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Effect of intralipid infusion on serum high- and low-density lipoprotein cholesterol, lecithin:cholesterol acyltransferase, and lipoprotein lipase in tumor-bearing rats

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Abstract. We compared the effects of 0.45% normal saline (NS), 5% Intralipid® (IL), and 16.7% glucose (Glu) infusions on total serum triglycerides and cholesterol, serum high-(HDL-c) and low-density lipoprotein cholesterol (LDL-c), and activity of serum lecithin:cholesterol acyltransferase (LCAT), and serum lipoprotein lipase (LPL) in rats implanted with a fibrosarcoma. In tumor-bearing rats given NS, a two-fold increase in total serum cholesterol, a four-fold increase in LDL-c, and a five-fold decrease in the HDL-c/LDL-c ratio were observed compared to tumor-free rats. In tumor-bearing rats administered IL, a two-fold increase in total serum triglyceride and cholesterol, a three-fold increase in HDL-c and HDL-c/LDL-c ratio, and a two-fold increase in LPL activity were observed compared to tumor-bearing rats administered NS. In tumor-bearing rats administered Glu, a two-fold decrease in total serum cholesterol, a two-fold decrease in HDL-c, and a three-fold decrease in LDL-c were observed compared to tumor-bearing rats administered NS. Tumor weights and LCAT activity did not differ significantly between treatment groups. Previous results have demonstrated that lipophilic compounds that interact with plasma lipoproteins have altered pharmacological effects when administered with IL. Therefore, this study suggests that IL infusions alter the HDL-c/LDL-c ratio and could affect the pharmacological behavior of anticancer compounds that predominantly distribute into the LDL fraction upon entrance into the bloodstream.
Key words. Intralipid; lipoproteins; fibrosarcoma.

Long-term intravenous infusions of lipid emulsions are sometimes given as part of a nutritional supplement program to debilitated patients unable to obtain sufficient nutrients orally. Different investigators have previously demonstrated that intravenous fat emulsions containing triglycerides are rapidly hydrolyzed into fatty acids and glycerol by lipoprotein lipase (LPL)[1], and that the exogenously supplied phospholipids and cholesterol accumulate primarily into low-density lipoproteins (LDL)[2–5]. In contrast, we have demonstrated that a continuous infusion of 5% Intralipid® to rats for five and fourteen days results in an increase in total serum cholesterol and high-density lipoprotein (HDL)-cholesterol without altering LDL-cholesterol[6]. This increase in HDL-cholesterol and HDL-cholesterol:LDL-cholesterol ratio may be due to the increase in lecithin:cholesterol acyltransferase (LCAT) activity, an enzyme which converts free cholesterol into cholesteryl esters within HDL.

Cancer cells presumably need additional cholesterol for the formation of new membrane material and the increased LDL uptake by these cells from the blood circulation may meet this requirement. Several established cancer cell lines cultured in vitro display elevated receptor-mediated uptake of LDL. When membranes prepared from these cell lines were inoculated into mice, the LDL binding was several-fold higher than membranes prepared from other tissues[7–11]. Cholesterol required for membrane synthesis in replicating cells is obtained through direct endogenous synthesis, or by preferential uptake of LDL from the bloodstream. In addition, hypocholesterolemia appears a frequent finding in patients with acute leukemia[12–14] and cholesterol levels are inversely correlated with the LDL receptor activities of the leukemic cells[12]. Since the presence of Intralipid® changes the HDL-cholesterol:LDL-cholesterol ratio, we have hypothesized that infusion of lipid emulsion may decrease tumor growth, presumably by altering the uptake of cholesterol.

In addition to the cell requirement for cholesterol, studies have shown that when lipophilic compounds such as amphotericin B (AmpB)[15–17] and cyclosporine[18] were mixed with Intralipid®, their-associated renal toxicities were decreased without diminishing their pharmacological efficacy.

Since Intralipid® is commonly used as an isotonic energy source in the total parental nutritional therapy of cancer patients and alters the pharmacological behavior of several lipophilic compounds, this study investigates the influence of Intralipid® and glucose infusions on total serum triglyceride and cholesterol, high- and low-density lipoprotein cholesterol, LCAT activity,

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LPL activity, and tumor weight in fibrosarcoma-bearing rats.

Materials and methods

Experimental design. Male Fischer 344 rats (220–250 g; n = 22) were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA). All the rats were allowed seven days to acclimatize to their environment. During the acclimatization period and throughout the study, the rats were given rat chow (Purina Chow 5001) and water ad libitum and kept in a 12-h light:dark cycle animal facility.

Following the acclimatization period, rats were randomized to remain tumor free (n = 5) or to be inoculated (n = 17) with a suspension of a methylcholanthrene-induced fibrosarcoma[19]. To prepare the fibrosarcoma suspension for inoculation, one gram of tumor tissue was homogenized in 2 ml of sterile normal saline. The resulting suspension (0.5 ml/rat; containing tumor cells) was injected subcutaneously into the right flank of rats that were under light ketamine anesthesia.

The tumor was allowed to grow for two weeks to a size of 1.0 cm in width at which time all the rats were anesthesized with Nembutal (50 mg/kg, i.p.) and a central venous catheter was inserted via the jugular vein[20]. 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, given tap water and rat chow (Ralston Purina Co. St. Louis, MO, USA) ad libitum. Saline (0.45% NS) was infused to keep the lines patent.

Following recovery from sugery, all rats were randomized to receive either no infusion (sham controls), 0.45% NS, 16.7% glucose, or 5% Intralipid® (Clinitect Nutrition Co., Deerfield, IL, USA) by continuous intravenous infusion for seven days at a flow rate of 1.2 ml/h. The Intralipid® dosage used reflected those used clinically in total parenteral nutrition for humans[21]. Intralipid® is a sterile non-pyrogenic fat emulsion prepared for intravenous administration as a source of calories and essential fatty acids, containing soybean oil (mixture of neutral triglycerides), egg yolk phospholipids, glycerin, and water. The purpose of the glucose administration was to correct for the possible influence that a generally increased caloric infusion may have on the parameters measured. Food intake of all animals was measured daily for seven days.

Blood collection. On day seven of the infusion whole blood samples (1.0 ml) were obtained from the abdominal aorta and placed in non-heparinized test-tubes. These test-tubes were centrifuged at 13,000 × *g* for 5 min and the serum (0.5 ml) was collected. Heparin was kept to a minimum because it caused an immediate release of LPL from the liver into the plasma, a decreased uptake of LPL in most extrahepatic tissues, and increased the fraction of LPL taken up in the liver[1].

Assays. The serum (200 µl) was then separated into its HDL and LDL fractions by size exclusion and heparin affinity chromatography as previously described[22]. Total, HDL, and LDL serum cholesterol levels in each sample were determined by enzymatic assays for cholesterol purchased from Sigma. Total serum triglycerides in each sample were determined by enzymatic assays for triglycerides, also purchased from Sigma. LCAT activity was determined by the percent conversion of ^{3}H-cholesterol-HDL to ^{3}H-cholesteryl ester-HDL (ref. 23). Cholesterol-HDL was radiolabelled by drying under nitrogen onto a disk of filter paper (Whatman Paper Ltd, Maidstone, England), 1.25 ml of ^{3}H-cholesterol (24 µCi/ml) and placing this disk in a vial containing disodium ethylenediaminetetraacetic acid (1.56 ml), bovine serum albumin (0.05%), paraoxin (20 µl) (antioxidant) and 2 mg HDL-cholesterol. This vial was then incubated overnight at 37 °C in a shaking water bath and the amount of ^{3}H-cholesterol appearing in HDL was determined. Then ^{3}H-cholesterol-HDL (25 µg of cholesterol; 7.0 µCi/ml) was incubated in serum samples (100 µl) for 3 h at 37 °C (percent conversion was linear from 1 to 4 h of incubation). Following incubation, lipids were extracted with an 95% ethanol, hexane, and water solvent (1/1/1 v/v/v) and the top layer was removed and fractionated on a thin layer chromatography plate (Whatman Paper Ltd, Maidstone, England; Silica Gel 60A; 20 × 20 cm 250 µm layer) in a mobile phase consisting of hexane, ether, and acetic acid (70/30/1 v/v/v). The cholesteryl ester and cholesterol bands were scraped and counted for radioactivity.

Serum LPL activity was determined by the percent of ^{3}H-triolein hydrolyzed to free fatty acids as previously described[24]. Briefly, ^{3}H-triolein (150 µg of triglyceride; 1.0 µCi/ml) was incubated in serum samples (100 µl) for 2 h at 37 °C, (precent hydrolyzed was linear for 1 to 4 h of incubation). After incubation, lipids were extracted, fractionated, and quantitated as described above with a 95% ethanol, hexane, water solvent (1:1:1 v/v/v). The top layer was removed and fractionated on a thin layer chromatography plate in a mobile phase consisting of hexane, ether, and acetic acid (70/30/1 v/v/v). The free fatty acid and triglyceride bands were scraped and counted for radioactivity. Following the removal of blood, tumors were removed and weighed.

Statistical analysis. Differences in total, HDL and LDL cholesterol levels, total triglyceride levels, LCAT activity, and tumor weight between tumor-free and tumor-bearing rats, and between the different treatment groups in tumor-bearing rats, were determined by multiple analysis of variation[25]. Critical differences were assessed by Newman-Keuls post-hoc test. Differences were considered significant if $p < 0.05$.

Results

To assess the influence of the tumor on the lipid profile, LCAT activity, and LPL activity, tumor-bearing rats (sham and 0.45% NS infusion group) 3 were compared to tumor-free rats following fourteen days of tumor growth and seven days of receiving no infusion of 0.45% NS infusion. Since the infusion of NS in tumor-bearing sham controls, these groups were combined. A two-fold increase in total serum cholesterol, a four-fold increase in LDL-cholesterol, and a five-fold decrease in the HDL-cholesterol/LDL-cholesterol ratio were observed in tumor-bearing rats (sham + infused on 0.45% NS) compared to tumor-free controls (table 1). LCAT and LPL activity were not significantly different between the two groups (table 1).

To assess the influence of Intralipid® and glucose infusions on the lipid profile, tumor growth, and LCAT and LPL activity, 0.45% NS, 5% Intralipid® or 16.7% glucose were infused over 7 days to tumor-bearing rats and compared to tumor-free rats. A two-fold increase in total serum triglycerides and cholesterol, a three-fold increase in HDL-cholesterol and HDL-cholesterol/LDL-cholesterol ratio (table 2), and a two-fold increase in serum LPL activity (table 3) were observed in tumor-bearing rats administered Intralipid® compared to tumor-bearing (sham + infused with 0.45% NS) controls. Previous results have demonstrated a two-fold increase in HDL-cholesterol and total cholesterol in tumor-free rats administered Intralipid® compared to tumor-free (infused with 0.45% NS) controls[6,37].

A two-fold decrease in total serum cholesterol and HDL-cholesterol, and a three-fold decrease in LDL-cholesterol were observed in tumor-bearing rats administered glucose compared to tumor-bearing (sham + infused with 0.45% NS) controls. Tumor growth, LCAT activity (table 3), and daily food intake (data not shown) were not significantly influenced by either Intralipid® or glucose treatment in these rats.

Table 1. Total serum triglycerides, total serum cholesterol, HDL-cholesterol, LDL-cholesterol, serum LCAT activity, and serum lipoprotein lipase activity in tumor-free or sarcoma-bearing (sham and infused with 0.45% NS) rats (220–240 g).

Parameters measured	Tumor-free rats	Sarcoma-bearing rats
Total serum triglycerides (mg/dl)	139.6 ± 19.5	97.0 ± 22.2
Total serum cholesterol (mg/dl)	28.8 ± 6.3	48.8 ± 10.5[a]
HDL-cholesterol (mg/dl)	20.8 ± 5.9	19.1 ± 4.0
LDL-cholesterol (mg/dl)	8.0 ± 3.7	29.7 ± 8.4[a]
HDL-cholesterol	3.14 ± 1.54	0.68 ± 0.21[a]
LDL-cholesterol		
LCAT activity (% cholesterol esterified)	5.17 ± 1.07	5.13 ± 1.27
Lipoprotein lipase activity (% of TG hydrolyzed to FFA)	6.91 ± 2.60	5.33 ± 1.44
	n = 5	n = 7

Abbreviations: TG, triglycerides; FFA, free fatty acids; LCAT, lecithin:cholesterol acyltransferase. All data are expressed as mean ± SD. [a]$p < 0.05$ vs tumor-free rats.

Discussion

Our study demonstrates that a sarcoma-bearing rat exhibits a significant increase in total serum cholesterol levels as a result of an increased LDL-cholesterol level. Furthermore, the administration of Intralipid® to these animals not only further increased total, HDL, and LDL serum cholesterol levels, but also total serum triglyceride levels and LPL activity. In contrast, the administration of glucose decreased total serum cholesterol levels as a result of a decreased LDL-cholesterol level. We have further demonstrated that the data presented in this study were not significantly different from the data acquired from preliminary studies where large rats (300–350 g) or rats inoculated with a Ward colon tumor were administered infusions of NS, Intralipid® or glucose (data not shown).

An increase in serum cholesterol following Intralipid® administration, has been reported in human[5,26] and animal studies[6,27–30], but the effect of Intralipid® administration on LCAT activity, remains controversial. Untracht demonstrated that Intralipid® administration

Table 2. Total serum triglycerides, total serum cholesterol, HDL-cholesterol, and LDL-cholesterol in sarcoma-bearing rats (220–240 g) receiving continuous infusion of 0.45% NS, 5% Intralipid® or 16.7% glucose for 7 days at a flow rate of 1.2 ml/h.

Treatment groups	Total serum triglycerides	Total serum cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol / LDL-cholesterol
0.45% NS + tumor	97.0 ± 22.2	48.8 ± 10.5	19.1 ± 4.0	29.7 ± 8.4	0.68 ± 0.21 n = 7
5% Intralipid® + tumor	204 ± 44[a]	95.0 ± 33[a]	60.0 ± 16[a]	34.9 ± 17.8	1.96 ± 0.69[a] n = 5
16.7% Glucose + tumor	91.6 ± 40.6[b]	18.3 ± 6.2[a,b]	8.5 ± 4.3[a,b]	9.8 ± 2.2[a,b]	0.84 ± 0.36[b] n = 5

All data are expressed as mean ± SD.
[a]$p < 0.05$ vs 0.45% NS + tumor.
[b]$p < 0.05$ vs 5% Intralipid® + tumor.

Table 3. Serum lecithin:cholesterol acyltransferase activity (LCAT), serum lipoprotein lipase activity, and tumor weight in sarcoma-bearing rats (220–240 g) receiving continuous infusion of 0.45% NS, 5% Intralipid®, or 16.7% glucose for 7 days at a flow rate of 1.2 ml/h.

Treatment groups	LCAT activity (% cholesterol esterified)	Lipoprotein lipase activity (% of TG hydrolyzed to free fatty acids)	Tumor weight (grams)
0.45% NS + tumor	5.13 ± 1.27	5.33 ± 1.44	7.5 ± 1.5 n = 7
5% Intralipid® + tumor	4.86 ± 0.94	12.0 ± 5.5[a]	5.1 ± 1.9 n = 5
16.7% Glucose + tumor	4.85 ± 1.33	3.84 ± 1.15[b]	4.8 ± 2.8 n = 5

All data are expressed as mean ± SD.
[a] $p < 0.05$ vs 0.45% NS + tumor.
[b] $p < 0.05$ vs 5% Intralipid® + tumor.

to adult humans decreases LCAT activity[4], others have observed that LCAT activity was unchanged[31] or increased[3]. We found no significant differences in LCAT activity in treatment groups when the fractionated esterification rate was corrected for the serum cholesterol level. Since HDL, particularly apoprotein A-I rich HDL is a substrate for LCAT[31], the lack of change in LCAT activity may be due to the corresponding lack of change in apoprotein A-I rich HDL following Intralipid® administration. The increase in HDL-cholesterol may be a result of an increased accumulation of cholesterol within Apo A-I rich HDL, or an increased appearance of lipoprotein X (ref. 5,27) within the bloodstream, which is not recognized as a substrate for LCAT[31]. Other investigators have reported that lower levels of the enzyme is associated with lower levels of the substrate[32].

Previously, investigators have reported that Intralipid® infusions increase serum lipoprotein lipase activity[30,33]. We have demonstrated an increase in serum LPL activity in tumor-bearing rats following Intralipid® administration, most likely the direct result of increased serum triglycerides from the Intralipid® infusion.

Elevated receptor-mediated uptake of LDL has been previously demonstrated in several established cancer cell lines[7,10]. We have observed a significant decrease in the HDL-cholesterol/LDL-cholesterol ratio, most likely the direct result of an increased LDL-cholesterol level and thus an elevated total serum cholesterol level in tumor-bearing versus tumor-free rats. This increase in serum LDL-cholesterol may be in response to an elevated receptor-mediated uptake of LDL by the fibrosarcoma, an increased production of LDL-cholesterol, or decreased metabolism of LDL-cholesterol by the liver. Studies addressing the effect of the tumor on the liver's metabolism of LDL-cholesterol remain to be completed. However, in contrast to our results, decreases in total serum cholesterol levels have been observed in cancer patients[12,13], though other investigators have observed decreased high-density lipoprotein cholesterol and increased levels in patients with colorectal adenomas[34]. Furthermore, this increase in serum LDL-cholesterol could have a significant influence on the pharmacokinetics and pharmacological behavior of lipophilic anticancer compounds that distribute into LDL upon entrance into the bloodstream. However, since LDL was separated from its other serum components by a heparin-agarose affinity column (which binds both apoprotein B and E-containing lipoproteins), it is possible that the retained fraction could contain apoprotein E-rich HDL as well as LDL.

Another limitation of this study concerns the use of this rat model and its relevance to humans. Several investigators have demonstrated that, unlike rats, cancer patients do not exhibit a four-fold increase in LDL-cholesterol as compared to healthy individuals. On the contrary, cancer patients exhibited lower total plasma cholesterol concentrations[35,36]. Additional studies exploring the effect of Intralipid® infusion on the metabolism of lipoproteins are continuing in rabbits which have similar lipoprotein profiles to humans.

The administration of Intralipid® in these tumor-bearing rats resulted in an increase of the HDL-cholesterol/LDL-cholesterol ratio through an increase in serum HDL-cholesterol. This increase in HDL-cholesterol may explain the enhanced therapeutic index of AmpB when administered concurrently with Intralipid®. Previous studies have suggested that, when AmpB was mixed with Intralipid®, a number of pharmacokinetic parameters were altered[37,38], and acute renal toxicity was decreased without changing the drug's pharmacological efficacy[15–17]. We have previously demonstrated that when AmpB is associated with HDL, AmpB-associated nephrotoxicity was decreased[39]. Therefore, the decreased toxicity of AmpB when administered concurrently with Intralipid® may be due to the increase in serum HDL-cholesterol.

The administration of glucose in tumor-bearing rats decreases LDL-cholesterol and normalizes LDL-cholesterol to the same level as observed in the normal controls. Since the Intralipid® and glucose infusions used in this study are isocaloric, it appears that the form in which the energy is supplied, and not the amount of energy supplied, affects the liver metabolism of LDL-cholesterol. The mechanism(s) controlling the differences in liver metabolism of LDL-cholesterol when a sarcoma-bearing animal is given Intralipid® versus glucose remain to be understood.

In conclusion, this study used tumor-bearing adult rats as an animal model for the infusion of Intralipid® to cancer patients maintained on total parental nutrition. Our results demonstrate that tumor-bearing rats have a decrease in the HDL-cholesterol/LDL-cholesterol ratio through an increase in serum LDL-cholesterol. Furthermore, the administration of Intralipid® leads to a marked increase in serum LPL activity, total serum, and HDL-cholesterol without a corresponding change in serum LCAT activity. Future studies will address the influence of total parental nutrition administration on the growth rate and proliferation of these tumors. In addition, this study suggests that Intralipid® infusions alter the HDL-cholesterol/LDL-cholesterol ratio and could have an impact on the pharmacological behavior of anticancer compounds that predominantly distribute into the LDL fraction upon entrance into the bloodstream.

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