Lipofundin 20% induces hyperlipidemia and oxidative stress in male Sprague Dawley rats

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Abstract

Lipofundin is a lipid emulsion used in parenteral nutrition. One of adverse effects reported for this kind of pharmaceutical products is the capacity to induce oxidative stress, which is an important contributor of many diseases, such as cardiovascular diseases. The aim of the present work was to evaluate the effects of Lipofundin administration on lipid profile and serum redox biomarkers, in order to determine if both events are responsible for the undesirable effects of this lipid emulsion. Male Sprague Dawley rats were intravenously administered with 2 mL/kg of Lipofundin 20% daily, for 8 days. Then, serum lipid profile and redox biomarkers were spectrophotometrically determined. A significant increase (p<0,05) of serum lipids and biomolecules damages was observed at the end of the experiment, while a reduction of antioxidant capacity was also detected in treated rats compared with controls. Our data demonstrated that Lipofundin 20% induces hyperlipidemia, which promotes an oxidative stress state in Sprague Dawley rats.
Key words: Lipofundin 20%, hyperlipidemia, oxidative stress, parenteral nutrition.

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Introduction

Artificial fat emulsions are widely used in parenteral nutrition. The soya oil-based fat emulsions represent a major part of energy and are also a necessary source of essential fatty acids in mentioned therapy (1). Lipofundin constitutes a usually indicated fat emulsion as a source of calories for patients requiring parenteral nutrition, but researches by Jellinek et al. (2) showed that Lipofundin induces atherosclerotic lesions in rabbits and recently, our group demonstrated that Lipofundin promotes a high susceptibility to lipid peroxidation (3) and also dyslipidemia and oxidative stress (OS) in New Zealand white rabbits after 8 days of treatment (non published data).

An imbalance between oxidants and antioxidants resulting from increased production of oxidants and/or reduction in the amounts of antioxidants generates a state of stress in the cell termed OS. Clearly, chronic OS encompasses a wide variety of patho-physiological processes that directly or indirectly affect the cellular redox state (4).

The impact of Lipofundin 20% administration on lipid levels and redox environment in male *Sprague Dawley* (SD) rats had not been studied previously. In the present study we demonstrated that Lipofundin 20% induces hyperlipidemia and a systemic oxidative stress after 8 days of treatment.

Materials and Methods

Animals : Adult male SD rats, weighing 250-300 g, were obtained from CENPALAB (Bejucal, Mayabeque, Cuba). Rats were housed in a temperature conditioned room (25 ± 1 ºC) with a relative humidity of 50-52% and exposed to light-dark cycle of 12 h with free access to water and

food. Animal studies were performed with approval of Pharmacy and Food Sciences College Institutional Animal Ethical Committee. All procedures were performed in accordance with the guidelines stipulated by the Institutional Animal Care Committee and the European Union Guidelines for animal experimentation.

Lipofundin composition: Lipofundin MCT/LCT 20% (Braun Melsungen AG, Melsungen, Germany) is a lipid emulsion containing soya oil 100 g, medium-chain triglycerides 100 g, glycerol 25 g, egg lecithin 12 g, -tocopherol 170 ± 40 mg, and sodium oleate/water for injection in sufficient quantity to 1000 mL.

Experimental design: Two groups of 5 rats were used in the study. The first group received an intravenous injection of phosphate-buffered saline (PBS), pH 7,4 (control group), and the second one received a slow intravenous injection of 2 mL/kg of Lipofundin MCT/LCT 20 %, as an infusion during 1-2 min (2). This procedure was repeated daily during a period of 8 days. On day 9, the animals were anesthetized with ketamine hydrochloride (5 mg/kg i.m.), and euthanized with an overdose of sodium pentobarbital (90 mg/kg, i.v.) (Abbott Lab., Mexico SA de CV, Mexico).

Serum sample collection : Blood samples (3 mL) were withdrawn from the ocular plexus on day 0 (before Lipofundin administration) and from the abdominal aorta on day 9 (at the end of the study), for biochemical analyses. These samples were immediately centrifuged at 2500*g*, at 4 ºC for 10 min. The serum was collected and aliquots were stored at -80 ºC until analysis.

Serum lipid assay: Serum total cholesterol (TC), triglycerides (TG), low-density lipoproteins (LDLc) and high density lipoprotein (HDLc) were determined using commercial enzymatic kits (Randox, Crumlin, UK).

Redox biomarkers determinations: All biochemical parameters were determined by spectrophotometric methods using a Pharmacia 1000 Spectrophotometer (Pharmacia LKB, Uppsala, Sweden). SOD activity was determined by using RANSOD kit (catalogue No. SD 125, Randox Labs, Crumlin, UK), where xanthine and xanthine oxidase were used to generate superoxide anion radicals ($O_2^{\cdot -}$), which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was measured by the inhibition degree of this reaction. Catalase (CAT) activity was determined by following the decomposition of hydrogen peroxide (H_2O_2) at 240 nm at 10 s intervals during 1 min (5).

After precipitation of thiol proteins, the reduced glutathione (GSH) levels were measured according to the method of Sedlak and Lindsay (6) with Ellman`s reagent (5,5`dithiobis-2-nitrobenzoic acid) (Sigma St Louis, MO, USA) and the absorbance was measured at 412 nm. Purified GSH (Sigma St Louis, MO, USA) was used to generate standard curves.

The advanced oxidation protein products (AOPP) were measured as described previously (7). Briefly, samples in PBS (1 mL) were treated with 50 µL of potassium iodide 1,16 M followed by the addition of 100 µL of acetic acid. The absorbance was immediately read at 340 nm. AOPP concentration was expressed as µM of chloramines-T.

Concentration of malondialdehyde (MDA) was determined using the LPO-586 kit obtained from Calbiochem (La Jolla, CA, USA). In the assay, the production of a stable chromophore after 40 min of incubation at 45 ºC was measured at 586 nm. For standards, freshly prepared solutions of malondialdehyde bis [dimethyl acetal] (Sigma St Louis, MO, USA) were employed and assayed under identical conditions (8).

In order to determine susceptibility to lipid peroxidation and total reactive antioxidant power (TRAP), the samples were incubated with a solution of copper sulphate (final concentration 2 mM) at 37 ºC for 24 h. The peroxidation potential (PP) was calculated by subtracting the MDA levels before the induction of lipid peroxidation from the one obtained at 24h (9).

Finally, the total antioxidant status (TAS) was determined using a Randox TAS kit Cat No. 2332. In the assay ABTS (2,2´-Azino-di-[3-

ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation $ABTS^{\cdot+}$. This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentrations.

Statistical analysis: Statistical analysis was performed using the SPSS program for Windows (version 11.5, SPSS Inc). Bartlett's Box-test was used to test the homogeneity of variance. Differences between groups were determined by student's t-test (two-tailed). Data were expressed as the mean ± standard deviation (SD). The level of statistical significance employed was at least $p < 0.05$.

Results

Serum lipids: Serum TC, TG, LDLc and HDLc levels showed a significant increase ($p<0.05$) in those animals who were treated during 8 days with the lipid-rich emulsion Lipofundin, while no significant changes in serum lipids were observed in the control rats throughout the study (Table 1).

Table-1. Effects of Lipofundin on serum lipid profile.

Parameters	Control (n=6)	Lipofundin (n=6)
TC, mmol/L	1.22 ± 0.08	3.01 ± 0.15*
TG, mmol/L	1.18 ± 0.09	2.33 ± 0.12*
HDLc, mmol/L	0.46 ± 0.03	1.31 ± 0.18*
LDLc, mmol/L	0.20 ± 0.02	0.73 ± 0.09*

Values are the mean ± standard deviation. Asterisks represent statistical differences ($p<0.05$)

Redox biomarkers: Table 2 shows the behavior of serum redox parameters in both groups at day 9. The markers of damages to biomolecules were significantly ($p<0.05$) modified after 8 days of Lipofundin administration compared to non treated group. At the end of the experimental period the MDA levels, one of the end-products of lipid peroxidation, were higher in Lipofundin treated animals compared with controls. Besides, Lipofundin treatment also caused a rise of AOPP levels in comparison with control group. The activity of both antioxidant enzymes SOD and CAT were significantly higher ($p<0.05$) in Lipofundin group at the end of the experiment compared to control rats. The GSH levels decreased significantly after 8 days of Lipofundin treatment in comparison to those of untreated animals ($p<0.05$). In addition, in treated rats, TAS was significantly lower ($p<0.05$) than in controls. Finally, the susceptibility to lipid peroxidation was higher in those rats who received Lipofundin. After 8 days, in these animals was observed a significant increase of PP ($p<0.05$), compared to the one calculated in controls.

Table-2. Effects of Lipofundin on redox biomarkers.

Parameters	Control(n=6)	Lipofundin group (n=6)
MDA, μM	2.69 ± 0.07	6.24 ± 0.28*
AOPP, μM of chloramines	11.50 ± 0.73	16.22 ± 0.47*
PP, μM of MDA	4.63 ± 0.18	9.13 ± 0.34*
CAT , U/L/min	351.13 ± 19.03	477.50 ± 30.46*
SOD , U/mL/min	22.03 ± 26.44	32.00 ± 1.60*
TAS , μM	3.18 ± 0.44	1.02 ± 0.29*
GSH, μM	309.03 ± 26.44	191.21 ± 8.26*

Values are the mean ± standard deviation. Asterisks represent statistical differences ($p<0.05$)

Discussion

At the end of the experiment we observed high serum levels of triglycerides, total cholesterol, LDLc and HDLc in the animals treated with Lipofundin 20% in comparison to control rats. Lipofundin-induced hyperlipidemia could be associated with the high content of triglycerides in this emulsion. High levels of exogenous triglycerides promote ApoB100 and cholesterol synthesis, and eventually the assembly of very low-density lipoproteins (VLDL) (10). In fact**,** Lipofundin 10% caused a 60% increase in total serum cholesterol after parenteral administration in a human study (11). On the other hand, the increase of HDLc may be determined by a physiological response against the elevated LDLc levels.

In this study we demonstrated that Lipofundin-induced hyperlipidemia was associated with a systemic OS. Strong evidences for the involvement of free radicals production in the onset of hyperlipidemia have been reported previously (4). Chronic generation and sustained high toxic levels of ROS are associated with several pathological conditions, and during

hyperlipidemia related diseases, such as cardiovascular diseases (12).

In hyperlipidemia, cellular damages take place through mechanisms involving lipid peroxidation and oxidative modifications of proteins (13). On the other hand, a disruption of antioxidant enzymes activity and a drastic reduction of non-enzymatic defenses are also observed (14). High levels of MDA in the sera of treated rats were observed, which suggest the role of LPO in the loss of redox cellular status in the former animals which were under Lipofundin treatment. MDA levels have been considered not only an indicator of OS, but also as a biochemical marker of atherogenesis and other diseases associated with the high levels of serum lipids (3,15-17).

Oxidative modifications of proteins have been also implicated in hyperlipidemia and atherosclerosis (18). Through AOPP determination we measured the chlorinated proteins levels, caused by myeloperoxidase-derived hypochlorous acid (HOCl). It has been shown that HOCl-modified proteins are present in hyperlipidemic patients and predict the progression of cardiovascular diseases such as atherosclerosis (19). The high levels of AOPP in those animals that received the lipid emulsion suggest an active role of macrophages activation and inflammation in the OS generation.

Antioxidant defenses, as expression of the balance between generation and inactivation of oxidized metabolites, represent a useful tool to examine the redox status. In our study the higher activity of extracellular SOD, detected in the animals treated with Lipofundin, could be associated with an increase in O_2^- generation, typically produced by an increase in vascular NADPH oxidase activity (20).

CAT is another antioxidant enzyme present in vertebrates, which plays an important role on redox environment maintenance (21). In our study we found a high activity of the enzyme in animals treated with Lipofundin, which may be associated with the high activity of SOD. This enzyme converts the O_2^- into H_2O_2 and water, and it is known that CAT assumes the detoxification of high levels hydroperoxides, such as H_2O_2 and lipid hydroperoxides. The high content of these metabolites may in turn induce the gene expression of the enzyme and also its activity is incremented in response to oxidant stimulus mediated by ox-LDL and other ROS (22).

During atherogenesis, the reactive molecules that are produced have the potential to deplete the surrounding cells of their GSH levels, affecting their antioxidant defenses and detoxification pathways (23). Our results showed a significant depletion of serum GSH levels in the animals treated with the lipid emulsion compared to the control rabbits. This fact could be associated with the Lipofundin-mediated ROS generation and with the high concentration of biomolecules damages detected in Lipofundin-treated animals. Finally, TAS was affected by Lipofundin, which is in accordance with other reports of the literature (17).

Conclusions

In summary, the present study demonstrated that Lipofundin 20% induces hyperlipidemia, thereby promoting a systemic oxidative stress. This work shows novel evidences of Lipofundin-induced oxidative damages on lipids and proteins and the deleterious effects on antioxidant status. These results reinforce the attractive characteristics of Lipofundin to be used as an inductor of experimental hyperlipidemia and oxidative stress.

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Conflict of interest

Authors declare that they have no conflict of interest.

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