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Effect of ibuprofen on monocyte activation by liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (CGP 19835A): can ibuprofen reduce fever and chills without compromising immune stimulation?

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Abstract. The purpose of this study was to determine the effects of ibuprofen on the ability of liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (L-MTP-PE) to activate human blood monocytes in vitro. We undertook these experiments because the major toxic side-effects following L-MTP-PE infusion, fever and chills, could be prevented when ibuprofen was given orally immediately before L-MTP-PE infusion. It was therefore important to determine whether ibuprofen interfered with the macrophage-activation properties of L-MTP-PE. Peripheral blood monocytes were isolated from normal donors, then incubated with L-MTP-PE in the presence or absence of ibuprofen. The cytotoxic properties of the monocytes were assessed by a radioisotope-release assay against A375 cells. Ibuprofen at dose levels of 40 μg/ml suppressed the generation of the cytotoxic phenotype but did not interfere with the killing process once the cells were activated. Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) production, as well as the mRNA expression of these cytokines, was suppressed by 40 μg/ml ibuprofen. Since IL-1 and TNF play a crucial role in the cytotoxic function of monocytes, these findings may explain the mechanism by which ibuprofen inhibited the generation of the cytotoxic phenotype by L-MTP-PE. By contrast, ibuprofen dose levels up to 10 μg/ml had no effect on the generation of monocyte-mediated cytotoxicity by L-MTP-PE and no effect on the production, secretion, or mRNA expression of TNF and IL-1. Therefore, we concluded that if ibuprofen is to be used to control the side-effects of L-MTP-PE, blood levels of up to 10 μg/ml are desirable. In two of three patients, we determined that an oral dose of 200 mg given immediately before L-MTP-PE infusion could achieve these desired blood levels.

Key words: Liposomal MTP-PE – Activated monocytes – Ibuprofen – Cytokine production

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Introduction

Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (L-MTP-PE) is a biological agent currently undergoing phase I and phase II testing at our institution in patients with melanoma or osteosarcoma. MTP-PE is a monocyte/macrophage activator and thus its use is classified as immunotherapy [7]. Encapsulation into liposomes allows the agent to be targeted to monocytes and pulmonary macrophages following intravenous infusion [23]. Only the liposomal form of MTP-PE (CGP 19835A) is currently available for clinical trials.

The selection of melanoma and osteosarcoma for clinical trials with L-MTP-PE stems from several preclinical investigations. In a murine B16 melanoma model, the administration of L-MTP-PE resulted in the activation of pulmonary macrophages and regression of lung and lymph node metastases [3, 20]. In addition, dogs with spontaneous osteosarcoma who received 8 weeks of therapy with L-MTP-PE alone following amputation survived significantly longer (median, 222 days) than those that did not receive L-MTP-PE (median, 77 days) [13]. Recent data suggest that L-MTP-PE can be combined with chemotherapy without interfering with the immunostimulatory capacity of the drug [4]. Furthermore, combination therapy consisting of cisplatin plus L-MTP-PE was found to be superior to cisplatin alone in the treatment of canine osteosarcoma [14]. Together these data make L-MTP-PE an obvious agent to add to current chemotherapy regimens to improve metastasis-free survival rates.

The major side-effects following L-MTP-PE infusion are fever and chills [17]. Fever as high as 40.5°C with shaking chills has been reported within 1–2 h after infusions (Kleinerman ES, Jia SF, Griffin J, et al., Phase II study of liposomal muramyl tripeptide (CGP 19835A Lipid) in osteosarcoma: the cytokine cascade and monocyte activation following administration, submitted). These uncomfortable symptoms can be lessened or prevented when ibuprofen is given immediately before L-MTP-PE infusion. Ibuprofen, however, had no effect when administered after the onset of symptoms. Before

recommending the use of ibuprofen to curb the side-effects of L-MTP-PE, however, we felt it important to determine whether ibuprofen suppressed the immunostimulatory capacity of L-MTP-PE. We therefore determined the effect ibuprofen had on several aspects of monocyte function, including tumoricidal activity and the production and secretion of interleukin-1 (IL-1) and tumor necrosis factor (TNF). These parameters are all stimulated by L-MTP-PE.

Materials and methods

Reagents. RPMI-1640, Hanks' balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+}, fetal bovine serum, human AB serum, and Eagle's minimum essential medium were purchased from M. A. Bioproducts (Walkersville, Md.). Recombinant interferon γ (IFNγ) was supplied by Genentech Inc. (South San Francisco, Calif.). Recombinant interleukin-1β (IL-1β) was purchased from Boehringer Mannheim (Indianapolis, Ind.). Lymphocyte-separation medium was purchased from Organon Teknika (Durham, N. C.). All reagents were free of endotoxins as determined by the *Limulus* amebocyte assay (sensitivity limit of 0.025 ng/ml). *Salmonella typhosa* lipopolysaccharide was purchased from Difco Laboratories (Detroit, Mich.). L-MTP-PE (CGP 19835A) was supplied by Ciba-Geigy Ltd. (Summit, N. J.). MTP-PE (1 mg) was prepared from the lyophilized product by hydrating the lipid with 2.5 ml phosphate-buffered saline and vortexing for 5 min (0.4 mg/ml), and then diluted in medium. Ibuprofen was purchased from Sigma Chemical Company (St. Louis, Mo.); 200 mg was dissolved with 1 ml absolute ethanol (EtOH) and then diluted in medium.

Cell lines. L929 murine transformed fibroblast cells were purchased from American Type Culture Collection (Rockville, Md.). Cell line A375, derived from a human melanoma, was the source of human tumor target cells [7, 8]. Monolayer cultures were maintained on plastic in Eagle's medium with sodium pyruvate, nonessential amino acids, vitamin solution, and L-glutamine (M. A. Bioproducts), and 10% fetal bovine serum at 37° C in an incubator with a humidified atmosphere containing 5% CO_2 in air.

Separation of monocytes. Mononuclear leukocytes were isolated from the peripheral blood of normal donors by separation on LSM (1500 *g* for 10 min) and washed three times in HBSS (300 *g* for 10 min, and then 50 *g* for 15 min ×2). Cells were suspended in RPMI-1640 medium containing 5% human AB serum. The percentage of monocytes in the MNL layer was assessed by morphology and peroxidase stain, and the cell suspension was adjusted to contain 1×10^6 monocytes/ml. Monocytes were added to each well of flat-bottomed Multiwell tissue-culture plates or Microtest II plates (Falcon Plastic, Oxnard, Calif.) and allowed to adhere for 1 – 1.5 h at 37° C. Nonadherent cells were removed by three washes with HBSS. Monocyte purity was above 97%, as previously described [7, 8].

In vitro activation of human monocytes. The monocyte cultures were incubated at 37° C for 4 – 24 h with control medium containing EtOH (negative control), LPS (0.4 μg/ml, positive control), or L-MTP-PE (1.5 μg/ml) plus IFNγ (50 U/ml) with and without ibuprofen at various concentrations. The adherent monocytes were washed with medium before the addition of A375 cells. The supernatants were collected, centrifuged to remove cells, and then assayed for TNF and IL-1 activity using the L929 and D10.G4.1 assays respectively. For preparation of intracellular IL-1, adherent monocytes were frozen and thawed three times and then suspended in medium and added to the D10.G4.1 cell line.

TNF bioassay. L929 cells are efficiently killed by TNF but are resistant to IL-1. The cells were harvested by a 1-min trypsinization with 0.25% trypsin (Difco) and 0.02% EDTA, washed, and resuspended in EMEM with 5% FBS. Cells (1×10^4) were plated into each well of 96-well flat-bottomed plates. After 6 h incubation the medium was aspirated off, and the freshly collected cell-free monocyte supernatants were added to the adherent L929 cells. In this assay, growth inhibition by TNF was quantified by labeling with 0.2 μCi [^{3}H]thymidine (dT) during the last 24-h of a 72-h culture period. The growth inhibition percentage was calculated as follows:

$$\text{Growth inhibition (\%)} = \frac{{}^3\text{H in target cells in medium} - {}^3\text{H in target cells in test sample}}{{}^3\text{H in target cells in medium}} \times 100$$

where ^{3}H is measured in cpm.

IL-1 bioassay. IL-1 activity was measured by the D10.G4.1 murine helper T cell line as previously described [6, 22]. D10.G4.1 cells were cultured in bulk quantities for 14 – 16 days following exposure to H-2b-antigen-presenting spleen cells (C57BL/6) and frozen at 2.5×10^6 cells/aliquot. On the day of the assay, the cells were quickly thawed, washed, and seeded at 2×10^4 cells/well in medium containing 2.5 μg/ml concanavalin A (Sigma Chemical Co.) with serial dilutions of test and positive control supernatants in 96-well microtiter plates. After 48 h incubation, 0.2 μCi [^{3}H]dT was added to each well. Cells were harvested 24 h later in a semiautomatic cell harvester (Skatron Inc., Sterling, Va.). The amount of [^{3}H]dT incorporated was quantified by a scintillation counter. IL-1 activity was calibrated against recombinant human IL-1β.

Assay of monocyte-mediated cytotoxicity. Monocyte-mediated tumor cytotoxicity was assessed as previously described [7, 8, 21]. Target cells in exponential growth phase were incubated for 24 h in supplemented medium containing 0.3 μCi ^{125}I-labeled iododeoxyuridine (New England Nuclear, Boston, Mass.). The A375 cells were washed once to remove unbound radiolabel and harvested by a 1-min trypsinization. The labeled cells were washed with medium and resuspended in the supplemented medium. Cells (1×10^4) were plated into each well of a 96-well culture plate containing 2×10^5 monocytes/well to obtain an initial target : effector cell ratio of 1 : 20. Radiolabeled target cells plated alone provided an additional control group. After 24 h, the cultures were aspirated to remove the medium, refed with fresh medium, and then cultured for an additional 48 h. The percentage of monocyte-mediated cytolysis was calculated according to the following formula:

$$\text{Cytotoxicity generated (\%)} = \frac{({}^{125}\text{I in target cells cultured with control monocytes}) - ({}^{125}\text{I in target cells with test monocytes})}{({}^{125}\text{I in target cells cultured with control monocytes})} \times 100$$

Statistical analysis. Experimental results were analyzed for their statistical significance by Student's *t*-test.

Isolation of monocyte RNA. Human MNL were isolated from leukocyte-rich buffy coats by the method already described. Into each well of a six-well tissue-culture cluster plate (Costar, Cambridge, Mass.), $(7-10) \times 10^6$ monocytes were plated. After 1 h of incubation, the nonadherent cells were removed by washing three times. The monocyte cultures were then incubated for 4 – 16 h with control medium, ibuprofen, L-MTP-PE, or ibuprofen plus L-MTP-PE.

Total RNA was isolated at designated times by the method of Chomczynski and Sacchi [2]. Briefly, the supernatant was aspirated, and the monocyte monolayer was lysed with 4 M guanidinium isothiocyanate. After transferring to a polypropylene tube, 2 M sodium acetate, phenol (water-saturated), and chloroform/isoamyl alcohol (49 : 1) were sequentially added. Total RNA was extracted by centrifugation at 10000 *g* for 20 min at 4° C.

mRNA dot-blot analysis. Total RNA (0.5 – 8 μg) was blotted onto a Nytran membrane (Schleicher & Schüll, Keene, N. H.) using the S & S Minifold I filtration apparatus. The membrane was baked at 80° C for 1.5 h under vacuum and prehybridized at 44° C overnight. Hybridization was performed in fresh prehybridization solution to which a ^{32}P-labeled human TNFα, IL-1α. IL-1β, or β-actin cDNA probe was added ($1.0 \times 10^6 – 2.5 \times 10^6$ cpm/ml hybridization mix). The ^{32}P-labeled probes were produced by a random-hexamer labeling kit from Amersham (Amersham, UK). The hybridization proceeded at 44° C for 24 – 48 h. The membrane was washed twice at 60° C for 30 min in 0.1 × standard

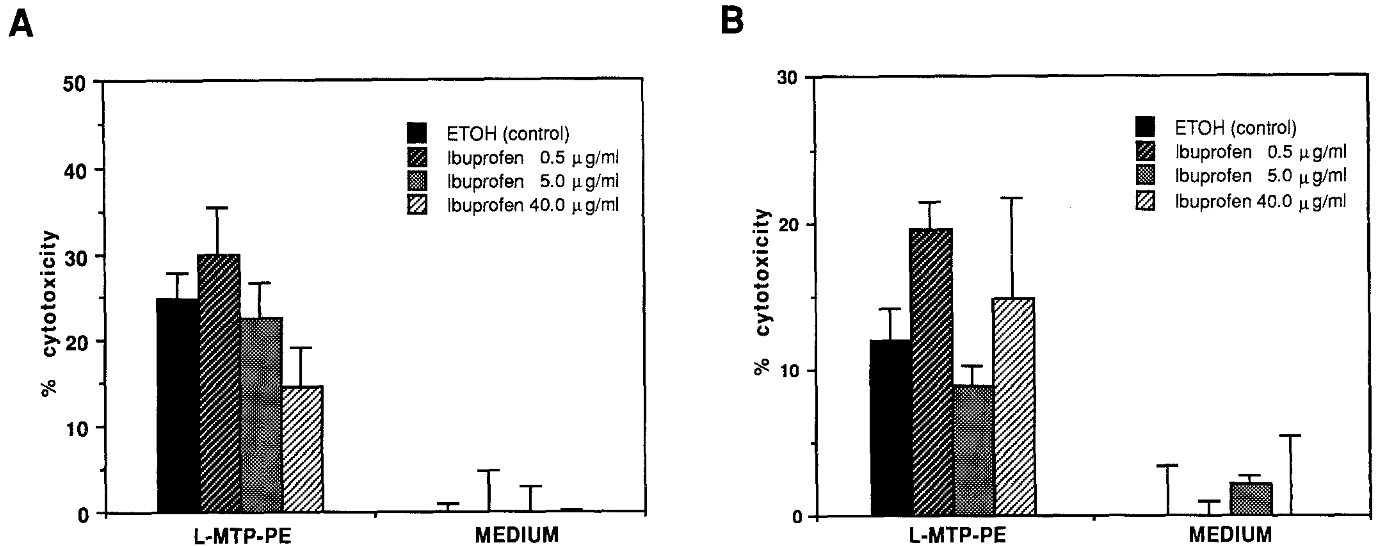

Fig. 1 A, B. Effect of ibuprofen on monocyte-mediated cytotoxicity induced by liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (*L-MTP-PE*). **A** Peripheral blood monocytes were incubated with L-MTP-PE plus interferon γ (IFNγ) or medium alone in presence or absence of ibuprofen for 24 h and then assayed for cytotoxicity against A375 tumor target cells. **B** Following 24 h incubation of monocytes with L-MTP-PE plus IFNγ, ibuprofen was added with A375 cells for the cytotoxicity assay

saline citrate and 0.1% sodium dodecyl sulfate, and then exposed to Kodak XAR-5 film with intensifying screens for adequate time at –70° C.

Results

Effect of ibuprofen on fever and chills

The fever and chills often experienced by patients following the first dose of L-MTP-PE usually diminish or disappear on subsequent administrations of the drug [17]. The symptoms are also usually well controlled by acetaminophen alone. However, we have found that some patients continue to experience these side-effects throughout the treatment course. Others have a resurgence of these symptoms late in the treatment period (2–3 months into therapy). For example, in one 45-yearl-old man who was receiving L-MTP-PE on the phase II osteosarcoma protocol, grade 2 fever with grade 1 shaking chills, unresponsive to acetaminophen, continued to be a problem following each L-MTP-PE infusion. Theses side-effects were interfering with his ability to work and drive. When 400 mg ibuprofen was administered after the L-MTP-PE infusion was completed, no relief was obtained; his fever rose from 38°C before therapy to 39.8°C (grade 2) with a grade 1 shaking chill. By contrast, when ibuprofen was given 30 min to 1 h before L-MTP-PE infusion, the patient experienced only grade 1 fever (38.9°C) with no chill. He was more comfortable and able to carry on with his usual tasks. Similar responses were seen in two additional patients. Since the shaking chill is often what causes the most discomfort for the patient, we have found this to be a potentially beneficial option to offer our patients. We hesitated to recommend it, however, until we could learn whether it interferes with the immune activation properties of L-MTP-PE.

Effect of ibuprofen on the monocyte-mediated cytotoxic activity stimulated by L-MTP-PE

The activation of the cytotoxic phenotype of human monocytes and the subsequent lysis of tumor cells can be divided into two distinct phases. The first 24-h period of the cytotoxic assay is referred to as the activation phase. During this phase, the activating agent is incubated with the monocytes and induces the expression of the killer phenotype. Increased mRNA expression of several monokines and their subsequent secretion have been documented during this phase [15]. These monokines (i.e. IL-1α, IL-1β, and TNF) have been shown to be important in the subsequent lysis of the tumor target cells [9, 11, 12, 15, 18, 19, 24, 25]. Suppression of monokine production during the activation phase results in the suppression of the cell's ability to lyse the tumor cells [5, 9, 16]. The subsequent 72-h period, when tumor cells are cocultivated with the "activated" monocytes, is referred to as the effector phase. During this phase, the monocyte binds to the target cell and delivers to it the lytic mediators produced during the activation phase. These lytic mediators then induce target-cell death.

To determine whether ibuprofen had any effect on the activation process of monocyte tumoricidal function, peripheral blood monocytes from normal donors were incubated with L-MTP-PE in the presence of various concentrations of ibuprofen (0.5–40 µg/ml) for 24 h. The monocyte cultures were then washed, and radiolabeled A375 melanoma cells were added; cytotoxicity was determined 72 h later. Ibuprofen was not present during the effector phase. Monocytes incubated with L-MTP-PE in the presence of 40 µg/ml ibuprofen showed a significant suppression of monocyte-mediated cytotoxicity ($P < 0.01$, Fig. 1 A), but up to 10 µg/ml ibuprofen had no effect on the generation of monocyte cytotoxic activity (Fig. 1 A; some data not shown). When present during the effector phase only, however, 40 µg/ml ibuprofen had no effect (Fig. 1 B).

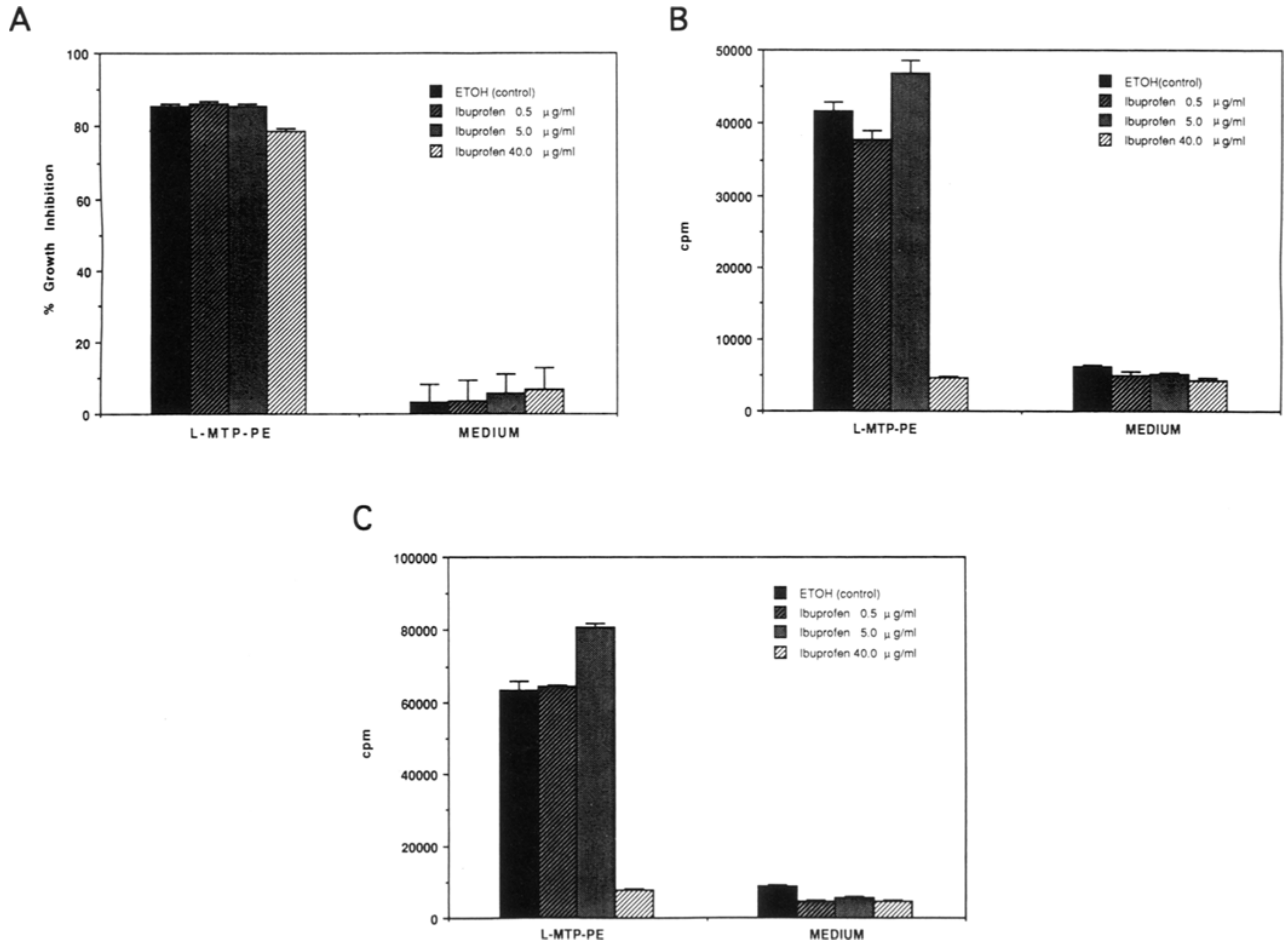

Fig. 2 A – C. Effect of ibuprofen on cytokine production by L-MTP-PE-treated monocytes. **A** TNF secretion; peripheral blood monocytes were incubated with L-MTP-PE plus IFNγ or medium alone in the presence or absence of ibuprofen for 24 h. Culture supernatants were collected and assayed for TNF secretion. **B** Interleukin-1 (IL-1) secretion; following 16 h incubation of monocytes with L-MTP-PE plus IFNγ or medium alone in the presence or absence of ibuprofen, culture supernatants were collected and assayed for IL-1 activity. IL-1 secretion was expressed as cpm ± standard deviation (*bars*). Background level of [^{3}H]thymidine incorporation was 4791 ± 332 cpm. From a standard curve using recombinant human IL-1β, 1×10^4 cpm was equivalent to 0.4 U/ml IL-1 activity, and 4×10^4 cpm was equivalent to 2.0 U/ml IL-1 activity. **C** Intracellular IL-1 production; following 16 h incubation of monocytes with L-MTP-PE or medium alone in the presence or absence of ibuprofen, adherent monocytes were frozen and thawed three times and suspended in medium, then assayed for intracellular IL-1 production. This assay was done at the same time as IL-1 secretion was measured and using cells from the same donor as in **B**

In these studies, monocytes were activated for 24 h with L-MTP-PE in the absence of ibuprofen. Ibuprofen was then added with the A375 target cells for 72 h. Thus, once the monocytes were activated, ibuprofen could not suppress their cytotoxic function or interfere with the subsequent lysis of the target cells. When ibuprofen was present during the entire cytotoxic assay (activation and effector phases), however, suppression of cytotoxicity was once again demonstrated, but only at 40 µg/ml (data not shown).

Effect of ibuprofen on the production of TNF and IL-1 by monocytes stimulated with L-MTP-PE

Monocyte-mediated tumoricidal activity is closely associated with TNF and IL-1 secretion [5, 9, 15]. We have recently demonstrated that L-MTP-PE stimulates both TNF and IL-1 [15]. We therefore tested the effect ibuprofen had on the monocytes' ability to produce TNF and IL-1 in response to L-MTP-PE. As shown in Fig. 2, L-MTP-PE stimulated TNF secretion and the production of both secreted and intracellular IL-1. Ibuprofen (40 µg/ml) had a profound effect on IL-1 production, suppressing both the secreted and intracellular forms, and caused a small but significant ($P \leq 0.01$) decrease in TNF secretion. As seen with the cytotoxicity assay (Fig. 1), ibuprofen up to 10 µg/ml had no effect on TNF and IL-1 production (Fig. 2; some data not shown). Therefore, the decreased production of TNF and IL-1 corresponds to the cytotoxicity data. Interfering with IL-1 and TNF production may be the mechanism by which ibuprofen suppresses monocyte cytotoxic activity at 40 µg/ml.

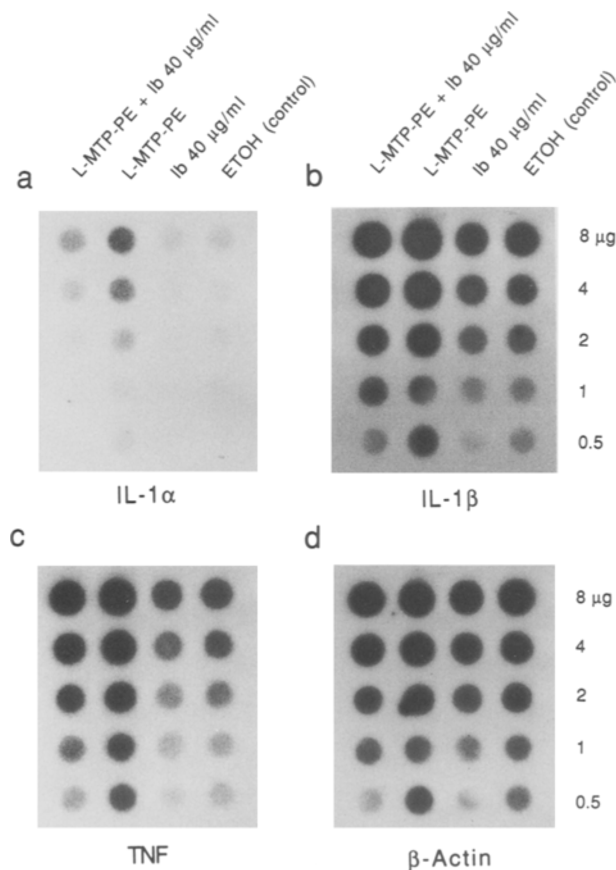

Fig. 3. Effect of ibuprofen (40 µg/ml) on cytokine mRNA expression induced by L-MTP-PE. Monocytes were incubated with L-MTP-PE in the presence or absence of ibuprofen (*Ib*) for 4 h. RNA was extracted and blotted onto a Nytran membrane in serial dilutions in microgram units as indicated, then hybridized to radiolabeled human IL-1α, IL-1β, TNF, or β-actin cDNA probe. The β-actin mRNA level does not change after L-MTP-PE stimulation. In this donor, IL-1α was suppressed by ibuprofen

Effect of ibuprofen on the monocyte IL-1 and TNF mRNA expression induced by L-MTP-PE

To determine whether the decreased production of TNF and IL-1 could be secondary to a decrease in the mRNA expression of these monokines, monocytes were incubated with L-MTP-PE in the presence of various doses of ibuprofen. Total cellular RNA was extracted and then analyzed, by serial twofold dilution, for its TNF, IL-1α, IL-1β, and β-actin mRNA by dot-blot analysis. We have previously shown that the specificity of the IL-1 and TNF messages measured by this assay corresponds to the reported molecular size of the mRNA when measured by Northern blot analysis [10, 15]. Figures 3–5 show representative experiments on monocytes derived from three different donors. As previously shown [15], L-MTP-PE stimulated increased expression of IL-1α, IL-1β, and TNF mRNA. Ibuprofen (40 µg/ml) suppressed this stimulated expression of IL-1α and IL-1β mRNA by two- to fourfold, depending upon the donor (Figs. 3, 4). No effect was seen at doses of 5–10 µg/ml ibuprofen (Figs. 4, 5). The effect of 40 µg/ml ibuprofen on TNFα expression was less impressive; in two of three donors tested, no effect was seen. Figures 3 and 4 show the results from two representative donors. Figure 3 shows a twofold decrease in TNF mRNA in monocytes incubated with 40 µg/ml ibuprofen, whereas no effect is seen in Fig. 4.

Once again the significant decrease in IL-1 mRNA and the equivocal decrease in TNF mRNA corresponded to the reduction in IL-1 and TNF protein measured in Fig. 2.

Plasma ibuprofen levels following oral administration

We have now determined that ibuprofen at dose levels of up to 10 µg/ml will not interfere with macrophage activation by L-MTP-PE, the production and secretion of IL-1 and TNF, or the lysis of tumor cells by the activated monocyte. We therefore wished to determine what dose of oral ibuprofen could be given to keep blood levels at or near the 10 µg/ml level. Anaya et al. reported that 400 mg ibuprofen given orally to healthy men produced peak levels after 1.5 h of 32.3 µg/ml [1]. We chose to investigate the dose of 200 mg ibuprofen. Three osteosarcoma patients participating in a phase II trial of L-MTP-PE were given 200 mg ibuprofen orally immediately before L-MTP-PE infusion. Serum was collected 1, 1.5, 2, and 3 h after administration of the ibuprofen and L-MTP-PE. In two patients (both 1.7 m^2), peak ibuprofen levels (11.5 µg/ml and 25.5 µg/ml) were seen after 2 h. The 25.5 µg/ml level had fallen to 7.4 µg/ml at 3 h. A third patient (1.9 m^2) had undetectable serum ibuprofen levels at all assay times following the 200-mg dose. All patients had minimal elevations in temperature and no chills. Therefore, although there is variability among patients with regard to metabolism and peak blood ibuprofen levels, we believe that 200 mg ibuprofen is a safe dose to employ to counteract the fever and chills induced by L-MTP-PE therapy.

Discussion

The major toxic effects encountered with L-MTP-PE therapy are fever and shaking chills. The present study indicates that ibuprofen may be a suitable agent to reduce the severity of these side-effects. When ibuprofen was administered before L-MTP-PE, fever and chills were reduced. Since L-MTP-PE is an immunostimulatory drug with potent monocyte- and macrophage-activating properties, choosing an agent that reduces toxic side-effects without compromising the stimulatory effects of L-MTP-PE is imperative. We therefore undertook these experiments to determine whether ibuprofen suppressed the monocyte activation by L-MTP-PE.

The present study demonstrated that the ability of L-MTP-PE to activate monocyte function was preserved when ibuprofen levels were at or below 10 µg/ml. At this level, the ibuprofen had no effect on L-MTP-PE's activation of monocytes to kill tumor cells, no effect on the killing process itself, no effect on the up-regulation of IL-1α, IL-1β, or TNF mRNA by L-MTP-PE, and no effect on the production and secretion of the above-mentioned cytokines. However, ibuprofen levels of 40 µg/ml interfered with all aspects of macrophage activation by

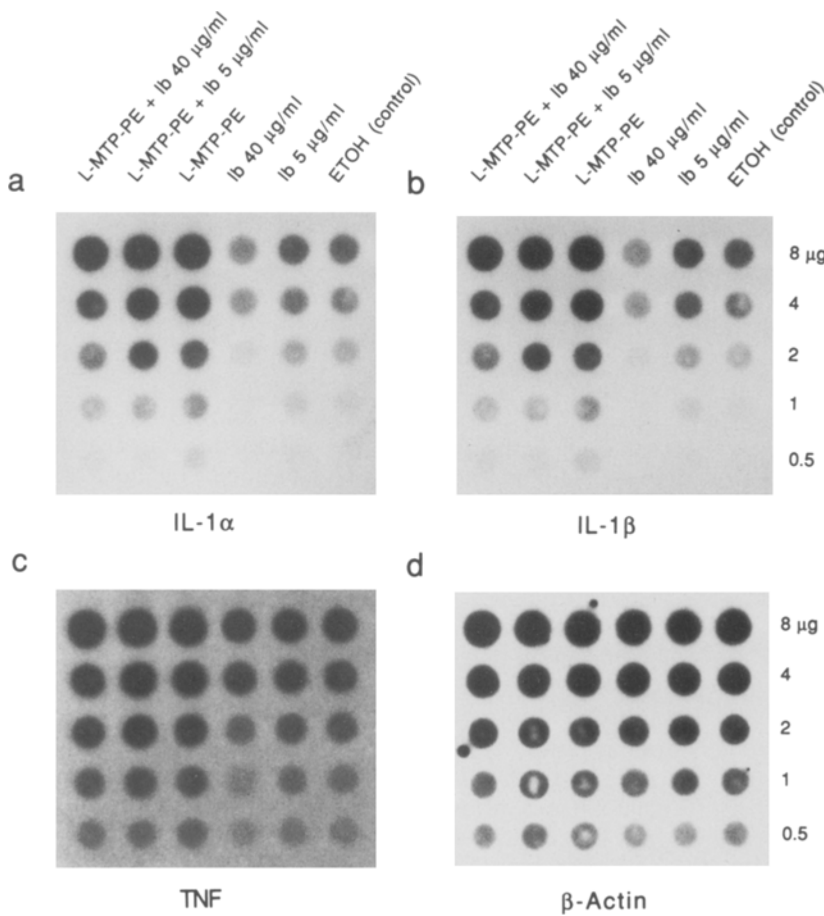

Fig. 4. Effect of ibuprofen (5 µg/ml and 40 µg/ml) on cytokine mRNA expression induced by L-MTP-PE. Monocytes were incubated with L-MTP-PE in the presence or absence of ibuprofen (*Ib*) for 4 h. RNA was extracted and analyzed by dot-blot analysis as in Fig. 3. The β-actin mRNA level does not change following L-MTP-PE stimulation

L-MTP-PE. We further demonstrated that blood levels compatible with the range up to 10 µg/ml could be achieved when 200 mg ibuprofen was given orally before L-MTP-PE infusion. A dose of 400 mg ibuprofen has been shown to produce a peak ibuprofen level of 32.3 µg/ml [12], which approaches the 40 µg/ml level that we found to be inhibitory to monocyte stimulation. Although there was patient variability with respect to drug metabolism and blood levels, we recommend 200 mg as the dose to employ to curb the toxic effects of L-MTP-PE without interfering with its immunostimulatory capacity. It may be possible to use higher doses of ibuprofen if blood levels are monitored.

The mechanism by which ibuprofen reduces fever and chills is poorly understood. We have previously demonstrated that the onset of fever and chills induced by L-MTP-PE correlated with elevated plasma IL-6 (Kleinerman, Jia, and Griffin, submitted). In 16 of 16 patients, induction of circulating IL-6 was detected 2–3 h after L-MTP-PE infusion. The onset of fever and chills began 30 min to 1 h before peak IL-6 levels. Although elevations in circulating TNF were seen 1–2 h after infusion, these did not correlate with the onset of fever and chills. Furthermore, no IL-1α nor IL-1β was detected in these patients. We therefore concluded that IL-6 was the major cause of fever.

In addition to elevated plasma TNF and IL-6, elevations in neopterin (a marker used for macrophage activation) and C-reactive protein were demonstrated 24 h after L-MTP-PE infusion in these patients. In one patient tested, the administration of ibuprofen before L-MTP-PE resulted in no detectable plasma IL-6. Two hours after infusion, plasma TNF rose from 50 pg/ml to 116 pg/ml, neopterin increased from 0.9 ng/ml to 2.3 ng/ml, and C-reactive protein from 0.4 ng/dl to 6.9 ng/dl. With the exception of IL-6, these levels are compatible with our previous findings (Kleinerman, Jia, and Griffin, submitted). Although these data are preliminary, they suggest that ibuprofen may control the fever and chills induced by L-MTP-PE through a mechanism involving IL-6. The finding that elevated TNF, neopterin, and C-reactive protein occurred when ibuprofen was given before L-MTP-PE would support our in vitro data that ibuprofen does not suppress the ability of L-MTP-PE to activate macrophages.

L-MTP-PE therapy is administered twice weekly to outpatients for 6 months in the two clinical trials now ongoing in our institution. It is unlikely that this extended drug administration time will decrease in future trials. Especially over such a lengthy period, persistent fever and shaking chills can be a hindrance to patients. Before considering reducing the dose of L-MTP-PE because of toxicity, or should these toxic side-effects severely compromise a patient's ability to carry out normal daily tasks, a trial of ibuprofen, administered as described, could be considered. Our data indicate that 200 mg ibuprofen given before

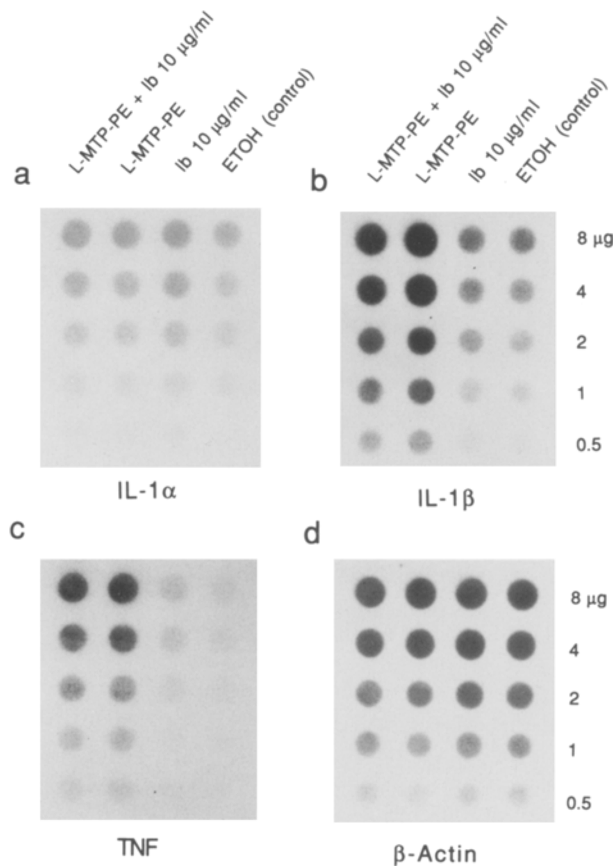

Fig. 5. Effect of ibuprofen (10 µg/ml) on cytokine mRNA expression induced by L-MTP-PE. Monocytes were incubated with L-MTP-PE in the presence or absence of ibuprofen (*Ib*). RNA was extracted and analyzed by dot-blot analysis as in Fig. 3. The β-actin mRNA level does not change following L-MTP-PE stimulation

L-MTP-PE can reduce the severity of fever and chills without compromising the immunostimulatory effects of L-MTP-PE.

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References

1. Anaya AL, Mayersohn M, Conrad KA, Dimmitt DC (1986) The influence of sucralfate on ibuprofen absorption in healthy adult males. Biopharm Drug Dispos 7: 443
2. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156
3. Fidler IJ, Sone S, Fogler WE, Barnes ZL (1982) Eradication of spontaneous metastases and activation of alveolar macrophages by intravenous injection of liposomes containing muramyl dipeptide. Yearbook of cancer
4. Hudson MM, Snyder JS, Jaffe N, Kleinerman ES (1988) In vitro and in vivo effect of Adriamycin therapy on monocyte activation by liposome-encapsulated immunomodulators. Cancer Res 48: 5256
5. Hudson MM, Markowitz AB, Gutterman JU, Knowles RD, Snyder JS, Kleinerman ES (1990) Effect of recombinant human interleukin 4 on human monocyte activity. Cancer Res 50: 3154
6. Kaye J, Porcelli S, Tite J, Jones B, Janeway CA Jr (1983) Both a monoclonal antibody and antisera specific for determinants unique to individual cloned helper T cell lines can substitute for antigen and antigen-presenting cells in the activation of T cells. J Exp Med 158: 836
7. Kleinerman ES, Erickson KL, Schroit AJ, Fogler WE, Fidler IJ (1983) Activation of tumoricidal properties in human blood monocytes by liposomes containing muramyl tripeptide. Cancer Res 43: 2010
8. Kleinerman ES, Schroit AJ, Fogler WE, Fidler IJ (1983) Tumoricidal activity of human monocytes activated in vitro by free and liposome-encapsulated human lymphokines. J Clin Invest 72: 304
9. Kleinerman ES, Murray JL, Snyder JS, Cunningham JE, Fidler IJ (1989) Activation of tumoricidal properties in monocytes from cancer patients following intravenous administration of liposomes containing muramyl tripeptide phosphatidylethanolamine. Cancer Res 49: 4665
10. Kleinerman ES, Knowles RD, Blick MB, Zwelling LA (1989) Lithium chloride stimulates human monocytes to secrete tumor necrosis factor/cachectin. J Leukocyte Biol 46: 484
11. Lachman LB, Dinarello CA, Llansa ND, Fidler IJ (1986) Natural and recombinant human interleukin 1-β is cytotoxic for human melanoma cells. J Immunol 136: 3098
12. Lovett D, Kozan B, Hadam M, Resch K, Gemsa D (1986) Macrophage cytotoxicity: interleukin 1 as a mediator of tumor cytostasis. J Immunol 136: 340
13. MacEwen EG, Kurzman ID, Rosenthal RC, Smith BW, Manley PA, Roush JK, Howard PE (1989) Therapy of osteosarcoma in dogs with intravenous injection of liposome-encapsulated muramyl tripeptide. J Natl Cancer Inst 81: 935
14. MacEwen EG, Kurzman ID, Rosenthal RC (1990) Randomized study using adjuvant liposome-encapsulated MTP-PE with cisplatin in the canine osteosarcoma model – preliminary results. Presented at NCI Conference, Combining Biological Response Modifiers with Cytotoxics in the Treatment of Cancer, Baltimore, Md
15. Maeda M, Knowles RD, Kleinerman ES (1991) Muramyl tripeptide phosphatidylethanolamine (MTP-PE) encapsulated in liposomes stimulates monocyte production of TNF and IL-1 in vitro. Cancer Commun 3: 305
16. Murray JL (1982) Prostaglandin E_2 modulation of human monocytes antibody – dependent cell-mediated cytotoxicity against human red blood cells. Cell Immunol 71: 196
17. Murray JL, Kleinerman ES, Cunningham JE, Tatom JR, Andrejcio K, Lepe-Zuniga J, Lamki LM, Rosenblum MG, Frost H, Gutterman JU, Fidler IJ, Krakoff IH (1989) Phase I trial of liposomal muramyl tripeptide phosphatidylethanolamine in cancer patients. J Clin Oncol 7: 1915
18. Old LJ (1985) Tumor necrosis factor (TNF). Science 230: 630
19. Onozaki K, Matsushima K, Aggarwal BB, Oppenheim JJ (1985) Human interleukin 1 is a cytocidal factor for several tumor cell lines. J Immunol 135: 3962
20. Poste G, Bucana C, Raz A, Bugelski P, Kirsh R, Fidler IJ (1982) Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. Cancer Res 42: 1412
21. Raz A, Fogler WE, Fidler IJ (1979) The effects of experimental conditions on the expression of in vitro mediated tumor cytotoxicity by murine macrophages. Cancer Immunol Immunother 7: 153
22. Saizawa M (1989) A comparison of frequencies between mouse T cells and B cells which are mutually interactive and specific to each other. J Nippon Med Sch 56: 472
23. Schroit AJ, Fidler IJ (1982) Effects of liposome structure and lipid composition on the activation of the tumoricidal properties of macrophages by liposomes containing muramyl dipeptide. Cancer Res 42: 161
24. Sugarman BJ, Aggarwal BB, Hass PE, Palladino MA Jr, Shepard HM (1985) Recombinant human tumor necrosis factro-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230: 943
25. Urban JL, Shepard HM, Rothstein JL, Sugarman BJ, Schreiber H (1986) Tumor necrosis factor: a potent effector molecule for tumor cell killing by activated macrophages. Proc Natl Acad Sci USA 83: 5233