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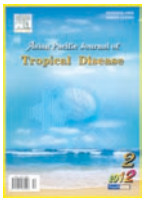

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Effects of intravenous palm oil-based lipid nanoemulsion on fat metabolism in rabbits

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ABSTRACT

Objective: To investigate the effects of a newly-developed nanoemulsion of palm oil in combination with medium chain triglyceride (MCT) oil (NEMS® MCT/LCT20%) on fat metabolism in male New Zealand white rabbits. **Methods:** Six rabbits were divided into two groups of three rabbits in each group. NEMS® MCT/LCT20%, Lipofundin® MCT/LCT 20% (a commercially-available lipid emulsion) and normal saline (control) were administered intravenously in these rabbits via the marginal ear vein for 6 h. Cross-over method was used in these rabbits with a wash-out period of two weeks in-between infusions. Triglyceride (TG), total cholesterol, low density lipoprotein and high density lipoprotein cholesterols were determined using enzymatic strip test at 0, 2, 4 and 6 h of infusion. Blood levels of free fatty acid (FFA) were measured at 0 and 6 h. **Results:** Serum TG levels increased in these rabbits after 2 and 4 h infusion of NEMS® MCT/LCT20% but returned to normal after 6 h. Concentration of FFA increased in a dose-related manner but remained within the normal range (315-535 μmol/L). Similar results were obtained with Lipofundin® MCT/LCT20%. All parameters of fat metabolism in these rabbits remained unchanged from baseline when normal saline was administered. Changes in the parameters of fat metabolism measured between NEMS® MCT/LCT20% and Lipofundin® MCT/LCT20% did not show any significant difference. Significant difference ($P<0.05$) was observed in these parameters when NEMS® MCT/LCT20% was compared to normal saline. **Conclusions:** Intravenous administration of NEMS® MCT/LCT20% did not cause a permanent increase in the TG level of rabbits while FFA remained within the normal range.

1. Introduction

Intravenous lipid emulsions (IVLEs) are used in parenteral nutrition therapy as providers of energy and essential fatty acids (EFADs). They are generally produced from soybean and safflower. These plant-based emulsions are sources of long-chain triglycerides (LCT)[1] and have been shown to increase lipid peroxidation[2]. Production of oxygen free radicals through lipid peroxidation can have a pathological impact such as increased erythrocyte fragility[3].

Since the mid-eighties, IVLEs comprising equal amounts of medium-chain triglycerides (MCTs) and LCTs have been used clinically especially in the intensive care units[4,5]. Studies have shown that MCT/LCT IVLEs produce less oxidative stress as compared to LCT IVLEs[6]. These emulsions are characterized by higher solubility into the phospholipid surface than LCT emulsions. Emulsions containing MCTs are more rapidly hydrolysed by lipoprotein lipase and provide rapidly available energy, sparing larger amounts of EFADs to be incorporated into cell membranes[6]. Interestingly, studies during the last 10 years have shown that lipid emulsions could reverse the cardiovascular toxicity of various lipophilic drugs, mainly anaesthetic and antiarrhythmic drugs[7,8].

Lipid emulsions have favourable characteristics that can be advantageous to patients. These emulsions are biodegradable and have low immunogenic tendency[9]. The administration of IVLEs consisting of LCTs has been shown to be safe in premature very-low-birth-weight infants receiving parenteral nutrition[10]. These findings provide

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fresh impetus into lipid emulsion research. New IVLEs are being developed combining various sources of fatty acids such as LCTs from soybean, MCTs from olive oil, and fish oil in a single emulsion formulation for use in the clinical settings either for parenteral nutrition therapy or as drug carriers[11-14].

Another source of LCTs is palm oil. Studies have reported that mice fed with palm oil-enriched diet had levels of total plasma cholesterol that were significantly lower than those fed with olive oil-enriched diets[15]. Palm oil is a natural antioxidant as it is rich in β carotene, tocopherol (vitamin E) and tocotrienols[16-18]. These antioxidants have been shown to exert protection at the cellular levels[19].

The use of palm oil as an intravenous source of lipids is being studied as it holds potential to be used clinically. Jufri *et al* have successfully used nanotechnology to develop a palm oil-based nanoemulsion in combination with MCTs for intravenous use (NEMS® MCT/LCT20%)[20]. The combination of LCT and MCT can enhance the potential of using an intravenous palm oil-based IVLE in patients.

The aim of this study was to investigate the effects of NEMS® MCT/LCT20% on fat metabolism in small animals.

2. Materials and methods

2.1. Determination of free fatty acid (FFA) or acidity

2.1.1. Determination of FFA in NEMS® MCT/LCT20%

A volume of 1 mL special reagent for FFAs (CDR, Italy) was incubated for 5 min at 25 ℃ and 5 μL NEMS® MCT/LCT20% added and homogenised. The sample was placed in a cuvette and fitted into the PalmOilTester (CDR, Italy). Wave length was set at 630 nm and measurements were taken in triplicate.

2.1.2. Determination of FFA in plasma of animals

Determination of FFA in animals used in the study was a modification of the method of Itaya and Ui[21]. Animal blood (1 mL) was filled in a tube containing sodium EDTA and centrifuged at 5 000 r/min for 10 min. Plasma portion was collected and mixed with phosphate buffer (pH 6.2) and 5 mL chloroform added. The chloroform layer was evaporated at 40 ℃ with the addition of nitrogen gas. A volume of 5 μL of the extracted sample was then analysed for FFA.

2.2. Determination of individual fatty acid contents in NEMS® MCT/LCT20%

Gas chromatography (GC) was used to analyse fatty acids which were present in the samples. Samples were extracted according to the standard method of the Malaysian Palm Oil Board (MPOB)[22]. Extracted fatty acid methyl esters (FAME) from samples were analysed using Shimadzu GC 2010 system (Shimadzu, Japan) with Supelco 238 column (Orbiter Scientific, USA) as the stationary phase. Helium gas was used as a carrier. FAME mix (Sigma-Aldrich, Malaysia) was used as the standard. The concentration of each fatty acid was determined from the area under the curve produced.

2.3. Measurement of lipid profile in animals

Lipid profile was determined by measuring triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol in blood samples. A drop of the animal's blood (about 30 μL) was placed on a test strip and measured using the Reflotron® blood analyser (Roche, Germany).

2.4. Animals

Male New Zealand white rabbits (2.6-3.0 kg) were used in this study. They were obtained from UKM animal house. Ethical approval for the use of animals in the study was obtained from the university's Animal Ethics Committee (Approval number: FF/FAR/2009/AHMAD/29-APRIL/256-MAY-2009-JUNE-2010). The rabbits were housed individually in stainless steel cages in an animal room for two weeks period of acclimatisation. Temperature was maintained at (23±2) ℃ and relative humidity of (55±10) % with 24 h circulating air ventilation and 12 h light-dark cycle initiated following the method of Kuwahara *et al*[23]. The rabbits were given standard pellet feed and drinking water *ad libitum*.

2.5. Infusing intravenous lipid emulsions in rabbits

The animals were divided into 2 groups with 3 rabbits in each group. NEMS® MCT/LCT20% was infused via the marginal ear vein at a rate sufficient to provide the total specified dose over a 6 h period. Doses of lipid emulsions administered were 2, 3 and 4 g/kg body weight[24]. Normal saline (NaCl 0.9%) solution was used as a negative control while Lipofundin® MCT/LCT20% (Braun, Germany), a soybean-based lipid emulsion was the positive control. Cross over method was used in these rabbits with two weeks of wash-out period from the last infusion. Blood samples were taken via the infusion site at an interval of 2 h.

2.6. Statistical analysis.

Values were obtained in triplicates and shown as mean ±SD. All data was analysed by SPSS 17 using two-way repeated Anova. P <0.05 was considered as significant.

3. Results

3.1. Free fatty acid (FFA) contents of NEMS® MCT/LCT20%

FFA content of NEMS® MCT/LCT20% remained stable even after six months storage at 25 ℃. The FFA content after preparation was (0.0014±0.0020) mEq/g. The content remained stable after six months at (0.0015±0.0030) mEq/g

(P>0.05).

3.2. Individual fatty acid contents of NEMS® MCT/LCT20%

The individual fatty acid contents of NEMS® MCT/LCT20% were shown to be stable after six months when stored at 4 ℃ (Table 1).

Table 1.
Individual fatty acid contents of NEMS® MCT/LCT20% after six months storage at 4 ℃.

Fatty acid	Fatty acid content in %	
	0 month	6 months
Capric acid (C8:0)	29.52±0.20	29.49±0.10
Caprylic acid (C10:0)	20.95±0.30	20.91±0.20
Lauric acid (C12:0)	0.38 ±0.40	0.37±0.30
Myristic acid (C14:0)	0.52±0.30	0.51±0.40
Palmitic acid (C16:0)	19.18±0.40	19.13±0.30
Stearic acid (C18:0)	2.05±0.30	2.01±0.40
Oleic acid (C18:1)	20.95±0.20	20.92±0.50
Linoleic acid (C18:2)	5.76±0.20	5.74±0.30
Linolenic acid (C18:3)	0.31±0.10	0.29±0.20
Saturated fatty acid	21.75±0.20	21.72±0.10
Mono unsaturated fatty acid	71.81±0.20	71.79±0.10
Poly unsaturated fatty acid	6.07±0.20	6.02±0.50

3.3. Lipid profile of rabbits after 6 h infusion of NEMS® MCT/LCT20%, Lipofundin® MCT/LCT20% and NaCl 0.9% solution

3.3.1. Lipid profile of animals after 6 h infusion of NEMS® MCT/LCT20%

The results obtained showed a significant increase in TG levels (P<0.05) which returned to baseline value after 6 h infusion (Figure 1). An increase in TG to a maximum level of 2.5 mmol/L was observed after 4 h infusion of NEMS® MCT/LCT20%. The TG content however fell to baseline level after 6 h of infusion. The difference between peak level and baseline was significant (P<0.05). There were no changes observed in LDL, HDL and total cholesterol levels (Figure 1).

3.3.2. Lipid profile of animals after 6 h infusion of Lipofundin® MCT/LCT20%

The results showed similar significant increase in TG levels (P<0.05) as compared to NEMS® MCT/LCT20%. The levels returned to baseline value after 6 h infusion. There were no changes observed in LDL, HDL and total cholesterol levels (Figure 2).

3.3.3. Lipid profile of animals after 6 h infusion NaCl 0.9% solution

Lipid profile in these rabbits remained unchanged from baseline when normal saline was administered (Figure 3).

3.4. Free fatty acid contents of animals after 6 h infusion of NEMS® MCT/LCT20% and Lipofundin® MCT/LCT20%

Blood concentration of FFA in both groups increased significantly (P<0.05) in a dose-related manner (Figure 4 and 5). These values were well within the normal blood level for fatty acids (normal range of 315 −535 μ mol/L).

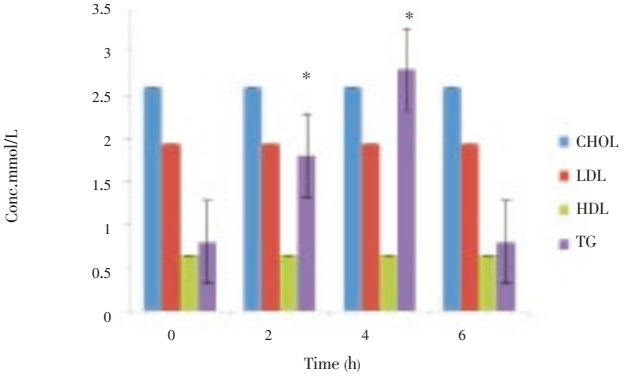

Figure 1. Lipid profile of blood samples of New Zealand White rabbits infused at 3 g/kg body weight of NEMS® MCT/LCT20% for 6 hours. (Data presented as mean±SD, *P<0.05).

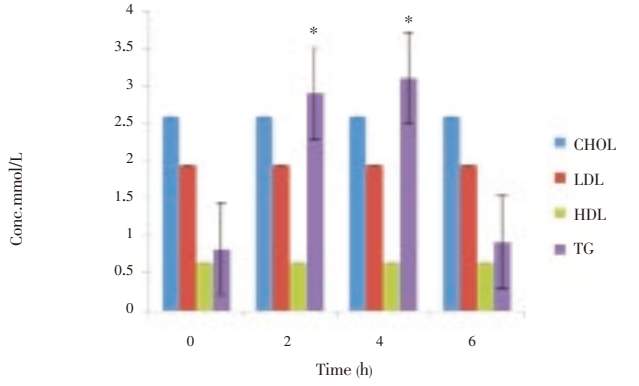

Figure 2. Lipid profile of blood samples of New Zealand White rabbits infused at 3 g/kg of Lipofundin® MCT/LCT20% for 6 hours. (Data presented as mean standard deviation, *P<0.05).

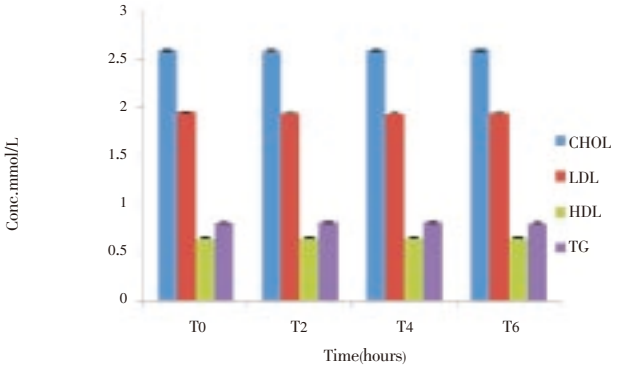

Figure 3. Lipid profile of blood samples of New Zealand White rabbits infused with NaCl 0.9% for 6 h.

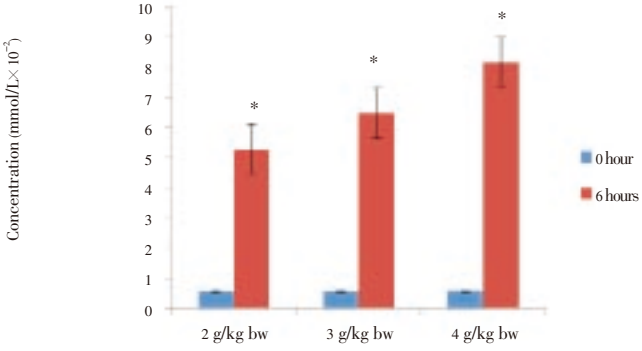

Figure 4. Levels of FFA at baseline and after 6 h infusion of NEMS® MCT/LCT20% at various doses (*P<0.05).

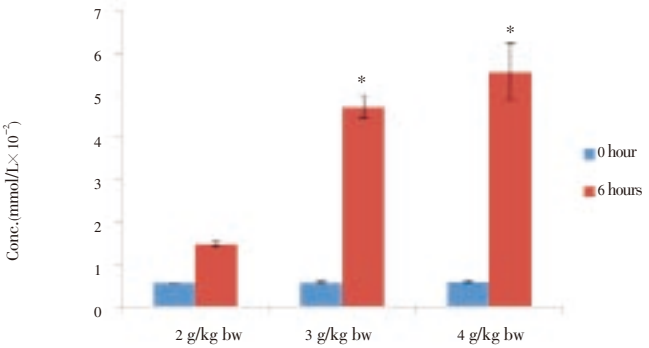

Figure 5. Levels of FFA at baseline and after 6 h infusion of Lipofundin® MCT/LCT20% at various doses (*P<0.05).

4. Discussion

NEMS® MCT/LCT20% showed a remarkable FFA stability in which the fatty acid content remained relatively unchanged after six months storage. The contents of FFA measured were lower than the standard limit of ≤0.07 mEq/g as stated in the United States Pharmacopeia[25]. Relative stability of FFA in IVLEs during storage is important to ensure safety of use in humans.

Palm oil has a palmitic acid (C16:0) content of 43.8%[26]. Reducing the quantity of this saturated fatty acid is desirable in a lipid emulsion for clinical use[27]. Introduction of MCT oil in NEMS® MCT/LCT20% has managed to reduce the palmitic acid content of this formulation to 19% (Table 1). The marked reduction of palmitic acid content will enhance the clinical use potential of NEMS® MCT/LCT20%.

The dose of 3 g/kg body weight was chosen to be administered in these rabbits as it was shown to be relatively safe as compared to other dosing regimens[24]. There were no changes to the other parameters of lipid profile except for TG when lipid emulsions were administered in the rabbits.

Triglyceride in emulsions containing MCTs and LCTs hydrolyses more rapidly than emulsions containing only LCTs. Emulsions containing MCTs are easily utilised and thus have a faster clearance rate than LCTs[4]. Following infusion, MCTs are rapidly cleared from the circulation because medium-chain fatty acids are not carnitine dependent for entry into the cell mitochondria as compared to LCT fatty acids[8,28]. This explains the subsequent decrease in TG level as seen in this study. Both NEMS® MCT/LCT20% and Lipofundin® MCT/LCT20% show similar results (P>0.05).

MCT/LCT IVLEs provide less polyunsaturated fatty acids as opposed to LCT IVLEs. Thus MCT/LCT IVLEs have lower tendency for lipid peroxidation and less pro-inflammatory as compared to LCT IVLEs[27,29]. In the present study, NEMS® MCT/LCT20% has an oleic acid (C18:1) concentration of 21%. Oleic acid is a monounsaturated fatty acid which does not affect plasma cholesterol concentration[30]. This explains the unchanged cholesterol levels in the blood of rabbits in this study.

The present study shows that the palm oil-based lipid nanoemulsion in combination with MCT oil (NEMS® MCT/LCT20%) is relatively safe as it did not cause any increase in the important components of blood cholesterols such as HDL, LDL and total cholesterol. An MCT/LCT IVLE has been shown to induce hyperlipidaemia in rats[31]. However, our results showed that fatty acid levels remained within normal limit in rabbits.

The results showed the potential of using palm oil-based nanoemulsion as pharmaceutical injections in terms of its safety profiles. However, more studies need to be conducted especially in bigger animals to confirm its reliability to be used clinically. The effects of long term administration or repeated injections of palm oil-based nanoemulsions should also be examined. The long term consequence of the presence of palmitic acid in injections should be addressed. The physico-chemical characteristics and stability profiles of palm oil-based nanoemulsions as formulations for injections should also be determined. Although results from this study showed that the newly-developed intravenous palm oil-based nanoemulsion does not exhibit unfavourable changes to fat metabolism, more studies need to be conducted before its use can be introduced in the clinical settings.

Conflict of interest statement

We declare that we have no conflict of interest.

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