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Effect of Genistein and L-carnitine and Their Combination on Lipid Profile and Inflammatory Cytokines in Experimental Nephrotic Syndrome

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Abstract

Background: Nephrotic syndrome is a disorder caused by kidney damage that results in severe leakage of protein from blood into urine. Hyperlipidemia is one complication of nephrotic syndrome. L-carnitine and genistein can control cardiovascular diseases by causing changes in lipid metabolism and cytokine production. This study was designed to examine the effects of genistein and L-carnitine on serum lipid and cytokine profiles in experimental nephrotic syndrome.

Methods: In this study, 50 male Sprague–Dawley rats were randomly divided into five groups of 10 animals each with similar mean body weights (300±50 g). The five groups were NC (normal-control), PC (patient-control), LC (L-carnitine), G (genistein), and LCG (L-carnitine-genistein). Serum HDL-cholesterol (HDL) LDL-cholesterol (LDL), triglyceride, cholesterol, IL-6, and TNF-α were measured. Statistics were analyzed using SPSS 18.0.

Results: At the end of the study, of the patient groups, HDL was significantly greater in the LC than in the PC or G groups (P<0.001). LDL was significantly less in the G than in the PC, LC, or LCG groups (P<0.001). Interleukin-6 was significantly greater in the PC than in the LC, G, or LCG groups, and significantly greater in the LC than in the G group. (P<0.001), but no significant differences were found for triglyceride, cholesterol, or TNF-α between the patient groups.

Conclusions: Genistein had less effect on HDL and triglyceride levels than LC or LCG. Regarding inflammatory cytokines, genistein and L-carnitine had less effect on TNF-α than on IL-6.

Keywords: Genistein, Hyperlipidemia, Interleukin 6, L-carnitine, Nephrotic syndrome, TNF-alpha

Introduction

Nephrotic syndrome is a disorder caused by kidney damage. A major symptom of nephrotic syndrome is severe leakage of protein from blood into urine. One disorder that results from this disease is hyperlipidemia (1), which can happen for two main reasons: hypoproteinemia could stimulate hepatic protein synthesis and cause excessive production of lipoproteins, and lipid catabolism can decrease as a result of low levels of lipoprotein lipase (LPL), which is the main enzyme involved in lipoprotein catalysis. These effects increase the risk for cardiovascular diseases and demonstrate the importance of lipid

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metabolism control in cardiovascular health.

Phytoestrogens are substances that can influence cardiovascular health through their effects on lipid metabolism (2). Studies on humans and animals have demonstrated the beneficial effects of dietary soy protein on serum lipid concentrations (4-7). Soybeans also contain important isoflavones such as genistein and daidzein (3). Recently, soy protein containing isoflavones has received much attention regarding hyperlipidemia management. Estrogens play a vital role in improvement and maturation of the immune system (8). Studies show that genistein reduces TNF-α through inhibition of tyrosine kinases (9, 10). Carnitine transports long-chain acyl groups from fatty acids into the mitochondrial matrix; hence, they can be broken down through β-oxidation to acetyl CoA and enter the citric acid cycle for energy production (11). Progression of dyslipidemia in some renal damage is caused by various factors including carnitine deficiency, which causes disorders in lipid metabolism (12, 13). In some studies, carnitine supplements were effective in managing the lipid profiles of patients with triglyceride levels greater than 200 mg/dl or HDL-C levels less than 35 mg/dl (14). The effect of carnitine on cytokine production has been controversial. One study showed that carnitine decreases cytokine production in leukocytes while later studies revealed this to be in response to TNF-α production inhibition (15, 16). While some studies indicated that carnitine reduces interleukin (IL) -1β, IL-6, and tumor necrosis factor (TNF) -α production (17-20), others have reported opposite results (21).

Considering the importance of a balanced lipid profile in preventing cardiovascular diseases in nephrotic syndrome patients, the aim of this study was to examine the effects of genistein and L-carnitine on serum lipids and cytokines in an experimental nephrotic syndrome model.

Materials and methods

Animals

The experiments were performed with male Sprague–Dawley rats obtained from the Iranian Pasteur Institute. Animal experiments were conducted in accordance with Ethics Committee of Tehran University of Medical Sciences (TUMS) guidelines, which conform to the provisions of the Declaration of Helsinki. The rats were maintained in an animal research facility under standard conditions of 25±3 ºC, 50% humidity, and 12-hour light and dark cycles (22). Rats were provided with AIN-93 rat food and tap water ad libitum. During the adaptation period to the new environment rats fed AIN-93 food for five days, then rats were randomly assigned to one of five groups of 10 animals each with similar mean body weights of 300±50 g as follows:

Group A: normal control (NC): no disease induction + AIN-93 diet,

Group B: nephrotic syndrome (patient control) (PC): induction of disease + AIN-93 diet + gavaged with carboxymethyl cellulose (CMC) as placebo,

Group C: nephrotic syndrome receiving L-carnitine (LC): induction of disease + AIN-93 diet + gavaged with L-carnitine diluted in CMC,

Group D: nephrotic syndrome receiving genistein (G): induction of disease + AIN-93 diet + gavaged with genistein diluted in CMC,

Group E: nephrotic syndrome receiving genistein plus L-carnitine (LCG): induction of disease + AIN-93 diet + gavaged with genistein and L-carnitine diluted in CMC.

Diet, disease induction, and intervention protocol

Throughout the eight-week experiment all animals received AIN-93 diet. After two weeks on the AIN-93 diet nephrotic syndrome was induced in all rats except NC by the intravenous injection into the tail vein of one dose of 7.5 mg/kg body weight of Adriamycin (Doxorubicin, Pharmacia Italia SPA Co.), an antibiotic/antineoplastic drug with nephrotoxic side effects. The PC rats were gavaged with 50 mg/kg/day body weight in 1% CMC at 2% concentration (100 mg/5 ml), the LC rats were gavaged with 50 mg/kg/day body weight L-carnitine (99%, Karen Pharma & Food Supplement Co.) diluted in 1% CMC at 2% concentration (100 mg/5 ml), the G rats were gavaged with 50 mg/kg body weight genistein (99%, LC-laboratories Co.) diluted in CMC, and the LCG rats were gavaged with 50 mg/kg body weight L-carnitine plus 50 mg/kg body weight

genistein diluted in CMC. Dietary intake and body weight were recorded once daily (23).

Sample preparation

During the experiment, urine samples were collected by spot urine method at the end of weeks 2, 3, and 7, and urine total protein and protein-to-creatinine ratios were measured. Pyrogallol-Red/Colorimetric End Point and Taussky (24) methods were used to measure protein and the Owen et al. (25) modified method was used to measure creatinine. In the last day of intervention. Twelve hours after the last gavage, the animals were kept overnight-fasted and euthanized under deep anesthesia with diethyl ether. Blood samples were directly collected via the abdominal aorta, and after coagulation centrifuged at 1500 rcf for 15 minutes. Serum samples were stored at -20°C until biochemical analysis.

HDL-cholesterol, LDL-cholesterol, triglyceride, and total cholesterol concentrations were determined using commercial kits (Pars Azmoon, Iran) and auto-analyzer method. Interleukin-6 and TNF-α were measured by enzyme-linked immunosorbent assays (ELISAs).

Statistical analysis

The data are expressed as means ± SDs. Statistical Package for the Social Sciences (SPSS, version 20, Chicago, IL) was used to analyze statistics. Quantitative variables between different groups were compared by analysis of variance (ANOVA), and Post hoc test by Scheffé test. The data was considered significant with $P<0.05$.

Results

Lipid Analysis

Serum HDL was significantly greater in the LC than in the PC or G patient groups. Serum LDL was significantly less in the G than in the PC, LC, or LCG patient groups. Neither triglyceride nor cholesterol were significantly different in any of the patient groups (Table 1).

Table 1. Serum lipid concentrations

Lipid	NC	PC	LC	G	LCG	P Value [a]
HDL (mg/dl)	1.10±19.10	6.57±47.16	29.38[bcd]±154.44	5.96[d]±60.55	30.60[b]±107.80	<0.001
LDL (mg/dl)	0.77±14.50	8.70[b]±84.25	5.65[bd]±112.49	9.96[cde]±38.45	17.78[be]±78.80	<0.001
Triglyceride (mg/dl)	5.94±66.30	109.23[b]±353.33	18.18±181.66	98.74[b]±409.55	79.05±219.40	0.004
Cholesterol (mg/dl)	2.71±68.20	74.83[b]±307.00	22.61[b]±295.23	42.74[b]±226.22	39.55±204.80	<0.001

NC: normal control, PC: patient control, LC: L-carnitine, G: genistein, LCG: L-carnitine+genistein
Values are based on mean ± standard error (SE).
[a] One Way ANOVA test between groups.
[b] the mean value has a statistically significant difference with the NC group (post hoc Scheffé statistical test with $P<0.05$).
[c] the mean value has a statistically significant difference with the PC group (post hoc Scheffé statistical test with $P<0.05$).
[d] the mean value for the LC group has a statistically significant difference with the G group (post hoc Scheffé statistical test with $P<0.05$).
[e] the mean value for the G group has a statistically significant difference with the LCG group (post hoc Scheffé statistical test with $P<0.05$).

Pro-inflammatory cytokine analysis

Serum IL-6 levels

Interleukin-6 was significantly greater in the PC than in the LC, G, or LCG patient groups and also significantly less in the G than in the LC group. Overall, the G group had the lowest IL-6 concentration of all the groups (Table 2).

Serum TNF-alpha levels

Tumor necrosis factor-alpha was not significantly different between any of the groups (Table 2).

Discussion

In this study we examined the separate and combined effects of genistein and L-carnitine in Sprague-Dawley rats. As shown elsewhere (22), rats with experimentally-induced nephrotic syndrome weighed significantly less than rats in the NC group. ; Reduction in food intake by nephrotic syndrome rats is the logical cause for this phenomenon Despite less food and subsequent protein intake in the nephrotic syndrome groups than in the NC group, the

proteinuria and the urine protein-to-creatinine ratio was greater in the nephrotic syndrome groups than in the NC group (22); this points to their suffering from nephrotic syndrome and the protein tissue lysis that led to greater weight loss among these groups, although such increase among a number of the patient groups was not statistically significant (22).

Table 2. Serum IL-6 and TNF-α concentrations

Variable	NC group	PC group	LC group	G group	LCG group	P Value [a]
IL-6 (pg/ml)	139.4±3865.00	154.56±4250.00	151.30[bcd]±3112.22	115.12[bcd]±2545.00	171.03[bc]±2862.00	<0.001
TNF-α (pg/ml)	19.38±540.91	21.76±582.48	5.66±537.47	10.46±556.43	3.36±525.00	0.066

NC: normal control, PC: patient control, LC: L-carnitine, G: genistein, LCG: L-carnitine – genistein
Values are based on mean ± standard error (SE).
[a] One Way ANOVA test between groups.
[b] the mean value has a statistically significant difference with the NC group (post hoc Scheffé statistical test with P<0.05).
[c] the mean value has a statistically significant difference with the PC group (post hoc Scheffé statistical test with P<0.05).
[d] the mean value for the LC group has a statistically significant difference with the G group (post hoc Scheffé statistical test with P<0.05).

Effects of genistein, L-carnitine, and combination on serum lipids

Lipid metabolism compromises a wide range of factors that could also be influenced by diet. The transcription factor sterol regulatory element-binding protein-2 (SREBP-2), binds the promoters of genes involved in cholesterol absorption and biosynthesis, including hydroxy methyl glutaryl-CoA (HMG-CoA) reductase and the LDL receptor (LDLr) (26). The isoflavones, particularly genistein in soy protein, stimulate SREBP-2 and by doing so, reduce serum cholesterol (27). When cellular cholesterol levels decreased in animals fed with soy protein, mature nuclear forms of SREBP-1 increased by 119% compared to rats fed with casein (168), which in itself would increase LDLr gene expression (28). The lower LDL in the G group may have been caused by increased LDLr expression, which would increase LDL absorption from blood into the liver. We have shown elsewhere that genistein, LC, and LCG reduce HMG-CoA reductase expression. Mild increment in LDLr expression has been observed following treatment with genistein, L-carnitine, and a combination of the two (22).

Consuming soy protein and its isoflavones inhibits triglyceride deposition in the liver and reduces the harmful effects of lipotoxicity (28). In rats fed soy protein diets containing genistein and casein diets, the soy-fed rats expressed greater PPARα CPT-1 mRNA than the casein-fed rats (29). Genistein, by reducing cholesterol and LDL-C in the liver, reduces the activity of enzymes involved in fatty acid synthesis, such as fatty acid synthase (FAS), resulting in decreased serum triglyceride and VLDL-C. However, we saw no reduction in triglyceride in group G, which could be due to lower PPAR-α and CPT-1 expression than in the LC and LCG groups (30, 31). While not statistically significant, the LC rats had lower serum triglyceride than the other nephrotic syndrome rats. This could be due to increased expression of PPAR-α and CPT-1, and as a result, the increase in fatty acid metabolism in the mitochondria reduces triglycerides and VLDL-C; this effect could be amplified by the effects of L-carnitine on HMG-CoA reductase and LDLr expression, reducing fatty acid synthesis in the liver.

Studies have shown that treatment with L-carnitine increases PPAR-α activation in renal tubule cells (32). Furthermore, eicosanoids are actually considered as a ligand for PPAR-δ and PPAR-α (33). It has been revealed prostaglandin production depends on carnitine, especially prostacyclin (PGI2) (34). Based on other articles in this subject, we propose that by affecting PGI2 production, LC indirectly causes PPAR-α activation. L-carnitine supplementation increases CPT-I and CPT-II transcription (35, 36). Mondola et al. (1992) showed that LC increases the binding of LDL to hepatocytes and inhibits HMG-CoA reductase activity (37). In our study, HDL was highest in the LC group, but so was LDL; consequently, although not statistically significant, serum cholesterol was also higher in this group than in the G or LCG groups. These higher levels of cholesterol and cholesterol-carrying lipoproteins may have been caused by greater HMG-CoA reductase expression than in the other nephrotic syndrome groups, as we

previously showed, which increased cholesterol and decreased LDLr expression, inhibiting potential LDL reduction by LC (22).

Evidently, genistein and L-carnitine have common pathways and purposes in lipid management; therefore, we expected their combined effect to be greater than either individually. However, in our study, simultaneous administration of genistein and L-carnitine resulted in greater concentrations of both HDL and LDL than with genistein alone, but lesser concentrations than with L-carnitine alone, lower triglycerides than with genistein alone, and lower cholesterol than with genistein or L-carnitine separately. Therefore, genistein and L-carnitine combined might cancel each other's possible harmful effects on serum lipids. This could be caused by unexpected negative effects of genistein on HDL and triglyceride and L-carnitine on LDL; possible beneficial effects of these compounds on lipid profiles in the long term needs further study. Although these differences were generally not significant, L-carnitine and genistein, alone or combined could be effective in lipid management.

Effects of genistein, L-carnitine and combination on pro-inflammatory cytokines

Generally speaking, by preventing tyrosine kinase phosphorylation, genistein inhibits its activity and consequently, prevents phosphorylation of IL-6 pro-inflammatory cytokine activators such as P38α and others. Yang et al. (2012) showed that rats receiving a soy protein diet for four weeks had significantly lower TNF-α, IL-1β, and IL-6 levels control rats in an alcoholic liver disease (ALD) model (38). In addition, L-carnitine activates glucocorticoid-response promoters and, as a result, genes encoding anti-inflammatory proteins; it also prevents production of TNF-α, IL-2, and interferon-gamma. Yuan et al.'s study (2011) of ischemic rats showed an increase in IL-10 and decreases in TNF-α, IL-1β, and IL-6 in the L-carnitine-receiving group relative to controls (39). Also, in a rat hypertension model, TNF-α and IL-6 expression decreased with L-carnitin administration (40); thus, we expected a reduction in IL-6 and TNF-α production. In our study, the genistein-receiving group had less IL-6 than the other nephrotic syndrome groups, especially LC; but TNF-α was essentially unaffected by either treatment, individually or combined. Adding genistein to L-carnitine slightly, but not significantly, reduced IL-6 in the nephrotic syndrome rats. Although TNF-α was lowest in the LCG rats, the differences between groups were not statistically significant. This may be due to the interference of genistein and L-carnitine in shared metabolic pathways. Also time-consuming production of TNF-α is another possible reason for this lack of observable effect; long-term studies are needed to examine this potential effect.

Limitations

To examine protein and creatinine in urine, weekly urine collection was required. Unfortunately, we did not have access to a metabolic cage; therefore, we were only able to collect urine three times during the six-week study. Also, measuring serum L-carnitine and genistein may have helped the final analysis with evaluation of their serum stability. Measuring IL-6 and TNF-α in kidney tissues, in addition to serum, could have also showed the effects of these two substances on these cytokines.

Conclusion

Genistein had less effect on HDL and triglyceride than LC or LCG; however, it reduced LDL more than LC or LCG and cholesterol more than LC. Rats that received genistein had less IL-6 than the other nephrotic syndrome rats. Neither L-carnitine nor genistein, alone or combined, significantly affected TNF-α levels in the nephrotic syndrome rats.

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Conflict of Interest: The authors report no conflicts of interest.

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