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Comparative Studies of NADPH and Ascorbate-Linked Lipid Peroxidation in Gossypol Treated Rat Liver

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ABSTRACT: This study was undertaken to investigate the possible influence of gossypol on enzymatic and non-enzymatic lipid peroxidation against the activity of glucose-6-phosphatase and, the level of total cholesterol in gossypol treated rat liver. Hydrogen peroxide formation was demonstrated by the use catalase and quantified by measuring malondialdehyde formed. Using the liver homogenate, the amount of malondialdehyde formed in NADPH-linked peroxidation is considerably greater than the amount formed in ascorbate-linked peroxidation. Gossypol by itself does not influence the level of protein content, has no inhibitory effect on glucose-6-phosphatase but significant reduction in total cholesterol in both high and low protein diets.

Key Words: Lipid peroxidation; Gossypol; Male infertility; Malondialdehyde.

Introduction

Several studies have been carried out to evaluate possible methods for controlling male fertility with little or no side-effects. Such studies have focused on testicular lipids of adult rats (Sheriff, 1988), metabolic enzymes activity (Vyas *et al*., 1990), cholesterol side chain (Cueller *et al*., 1990) and estimating low-density lipoprotein cholesterol (Warnick *et al*., 1990).

Lipid peroxidation is a measure potentially useful as a diagnostic tool in the detection of cardiovascular disease in human because of the extent of structural damage that can be caused by toxic effects of free oxygen radicals (Pellssier *et al*., 1990). It follows therefore that biomembranes and subcellular organelles are susceptible to lipid peroxidation. Such damage impairs the integrity of their structure and function. Our previous study showed that the composition of rat liver homogenate can be altered considerably by changes in the dietary pattern of food (Achudume *et al*., 1994). This leads to changes in the activity of several hepatic enzymes and ascorbate-dependent lipid peroxidation.

Since gossypol appears to decrease the level of cholesterol in rat liver (Achudume *et al*., 1997, 2000), this experiments is aimed at studying the possibility of peroxidative effect caused by gossypol consumption which is likely to be due to direct consequence of the changes associated with nutrition. The specific objective therefore is to compare the incidence of NADPH-dependent and ascorbate-dependent lipid peroxidation in normal and protein deficient rat liver.

Materials and Methods

Ascorbic acid, NADPH, ADP, Cytochrome C and catalase were obtained from Sigma Chemical Company (St. Louis, Mo). Gossypol acetic acid was obtained from WHO, Geneva, Switzerland. Thiobarbiturate was obtained from Eastman Kodak Co., (Rochester, N.Y.). Male albino Wistar rats (Vom Ife strains) weighing between 190-210g were used for all the studies. Five animals were allocated to two groups fed 25% and 3% protein. Gossypol was dissolved in olive oil and administered at a dose of 20 mg/kg body wt/rat/day for eight weeks. The control received equivalent of the vehicle (olive oil). At the end of the experiment, the animals were sacrificed, the livers were removed and suspended in cold 0.25mM Tris HCl buffer pH 7.6.

Rat liver homogenate containing microsomes were prepared as described by Shimada and Yasuda, 1977. All subsequent operations were carried out at 27°C.

Enzyme Assays

Lipid peroxidation was determined by measuring spectrophotometrically the malondialdehyde formation. The peroxidase activity of the homogenate was determined in the reaction mixtures containing 0.25mM Tris HCl buffer, pH 7.6, 0.6M ADP and 6mM $FeCl_3$ (ADP-Fe and the homogenate containing 2-4mgprotein in a final volume of 3ml with 0.15M KCl. The mixture was incubated at 37°C for 5 min., the reactions were initiated separately by addition of 50ul of ADP-Fe. The controls were incubated without the enzyme or cofactors and any peroxide formed was subtracted from the experimental values. After 10 minutes of incubation, 0.1ml of 100% trichloroacetic acid (TCA) was added to the reaction mixture containing 0.2ml of 5M HCl and 2ml of 0.75% thiobarbituric acid (TBA). The mixture was boiled for 15 min. for the indicator colour to develop. The mixture was centrifuged and the clear colour was measured at 535mu (Shimada and Yasuda, 1977), using an extinction coefficient of $1.56 \times 10^3\ M^{-1}\ Cm^{-1}$.

Ascorbic acid-linked lipid peroxidation was assayed following the methods of Shimada *et al*., 1979. The resultant supernatant was assayed for malondialdehyde by thiobarbituric acid method as outlined above.

Glucose-6-phosphatase activity was determined by measuring inorganic phosphate released (Nordlie and Arion, 1966). Cholesterol level was determined by the methods of Abel *et al*., 1962). Protein was estimated by the method of Lowry *et al*., 1951. The statistical significance of the differences between two averages was determined by calculating the Student t-value of Fisher-Student.

Results

Administration of gossypol to a high protein diet (25%) fed rat and a malnourished rat (3%) resulted in a marked increases in the level of lipid peroxidation as detected in the increases in malondialdehyde formation. Hydrogen peroxide is formedas an oxidation product of NADPH which was clearly demonstrated in this study by the addition of catalase to the reaction mixtures and measured by malondialdehyde produced. Incubation of rat liver homogenate containing microsomes at 37°C in the presence of NADPH and ADP-Fe^{3+} also resulted in varying increases over the control. The amount of malondialdehyde formed in NAD-PH-linked peroxidation is considerably greater than those formed in ascorbate-linked peroxidation (Table 1). The formation of lipid peroxidation in ascorbate-linked peroxidation was much slower, and the amount decreases in comparison with NADPH-linked peroxidation. In high protein diet; the amount of peroxidation formed is greater than those of low protein diet. The increase observed for the enzymatic of NADPH peroxidation did not follow the same pattern as that of ascorbic acid peroxidation. Addition of cytochrome C to ascorbic acid though, increased the level of malondialdehyde formed, greater increase was observed when ADP-FE^{3+} was added.

The results presented in Table 2 show that gossypol alone does not have much influence on the protein content and virtually no inhibition on glucose-6-phosphatase, regardless of the diet composition. However, the consumption of gossypol by animals produced significant reduction in total cholesterol in the two formulated diets.

Table 1: Effect of gossypol consumption on NADPH-linked and Ascorbate-linkedlipid peroxidation in rat liver homogenate.

Treatment	Malondialdehyde nmol/mg. NADPH-linked		Ascorbate-linked	
Protein diet	25%	3%	25%	3%
Control	1.62 ± 0.11	0.23 ± 0.04	1.28 ± 0.91	0.67 ± 0.41
+ ADP-Fe^{3+}	2.72 ± 0.01	2.30 ± 0.11	2.30 ± 0.18	0.86 ± 0.14
+ NADPH	3.01 ± 0.71	2.44 ± 0.25	-	-
+ Catalase	3.33 ± 0.32	2.06 ± 0.11	-	-
+ Ascorbic acid	-	-	0.64 ± 0.08	0.64 ± 0.18
+ Cytochrome C			1.92 ± 0.12	0.81 ± 0.15

The values are mean of four separate preparations ± SD Malondialdehyde formation was determined after 10 min. of incubation.

Table 2: Effect of gossypol consumption on total cholesterol and glucode-6-phosphatase in rat liver homogenate.

Treatment	Glucose-6-Phosphatase	mg/ml	Cholesterol nmd/mg/protein		% protein in	the tissue
Protein diet	25%	3%	25%	3%	25%	3%
Control	3.73 ± 0.13	3.11 ± 0.60	12.65 ± 2.49	1.8 ± 0.33	29.7 ± 0.29	1.2 ± 0.61
Gossypol	3.55 ± 0.20	3.11 ± 0.28	2.7 ± 0.2+	0.92 ± 0.11	27.5 ± 0.59	1.03 ± 0.74

$^{+}$Significantly different from control $P < 0.05$
The values are mean of four separate preparations ± SD.

Discussion

These results demonstrate that changes in dietary pattern can influence the activity of NADPH-linked lipid peroxidation (LP) in liver homogenate containing microsomes. Similar effect is observed in the ascorbate-linked peroxidation system (Table 1). The increase in peroxidation produced by consumption of gossypol is associated with 10 percent reduction in high protein diet and 16 percent reduction for low protein diet. Taken together, these findings suggest that changes in dietary protein during gossypol therapy after lipid peroxidation by modifying the cholesterol composition particularly as observed in low protein diet (Table 2).

It is quite reasonable to suggest that the increase in peroxidation activity may not entirely be due to the diet, but the gossypol. At any rate, the high protein diet contains less of the enzymes involved in this reaction. When catalase was incubated in the presence of NADPH there was 18-fold increase in peroxidation and the maximum activity achieved was 38 percent of the low protein diet (Table 1). Furthermore, when ADP-Fe^{3+} was present in the reaction mixtures, high peroxidation rates were observed.

Therefore, gossypol is capable of inducing peroxidation at this given rates provided that substrate is made available.

Although factor that argues against any difference in diet content is the observation that glucose-6-phosphatase and protein contents were not affected appreciately in both diets. Thus any change could have been due to the presence of gossypol that was involved in the initiation and propagation of the peroxidation process. Direct measurement of peroxidation in NADPH-linked and ascorbate-linked processes revealed that the two preparations were not significantly different.

Similar study had shown that animals maintained on protein deficient diet and treated with gossypol had a significant reduction in body weight, decreased sperm motility and disorganisation of testicular tubules (Kalla *et al*., 1990). It is clear from this presentation that the stimulation of peroxidation may be due to gossypol treatment and activated by NADPH, catalase, ADP-Fe^{3+} and cytochrome C.

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