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Abnormal incorporation and utilization of α-tocopherol in erythrocyte membranes of streptozotocin-induced diabetic rats

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Abstract

α-Tocopherol is a well-known lipophilic-free radical scavenger that is mainly localized in biomembranes. In this study, we investigated the changes in the incorporation and utilization of α-tocopherol in erythrocyte membranes of streptozotocin-induced diabetic rats and the effects of insulin to control hyperglycemia on these changes. Diabetes was experimentally induced by the injection of streptozotocin (60 mg/kg, i.v.). Blood was collected to determine the concentrations of α-tocopherol and its oxidative metabolite (α-tocopherolquinone) in plasma or erythrocyte membranes after streptozotocin injection. In streptozotocin-induced diabetic rats, α-tocopherol concentrations were decreased in erythrocyte membranes and increased in plasma. The ratio of α-tocopherol in erythrocyte membranes to that in plasma, which reflects the incorporation of α-tocopherol into erythrocyte membranes, was dramatically decreased in streptozotocin-induced diabetic rats. Moreover, the ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes, which reflects the utilization of α-tocopherol, was increased in streptozotocin-induced diabetic rats. These changes were prevented by insulin to control hyperglycemia. These findings suggest that the abnormalities in the incorporation and utilization of α-tocopherol in erythrocyte membranes of streptozotocin-induced diabetes can be restored to normal by insulin therapy to control hyperglycemia.

Keywords: α-Tocopherol; Erythrocyte membrane; Diabetes; Free radical; Insulin

1. Introduction

Oxygen-derived free radicals are generated in aerobic organisms during physiological or physiopathological oxidative metabolism of mitochondria. Free radicals may react with a variety of biomolecules, including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue, thereby interfering with cell function. Under normal physiological conditions, there is a critical balance in the generation of oxygen-free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity (Sies, 1997). Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production, reduced activity of antioxidant defenses or both. Oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide range of human diseases (Halliwell, 1994).

Diabetes mellitus is a complex, progressive disease that is accompanied by accelerated arteriosclerosis. The Diabetes Control and Complications Trial (DCCT) Research Group has demonstrated that intensive therapy effectively delays the onset and slows the progression of diabetic complications (nephropathy, retinopathy and neuropathy) in patients with Type 1 diabetes mellitus (The Diabetes Control and Complications Trial Research Group, 1993). There has recently been interest in the hypothesis that oxidative stress may contribute to the development of complications in diabetes mellitus (Baynes, 1991; Lyons, 1991). The increase of oxidative stress in diabetes leads to a shortening of the erythrocyte life span, increased thrombocyte aggregation, changes in membrane lipid structure and increased adhesiveness to endothelial cells (Jain, 1989). Hyperglycemia in diabetes can generate free radicals, hydrogen peroxide and reactive ketoaldehydes by the autooxidation of glucose or from glycated proteins (Jain, 1989; Mullarkey et al., 1990;

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Baynes, 1991; Wolff et al., 1991; Tesfamariam and Cohen, 1992; Ceriello et al., 1997).

Vitamin E has been proposed to be the major lipid-soluble chain-breaking antioxidant and protects biologic membranes from lipid peroxidation (Scholz et al., 1997). Vitamin E deficiency reduces erythrocyte deformability and survivability and increases susceptibility to oxidant damage and adhesiveness to endothelial cells (Lubin and Chiu, 1982; Krishnamurthy et al., 1984; Kay et al., 1986; Tamai et al., 1986). Vitamin E scavenges lipid hydroperoxide radicals produced in the membrane lipid hydroperoxide reaction and changes into the vitamin E radical, which can in turn change into vitamin E quinone (oxidative metabolite) or vitamin E (Svanholm et al., 1974). Native vitamin E consists of eight fractions, α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. α-Tocopherol, which has the strongest physiological activity of all of the vitamin E fractions and comprises 90% of vitamin E, has important biomedical significance. There have been many studies on diabetes related to vitamin E. Improved insulin sensitivity and glucose control were found in elderly or Type 2 diabetic patients treated by high doses of vitamin E (Paolisso et al., 1993, 1994). Other studies have shown that vitamin E can prevent oxidative damage by free radicals in Type 1 diabetes mellitus (Wautier et al., 1981; Bono et al., 1987; Baynes, 1991).

Recently, we reported a simple, selective and highly sensitive assay for the simultaneous determination of α-tocopherol and oxidative metabolite α-tocopherolquinone in plasma and erythrocyte membranes by reversed-phase high-performance liquid chromatography (HPLC) with a redox detection mode using a series of four coulometric working electrodes (Takeda et al., 1996; Yanagawa et al., 1999). This assay model can be used to study the biological effect of α-tocopherol on lipid peroxidation.

In this study, we investigated the changes in the incorporation and utilization of α-tocopherol in erythrocyte membranes of streptozotocin-induced diabetic rats, and the effects of insulin to control hyperglycemia on these changes.

2. Materials and methods

2.1. Materials

Streptozotocin was purchased from Sigma (St. Louis, MO, USA). Insulin (Humalin N) was purchased from Lilly (Indianapolis, IN, USA). Plasma samples were assayed for total cholesterol and triglyceride with enzymatic kits (WAKO, Osaka, Japan). Reagents of the highest purity available were used without further purification. α-Tocopherol was purchased from Eisai (Tokyo, Japan) and α-tocopherolquinone was purchased from ICN Biomedicals (Costa Mesa, CA, USA). Analytical-reagent grade chemicals for sample preparation and chromatography were obtained from WAKO and Nacalai Tesque (Kyoto, Japan). A stock standard solution containing α-tocopherol and α-tocopherolquinone was prepared by dissolving 1 mg of each in 1 ml of methanol deoxygenated with nitrogen. Working standard solutions were prepared daily by diluting the stock standard solution with methanol deoxygenated with nitrogen.

2.2. Animals

Male Wistar rats weighing 260–320 g were purchased from Charles River (Yokohama, Japan). Rats were maintained in a temperature-controlled (25 °C) facility with a strict 12-h light/dark cycle and were given free access to water and standard rat food throughout the experiment. Diabetes was induced by injection of streptozotocin (60 mg/kg, i.v.) in 0.05 M of citrate buffer (pH 4.5) into the tail vein while rats (8 weeks old) were under light ether anesthesia. Age-matched control rats were injected with 0.05 M of citrate buffer (pH 4.5). Determination of blood glucose concentrations was performed with a Medisafe leader (Terumo, Japan). Experiment 1: diabetic rats were killed on day 3 and 1, 2, 4 and 12 weeks after streptozotocin injection. The control rats were age matched accordingly. Experiment 2: diabetic rats were subdivided into two groups (vehicle treatment and insulin treatment groups). Insulin-treated diabetic rats were treated with subcutaneous injections of insulin (Humalin N: 5 U/rat/day) to control hyperglycemia started 24 h after streptozotocin injection and repeated for 1 week. Vehicle treatment control and vehicle treatment diabetic rats were injected with an equivalent volume of saline. Rats were killed by decapitation, and trunk blood was collected to determine α-tocopherol, α-tocopherolquinone, serum total cholesterol and triglyceride and blood glucose levels. Serum total cholesterol and triglyceride were measured by enzymatic methods.

2.3. Sample preparation

Blood samples were collected in tubes containing both dry sodium heparin and disodium ethylenediaminetetraacetic acid as anticoagulants. Plasma was prepared immediately from fresh heparinized blood by centrifugation at 100 × *g* for 20 min at 4 °C. The plasma sample (0.1 ml) was pipetted into a tube containing 6 μl of 0.5 M sodium hydrogensulphite and deoxygenated with nitrogen. The erythrocyte layer was washed three times with three volumes of isotonic sodium chloride solution (pH 7.4) and pipetted into a tube containing 0.05 mg of butylated hydroxytoluene. An erythrocyte membrane sample was prepared from 1.0 ml of erythrocyte suspension according to the method of Dodge et al. (1963) under a stream of nitrogen. Extraction of α-tocopherol and α-tocopherolquinone was based on the procedure of Pascoe et al. (1987). The plasma (0.1 ml) or erythrocyte membrane sample (0.1 ml) was pipetted into a tube containing 0.5 ml of ethanol deoxygenated with nitrogen and mixed vigorously for 3 min. Two milliliters of *n*-hexane deoxygenated with nitrogen was then added, and this was followed by remixing and centrifugation at 2000 × *g* for 5 min at 4 °C. The hexane

Table 1
Changes in body weight and blood glucose levels in age-matched control and streptozotocin-induced diabetic rats

Period[a]	Group	*n*	Body weight (g)	Blood glucose (mg/dl)
Day 3	Control	8	293.12 ± 4.43	123.88 ± 4.79
	DM	8	269.38 ± 5.55[b]	399.88 ± 23.99[c]
1 week	Control	8	333.75 ± 6.25	132.75 ± 5.08
	DM	8	290.62 ± 7.41[c]	381.12 ± 16.43[c]
2 weeks	Control	8	373.75 ± 9.48	141.25 ± 5.36
	DM	8	298.12 ± 6.61[c]	498.00 ± 21.08[c]
4 weeks	Control	8	410.00 ± 8.45	141.50 ± 8.65
	DM	8	290.62 ± 12.83[c]	507.00 ± 31.39[c]
12 weeks	Control	5	538.00 ± 24.78	133.20 ± 11.77
	DM	10	349.00 ± 11.13[c]	543.50 ± 18.65[c]

Each value represents the mean ± S.E.
DM: streptozotocin-induced diabetic rats, *n*: number of animals.
[a] The period after streptozotocin (60 mg/kg, i.v.) injection.
[b] $P<0.01$ compared to age-matched control rats.
[c] $P<0.001$ compared to age-matched control rats.

layer was carefully transferred to a new tube and dried under a stream of nitrogen at 40 °C. The residue was dissolved in 100 μl of mobile-phase solution, 10 μl of which was injected into the HPLC-electrochemical detection system.

2.4. Determination of α-tocopherol and α-tocopherolquinone

For the selective, highly sensitive and simultaneous determination of α-tocopherol and α-tocopherolquinone in plasma or erythrocyte membrane, reversed-phase HPLC with redox detection using a series of four coulometric high-efficiency flow-through cells (Takeda et al., 1996) was used. This assay system consisted of a Model LC-9A solvent-delivery system (Shimadzu, Kyoto, Japan) equipped with an extra damper, a Model 7125 sample injector (Rheodyne, Cotani, CA, USA), an MC MEDICAL C18 reversed-phase column (80 × 4.6 mm ID, 3-μm particle size) (MC Medical, Tokyo, Japan) and multiple coulometric detectors (ESA, Bedford, MA, USA). The analytical column was maintained at a constant temperature (35 °C). A mobile phase consisting of 96% methanol [methanol-HPLC-grade distilled water (96:4, v/v)] containing 40-mM sodium perchlorate as the supporting electrolyte was used (Yanagawa et al., 1999). The mobile phase was kept oxygen-free by continuous bubbling with nitrogen. Standard α-, β-, γ- and δ-tocopherol for quantitative analysis were purchased from Eisai, and α-tocopherolquinone was purchased from ICN Biomedicals.

2.5. Statistical analysis

Statistical analysis of the data was performed using Student's *t*-test or an analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. The results were considered statistically significant when *P*-values were less than 0.05 (two-tailed).

3. Results

Changes in body weight and blood glucose levels at day 3 and 1, 2, 4 and 12 weeks after streptozotocin injection are shown in Table 1. The body weight at day 3 and 1, 2, 4 and 12 weeks after streptozotocin injection was significantly lower than that in age-matched control rats. The blood

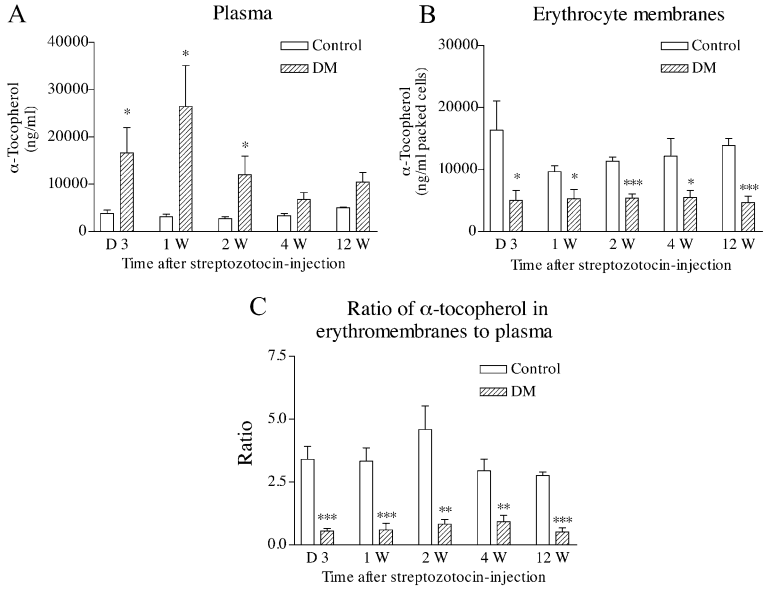

Fig. 1. Concentrations of α-tocopherol in plasma (A) and in erythrocyte membranes (B) of age-matched control and streptozotocin-induced diabetic rats. The ratios of α-tocopherol in erythrocyte membranes to plasma in control and streptozotocin-induced diabetic rats (C). The concentration of α-tocopherol in erythrocyte membrane and in plasma is shown as ng/ml packed cells and ng/ml, respectively. Each value represents the mean ± S.E. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ vs. control rats. D: days, W: weeks and DM: streptozotocin-induced diabetic rats.

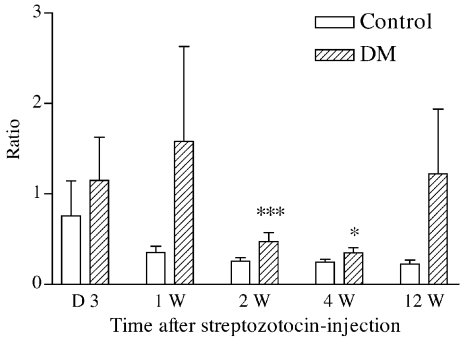

Fig. 2. Changes in the ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes of age-matched control and streptozotocin-induced diabetic rats. Each value represents the mean ± S.E. *$P<0.05$, ***$P<0.001$ vs. control rats. D: days, W: weeks and DM: streptozotocin-induced diabetic rats.

glucose levels at day 3 and 1, 2, 4 and 12 weeks after streptozotocin injection were significantly higher than those in age-matched control rats. Plasma total cholesterol and triglyceride were markedly increased in streptozotocin-induced diabetic rats compared to those in age-matched control rats (data not shown).

Fig. 1A shows the concentrations of α-tocopherol in plasma of control and streptozotocin-induced diabetic rats. There was a significant increase in α-tocopherol levels in plasma of streptozotocin-induced diabetic rats ($P<0.05$) compared to those in control rats on day 3 and 1 and 2 weeks after streptozotocin injection. At 4 and 12 weeks after streptozotocin injection, there were no statistically significant differences in α-tocopherol levels in plasma of control and streptozotocin-induced diabetic rats. Fig. 1B shows the concentrations of α-tocopherol in erythrocyte membranes of control and streptozotocin-induced diabetic rats. There was a significant decrease in α-tocopherol levels in erythrocyte membranes of streptozotocin-induced diabetic rats compared to those in control rats on day 3 and 1, 2, 4 and 12 weeks after streptozotocin injection. Fig. 1C shows changes in the ratio of α-tocopherol in erythrocyte membranes to α-tocopherol in plasma, which is thought to reflect the rate of α-tocopherol transport or uptake from plasma to erythrocyte membranes. This ratio in streptozotocin-induced diabetic rats was significantly lower than that in control rats on day 3 and 1, 2, 4 and 12 weeks after streptozotocin injection.

Fig. 2 shows changes in the ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes, which is thought to reflect the rate of the utilization of α-tocopherol in erythrocyte membranes. This ratio in streptozotocin-induced diabetic rats was significantly higher than that in control rats 2 and 4 weeks after streptozotocin injection. At day 3 and 1 and 12 weeks after streptozotocin injection, there were no statistically significant differences between control and streptozotocin-induced diabetic rats.

Fig. 3 shows the effects of insulin on the incorporation and utilization of α-tocopherol in streptozotocin-induced diabetic rats. There was a marked increase in α-tocopherol in the plasma of streptozotocin-induced diabetic rats compared to that in control rats. This increase was significantly ($P<0.05$)

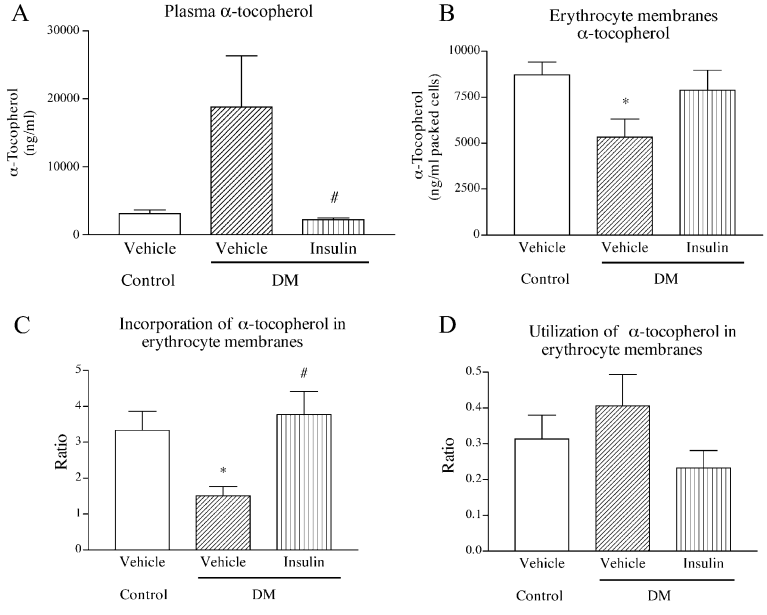

Fig. 3. Effect of insulin on the incorporation and utilization of α-tocopherol in streptozotocin-induced diabetic rats. Concentrations of α-tocopherol in plasma (A) and erythrocyte membranes (B) of control, streptozotocin-induced diabetic and insulin-treated streptozotocin-induced diabetic rats. The ratio of α-tocopherol in erythrocyte membranes to that in plasma (C) and the ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes (D) in control, streptozotocin-induced diabetic and insulin-treated streptozotocin-induced diabetic rats. The concentration of α-tocopherol in erythrocyte membrane and in plasma is shown as ng/ml packed cells and ng/ml, respectively. Each value represents the mean ± S.E. *$P<0.05$ vs. vehicle-treated control rats. $^{\#}P<0.01$ vs. streptozotocin-induced diabetic rats. DM: streptozotocin-induced diabetic rats.

Table 2
Effects of insulin on body weight and blood glucose levels in streptozotocin-induced diabetic rats

Group	Treatment	*n*	Body weight (g)	Blood glucose (mg/dl)
Control	Vehicle	8	335.62 ± 6.25	132.75 ± 5.08
DM	Vehicle	7	284.29 ± 14.24[a]	331.00 ± 30.95[b]
DM	Insulin	7	310.00 ± 10.63	103.00 ± 27.69[c]

Each value represents the mean ± S.E.
Control: age-matched control rats, DM: streptozotocin-induced diabetic rats and *n*: number of animals.
[a] $P<0.01$ compared to age-matched control rats.
[b] $P<0.001$ compared to age-matched control rats.
[c] $P<0.001$ compared to streptozotocin-induced diabetic rats.

prevented when streptozotocin-induced diabetic rats were treated with insulin to control hyperglycemia (Fig. 3A). However, α-tocopherol in erythrocyte membranes and the ratio of α-tocopherol in erythrocyte membranes to α-tocopherol in plasma were significantly ($P<0.05$) lower in streptozotocin-induced diabetic rats than in control rats. This decrease was prevented when streptozotocin-induced diabetic rats were treated with insulin (Fig. 3B and C). The ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes of streptozotocin-induced diabetic rats was slightly greater than that in control rats. This increase was prevented when streptozotocin-induced diabetic rats were treated with insulin (Fig. 3D). The effects of insulin on body weight and blood glucose levels in control, streptozotocin-induced diabetic and insulin-treated streptozotocin-induced diabetic rats are shown in Table 2. Treatment of streptozotocin-induced diabetic rats with insulin resulted in the normalization of body weight and blood glucose levels ($P<0.001$). Furthermore, plasma total cholesterol and triglycerides in insulin-treated streptozotocin-induced diabetic rats also recovered to control levels (data not shown).

4. Discussion

Recently, it has been proposed that oxidative stress contributes to the development of diabetic complications (Baynes, 1991; Lyons, 1991). Diabetic patients are exposed to increased oxidative stress due to several mechanisms, including glucose autooxidation and non-enzymatic protein glycation (Sakurai and Tsuchiya, 1988; Wolff, 1993). In diabetes mellitus, the high incidence of microvascular and atherosclerotic disease has been associated with abnormalities of erythrocyte composition and rheological function and with increased oxidative stress, among many other factors. The increased blood viscosity seen in diabetes (McMillan, 1976) and more so in patients with established complications (Schut et al., 1992) has been ascribed to a decrease in erythrocyte deformability (McMillan et al., 1978) and to changes in erythrocyte membrane fluidity (Bryszewska and Leyko, 1983; Juhan-Vague et al., 1986). There have been many studies on diabetes related to vitamin E, which is a potent-free radical scavenger. In this study, we observed an increase in plasma α-tocopherol levels in diabetic rats compared to those in control rats. These results are similar to those reported by Vatassery et al. (1983) and McMasters et al. (1967), but differ from those reported by Vandewoude et al. (1987), who found no difference in plasma vitamin E levels between control and diabetic groups. Vatassery et al. (1983) found that plasma vitamin E levels in patients with Type 1 diabetes mellitus and Type 2 diabetes mellitus were higher than those in the control group. However, they did not explain the mechanism that increases vitamin E levels in diabetes. In addition, Vandewoude et al. (1987) found an increase in plasma α-tocopherol concentrations only in hypercholesterolemic diabetes or controls. Moreover, they have shown that plasma α-tocopherol was correlated with plasma lipids, especially with total and low-density lipoprotein cholesterol. We have demonstrated that plasma total cholesterol and triglyceride were markedly increased in streptozotocin-induced diabetic rats. Since α-tocopherol is known to be a transport in plasma lipoproteins. Further studies are needed to examine in detail the relation between the transport of α-tocopherol to plasma membranes and concentration of plasma lipids or lipoproteins in diabetes.

In erythrocyte membranes, the concentrations of α-tocopherol in diabetic rats were significantly lower than those in control rats. There have been a few reports on the vitamin E levels in erythrocytes in diabetes. Vitamin E is the major lipid antioxidant in human erythrocyte membranes, where it plays an important role in the suppression of free radical-induced lipid peroxidation (Tappel, 1962; Traber and Packer, 1995). Hence, the regulation of α-tocopherol concentrations in membranes is critically important to maintain erythrocyte membrane structure and function. Efficient defense systems in human erythrocytes are located in both the cytosol and membrane (Burton et al., 1985). Yanagawa et al. (2001) reported that in erythrocyte membranes, the concentrations of α-tocopherol in elderly Type 2 diabetic patients were significantly lower than those in healthy subjects. Jain et al. (1991) reported that hyperglycemia reduces erythrocyte vitamin E levels and increases the production of lipofuscin in diabetic rats. Our results are consistent with these findings. The decreased erythrocyte vitamin E levels can be attributed either to increased consumption through the destruction of oxygen-free radicals caused by hyperglycemia or to a reduction in the activity of enzymes that detoxify oxygen radicals.

In addition, we found that the ratio of α-tocopherol in erythrocyte membranes to that in plasma was significantly decreased in diabetic rats. This ratio is thought to reflect the rate of α-tocopherol transport or uptake from plasma to erythrocyte membranes. Previous studies have indicated that tocopherols were incorporated into lipoproteins through lipid and lipoprotein transport and catabolism, and finally delivered to erythrocyte membrane in low-density lipoprotein and high-density lipoprotein (Traber et al., 1988) or that there was direct cell-to-cell transfer of tocopherols (Tanaka and Mino, 1986). Erythrocytes require a constant supply of

α-tocopherol from plasma lipoproteins to prevent membrane lipid peroxidation and thereby maintain membrane integrity. Accordingly, in diabetes, the above-mentioned decrease in the α-tocopherol concentration in erythrocyte membranes likely occurs due to a disturbance of α-tocopherol transfer or uptake from plasma to erythrocyte membranes. The precise mechanisms responsible for disturbing this transfer system have not yet been fully elucidated and appear to be complex and multifactorial. Some possible causes of the disturbance of the transfer or uptake and antioxidative status of α-tocopherol in erythrocyte membrane include the generation of membrane lipid peroxide and the degeneration of cell surface receptors (Traber and Kayden, 1984). Further studies are needed to examine the influence of hyperglycemia and other factors of diabetes on receptor-mediated uptake of lipoproteins in cells.

Since the depletion of cellular α-tocopherol increases the potential for peroxidative damage of cell membranes, its loss through oxidation to the tocopherol radical would predispose the cell to chemical or oxidant-induced injury. Furthermore, the α-tocopherol radical may be oxidized to α-tocopherolquinone. We demonstrated that the ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes, which reflects the utilization of α-tocopherol, was higher in diabetic rats than in controls. Therefore, this ratio is also thought to reflect the rate of the oxidation of α-tocopherol in erythrocyte membranes. Recent studies have shown that free oxygen radicals and erythrocyte membrane lipid peroxidation are significantly increased in diabetic patients and experimentally induced diabetic rats (Pitkanen et al., 1992; Jain et al., 1989; Uzel et al., 1987). Recently, we reported that the ratio of α-tocopherolquinone to α-tocopherol in erythrocyte membranes was not different between healthy and elderly patients of Type 2 diabetes mellitus (Yanagawa et al., 2001). This explains why α-tocopherol is normally used to reduce oxidative stress in elderly Type 2 diabetic patients. These discrepancies could be related to the different blood glucose levels in Type 1 diabetic model rats and Type 2 diabetic patients. We thought that oxidative stress in erythrocyte membranes of Type 1 diabetic model rats was greater than that in Type 2 diabetic patients. In fact, hyperglycemia can cause the peroxidation of membrane lipids in human red blood cells (Jain, 1989). The utilization of α-tocopherol and the above-mentioned disturbance of α-tocopherol transfer may be related to diabetic complications.

We also examined the effects of insulin therapy on the incorporation and utilization of α-tocopherol in erythrocyte membranes of streptozotocin-induced diabetic rats. There has been no previous report on the effect of insulin therapy on these changes. In this study, α-tocopherol levels in plasma and erythrocyte membranes, and the ratio of α-tocopherol in erythrocyte membranes to plasma, returned to control levels upon treatment with insulin. These results suggest that the changes in the incorporation and utilization of α-tocopherol were prevented by the use of insulin to control hyperglycemia. Thus, hyperglycemia may contribute to the changes in erythrocyte membrane structure and function.

In conclusion, our results showed that the incorporation of α-tocopherol in erythrocyte membranes was extremely decreased in streptozotocin-induced diabetic rats. Furthermore, the present data also indicate that the utilization of α-tocopherol in erythrocyte membranes was increased in streptozotocin-induced diabetic rats, and this reflects the oxidation of α-tocopherol. Abnormalities in the incorporation and utilization of α-tocopherol in erythrocyte membranes in diabetes are restored to normal by insulin therapy to control hyperglycemia.

References

Baynes, J.W., 1991. Role of oxidative stress in development of complications in diabetes. Diabetes 40, 405–412.

Bono, A., Caimi, G., Catania, A., Sarno, A., Pandolfo, L., 1987. Red cell peroxide metabolism in diabetes mellitus. Horm. Metab. Res. 19, 264–266.

Bryszewska, M., Leyko, W., 1983. Effect of insulin on human erythrocyte membrane fluidity in diabetes mellitus. Diabetologia 24, 311–313.

Burton, G.W., Webb, A., Ingold, K.U., 1985. A mild, rapid, and effective method of lipid extraction for use in vitamin E-lipid ratios. Lipids 20, 29–39.

Ceriello, A., Bortolotti, N., Pirisi, M., Crescentini, A., Tonutti, L., Motz, E., Russo, A., Giacomello, R., Stel, G., Taboga, C., 1997. Total plasma antioxidant capacity predicts thrombosis prone status in NIDDM patients. Diabetes Care 20, 1589–1593.

Dodge, J.T., Mitchell, C., Hanahan, D.J., 1963. The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch. Biochem. Biophys. 100, 119–130.

Halliwell, B., 1994. Free radicals, antioxidants, and human disease: cause or consequence? Lancet 344, 721–724.

Jain, S.K., 1989. Hyperglycemia can cause membrane lipid peroxidation and osmotic fragility in human red blood cells. J. Biol. Chem. 264, 21340–21345.

Jain, S.K., Mc Vie, R., Duett, J., Meachum, Z.D., Herbst, J.J., 1989. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes 38, 1539–1543.

Jain, S.K., Levine, S.N., Duett, J., Hollier, B., 1991. Reduced vitamin E and increased lipofuscin products in erythrocytes of diabetic rats. Diabetes 40, 1241–1244.

Juhan-Vague, I., Rahmani-Jourdheuil, D., Mishal, Z., Roul, C., Mourayre, Y., Aillaud, M.F., Vague, P., 1986. Correction by insulin added in vitro of abnormal membrane fluidity of the erythrocyte from type 1 (insulin-dependent) diabetic patients. Diabetologia 29, 417–420.

Kay, M.M.B., Bosman, G.J., Shapiro, S.S., Bendich, A., Bassel, P.S., 1986. Oxidation as a possible mechanism of cellular aging: vitamin E deficiency causes premature aging and Ig G binding to erythrocytes. Proc. Natl. Acad. Sci. U. S. A. 83, 2463–2467.

Krishnamurthy, S., Bai, N.J., George, T., 1984. Mechanism of oxidative lysis and lipid peroxidation of vitamin E deficient erythrocytes. Indian J. Biochem. Biophys. 21, 361–364.

Lubin, B., Chiu, D., 1982. Properties of vitamin E-deficient erythrocytes following peroxidant injury. Pediatr. Res. 16, 928–932.

Lyons, T.J., 1991. Oxidized low density lipoproteins: a role in the pathogenesis of atherosclerosis in diabetes? Diabet. Med. 8, 411–419.

McMasters, V., Howard, T., Kinsell, L.W., Van Der Veen, J., Olcott, H.S., 1967. Tocopherol storage and depletion in adipose tissue and plasma of normal and diabetic human subjects. Am. J. Clin. Nutr. 20, 622–626.

McMillan, D.E., 1976. Plasma protein changes, blood viscosity and diabetic microangiopathy. Diabetes 25 (Suppl. 2), 858–864.

McMillan, D.E., Utterback, N.G., La Puma, J., 1978. Reduced erythrocyte deformability in diabetes. Diabetes 27, 895–901.

Mullarkey, C.J., Edelstein, D., Brownlee, L., 1990. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem. Biophys. Res. Commun. 173, 932–939.

Paolisso, G., D'Amore, A., Giugliano, D., Ceriello, A., Varricchio, M., D'Onofrio, F., 1993. Pharmacologic doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. Am. J. Clin. Nutr. 57, 650–656.

Paolisso, G., Di Maro, G., Galzerano, D., Cacciapuoti, F., Varricchio, G., Varricchio, M., D'Onofrio, F., 1994. Pharmacological doses of vitamin E and insulin action in elderly subjects. Am. J. Clin. Nutr. 59, 1291–1296.

Pascoe, G.A., Duda, C.T., Reed, D.J., 1987. Determination of α-tocopherol and α-tocopherolquinone in small biological samples by high-performance liquid chromatography with electrochemical detection. J. Chromatogr. 414, 440–448.

Pitkanen, O.M., Martin, J.M., Hallman, M., Akerblom, H.K., Sariola, H., Andersson, S.M., 1992. Free radical activity during development of insulin dependent diabetes mellitus in the rat. Life Sci. 50, 335–339.

Sakurai, T., Tsuchiya, S., 1988. Superoxide production from nonenzymatically glycated protein. FEBS Lett. 236, 406–410.

Scholz, R.W., Reddy, P.V., Wynn, M.K., Graham, K.S., Liken, A.D., Gumpricht, E., Reddy, C.C., 1997. Glutathione-dependent factors and inhibition of rat liver microsomal lipid peroxidation. Free Radic. Biol. Med. 23, 815–828.

Schut, N.H., Van Arkel, E.C., Hardemann, M.R., Bilo, H.J.G., Michels, R.P.J., Vreeken, J., 1992. Blood and plasma viscosity in diabetes: possible contribution to late organ complications? Diabetes Res. 19, 31–35.

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82, 291–295.

Svanholm, U., Bechgaard, K., Parker, V.D., 1974. Electrochemistry in media of intermediate acidity: Part VIII. Reversible oxidation products of the α-tocopherol model compound. Cation radical, cation, and dication. J. Am. Chem. Soc. 96, 2409–2413.

Takeda, H., Shibuya, T., Yanagawa, K., Kanoh, H., Takasaki, M., 1996. Simultaneous determination of α-tocopherol and α-tocopherolquinone by high-performance liquid chromatography and coulometric detection in the redox mode. J. Chromatogr., A 722, 287–294.

Tamai, H., Miki, M., Mino, M., 1986. Hemolysis and membrane lipid changes induced by xanthine oxidase in vitamin E deficient red cells. J. Free Radic. Biol. Med. 2, 49–56.

Tanaka, H., Mino, M., 1986. Membrane-to-membrane transfer of tocopherol in red blood cells. J. Nutr. Sci. Vitaminol. 32, 463–474.

Tappel, A.L., 1962. Vitamin E as the biological lipid antioxidant. Vitam. Horm. 20, 493–510.

Tesfamariam, B., Cohen, R.A., 1992. Free radicals mediate endothelial cell dysfunction caused by elevated glucose. Am. J. Physiol. 263, H321–H326.

The Diabetes Control and Complications Trial Research Group, 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N. Engl. J. Med. 329, 977–986.

Traber, M.G., Kayden, H.J., 1984. Vitamin E is delivered to cells via the high affinity receptor for low-density lipoprotein. Am. J. Clin. Nutr. 40, 747–751.

Traber, M.G., Packer, L., 1995. Vitamin E: beyond antioxidant function. Am. J. Clin. Nutr. 62, 1501–1509.

Traber, M.G., Ingold, K.U., Burton, G.W., Kayden, H.J., 1988. Absorption and transport of deuterium-substituted 2′*R*, 4′*R*, 8′*R*-α-tocopherol in human lipoproteins. Lipids 23, 791–797.

Uzel, N., Sivas, A., Uysal, M., Oz, H., 1987. Erythrocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes mellitus. Horm. Metab. Res. 19, 89–90.

Vandewoude, M.G., Van Gaal, L.F., Vandewoude, M.F., De Leeuw, I.H., 1987. Vitamin E status in normocholesterolemic and hypercholesterolemic diabetic patients. Acta Diabetol. Lat. 24, 133–139.

Vatassery, G.T., Morley, J.E., Kuskowski, M.A., 1983. Vitamin E in plasma and platelets of human diabetic patients and control subjects. Am. J. Clin. Nutr. 37, 641–644.

Wautier, J.L., Paton, R.C., Wautier, M.P., Pintigny, D., Abadie, E., Passa, P., Caen, J.P., 1981. Increased adhesion of erythrocytes to endothelial cells in diabetes mellitus and its relation to vascular complications. N. Engl. J. Med. 305, 237–242.

Wolff, S.P., 1993. Diabetes mellitus and free radicals. Br. Med. Bull. 49, 642–652.

Wolff, S.P., Jiang, Z.Y., Hunt, J.V., 1991. Protein glycation and oxidative stress in diabetes mellitus and aging. Free Radic. Biol. Med. 10, 339–352.

Yanagawa, K., Takeda, H., Egashira, T., Sakai, K., Takasaki, M., Matsumiya, T., 1999. Age-related changes in alpha-tocopherol dynamics with relation to lipid hydroperoxide content and fluidity of rat erythrocyte membrane. J. Gerontol., Ser. A, Biol. Sci. Med. Sci. 54, B379–B383.

Yanagawa, K., Takeda, H., Egashira, T., Matsumiya, T., Shibuya, T., Takasaki, M., 2001. Changes in antioxidative mechanisms in elderly patients with non-insulin dependent diabetes mellitus: investigation of the redox dynamics of α-tocopherol in erythrocyte membranes. Gerontology 47, 150–157.