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THE ALTERATIONS IN LIPID AND PROTEINS IN ERYTHROCYTE MEMBRANES OF ENDOTOXIN/D-GALACTOSAMINE (LPS/GaIN)-ADMINISTERED RATS

ENDOTOKSİN/D-GALAKTOZAMİN (LPS/GaIN) VERİLEN SIÇANLARIN ERİTROSİT ZARLARINDA LİPİT VE PROTEİN DEĞİŞİKLİKLERİ

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ABSTRACT

Objective: To clarify the effects of endotoxin/D-galactosamine (LPS/GaIN) on erythrocyte membranes (ghost) of rats.

Materials and methods: After 18 hours of intraperitoneal coadministration of 200 mg/kg D-galactosamine (GaIN) and 200 µg/kg lipopolysaccharide (LPS), male Wistar rats were decapitated and ghost was prepared by ultracentrifugation. Malondialdehyde (MDA), sulfhydryl (-SH) and protein carbonyl (PC) contents and Na^+,K^+-ATPase activity were measured in ghost, spectrophotometrically. MDA and PC were measured in also plasma of rats. Furthermore, sodium dodesyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique was used for the determination of the modifications in cytoskeletal ghost proteins .

Results: LPS/ GaIN administration resulted in hepatitis in male Wistar rats as evaluated by assessments of serum alanine transaminase (ALT) and aspartate transaminase (AST) activities. While plasma MDA levels were determined to increase, ghost MDA and PC contents were observed unchanged. However, -SH content as well as Na^+,K^+-ATPase activity of ghost were found decreased. As shown by the electrophoretic gel patterns, significant reduction in the intensities of some cytoskelatal membrane proteins, especially in α–spectrin and β–spectrin, was observed in erythroctes of LPS/ GaIN-administered rats.

Conclusion: From these findings, we concluded that reduced Na^+,K^+-ATPase activity may be associated with the diminished –SH content in ghost of LPS/GaIN-administered rats. Furthermore, alterations seen in cytoskeletal proteins of ghost may participate to LPS/GaIN-induced hepatotoxicity by causing the deformation of cell membrane structure and abnormality of membrane functions, ultimately.

Key words: Lipid/protein oxidation, cytoskeletal proteins, LPS/GaIN, ghost, rat.

ÖZET

Amaç: Endotoksin/D-galaktozamin (LPS/GaIN)'in sıçan eritrosit zarı üzerine etkilerini aydınlatmak.

Gereç ve yöntem: Erkek Wistar sıçanlar, intraperitoneal 200 mg/kg D-galaktozamin (GaIN) ve 200 µg/ kg lipopolisakkarit (LPS) uygulamasından 18 saat sonra kesilerek ultrasantrifüj yöntemi ile eritrosit zarı (ghost) elde edildi. Sıçanların eritrosit zarlarında, malondialdehit (MDA), sülfidril (-SH) ve protein karbonil (PC) içeriği ve Na^+,K^+-ATPaz aktivitesi spektrofotometrik olarak ölçüldü. MDA ve PC sıçanların plazmalarında da tayin edildi. Bunun yanısıra, eritrosit zar iskeletinin proteinlerindeki değişiklikler sodyum dodesil sülfat gel elektroforezi (SDS-PAGE) tekniği ile saptandı.

Bulgular: LPS/GaIN uygulamasının, erkek Wistar sıçanlarda, serum alanin transaminaz (ALT) ve aspartat transaminaz (AST) ölçümleri sonucu, hepatit oluşturduğu belirlendi. Sıçanların plazma MDA düzeylerinde artış bulunurken, eritrosit zarlarında MDA ve PC düzeylerinin değişmediği gözlendi. Bununla birlikte sıçan eritrosit zarlarının –SH içeriği ve Na^+,K^+-ATPaz aktivitesinde azalma olduğu saptandı. Eritrosit zar iskeleti proteinlerinin SDS-PAGE örneklerinde ise, proteinlerin bir kısmında, özellikle de, α-spektrin ve β-spektrin band yoğunluklarında azalmalar olduğu gözlendi.

Sonuç: Bulgularımız, Na^+,K^+-ATPaz aktivitesindeki azalmadan zar –SH içeriğindeki azalmanın sorumlu olabileceğini düşündürmektedir. Bunun yanısıra, eritrosit zar iskeletinin proteinlerinde görülen değişiklikler, hücre zar yapısında ve dolayısıyla fonksiyonlarında anormalliklere neden olarak LPS/GaIN hepatotoksisitesine katkıda bulunabilir.

Anahtar kelimeler: Lipit/protein oksidasyonu, zar iskelet proteinleri, LPS/GaIN, eritrosit zarı, sıçan.

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INTRODUCTION

Endotoxin from gram negative bacteria (lipopolysaccharide:LPS) induces septic shock and finally wide variety of several organ disorders in human and animals (9,20). On the other hand, D-galactosamine (GalN) highly sensitizes animals to develop lethal liver injury mimicking fulminant hepatitis when given together with a sublethal dose of lipopolysaccharide (LPS) (6,23,25). For this reason, GaIN sensitization to endotoxin is a useful model for studying in the mechanisms of hepatotoxicity of LPS in the means of minimizing the other organ injuries.

Although the pathogenesis of LPS/GaIN-induced tissue damage is still unclear, reactive oxygen species formed during endotoxic shock is thought to be involved in tissue damage by stimulating lipid peroxidation (5,21,25).

Erythrocyte membrane cytoskeleton is a filamentous network of proteins which is essential to keep the cell shape and integrity. The major cytoskeletal contituents are spectrin, actin, band 4.1 and ankyrin (7). Several studies have demostrated that any defect in major erythrocyte membrane proteins caused by oxidative stress results in abnormal cell shape, altered deformability (1,7,22).

The purpose of the peresent study was to investigate the participation of the changes in erythrocytes to LPS/GaIN hepatotoxicity in rats *in vivo*, since inadequate information has been found in the literature. For this reason, we assessed the lipid and protein oxidation as well as structural modifications of proteins in erythrocyte membranes (ghost) of rats intoxicated by LPS/GaIN.

MATERIAL and METHODS

In this study male Wistar rats weighing 250-300 g were used. Animals were obtained from the Experimental Medical Research Institute of Istanbul University. Rats were fed a standard laboratory chow and had free access to water. They were kept in wire-bottomed stainless stell cages. Rats were divided into two groups. LPS/GaIN group (n=12) were injected intraperitoneally at the dose of 200 mg/kg GaIN with 200 µg/kg E. coli LPS in saline. Control group (n=8) were given only saline. The animals were killed by decapitation 18 hours after the injection, blood was withdrawn by heart puncture and plasma was separated. Liver injury was determined by measurements of plasma ALT and AST activities with BioSystem Diagnostic Kit.

Measurements in plasma:

Plasma MDA levels, one of the end products of lipid peroxidation, were measured by the method described by Yagi et al. (26). Protein carbonyl (PC) content of plasma was measured as an index of protein oxidation by the procedure of Levine et al. (14) .

Measurements in ghost:

For ghost preparation, washed and packed erytrocytes were hemolyzed by 10mM Tris/0.1mM EDTA (pH 7.4) and centrifuged at 20000 g for 30 minutes. Pellets were washed three times and obtained ghosts were resuspended in 2-3 ml of hemolysis tampon (16). Ghost MDA levels was determined by the method of Bidlack and Tappel (3). PC content of ghost was assayed by the same method of that described above (14). The sulfhydryl (-SH) content of ghost proteins were measured by Ellman reagent [5,5'ditiobis-(2-nitrobenzoic acid)] (11). Na^+,K^+-ATPase activity of ghost was assayed according to Kovachich and Misra's method (12). For the determination of the modifications in ghost proteins, SDS-PAGE was carried out by the method of Laemmli (13). The bands of ghost proteins were confirmed by using molecular weight standart ranging from 29 kDa-205 kDa.

Protein content of ghost was determined by the method of Lowry et al. (15).

Statistics: Data were expressed as the means ± SD and the statistical analyses were performed by using Student t-test. Correlation coefficients were determined by the Pearson method.

Table 1. MDA levels, -SH and PC contents and Na^+, K^+-ATPase activity of ghosts; MDA and PC content of plasma of GaIN/ LPS- administered rats (Mean ± SD)

PARAMETER	CONTROL (N=8)	LPS/GaIN (N=12)
GHOST		
MDA (nmol/mg protein)	1.34 ± 0.21	1.21 ± 0.26 ns
-SH (nmol/mg protein)	160.00 ± 18.31	130.00 ± 24.12 *
PC (nmol/ mg protein)	3.18 ± 0.89	3.12 ± 1.23 ns
Na^+,K^+-ATPase (nmol/mg protein/ hour)	413.2 ± 45.84	280.40 ± 66.68 **
PLASMA		
AST (U/l)	39.6 ± 33.2	1115.00± 693.41**
ALT (U/l)	42.50 ± 12.81	1114.30 ± 451.10 **
MDA (nmol/ml plasma)	2.23 ± 0.34	3.51 ± 0.32 **
PC (nmol/ mg protein)	0.81 ± 0.09	0.79 ± 0.06 ns

*p< 0.01; ** p< 0.001 compared to controls; ns: nonsignificant

RESULTS

In this study, liver damage was confirmed by assessements of plasma ALT (1150.0 U/l) and AST (1114.3 U/l) activities 18 hr after the LPS/GaIN injection. In these conditions:

Lipid peroxidation assayed as MDA levels was found significantly to be increased (57.4%) in plasma but not to be changed in ghost. PC content in both plasma and ghost was observed to be unchanged. Sulfhydryl content and Na^+,K^+-ATPase activity were found significantly decreased (18.7% and 32%, respectively) in ghost (Table 1). Furthermore, a significant positive correlation was found between –SH content and Na^+,K^+-ATPase activity ($r=0.668$; $p<0.05$) (Figure 1) .

As illustrated in electrophoretic gel patterns, reduction in the intensities of some cytoskelatal membrane proteins was observed in 8 (Numbers: 2, 5-9, 10,12) of 12 experimental animals. In detail, a general decrease was seen in the intensities of whole protein bands of number 2,5,9. The bands of both α–spectrin and β-spectrin were observed to reduce in number 2,5,6,9,10. Alpha–spectrin was observed to be reduced in especially number 7,8,12 (Figures 2 A-C).

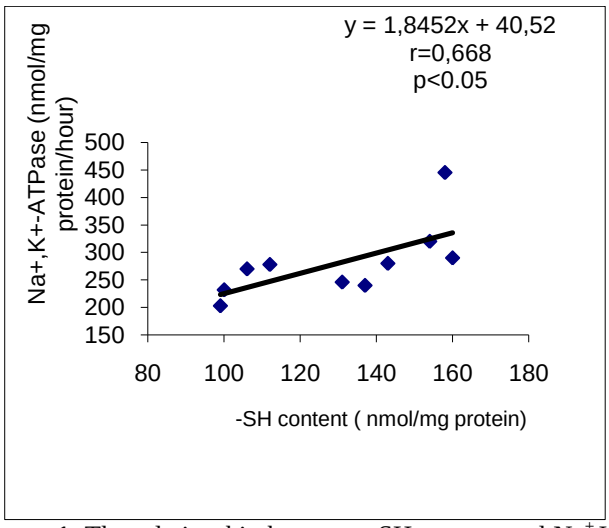
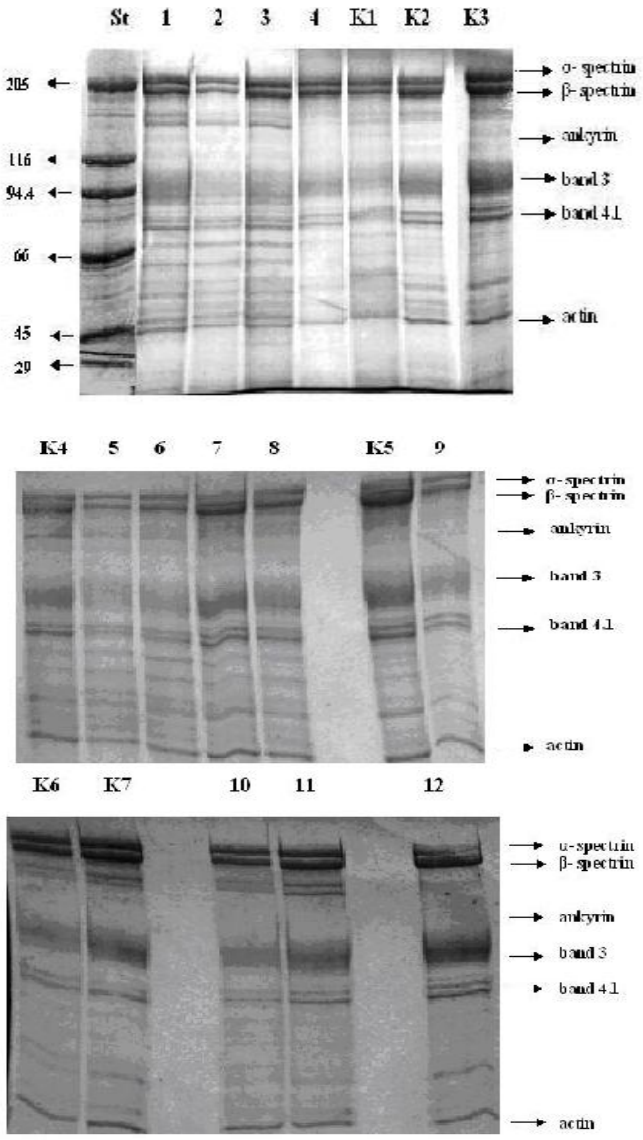


Figure 1. The relationship between –SH content and Na^{+},K^{+}-ATPase activity (regression line and correlation coefficient)



Figures 2A-C: SDS-PAGE of ghost proteins from LPS/GaIN- administered (numbers 1-12) and control rats (C1-C7). 40 µg of membrane proteins were applied to 7.5 % acrylamide gels and subjected to electrophoresis according to Laemmli's method

DISCUSSION

Several studies have shown that endotoxin administration results in diverse effects such as hypotension, hypoglycemia, activation of immune system and intravascular coagulation (10). Although the mechanism underlying these effects is not clearly known it may be related to the LPS-induced alterations of cell membrane structure and functions.
Altered erythrocyte membrane properties such as decreased membrane fluidity, decreased red cell deformability, increased osmotic fragility and the conformational changes in proteins after LPS treatment have been reported by several in vitro studies (8,18).

On the other hand, lipid peroxidation has been suggested to be one of the underlying destructive factor in LPS-induced tissue damage (2,4). Indeed, we demonstrated liver and plasma lipid peroxidation to be enhanced in both LPS (17) and LPS/GaIN administered rats (not published) in previous studies performed in our laboratory. In this study, plasma MDA levels was observed to increase while ghost MDA levels remained unchanged. This increase seen in plasma is likely to be originated from the liver damage. Lipid peroxidation is also known to decrease fludity by forming cross-links with membrane components and/or by comsuming polyunsaturated fatty acids (PUFAs) in the membranes (19). Unchanged levels of ghost MDA may be due to the leakage of the lipid peroxide products to the plasma as a consequence of destruction of the membrane and/or consumption of PUFAs.
Beside lipid oxidation, -SH content of ghost proteins was observed significantly decreased. Na^{+},K^{+}-ATPase activity, a sulfhydryl protein which takes play in the ion tranport in erythrocyte membrane, was also found to be diminished significantly in LPS/GaIN intoxicated rats**.** Furthermore, a significant positive correlation (r=0.668; p>0.05) was detected between these two parameters. Hence, the reduced Na^{+},K^{+}-ATPase activity may be associated with the diminished -SH content in LPS/GaIN-intoxicated rats.
Regarding the structural changes in membrane proteins, we observed significant reductions in the intensities of some cytoskelatal membrane proteins of 8 of 12 LPS/GaIN intoxicated rats. These changes were especially in the bands of α-spectrin and β-spectrin of those numbered 2, 5-9,10,12. As known, cytoskeleton proteins of ghost, especially spectrin seem to be important in the maintenance of the mechanical stability and the deformability of the erythrocytes (1,7,24,26).

Therefore, alterations seen in these proteins, which are connected the vital functions of the cell membranes, may lead the loss of erythrocyte membrane integrity.
As conclusion, oxidative and /or structural changes seen in membrane lipids and proteins of erythrocytes may participate to hepatic damage by causing the deformation of cell membrane structure and abnormality of membrane functions, ultimately, in LPS/GaIN intoxication.

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