Use of dietary saturated fatty acids and vitamin E in the treatment of alcoholic liver disease

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Several lines of evidence indicate that dietary fat has the potential to affect the severity of alcohol-induced liver injury. Aside from altering the threshold for alcoholic liver injury, saturated fats can be used to treat experimental alcoholic liver disease. Diets enriched in saturated fatty acids (palm oil and medium chain triglycerides) inhibit cytochrome P450 2E1 and lipid peroxidation and ameliorate established alcoholic liver disease. Vitamin E may also play a role in modulating lipid peroxidation and liver injury. Additionally, plasma levels of endotoxin and liver mRNAs for pro-inflammatory cytokines are also downregulated after treatment with saturated fatty acids. Thus saturated fatty acids are a potential therapeutic intervention in inflammatory liver injury.

Introduction

Evidence shows that dietary lipid is an important determinant of alcohol-induced liver injury. For example, epidemiologic observations suggest that saturated fat is relatively protective against alcoholic liver disease[1]. Also, dietary lipid can modulate the severity of alcoholic liver injury in rats[2-5]. None of the histologic features of alcoholic liver injury develop in rats fed ethanol and saturated lipid whereas fatty liver, necrosis, inflammation and fibrosis develop in rats fed ethanol and lipid enriched in polyunsaturated fatty acids. Several investigators have proposed that polyunsaturated fatty acids promote alcoholic liver injury by inducing cytochrome P450 2E1 (CYP 2E1) and lipid peroxidation[6-8].

With these facts in mind, different strategies could be employed to decrease lipid peroxidation and treat alcoholic liver disease. One approach would be to use dietary saturated fatty acids because both CYP 2E1, fatty acid composition of the liver and lipid peroxidation are sensitive to dietary manipulation[9,10]. Therefore, studies were carried out in which rats with alcoholic liver injury were treated with diets enriched in saturated fatty acids (palm oil and medium chain triglycerides) or a diet enriched in polyunsaturated w-3 fatty acids (fish oil). In addition to being a rich source of saturated fatty acid, palm oil contains tocopherols and tocotrienols which inhibit lipid peroxidation[11]. Palm oil also modulates eicosanoid metabolism in a manner in which the ratio of vasodilator to vasoconstrictor prostanoids is increased[12].

Materials and methods

Experimental design

Male Wistar rats weighing between 225 and 250 grams were fed a liquid diet by continuous infusion through permanently implanted gastric tubes as previously described[13,14]. The rats were administered their total nutrient intake by intragastric infusion. The percentage of calories derived from fat was 35% of total calories. Vitamins and minerals were given as described previously[13,14]. The amount of ethanol given was modified to maintain high levels of blood ethanol (150-300 mg/dL) throughout the day. This amount was initially 8 g/kg/day and was increased up to 16 g/kg/day as tolerance developed. Each ethanol-fed rat had at least two measurements of blood alcohol level.

Experiment 1. Three groups of rats (5 rats/group) were studied. Rats in group 1 were fed a fish oil-ethanol diet for 6 weeks (FE group), after which they were killed. Rats in groups 2 and 3 were fed the same fish oil-ethanol diet for 6 weeks, after which they were switched to a diet containing either fish oil with dextrose (FE-FD group) or palm oil with dextrose (FE-PD group) for 2 more weeks and then killed. A liver biopsy specimen was obtained for assessment of histopathology before switching the animals to the dextrose-containing diets. The percentage of calories derived from either fish oil or palm oil was 35% of total calories. The caloric intake was identical in all groups. When the animals were killed, a sample of the liver was obtained for histopathological analysis, and the remainder of the liver was rapidly excised, washed with ice-cold 1.15% (wt/vol) KCl, and cut into small pieces, which were transferred to plastic vials and placed in liquid nitrogen. The vials were stored at –80°C. The studies were conducted according to the guidelines on care and use of laboratory animals (National Institute of Health).

Experiment 2. Four groups of rats (five rats/group) were studied. Rats in group 1 were fed a fish oil-ethanol diet for 6 weeks, at which time they were sacrificed. Rats in groups 2, 4 and 5 were fed the same fish oil-ethanol diet for 6 weeks, after which they were switched to a diet containing either fish oil with dextrose, fish oil with dextrose and vitamin E (300 U of a-tocopherol per kg of diet) or MCT with dextrose for 2 more weeks and then sacrificed using ketamine and xylazine. A liver biopsy was performed for histopathology before the animals were switched to the dextrose-containing diets. The percentage of calories derived from either fish oil or MCT was 35% of total calories. The caloric intake was identical for all groups. When the animals were sacrificed, a sample of liver was taken for histopathology and the remainder of the liver was rapidly excised, washed with ice-cold 1.15% (w/v) KCl and cut into small pieces, which were transferred to plastic vials and placed in liquid nitrogen. The vials were stored at -80°C.

Histologic analysis

A small sample of liver was obtained by biopsy or when the rats were killed and formalin-fixed. H&E stain was used for light microscopy. The severity of liver pathology was assessed as

Note: Portions of these studies have been previously reported in Gastroenterology 1995; 109: 547-554 and J Pharmacol. Exp. Ther. 1996; 277: 1694-1700.

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follows: steatosis (the percentage of liver cells containing fat) was scored 1+ with <25% of the cells containing fat; 2+, with 26-50% fat, 3+, with 51-75% fat; and 4+, with >75%. Necrosis was evaluated as the number of necrotic foci per square millimetre, and inflammation was scored as the number of inflammatory cells per square millimetre. At least three different sections were examined per sample of liver. The pathologist evaluating the sections was unaware of the treatment groups when assessing the histology.

Measurements of blood alcohol

Blood was collected from the tail vein, and ethanol concentration was measured using an alcohol dehydro-genase kit from Sigma Chemical Co (St Louis, MO).

Determination of thiobarbituric acid reactive substances

Levels of liver thiobarbituric acid-reactive substances (TBARS) were measured according to the method of Ohkawa *et al*[15]. Briefly, 0.2 mL sodium dodecyl sulfate (8.1%), 1.5 mL 20% acetic acid, and 1.5 mL 0.8% thiobarbituric acid were added to 200 mL of liver homogenate. After addition of distilled water, the tubes were vortexed and placed in boiling water for 1 hour. The reaction was stopped by immersion of tubes in a cold water bath. After addition of 15:1 (vol/vol) butanol-pyridine and centrifugation, the upper phase was removed and absorbance at 532 nm was determined. Butylated hydroxytoluene (BHT) (90 mM) was added to prevent the formation of TBARS *in vitro*.

Measurement of conjugated dienes

Conjugated dienes in the total lipid extracted from liver homogenates were identified by their optical density of between 220 nm and 300 nm as described by Recknagel and Glende[16].

Aniline hydroxylase activity

Aniline hydroxylase activities were performed according to the method of Imai *et al*[17] with the following modification[18]. Liver microsomes were incubated for 60 minutes at 37°C in 0.45 mL of 0.1 mol/L KPi (pH 7.4) containing 8 mmol/L aniline and 1 mmol/L NADPH. Reactions were terminated with 90 mL of 40% trichloroacetic acid. Samples were then placed on ice for 10 minutes followed by 10 minutes of centrifugation. An aliquot of the supernatant (0.36 mL) was mixed with 10% Na_2CO_3 (0.24 mL) and 2% phenol (0.36 mL). A_{630} values were determined after incubation for 45 minutes in the dark. Specific activities were calculated from a standard curve prepared with the reaction product 4-aminophenol (Aldrich, Milwaukee, WI).

Results

No differences were found in the amount of weight gained during the 6 week period of ethanol feeding or during the 2 week period after switching to the experimental diets. No significant difference was found in blood alcohol levels.

Effects of experimental diets on liver pathology

Feeding the fish oil-ethanol diet for 6 weeks resulted in fatty infiltration, inflammation and necrosis (Table 1, Figure 1). There was minimal improvement when the ethanol was stopped and the rats switched to the fish oil-dextrose diet for 2 weeks. Addition of vitamin E to the fish oil-dextrose diet resulted in an improvement in the severity of fatty liver, necrosis and inflammation. The degrees of fatty liver, necrosis and inflammation were all markedly improved when the rats were switched to palm oil-dextrose or MCT-dextrose diets. In fact, treatment with diets enriched in saturated fatty acids led to almost complete normalisation of liver histology (Figure 2).

Dietary modulation of lipid peroxidation

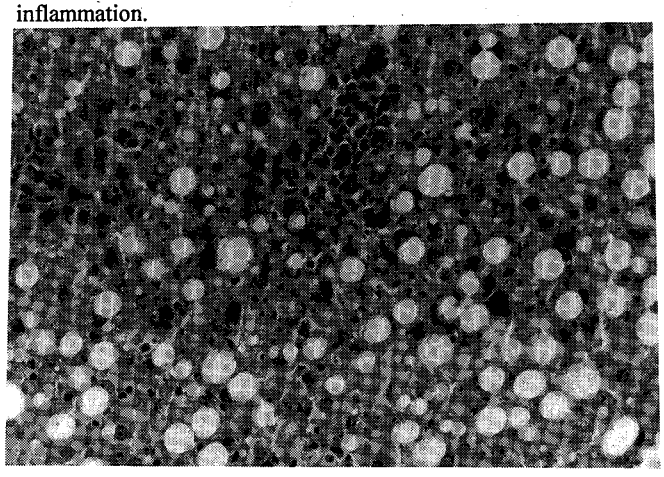
We had hypothesised that feeding saturated fatty acids would result in decreased levels of lipid peroxidation. The levels of TBARS and conjugated dienes were significant lower in the dietary groups treated with saturated fatty acids (Table 2). Part of the explanation for the decrease in lipid peroxidation could be related to changes in CYP 2E1 activity. The activity of aniline hydroxylase, which reflects the activity of CYP 2E1, is shown in Table 2. The activity of aniline hydroxylase in the MCT-dextrose and palm oil-dextrose treated groups was significantly lower than in other treatment groups.

Table 1. Pathologic changes in the different experimental groups.

Treatment group	Duration of feeding (weeks)	Fatty liver 0-4	Necrosis foci/mm^2	Inflammation (cells/mm^2)
Group 1				
Fish oil-ethanol (FE)	6	4.0±0.0	1.4±0.4	32.4±7.4
Group 2				
Fish oil-ethanol	6	3.8±0.4	1.2±0.4	29.3±9.1
Fish oil-dextrose (FE-FD)	2	2.2±0.4	0.7±0.3	22.5±5.9
Group 3				
Fish oil-ethanol	6	3.6±0.5	1.4±0.5	30.1±8.8
Palm oil-dextrose (FE-PD)	2	1.6±0.5[a]	0.5±0.1[a]	15.7±6.2[b]
Group 4				
Fish oil-ethanol	6	3.8±0.4	1.3±0.3	32.0±10.6
Fish oil-dextrose-vitamin E (FE-FD-Vit E)	2	1.0±0.6[a]	0.2±0.2[a]	1.9±1.6[c]
Group 5				
Fish oil-ethanol	6	3.6±0.5	1.0±0.6	30.6±12.6
MCT (FE-MCT)	2	0.8±0.7[a]	0.2±0.1[a]	2.0±1.0[c]

a: p<0.02 vs. fish oil-ethanol in the same group
b: p<0.05 vs. fish oil-ethanol in the same group
c: p<0.01 vs. fish oil-ethanol in the same group

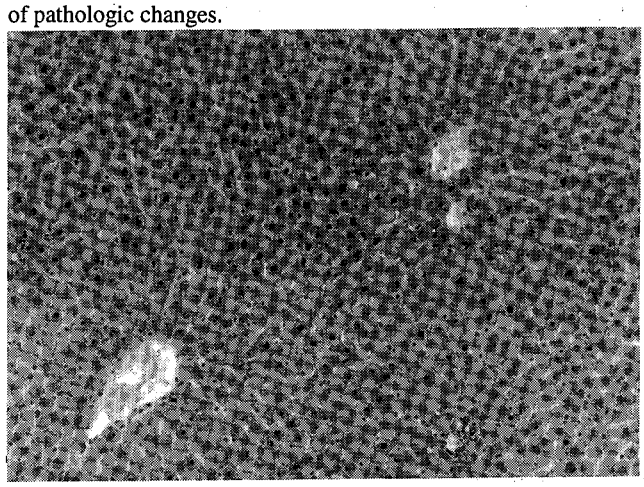
Figure 1. Liver section from a rat fed fish oil and ethanol for 6 weeks showing evidence of fatty infiltration, necrosis and inflammation.



Discussion

The problem of treating alcoholic liver injury has remained intractable. Although diets high in protein and calories have been used to reverse the protein-calorie malnutrition that often accompanies alcoholic liver disease, little effort has been directed toward developing a dietary strategy that might treat the underlying disease. Our results show that when the dietary fat was switched from fish oil that is rich in polyunsaturated fatty acids to diets rich in saturated fatty acids and tocols, the alcohol-induced liver injury was reversed to normal. When rats were continued on fish oil, the liver pathology persisted.

Figure 2. Liver section from a rat treated with palm oil dextrose for 2 weeks after 6 weeks of fish oil-ethanol. There is no evidence of pathologic changes.



Palm oil and MCT were effective in treating alcoholic liver injury probably because of their low content of polyunsaturated fatty acids and/or tocopherol content. Saturated fatty acids are not targets of free radical attack[19]; therefore, lipid peroxidation was minimised in rats fed the saturated fatty acid diets, especially palm oil with its extra vitamin E. In fact vitamin E alone was able to accomplish what palm oil did when the vitamin E was added to fish oil. Thus, the main protection would seem to be an antioxidant issue. In the intragastric feeding rat model for alcoholic liver disease, CYP 2E1 induction is associated with an increase in lipid peroxidation[6-8]. The reduction in lipid peroxidation in the saturated fat-treated groups was accompanied by a decrease in CYP 2E1 activity.

Table 2. Lipid peroxidation and aniline hydroxylase activity in the different experimental groups

Experimental group	TBARS (nmol/mg protein)	Conjugated dienes	Aniline hydroxylase activity (nmol/mg/min)
FE (6 wks)	1.37±0.26	0.46±0.16	0.75±0.11
FE-FD	0.74±0.19[a]	0.29±0.08	0.39±0.03[a]
FE-PD	0.28±0.08[b]	0.14±0.01[b]	0.32±0.04[c]
FE-FD-Vit E	0.30±0.11[b]	0.07±0.07[b]	0.35±0.02[a]
FE-MCT	0.22±0.07[b]	0.09±0.03[b]	0.29±0.01[c]

(a) $p<0.02$ vs. FE group; (b) $p<0.01$ vs. FE and FE-FD gp; (c) $p<0.01$ vs. other groups except FE-MCT, FE-PD

The therapeutic strategies employed in these studies are based on prior studies in which ethanol fed to rats with saturated fatty acids prevented both the induction of CYP 2E1, lipid peroxidation and liver injury. Polyunsaturated fatty acids, on the other hand, promote CYP 2E1 induction, lipid peroxidation and liver injury[4,20]. Regardless of the mechanisms involved, feeding saturated fatty acids or vitamin E represents a simple and effective means of reversing alcoholic liver injury. It is important to determine whether a lipid-based strategy will be effective in clinical alcoholic liver disease.

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膳食飽和脂肪酸在治療酒精性肝臟疾病的應用

摘要

若干證明指出膳食脂肪可影響酒精引起肝臟損害的嚴重程度. 飽和脂肪可用以治療實驗的酒精性肝臟疾病. 富含飽和脂肪(棕櫚油和中鏈甘油三酯)的膳食, 可抑制細胞色素P450 2E1和脂類過氧化作用, 并改善酒精性肝臟疾病. 再者, 用飽和脂肪治療后, 血漿的內毒素濃度和肝臟信使核糖核酸覆制炎症細胞活素(Pro - inflamation cytokine)均減少. 因此作者認爲飽和脂肪酸可能用于肝臟炎症損害的治療.

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