Inhibition of Dehydroascorbic Acid Transport across the Rat
Blood–Retinal and –Brain Barriers in Experimental Diabetes

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Vitamin C is mainly transported across the blood–retinal and –brain barriers as dehydroascorbic acid (DHA) via a facilitative glucose transporter, GLUT1, and accumulates as ascorbic acid in the retina and brain. To investigate whether DHA transport to the retina and brain is changed by hyperglycemia, [14C]DHA transport across the blood–retinal and –brain barriers was examined using in vivo integration plot analysis in streptozotocin-induced diabetic rats with a 3-week duration of diabetes and in normal rats. Blood-to-retina and -brain transport of [14C]DHA was reduced by 65.5% and 84.1%, respectively, in diabetic rats compared with normal rats, whereas there was no major difference in the heart. Therefore, we propose that hyperglycemia reduces the supply of vitamin C to the retina and brain.

Key words dehydroascorbic acid; GLUT1; diabetes; blood–retinal barrier; blood–brain barrier

MATERIALS AND METHODS

Animals Male Wistar rats at 3 weeks of age (body weights: 50–55 g) were purchased from SLC (Shizuoka, Japan). The investigations using rats described in this report conformed to the provisions of the Animal Care Committee, Toyama Medical and Pharmaceutical University (currently University of Toyama) (#2004-48) and the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

Regents L-1-[1,4-C]Ascorbic acid ([14C]AA, 13 mCi/mmol) was purchased from Perkin-Elmer Life Sciences (Boston, MA, U.S.A.). L-1-[14C]Dehydroascorbic acid ([14C]DHA) was generated in all experiments by incubating [14C]AA (1 μM in saline) with ascorbate oxidase (1 unit/1 mmol AA in saline, Sigma, St. Louis, MO, U.S.A.) at 37°C for 90 s as described in a previous report. All other chemicals were of reagent grade and available commercially.

Animal Model of Diabetes Mellitus Rats at 3 weeks of age were randomly assigned to become diabetic (n=5) or to remain non-diabetic controls (n=5). Diabetes was induced by the injection of a freshly prepared solution of streptozotocin in citrate buffer (pH 4.5) at a dose of 45 mg/kg into the tail vein, whereas non-diabetic control rats were treated with citrate buffer alone. A sample of blood was obtained from this vein over a period from 1 to 3 weeks after streptozotocin injection for measurement of the blood D-glucose concentration to confirm the presence of hyperglycemia. Blood glucose concentrations were determined by the glucoseoxidase method (Nipro Freestyle, Osaka, Japan).

Determination of Influx Permeability Clearance The rats were anesthetized with an intramuscular injection of ketamine–xylazine (1.22 mg xylazine and 125 mg ketamine/kg) and then [14C]DHA (5 μCi/rat, approximately 200 μmol DHA in 200 μl/head) was injected via the femoral vein. At the times designated (0.5, 1, 3 or 5 min) after administration, rats were sacrificed and the plasma and tissues were removed. Tissue sampling and determination of radioactivity were performed as described in a previous report. The apparent influx permeability clearance (K_in) of [14C]DHA in the tissue (K_in tissue) [μl/(min·g tissue)] was determined by integration plot analysis. As an index of the tissue distribution characteristics of [14C]DHA, the apparent tissue-to-plasma concentration ratio (V_t) was used. This ratio [V_t(t) (ml/g tissue)] was defined as the amount of [14C] per gram tissue divided by that per millilitre plasma, calculated over the time-period of the experiment. In brief, the tissue uptake rate of [14C]DHA can be described by equation (Eq. 1):
Data Analysis Unless otherwise indicated, all data represent means±S.E.M. An unpaired, two-tailed Student’s t-test was used to determine the significance of differences between two group means.

RESULTS

The blood d-glucose concentration was 25.6±0.4 mm (n=5) in the streptozotocin-induced diabetic rats with a 3-week duration of diabetes, and 4.42±0.07 mm (n=10) and 8.01±0.32 mm (n=5) at 3 and 6 weeks age, respectively, in non-diabetic control rats. It was increased 3.2-fold in diabetic rats compared with controls. The final body weights were not appreciably increased in the streptozotocin-induced diabetic rats with a 3-week duration of diabetes (97.6±4.0 g, n=5), whereas the final body weights of the control rats increased to 215±5 g (n=5) from 52.0±1.0 g (n=10).

The DHA transport into the retina and brain across the BRB and BBB was evaluated and compared with the heart by means of the integration plot analysis after intravenous administration of [14C]DHA to the streptozotocin-induced diabetic rats. The K_{in retina} of [14C]DHA in diabetic and normal rats was determined to be 841±243 μl/(min·g retina) and 2.44×10^{7}±0.05×10^{3} μl/(min·g retina), respectively. The K_{in brain} of [14C]DHA in diabetic and normal rats was determined to be 49.1±11.5 μl/(min·g brain) and 309±53 μl/(min·g brain), respectively. The K_{in retina} and K_{in brain} of [14C]DHA in diabetic rats was reduced by 65.5% and 84.1%, respectively, compared with normal rats, whereas no significant difference was found between the K_{in heart} of diabetic and normal rats [50.0±12.6 μl/(min·g heart) and 49.7±15.1 μl/(min·g heart), respectively].

DISCUSSION

The present study produces, for the first time, in vivo evidence that hyperglycemia reduces blood-to-retina and -brain transport of [14C]DHA across the BRB and BBB (Fig. 1). DHA is transported by GLUT1, which is expressed at the luminal (blood) and abluminal (retina or brain) side of the inner BRB (retinal capillary endothelial cells), outer BRB (retinal pigment epithelial cells), and BBB (brain capillary endothelial cells).^{9–11} On the other hand, there was only a small difference between the K_{in heart} of diabetic and normal rats since there is no barrier in the heart. Therefore, DHA transport from blood to the retina and brain decreases with increasing blood d-glucose concentration because of prevention of DHA uptake by GLUT1 at the BRB and BBB. We previously reported that [14C]DHA uptake by a retinal endothelial cell line (TR-iBBR2 cells) expressing GLUT1 was inhibited by d-glucose in a concentration-dependent manner with a 50% inhibition of 5.56 mM.^{5} Although DHA transport via GLUT1 at the BRB and BBB is not completely inhibited under normal conditions, it is markedly inhibited under diabetic conditions. Indeed, the K_{in retina} and K_{in brain} of [14C]DHA in diabetic rats was reduced by 65.5% and 84.1%, respectively, compared with normal rats (Fig. 1). Badr et al. reported that streptozotocin-induced diabetic rats with a 8-week duration of diabetes exhibited reduced GLUT1 expression by >60% at the inner BRB compared with non-diabetic rats, but this resulted in no reduction in GLUT1 expression at the outer BRB and BBB.^{12} On the other hand, Pardridge et al. reported that streptozotocin-induced diabetic rats with a 1-week duration of diabetes exhibited reduced GLUT1 expression by 77% at the BBB compared with non-diabetic rats.^{13} Although down-regulation of GLUT1 expression is not constant under different experimental conditions, reduction of GLUT1 expression at the BRB and BBB cannot be ruled out as a contributor to DHA transport to the retina and brain in the present study. When the GLUT1 expression at the BBB was reduced by 77%, the GLUT1 transport activity at the BBB was only reduced by 44%.^{13} Therefore, hyperglycemia plays a role in reducing the supply of DHA to the brain and retina even although GLUT1 is down-regulated.

Since there is no compensatory down-regulation of GLUT1 at the inner BRB in an animal model and in patients with long-standing diabetes,^{14,15} reduction of DHA transport by hyperglycemia is more involved in the chronic hyperglycemia of long-standing diabetes than acute hyperglycemia. The total vitamin C concentration in the retina and brain is not changed in short-term diabetics, since AA/DHA could be recycled by glutathione.^{16,17} However, the chronic hyperglycemia of long-standing diabetes is associated with “glucose toxicity” leading to oxidative stress resulting from increased production of reactive oxygen species and, often, down-regulation of anti-oxidative defense mechanisms.^{18,19} Hyperglycemia reduces DHA transport via GLUT1 at the BRB and BBB. DHA is reconverted into AA in the retina and brain.^{4,5} Reduction of the supply of AA to the retina and brain through competition of d-glucose and DHA for a common transporter would reduce the antioxidant and could lead to accumulation of reactive oxygen species followed by acti-
vation of the protein kinase C and aldose reductase pathway and advanced glycation end-products in the retina and brain. Moreover, AA is a required cofactor for several intracellular hydroxylases, including proline hydroxylase and dopamine-β-hydroxylase. Therefore, chronic hyperglycemia appears to cause disregulation of catecholamine metabolism in the retina and brain. In the light of these findings, diabetic patients may experience enhanced oxidative stress and metabolic perturbations in the retina and brain by reducing the influx transport of DHA, leading to the hypothesis that diabetic retinopathy and encephalopathy involve a dysfunction of DHA influx transport at the BRB and BBB.

In conclusion, hyperglycemia reduces blood-to-retina and -brain transport of DHA across the BRB and BBB. These findings provide important information to help us understand the implications of increasing the antioxidant potential in the retina and brain.

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REFERENCES