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DIETARY LINOLEIC ACID IS REQUIRED FOR DEVELOPMENT OF EXPERIMENTALLY INDUCED ALCOHOLIC LIVER INJURY

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Summary

We had previously hypothesized that linoleic acid (LA) was essential for development of alcoholic induced liver injury in our rat model. Male Wistar rats were fed a nutritionally adequate diet (25% calories as fat) with ethanol (8-17 g/kg/day). The source of fat was tallow (0.7% LA), lard (2.5% LA) or tallow supplemented with linoleic acid (2.5%). Liver damage was followed monthly by obtaining blood for alanine aminotransferase assay and liver biopsy for assessment of morphologic changes. Enzyme and histologic changes (fatty liver, necrosis and inflammation) in the tallow-linoleic acid-ethanol fed animals were more severe than in the lard-ethanol group. The tallow ethanol group did not show any evidence of liver injury. Our results strongly support our hypothesis that LA is essential for development of alcoholic liver disease in our rat model.

Our previous epidemiologic and experimental studies have identified an important role for dietary fat in the pathogenesis of alcoholic liver disease (ALD) in man and experimental animals (1,2,3). A study of various populations indicated that in those countries with a high intake of saturated fat had a lower than expected mortality rate for alcoholic liver disease; higher than expected rates were associated with a high intake of polysaturated fats (2). A highly significant correlation was also observed between mortality rates from cirrhosis and the product of ethanol and pork consumption (1). Using this background data, we conducted experiments to determine the effect of tallow, lard and corn oil on experimentally induced alcoholic liver injury in the rat. We showed that beef fat completely prevented the occurence of ALD, minimal to moderate disease was found in lard and ethanol fed animals and the most severe pathology was found in the corn oil-ethanol fed animals (4). We hypothesized that the most likely cause for this difference in the different dietary groups was the amount of linoleic acid in tallow (0.7%) lard (2.5%) and corn oil (56.6%). To test our hypothesis, we designed an experiment in which the occurrence of alcohol induced liver injury was followed in rats in whom the tallow diet was supplemented with linoleic acid at the level found in lard (2.5%). Development of ALD in the linoleic acid supplemented diet would conclusively show that linoleic acid was a requirement for the pathogenesis of ALD in the rat.

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Materials and Methods

The experimental groups consisted of Charles River rats, male, weighing 220-355 grams. Each of the animals in the different experimental groups were fed, for periods ranging from 2 to 6 months, a totally liquid, nutritionally adequate diet in which protein comprised 25%, fat 25% and carbohydrate 50% of calories. This diet was supplemented with minerals and vitamins (5). The experimental liquid diet was fed continuously through a permanent intragastric cannula so as to maintain a high blood ethanol concentration and perfect pair feeding with a control diet where glucose was substituted for alcohol calories on an isocaloric basis (6,7,8). The amount of ethanol fed ranged from 8 to 17 g/kg/day to maintain blood ethanol levels between 200 mg% and 400 mg%. Ethanol was measured once a month by flouresence polarization using the TDX analyzer (Abbott Laboratories, North Chicago, Illinois). All animals in the different dietary groups received the same dietary regimen except for the type of dietary fat (25% of total calories). They were: tallow, tallow supplemented with 2.5% linoleic acid and lard. Since our hypothesis for experimental alcohol liver injury is related to some aspect of eicosanoid metabolism, we used free linoleic acid rather than the naturally occurring triglyceride because this simplifies the incorporation of linoleic acid into the biosynthetic pathway of eicosanoids (9).

Liver damage was followed monthly by obtaining blood for alanine aminotransferase assay (10) and whenever possible liver biopsies for assessment of morphologic changes. In addition to hematoxylin and eosin, collagen was stained using sirius red (11). Livers were graded for degree to fatty change (0-4+) using the percentage of liver cells containing fat: $<$ 25% = 1+, 25-50% = 2+, 50-75% = 3+ and $>$ 75% = 4+. Necrosis, inflammation and fibrosis were graded as 0-2+ as follows: 1+ = 1 focus per low power field, 2+ = $>$ 1 foci per low power field. One point was given for each grade of histologic abnormality as described above; a total liver score was then calculated.

Results

The preliminary results obtained in some of the animals in our study of tallow (no linoleic acid supplementation) and lard fed animals have been reported elsewhere (4).

The results obtained with the rats fed ethanol and tallow supplemented with linoleic acid indicate the requirement for linoleic acid in the pathogenesis of alcoholic liver injury. (Tables 1 and 2).

TABLE I

Mean ± SD of serum ALT (U/L) at different months

Dietary group	Baseline	1	2	3	4
Tallow	52.2±21.2(5)**	37.4±17.6(5)	54.5±36.3(4)	34‡	42
Tallow + E	57.2±24.8(5)	66.2±19.7(5)	71±21(4)	57.5	34.5
Lard	36.8±11(5)	25.6±14.3(5)	28±11(4)	42.2±10(3)	31
Lard + E	47±12(5)	100.6±71.5(5)	53.5±10(4)	51±12(3)	55
Tallow + linoleic acid	36.7±4.0(4)	29±8.2(4)	35.8±12.2(3)	59.5	28
Tallow + linoleic acid +E	39.3±6.4(4)	52.5±18*(4)	117.5±38.4+(3)	307	97

* $p < 0.05$ when compared to control
\+ $p < 0.01$ when compared to control
‡ where numbers without SD are shown, this represents the average of two results
** numbers in parentheses refer to number of animals

Serum alanine aminotransferase levels (ALT) in rats fed tallow or lard or tallow with linoleic acid with and without ethanol (E).

TABLE II

Mean ± SD of liver score at different months

Dietary group	1	2	3	4
Tallow + E	0.2±0.4(5)	0.2±0.4(5)	0.1±0.2(3)	0.0±0.0(3)
Lard + E	0.6±0.5(5)	1.8±1.1(5)	1.6±1.0+(3)	1.0±0.9+(3)
Tallow + linoleic acid + E	1.7±0.9*+(4)	2.0‡	3.5	3.0

* $p < 0.01$ compared to lard and tallow group
\+ $p < 0.01$ compared to tallow group
‡ where numbers without SD are shown, this represents the average of two results

Liver score in rats fed ethanol and tallow or lard or tallow with linoleic acid.

The progressive increase in serum levels of ALT (Table 1) in the ethanol-tallow-linoleic acid group is accompanied by an increase in the liver score (Table 2). The histologic lesions seen in this group include fatty change, inflammation and necrosis. The control livers were histologically normal (liver score equal to zero).

Discussion

Our previous study had clearly shown that tallow spared the liver from the pathologic effects of ethanol. We had hypothesized that dietary linoleic acid was a key factor in the pathogenesis of ALD. Our present study confirms this hypothesis that dietary linoleic acid in adequate amounts is an essential requirement for ALD in the rat. The higher serum ALT and liver scores when compared to the lard-ethanol group are consistent with both the observation that free linoleic acid is better incorporated into eicosanoid metabolism than the naturally occurring triglyceride and our hypothesis that experimental alcoholic injury is related to disordered eicosanoid metabolism.

The mechanism(s) by which linoleic acid promotes ALD is unknown; there are however many possibilities. A decrease in microsomal phospholipid concentration of arachidonic acid after ethanol feeding has been observed by many workers (12,13,14). The mechanisms by which this occurs could be due either to inhibition of $\Delta 6$ desaturase activity needed for arachidonic acid synthesis (15) or enhancement of lipid peroxidation of polyunsaturated fatty acids including arachidonic acid. In addition to the cyclo-oxygenase and lipoxygenase branches of the arachidonic acid cascade, it is now recognized that cytochrome P_{450} plays an important role in arachidonic acid metabolism (16,17). The metabolism of ethanol by cytochrome P_{450} (18) provides a possible link to the metabolism of arachidonic acid via this system which is known to produce superoxides and free radicals (19). Alternatively, it could be that linoleic acid metabolism is diverted away from arachidonic acid leading to generation of free radicals by the action of lipoxygenase on linoleic acid (20).

Our study clearly demonstrates that the composition as opposed to the amount of fat per se is critical in the pathogenesis of ALD in the rat. A sufficient amount of linoleic acid is a requirement, however, the exact mechanism by which the combination of linoleic acid and ethanol promote liver injury requires further investigation.

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