Vitamin- E For Treatment Of Early Stages Of Ethanol Induced Liver Damage

V Raghesh, R Rajasree Pai

Abstract

The association of alcohol abuse and liver damage was known from ancient times. No systematic studies have been carried out to evaluate the role of Vitamin-E or the drug in the prevention of alcoholic liver disease. So it was considered worthwhile to study the role of Vitamin E for the treatment of alcohol induced fatty liver.

ABBREVIATIONS

gm - gram
TG - triglyceride

INTRODUCTION

The amount of a fatty acids in the Liver depends on the balance between the processes of delivery and removal. Ingestion of ethanol alters these processes ([7]).

Ethanol inhibits the oxidation of fatty acids to co_2. This can be explained, in part by ethanol induced enhancement of esterification of fatty acids. Ethanol increases the ratio of NADH to NAD^+, which when elevated inhibits several of the enzymes of the tri carboxylic acid cycle([8]).Ethanol via production of acetate is a good promoter of fatty acid synthesis and so accumulation of triglycerides in liver can occur due to ingestion of ethanol. Ethanol induced fatty liver can occur in the absence of abnormalities of liver function and independent of nutritional state([9].)

Role of Vitamin E: Anti oxidants like Vitamin E detoxify free radicals, by abstracting an electron from them. Deficiency of Vitamin E is associated with enhanced lipid peroxidation. Vitamin E is a chain breaking anti-oxidant. It scavenges intermediate radicals to prevent continued hydrogen abstraction. Being hydrophobic it is concentrated in the interior of membranes. It quenches and react with singlet oxygen([10]). Alpha tocopherol is oxidized by free-radical generating systems. But mainly they react with lipid peroxy and alcoxy radicals, donating labile hydrogen to them, and so terminating the chain reaction of peroxidation by scavenging chain propagating radicals.

MATERIALS AND METHODS

To assess the extent of fatty changes in the liver the hepatic cholesterol and triglyceride levels were studied on rats fed with alcohol and alcohol with Vitamin E. Histopathological study of the liver specimen from control and experimental rats were done to substantiate the extent of liver damage in various groups.

ANIMAL MAINTENANCE AND CARE

Albino rats where used through out the study. Rats were housed under hygienic conditions in polypropylene cages. They were maintained on synthetic pellet GOLD MOHUR LABORATORY ANIMAL DIET, a product of Hindusthan Lever Limited. Rats had free access to water. Each rat consumed about 150 gm of pellet per day. At the end of 6 weeks they had 80-100 gm weight. Male albino rats weighing 180-200 gm were used for producing experimental models. 18 rats were divided to 3 groups of 6 rats each Group –1. A group of 6 rats maintained on synthetic diet and water has been taken as control. Group 2 – A group of 6 rats received 8 ml of 20 % ethanol once daily orally for 12 weeks. Group3 -A group of 6 rats received 8ml of 20% alcohol orally once daily for 10 weeks, in the subsequent 2 weeks rats where given 10 mg of alfatocopherol acetate per 100gm body weight along with alcohol.

ADMINISTRATION OF VITAMIN E AND ALCOHOL

Aqueous solution of alcohol and oil suspension of alpha tocopherol acetate was administered using metal cannula attached to syringe.

MODE OF SACRIFICE OF RATS

Rats were sacrificed under light ether anaesthesia at the same time after 12 hr fasting. They were cut open.

EXTRACTION OF TISSUE LIPIDS

Lipids were extracted by the method of Folch et al[1].Liver was homogenized in choloroform: methanol (2;1 v/v) and extracted twice with the same solvent mixture. Phospholipids were broken by adding $1/10^{th}$ volume of 1% KCl.

Choloroform layer dried evaporated to dryness under reduced pressure and made up to a known volume with choloroform. Triglycerides and total cholesterol were estimated using suitable aliquots of the above choloroform solution as detailed below.

ESTIMATION OF TG

Principle: Triglycerides are hydrolysed by microbial lipase to produce glycerol and fatty acids.The glycerol participates in a series of coupled enzymatic reactions, the last of which results in the formation of stable red quinoneimine dye that is red at 520 nm. This is proportional to concentration of triglyceride in samples [2]

Lipase Trigyceride----------------Glycerol+FFA

Kinase Glycerol+ATP----------------------Glycerol-3-Phosphate + ADP Glycerol-3-Phosphate+O_2 ---------Oxidase ----------------Dihydroxy acetone - Phosphate+H_2O_2

H_2O_2 +4-Amino anti pyrene+ Sodium-2-hydroxy 3,5-dicholorobenzene Sulphate-------Quinoneimine Dye

REAGENTS

The working reagent was prepared in one litre of Tris-Hcl buffer (50 Mm/L,Ph7.6) containing 0.1gm Triton x-100,1 mM ADP, Alpha cytodextrin, 1.5mM Sodium-2-hydroxy-3,5 dicholorobenzene sulphonate, 5mM gcl_2,0.5 mM ATP, 10^4 Units peroxidase, $.25 \times 10^3$ Units glycerol kinase and 10^5 Units Lipase. Triglycerides standard contains glycerol with surfactant to yield 20 mg/dl triglyceride as Triolein. Sodium azide 0.1% is added as preservative.

PROCEDURE

1 ml of the reagent was taken in the reaction cuvette, added 20 microlitre of the sample. After 10 minutes incubation at 37 degree Celsius, 2ml of distilled water was added, mixed well and read immediately at 520nm.

TG mg/dl = A.T/ A.S X 200 [3, 4]

ESTIMATION OF TOTAL CHOLESTEROL

Principle: The cholesterol esters present in the sample are hydrolysed quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen free cholesterol is then oxidized by cholesterol oxidase to cholest-4 ene-3 one and H_2O_2.The H_2O_2 reacts with Phenol and 4-Aminophenasone (4-Aminoantipyrene) in the presence of peroxidase to form an 0-Quinoneimine dye. The intensity of the color formed is proportional to the cholesterol concentration and can be measured photometrically between 480 nm and 520 nm.

REAGENTS

The concentration of the substrate mixture is Tris-Hcl (100 mM/L ph 7.7), 50 mM/L of Magnesium acetate, 1 mM/L Phenol,4mM/L 3,4 dichorophenol, 10mM/L Sodium cholate,3gm/L detergent (Fatty alcohol polyglycol ether), 400U/L cholesterol oxidase (Nocardia) and 200 U/L peroxidase. Concentration of cholesterol standard is 200 mg/100ml.

PROCEDURE

To 1 ml of the substrate mixture add 25 microlitres of sample, standard or control. Incubate at 37 degree Celsius for 10 minutes.Then 3 ml of distilled water added and absorbance was measured at 500 nm against a blank.

Cholesterol mg/dl = A.T/A.S X 200

STATISTICAL INTERPRETATIONS

Normal Deviate =(x - ⏹) / (⏹ /⏹n)

Where ⏹ = mean, ⏹= standard deviation and n= number of variables

Arithmetic mean=⏹ (x-⏹x)/n
n= frequency, x= variable and ⏹x = mean

Level of significance was determined by p value.

RESULTS AND DISCUSSION

In the present study animal model has been selected for understanding alcohol induced biochemical changes. Rats were used as the animal for producing experimental model of alcohol injury.The replacement of dietary triglycerides containing long chain fatty acids by fat containing medium chain fatty acids markedly reduces the capacity of alcohol to produce fatty liver in rats[5,6].The pellet diet given to the rats in the study was Gold Mohur Laboratory Animal feeds a

product of Hindustan Lever Limited(Bombay).

The composition of the pellet diet according to the manufacture is

Figure 1

Ingredient	Percentage
Crude protein	21
Ether extract	5
Ash	8
Calcium	1
Phosphates	0.6
Nitrogen free extract	53

Each rat consumed average 15 gm of diet per day. Rats received proteins, carbohydrates, fats in proper proportions. Diet was not deficient in choline or other lipotropic factors. Table I shows the dietary regimen and designation of groups. Fatty liver is the earliest reproducible lesion of alcoholic liver disease. This had been noticed in 90% of chronic alcoholics. Fatty liver develops following moderate alcohol ingestion and appears within a few days. The change is reversible following alcohol withdrawal and the fat is rapidly mobilized and disappears within 3-4 days. Table II shows the hepatic levels of total cholesterol and triglycerides. Vitamin E administration has lowered the hepatic content of total cholesterol and triglycerides in alcohol treated rats. Table III shows statistical significance between various groups. Microscopic observation of histopathological changes in liver of the control and experimental rats are given in Table IV. Thus observations indicate that Vitamin E is an effective drug to revert back the changes induced by alcohol on the liver. Vitamin E supplementation has prevented the development of fatty liver in group III rats as shown by histopathological examination (no vacuolations). Vitamin E has also decreased the cholesterol and triglyceride levels in the liver tissue of group III rats to a statistically significant level.

Figure 2

Table 1

DIETARY REGIME	GROUP
Gold Mohur Pellet diet through out experimental period	I
8ml 20% alcohol Once daily for 12 weeks	II
8ml 20% alcohol Once daily for 12 weeks of which last 2 weeks supplemented with Vitamin E 10 mg/gm body weight along with alcohol	III

Figure 3

Table 2

	cholesterol	triglycerides
GROUP I&II	< 0.01	< 0.01
GROUP II &III	< 0.01	< 0.01

Figure 4

Table 3: Statistical Significance Between Various Groups

GROUP	HEPATIC CHOLESTEROL(mg/gm Tissue)	HEPATIC TRIGLYCERIDES(mg/gm Tissue)
I	2.87 0.13	2.07 0.14
II	5.37 0.12	4.12 0.21
III	4.28 0.18	3.54 0.28

Figure 5

Table 4: Histological Changes

HISTOLOGICAL CHANGES	Control	Group I	Group II
Necrosis	NIL	Marked changes	Moderate changes
Dysplasia	NIL	Minimal changes	Minimal changes
Vacuolation	NIL	Minimal changes	NIL
Inflammation	NIL	Marked changes	Moderate changes
Hyaline bodies	NIL	Minimal changes	Minimal changes

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Author Information

V. K. Raghesh, MBBS
Tutor, Department of General Surgery, Dr.SMCSI Medical College

R. Rajasree Pai, MBBS
Tutor, Dr.SMCSI Medical College