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Research Article

The Improving Effects of Simvastatin on Erythrocyte Membrane Proteins in Endotoxemic Rats

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Abstract

The recent studies in patients with inflammation reported that simvastatin inhibited the vascular hyporeactivity and reduced the risk of the sepsis. The membrane proteins spectrin, ankyrin, actin, band-3, protein 4.1, and glycophorin have important roles in maintaining the structural and functional of the red blood cells (RBC). In the present study, we aimed to investigate the effects of simvastatin on the membrane proteins of rats treated with lipopolysaccharide (LPS). Rats were divided into four groups as the control, Endotoxemic (20 mg/kg, i.p. LPS), Simvastatin (20 mg/kg, p.o.), and Simvastatin+Endotoxemic group. Erythrocyte membrane proteins were detected using the SDS-PAGE. In the endotoxemic group, while the levels of proteins band-3, Protein 4.1, ankyrin were to decreased, spectrins, Actin, Glycophorins were extremely lower compared to other groups. In addition, simvastatin administration in endotoxemic animals was found to improve the distrupted membrane proteins which may suggest a compensatory defense mechanism supporting the RBC function.

INTRODUCTION

Sepsis causes the deterioration of the microcirculation homeostasis, and physiological changes in the blood vessels, which leads to the death of the organism as a result of organ failure. The imbalances between the blood circulation, and perfusion together with increased free radicals led to inadequate oxygenation of the tissues by causing oxidative damage in erythrocytes [1].

The red cell hemorheology is known to have a highly clinical significance in various disorders including diabetes mellitus, sepsis, and atherosclerosis [2-6]. The red blood cell (RBC) membrane consists of a complex of 40% lipid bilayer, 52% proteins, and 8% carbohydrates. The RBC membrane proteins are classified integral and peripheral proteins as depending on the positioning of lipid bilayer. The integral membrane proteins are composed of Protein band 3, glycophorins (A,B, and C), glycoproteins. The second group is peripheral membran proteins are linked to inner membrane skeleton, consisting of spectrin (α and ß spectrin), protein 4.1, ankyrin, and actin [7]. The interactions of both the plasma membrane, and protein skeletal structure are critical in maintaining the shape, and elasticity of the RBC membrane.

In in vivo models, systemic lipopolysaccharide (LPS) injection is commonly used in the acute systemic inflammation, and endotoxemic shock studies. The infection related injury in the erythrocyte membrane structure is known to cause damage in the microcirculation of the patients diagnosed with sepsis [1]. Implementation of statins decreased the inflammatory response of cytokines, and mediators both in the experimental, and clinical sepsis [8].

Researchers in some studies used statins which prevented the vascular hyporeactivity, and decreased the progression of serious sepsis in patients with acute systemic inflammation [9,10].

Simvastatin is a member of statin family, which affects a rate-limiting enzyme on 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis, and also has pleitropic effects in addition to cholesterol lowering effect [11,12]. Simvastatin was demonstrated to have anti oxidant, anti inflammatory, and pro apoptotic effects [13-15].

However, no study was found that investigated the effects of inhibitors of HMG-CoA reductase on disrupted erythrocyte membrane proteins in sepsis. In the light of these information, we aimed to research the effects of simvastatin on membrane proteins in lipopolysaccharide (LPS) applied Wistar Albino rats.

MATERIAL AND METHODS

Wistar albino adult rats were divided into 4 groups including the control, endotoxemic, simvastatin, and simvastatin plus endotoxemic groups after the approval of the ethical commitee. 0.9% NaCl (i.p) (n=10) was administered to the control group. LPS (*E.coli*: O127:B8; 20 mg/kg, i.p.) was given into to create endotoxemic group, and 4 hours later, animals were taken into the experiment (n=10). In the Simvastatin group; simvastatin was given (20 mg/kg, p.o.) for 5 days, and animals were decapitated (n=10) at the end of the 5th days. Simvastatin was given for 5 days in the Simvastatin plus Endotoxemic group. In the 5th day, LPS was given, and 4 hours later, animals were taken into the experiment (n=10). At the end of the experiment, blood samples were taken into tubes with Ethylenediaminetetraacetic acid (EDTA) and the erythrocytes were separated.

Preparation of RBC Ghost

Red blood cell membrane ghosts were prepared in accordance with the method of Dodge et al. [16]. The erythrocytes were isolated by centrifugation for 20 min at 2000 rpm. After the plasma, and buffy coat were removed by aspiration; RBC were washed 3 times in 5 *M* sodium phosphate / 0.15 *M* sodium chloride, and centrifugation was repeated. Aspiration was performed carefully after each wash, sacrificing packed cells at the interphase to ensure removal of the buffy coat. Packed cells were then lysed in cold 5 m*M* sodium phosphate. Further centrifugation was performed at 16000 rpm at 4°C (Heraeus Megafug 1.0R, Hanau, Germany). The resultant deep red supernatant lysates were discarded, leaving a red pellet of packed ghost over a creamy white layer of leukocyte debris. The ghost was carefully separated from this layer by tilting, and rotating the tube to free the ghost. Samples were then stored at –20°C until they were used in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE of RBC Membrane

We performed SDS-PAGE by a mini-gel system (Bio-Metra, Minigel- Twin, Göttingen, Germany) using a 10% gel, and a discontinuous buffer system. Samples were diluted with an equal volume sample buffer (125 m*M* Tris-HCl buffer, pH 6.8, containing 2% SDS, 0.05% 2-mercaptoethanol, and bromophenol blue). The gels were stained with Coomassie brilliant blue R-250 in methanol/acetic acid. The gels were destained until the protein bands were clear. A mixture of molecular mass standards was also run. Bovine serum albumin (66.2 kD), ovalbumin (45 kD), carbonic anhydrase (31 kD), and lysozyme (14.4 kD) were used as standards (Sigma MW-SDS-70, Mo., USA).

After gel electrophoresis, staining was applied 30 min at 4 ºC, removal of the dye at the same time, and temperature was done, after gels were incubated 10% acetic acid solution in 30 min, and then 15 min in 1% glycerol solution. Then, the gels were evaluated after drying by applying vacuum, and heated in the Bio-Rad Model gel dryer between Whatman paper. The intensities of each band were quantified by comparing with the densities of molecular mass standard using Image J Program for each applied wells.

Statistical analysis

The Statistical package for the Social Sciences (SPPS) 21.0 statistical software (SPSS, Inc., Chicago, IL, U.S.A.) was used in the statistical analysis. The mean values were compared using Tukey's multiple comparison test followed by one-way ANOVA. The data were presented as the mean ± standard deviation (SD). $P<0.05$ was considered as statistically significant.

RESULTS

In the endotoxemic group, ankyrin (MW: 210 kDa), protein 4.1 (MW: 66 kDa), and Band 3 (MW: 100 kDa) were decreased, however, the intensities of actin (band 5) (MW: 43 kDa), and glycophorin A,B,C (MW: 30 kDa), α (MW: 280 kDa), and ß spectrin (MW: 246 kDa) were extremely lower in the endotoxemic group compared to controls. Similar protein band compositions were detected between the control and simvastatin groups. The examination of SDS-PAGE electrophoresis assay showed to improve band compositions decreased membrane proteins band 3, Protein 4.1, ankyrin and also extremely lower α spectrin, β spectrin, Actin, Glycophorin A,B,C proteins in the simvastatin treated endotoxemic group compared to endotoxemic group (Figure 1,2).

DISCUSSION

Erythrocytes can easily pass through capillary vessels due to erythrocyte membrane flexibility, which is an important factor that determines the flow characteristics of blood. Sepsis is a

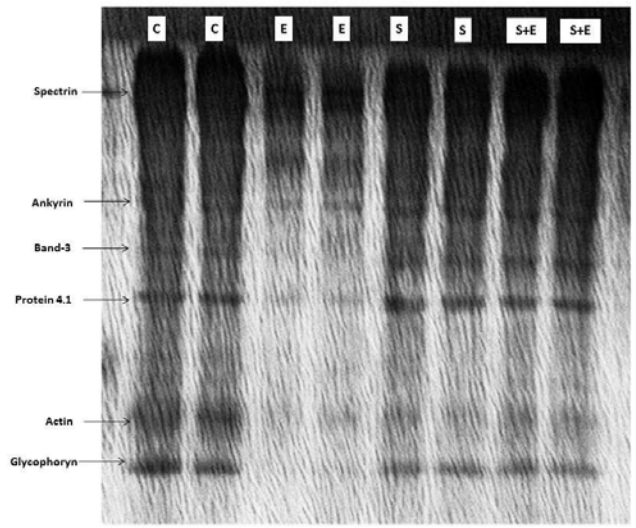

Figure 1 Levels of Erytrocyte membrane protein in groups. Endotoxemia group versus other experimental groups; *: *P<0.01*.

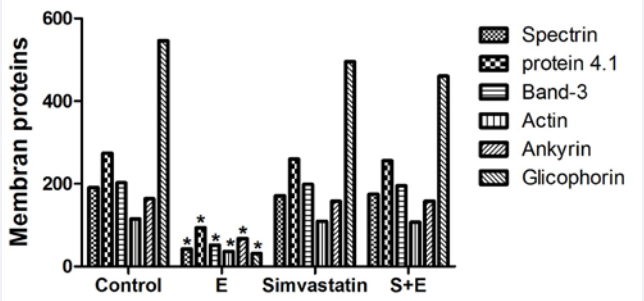

Figure 2 The electrophoresis gel appearance of Erythrocyte membrane proteins related to each experimental groups. Control (C), Endotoxemia (E), Simvastatin (S), Simvastatin+ Endotoxemia (S+E).

pathological condition that is involved in the formation of the biochemical, and physiological changes in blood vessels, causing disruption of microcirculation homeostasis [17]. Increased free radicals result with oxidative damage in erythrocytes, causing the tissues inadequate oxygenation in sepsis due to insufficient balance between blood flow, and perfusion, and develops organ failure. It is known that membrane proteins, which have an important role in preserving the structural, and functional integrity of erythrocytes, are peroxidized with increasing free radicals in sepsis, and the deformability properties of erythrocytes are impaired [3,17].

Experimental and clinical studies showed that statin administration reduced the inflammatory response of cytokines [18]. Some studies showed that statins prevented vascular hyporeactivity, and reduced severe sepsis in patients with acute systemic inflammation [4,11]. The damage to erythrocyte membranes caused by infection is known to cause disruption of microcirculation in patients with sepsis [15]. However, there are no studies reported the effect of HMG-CoA reductase inhibitors on erythrocyte membrane proteins that have been impaired in sepsis.

Membrane proteins of α and β spectrin, ankyrin, actin (band 5) and band 3, protein 4.1 and glycophorin A,B,C have important roles in maintaining the structural, and functional integrity, and in the preservation of the shape of red blood cells. Tse and Lux demonstrated that the decreaments in both of function and structure in RBC membrane proteins were observed in clinical disorders [17].

In the endotoxemic group, while the levels of proteins band 3, Protein 4.1, ankyrin were to decreased, α spectrin, β spectrin, Actin, Glycophorin A,B,C were extremely lower compared to other experimental groups. We also observed that the administration of simvastatin in LPS treated animals improved the distrupted red cell membrane proteins in the study [17,19,20].

Our findings showed that the simvastatin may be repairing the defensive mechanism to support the erythrocyte function in simvastatin treated endotoxemic group in case of the damage in the regulation of the erythtrocyte membrane proteins. The results of our study has clinical importance appearing of complications and in terms of treatment of disease in septic patients.

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