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Research Report

Anti-oxidative nutrient rich diet protects against acute ischemic brain damage in rats

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ABSTRACT

We evaluated the neuroprotective effects of an anti-oxidative nutrient rich enteral diet (AO diet) that contained rich polyphenols (catechins and proanthocyanidins) and many other anti-oxidative ingredients. Wistar rats were treated with either vehicle, normal AO diet (containing 100 kcal/100 mL, catechin 38.75 mg/100 mL and proanthocyanidin 19 mg/100 mL, 1 mL/day), or high AO diet (containing 10 times the polyphenols of the normal AO diet) for 14 days, and were subjected to 90 min of transient middle cerebral artery occlusion. The AO diet improved motor function, reduced cerebral infarction volume, and decreased both peroxidative markers such as 4-hydroxynonenal, advanced glycation end products, 8-hydroxy-2-deoxyguanosine and inflammatory markers such as monocyte chemotactic protein-1, ionized calcium-binding adapter molecule-1, and tumor necrosis factor-α. Our study has shown that an AO diet has neuroprotective effects through both anti-oxidative and anti-inflammatory mechanisms, indicating that nutritional control with polyphenols could be useful for patients with acute ischemic stroke.

1. Introduction

Because ischemic stroke is a major cause of neurological disorders and death in the world (Truelsen et al., 2007), effective therapies for preventing cell death by cerebral blood flow restoration and neuroprotection are urged in the acute stage. Oxidative stress is one important factor that can aggravate ischemic brain damage during such an acute stage (Abe et al., 1995; Hayashi et al., 1999). After reperfusion of acute ischemic stroke, reactive oxygen species (ROS) are excessively generated, which promote apoptotic cell death through protein, lipid, and DNA peroxidation (Schaller and

Abbreviations: AGEs, advanced end glycation products; ANOVA, analysis of variance; AO, anti-oxidative; BP, blood pressure; CV, cresyl violet; DBP, diastolic blood pressure; h, hour; Iba-1, ionized calcium-binding adapter molecule 1; IR, ischemic-reperfusion; MCA, middle cerebral artery; MC, methylcellulose; MCP-1, monocyte chemotactic protein-1; min, minute; OCT, optimal cutting temperature; PBS, phosphate buffered saline; rCBF, regional cerebral blood flow; ROS, reactive oxygen species; SBP, systolic blood pressure; Sirt-1, sirtuin-1; tMCAO, transient middle cerebral artery occlusion; TNFα, tumor necrosis factor α; 4-HNE, 4-hydroxynonenal; 8-OHdG, 8-hydroxy-2-deoxyguanosine; W, week

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Graf, 2004; Zhang et al., 2004). Inflammation also plays an important role in acute ischemic stroke through activating macrophage and tumor necrosis factor-α (TNF-α) (Dirnagl et al., 1999). Thus, anti-oxidative and anti-inflammatory actions could be an indispensable strategy for ameliorating ischemic brain damage (Sun et al., 2002; Villegas et al., 2004).

We have previously reported that a free radical scavenger, edaravone, strongly reduced brain edema after cerebral ischemia in rats (Abe et al., 1988), which is not only in clinical use for stroke patients in Japan, but also currently showing good clinical effects in Europe (Kaste et al., 2013). Meanwhile, polyphenols are contained in many foods such as green tea (catechins), grape seed (proanthocyanidins), and red wine (resveratrol), which also show both anti-oxidative and anti-inflammatory effects (Landete, 2012). Polyphenols have been shown partly to prevent coronary heart disease, dementia, cancer, and arteriosclerosis (Ghosh and Scheepens, 2009). In the present study, therefore, we investigated whether an anti-oxidative nutrient rich enteral diet (AO diet) therapy could reduce ischemic rat brain damage through anti-oxidative and anti-inflammatory mechanisms after transient middle cerebral artery occlusion (tMCAO) in rats.

2. Results

2.1. Physiological parameters

Body weights were not significantly different among the three diet groups (vehicle, 270.5±12.2 g; normal AO diet, 280.5±4.6 g; high AO diet, 273.7±14.7 g). The time-dependent changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and regional cerebral blood flow (rCBF) are shown in Table 1. SBP (vehicle, 124.4±7.6 mmHg; normal AO diet, 121.5±7.8; high AO diet, 123.0±3.2) and DBP (vehicle, 95.7±4.4 mmHg; normal AO diet, 91.9±11.2; high AO diet, 91.8±6.8) before tMCAO showed no significant difference between the three diet groups. SBP 24 h after reperfusion also showed no significant difference between the three diet groups (vehicle, 151.2±6.5 mmHg; normal AO diet, 120.5±12.3; high AO diet, 135.4±4.2). However, DBP 24 h after reperfusion was significantly lower in the high AO diet group than the vehicle group (vehicle, 111.2±11.2 mmHg; normal AO diet, 99.5±6.3; high AO diet, 88.4±9.5, $p<0.05$ versus vehicle) (Fig. 1).

Although rCBF rates among the three diet groups were not significantly different, rCBF seemed to be higher in the two AO diet groups (vehicle, 79.5±36.1%; normal AO diet, 82.6±20.9; high AO diet, 91.2±27.3; Fig. 2).

2.2. Motor function and infarct volume

As compared to the vehicle group (2.4±0.6), Bederson scores showed an improvement in normal and high AO diet groups (normal AO diet, 1.7±0.7; $p<0.05$; high AO diet, 1.6±0.7, $p<0.01$; Fig. 3A).

Compared with the vehicle group (75.9±12.1 mm^3), the AO diet groups showed a reduction in the infarct volume (normal AO, 67.5±14.6 mm^3; p=n.s. versus vehicle) with significant reductions in the high AO diet group (54.6±10. 3 mm^3; $p<0.05$ versus vehicle; Fig. 3B). The examples of CV sections are shown in Fig. 3C.

2.3. Oxidative stress markers

Typical immunohistochemical stains for 4-hydroxynonenal (4-HNE), advanced end glycation products (AGEs), and 8-hydroxy-2-deoxyguanosine (8-OHdG) at the peri-ischemic areas are shown in Fig. 4. Compared with the vehicle group (4-HNE, 192.7±325/mm^2; AGEs, 243.2±30.1; 8-OHdG, 147.5±24.7), the normal AO diet group significantly reduced the number of positive cells for each of the three antibodies (4-HNE, 148.2±31.7/mm^2; AGEs, 157.3±21.1; 8-OHdG, 97.2±27.8; $p<0.01$ versus vehicle). The high AO diet group showed further reductions in the number of positive cells in 4-HNE (114.3±26.2/mm^2; $p<0.01$ versus vehicle), AGEs (121.8±32.7 mm^2; $p<0.01$ versus vehicle), and 8-OHdG (74.4±12.0/mm^2; $p<0.01$ versus vehicle).

Table 1 – Time-dependent changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and rCBF.

	Before tMCAO	During tMCAO	Just after reperfusion	24 h After repurfusion
Systolic blood pressure (mmHg)				
Vehicle	124.4±7.6			151.2±6.5
Normal AO	121.5±7.8			120.5±12.3
High AO	123.0±3.2			135.4±4.2
Diastolic blood pressure (mmHg)				
Vehicle	95.7±4.4			111.2±11.2
Normal AO	91.9±11.2			99.5±6.3
High AO	91.8±6.8			88.4±9.5*
Regional cerebral blood flow(%)				
Vehicle	100	55.4±20.3	91.6±36.6	79.5±36.1
Normal AO	100	65.1±21.1	82.8±24.4	82.6±20.9
High AO	100	66.3±29.3	89.0±14.5	91.2±27.3

Time dependent change in systolic blood pressure, diastolic blood pressure and rCBF. Diastolic blood pressure of high AO at 24 h after the reperfusion was significantly decreased.
* $p<0.05$ vs vehicle.