Effects of Intralipid infusion on rat serum lipoproteins

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Summary

The purpose of this investigation was to study the effects of continuous Intralipid® infusions on serum HDL and LDL levels in the rat. Male Fischer 344 rats were infused continuously via central venous catheter with 10% Intralipid® for 96 h and 5, or 2.5% Intralipid for 14 days. Blood samples were collected during the infusion period for total serum cholesterol, HDL-, and LDL-cholesterol measurements. Food intake was monitored during the studies. Total cholesterol, HDL-cholesterol and LDL-cholesterol levels were significantly elevated following 96 h of infusion with 10% Intralipid with food intake significantly decreased compared to a control group. In a second experiment, animals received a continuous infusion of either 5% Intralipid, 2.5% Intralipid or 0.45% saline for 14 days. Total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly elevated following 14 days of infusion with 5% Intralipid group compared to controls but food intake remained constant for 12 days with no evident toxicity.

Keywords Serum lipoproteins; Intralipid; chronic infusion; rats; high density lipoproteins

The infusion of Intralipid has been shown to alter the intravascular metabolism of cholesterol and lipoproteins (Chait et al. 1981, Judd et al. 1984, MacFadyen et al. 1973, Rigaud et al. 1984, Taskinen et al. 1981, 1983, Thompson et al. 1975, Untracht 1982). The data are conflicting with regard to the magnitude and direction of changes that occur in serum cholesterol and lipoprotein fractions. Little is known with regard to changes in plasma lipoprotein induced by utilizing both glucose and Intralipid as a caloric source. In man, combined infusions of glucose and Intralipid have resulted in an increase [Innis et al. 1985, Judd et al. 1984, Taskinen et al. 1981, Thompson et al. 1975] or decrease [Taskinen et al. 1983] of serum low density lipoproteins (LDL). Given intravenously, artificial fat droplets are distributed in the blood and metabolized by essentially the same pathways as for chylomicrons [Turco & King 1987]. Most of this lipid or lipoprotein is hydrolyzed by lipoprotein lipase and the hydrolytic products, free fatty acids and monoglycerides, are taken up and metabolized by cells.

In the rat attempts to maintain a chronic elevation of total cholesterol, high density lipoproteins (HDL)-cholesterol, and LDL-cholesterol levels have been unsuccessful because of the excessive accumulation of bile acid [Delzenne et al. 1992] and linoleic acid by-products [a major fatty acid component found in Intralipid] [Anderson et al. 1986] which may lead to toxicity. The purpose of this study was to determine the effects of continuous...
Intralipid infusion on serum HDL and LDL in the rat where the Intralipid dosage used reflected those used clinically in total parenteral nutrition for humans (Turco & King 1987).

Materials and methods

Animals and cannulation

Seventeen male Fischer 344 rats (350–370 g; Harlan Breeders; Indianapolis, Indiana, USA) were anaesthetised with Nembutal (50 mg/kg; intraperitoneally) and a central venous catheter inserted via the jugular vein for total parenteral nutrition (Steiger et al. 1972). Rats were housed individually in stainless steel metabolism cages modified to accommodate the infusion apparatus. The animals were allowed to recover for 48 h after the surgical procedure, and given tap water and rat chow (Ralston Purina Co. St Louis Mo., USA) ad libitum. To keep the infusion lines patent during the study 0.45% normal saline (NS) was infused. Animal food intake was determined daily.

Experimental design

Following recovery, rats \( n = 4 \) per group) received 0.45% normal saline (NS) or 10% Intralipid (Clinitec Nutrition Co, Deerfield, Illinois, USA) by continuous intravenous infusion for 96 h at a flow rate of 1.2 ml/h. In a second experiment, rats \( n = 3 \) per group) were infused with either 5% Intralipid, 2.5% Intralipid, or 0.45% NS continuously for 14 days at a rate of 1.2 ml/h. Intralipid 2.5 and 5.0% were formulated by diluting Intralipid 10% with 0.45% NS. Intralipid 10% is bottled under nitrogen and stored under refrigeration and it is stable for one year following manufacture (Turco & King 1987). The osmolality of Intralipid of 10% is 280 milliosmols, which is comparable to that of blood (Turco & King 1987). Emulsions of the Intralipid placed in sterile infusion bottles were checked daily to determine if the emulsion remained stable and did not separate into its oil and aqueous phases.

Blood collection

Whole blood samples \( 1 \) ml were removed through the catheter at 0, 24, 48 and 96 h during the Intralipid 10% infusion, and at 0, 5 and 14 days during the 2.5 and 5% Intralipid infusion; lines were flushed with 0.9% NS and the initial 0.1 ml of blood was discarded. Fluid replacement with 0.9% NS \( 1 \) ml was performed after each blood collection, the line was flushed with a 1:10 v/v heparin: 0.9% NS solution, and the respective infusion continued. The blood was then centrifuged at 13 000 g for 5 min and the serum \( 0.5 \) ml was collected.

Serum assays

Serum \( 200 \mu l \) was separated into its HDL and LDL fractions by the LDL-direct cholesterol chromatographic column (LDL-Direct, Isolab Inc, Akron Ohio, USA. Bentzen et al. 1982). This column is a heparin-manganese polyacrylamide matrix that separates lipoproteins based on their apolipoprotein content. Serum components that contain apolipoprotein B (mainly LDL) are retained on the column, while all other components are eluted. Once the virgin gel was fully hydrated with 1 ml of a preparatory solution \( 0.7 \) sodium chloride + 0.002% chloramphenicol), serum samples were placed onto the column. LDL-eluting agent \( 1 \) ml containing 0.7% sodium chloride + 0.002% chloramphenicol) was used to collect the flow-through fraction, which contains HDL \( 1.2 \) ml, and an LDL-eluting agent \( 2.9 \) sodium chloride + chloramphenicol) was then added to the column to collect the LDL fraction \( 2.4 \) ml.

The HDL fraction was further separated from other serum components by size exclusion chromatography. A 50 cm glass column \( 0.085 \) inner diameter; Biorad) was packed with 11 ml \( 30 \) cm of agarose beads (Biogel-A 5 M) and the column was equilibrated with Tris-HCl buffer \( pH = 7.4 \) at a rate of 0.12 ml/min for 24 h using a peristaltic pump (Masterflex; Rainin Instruments, Woburn Mass. USA). Following equilibration, lipoprotein standards and HDL fractions separated by the Isolab* procedure \( 75 \mu l \) injection volume) were
injected onto the column, and 200 μl fractions of eluate were collected. A total of 11 ml were collected per run and each of the 200 μl fractions was measured spectrophotometrically (at 213 nm, with). The volume fractions at which peak absorbance occurred were similar to those obtained with HDL standards, and these fractions (representing HDL) were assayed for cholesterol. All of this work was performed at 37°C. Total serum cholesterol, and LDL cholesterol were determined by the same enzymatic assay used for HDL-cholesterol (Whitaker et al. 1986).

Statistical analysis

Differences in total serum cholesterol, HDL-cholesterol and LDL-cholesterol between the treatment groups and control group were determined using one-way analysis of variance [Zar 1984]. Critical differences were assessed by Newman-Keuls post hoc test. Differences were considered significant if \( P<0.05 \).

Results

10% Intralipid infusion

When rats \( n=4 \) were infused with 10% Intralipid at 1.2 ml/h for 96 h, total cholesterol, HDL-cholesterol, and LDL-cholesterol were significantly elevated compared to the 0.45% NS controls. The food intake of the Intralipid group was significantly decreased compared to the 0.45% NS control group by day 4 (Fig 1).

5% Intralipid infusion

When animals \( n=3 \) were continuously infused with 5% Intralipid at 1.2 ml/h for 14 days, total cholesterol, HDL-cholesterol,
Effect of Intralipid infusion on rat serum lipoproteins

and LDL-cholesterol were significantly elevated compared to the 0.45% NS control group. The food intake of this Intralipid group was significantly different compared to the 0.45% NS control group at day 14 but there was no significant difference in food intake between the groups up to 12 days of continuous infusion (Fig 2).

2.5% Intralipid infusion

For animals (n = 3) infused with 2.5% Intralipid at 1.2 ml/h for 14 days, HDL-cholesterol, and LDL-cholesterol were significantly elevated but total cholesterol was not significantly different compared to the 0.45% NS control group. The food intake of the Intralipid group was significantly decreased compared to the 0.45% NS control group at day 14 but there were no significant differences in food intake up to 12 days of continuous infusion (Fig 2).

Discussion

Administration of Intralipid at 5% for 14 days, and 10% for 96 h by continuous infusion to healthy rats led to elevations of serum cholesterol, HDL-cholesterol, and LDL-cholesterol levels. Intralipid 2.5% infused for 14 days resulted in a significant elevation in HDL and LDL-cholesterol but no significant changes in total serum cholesterol.

No significant changes in food intake were observed following continuous infusion of Intralipid 2.5 and 5% for up to 12 days but Intralipid 10% administration was stopped after 4 days because of significant decreases in food intake. These
Results are consistent with previous observations which show that rats are unable to handle such quantities of Intralipid or Intralipid by-products when infused rapidly. (Anderson et al. 1986, Delzenne et al. 1992) This amount of Intralipid causes a build-up of intracellular cholesterol, which is converted in the rat to bile acids by cholesterol 7-alpha hydroxylase. The resultant accumulation of bile acids and bile acid by-products in the vasculature leads to toxicity and eventual death.

Typically, Intralipid is administered as a nutritional supplement in debilitated patients undernourished and bedridden by malignancy. Elevations of lipoproteins following the chronic administration of Intralipid may significantly influence the pharmacological behaviour of drugs in patients. Monitoring of lipoprotein levels in these patients during treatment may be important for drug efficacy and animal models may help in an understanding of these problems. These studies show that the period of Intralipid infusion is limited by the concentrations of Intralipid used.

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