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EFFECTS OF INTRAVENOUS LIPID AS A SOURCE OF ENERGY IN PARENTERAL NUTRITION ASSOCIATED HEPATIC DYSFUNCTION AND LIDOCAINE ELIMINATION: A STUDY USING ISOLATED RAT LIVER PERFUSION*

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ABSTRACT

The effects on liver function and hepatic lidocaine elimination using 20% Intralipid® as a source of non-protein calories (30%) in parenteral nutrition were studied using an isolated rat liver perfusion procedure. Rats were randomly assigned to one of the three treatment groups: PNL group ($n = 6$), consisting of 16·94% dextrose, 2·46% Intralipid®, and 5·2% amino acids; PN group ($n = 5$), consisting of 24·2% dextrose and 5·2% amino acids; and CF group ($n = 6$), chow fed (rat chow and water). The rate of lidocaine metabolism was significantly reduced after 7 d in the two PN treated groups when compared to CF. Steatosis was observed in five out of six PNL treated animals and two out of five PN treated animals. Intrinsic clearance was reduced by 80% in the PNL group and by 60% in the PN animals ($p < 0·05$). Molar metabolite to drug ratios revealed significant reductions in N-dealkylation, *m*-hydroxylation, and aryl methyl hydroxylation in groups PNL and PN; these values amounted to 67–92% ($p < 0·05$). These findings suggest that a dextrose–amino acid solution induced steatosis and reduced the rate of lidocaine metabolism. The incorporation of Intralipid® caused further deterioration. ©1997 John Wiley & Sons, Ltd.

KEY WORDS: Intralipid®; parenteral nutrition; hepatosteatosis; lidocaine metabolism

INTRODUCTION

Parenteral nutrition (PN) is widely used to provide adequate calories and essential nutrients to critically ill patients who are unable to eat, and to low-birth-weight neonates for their sustenance. Although nutritional rehabilitation

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can be successfully and safely achieved with PN in most patients, the procedure is not free of complications. One of the most frequently reported problem is hepatic dysfunction.[1–4] In adults hepatic dysfunction is mostly characterized by an elevation of serum liver function test (LFT) values.[5,6] Hepatic steatosis, severe cholestasis, portal inflammation, and fibrosis of the liver may develop; these can lead to hepatic failure and death.[7,8]

Although the clinical manifestations and the pathologic changes in the liver have been extensively studied, the etiology and pathogenesis of PN associated hepatic dysfunction remain obscure. Several postulations have been suggested to explain the underlying causes; these include enteral fasting,[9] amino acid and sugar overload,[10] photo-degradation of amino acids,[11] hormonal imbalance,[12] and essential fatty acid deficiency.[13]

Intravenous fat emulsions are now a popular source of essential fatty acids in patients receiving PN. Initially a fat emulsion was administered separately from the amino acid and dextrose mixture to avoid catheter occlusion and solution instability. Recent studies,[14,15] however, have shown that a mixture of amino acids, dextrose, and lipid or the 'three-in-one' solutions is clinically safe, stable, and economical. Improved patient compliance was also reported because of ease of administration.[15] Despite these findings, controversial results are reported in the literature linking intravenous lipid to changes in hepatic function. Reif *et al.*[16] and Zohrab *et al.*[17] reported a reversal of hepatic steatosis with lipid infusions; whereas Allardyce[1] and Gerard-Boncompain *et al.*[18] observed hepatic steatosis and progressive cholestasis during a course of PN with lipid in humans. A similar steatotic effect of lipids was also observed in rats.[19] A study conducted in our laboratory has shown that the use of an amino acid and glucose based parenteral nutrition results in significant reduction in the rate of lidocaine (LIDO) metabolism.[20] Ross *et al.*[21,22] observed that antipyrine pharmacokinetics was restored to normal when a lipid based PN was used. It is not clear whether a lipid based PN solution would have the same effect on the rate of LIDO metabolism.

The aims of this study were twofold: (i) to investigate the effects of intravenous lipid on hepatic function and (ii) to study the effect of intravenous lipid on hepatic elimination of a model compound, lidocaine, using rat as an animal model.

METHODS

Male Sprague–Dawley rats weighing 200–230 g were supplied by Biosciences Animal Services, University of Alberta. The protocol of this study met the guidelines of the Canadian Council of Animal Care and the use of animals was approved by the Health Sciences Animal Welfare Committee at the University of Alberta. Each rat was anaesthetized with methoxyflurane and its right jugular vein was catheterized with a silastic tubing (o.d., 0·047 in; i.d., 0·025 in;

Dow Corning, Midland, MI). The catheter was then tunnelled subcutaneously and exteriorized in the scapular region where it was connected to a coiled metallic spring mounted on a swivel (Rodent Single Channel Swivel, Alice King Chatham Medical Arts, Los Angeles, CA). This connection permitted free mobility of the subject. Surgical equipment was sterilized prior to each surgery and the entire surgical procedure was carried out aseptically under a laminar flow hood. After each surgery, the animal was weighed and placed in a metabolic cage which was housed in a well ventilated room where a 12 h light and dark cycle was maintained. Rats were allowed to recover for at least 2 d during which time they had free access to a standard laboratory rodent chow (Richmond Standard, PMI Feed, St. Louis, MO) and water. On day three, the weight of the animal was recorded.

Animals were randomly assigned to one of the three treatment groups: PNL group, recipients of dextrose, amino acid, and lipid nutrition; PN group, recipients of dextrose and amino acid nutrition, CF group, chow fed, animals that received rat chow and water. These animals were infused for 7 d with saline or PN with or without Intralipid® at a rate of $3\,mL\,h^{-1}$, delivered by a volumetric infusion pump (model 927, IMED Corporation, San Diego, CA). Only chow-fed animals had free access to laboratory chow and water; PN and PNL animals had free access to water only. Each rat was weighed before and after a 7 d course of the assigned infusion; the liver was isolated and studied on the eighth day.

The composition of the PN solution for the PN group was identical to that described by Ke *et al.*[20] Each litre of PN contains 242 g 50% dextrose, 52 g amino acids (10% Travasol blend B with electrolytes, Baxter-Travenol Laboratory, Malton), 2 mL multivitamin (MVI 1000, aqueous multivitamin IV infusion, USV Canada), and 2·25 mmol calcium gluconate (10% calcium gluconate injection, Squibb Canada, Montreal). Animals in the PNL group received a PN solution which was isocaloric to that of the PN group. The only difference was that 30% of non-protein calories were replaced by 20% Intralipid® (Kabi Pharmacia Canada). The 20% Intralipid® contains 20% purified soybean oil with 1·2% purified egg phospholipids and 2·3% anhydrous glycerin. PN and PNL groups were isocaloric and the average calories provided by these PN formulations are $\sim 330\,kcal\,kg^{-1}\,d^{-1}$. PN solutions were prepared aseptically in a laminar flow hood and containers were immediately covered with aluminium foil to avoid exposure to light. To preserve fat emulsion stability, the order of mixing the PN solution was as follows: dextrose was first mixed with Travasol, followed by the addition of calcium gluconate, multivitamin, and lipid in sequence. The pH of the final emulsion was between 5·5 and 6·6.[23] Freshly prepared lipid containing total parenteral nutrient mixtures, especially those containing multivitamins, were off-white or banana coloured as opposed to slightly yellowish colour of the PN solution without lipid. These solutions were found to be devoid of signs of creaming or cracking, even when infusion bags were stored at 4 °C for up to a period of 2 weeks.

Isolated rat liver perfusion

A 'one-pass' liver perfusion method was used in this study. Details of the isolated liver perfusion procedure have been reported previously.[24–26] Concisely, the portal vein was cannulated with an intravascular 'over-the-needle' 16 G Teflon® catheter (Quick-cath, Baxter Healthcare, Deerfield, IL), which was used to provide oxygenated Krebs bicarbonate buffer. The effluent perfusate sample was collected from a catheter located in the thoracic inferior vena cava. The catheter from the posterior vena cava was hooked up to an oxygen monitor (Biological Oxygen Monitor YSI, Yellow Springs, OH). Liver function was monitored by measuring oxygen consumption, aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) levels in the effluent perfusate at zero and 70 min and the hydrostatic pressure (measured continuously using a pump, Rosemount Instruments, Calgary) and visually checked by the organ's general physical appearance. The rate of oxygen consumption was 2–3 $mL\,h^{-1}\,g^{-1}$ and was within the normal range reported.[27] AST, ALT, and ALP levels and the hydrostatic pressure were constant throughout the experiment. It has been reported that liver will remain viable for at least 3–4 h after its removal from the animal.[28] Our experiment was completed within 90 min. The viability of the liver was further confirmed by the stable concentrations of LIDO and its metabolites at steady state during the infusion period. Each liver was infused with LIDO (3·5 $\mu g\,mL^{-1}$ at a constant rate of 30 $mL\,min^{-1}$) via a Vario pump (Cole Parmer, Chicago, IL) for 70 min for all experimental subjects. This period was found to be sufficient for LIDO and its metabolites to approach a steady state.[24] The inlet concentration of LIDO (C_{in}) was determined by obtaining six samples (2 mL each) directly from the inlet reservoir at 0, 12, 22, 33, 44, and 70 min after the initiation of LIDO infusion. The outlet concentration of LIDO and its metabolites (C_{out}) were measured in the effluent samples at 0, 1, 3, 5, 7, and 10 min and at 5 min intervals up to 50 min and then at 10 min intervals up to 70 min. After each experiment the liver was blotted dry and its weight recorded.

Histological examination. A portion of each liver was stored in 10% formalin prior to being stained with haematoxylin-eosin, trichome stain for connective tissue and evaluated by Perl's reaction for iron. Histological examination was performed in a blind fashion. The degree of steatosis was rated on a scale of 1–4 (1, no steatosis; 2, mild steatosis in $<25\%$ of the liver; 3, moderate steatosis in 25–75% of the liver; 4, severe steatosis in $>75\%$ of the liver).

Serum enzymes and amino acid assays. Blood ($\sim$1·5 mL) was collected from the posterior vena cava of all the animals immediately after catheterizing the portal vein and the serum was separated by centrifugation. Serum levels of AST, ALT, ALP, gamma glutamyl transpeptidase (γ-GT), and total bilirubin

(BILI) were determined by an individual colorimetric method using a Multistat 3 Micro Centrifugal Analyzer. The coefficient of variation of each assay was within $\pm 3\%$. Individual serum amino acid concentrations were quantified on a Varian 5000 high-performance liquid chromatograph (HPLC) coupled to a Varian Fluorichrom detector.[29] The reproducibility of the assay was within an average deviation of $\pm 2\%$

HPLC assay

Chemicals and reagents. Samples of LIDO hydrochloride and four of its metabolites, *N*-(*N*-ethylglycyl)-2, 6-xylidide (MEGX), *N*-glycyl-2, 6-xylidide (GX), 3-hydroxylidocaine (3-OH-LIDO), 3-hydroxy-*N*-(*N*-ethylglycyl)-2, 6-xylidide (3-OH-MEGX), and the internal standard, *N*-ethyl-*N*-methylglycyl-2, 6-xylidide (EMGX), were gifts from Astra Pharmaceuticals (Mississauga). The remaining two metabolites, *N*-(*N*, *N*-diethylglycyl)-2-hydroxymethyl-6-methylanilide (MeOH-LIDO) and *N*-(*N*-ethylglycyl)-2-hydroxymethyl-6-methylanilide (MeOH-MEGX) were synthesized in our laboratory based on the method of Nelson *et al*.[30] Their chemical structures and purity were confirmed using NMR, elemental analysis, and GC–MS. HPLC grade solvents and reagents were obtained from BDH, Toronto.

Apparatus. The HPLC system consisted of two M-45 pumps, a model 441 UV detector (set at 214 nm), a model 840 data processing station (Waters, Mississauga) and a Shimadzu automatic sampler (model SIL-9A, Shimadzu, Kyoto). Separation of LIDO and its six metabolites was achieved on a C_{18} LiChrospher® 60 RP-select B column (5 μm, 125 mm × 4 mm, Chromatography Merck, Darmstadt). The HPLC method developed in our laboratory[31] was used to separate and quantify LIDO and its metabolites.

Standard curves. Standard solutions were prepared by spiking 1 mL volumes of the blank perfusate with various concentrations (0·02–3·5 $\mu g\,mL^{-1}$) of LIDO and its six metabolites. Calibration curves, constructed by plotting the peak area ratio of LIDO and each of its metabolites to that of the internal standard versus the respective concentration of the drug or metabolite, were linear over the range studied ($r > 0{\cdot}99$). Quality control samples, prepared by non-involved personnel in the laboratory were used to validate the assay. The quantifiable limit of this assay was 0·02 $\mu g\,mL^{-1}$ for LIDO and its six metabolites when a 1 mL aliquot of perfusate sample was used. The percentage deviation from expected values as a measure of accuracy was less than 15% for the standards and less than 10% for the quality control samples. The *intra*-day and *inter*-day coefficients of variation for standard and quality control samples of LIDO and its metabolites were within 10%. A stability study revealed that stock solutions of LIDO and its metabolites were stable for 8 weeks when stored at $-20\,^{\circ}C$.

Pharmacokinetic analysis

The times for LIDO and its metabolites to reach steady state (T_{ss}) were statistically determined according to the method reported by Saville *et al.*[32] The efficiency of the liver in removing LIDO at steady state was expressed by the following equations:

$$E_H = \frac{(C_{in} - C_{out})}{C_{in}} \quad (1)$$

$$Cl_{int} = \frac{QE_H}{(1 - E_H)} \quad (2)$$

where Q is the buffer perfusion rate, C_{in} is the inlet drug concentration, C_{out} is the effluent drug concentration, E_H is the drug extraction ratio, and Cl_{int} is hepatic intrinsic clearance.

Statistical analysis

The Shapiro–Wilks test and the Kolmogorov–Smirnov goodness of fit test were first used to test for the normality of the data.[33] It was found that the data were not normally distributed; therefore a Kruskal–Wallis one-way ANOVA was used to evaluate the difference. When a difference was detected, Duncan's multiple-comparison test was used to evaluate the differences among the groups. Significance was set at the $p = 0{\cdot}05$ level. Values are expressed as means $\pm$ S.D. The SPSS® for Windows™ computer program was used for these statistical calculations.

RESULTS

Body and liver weight changes

Animals in the PNL, PN, and CF groups had similar body weights (PNL, $220{\cdot}33 \pm 15{\cdot}42$ g; PN, $213{\cdot}80 \pm 19{\cdot}59$ g; CF, $224{\cdot}50 \pm 22{\cdot}06$ g) before initiation of the experiment. Despite healthy appearances of all the animals at the end of each experiment, only animals in the CF group exhibited a significant weight gain of $7{\cdot}55 \pm 1{\cdot}52\ g\ d^{-1}$. Animals in the two PN groups, on the other hand, showed no change in body weight at the end of the experiment. The absolute liver weights were significantly lower in the PNL and PN groups, the PN group being the lowest (Table 1). A difference in liver weight in terms of percentage body weight was found only between the PNL and PN group (PNL, $4{\cdot}49 \pm 0{\cdot}68\%$; PN, $3{\cdot}69 \pm 0{\cdot}31\%$; CF, $3{\cdot}98 \pm 0{\cdot}30\%$; $p < 0{\cdot}05$).

Table 1. Steady state kinetic parameters of LIDO in perfused rat livers after 7 d parenteral nutrient (with and without lipids) treatments. Values are represented as mean ± SD

Kinetic parameter	CF ($n = 6$)	PN ($n = 6$)	PNL ($n = 6$)
Q (mL min^{-1} g^{-1})	2·83 ± 0·35[a]	3·95 ± 0·40[a]	3·23 ± 0·23[a]
C_{in} (μM)	15·53 ± 1·62	15·35 ± 1·48	16·04 ± 186
C_{out} (μM)	1·77 ± 0·81[a]	3·99 ± 0·47[a]	6·26 ± 1·41[a]
Liver (g)	11·08 ± 1·51[a]	7·95 ± 0·84[a]	9·63 ± 0·81[a]
Cl_{int} (mL min^{-1} g^{-1})	28·06 ± 16·40[a]	11·33 ± 1·62[a]	5·46 ± 2·40[a]

[a]Values of the three groups are significantly different ($p < 0·05$) from each other.

Serum liver function test values and serum amino acid levels

There were no significant differences between any of the LFT values, AST, ALT, ALP, and γ-GT, among the groups (Table 2). However, two rats in the PNL group had total bilirubin (BILI) values above the normal range (2·21 and 7·37 mg dL^{-1}; the normal range is 0–1·45 mg dL^{-1}), suggesting lipid incorporation in PN may cause cholestasis. Serum amino acids (μmol L^{-1}) such as serine, glycine, methionine, and phenylalanine were significantly elevated in PNL and PN groups compared to the CF group (Table 3).

Liver histology

Examination of liver specimens from PNL animals revealed that five out of six had developed mild to moderate steatosis and their severity was rated between 2 and 3. Steatosis was characterized by fatty degeneration of hepatocytes and numerous clear intracytoplasmic fat globules, extended diffusely from the perilobular regions to the central veins (Figure 1(C)). Two out of five liver specimens in group PN showed mild steatosis and received a score of 2 (Figure 1(B)). No evidence of cell injury or inflammation was

Table 2. Concentration of serum liver function values in the three treatment groups on day 8. AST, aspartate amino transferase; ALT, alanine amino transferase; ALP, alkaline phosphatase; BILI, bilirubin; γ-GT, gamma glutamyl transpeptidase (values represented as mean ± SD)

Tests	CF ($n = 6$)	PN ($n = 5$)	PNL ($n = 6$)
AST (IU L^{-1})	115·17 ± 21	120·9 ± 21·99	191·33 ± 129·38
ALT (IU L^{-1})	35·10 ± 4·19	29·4 ± 11·07	50·55 ± 31·49
ALP (IU L^{-1})	364·98 ± 226·73	295·78 ± 219·11	222·88 ± 122·37
BILI (mg dL^{-1})	0·74 ± 0·38	1·22 ± 0·71	2·43 ± 2·84[a]
γ-GT (IU L^{-1})	56·03 ± 37·91	35·26 ± 20·91	31·30 ± 29·84

[a]Two rats in the PNL group had high bilirubin values of 2·21 and 7·37 mg dL^{-1}.

Table 3. Serum amino acid concentrations in ($\mu mol\,L^{-1}$) on day 8 in each group. Values are represented as mean ± SD ($n = 4$ in each group)

Amino acids	CF	PN	PNL
Aspartic acid	15·65 ± 2·40	21·50 ± 5·61	35·06 ± 32·99
Glutamic acid	74·92 ± 22·68	89·90 ± 15·64	95·19 ± 67·38
Asparagine	102·54 ± 34·21	59·15 ± 26·03	44·30 ± 6·11[a]
Serine	276·38 ± 83·77	551·04 ± 92·16[a]	529·07 ± 216·41[a]
Glutamine	662·30 ± 21·98[b]	750 ± 133·33[b]	371·40 ± 40·36
Histidine	70·54 ± 4·14[c]	218·03 ± 61·95[c]	118·07 ± 13·79[c]
Glycine	272·64 ± 36·71[c]	1439·07 ± 319·89[c]	828·37 ± 330·46[c]
Threonine	272·04 ± 56·84	463·20 ± 269	413 ± 186·72
Citrulline	53·07 ± 5·05	46·26 ± 31·46	55·66 ± 15·36
Arginine	221·85 ± 27·67	269·04 ± 89·47	131·18 ± 88·20
Taurine	208·77 ± 57·87	475·15 ± 205·48[a]	282·52 ± 73·36
Alanine	631·91 ± 102·35	1286 ± 616·65	719·80 ± 377·63
Tyrosine	91·59 ± 15·24	99·0 ± 74·24	106·61 ± 25·84
Tryptophan	78·66 ± 14·20[d]	125·79 ± 14·22	83·8 ± 7·28[d]
Methionine	58·30 ± 7·21[c]	210·29 ± 52·0[c]	119·75 ± 9·62[c]
Valine	187·95 ± 31·35	224·57 ± 33·28	207·99 ± 51·39
Phenylalanine	60·0 ± 8·04	141·87 ± 49·1[a]	119·67 ± 23·6[a]
Isoleucine	98·25 ± 18·55	101·46 ± 31·2	110·64 ± 46·84
Leucine	153·87 ± 17·38	133·13 ± 44·99	166·62 ± 80·88
Ornithine	128·33 ± 60·19	281·93 ± 205·4	523·39 ± 63·76[a]
Lycine	1060·76 ± 402·8	948·54 ± 430·85	1117·31 ± 342·53

[a] $p < 0·05$, significant against CF group.
[b] $p < 0·05$, significant against PNL group.
[c] Values of the three groups are significantly different ($p < 0·05$) from each other.
[d] $p < 0·05$, significant against PN group.

observed in steatotic animals. Liver specimens from the CF animals received a score of 1, indicating an absence of steatosis and a normal cellular architecture (Figure 1(A)).

Lidocaine elimination

Representative concentration–time profiles of LIDO and its metabolites in the three treatment groups are shown in Figure 2. The effluent LIDO concentration is the highest in the PNL group, followed by the PN and CF group; these values are significantly different from each other (Table 1).

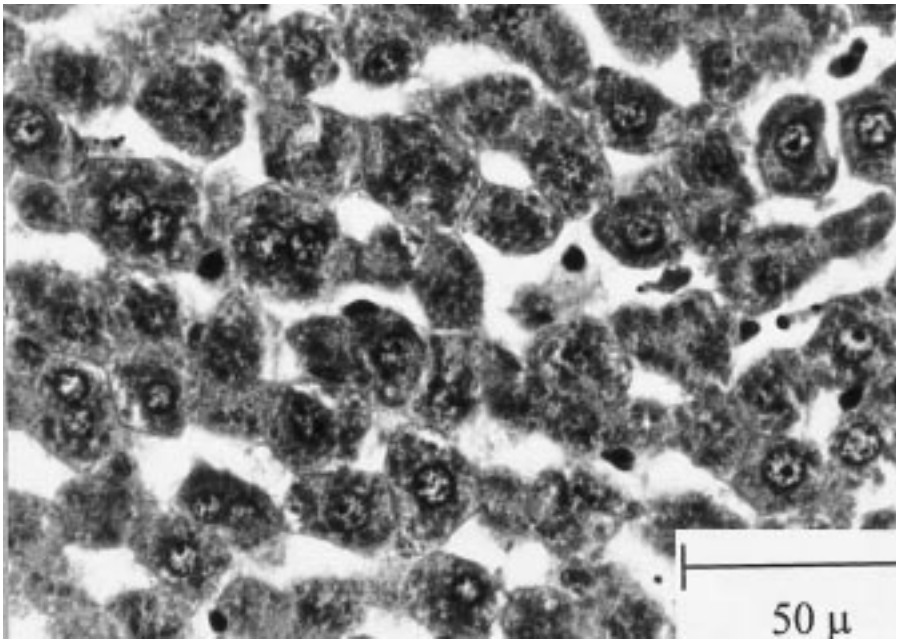
The kinetic parameters in Table 1 reveal that the liver weight normalized buffer perfusion rate (Q) is significantly higher in PN and PNL groups because of the lower liver weights in these two groups. The average intrinsic clearance (Cl_{int}) values per gram of liver weight were lower in both PN and PNL animals, the PNL group being the lowest. The mean value of Cl_{int} in the PNL group is one-fifth of that of the CF group ($p < 0·05$). These data clearly suggest that the rate of LIDO metabolism is severely compromised, particularly in the PNL group.

Steady state percentage recoveries of LIDO and its metabolites are shown in Table 4. The recovery of unchanged LIDO was the highest and MEGX level the lowest in the PNL group ($p < 0{\cdot}05$). The MeOH-LIDO level was reduced in both PNL and PN groups compared to that of the CF group. MeOH-MEGX was not detected in the PN group. The mean levels of this metabolite were similar between PNL and CF animals. When molar ratios of metabolite to LIDO were used for comparison (Table 5), a significant trend of reduction in *N*-dealkylation, *m*-hydroxylation, and aryl methyl hydroxylation was observed in the two PN treated groups. Consistent with the kinetic parameters obtained for lidocaine, animals receiving intravenous lipid had the most pronounced reduction in the rate of LIDO metabolism.

DISCUSSION

Results of the present study indicate that animals receiving a lipid based PN infusion had a higher frequency of hepatosteatosis and a more severe reduction of hepatic metabolic enzyme activities than animals who received an intravenous infusion of an amino acid–dextrose mixture.

The C_{out} data showed that the mean LIDO steady state level (Table 1) increased almost fourfold in the PNL group and twofold in the PN group when compared to the CF group, suggesting impaired drug elimination. Metabolic reduction was indicated by a decrease in the mean Cl_{int} values, 80% in the PNL group and 60% in the PN group ($p < 0{\cdot}05$). The changes observed in Cl_{int} are due to the ability of the liver to eliminate LIDO, since there is no protein in the perfusate media (Krebs buffer). Reduced metabolic clearance also resulted in an increase in the material balance of LIDO, 30% in the PNL group and 16% in the PN group when compared to that of the CF group (Table 4). The formation of the *N*-dealkylated metabolite of LIDO, MEGX, is catalysed by CYP2C11 in rats,[34,35] and accounts for approximately 40–50% of the measured mass balance. The molar ratios of metabolite to drug (MEGX/LIDO) (Table 5) reveal an 83% reduction in the PNL group and a 67% reduction in the PN group compared to the CF group ($p < 0{\cdot}05$). This is consistent with the *in vitro* microsomal findings reported by Knodell *et al.*,[36] who showed that *N*-dealkylations of benzphetamine, ethylmorphine, and erythromycin, which are mediated by CYP2C11, were significantly reduced in intravenously fed animals when compared to those fed intragastrically. In addition to a reduced rate of *N*-dealkylation, significant reductions in ring hydroxylation and aryl methyl hydroxylation also occurred in both PNL and PN animals. The fact that LIDO metabolism involves unidentified primary and sequential metabolic pathways makes it impossible to deduce the rate or extent of reduction of each metabolic pathway from this study. From the mass balance data, it is quite clear that the rate of one or more unknown pathways has been reduced.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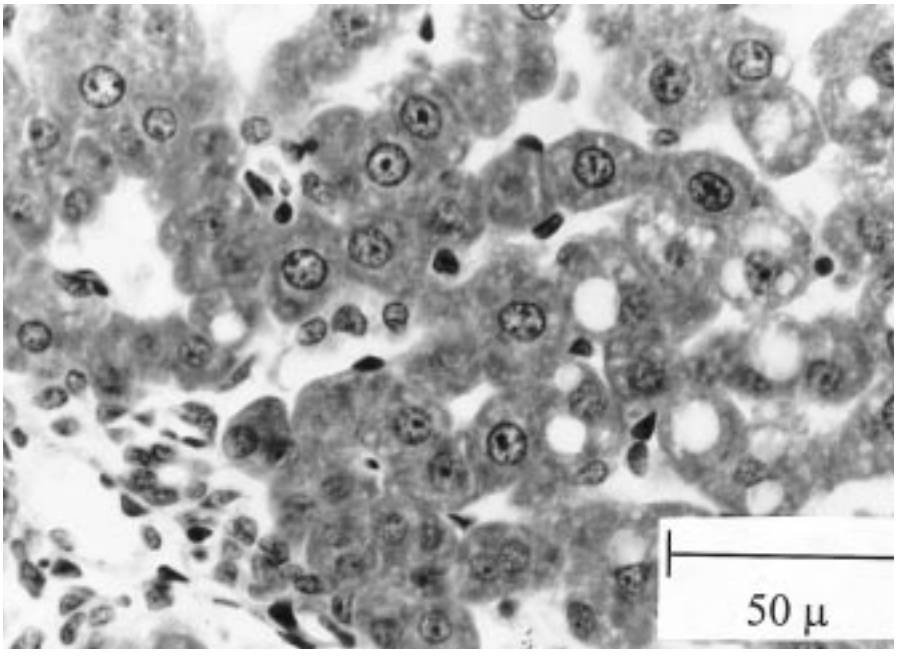
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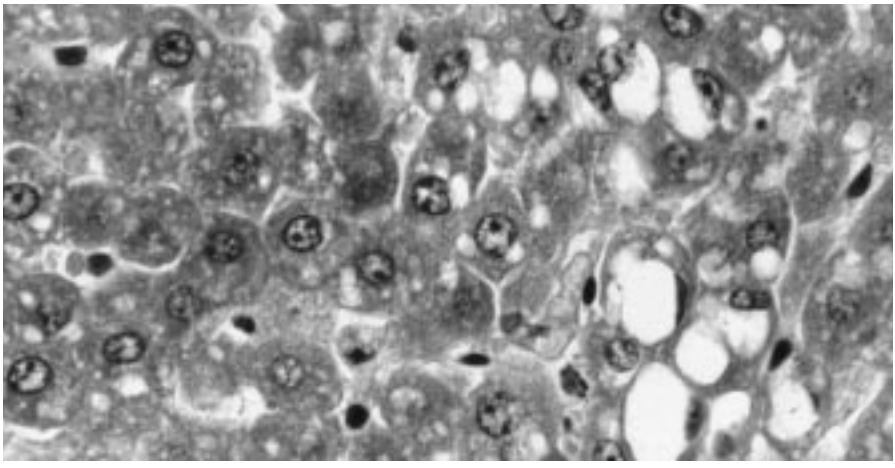

Figure 1. Representative photomicrographs of rat livers: (A) chow-fed, showing a normal overall cellular architecture; (B) PN, a few fatty globules (F) are present, however with no evidence of cell injury of inflammation; (C) PNL, steatosis was present in this group. Numerous intracytoplasmic fat globules are present throughout the hepatocytes

This set of data clearly shows that PN reduces the rate of LIDO elimination, and inclusion of lipid in PN exacerbates the reduction. This is the first report of an inhibitory effect of lipid based PN infusion on drug metabolism. The findings in the PN animals (without lipid) were consistent with those observed by Ke *et al.*[20] Previous reports by Ross *et al.*[22] and Knodell *et al.*[37] have reported either a restoration or an unchanged effect of lipid on metabolic changes of drug induced by amino acid–dextrose infusion. Lipid based PN restored microsomal membrane fluidity and lipid profile,[37] and this change failed to reverse the reduction of meperidine demethylase activity induced by amino acid–dextrose infusion. These findings suggest that the effect of lipid on membrane composition may have selective effects on drug metabolizing enzyme activities. However, the mechanism(s) is (are) not well understood. This phenomenon may explain the discrepancy in results between the study of Ross *et al.*[22] and our findings.

It may be argued that reduced metabolic enzyme activities in PNL and PN animals could be due to a lower liver weight and a poor body weight gain. The decline may be attributable to a lower total calorie intake by the PN animals. This argument is unlikely because the CF and the PN animals had similar energy consumption. The CF animals consumed between 15 and 20 $g\,d^{-1}$. Each gram of rat chow contains 4 kcal. A rat weighing 200–230 g, such as those used in our study, consumes an average of 300–400 $kcal\,kg^{-1}\,d^{-1}$ and gains 5–10 $g\,d^{-1}$.[38] The weight gain of the CF animals was within this range ($7{\cdot}55 \pm 1{\cdot}52\,g\,d^{-1}$). The PN rats consumed an average of 300–360 $kcal\,kg^{-1}\,d^{-1}$ or $\sim 33\,kcal\,100\,g\,d^{-1}$ which is similar to that of the CF animals; this energy supply is sufficient to provide the right nutrition.[21,22,39]

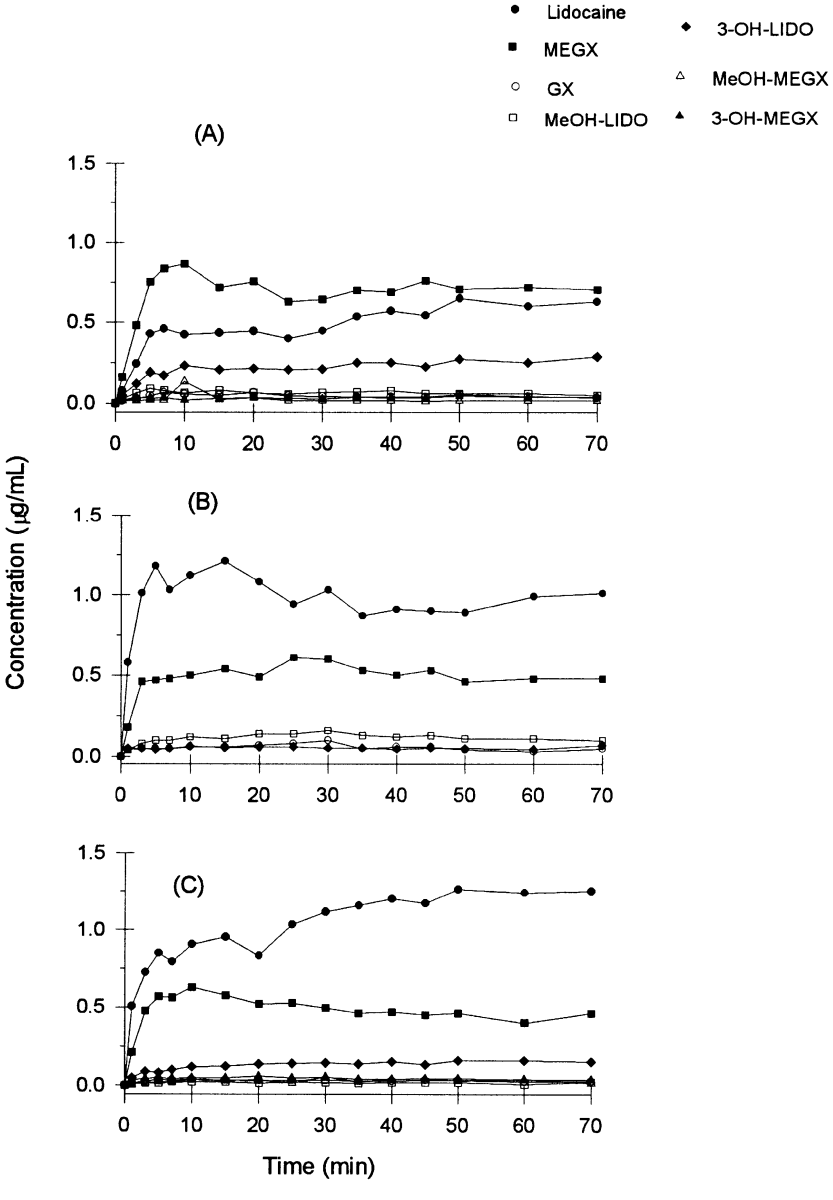

Figure 2. Representative concentration–time profiles of LIDO and its metabolites in the effluent of perfused rat livers: (A) CF (C_{in} = 16·21 μM); (B) PN (C_{in} = 14·56 μM); (C) PNL (C_{in} = 13·92 μM)

Table 4. Mean steady state recoveries (% LIDO dose) of LIDO and its metabolites in the three treatment groups. Values are represented as mean ± SD

Compounds	CF ($n = 5$)	PN ($n = 5$)	PNL ($n = 6$)
LIDO	11·35 ± 5·28[a]	25·92 ± 2·09[a]	39·75 ± 11·36[a]
MEGX	21·34 ± 3·16[b]	19·85 ± 5·48[b]	13·32 ± 3·9
GX	1·74 ± 0·76	1·58 ± 0·69	1·07 ± 0·33
3-OH-LIDO	5·83 ± 1·70	5·23 ± 2·70	7·34 ± 1·66
MeOH-LIDO	2·48 ± 0·83	0·65 ± 0·48[c]	0·58 ± 0·14[c]
3-OH-MEGX	1·31 ± 0·35	1·76 ± 1·01	1·07 ± 0·34
MeOH-MEGX	0·76 ± 0·64	ND[d]	0·51 ± 0·43
Total	44·82 ± 5·44[a]	53·46 ± 6·02[a]	63·64 ± 6·75[a]

[a]Values of the three groups are significantly different ($p < 0·05$) from each other.
[b]$p < 0·05$; significant against PNL group.
[c]$p < 0·05$; significant against CF group.

Table 5. Molar ratios of MEGX, 3-OH-LIDO, and MeOH-LIDO to LIDO in three treatment groups. Values are represented as mean ± SD

Molar ratios	CF ($n = 6$)	PN ($n = 5$)	PNL ($n = 6$)
MEGX/LIDO	2·32 ± 1·32[a]	0·76 ± 0·21[a]	0·38 ± 0·19[a]
3-OH-LIDO/LIDO	0·64 ± 0·42	0·20 ± 0·11[b]	0·20 ± 0·08[b]
MeOH-LIDO/LIDO	0·26 ± 0·14	0·024 ± 0·018[b]	0·02 ± 0·01[b]

[a]Values of the three groups are significantly different ($p < 0·05$) from each other.
[b]$p < 0·05$; significant against CF group.

Despite similar caloric intakes, the weight gain of intragastrically feed animals was greater than those of intravenously fed ones. This observation has also been made by previous researchers.[19,40,41]

Mild to moderate hepatosteatosis was observed in five out of six rat livers in PNL animals, although most of the LFT values were not altered. Despite a high BILI level that was observed in two rats in the PNL group, these livers did not show any histological signs of cholestasis. This observation was consistent with those of previous investigators.[42–44] The reason for this is not clear. There have been controversial reports, both in animals and humans, on the effect of intravenous lipid on hepatic morphology and test markers. Some studies[10,16,17] showed that intravenous lipid can reverse steatosis; however, other studies[1,19,45–48] have shown the opposite. In a recent study, Balderman *et al*. compared ultrasound results for two groups of patients who received a PN formulation containing either long-chain triglycerides (LCTs) or a mixture of medium-chain triglycerides (MCTs) and LCTs. After 7 d infusions, a significant increase in liver size and grey-scale value was observed in those patients who received LCTs. These changes were not apparent in the group that received MCTs–LCTs. The increased liver size in the LCT group could be

due to an increased deposition of glycogen and fat in the liver. Perhaps this increased level of liver glycogen would enhance the process of lipogenesis, and this reaction requires NADPH. There is a possibility that there would be a competition between CYP enzymes and the enzymes responsible for lipogenesis for NADPH which in turn could reduce the rate of liver drug metabolism.[50]

Potential benefits of using MCTs–LCTs over LCTs in preventing hepatomegaly and fatty liver infiltration were also demonstrated by Clarke *et al*.[47] In the present study, animals in the PN group displayed evidence of steatosis but the result was less prominent than that observed in the PNL group. These results imply that the pathogenesis of PN induced steatosis in the rat is multifactorial, and the lack of MCTs in intravenous lipid may be one of the contributing factors.

Possible explanations of the observed steatosis in PN group animals are (i) the instability of the emulsion; (ii) the procedure of lipid incorporation; (iii) the amount of lipid infused; and (iv) the type of lipid infused. A major argument against emulsion instability is that infusions were freshly prepared. No marked changes in their colour or consistency were observed in lipid containing PN solutions. The 'three-in-one' media used are reported to be clinically safe, stable, and economical.[14,15] The amount of lipid that was used provided 30% of non-protein calories and this is also consistent with amount used in previous studies.[21,51–53]

The steatosis observed in PNL animals may be attributable to the type of fatty acid infused. A higher percentage of 20% Intralipid® is composed of long-chain fatty acids or linoleic acid and does not contain any MCTs. LCT utilization may become a problem because its entry into the cell is dependent on carnitine.[54,55] The latter is often found to be depleted in PN treated patients. This situation may eventually lead to a reduced capacity to metabolize LCTs[54] and cause fatty deposition. In contrast, MCTs can enter mitochondria directly and can be oxidized quickly to provide energy.[54–58] Therefore, it may be advantageous to use an MCT or a mixture of LCTs–MCTs in place of LCTs to prevent liver dysfunction and reduce the risk of PN associated reduction in hepatic drug metabolism.

Cohen[7] performed an *in vitro* guinea pig liver explant study and observed that the amino acids leucine, threonine, isoleucine, and particularly glycine were hepatotoxic. This observation may not have any relevance to current findings, because serum levels of glycine were elevated almost fivefold in PN animals, and were doubled in PNL animals. This change bears no correlation with the frequency and severity of steatosis observed. Photo-decomposition of amino acids results in products which are hepatotoxic[5,11,59] but formation of such products was prevented in this study by protecting infusion liquids from the damaging effects of light. The exact mechanism(s) by which amino acids in PN solutions interact with hepatic drug metabolism is (are) still not understood.

In summary, it has been determined that PN, with and without Intralipid®, produces a reduction in the rate of LIDO metabolism in rats, and that incorporation of lipid in PN solutions promotes steatosis and exacerbates the reduction of drug metabolism rate. Therefore, these animal data may be helpful in the design and interpretation of human PN studies.

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