Effects of Added Dietary Taurine on Erythrocyte Lipids and Oxidative Stress in Rabbits Fed a High Cholesterol Diet

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Lipid peroxidation leads to damage of polyunsaturated fatty acids of membrane phospholipids. The contribution of oxidative stress to hypercholesterolemia-induced hemolytic anemia and the effects of addition of taurine on erythrocyte lipid composition, oxidative stress, and hematological data were studied in rabbits fed on a high cholesterol (HC) diet (1%, w/w) for 2 months. The effects of taurine on erythrocyte hemolysis and H2O2-induced lipid peroxidation were investigated in normal rabbit erythrocytes in vitro. The HC diet resulted in increases in plasma lipids and lipid peroxide levels as well as increases in cholesterol levels and the cholesterol:phospholipid ratio in the erythrocytes. This diet caused a hemolytic anemia, but lipid peroxide levels remained unchanged in the erythrocytes of the rabbits. Taurine (2.5%, w/w) added to the food has an ameliorating effect on plasma lipids and lipid peroxide levels in rabbits fed on a HC diet. This treatment also caused decreases in elevated erythrocyte cholesterol levels and cholesterol:phospholipid ratio due to the HC diet, but it did not prevent the hemolytic anemia and did not change erythrocyte lipid peroxide levels. In addition, in an in vitro study, taurine did not protect erythrocytes against H2O2-induced hemolysis or lipid peroxidation. These results show that the HC diet causes hemolytic anemia without any changes in erythrocyte lipid peroxidation, and taurine treatment was not effective against hemolytic anemia caused by the HC diet.

Key words: taurine; lipid peroxidation; erythrocyte; high cholesterol diet; rabbit

There is a continuous exchange of lipids between plasma lipoproteins and erythrocyte membrane. Severe hypercholesterolemia is known to lead to alterations in the erythrocyte lipid composition.1 It has been reported that cholesterol-enriched erythrocytes have several abnormal properties such as decreased membrane fluidity, increased hemolysis, decreased permeability to non-electrolytes and electrolytes, and decreased activities of some membrane-bound enzymes.1,2) As it is known, free radicals and oxidative stress have deleterious effects on membrane function and stability.3) Therefore, one of the mechanisms responsible for the structural changes of erythrocytes in hypercholesterolemia has been reported to be oxidative stres.4–9) However, some investigators have suggested that cholesterol may play an antioxidant role in biological membranes9) and so may protect the erythrocytes from oxidative stress.9,10

Rabbits are susceptible to the development of atherosclerosis, and a high cholesterol diet (HC) in rabbits resulted in alterations in prooxidant-antioxidant status in several tissues as well as typical atherosclerotic changes in the aorta.11–15) Erythrocyte lipid peroxidation also increased4) and the antioxidant system was affected by an HC diet in rabbits.14) This diet has been found to cause hemolytic anemia in rabbits.4,16) An increase in oxidative stress has also been suggested as a cause of the hemolytic anemia which was seen following an HC diet.4

Taurine (2-aminoethane sulfonic acid), a non-protein amino acid, is present in most mammalian tissues and cells and has a variety of physiological and biochemical functions.17) Some investigators have suggested that taurine has hypolipidemic, antiatherosclerotic18,19) and hepatoprotective effects.20,21) Although the mechanism of these effects of taurine is not clear, these effects may be related to its antioxidant effect.17) We have recently reported that taurine ameliorates cholesterol accumulation and cholesterol-induced oxidative stress in the plasma, liver, and aorta of rabbits on fed an HC diet, and that this effect may be related to its antioxidant effect besides the reducing effect on serum lipids.20) In addition, some investigators have reported that taurine has an ability to suppress erythrocyte hemolysis caused by oxidative damage in in vitro conditions.22–25) However, there is no adequate report about the effects of taurine on erythrocyte hemolysis.
in *in vivo* conditions. Therefore, the first aim of this study was to estimate the contribution of oxidative stress to hypercholesterolemia-induced hemolytic anemia in rabbits. The second aim of this study was to examine the effects addition of taurine on lipid composition, oxidative stress, and hematological data in rabbits fed on an HC diet.

**In vivo study.** Male New Zealand white rabbits from Eczacıbaşı Pharmaceutical Company, İstanbul, Turkey weighing 2.0–2.5 kg, were used for all experiments. The animals were kept on a basal diet (control), a high cholesterol diet (HC) of cholesterol 10 g/kg, an HC diet with added taurine at 25 g/kg (HCHT) for 2 months. The commercially basal diet contained 11% moisture, 10% crude ash, 15% protein, 3.5% crude fat, 47% carbohydrate, and 7.5% cellulose. Cholesterol and taurine were supplied by Sigma. The food was stored at 4°C. The animals were allowed free access to food and water. The weight gain was not significantly different among groups fed on control, HC, and HCHT diets during the 2-month period (data not shown). The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of İstanbul. At the end of the feeding period, the animals were without food overnight, and were anesthetized with sodium pentobarbital. Blood was collected in tubes containing EDTA. The following measurements were made on whole blood: the erythrocyte and reticulocyte numbers, hemoglobin (Hb),26) hematocrit (Htc),26) and osmotic fragility values.26) Plasma cholesterol and triglyceride levels were measured with kits from Sigma Diagnostics (Katalog no: 402-20, 334-A, respectively). Plasma phospholipids were measured in plasma lipid extracts.27) Lipid extracts were exposed to oxidative digestion and liberated inorganic phosphorus was measured. Endogenous lipid peroxidation in plasma was assessed by measuring both malondialdehyde (MDA) levels and diene conjugate (DC) formation by the method of Buege and Aust.28) Extracted lipids (MDA) levels and diene conjugate (DC) formation by the method of Buege and Aust.28) Extracted lipids were evaporated and dissolved in cyclohexane, and DC was measured at 233 nm.20) Lipids of the erythrocyte were extracted with isopropanol-chloroform (11:7, v/v) by the method of Rose and Oklander.29) Cholesterol, total phospholipids, and DC levels were measured in erythrocyte lipid extracts as described for plasma. Erythrocyte susceptibility to lipid peroxidation was measured by the method of Stocks and Dormandy.30) The final composition of the incubation mixture was 5 mM H$_2$O$_2$, 2 mM sodium azide, and erythrocyte suspension in phosphate-buffered saline, pH 7.4 (30 mg Hb/ml incubation mixture). Lipid peroxidation was assayed by measurement of MDA production during a 2-h incubation period at 37°C. Values were expressed as nanomoles of MDA per gram of Hb. Hb concentration of erythrocyte suspensions was measured by Drabkin’s reagent. A spontaneous hemolysis test of erythrocytes was done after 4 h of incubation at 20°C in phosphate saline buffer, pH 7.4. The percentage of hemolysis was expressed as the ratio of the absorbance at 410 nm in isotonic buffer to the completely hemolysed samples in water.31) The results were expressed as mean ± SD. Statistical analysis was done by a one-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference post-hoc test.

**In vitro study.** We investigated the effects of taurine on spontaneous hemolysis and osmotic fragility tests as well as erythrocyte H$_2$O$_2$-induced hemolysis and lipid peroxidation in normal rabbit erythrocytes. For this reason, a) an erythrocyte spontaneous hemolysis test was done in the presence and absence of taurine at 80 and 160 mM final concentrations in the incubation medium.30) b) Erythrocytes were incubated with taurine (100 mM final concentration) at room temperature for 30 min and osmotic fragility was measured thereafter by using buffered NaCl solutions at various concentrations.26) c) Erythrocytes were exposed to the actions of H$_2$O$_2$ (5 and 10 mM final concentrations) in the presence and absence of taurine at 20 mM and 40 mM final concentrations in the incubation medium, and osmotic fragility26) and MDA formation29) were measured thereafter.

There is limited and conflicting knowledge in the literature about the erythrocyte lipid peroxidation and antioxidant system in hypercholesterolemic subjects5–10) and experimental animals.4,11,12,32) Erythrocytes are an appropriate model for lipid peroxidation studies.3) However, it is difficult to demonstrate MDA accumulation in freshly drawn erythrocytes, Stocks and Dormandy30) adapted the TBA-test to measure the susceptibility of erythrocytes under exogenous oxidative stress. In this study, lipid peroxidation was evaluated by measuring both endogenous DC levels and H$_2$O$_2$-induced MDA levels in erythrocytes. The HC diet caused significant increases in plasma cholesterol, triglyceride, and phospholipid levels as well as plasma DC and MDA levels. This diet resulted in an increase in erythrocyte cholesterol levels, but it did not affect erythrocyte phospholipid levels, compared to controls. The cholesterol: phospholipid ratio was also elevated by the HC diet. In addition, the number of erythrocytes and Hb and Htc values showed significant decreases after the HC diet. Contrarily, the reticulocyte numbers and *in vitro* spontaneous hemolysis increased, but no significant changes in either endogenous DC levels or H$_2$O$_2$-induced MDA levels were observed in erythrocytes of rabbits fed on an HC diet (Table 1). In addition, the HC diet resulted in an increase in erythrocyte osmotic fragility (Fig. 1A). Therefore, according to our results, lipid peroxidation did not accompany the increases in erythrocyte hemolysis, osmotic
Table 1. The Effects of Taurine on Plasma and Erythrocyte Lipid Concentrations and Diene Conjugates (DC) and Malondialdehyde (MDA) Levels, Haematological Data, and Spontaneous Hemolysis Values in Rabbits Fed on a High Cholesterol Diet (means ± SD, n = 6 each)

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Control diet</th>
<th>HC diet¹</th>
<th>HCHT diet²</th>
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<tbody>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.15 ± 0.49</td>
<td>39.9 ± 7.07ᵃ</td>
<td>31.1 ± 5.96ᵇ</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.63 ± 0.21</td>
<td>1.92 ± 0.54ᵃ</td>
<td>1.20 ± 0.14ᵇ</td>
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<tr>
<td>Phospholipid (mmol/L)</td>
<td>1.54 ± 0.14</td>
<td>14.51 ± 2.83ᵃ</td>
<td>10.7 ± 1.40ᵇ</td>
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<tr>
<td>DC (µmol/L)</td>
<td>116.2 ± 13.7</td>
<td>302.8 ± 44.6ᵃ</td>
<td>225.8 ± 31.1ᵇ</td>
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<tr>
<td>MDA (µmol/L)</td>
<td>3.13 ± 0.28</td>
<td>7.33 ± 0.58ᵃ</td>
<td>5.68 ± 0.56ᵇ</td>
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<table>
<thead>
<tr>
<th></th>
<th>Erythrocytes</th>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.18 ± 0.17</td>
<td>2.98 ± 0.66ᵃ</td>
<td>2.42 ± 0.34ᵇ</td>
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<tr>
<td>Phospholipid (mmol/L)</td>
<td>2.20 ± 0.28</td>
<td>1.88 ± 0.40</td>
<td>1.87 ± 0.32</td>
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<tr>
<td>Cholesterol:Phospholipid (mol:mol)</td>
<td>1.00 ± 0.09</td>
<td>1.59 ± 0.26ᵃ</td>
<td>1.30 ± 0.17ᵇ</td>
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<tr>
<td>DC (µmol/L erythrocyte)</td>
<td>240.5 ± 25.4</td>
<td>313.3 ± 63.1</td>
<td>258.5 ± 68.4</td>
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<tr>
<td>MDA (nmol/g Hb)</td>
<td>255.7 ± 22.3</td>
<td>204.5 ± 51.0</td>
<td>211.4 ± 29.7</td>
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<tr>
<td>Erythrocyte numbers (million/mm³)</td>
<td>6.16 ± 0.27</td>
<td>3.59 ± 0.70ᵃ</td>
<td>3.92 ± 0.21ᵇ</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.3 ± 0.92</td>
<td>8.38 ± 1.61ᵃ</td>
<td>9.28 ± 1.21ᵇ</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.0 ± 1.70</td>
<td>24.8 ± 4.19ᵃ</td>
<td>27.0 ± 2.49ᵇ</td>
<td></td>
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<tr>
<td>Reticulocyte number (%)</td>
<td>0.40 ± 0.15</td>
<td>6.30 ± 0.54ᵃ</td>
<td>4.90 ± 1.73ᵇ</td>
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<tr>
<td>Hemolysis (%)</td>
<td>8.70 ± 1.60</td>
<td>27.5 ± 6.12ᵃ</td>
<td>25.2 ± 5.82ᵇ</td>
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ᵃ p<0.05 as compared to control group; ᵇ p<0.05 as compared to cholesterol group.
¹ HC diet: High cholesterol.
² HCHT diet: High cholesterol plus taurine.

Fig. 1. A In Vivo Effect of Taurine on Erythrocyte Osmotic Fragility in Rabbits Fed on an HC Diet (Values: the means ± SD, n = 6 each, HC diet: high cholesterol diet; HCHT diet: HC diet supplemented with taurine).

B The Changes in Osmotic Fragility of Erythrocytes Incubated with H₂O₂ in the Presence or Absence of Taurine for 2 h (Values: the means ± SD, n = 4 each).

Fragility, and the development of hemolytic anemia in cholesterol-fed rabbits.

Taurine treatment decreased plasma lipids and lipid peroxide levels as well as erythrocyte cholesterol levels and the cholesterol:phospholipid ratio in rabbits fed on an HC diet. We²² and other investigators²⁻⁸,¹⁹ have reported that taurine administration to cholesterol-fed animals had a reducing effect on cholesterol and triglyceride levels in plasma and liver. Therefore, a decrease in erythrocyte cholesterol levels may depend on a decrease in serum cholesterol levels which arose following taurine treatment in rabbits fed on an HC diet. However, there were no changes in Hb or Hct values, erythrocyte and reticulocyte numbers, osmotic fragility and spontaneous hemolysis values in rabbits of the HCHT group, compared to the HC group. In addition, endogenous DC and H₂O₂-induced MDA levels remained unchanged in erythrocytes of rabbits fed on an HC diet following taurine treatment.

On the other hand, some investigators have reported that taurine can suppress erythrocyte hemolysis which was induced by using 2,2'-azobis (2-amidinopropan)²³ H₂O₂²⁴ and phenylhydrazine²⁵ under in vitro conditions. Taurine has also been reported to restore the depletion of erythrocyte membrane Na-K-ATPase activity after ozone exposure or cholesterol enrichment.³³ Therefore, it has been suggested that these effects of taurine may be related to its inhibitor effect on lipid peroxidation and/or its stabilizer effect on membranes.²⁻₂⁵,³³ However, when normal rabbit erythrocytes were incubated with H₂O₂ in the presence of taurine, no changes were observed in the osmotic fragility test of erythrocytes (Fig. 1B) and MDA levels (Fig. 2) as compared to non-taurine-containing conditions. In addition, there were no changes in osmotic fragility and spontaneous hemolysis values in erythrocytes incubated with taurine compared to those incubated without taurine.
Fig. 2. The Malondialdehyde (MDA) Levels in Erythrocytes Incubated with H2O2 in the Presence or Absence of Taurine (n = 4, each).

(data not shown). Therefore, our in vitro and in vivo findings are in accordance.

In conclusion, an HC diet resulted in hemolytic anemia without any changes in lipid peroxide levels in rabbits, and taurine treatment could not protect against the increases in erythrocyte hemolysis in hypercholesterolemic rabbits, while this treatment decreased the increases in erythrocyte cholesterol levels and cholesterol:phospholipid ratio as well as plasma lipids and lipid peroxides.

Acknowledgment

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References

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