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Lycopene stabilizes lipoprotein levels during D-galactosamine/lipopolysaccharide induced hepatitis in experimental rats

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PEER REVIEW

Peer reviewer

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Comments

This important study evaluates the ability of lycopene to maintain liver health particularly, lipid metabolism during experimentally induced hepatitis by measuring the lipid metabolizing enzymes and lipoprotein levels. The clear findings of results suggest that lycopene is a potential candidate to restore the derangement of lipid metabolism during hepatitis.
(Details on Page 978)

ABSTRACT

Objective: To investigate the effect of lycopene on lipoprotein metabolism during D-galactosamine/lipopolysaccharide (D-Gal/LPS) induced hepatitis in experimental rats. **Methods:** The efficacy of lycopene was validated during D-Gal/LPS induced hepatitis by analyzing the activity of lipid metabolizing enzymes such as lipoprotein lipase (LPL), lecithin-cholesterol acyl transferase (LCAT) and hepatic triglyceride lipase (HTGL). Lipo protein analyses were done by the estimation of very low density lipoprotein cholesterol (VLDL), low density lipoprotein cholesterol (LDL) and high density lipoprotein cholesterol (HDL). **Results:** The toxic insult of D-galactosamine/lipopolysaccharide (D-Gal/LPS) in experimental group of animals reduces the normal values of lipid metabolizing enzymes due to liver injury. The significant drop in the levels of HDL and concomitant increase in the values of VLDL and LDL were observed. The pretreatment of lycopene restore these altered values to near normal level in experimental group of animals. **Conclusions:** In the light of results, it can be concluded that administration lycopene stabilizes the lipoprotein levels by regulating the lipid metabolizing enzymes through its antioxidant defense and helps to maintain the normal lipid metabolism during toxic injury in liver.

KEYWORDS

Lycopene, Dyslipidemia, Galactosamine, Antioxidants, Lipoproteins

1. Introduction

The liver is the major site of biochemical modifications for both endogenous and exogenous substances including drugs. The functional integrity of liver is essential to maintain a normal metabolism and homeostasis of carbohydrates, lipids, and amino acids[1]. The insoluble lipid molecules are transported through the blood stream along the plasma with proteins and are termed as lipoproteins. Since the liver plays a crucial role in lipid and lipoprotein metabolism, the significant impairment of the hepatic function occur during liver diseases, including hepatitis[2]. The viral hepatitis particularly, Hepatitis B is the most common form of acute hepatitis. Hepatitis B virus (HBV) is a major cause of acute hepatitis, cirrhosis and hepatocellular carcinoma worldwide[3]. HBV continues to be the single most important cause of viral hepatitis in the developing and underdeveloped world[4]. To date there are nearly 360 million HBV carriers in the world with highest incidence of 10%-20% in the tropical countries. Liver diseases due to HBV infection is considered to be the fourth or fifth important cause of mortality in the most productive period of life (15 to 45 years). Hepatitis remains as a clinical challenge and a problem of great importance. Acute hepatitis

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can have serious health effects[5] including disturbance in lipid metabolism. Despite considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations[6]. Hence, crude drugs or natural food diet which possesses antioxidant or free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health.

Carotenoids are a class of more than 600 natural pigments that are present in fruits and vegetables[7]. These carotenoids are ubiquitous in the plant kingdom, fruits and vegetables that are a rich source of carotenoids are thought to provide health benefits by decreasing the risk of various diseases. Lycopene is a potent antioxidant and member of the carotenoid family[8]. It is a naturally occurring compound that gives the characteristic red color to the tomato, watermelon, pink grapefruit, orange, and apricot. A number of studies have indicated the health benefits of consuming lycopene[9-14]. As a major carotenoid in human blood, lycopene protects against oxidative damage to lipids, proteins and DNA[15,16]. Lycopene is a potent quencher of singlet oxygen which suggests that it may have comparatively stronger antioxidant properties, than other major plasma carotenoids[17].

Among the numerous models of experimental hepatitis, D-GalN induced liver damage is very similar to human viral hepatitis in its morphological and functional features and widely used for pharmacokinetic investigations and inflammatory processes of the liver[18-19]. Furthermore, D-GalN-induced liver injury served as a model for testing and elucidating the protective and even therapeutic value of flavones, quinines and carotenoids that are known as antioxidants and of other plant products[20-22]. Altered lipid metabolism and dyslipidemia is the characteristic feature of D-Gal/LPS induced liver injury in experimental animals[2-23]. The scientific research to date has demonstrated an array of health benefits clearly associated with lycopene. It offers important health benefits particularly in regard to prostate, lung, heart and skin health. We have earlier reported that the antioxidant potential of lycopene proves to be valid reason for its hepatoprotective and hypolipidimic role in experimental animals[24].

The present investigation further explores the antidyslipidimic effect of lycopene during D-galactosamine/lipopolysaccharide induced hepatitis in rats.

2. Materials and methods

2.1. Chemicals

D-GalN and LPS (Sero type 011. B4 extracted by phenol water method from *Escherichia coli*) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals (acids, bases, solvents and salts) used were of analytical grade obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Glaxo Laboratoreis, CDH division, Mumbai, India. Lycopene was kindly provided as a gift by Jagsonpal Pharmaceuticals, New Delhi, India.

Lycopene (100 mg) was mixed in 2 mL Tween-80 at room temperature until a homogeneous paste was obtained. Physiologic saline at room temperature was added, drop wise and with vigorous stirring, to a final concentration of 10 mg/mL lycopene of suspension[25].

2.2. Animals

Adult male albino rats of Wistar strain weighing around 120 g to 150 g obtained from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai, India were used in this study. They were housed in polypropylene cages over husk bedding and a 12-hour light and dark cycle was maintained throughout the experimental period. Rats were fed a commercial pelleted diet (Hindustan Lever Limited, Bangalore, India) and water *ad libitum*. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines (IAEC No. 01/026/08).

2.3. Experimental design

The animals were divided into four groups of six animals each.

Group 1 was served as vehicle control and administered with Tween-80 in saline. Rats in Group 2 were given lycopene alone (10 mg/kg body weight for 6 d, *i.p.*). Rats in Group 3 were induced with D-GalN and LPS (300 mg/kg body weight and 30 μg/kg body weight, *i.p.*, 18 h before the experiment)[26]. Rats in Group 4 were pretreated with lycopene for 6 d prior to the induction of D-GalN/LPS.

2.4. Collection of samples for analysis

After the experimental period, the animals were anaesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg body weight) and sacrificed. Blood was collected and the liver tissue was excised quickly. The tissues were washed in physiological saline to remove blood clot and other tissue materials.

2.5. Separation of serum

The blood samples collected in plain centrifuge tubes were kept in inclined position to allow complete clotting of blood and then centrifuged at 2500 r/min for 30 min. The resultant clear supernatant was pipetted out and preserved in small vials in the freezer for the purpose of biochemical investigations.

2.6. Preparation of liver homogenate

Within 3 h after sacrifice, liver samples were blotted to dryness. From this, a piece weighing about 100 mg was taken and homogenized at 4 °C in Tris-HCl buffer (0.1 mol/L, pH 7.4). The tissue homogenates were centrifuged at 2 500 r/min for 30 min. The resultant supernatant was kept under refrigeration until further biochemical analysis. All the assay procedures were carried out within 48 h after sample collection.

2.7. Lipid metabolising enzymes

Activity of lipoprotein lipase was determined by the method described by Korn[27]. The activity was expressed as mmol of glycerol liberated/h/min/mL plasma. The activity of lecithin-cholesterol acyl transferase was assayed by the method of Hitz

et al[28]. LCAT activity in serum was expressed as μg of cholesterol esterified/min/L. Assay of the activity of the hepatic triglyceride lipase was done by the method of Schmidt *et al*[29]. The enzyme activity was expressed as μg of free fatty acids released/min/mg protein.

2.8. Lipoprotein analysis

Lipoproteins were fractionated by dual precipitation technique of Burstein and Scholnick[30]. The values were expressed as mg/dL.

2.8.1. HDL fractionation

To 0.1 mL of serum, 0.09 mL of heparin-manganese chloride reagent was added and mixed well. The solution was allowed to stand at 4 °C for 30 min and then centrifuged at 2500 r/min for 30 min. The supernatant represented HDL fraction. Aliquot was taken from HDL fraction for the estimation of cholesterol.

2.8.2. LDL fractionation

To 0.1 mL of serum, 0.15 mL of sodium dodecylsulphate was added, mixed well and incubated at 37 °C for 2 h. The contents were centrifuged in the refrigerated centrifuge at 10000 r/min for 15 min. VLDL was aggregated as pellet. The supernatant contained the HDL and LDL fractions. Cholesterol was estimated from this fraction in relation with each other.

2.9. Statistical analysis

All the grouped data were statistically evaluated with SPSS, Version 10.0. Hypothesis testing methods included One-way ANOVA followed by LSD test. $P<0.05$ is considered to indicate statistical significance. All the results were expressed as mean ±SD for six animals in each group.

3. Results

3.1. Lipid metabolising enzymes

Figure 1 indicates the activity of lipoprotein lipase, an important lipid catabolizing enzyme in control and experimental groups of rats. A significant decrease in the activities LPL was observed in rats injected with D-GalN/LPS when compared to control rats. Lycopene pretreatment in Group 4 animals restored the activity of LPL to near normal when compared with that of Group 1 control animals. Lycopene alone treated Group 2 rats did not exhibit significant variation with that of control ones.

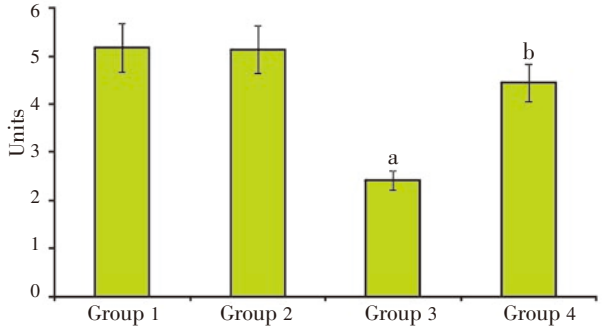

Figure 1. Activity of lipoprotein lipase in control and experimental group of animals.

Figure 2 depicts the activity of hepatic triglyceride lipase, a predominant lipoprotein catabolizing enzyme in the liver sample of control and experimental group of animals. The administration of D-GalN/LPS resulted in the decreased activity of HTGL in Group 3 animals in comparision with control group. A significant restoration in the activity of HTGL was achieved in Group 3 rats due to the pretreatment of lycopene. Group 2 animals treated with lycopene alone exhibited similar activity with that of control Group 1 animals.

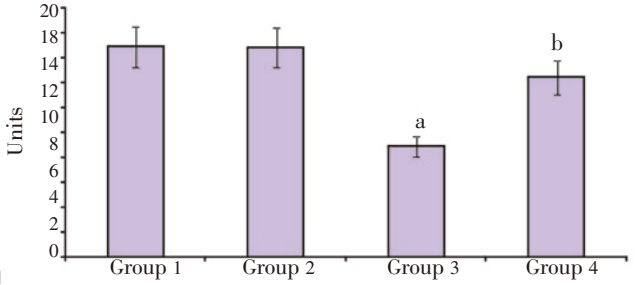

Figure 2. Activity of hepatic TG lipase in control and experimental group of animals.

Figure 3 represents the activity of LCAT, an important cholesterol metabolizing enzyme in various group of control and experimental set of rats. The hepatotoxic nature of D-GalN/LPS resulted a statistically significant drop ($P<0.05$) in the activity of LCAT in Group 3 rats in comparison with that of group one control animals. Group 2 animals treated with lycopene alone did not show any significant variation from that of control group. However Group 3 rats pretreated with lycopene prior to the administration D-GalN/LPS showed a considerable increase in the activity of LCAT which was near normal to that of control Group 1 animals.

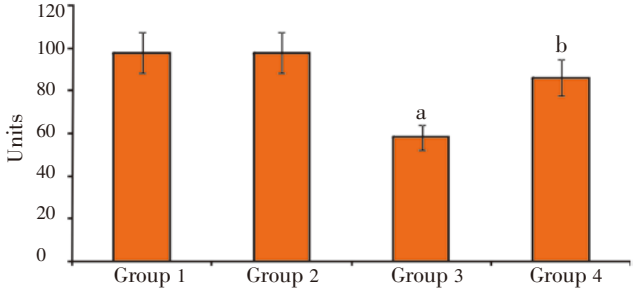

Figure 3. Activity of liver LCAT in control and experimental group of animals .

3.2. Lipoproteins

Figure 4 exhibits the levels of lipoproteins (HDL, LDL and VLDL) in plasma of control and experimental groups of rats. A typical reduction in the levels of HDL along with a marked increase in the levels of LDL and VLDL were recorded in rats that were administered with a toxic dose of D-GalN/LPS in Group 3 animals in comparison with Group 1 control rats. Upon pretreatment with lycopene in Group 4 animals, the altered levels of lipoproteins were reversed back to their normal values in comparison to that of control ones. There was no marked difference between lycopene alone treated rats (Group 2) and control.

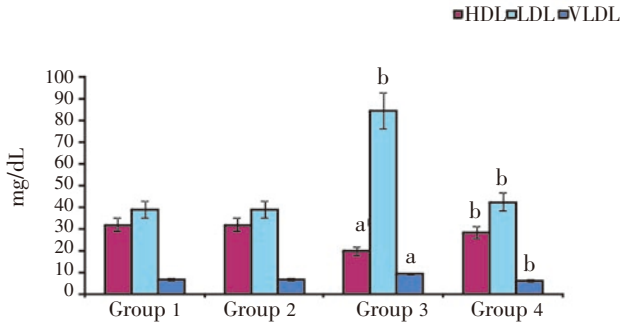

Figure 4. Levels of plasma lipoproteins in control and experimental group of animals.

4. Discussion

The lipid metabolizing enzymes such as lipoprotein lipase, hepatic triglyceride lipase and 1ecithin: cholesterol acyltransferase are the key players involved in the Lipoprotein metabolism. LPL and HTGL are equally important for normal lipoprotein catabolism[31-34]. Lipases regulate lipoprotein catabolism by two mechanisms, as lipolytic enzymes and as ligands for lipoprotein receptors. These enzymes catalyze the hydrolysis of triglycerides and cause the conversion very low density lipoprotein (VLDL) particles into cholesterol-rich remnant particles[35]. LPL or HTGL with VLDL results in hydrolysis of triglycerides and the release of free fatty acids. Studies suggest that lipases may exert their stimulatory effect on VLDL catabolism by both lipolytic and adapterlike functions.

The decreased activity of these enzymes observed in our investigation during D-GalN/LPS toxicity was on par with the earlier findings[36-38]. LPL activity varies within tissues according to the nutritional and stress state of an animal. LPL is involved in the initial catabolism of triglyceride rich lipoproteins, VLDL and chylomicrons[34,38]. Thus the decreased activity of LPL indicates decreased uptake of these lipoproteins by the tissues resulting in hypertriglyceridemia in D-GalN/LPS-induced rats[2,24]. HTGL is said to have a role in the catabolism of VLDL and chylomicron remnants. The efficient clearance of VLDL and chylomicron from circulation by the liver requires the action of HTGL. Hence decreased activities of hepatic lipases results in increased accumulation of lipoproteins (LDL, VLDL) and triglycerides during D-GalN liver injury[2,38].

Among the activities most drastically reduced after D-GalN/LPS challenge is that of plasma LCAT. Plasma LCAT is involved in the "reverse cholesterol transport" where tissue choleterol is transported to the liver for catabolism. This deficiency results in low levels of plasma cholesterol esters and in severe damage, the fall in cholesterol ester content in the plasma was attributed to the lack of LCAT[39-41]. Earlier report also suggests that D-GalN injection results in severe LCAT deficiency in rats[36].

The activities of above lipid metabolizing enzymes were reverted to near normal upon pretreatment with lycopene. This assures the hypolipidemic effect of lycopene[24,42].

The formation of serum lipoproteins is dependent upon the metabolism of precursor (nascent) lipoproteins secreted from the liver and intestine. The conversion of nascent to mature lipoproteins involves enzymatic modification of lipids by LPL, HTGL and LCAT and the exchange of apoproteins and lipids with other lipoproteins and cells[34,41]. It was shown that D-GalN toxicity results in LDL accumulation in plasma [2,43,44].

Lipid disorders, most often encountered in liver diseases also include increased levels of LDL and VLDL[2,44,45]. These abnormalities appear to result from increased hepatic secretion of VLDL particles due to increased concentration of free fatty acids and glucose, reduced VLDL clearance due to reduced activity of LPL and reduced LDL clearance due to glycation of ligand protein. The high level of VLDL triglycerol on D-GalN/LPS induction has been due to the decreased activity of extrahepatic LPL.

HDL is considered to be a beneficial lipoprotein[46]. It helps in the scavenging of cholesterol from the extra hepatic tissues in the presence of LCAT and brings it to the liver[34]. The lowered HDL levels can be attributed to decreased serum LPL and LCAT activity. In this context, it has been shown that elevated activity of plasma LPL leads to an increase in HDL production and reduction in LDL constituents[41]. Therefore the decreased HDL levels in D-GalN/LPS-induced rats might be due to the reduced activities of LPL and LCAT[44]. Numerous studies have already reported the increased levels of LDL, VLDL and concomitant reduction in the HDL values during GalN/LPS induced liver injury[2,47-49]. Our present findings clearly match with the above findings. The consumption of tomoto based products and/or administration of lycopene have been reported to have profound effect on the lipid and lipoprotein metabolism[24,42,50-53]. The ability of lycopene to normalize the abnormal levels of lipoproteins during D-GalN/LPS liver injury in our investigation justifies the previous findings and confirms its antidyslipidimic effect. This may be due to the prevention of free radical generation responsible for lipid peroxidation of β-lipoprotein lipids, regulation of lipoprotein metabolism by restoring the defective lipid metabolizing enzymes as well as stabilization of hepatocytes to neutralize the cytotoxic action of lipid peroxide rich lipoproteins during liver injury.

The results of the present investigation clearly suggest that lycopene is able to influence the lipoprotein metabolism by restoring the altered levels of lipid metabolizing enzymes and stabilizing the derangement of lipoprotein levels during experimentally induced hepatitis. The antidyslipidemic effect of lycopene during D-GalN/LPS liver injury may be attributed due to its ability to combat the oxidative stress and or the involvement of some cellular regulatory mechanism that preserve the cellular integrity against chemically induced toxicity.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

The viral hepatitis particularly, Hepatitis B is the most common form of acute hepatitis. Hepatitis B virus

(HBV) is a major cause of acute hepatitis, cirrhosis and hepatocellular carcinoma worldwide. HBV continues to be the single most important cause of viral hepatitis in the developing and underdeveloped world. To date there are nearly 360 million HBV carriers in the world with highest incidence of 10%-20% in the tropical countries. Liver diseases due to HBV infection is considered to be the fourth or fifth important cause of mortality in the most productive period of life (15 to 45 years). Hepatitis remains as clinical challenge and a problem of great importance. Acute hepatitis can have serious health effects including disturbance in lipid metabolism.

Research frontiers

The experimental hepatitis model of D-GalN induced liver damage is very similar to human viral hepatitis in its morphological and functional features and widely used for pharmacokinetic investigations and inflammatory processes of the liver. D-GalN-induced liver injury served as a model for testing and elucidating the protective and even therapeutic value of antioxidants and of other plant products. Altered lipid metabolism and dyslipidemia is the characteristic feature of D-Gal/LPS induced liver injury in experimental animals The present investigation explores the antidyslipidimic effect of lycopene a antioxidant carotenoid during D-galactosamine/lipopolysaccharide induced hepatitis in rats.

Related reports

The altered activities of lipid metabolizing enzymes were reverted to near normal upon pretreatment with lycopene. This finding and the hypolipidemic effect of lycopene is coinciding and confirming the earlier reports of Shivashangari *et al.* (2006) and Lorenz *et al.* (2012).

Innovations and breakthroughs

Even though the scientific research to date has demonstrated an array of health benefits to prostate, lung, heart and skin health there is no exclusive reports about the role lycopene on liver health in relation with hepatitis and particularly regarding the lipid metabolizing enzymes. This design and data are innovative exploration in this field of study.

Applications

The findings of the research prove worthy to be explored further for the molecular mechanisms underlying on lycopene's ability to alter the lipid metabolism and antidyslipidimic effect during hepatitis.

Peer review

This important study evaluates the ability of lycopene to maintain the liver health particularly lipid metabolism during experimentally induced hepatitis by measuring the lipid metabolizing enzymes and lipoprotein levels. The clear findings of results suggest that lycopene is a potential candidate to restore the derangement of lipid metabolism during hepatitis.

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