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Original Paper

Evidence that Cisplatin Induces Serotonin Release from Human Peripheral Blood Mononuclear Cells and that Methylprednisolone Inhibits this Effect

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Corticosteroids counteract cisplatin(CDDP)-induced acute emesis but the mechanism involved is still unknown. Therefore, the aim of this study was to verify whether CDDP can induce serotonin (5HT) release from peripheral blood mononuclear cells (PBMC) and determine whether methylprednisolone (MP) can inhibit such release. Blood from 10 healthy volunteers was used. Our study showed that CDDP did induce 5HT release from PBMC dose-dependently (10 ± 1 nM for controls, 18 ± 4 nM for CDDP 0.01 μg and 30 ± 4 nM for CDDP 0.1 μg, $P < 0.001$) and that the addition of MP to cultures of PBMC in the presence of CDDP induced a significant decrease of 5HT concentrations. Our results highlight a new mechanism through which CDDP could induce emesis and suggest a further mechanism by which corticosteroids mediate their anti-emetic effect.

Key words: anti-emetic effect, cisplatin, emesis, methylprednisolone, peripheral blood mononuclear cells, serotonin

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INTRODUCTION

NAUSEA AND vomiting are side-effects often associated with chemotherapeutic treatments. Cisplatin (CDDP) is the most powerful emesis-inducing antineoplastic drug [1]. This is due to its action on enterochromaffin cells which leads to a direct release of serotonin (5HT). The key role of 5HT in CDDP-associated acute emesis has been highlighted by the introduction of $5HT_3$ receptor antagonists in clinical practice [2, 3]. Randomised clinical trials have demonstrated that corticosteroids have an anti-emetic effect and are capable of increasing the activity of $5HT_3$ receptor antagonists [4], but it is still not clear which mechanism mediates their action. Several studies have reported the role of CDDP in enhancing immune functions, such as the induction of LAK (lymphokine-activated killer) cells and the production of cytokines [5–7]. It has recently been demonstrated that both T cells and monocytes release 5HT after mitogen stimulation [8], and that CDDP may be responsible for 5HT release by immune cells. Furthermore, as corticosteroids strongly inhibit lymphocyte activation, it can be hypothesised that their anti-emetic activity may take place via the inhibition of CDDP-induced 5HT lymphocyte/monocyte release. The aim of this study was to verify whether CDDP can induce, *in vitro*, 5HT release from peripheral blood mononuclear cells (PBMC) of normal subjects and whether methylprednisolone (MP) can inhibit CDDP-induced 5HT lymphocyte/monocyte release from PBMC.

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MATERIALS AND METHODS

Mononuclear cells were separated by Ficoll-Hypaque density gradient from freshly drawn heparinised peripheral blood of 10 healthy, volunteer donors (mean age 23.6 years, range 18–31, 4 males and 6 females). The cells were then washed and suspended at a concentration of 1×10^6 cells/ml in RPMI 1640 medium (GIBCO, Paisley, U.K.), supplemented with 10% fetal calf serum (FCS) (Boehringer Mannheim, Germany), 20 nM L-glutamine (GIBCO) and 10 μg/ml gentamicin (GIBCO), hereafter referred to as 'complete medium'.

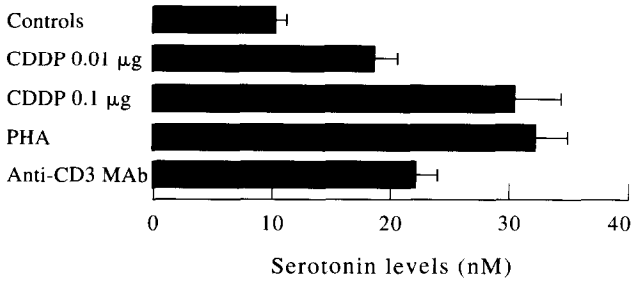

Figure 1. Serotonin (5HT) levels in culture supernatants of peripheral blood mononuclear cells in the absence (controls) or presence of mitogens or cisplatin (CDDP) at different concentrations. The values are expressed as nM (mean ± standard error of mean). PHA, phytohaemagglutinin.

The levels of 5HT were assessed in culture supernatants of PBMC stimulated with phytohaemagglutinin (PHA) or anti-CD3 monoclonal antibody (MAb) or CDDP at different concentrations in the presence or absence of MP. PBMC were cultured in flat-bottomed 96-microwell microtitre plates (Costar, Cambridge, Massachusetts, U.S.A.). Each well contained 1×10^5 cells plus 0.5 μg PHA (PHA-M, Boehringer) or 0.5 μg anti-CD3 MAb (Boehringer) or 0.01 μg or 0.1 μg CDDP (Platamine, Pharmacia, Milan, Italy) in a volume of 200 μl complete medium. Therefore, the CDDP concentration in cultures was 0.05 μg/ml or 0.5 μg/ml, respectively, i.e. in a range lower than that reached in plasma after clinical administration of CDDP 100 mg/m^2. MP sodium succinate (Solu-Medrol, Upjohn, Milan, Italy) was initially reconstituted with the manufacturer's diluent (sodium biphosphate, sodium phosphate and benzyl alcohol in distilled water), further diluted in complete medium and 0.1 μg were added to the appropriate wells. Therefore, the MP concentration in culture was 0.5 μg/ml, i.e. in the same range as that reached in plasma after clinical administration of MP 150–200 mg. Control wells were represented by 1×10^5 cells in 200 μl complete medium. The cultures were set in triplicate and kept at 37°C in 5% CO_2 atmosphere. After 24 h, the cultures were centrifuged at 2000 rpm for 10 min to remove cells, and supernatants were frozen until assayed. 5HT levels were determined by an ELISA test (Immunotech SA, Marseille, France). The intra-assay variation was 9%, the inter-assay 8% and the results are expressed in nM.

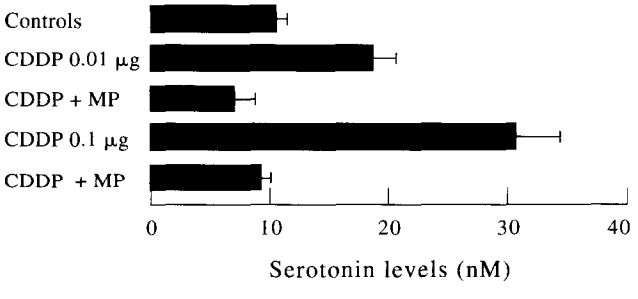

Figure 2. Effect of methylprednisolone sodium succinate (MP) on serotonin released from peripheral blood mononuclear cells cultured in the presence of CDDP at different concentrations.

All results are expressed as mean ± standard error of mean (M ± SE). Statistical analysis of data was performed using *t*-test for paired data and ANOVA test.

RESULTS

The level of 5HT in culture supernatants of unstimulated PBMC was 10 ± 1 nM. When PBMC were stimulated with PHA or anti-CD3 MAb, we observed a significant increase of 5HT levels (32 ± 3 nM for PHA, $P < 0.001$, and 22 ± 2 nM for anti-CD3 MAb, $P < 0.001$). Similarly, CDDP was able to induce 5HT release from PBMC in a dose-dependent manner (18 ± 4 nM for CDDP 0.01 μg and 30 ± 4 nM for CDDP 0.1 μg, $P < 0.001$) (Figure 1). The addition of MP to the culture in the presence of the two different doses of CDDP induced a significant decrease of 5HT levels: from 18 ± 2 to 6.8 ± 2 nM ($P < 0.01$) for CDDP 0.01 μg and from 30 ± 4 to 9.2 ± 1 nM ($P < 0.01$) for CDDP 0.1 μg (Figure 2).

DISCUSSION

To our knowledge, this is the first report showing the ability of CDDP to induce 5HT release from PBMC and of MP to inhibit this effect. Recent studies have shown that 5HT can be released, along with several cytokines, by activated T lymphocytes and monocytes. Furthermore, inhibitors of 5HT synthesis and antagonists of $5HT_{1A}$ receptors inhibit T cell proliferation and the production of interferon (IFN)γ and interleukin(IL)-2 by T cells after antigenic stimulation [8]. Our study was based upon the previously demonstrated immunomodulating activities of CDDP to verify its ability to induce 5HT release from PBMC. In fact, CDDP induces a series of intracellular changes in PBMC, similar to those demonstrated after PHA or anti-CD3 MAb stimulation, such as an increase of intracellular Ca^{2+} and of PK activity [5]. Furthermore, CDDP enhances the induction of LAK activity [6] and stimulates lymphomonocyte production of IL-1β and tumour necrosis factor alpha (TNFα) in a dose- and time-dependent manner [7].

Our data confirm the previous reports on PBMC ability to release 5HT after stimulation with PHA or anti-CD3 MAb. Furthermore, they show that CDDP is able to induce 5HT release from PBMC dose-dependently. From these findings, the question may arise of whether CDDP-induced emesis in cancer patients undergoing high-dose CDDP could be due to the release of 5HT, not only from enterochromaffin cells, but also from PBMC. Based on many physiological and biochemical considerations, it can be argued that the contribution of 5HT released by PBMC is minimal, if any, to the clinical CDDP-induced emesis. Since: (i) although chemotherapy-induced emesis is associated with increases in the urinary excretion of 5-hydroxyindole-acetic acid (the main metabolite of 5HT [9]), increases in plasma 5HT levels have been observed in only a few patients [10, 11], which means that the mechanism of activation of $5HT_3$ receptors is mediated exclusively by 5HT locally released by enterochromaffin cells [12]; (ii) 5HT release occurs by direct cytotoxicity of CDDP on enterochromaffin cells of gastrointestinal mucosa [13]; (iii) no evidence of 5HT depletion has been obtained after high-dose CDDP [13]; and (iv) animal experiments ruled out a role of circulating substances [13]. However, the question of a possible causative role of CDDP-induced 5HT release by

lymphocytes of the gastrointestinal mucosa, which constitute the mucosa-associated lymphoid tissue (MALT), still remains. It is very difficult to assess the number of these cells, which is subject to wide-ranging differences between individuals, and therefore their possible relative contribution to the phenomenon of clinical emesis. Based on the knowledge that more than 90% of gastrointestinal 5HT is present in enterochromaffin cells [10, 14], it can be suggested that the same cells are by far the major, if not even the unique, source of 5HT involved in clinical emesis.

Interestingly, our study shows that MP, well known for its potent anti-emetic effect, is able to inhibit *in vitro* CDDP-induced 5HT release by PBMC. Thus, the anti-emetic effect of corticosteroids may be, at least partly, due to their specific counteraction on the effects of CDDP on 5HT production and release by MALT cells. This counteraction may be considered as a particular mechanism by which corticosteroids act as immunosuppressing agents, influencing in an inhibitory way most immune functions, such as the cytokine IL-1, IL-2, TNF, IFNγ production, the proliferative response of PBMC to antigenic/mitogenic stimuli and the generation of LAK activity [15].

In conclusion, our results highlight a new mechanism through which CDDP could induce emesis, and moreover suggest a further mechanism by which corticosteroids mediate their anti-emetic effect.

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