other authors, showing that MPA, like MA, is able to hinder the activity of some cytokines, such as IL-1β, IL-6 and TNFα, which have a key role in the pathogenesis of ACS by inhibiting their production and/or release. This mechanism may explain the clinical beneficial effect of MPA administration in cancer patients with ACS. A further interesting and, to our knowledge, not previously known finding highlighted by our study is the ability of MPA to inhibit the 5-HT release by lymphocytes. The broad spectrum of biological activities of 5-HT, including its role in chemotherapy-induced and non-chemotherapy-induced emesis and its activity in the immune regulation of T lymphocyte function both in vitro and in vivo, suggests that further studies on the relationships between MPA and 5-HT production and/or release may help to clarify some important issues of not yet well understood clinical situations, such as emesis, ACS and immune impairment of advanced stage cancer patients.

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REFERENCES