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Effect of Liv.52 on liver lipids

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Effect of oral feeding of Liv.52, an Ayurvedic liver tonic, on liver lipids of weanling rats was studied. While the gross levels of total lipids, neutral lipids, phospholipids did not show any appreciable change, incorporation of ^{14}C acetate *in vivo* into these lipid classes was significantly decreased in Liv.52-fed animals. As evident by per cent incorporation of radioactivity, biosynthesis of triglycerides was inhibited while that of cholesterol was stimulated in Liv.52-fed rats. Levels of lysophosphatidyl choline and sphingomyelin decreased and those of phosphatidyl serine and phosphatidyl ethanolamine increased while incorporation of ^{14}C-acetate decreased in all the individual phospholipids in Liv.52-fed animals. Lipid peroxidation *in vitro* showed significant decrease followed by an increase in the level of tocopherols in Liv.52-fed group.

Oral feeding[1] of Liv.52 (an Ayurvedic liver tonic, manufactured by The Himalaya Drug Co., Bombay) stimulates growth in young rats and increases activities of mitochondrial succinate dehydrogenase, cytochrome oxidase and ATPase as well as microsomal drug metabolising aniline hydroxylate and aminopyrine N-demethylase but inhibits lysosomal acid phosphatase, cathepsin B, acid ribonuclease and acid deoxyribonuclease. Liv.52 also provides protection against decrease in levels of mitochondrial and microsomal enzymes in carbon tetrachloride hepatotoxicity. Since these enzymes are membrane-bound and phospholipids and cholesterol are essential constituents of hepatic intracellular membranes and modulate membrane functions, the effect of prolonged oral feeding of Liv.52 on lipid composition and *in vitro* lipid peroxidation in liver has been studied.

Weanling rats (Druckrey strain) inbred in CDRI animal house were divided into 2 groups of 6 rats each, one group received orally Liv.52 (0.125 ml/kg body weight/day) and the other normal saline (control). The animals had free access of pellet diet (Hindustan Lever's, Bombay) and water. After 11 weeks, rats were killed and liver excised. Lipids were extracted from the liver by the method of Folch *et al*[2]. Lipid P and total cholesterol was assayed[3,4]. Phospholipids were separated by TLC on silica gel-G using chloroform-methanol-acetic acid-water (65:25:4:2) as solvent system[5]. Individual phospholipids were identified by simultaneously running standard phospholipids. Phosphorus in iodine-marked spots was measured according to Wagner *et al*[3]. *In vitro* lipid peroxidation and levels of tocopherols (antioxidant) were measured in liver[6,7].

1-^{14}C-acetate (56 μCi; sp. Activity 27.8 mCi/mmole) was given (2 μmole, ip) to each rat.. In a pilot experiment normal animals were killed after 30 min, 1, 1-½, 2, 2-½ and 3 hr after administration of ^{14}C-acetate and it was found that maximum incorporation of radioactivity in liver lipids was at 2 hr. Therefore, in all the experiments, rats were killed after 2 hr and liver lipids prepared as described above. Neutral lipids were separated by thin TLC using 2 solvent systems[8] and phospholipids were separated as above. Counts per minute (cpm) in each fraction were recorded using scintillation liquid[9] in Packard liquid scintillation spectrometer (model 3330; maximum efficiency for ^{14}C-90%).

Levels of different lipids in liver of Liv.52 fed rats were more or less same as in normal rats but incorporation of ^{14}C-acetate *in vivo* in all these lipid classes was significantly reduced (Table I). Inhibition of acetate incorporation in the phospholipid fraction of Liv.52 fed group was about 8-fold and less than 4-fold in the neutral lipid fraction while the incorporation in cholesterol was inhibited slightly.

Table I: Changes in liver lipids			
		Normal	Liv.52 fed
Total lipid	mg/g liver	50.75 ± 1.08	50.50 ± 0.50
	cpm/mg lipid	7713 ± 129	1192 ± 152
Phospholipid	mg/g liver	28.65 ± 1.61	29.17 ± 0.95
	cpm/mg phospholipid	8052 ± 910	951 ± 141
Neutral lipid	mg/g liver	22.10 ± 1.56	22.00 ± 1.30
	cpm/mg neutral lipid	5750 ± 492	1530 ± 254
Cholesterol	mg/g liver	7.47 ± 0.23	6.93 ± 0.31
	cpm/mg cholesterol	2122 ± 397	1683 ± 191

Table II: Distribution of radioactivity in different neutral lipids (Values represent per cent of total radioactivity in neutral lipids)		
	Normal	Liv.52 fed
Free fatty acids	2.43 ± 0.38	2.73 ± 0.50
Monoglycerides	1.65 ± 0.18	2.85 ± 0.36
Diglycerides	2.26 ± 0.38	2.32 ± 0.79
Triglycerides	78.96 ± 1.95	47.88 ± 2.31
Cholesterol	12.15 ± 1.78	35.21 ± 3.38
Cholesterol esters	2.56 ± 0.40	9.28 ± 0.44

Table III: Changes in individual phospholipids			
		Normal	Liv.52 fed
Lysophosphatidyl choline	Mg*	2.37 ± 0.19	1.60 ± 0.13
	Sp. act$	1.95 ± 0.13	0.89 ± 0.18
Sphingomyelin	Mg	2.67 ± 0.17	2.25 ± 0.10
	Sp. act	2.56 ± 0.25	0.74 ± 0.10
Phosphatidyl choline	Mg	11.09 ± 0.47	10.64 ± 0.28
	Sp. act	14.97 ± 0.80	1.71 ± 0.28
Phosphatidyl serine	Mg	2.58 ± 0.14	3.25 ± 0.39
	Sp. act	21.22 ± 2.4	2.15 ± 0.39
Phosphatidyl ethanolamine	Mg	2.70 ± 0.29	3.62 ± 0.23
	Sp. act	1.40 ± 0.40	0.30 ± 0.04
* mg phospholipids/g liver. $ cpm × 10^{-3}/mg individual phospholipids			

Table IV: Lipid peroxidation and tocopherol levels in the liver			
		Normal	Liv.52 fed
Lipid peroxidation*	0 hr	0.58 ± 0.10	0.45 ± 0.20
	3 hr	3.33 ± 0.50	2.26 ± 0.60
Tocopherol$		663 ± 49.3	846 ± 50

Per cent distribution of radioactivity in different classes of lipids is given in Table II. In normal animals incorporation of radioactivity in triglycerides was 78.96% of the incorporation in neutral lipids followed by cholesterol (12.1%) while in Liv.52 fed group the corresponding values were 47.88% and 35.31% respectively. This shows that feeding of Liv.52 caused a shift in lipid biosynthesis from triglycerides to cholesterol.

Although the gross levels of phospholipids in liver of normal Liv.52 fed rats remained unchanged considerable differences were observed in the phospholipid composition and incorporation of ^{14}C-acetate into individual phospholipids (Table III). Contents of lysophosphatidyl choline and sphingomyelin as well as the specific radioactivity of these fractions in Liv.52 fed group were significantly lower while the level of phosphatidyl choline remained more or less the same in the 2 groups; specific radioactivity of this phospholipid was reduced by more than 8-fold in Liv.52 fed group. On the other hand, the phosphatidyl serine and phosphatidyl ethanolamine contents in Liv.52

fed group were higher than those in normal but the incorporation of ^{14}C-acetate in these fractions was considerably lower.

Level of lipid peroxides *per se* remained the same in both the groups but lipid peroxidation *in vitro* was considerably decreased in Liv.52 fed group (Table IV). This appears to be due to increase in the levels of tocopherols observed in Liv.52 fed animals. It is well known[10,11] that tocopherols exert antioxidant action on lipids with both *in vivo* and *in vitro*.

These results show that the turnover of various lipid classes in rat liver is affected by the feeding of Liv.52 which may in turn affect the lipid composition of hepatic intracellular membranes such as mitochondria and microsomes.

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