Research Article

Lipofundin Affects Cytokine Release by Peripheral Blood Mononuclear Cells from Healthy Individuals

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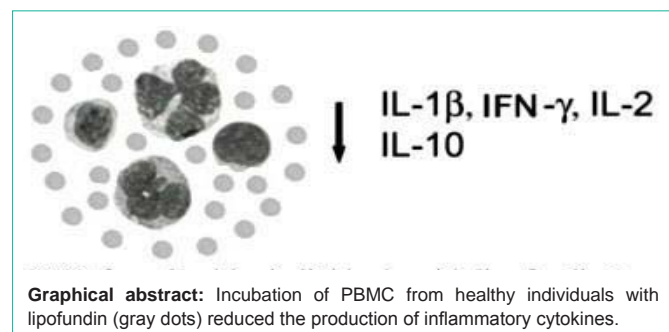
Abstract

Background: Administration of intralipid to critically ill patients is a common therapeutic modality. Studies have shown that patients with life-threatening diseases expressed a marked improvement of their clinical and immunology features following feeding by parenteral intralipid regimens. Considering the important role of the peripheral blood mononuclear cells (PBMC) as immune system constituents, the question has been raised if intralipid added to the cells *in vitro* will affect their capacity for inflammatory cytokine generation.

Methods: Non-stimulated and LPS or PMA stimulated human PBMC were incubated with intralipid (Lipofundin) and the production of TNFα, IL-1β, IL-6, IL-1ra, IL-10, IL-2 and IFNγ was evaluated.

Results: Following 24 hrs of incubation a concentration dependent decreased production of IL-1β and IL-10 in LPS-stimulated and non-stimulated cells were observed, whereas that of TNF-α, IL-6 and IL-1ra was not affected. Intralipid suppressed the production of IFNγ and IL-2 following incubation with stimulated cells only.

Conclusions: The results indicate that intralipid modulates immune cells' function, an observation that may explain the favorable effect of parenteral nutrition on patients with life-threatening diseases.

Keywords: Cytokines; Lipofundin; Immunity; Mononuclear cells; Parenteral nutrition

Graphical abstract: Incubation of PBMC from healthy individuals with lipofundin (gray dots) reduced the production of inflammatory cytokines.

Abbreviations

IL – InterLeukin; PBMC - Peripheral Blood Mononuclear Cells; PBS – Phosphate Buffered Saline; CM – Complete Medium; LPS – LipoPolySaccharides; TNF – Tumor Necrosis Factor; IFN – InterFeron; PMA – Phorbol Myristate Acetate

Introduction

Parenteral nutrition is a widely accepted therapeutic modality in cases with intestinal failure. It is indicated in patients ranging from preterm infants up to adults suffering from disabling or incurable diseases requiring nutritional support for life maintenance [1, 2]. It is imperative to keep-in-mind that applying short- term, long-term or even cyclic parenteral nutrition necessitates the existence of a close collaboration between trained medical personnel, pharmacists and dietitians, as well as profound proficiency on the variety of nutritional lipid emulsions [2-4]. Inappropriate dosage, rapid intravenous administration, lack of carbohydrate addition may cause undesirable effects leading to fat overload syndrome [5, 6]. It is notable that intravenous administration of lipid emulsions has been used for purposes others than parenteral nutrition, such as reverse of life threatening drug toxicities, reducing heart's ischemia-reperfusion injury and infarct-size, along with treatment for local anesthetic systemic toxicity [7-9]. Malnutrition in patients in need for parenteral nutrition is linked to conspicuous metabolic, hormonal and immune alterations, such as increased levels of the pro-inflammatory cytokines IL-1, IL-6, IL-8 and TNF-α [10]. Studies have proven that intralipid administration to critically ill patients with potentially impaired immune system may improve the outcome of the underlying disease. Muller et al. [11] have reported that patients with gastrointestinal carcinoma given preoperatively parenteral nutrition for 10 days showed lower postoperative complications than matched patients receiving regular hospital diet. Similar observations have been reported in other studies [12]. According to Ziegler [10] critically ill patients maintained on parenteral nutrition showed lower mortality compared to those given oral nutrition. On the other hand, in some cases the immune response may be impaired following parenteral nutrition. Cruccetti et al. [13] have found that LPS stimulated monocytes from parenteral fed infants confronted with microbial infections produced lower levels of TNF-α and IL-6 compared to enterally fed control infants and adults. Similar observations have been reported by others [14]. However, the impression from majority

of the studies on the subject is that intralipid administration activates immune cell functions. Accordingly, it has been shown that intralipid particles have been vividly ingested by human monocytes and remained within the cells up to three weeks. In addition, it has been observed that intralipid induces an increased phagocytosis of zymozan particles, without affecting other normal monocyte functions, such as superoxide production, glucose consumption and lyzozyme secretion [15]. A similar increase in monocyte lipid phagocytic ability has been reported in healthy subjects given intralipid [16]. Prolonged parenteral intralipid administration in pigs induced a lipid uptake by their lung macrophages, as well as an increase in both cell size and number [17]. In addition, increased lipid phagocytosis has been observed following 24 hrs incubation of their peripheral blood mononuclear cells with intralipid. According to Padeh et al. [18] cultured monocytes treated with intralipid showed an increased number of engulfed Candida albicans microorganisms, although their killing activity did not differ from that of control cells. Bearing in mind that mononuclear cells participate actively in the immune processes by functions other than phagocytosis, such as production of pro- and anti-inflammatory cytokines, the aim of the present work was to examine the in vitro effect of lipid emulsion (lipofundin), on the capacity for cytokine production by human peripheral mononuclear blood cells (PBMC) from healthy individuals.

Materials and Methods

Lipofundin

A 20% solution of lipofundin MCT/LCT emulsion (B. Braun, Melsingen AG, Germany) containing 100gr/L soybean oil, 100gr/L medium chain triglycerides, 25gr/L glycerol, 12gr/L egg lecitin and 0.02% α-tocopherol, pH 6.5-8.5 was used. 1:3 dilutions of lipofundin were prepared in medium and added at a final volume of 5μl/ml of cell suspension. A concentration of 5μl/ml of 20% lipofundin MCT/LCT emulsion was considered as 0.5% and designated as lipofundin solution

Cell preparation and culture conditions

PBMC were separated from healthy adult blood bank donors' venous blood by gradient centrifugation using Lymphoprep-1077 (Axis-Shield PoC AS, Oslo, Norway). The cells were washed twice in phosphate buffered saline (PBS) and suspended in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 1% penicillin, streptomycin and nystatin and supplemented with 10% fetal calf serum (designated as complete medium CM).

Effect of lipofundin on cytokine production

$2x10^6$/ml of PBMC suspended in CM were incubated for 24 hrs without or with 20ng/ml lipopolysaccharide (LPS, E. coli, Sigma) to determine the secretion of TNFα, IL-1β, IL-6, IL-1ra and IL-10, or with 0.5μg/ml of phorbol myristate acetate (PMA-Sigma, Israel) and 0.25μg/ml of ionomycin (Sigma, Israel) to evaluate IL-2 and IFNγ production. Lipofundin (20% solution) was added at the onset of the cultures at final concentrations between 0.02% and 0.5%. Cultures without lipofundin served as control. The plates were incubated at 37⁰C in a humidified atmosphere containing 5% CO2. At the end of the incubation period, the culture media were collected, the cells were removed by centrifugation and the supernatants were kept at -70ºC until assayed for cytokine content.
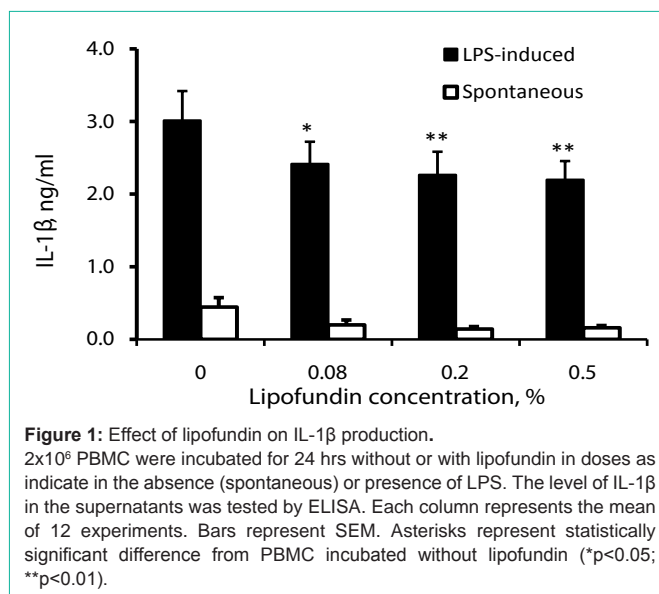

Figure 1: Effect of lipofundin on IL-1β production.
$2x10^6$ PBMC were incubated for 24 hrs without or with lipofundin in doses as indicate in the absence (spontaneous) or presence of LPS. The level of IL-1β in the supernatants was tested by ELISA. Each column represents the mean of 12 experiments. Bars represent SEM. Asterisks represent statistically significant difference from PBMC incubated without lipofundin (*p<0.05; **p<0.01).

Cytokine content in the supernatants

The concentration of cytokines in the supernatants was tested using ELISA kits specific for human cytokines (Biosource International, Camarillo, CA) as detailed in the guide-line provided by the manufacturer. Each kit is specific for one individual cytokine. The detection level of all cytokines was 30pg/ml. The percentage of the CV at the ELISA assay for the cytokines examined was 2% to the mean or less.

The possible effect of lipofundin on the ELISA assay was tested by comparing cytokine levels in the supernatants without and with lipofundin at final concentrations as described above.

Statistical analysis

Data was analyzed using one way ANOVA with repeated measures to evaluate the effect of lipofundin on each cytokine and two tailed paired Student's t-test to compare the effect of various lipofundin concentrations on the controls (incubated without lipofundin). The results are expressed as mean ± SEM. P value of <0.05 was considered as statistically significant.

Results

Effect of lipofundin on ELISA assay

The levels of all cytokines examined and tested in supernatants obtained from either LPS or PMA/ionomycin stimulated PBMC was not affected by addition of lipofundin at concentrations between 0.03% and 0.5% (data not shown).

Effect of lipofundin on IL-1β production (Figure 1)

Lipofundin caused a concentration dependent inhibition of IL-1β secretion by both non-stimulated and LPS-stimulated PBMC (p=0.04). At lipofundin doses of 0.08%, 0.2% and 0.5%, the secretion of IL-1β by non-stimulated PBMC was lowered by 55%, 78% and 73%, respectively (p=0.05) and that by LPS-stimulated cells was reduced by 20%, 25% and 27%, respectively (p=0.04, p=0.014 and p=0.007, respectively).

Effect of lipofundin on TNFα and IL-6 production

The results are shown in Table 1. Incubation of PBMC with

Table 1: Effect of lipofundin on cytokine production by PBMC.

Lipofundin concentration	0	0.08%	0.2%	0.5%	p
TNFα, pg/ml					
Spontaneous	322±86	197±52	176±37	217±64	NS
LPS-induced	595±59	614±66	634±69	559±63	NS
IL-6, ng/ml					
Spontaneous	1.49±0.16	1.43±0.19	1.30±0.14	1.00±0.21	NS
LPS-induced	28.5±4.7	31.5±5.0	31.5±5.1	31.3±4.5	NS
IL-1ra, pg/ml					
Spontaneous	336±59	374±71	376±89	372±86	NS
LPS-induced	567±62	563±57	608±56	691±75	NS

The results are expressed as Mean ± SEM (n=12 for LPS-induced cytokine secretion and n=7 for spontaneous production). P calculated by ANOVA with repeated measures represents statistically significant difference between cells incubated without or with the various doses of lipofundin as indicated. NS- not significant.

various lipofundin doses did not cause any significant effect on either TNF-α or IL-6 production by non-stimulated PBMC (p=0.871, p=0.246, respectively) or LPS-stimulated PBMC (p=0.832, p=0.966, respectively).
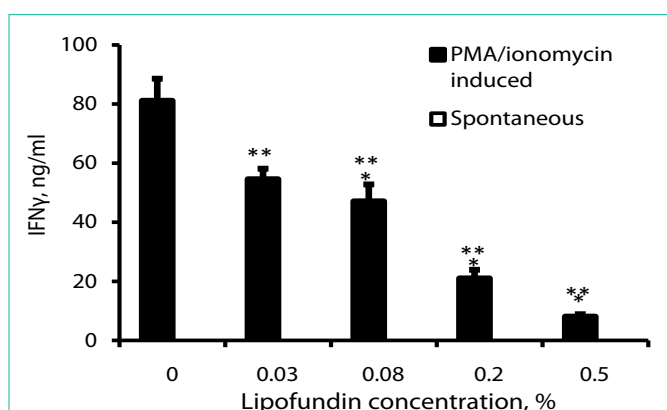
Effect of lipofundin on IFNγ secretion (Figure 2)

IFNγ could not be detected in supernatants obtained from non-stimulated PBMC incubated without or with lipofundin. A concentration dependent inhibition of IFNγ secretion was observed when lipofundin was added to PMA/ionomycin stimulated PBMC (p=0.001). At lipofundin doses of 0.02%, 0.08%, 0.2% and 0.5% the production of IFNγ was inhibited by 33% (p=0.01), 42% 74% and 90% (p<0.001), respectively.
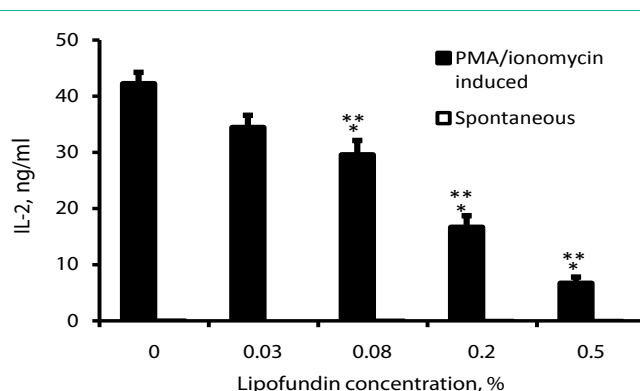
Effect of lipofundin on IL-2 production (Figure 3)

IL-2 could not be detected in supernatants obtained from non-stimulated PBMC incubated without or with lipofundin. A concentration dependent inhibition of IL-2 production was found when lipofundin was added to PMA/ionomycin stimulated PBMC (p=0.001). At lipofundin doses of 0.08%, 0.2% and 0.5% the production of IFNγ was inhibited by 32%, 60% and 84% (p<0.001), respectively.
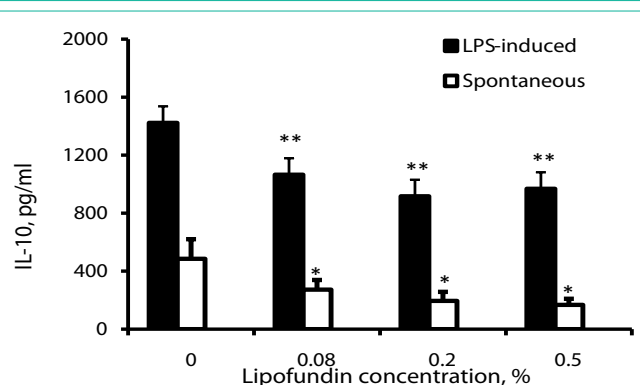
Effect of lipofundin on IL-10 production (Figure 4)

Figure 2: Effect of lipofundin on IFNγ production.
2×10^6 PBMC were incubated for 24 hrs without or with lipofundin in doses as indicate in the absence (spontaneous) or presence of PMA/ionomycin. The level of IFNγ in the supernatants was tested by ELISA. Each column represents the mean of 12 experiments. Spontaneous secretion of IFNγ by non-stimulated PBMC could not be detected. Bars represent SEM. Asterisks represent statistically significant difference from PBMC incubated without lipofundin (**p<0.01; ***p<0.001).

Figure 3: Effect of lipofundin on IL-2 production.
2×10^6 PBMC were incubated for 24 hrs without or with lipofundin in doses as indicate in the absence (spontaneous) or presence of PMA/ionomycin. The level of IL-2 in the supernatants was tested by ELISA. Each column represents the mean of 12 experiments. Spontaneous secretion of IL-2 by non-stimulated PBMC could not be detected. Bars represent SEM. Asterisks represent statistically significant difference from PBMC incubated without lipofundin (***p<0.001).

Figure 4: Effect of lipofundin on IL-10 production.
2×10^6 PBMC were incubated for 24 hrs without or with lipofundin in doses as indicate in the absence (spontaneous) or presence of LPS. The level of IL-10 in the supernatants was tested by ELISA. Each column represents the mean of 12 experiments. Bars represent SEM. Asterisks represent statistically significant difference from PBMC incubated without lipofundin (*p<0.05; **p<0.01).

Lipofundin at the doses tested caused a concentration dependent reduced secretion of IL-10 by non-stimulated and LPS stimulated PBMC (p=0.007). At lipofundin doses of 0.08%, 0.2% and 0.5% the secretion of IL-10 by non-stimulated PBMC was reduced by 44%, 60% and 65% (p=0.076, p=0.04, p=0.0228), respectively and that by LPS-stimulated PBMC by 25%, 35% and 32% (p<0.005), respectively.

Effect of lipofundin on IL-1ra production (Table 1)

At doses between 0.08% and 0.5%, lipofundin did not exert any significant effect on the production of IL-1ra by non-stimulated or LPS-stimulated PBMC, p=0.979, p=0.45, respectively.

Discussion

The results of this study demonstrate that incubation of PBMC from healthy individuals with lipofundin modulates their immune response expressed by modification of their capacity for cytokine production. This alteration was dose-dependent and related to the stimulant inducing their activation. Thus, lipofundin caused a

decrease production of IL-1β and IL-10 in LPS-stimulated and non-stimulated cells. On the other hand, it did not affect the production of TNF-α, IL-6 and IL-1ra in both stimulated and non-stimulated PBMC. As for IFNγ and IL-2, their production was suppressed by lipofundin following incubation with stimulated cells only. The suppressive activity of lipofundin on the pro-inflammatory cytokines generation, i.e. IL-1β, IFNγ and IL-2, merits attention. Even though this effect was observed *in vitro*, it is conceivable that at least a part of the beneficial effect of intralipid given to patients with life-threatening diseases will proceed via inhibition of pro-inflammatory responses by PBMC. Inhibition of IL-2, TNF-α and IL-1β release by stimulated peripheral white blood cells from healthy individuals following incubation with lipid emulsions with various fatty acid compositions has been reported by Granato et al. [19]. In patients with malignant diseases of the alimentary tract, parenteral nutrition post surgery induced a significant reduction in plasma concentration of the pro-inflammatory cytokines IL-6 and IL-8 [20]. In a previous study from our laboratory we have found that intralipid caused a dose-dependent inhibition on IL-2 production by cord blood mononuclear cells, as well as a decreased capacity of IL-2 dependent CTLL-2 cell line to respond to IL-2 [21]. On the other hand, it has been reported that in patients receiving lipid emulsion during total parenteral nutrition, there was a significantly enhanced IL-2 production concurrently with increased peripheral blood T cell subsets and antibody dependent cellular cytotoxicity [22]. It appears therefore, that the immunomodulatory properties of various lipid emulsions and the pattern of their efficacy on cytokine production depends on a few factors, such as emulsion's ingredients, intralipid concentration and longevity of intralipid administration. Arias-Diaz et al. [23] have treated patients undergoing surgery because of gastro-intestinal tract malignancies with three total parenteral nutrition regiments i.e. hypertonic glucose solution, 45% glucose and 20% long chain triglycerides and the third – as that in group two, but with addition of 20% emulsion of a mixture of medium- and long chain triglycerides The results showed a similar increase in TNF-α, IL-1, IL-6 and nitrate/nitrite plasma levels in all three regiments. A similar dependency of immunomodulatory responses on the type of intralipid composition has been observed by other investigators [19, 24-26]. It has been reported that feeding stressed rats with fat emulsions containing omega-3 and omega-6 polyunsaturated fatty acids resulted in reducing IL-8 and IL-10 production by the first emulsion, where as the second one caused increased pro-inflammatory cytokines levels [27]. Administration of fish oil-enriched emulsion to patients during the postoperative period increased the production of the pro-inflammatory cytokines TNF-α, IFNγ and IL-2 by peripheral blood lymphocytes [28]. In disparity with these reports, lipofundin in the present work induced a decreased production of the pro-inflammatory cytokines by both non-stimulated and stimulated PBMC, while the production of TNF-α, IL-6, IFNγ and IL-2 by non-stimulated PBMC was not affected. Here again, the role of the brand of lipid emulsion on the capacity of immune cells to exert their activity for cytokine production is emphasized. However, stimulated PBMC did produce decreased levels of IFNγ and IL-2 following incubation with lipofundin. Ooi et al. [29] have reported that the levels of IL-1β, TNF-α, IL-6, IFNγ and IL-2 by phytohemagglutinin stimulated lymphocytes from parenterally fed healthy volunteers and patients with colorectal cancer did not differ before and after total

parenteral nutrition. Considering the beneficial effects of lipofundin on immune responses, the decreased IL-10 production by PBMC following incubation with lipofundin observed in the present work is rather unexpected, since this is an anti-inflammatory cytokine. However, similar observation has been reported in other studies showing that IL-10 expression remained either unchanged after 14 days of parenteral nutrition in preterm infants [30], or its production by intestinal intraepithelial lymphocytes in mouse models has been suppressed [31, 32]. Accumulating evidence has demonstrated that intralipid may affect immune mononuclear cells' functions other than cytokine production. Experiments with rats given intralipid emulsion for five days showed an increased NK activity observed only with 10% and not with 5% concentration [33]. Following reports that impaired reproduction ability in women linked with increased NK activity may be improved by administration of intralipid infusions, Roussev et al. [34] have reported that intralipid given to fifty women with increased NK activity resulted in suppression and normalization of this cell function, indicating the possibility of applying intralipid as an alternative therapeutic modality in these cases.

In short, the present work demonstrates that lipofundin modifies the capacity for inflammatory cytokine production by peripheral blood mononuclear cells from healthy individuals emerging to be concentration dependent and related to the type of cell stimulation. These observations may clarify the favorable therapeutic effect of lipofundin administration to critically ill patients.

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