

**TOTAL, LDL AND VLDL CHOLESTEROL, TRIGLYCERIDES AND PHOSPHOLIPIDS
IN THE SERUM OF GUINEA PIGS WITH EXPERIMENTAL ALLERGIC
ENCEPHALOMYELITIS**

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Total, LDL and VLDL lipids — cholesterol, triglycerides and phospholipids were determined in the serum of guinea pigs with experimental allergic encephalomyelitis (EAE). EAE was induced by immunization with myelin basic protein (MBP) and galactocerebroside (GC). The results showed no differences in the total cholesterol, triglycerides and phospholipids. The content of these lipids was increased in the LDL fraction of lipoproteins (LP) and decreased in the VLDL fraction. A possible explanation is absorption of the break-down products of myelin lipid degradation by increased activity of lipolytic enzymes and rapid conversion of plasma high density lipoproteins (HDL) to very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the serum of guinea pigs in EAE. The lipoprotein lipids of myelin possible have a role in the processes of demyelination in the immune reaction in EAE.

Key words: lipids, phospholipids, cholesterol, lipoproteins, encephalomyelitis

INTRODUCTION

Besides their transport function, serum lipoproteins (LP) possess the capacity to regulate selected functions. The immunoregulatory activity of serum lipoproteins may be attributed to the protein or lipid components of the lipoprotein molecule or be the result of the action of the protein-lipid complex (Curtiss and Edgington, 1976).

A possible role for the lipid component of the LP molecule in immune reactions has also been suggested (Stuart et al., 1960; Stuart, 1962; Mertin and Hughues, 1975). They may have an effect on the fluidity of membranes (Erickson et al., 1983). The results of a study of the role of myelin lipids in experimental allergic encephalomyelitis (EAE) suggested that myelin lipids induced immunological changes and potentiated the im-

mune responses to myelin basic protein (MBP) (Hosein et al., 1984; Hosein et al., 1986; Kržalić et al., 1989).

Since EAE is an autoimmune disease accompanied by degradation of myelin proteins and lipids, the aim of our work was to determine the total and lipoprotein content of cholesterol, triglycerides and phospholipids in the serum of guinea pigs with EAE to determine whether or not a disturbance of lipid metabolism in the central nervous system (CNS) can affect the content of lipoprotein lipid components or turnover of the blood plasma.

MATERIALS AND METHODS

Guinea pigs of the *Cavia porcellus* species, line 2 from our own breeding animals established 20 years ago, of both sexes, weighing 180—200 g, were used at an age of about 3—4 weeks.

Experimental allergic encephalomyelitis was induced by immunization with myelin basic protein (isolated from bovine spinal cord) and galactocerebroside (from bovine brain; type II, Sigma) emulsified in complete Freund's adjuvant (CFA) (Difco Bacto adjuvant containing *Mycobacterium tuberculosis*). The ratio of the aqueous-to-oily phase was 1:2 and the total volume injected was 0.25 ml divided between the two hind foot pads. Each dose contained 75 µg MBP and 180 µg GC. The second group (controls) were unimmunized animals. Each animal was monitored for the appearance of clinical signs of disease and body weight was measured. Blood for analysis was withdrawn from the heart during the period of over clinical signs of the disease. We determined the total and LDL cholesterol, triglycerides and phospholipids.

Quantitative determination of cholesterol concentration in the serum was made using Gilford diagnostic cholesterol reagent (Color) which measures cholesterol enzymatically. The intensity of the red color of quinonemine produced is directly proportional to the amount of total cholesterol in the serum sample which is measured spectrophotometrically with a Gilford System instrument.

Determination of triglycerides was made by Gilford Diagnostic Triglyceride Reagent (Color) which involves enzymatic hydrolysis followed by measurement of the glycerol released by coupled enzymatic reactions and the formation of Formazan. The intensity of the red color of formazan, as measured spectrophotometrically, is proportional to the concentration of glycerol in the reaction mixture and hence to the level of triglycerides in the sample.

Phospholipids were measured by Bio Mérieux phospholipid enzymatic reagent.

LDL lipoproteins were isolated by ultracentrifugation with the Airfuge.

The content of VLDL was determined as the difference between the total and LDL content of lipids because the serum of guinea pigs contains only traces or no HDL.

RESULTS

The concentrations of total cholesterol, triglycerides and phospholipids in the serum of control and treated guinea pigs are presented in Table 1. The values for the total levels of these lipids showed no differ-

Table 1. The content of total cholesterol, triglycerides and phospholipids in the serum of non-immunized guinea pigs and after immunization with MBP and GC
Values are mean \pm SD

	Number of guinea pigs	Cholesterol	Triglycerides	Phospholipids
Non-immunized	14	2.10 ± 0.88	1.16 ± 0.49	0.95 ± 0.23
Immunized with MBP and GC	9	2.13 ± 0.49	1.18 ± 0.31	1.00 ± 0.24
P		n.s.	n.s.	n.s.

Values are expressed in mmol/L
n.s. not significant.

ces. The mean values of the results obtained for cholesterol, triglycerides and phospholipids in the LDL fraction in the serum of guinea pigs with EAE and in the serum of unimmunized guinea pigs (controls) are given in Table 2. The content of all LDL lipids were increased in animals with EAE

Table 2. The content of LDL cholesterol, triglycerides and phospholipids in the serum of non-immunized guinea pigs and after immunization with MBP and GC
Values are mean \pm SD

	Number of guinea pigs	Cholesterol	Triglycerides	Phospholipids
Non-immunized	14	1.06 ± 0.14	0.05 ± 0.01	0.08 ± 0.03
Immunized with MBP and GC	9	1.86 ± 0.42	0.53 ± 0.27	0.86 ± 0.23
P		0.01	0.01	0.001

Values are expressed in mmol/L

with comparison with control values. Table 3 presents the mean values for cholesterol, triglycerides and phospholipids in the VLDL fraction in the serum of guinea pigs. The content of all lipids were decreased in the animals with EAE compared to the serum of controls.

Table 3. The content of VLDL cholesterol, triglycerides and phospholipids in the serum of non-immunized guinea pigs and after immunization with MBP and GC
Values are mean \pm SD

	Number of guinea pigs	Cholesterol	Triglycerides	Phospholipids
Non-immunized	14	0.97 ± 0.69	1.11 ± 0.49	0.87 ± 0.21
Immunized with MBP and GC	9	0.26 ± 0.12	0.63 ± 0.24	0.13 ± 0.03
P		0.01	0.01	0.001

Values are expressed in mmol/L

Statistical evaluation of the results was made using Student's *t* test.

It is possible that the findings of increased concentrations of LDL lipids and decreased serum VLDL lipid concentrations could be explained by the increased lipid catabolism in the CNS and by increased conversion of VLDL to LDL in EAE.

DISCUSSION

Serum lipoproteins have both transport and bioregulatory roles. It is considered that serum lipoproteins act as antigens the reactivity of which depends on the presence of apolipoproteins. It is known that subspecies of LDL have an immunoregulatory role such as the immunosuppressive action of the rosette inhibitory factor (RIF) and low density lipoprotein inhibitor (LDL-in) (Chisary and Edgington 1975; Curtis and Edgington 1976; Curtis et al., 1977).

However, lipids of lipoproteins are also included among the modulators of the immune functions. Simple lipid complexes such as cholesterol oleate and ethyl stearate have a role in depression of the functional capacity of the reticuloendothelial system (Stuart et al., 1960; Stuart 1962). Several workers have shown that administration of the essential fatty acid (EFA) inhibits the clinicohistopathological signs of EAE (Meade et al., 1978). It was reported that lipoproteins could have an important modulating effect on the development of clinical symptoms in EAE (Shore et al., 1987). Lipids of lipoproteins can have immune modulatory influence on the CNS reactive T-cells. They may have an effect on the fluidity and other membrane properties by exchange with cell membranes. It was shown that selected myelin lipids augment the effect of MBP in guinea pigs to produce an autoimmune encephalomyelitis. Lesions were characterized by demyelination similar to that obtained with whole white matter. The mechanism by which lipids enhance autoimmune demyelination in the CNS is far from clear. It seems that their effect must be mediated by modulating the immune response (Erickson et al., 1983; Hosein et al., 1984; Morre et al., 1984; Kržalić et al., 1989).

It is known that the process of demyelination is accompanied by degradation of myelin lipids and proteins due to increased activity of lipolytic and proteolytic enzymes of lysosomal origin (Einstein et al. 1972; Eto and Suzuki, 1973a, b; Smith et al., 1974; Hirsch, 1976). Alterations in lipid metabolism, such as a decrease in phospholipids, changes in fatty acids and an increase in cholesterol esters have already reported (Cumings, 1955; Bernson 1955; Davison and Cuzner, 1977; Kržalić et al., 1982; Wender et al., 1984).

In our experiments the content of total lipids of lipoproteins showed no differences. Several other authors have found no differences in the total amount of cholesterol and phospholipids in the serum of patients with multiple sclerosis (Gerst et al., 1957; Freeman and Siegel, 1957; Plum and Fog, 1959; Cumings et al., 1965).

Our results showed higher contents of cholesterol, triglyceides and phospholipids in the lipoproteins of the LDL fraction in EAE in comparison

to the levels in the serum of normal guinea pigs (controls). The high content of lipid components in LDL may merely reflect an absorption of the break-down products of myelin degradation. The increased vascular permeability of the CNS in EAE animals might facilitate entry of degraded myelin lipids into the blood. Rapid conversion of HDL to VLDL and LDL can explain also the greater content of lipoprotein lipids in the LDL fraction. It is known that the guinea pig is an animal in which LDL represents the major plasma lipoprotein fraction. The concentration of HDL is very low. The serum of guinea pigs contains traces or no HDL. This low concentration of HDL can be explained by rapid transference of HDL to VLDL and LDL. (Puppione et al., 1971; Sardet et al., 1972; Kržalić and Čupić, 1981; Böhmer et al., 1972).

Having in mind the possible immunoregulatory role of lipoprotein lipids it may be speculated that the alterations of lipoprotein lipids in EAE may interfere with neuroimmunoregulatory mechanisms responsible for neuroimmunological disease such as EAE. Our results may indicate an important role of lipid components of lipoprotein in the immune mechanisms which are connected to the demyelinating process in EAE.

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**UKUPNI, LDL I VLDL HOLESTEROL, TRIGLICERIDI I FOSFOLIPIDI U SERUMU
ZAMORČIĆA SA EKSPERIMENTALNIM ALERGIJSKIM ENCEFALOMIJELITISOM**

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SADRŽAJ

Određivani su ukupni, LDL i VLDL holesterol, trigliceridi i fosfolipidi u serumu zamorčića sa eksperimentalnim alergijskim encefalomijelitisom.

Eksperimentalni alergijski encefalomijelitis je izazivan imunizacijom sa mijelin baznim proteinom i galaktocerebrozidima. Lipoproteini male gustine (LDL) izolovani su ultracentrifugovanjem. Holesterol, trigliceridi i fosfolipidi su određivani enzimskim metodama.

Rezultati određivanja ukupnih lipida-holesterol, triglicerida i fosfolipida nisu pokazali razlike u odnosu na sadržaj istih lipida u serumu kontrolnih životinja. Sadržaj holesterola triglicerida i fosfolipida bio je značajno povišen u LDL lipoproteinima i snižen u VLDL lipoproteinima.

Moguće objašnjenje moglo bi biti absorpcija razgrađenih lipida mijelina usled povećane aktivnosti lipopolitičnih enzima kao i povećana konverzija VLDL-a u LDL.