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Ischemic Neurodegeneration in Stroke-Prone Spontaneously Hypertensive Rats and Its Prevention with Antioxidants Such as Polyphenols

Kazuo Yamagata
Laboratory of Molecular Health Science of Food,
Department of Food Bioscience and Biotechnology,
College of Bioresource Sciences, Nihon University (NUBS),
Japan

1. Introduction

Stroke involves cerebral infarction and hemorrhaging and is associated with very high mortality. Previous reports have indicated that ischemic stimulation such as the reoxygenation that occurs after hypoxia produces a large quantity of reactive oxygen species (ROS) that strongly induces neuronal death *in vivo* and *in vitro* (Negishi et al., 2001). Indeed, this is considered to be the factor that most strongly induces cell death in cerebral ischemia. In recent years, apoptosis has been suggested to be the mechanism responsible for ischemic neuronal death in animal stroke models (Tagami et al., 1998).

Stroke-prone spontaneously hypertensive rats (SHRSP) are widely used as a model of human stroke (Yamori et al., 1974). In this model, blood pressure is elevated as age increases, as is found in humans; and the rats eventually die of stroke. One feature of this model is that strokes develop spontaneously following severe hypertension (more than 150 mmHg). Therefore, in SHRSP, because strokes develop after the onset of elevated blood pressure, elevated blood pressure is considered to be the most critical factor for stroke induction. However, interestingly, the neuronal cells of this model exhibit a great vulnerability compared with normal control WKY/Izm rats during the reoxygenation conditions following hypoxia (Tagami et al., 1998; Yamagata et al., 2010c). In addition to the influence of blood pressure in SHRSP/Izm rats, the neuronal vulnerability of this model strongly contributes to stroke development. SHRSP/Izm rats are susceptible to apoptosis under conditions of hypoxia and reoxygenation (H/R) (Tagami et al., 1998). The expression of antioxidant enzymes in SHRSP/Izm rats is attenuated in comparison with that in WKY/Izm rats. We highlight that this attenuation of antioxidant enzymes is related to the vulnerability of neuronal cells (Yamagata et al., 2000b). Furthermore, an altered susceptibility to apoptosis was detected in the astrocytes of SHRSP/Izm rats compared with those of WKY/Izm rats (Yamagata et al., 2010a).

Epidemiologic study indicated the possibility of preventing stroke using antioxidants such as dietary polyphenols (Vita, 2005). Polyphenols are substances produced by plants via photosynthesis, and their structures contain many hydroxyl groups (–OH). Polyphenols are found in vegetables, fruit, and processed products. They are also found abundantly in red wine, tea, soybeans, and coffee. The preventive effects of polyphenols include the inhibition of blood pressure elevation, cholesterol-lowering activity, hypoglycemic activity, antioxidant activity, and antimutagen activity (Sies et al., 2010). The effects of polyphenols differ between substances, but most are capable of "antioxidation". It is considered that the antioxidative effects of polyphenols are advantageous in their roles as defensive substances that protect plant components from oxidation. Polyphenols are found in trace amounts in our diet and have been demonstrated to prevent degenerative diseases such as cancer and cardiovascular disease (Manach et al., 2004). This review describes the vulnerability of neuronal cells and susceptibility of astrocytes in SHRSP in stroke conditions. Furthermore, we describe the prophylactic effects of apigenin, epigallocatechin-3-gallate (EGCG), and resveratrol on endothelial cells as well as their stroke preventive effects.

2. Susceptibility of neuronal cells and astrocytes of SHRSP/Izm rats during cerebral ischemia

The reoxygenation after cerebral ischemia rapidly generates a large quantity of ROS. The following chain of events leads to neuronal cell injury (Love, 1999). Free radicals are generated early in the period of the reperfusion and cause neuronal damage (Bolli, 1991). Cerebral ischemia–reperfusion induced neuronal cell death is usually apoptotic (Rothstein et al., 1994). Here, we describe alteration in neuronal cells and astrocytes related to apoptosis in SHRSP/Izm rats during H/R.

2.1 Neuronal vulnerability of SHRSP during stroke and oxidative stress

Neuronal death because of cerebral ischemic stress strongly induces apoptosis (Rothstein et al., 1994). Reports indicate that the production of hydroxyl radicals is strongly induced in SHRSP/Izm rats during H/R (Negishi et al., 2001). SHRSP/Izm and WKY/Izm rats produce hydroxyl radicals in their hippocampi when subjected to reoxygenation after 20 minutes of hypoxia. However, SHRSP/Izm rats display significantly increased hydroxyl production when compared with normal WKY/Izm control rats (Tagami et al., 1998). In SHRSP/Izm rats the production of hydroxyl radicals is strongly induced during H/R (Negishi et al., 2001). The increased levels of hydroxyl radicals produced by SHRSP/Izm rats may induce neuronal injury. These findings suggest that capturing the hydroxyl radicals produced during H/R, in which the level of antioxidant substances is decreased, would be beneficial for preventing neuronal injury (Yamagata et al., 2010c).

2.2 The neuronal cells of SHRSP/Izm rats strongly induce apoptosis during H/R

Neuronal cells are easily damaged during H/R. We examined neuronal cells during hypoxia using SHRSP/Izm and WKY/Izm rats. After 24 hours of hypoxia, neuronal cell death was not observed in WKY/Izm or SHRSP/Izm rats. However, after 36 hours of hypoxia, neuronal cell death increased in SHRSP/Izm rats. This was not observed in WKY/Izm rats. The findings of a morphologic examination of SHRSP/Izm rats indicated that most neuronal cell death was

apoptotic. About 41% of the WKY/Izm neurons died 1.5 hours after reoxygenation (necrosis = 12%, apoptosis = 29%). On the other hand, 78% of SHRSP/Izm neurons died (necrosis = 15%, apoptosis = 63%). Following three hours of reoxygenation, 99% of cells from both strains had died. In SHRSP/Izm rat neurons, fragmentation of DNA was strongly induced by 36 hours of hypoxia and reoxygenative stimulation for three hours (Tagami et al., 1998). The H/R induced apoptosis of neuronal cells in SHRSP/Izm rats (Yamagata et al., 2010c). The neuronal cells of SHRSP/Izm rats were strongly induced into apoptosis with 3 or 5 hours of reoxygenation following hypoxia. When DNA fragmentation was examined using a TUNEL method, few of the SHRSP/Izm rat neurons displayed DNA fragmentation when incubated under normal oxygen concentrations (data not shown). However, after 3 hours of reoxygenation following 36 hours of hypoxia, marked DNA fragmentation was seen. At the same time, many lipid droplets were detected in the cells (Tagami et al., 1998). We classified the apoptotic levels in H/R conditions via a morphologic analysis of neuronal death (Tagami et al., 1998, 1999). We demonstrated the criteria for neuronal apoptosis in the SHRSP/Izm rats in Table 1 and Figure 1. Neuronal axons and dendrites are lost in the early stages of apoptosis, and many lipid droplets are seen in the neuronal cell body (A, initial stage of apoptosis). Furthermore, cells shrink as apoptosis advances (B, second stage of apoptosis; C, third stage of apoptosis). The neuronal cell membrane is lost in the advanced stage of apoptosis, and the nucleus disappears (D). Figure 2 is considered to show the second stage of apoptosis (Tagami et al., 1998: Yamagata et al., 2010c). These processes eventually lead to cell death. From these results, it is suggested that the neuronal weakness of SHRSP/Izm rats is associated with stroke development (Fig. 4).

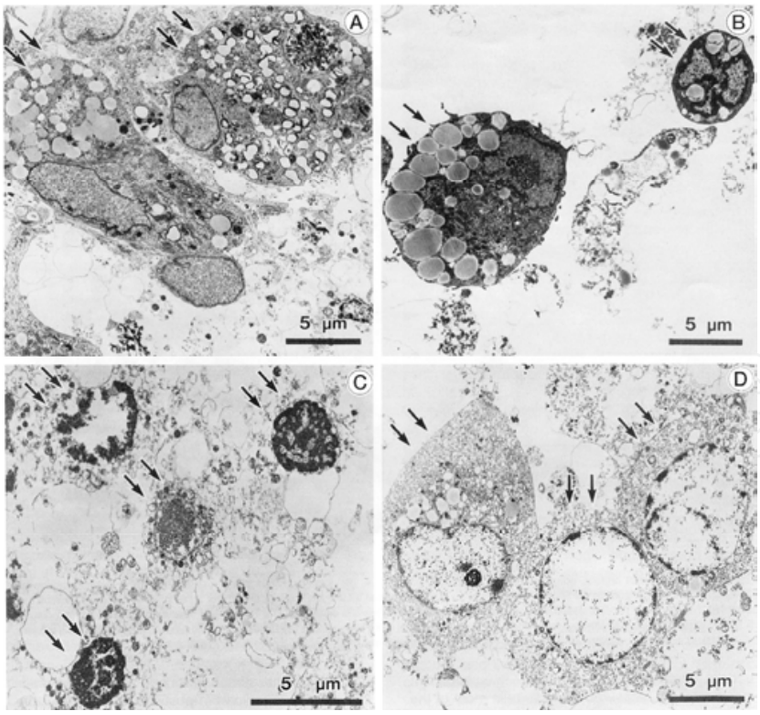


Fig. 1. Our criteria to determine apoptosis and necrosis in neurons during H/R in SHRSP/Izm rats.
A. initial stage, B, second stage, C. third stage and D necrosis (Tagami et al., 1998)

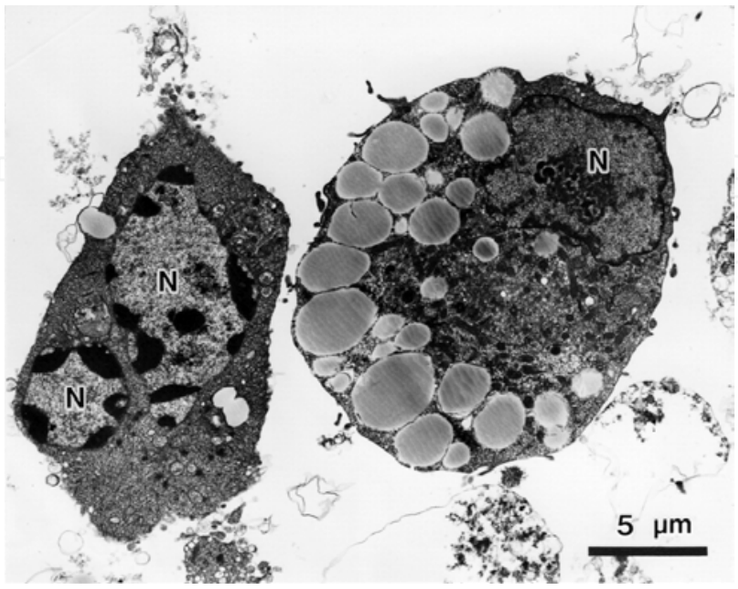


Fig. 2. Second stage of apoptosis in neurons during H/R in SHRSP/Izm rats. N: nucleus

Stage	Criteria of neuronal death	Features of morphological
1	Initial stage	The cells lose their axons and dendrites, and numerous lipid droplets appear in the cell bodies, although cell organelles remain intact
2	Second stage	The cells become round, small, and electron-dense, and their nuclei demonstrate prominent invagination
3	Advanced stage	The cells lose their cytoplasm and cell membrane, and their nuclei become small and dark before disappearing
4	Final stage	The cells become electron-lucent, organelles decrease in number, and nuclei contain abnormal clusters of chromatin (the cells lose their cytoplasm and cell membrane, and their nuclei become small and dark before disappearing)

Cited references (Tagami et al., 1998, 1999).

Table 1. The morphological criteria for neuronal apoptosis in the SHRSP/Izm rats.

2.3 Gene expression of Bcl_2 and thioredoxin II in neuronal cells of SHRSP/Izm rats during H/R

The apoptosis in neuronal cells of SHRSP/Izm is strongly induced by reperfusion after ischemia (Tagami et al., 1998). Simultaneously, oxidative stress can induce antioxidant enzymes in neuronal cells. Antioxidant enzymes can prevent the apoptosis caused by oxidation stress. Furthermore, the Bcl_2 gene is an oncogene related to human lymphoma and is able to inhibit the apoptosis induced by neurodegeneration stimuli (Akhtar et al, 2004). We highlight that the Bcl_2 gene expression in SHRSP/Izm rat neuronal cells is significantly attenuated after 30 minutes of reoxygenation following hypoxia in comparison with that in WKY/Izm rats (Yamagata et al., 2000b). The decrease in the expression of Bcl_2 leads to release of the cytochrome C from mitochondria. Thereafter, caspase activity increases and can strongly induce apoptosis. In SHRSP/Izm rat neurons, gene expression of thioredoxin II (Txn2) and mitochondrial cytochrome c oxidase III (CO III) decreased in a fashion similar to Bcl_2 30 minutes after reoxygenation following hypoxia (Yamagata et al., 2000b). Txn2 provides protection against ROS via its SH group. In addition, these proteins have many functions that contribute to intracellular signal transduction. Namely, CO III is associated with energy metabolism in mitochondria. It transfers electrons from the reduced form of cytochrome C to molecular oxygen. Vitamin E and CO III are present in mitochondria where they protect the cell from injury by free radicals (Yang & Korsmeyer, 1996). Attenuation of Bcl_2 and CO III gene expression in SHRSP/Izm rat neuronal cells may reduce energy metabolism and redox control during posthypoxic reoxygenation. The decrease of viability in SHRSP/Izm rat neurons, unlike that in WKY/Izm rat neurons, may be associated with their vulnerability.

2.4 Characteristics of SHRSP/Izm rat astrocytes during stroke

The functions of the astrocytes regulate outbreaks of cerebropathy (Chen & Swanson, 2003). In brain lesions, reactive astrocyte numbers increase and promote the development of stroke (Pekny & Nilsson, 2005). This characteristic of the astrocytes of SHRSP/Izm rats may be related to brain disease (Chen & Swanson, 2003). We separated astrocytes from the brain of fetal SHRSP/Izm rats and cultured them. We compared the proliferation of astrocytes from WKY/Izm with SHRSP/Izm rats under various culture conditions (Yamagata et al., 1995). The astrocytes isolated from fetuses are not influenced by blood pressure. We examined the characteristics of astrocytes from SHRSP/Izm rats in environments that were not influenced by blood pressure. We found that the growth of astrocytes from SHRSP/Izm rats was increased in comparison with those from WKY/Izm rats (Yamagata et al., 1995). We suggest that the numbers of astrocytes of the SHRSP/Izm rats are increased and that this strongly leads to the gliosis following damage. In the rat brain transient cerebral ischemia model, epidermal growth factor (EGF) receptor is related to mechanism of astrocyte reactivity. The details are not known, but astrocyte numbers of SHRSP/Izm rats may increase by cell division through EGF stimulation during the appearance of cerebral blood vessel pathogenesis. This proliferation of astrocytes is enhanced by vascular smooth muscle cells in SHRSP/Izm rats (Yamori et al., 1981). In fibrinoid necrosis degeneration by hypertension, the barrier function of endothelial cells diminishes and blood plasma components leak out of the circulation (Johansson, 1999). In SHRSP/Izm rats, there is denaturation of smooth muscle cells of the media, necrosis with

a rise in blood pressure, and destruction of the blood–brain barrier (BBB) in perforating branch arteries (Tagami et al.,1987). We have indicated the possibility that attenuated endothelial barrier functions might be induced by comparing the astrocytic potency of SHRS/Izm rats (Yamagata et al., 1997b).

Glutamate is released as a neurotransmitter by nerve terminals and activates astrocytes. Furthermore, glutamate uptake via a glutamate transporter in the cell membrane is mediated by astrocytes. Glutamate produces lactate in astrocytes and the lactate produced by astrocytes is supplied as an energy source to neuronal cells (Pellerin & Magistretti 1994). Concurrently, the lactate supplied by astrocytes is important for the recovery of the neuronal cells after ischemia (Schurr et al., 1997; Dringen et al., 1995). We demonstrated that there is decreased lactate produced in cultured astrocytes from SHRSP/Izm rats when compared with that from WKY/Izm rat astrocytes during hypoxia (Yamagata et al., 2000a). The decreased lactate production by SHRSP/Izm rat astrocytes may cause neuronal cell death through reduced energy supply.

Furthermore, we examined characteristics of SHRSP/Izm rat astrocytes during stroke. In H/R, the expression levels of intercellular adhesion molecule-1 (ICAM1), monocyte chemotactic protein-1 (MCP1), and vascular cell adhesion molecule-1 (VCAM1) in astrocytes from SHRSP/Izm rats were increased in comparison with that in astrocytes from WKY/Izm rats (Yamagata et al., 2010a). In addition, production of glial cell line-derived neurotrophic factor (GDNF) by adenosine, H_2O_2, glutamate, sphingosine-1-phosphate (S1P) was decreased during H/R in astrocytes from SHRSP/Izm rats in comparison with that from astrocytes from WKY/Izm rats (Yamagata et al., 2002; 2003; 2007a) (Fig. 3). Moreover, production of l-serine by nitric oxide (NO) stimulation decreased in SHRSP/Izm rats in comparison with that in WKY/Izm rats (Yamagata et al., 2006). Not all of the differences seen in SHRSP/Izm rats compared with WKY/Izm rats may be related to the generation of neuronal dysfunction in SHRSP/Izm rats. However, decreased astrocytic lactate and GDNF production may worsen energy conditions and nutrition status of SHRSP/Izm rat neurons (Yamagata et al., 2008). We suggest that attenuation of astrocyte functions accelerates neuronal cell death during stroke and may participate in its appearance (Fig. 4).

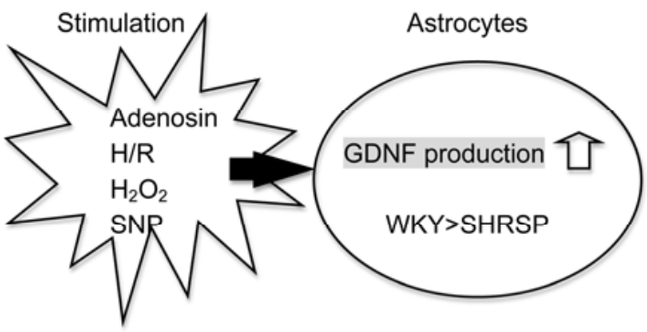


Fig. 3. Expression of GDNF in SHRSP/Izm rats by H/R stimulation.
H/R: hypoxia and reoxygenation: S1P: sphingosine-1-phosphate,
GDNF: glial cell line derived neurotrophic factor

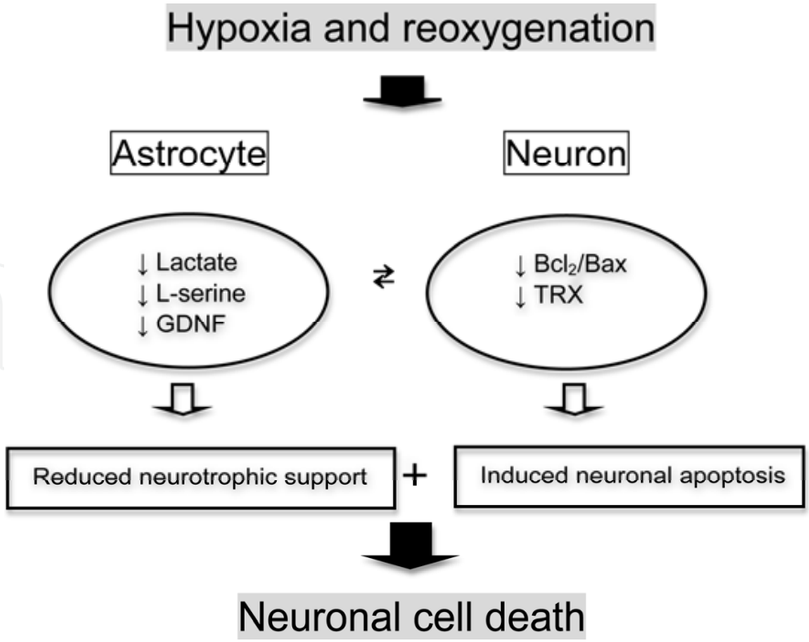


Fig. 4. Alteration of astrocytes and neuronal apoptosis by H/R stimulation.

3. Endothelial dysfunction and importance of stroke prevention through nutrition

The risk of stroke increases with the presence of arteriosclerosis in cerebral blood vessel endothelial cells. Here, we describe preventive action for endothelial cell disorders by food components. The secretion of cytokines by initial lesions strongly activates endothelial cells, vascular smooth muscle cells, and blood cells. For example, endothelial cells are strongly influenced by the effects of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin beta (IL-1β) (Kofler et al., 2005). As a consequence, monocytic adhesion to endothelial cells is induced, which promotes various arteriosclerotic processes. Among these, the oxidative stress produced during the early period of the disorder triggers arteriosclerosis. When the various arteriosclerotic reactions begin simultaneously, they are very difficult to inhibit. Therefore, it is best to inhibit ROS production in the early stages of the disorder in order to avoid arteriosclerosis (Kondo et al., 2009). Indeed, the effects of nutritional components with antioxidant activity on the redox regulation of ROS in stroke conditions have been reported previously. It is considered to be possible to inhibit blood vessel disorders in the early stages and that the inhibition of ROS production using polyphenols prevents the development of arteriosclerosis (Manach et al., 2004). Therefore, it is very likely that arteriosclerosis prevention via the consumption of appropriate foods such as antioxidant nutrients can be used to reduce the risk of stroke.

3.1 Possible role of polyphenols against cerebral ischemia injury

Cerebral ischemia induces the rapid production of a large quantity of ROS and induces cell injury through self-perpetuating reactions. Free radicals are produced within several minutes of reoxygenation after cerebral ischemia and induce brain cell injury (Bolli, 1991). Cerebral ischemia elevates the intracellular level of calcium ions and activates calcium-

dependent proteases. Moreover, these reactions activate xanthine dehydrogenase (XDH) and produce xanthine oxidase (XOD) (Thompson-Gorman & Zweier, 1990). It is considered that the superoxide anion radicals produced via this pathway cause neuronal death. However, the consumption of polyphenol-rich foods, such as fruits and vegetables, is beneficial for preventing vascular disorders (Manach et al., 2004). Epidemiological studies have indicated that an inverse correlation exists between polyphenolic consumption and the risk of having to undergo a cardiovascular procedure (Arts & Hollman, 2005). Polyphenols induce the production of vasodilatory factors such as NO (Auger et al., 2010) and prostacyclin (PGI_2) (Mizugaki et al., 2000) and inhibit the synthesis of endothelin-1, which induces vasoconstriction in endothelial cells (Reiter et al., 2010). On the other hand, the polyphenols present in the skin of grapes and in wine inhibit the proliferation and migration of smooth muscle cells (Lee et al., 2009). Polyphenols may eliminate the active oxygen produced by reoxygenation after cerebral ischemia via their antioxidative effects.

3.2 Vasorelaxant effects of polyphenols on endothelial cells

Epidemiological analysis has suggested that polyphenols have protective effects against heart disease. The polyphenols that protect against heart disease are found in foods including cocoa, wine, grape pips, berries, tea, tomatoes, soybeans, and pomegranates (Chong et al., 2010). The mechanisms by which polyphenols reduce the risk of heart disease are associated with the prevention of endothelial cell disorders. Endothelial cell disorders strongly induce arteriosclerosis, which subsequently progresses to heart disease and stroke. Therefore, the prevention of endothelial cell disorders by polyphenols is effective in preventing heart disease and stroke. Table 2 shows the effects of the typical polyphenols apigenin, EGCG, and resveratrol on endothelial cells. Jin et al. (2009) demonstrated that apigenin (0.5 – 72.0 μM) enhanced concentration-dependent relaxation in aortas. Apigenin action is mediated by weakening the oxidative stress and by NO reduction. On the other hand, it has been shown that stimulation of expression of endothelial NOS (eNOS) by apigenin occurs through phosphatidylinositol 3-kinase/Akt (PI3K/Akt) for Ca^{2+} dependence (Chen et al., 2010). Moreover, the blockade of adhesion of monocytes and cyclooxygenase (COX)-2 expression in endothelial cells by apigenin has been reported (Lee et al., 2007). We have shown that apigenin strongly inhibits high glucose- and TNF-α-induced VCAM1 expression and the adhesion of U937 in human endothelial cells (Yamagata et al., 2010b). These effects of apigenin are caused by the inhibition of Iionkinase (IKK) α and IKKε/IKKi. From these findings, we suggested that the mechanism by which apigenin inhibits the expression of adhesion molecules and the adhesion of monocytic U937 to endothelial cells involves nuclear factor kappa beta (NF-κβ). From the structure and inhibitory activity profiles of dietary flavonoids, it was recognized that the double bond found in the C-ring of flavonoids and the third hydroxyl group (A-ring) are required for the inhibition of VCAM1 gene expression (Yamagata et al., 2010b). Apigenin may inhibit monocytic adhesion caused by superoxide anions as well as block reductions in NO activity. From these reports, it is considered that apigenin reduces the levels of ROS, promotes NO activity, and inhibits cell adhesion. Moreover, apigenin strongly inhibited the TNF-α-stimulated expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) (Yamagata et al., 2011) and the double bond of the C ring of apigenin is essential for this action (Fig. 5). As shown in Figure. 5, the inhibition of LOX-1 expression by apigenin requires a flavone frame, a double bond in the C-ring, and the absence of a third hydroxyl group in the B- and C-rings, which are not found in naringenin (not active) (Yamagata et al., 2011).

Polyphenol(s)	Effects on pathological condition(s)	Ref (authors and issue)
Apigenin	Endothelium-dependent vasorelaxant and antiproliferative effect	Zhang et al (2000)
	Inhibiton of VEGF	Osada et al., (2004)
	Inhibiton of laser-induced choroidal neovascularization	Zou and Chiou (2006)
	Inhibition of COX-2 expression and adhesion of monocytes	Lee et al., (2007)
	Inhibition of superoxide anion-mediated impairment	Ma et al., (2008)
	Inhibition of platelet adhesion and thrombus formation	Navarro-Nunez et al., (2008)
	Protection against the oxidative stress by the NO	Jin et al., (2009)
	Induction of calcium dependent activation of the NO	Chen et al., (2010)
	Inhibition of high glucose and TNFα-induced adhesion molecule expression	Yamagata et al., (2010b)
	Inhibition of TNFα-induced LOX-1 expression	Yamagata et al., (2011)
EGCG	Increase of the prostacyclin production	Mizugaki et al., (2000)
	Inhibition of the vascular-endothelial growth factor-induced intracellular signaling and mitogenesis	Neuhaus et al., (2004)
	Inhibits the angiotensin II-induced adhesion molecule expression	Chae et al., (2007)
	Inhibiton of MCP-1 expression	Hong et al., (2007)
	Improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR	Potenza et al., (2007)
	Inhibiton of TNFα-induced MCP-1 production	Ahn et al., (2008)
	Protection against linoleic-acid-induced endothelial cell activation	Zheng et al., (2009)
	Decrease of caveolin-1 expression	Li et al., (2009)
	Decrease of endothelin-1 expression and secretion	Reiter et al., (2010)
	Induction of the NO	Auger et al., (2010)
	Protection of against oxidized LDL-induced endothelial dysfunction	Lee et al., (2010)
	Protects against oxidized LDL-induced endothelial dysfunction by inhibiting LOX-1-mediated signaling	Ou et al., (2010)
	Decreases thrombin/paclitaxel-induced endothelial tissue factor expression	Wang et al., (2010)
	inhibiton of angiotensin II-induced endothelial barrier dysfunction	Yang et al., (2010)

Resveratrol	Inhibition of angiogenesis, tumor growth, and wound healing	Brakenhielm et al., (2001)
	Prevention of superoxide-dependent inflammatory responses induced by I/R, PAF, or oxidants.	Shigematsu et al., (2003)
	Inhibition of VEGF-induced angiogenesis	Lin et al., (2003)
	Protection against peroxynitrite-mediated endothelial cell death	Brito et al., (2006)
	Attenuation of TNF alpha-induced activation; inhibition of NF-kappaB	Csiszar et al., (2006)
	Inhibition of MCP-1 synthesis and secretion	Cullen et al., (2007)
	Attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages.	Chow et al (2007)
	Prevention of concentric hypertrophy and diastolic impairment	Juric et al., (2007)
	Induction of NO production by increasing estrogen receptor alpha	Klinge et al., (2008)
	Induction of NADPH oxidases 1 and 4 mediate cellular senescence	Schilder et al., (2009)
	Reduces oxidative stress by modulating the gene expression of SOD1, GPx1 and Nox4	Spanier et al (2009)
	Decrease of mitochondrial oxidative stress	Ungvari et al., (2009)
	Prevention of hyperglycemia-induced endothelial dysfunction	Xu et al., (2009)
	Decrease of oxidized LDL-evoked LOX-1 signaling	Chang et al., (2011)
	Protecton of H 2O2-induced oxidative stress	Kao et al., (2010)
	Protecton of oxidized LDL-induced breakage of the blood-brain barrier	Lin et al., (2010)

COX; cyclooxygenase, GPx1; glutathione peroxidase 1, I/R; ischemia/reperfusion, LDL; low density lipoprotein, LOX-1; lectin-like oxidized low-density lipoprotein receptor-1, MCP-1; monocyte chemotactic protein-1, NO; nitric oxide, Nox4; NADPH oxidase subunit, PAF; platelet-activating factor, SOD1; superoxide dismutase 1, SHR, spontaneously hypertensive rats, TNF; tumor necrosis factor, VEGF; vascular endothelial growth factor,

Table 2. Studies on the protective effects of apigenin, EGCG and resveratrol in endothelial cells

EGCG is a catechin that is found in green tea. The catechins found in tea include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate

(EGCG), and the content order of these compounds is as follows: EGCG>EGC>ECG>EC. Catechins are also responsible for the bitter taste of green tea. Catechins account for around 13%–30% of the dry weight of tea leaves (Wolfram, 2007). EGCG suppresses the expression of adhesion molecules such as MCP1 (Ahn et al., 2008; Chae et al., 2007; Hong et al., 2007) and expression of endothelin-1 (Reiter et al., 2010). Like apigenin, EGCG inhibits the expression of monocyte adhesion molecules in endothelial cells stimulated with TNF-α (Ahn et al., 2008; Zheng et al., 2010) and it has been reported that EGCG inhibits the TNF-α-induced expression of activator protein-1 in endothelial cells and increased the expression of HO-1. These findings suggest that EGCG inhibits the expression of activator protein-1 and increases the expression of HO-1, both of which aid endothelial protection. Furthermore, a least one study demonstrated that EGCG downregulated the endothelial cell activation induced by linoleic acid via caveolin-1 (Zheng et al., 2009). Six hours of linoleate exposure induced the expression of caveolin-1 and COX-2 in caveolae. However, pretreatment with EGCG inhibited the expression of caveolin-1 and COX-2 induced by linoleic acid. Exposure to linoleic acid also increased the levels of several kinases (p38 MAPK, extracellular signal regulated kinase 1/2 8ERK1/2), and amino kinase terminal (Akt). According to these findings, EGCG activates several enzymes in endothelial cell caveolae and may have many preventive effects for vascular disorders.

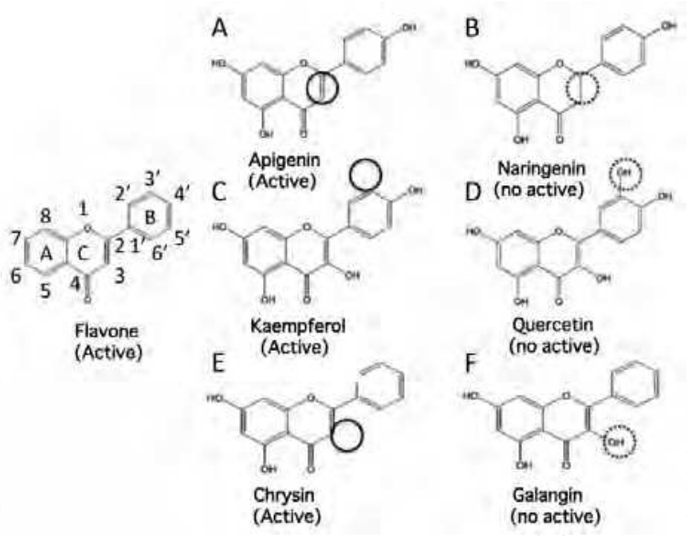


Fig. 5. Structures and LOX-1 inhibitory activities of apigenins.
○: active (indicates that the compound dose-dependently inhibited TNFα-induced LOX-1 gene expressions).

Many studies have demonstrated that ischemic heart disease is decreased by wine intake, and in particular, it has been shown that the antioxidative effects of the polyphenols found in red wine are important for cardioprotection. It was shown that this cardioprotective effect is caused by the actions of resveratrol. It has been confirmed that resveratrol displays various pharmacologic actions such as antioxidant activity in humans

(Brito et al., 2006; Chow et al., 2007; Spanier et al., 2009; Ungvari et al., 2009). Resveratrol is considered to decrease circulating low-density lipoprotein (LDL) cholesterol levels and thereby reduce the risk of cardiovascular disease (CVD) (Ramprasath & Jones, 2010). Resveratrol inhibits atherosclerosis and improves the function of endothelial cells in animal models. There have been many studies of resveratrol actions, which have shown that it has various effects on endothelial cells, as shown in Table 2. The effects of resveratrol and red wine on endothelial cells were investigated using experimental hypercholesterolemic rabbits (Zou et al., 2003). It was found that hypercholesterolemic rabbits displayed significant improvements in the functions of their endothelial cells after the administration of resveratrol (3 mg/kg/day), red wine (4 ml/kg/day), or nonalcoholic red wine (4 ml/kg/day) for 12 weeks. Moreover, they demonstrated decreased levels of plasma endothelin-1 and NO, which are increased by hypercholesterolemia. On the other hand, it was also shown that resveratrol protects against injury to the BBB caused by oxidized LDL (oxLDL) (Lin et al., 2010). It is considered that the mechanism behind these effects of resveratrol involves amelioration of the effects of oxLDL on the expression of occludin and ZO-1, which aids the stability of tight junctions. Resveratrol regulates the expression of tight junction proteins as a means of protecting against the disruption of the BBB induced by oxLDL. In a rat postischemic reoxygenation model, resveratrol decreased ROS generation (Shigematsu et al., 2003), and the effect of resveratrol on cerebral infarction was also examined in a rat middle cerebral artery occlusion (MCA) model (Sinha et al., 2002). In addition, after MCA and 2 hours of reperfusion, the rats were evaluated for motor disorders, malondialdehyde (MDA), reduced glutathione, and infarct volume. After MCA, increases in the frequency of functional motility disorders and the levels of MDA and reduced glutathione were observed. On the other hand, the administration of resveratrol prevented these increases and significantly decreased the infarct volume. These findings indicate that resveratrol inhibits the organ injuries produced by ischemia–reperfusion. The other polyphenols found in wine are not known to have this effect. Correspondingly, resveratrol prevents myocardial infarction by reducing peroxide levels. It is suggested that this effect can be attributed to the antioxidative effects of resveratrol (Dudley et al., 2008).

4. Preventive effects of antioxidant drugs and polyphenols for SHRSP rat neurons during stroke

We indicated that high dose vitamin E induced neutral gamma glutamylcystenyl synthase (γ-GCS), GSH levels, and strongly prevented neuronal death (Yamagata et al., 2009). Furthermore, we have shown that ebselen, a seleno–organic antioxidant (Yamagata et al., 2008), amlodipine, and carvedilol (Yamagata et al., 2004) prevented neuronal cell death in SHRSP/Izm rats. Another study demonstrated that the expression of VCAM1 by TNF-α in astrocytes isolated from SHRSP/Izm rats was increased compared with that in those from WKY/Izm rats. However, apigenin strongly attenuated TNF-α-induced VCAM1 mRNA and protein expression and suppressed the adhesion of U937 cells and SHRSP/Izm astrocytes (Yamagata et al., 2010a). It is suggested that apigenin regulates adhesion molecule expression in reactive astrocytes during ischemia and prevents neuronal death.

5. Conclusion

Endothelial cell dysfunction causes arteriosclerosis and promotes neuronal demise after stroke. Enhanced neuronal sensitivity to oxidative stress contributes to the neuronal death observed in SHRSP/Izm rats. Also, enhanced oxidative stress after hypoxia-reoxygenation is important in ischemic stroke. Polyphenols reduce oxidation stress and have a protective effect on endothelial and neuronal cells. Antioxidant nutrients such as polyphenols may prevent or reduce endothelial dysfunction and neuronal cell injury during cerebral ischemia.

6. Abbreviations

BBB; blood–brain barrier, CO III; cytochrome c oxidase III, COX; cyclooxygenase, CVD; cardiovascular disease, EC; epicatechin, ECG; epicatechin gallate, EGC; epigallocatechin, EGCG; epigallocatechin-3-gallate, EGF; epidermal growth factor, eNOS; endothelial NOS, γ-GCS; gamma glutamylcystenyl synthase, GDNF; glial cell line-derived neurotrophic factor, GSH: glutathione, HO-1; hemoxigenase-1, H/R; hypoxia and reoxygenation, ICAM1; intercellular adhesion molecule-1, IL-1β; interleukin beta, IKK; IIKKkinase, LDL; low-density lipoprotein, LOX-1; lectin-like oxidized low-density lipoprotein receptor-1, MCP1; monocyte chemotactic protein-1, NO; nitric oxide, NF-κβ; nuclear factor kappa beta, oxLDL; oxidized LDL, PGI_2; prostacyclin, PI3K/Akt; phosphatidylinositol 3-kinase/Akt, ROS; reactive oxygen species, SHRSP/Izm; spontaneously hypertensive rats/Izm, S1P; sphingosine-1-phosphate, TNF-α; tumor necrosis factor alpha, TRX; thioredoxin, VCAM1; vascular cell adhesion molecule-1, WKY/Izm; Wistar Kyoto rat/Izm, XDH; xanthine dehydrogenase, XOD; xanthine oxidase.

Keywords; Endothelial cells, Ischemic stroke, Polyphenol, SHRSP.

7. References

Ahn, H.Y., Xu, Y. & Davidge, S.T. (2008). Epigallocatechin-3-O-gallate inhibits TNFalpha-induced monocyte chemotactic protein-1 production from vascular endothelial cells. Life Sci 82 (17-18): 964-968.

Akhtar, R.S., Ness, J.M. & Roth, K.A. (2004). Bcl-2 family regulation of neuronal development and neurodegeneration. Biochim Biophys Acta 1644(2-3): 189-203.

Arts, I.C., & Hollman, P.C. (2005). Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 81 (Suppl 1): 317S-325S.

Auger, C., Kim, J.H., Chabert, P., Chaabi, M., Anselm, E., Lanciaux, X., Lobstein, A. & Schini-Kerth, V.B. (2010). The EGCg-induced redox-sensitive activation of endothelial nitric oxide synthase and relaxation are critically dependent on hydroxyl moieties. Biochem Biophys Res Commun 393 (1): 162-167.

Bolli, R. (1991). Oxygen-derived free radicals and myocardial reperfusion injury: an overview. Cardiovasc Drugs Ther (Suppl 2): 249-268.

Bråkenhielm, E., Cao, R. & Cao, Y. (2001). Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. FASEB J 15 (10): 1798-1800.

Brito, P.M., Mariano, A., Almeida, L.M. & Dinis, T.C. (2006). Resveratrol affords protection against peroxynitrite-mediated endothelial cell death: A role for intracellular glutathione. Chem Biol Interact 164 (3): 157-166.

Chae, Y.J., Kim, C.H., Ha, T.S., Hescheler, J. & Ahn, H.Y. & Sachinidis A. (2007). Epigallocatechin-3-O-gallate inhibits the angiotensin II-induced adhesion molecule expression in human umbilical vein endothelial cell via inhibition of MAPK pathways. Cell Physiol Biochem 20 (6); 859-866.

Chang, H.C., Chen, T.G., Tai, Y.T., Chen, T.L., Chiu, W.T. & Chen, R.M. (2011). Resveratrol attenuates oxidized LDL-evoked Lox-1 signaling and consequently protects against apoptotic insults to cerebrovascular endothelial cells. J Cereb Blood Flow Metab 31 (3): 842-854.

Chen, C.C., Ke, W.H., Ceng, L.H., Hsieh, C.W. & Wung, B.S. (2010). Calcium- and phosphatidylinositol 3-kinase/Akt-dependent activation of endothelial nitric oxide synthase by apigenin. Life Sci 87 (23-26); 743-739.

Chen, Y., & Swanson, R.A. (2003). Astrocytes and brain injury. J Cereb Blood Flow Metab 23 (2): 137-149.

Chong, M.F., Macdonald, R. & Lovegrove, J.A. (2010). Fruit polyphenols and CVD risk: a review of human intervention studies. Br J Nutr 104 (Suppl 3): S28-S39.

Chow, S.E., Hshu, Y.C., Wang, J.S. & Chen, J.K. (2007). Resveratrol attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages. J Appl Physiol 102 (4): 1520-1527.

Csiszar, A., Smith, K., Labinskyy, N., Orosz, Z., Rivera, A. & Ungvari, Z. (2006). Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition. Am J Physiol Heart Circ Physiol 291 (4): H1694-H1699.

Cullen, J.P., Morrow, D., Jin, Y., Curley, B., Robinson, A., Sitzmann, J.V., Cahill, P.A. & Redmond, E.M. (2007). Resveratrol, a polyphenolic phytostilbene, inhibits endothelial monocyte chemotactic protein-1 synthesis and secretion. J Vasc Res 44 (1): 75-84.

Dringen R, Peters H, Wiesinger H, Hamprecht B. (1995). Lactate transport in cultured glial cells. Dev Neurosci 17 (2): 63-69.

Dudley, J.I., Lekli, I., Mukherjee, S., Das, M., Bertelli, A.A. & Das, D.K. (2008). Does white wine qualify for French paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol. J Agric Food Chem 56 (20); 9362-9373.

Hong, M.H., Kim, M.H., Chang, H.J., Kim, N.H., Shin, B.A., Ahn, B.W. & Jung, Y.D. (2007). (-)-Epigallocatechin-3-gallate inhibits monocyte chemotactic protein-1 expression in endothelial cells via blocking NF-kappaB signaling. Life Sci 80 (21): 1957-1965.

Jin, B.H., Qian, L.B., Chen, S., Li, J., Wang, H.P., Bruce, I.C., Lin, J, & Xia, Q. (2009). Apigenin protects endothelium-dependent relaxation of rat aorta against oxidative stress. Eur J Pharmacol 616 (1-3): 200-205.

Johansson, B.B. (1999). Hypertension mechanisms causing stroke. Clin Exp Pharmacol Physiol 26 (7): 563-565.

Juric, D., Wojciechowski, P., Das, D.K. & Netticadan, T. (2007). Prevention of concentric hypertrophy and diastolic impairment in aortic-banded rats treated with resveratrol. Am J Physiol Heart Circ Physiol 292 (5): H2138-H2143.

Kao, C.L., Chen, L.K., Chang, Y.L., Yung, M.C., Hsu, C.C., Chen, Y.C., Lo, W.L., Chen, S.J., Ku, H.H. & Hwang, S.J. (2010). Resveratrol protects human endothelium from H(2)O(2)-induced oxidative stress and senescence via SirT1 activation. J Atheroscler Thromb 17 (9); 970-979.

Klinge, C.M., Wickramasinghe, N.S., Ivanova, M.M. & Dougherty, S.M. (2008). Resveratrol stimulates nitric oxide production by increasing estrogen receptor alpha-Src-

caveolin-1 interaction and phosphorylation in human umbilical vein endothelial cells. FASEB J 22 (7): 2185-2197.

Kofler, S., Nickel, T. & Weis, M. (2005). Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. Clin Sci (Lond) 108 (3): 205-513.

Kondo, T., Hirose, M. & Kageyama, K. (2009). Roles of oxidative stress and redox regulation in atherosclerosis. J Atheroscler Thromb 16 (5): 532-528.

Lee, B., Lee, E.J., Kim, D.I., Park, S.K., Kim, WJ. & Moon, S.K. (2009). Inhibition of proliferation and migration by piceatannol in vascular smooth muscle cells. Toxicol In Vitro 23 (7): 1284-1291.

Lee, J.H., Zhou, H.Y., Cho, S.Y., Kim, Y.S., Lee, Y.S. & Jeong, C.S. (2007). Anti-inflammatory mechanisms of apigenin: inhibition of cyclooxygenase-2 expression, adhesion of monocytes to human umbilical vein endothelial cells, and expression of cellular adhesion molecules. Arch Pharm Res 30 (10): 1318-1327.

Lee, Y.W., Lee, W.H. & Kim, P.H. (2010). Role of NADPH oxidase in interleukin-4-induced monocyte chemoattractant protein-1 expression in vascular endothelium. Inflamm Res 59 (9): 755-765.

Li, Y., Ying, C., Zuo, X., Yi, H., Yi, W., Meng, Y., Ikeda, K., Ye, X., Yamori, Y. & Sun, X. (2009). Green tea polyphenols down-regulate caveolin-1 expression via ERK1/2 and p38MAPK in endothelial cells. J Nutr Biochem 20 (12): 1021-1027.

Lin, M.T., Yen, M.L., Lin, C.Y. & Kuo, M.L. (2003). Inhibition of vascular endothelial growth factor-induced angiogenesis by resveratrol through interruption of Src-dependent vascular endothelial cadherin tyrosine phosphorylation. Mol Pharmacol 64 (5): 1029-1036.

Lin, Y.L., Chang, H.C., Chen, T.L., Chang, J.H., Chiu, W.T., Lin, J.W. & Chen. R.M. (2010). Resveratrol protects against oxidized LDL-induced breakage of the blood-brain barrier by lessening disruption of tight junctions and apoptotic insults to mouse cerebrovascular endothelial cells. J Nutr 140 (12): 2187-2192.

Love, S. (1999). Oxidative stress in brain ischemia. Brain Pathol 9 (1): 119-131.

Ma, X., Li, Y.F., Gao, Q., Ye, Z.G., Lu, X.J., Wang, H.P., Jiang, H.D., Bruce, I.C. & Xia, Q. (2008). Inhibition of superoxide anion-mediated impairment of endothelium by treatment with luteolin and apigenin in rat mesenteric artery. Life Sci 83 (3-4): 110-117.

Manach, C., Scalbert, A., Morand. C., Remesy, C. & Jimenez, L. (2004). Polyphenols: food sources and bioavailability. Am J Clin Nutr 79 (5): 727-747.

Mizugaki, M., Ishizawa, F., Yamazaki, T. & Hishinuma T. (2000). Epigallocatechin gallate increase the prostacyclin production of bovine aortic endothelial cells. Prostaglandins Other Lipid Mediat 62 (2): 157-164.

Navarro-Nunez, L., Lozano, M.L., Palomo, M., Martinez, C., Vicente, V., Castillo, J., Benavente-Garcia, O., Diaz-Ricart, M., Escolar, G. & Rivera, J. (2008). Apigenin inhibits platelet adhesion and thrombus formation and synergizes with aspirin in the suppression of the arachidonic acid pathway. J Agric Food Chem 56 (9): 2970-2096.

Negishi, H., Ikeda, K., Nara. Y. & Yamori, Y. (2001). Increased hydroxyl radicals in the hippocampus of stroke-prone spontaneously hypertensive rats during transient ischemia and recirculation. Neurosci Lett 306 (3): 206-208.

Neuhaus, T., Pabst, S., Stier, S., Weber, A.A., Schrör, K., Sachinidis, A., Vetter, H. & Ko, Y.D. (2004). Inhibition of the vascular-endothelial growth factor-induced intracellular signaling and mitogenesis of human endothelial cells by epigallocatechin-3 gallate. Eur J Pharmacol 483 (2-3): 223-227.

Osada, M., Imaoka, S. & Funae, Y. (2004). Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1alpha protein. FEBS Lett 575 (1-3): 59-63.

Ou, H.C., Song, T.Y., Yeh, Y.C., Huang, C.Y., Yang, S.F., Chiu, T.H., Tsai, K.L., Chen, K.L., Wu, Y.J., Tsai, C.S., Chang, L.Y., Kuo, W.W. & Lee, S.D. (2010). EGCG protects against oxidized LDL-induced endothelial dysfunction by inhibiting LOX-1-mediated signaling. J Appl Physiol 108 (6): 1745-1756.

Potenza, M.A., Marasciulo, F.L., Tarquinio, M., Tiravanti, E., Colantuono, G., Federici, A., Kim, J.A., Quon, M.J. & Montagnani, M. (2007). EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 292 (5): E1378-E1387.

Pekny, M. & Nilsson, M. (2005). Astrocyte activation and reactive gliosis. Glia 50 (4): 427-434.

Pellerin, L. & Magistretti, J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci USA 91 (22): 10625-10629.

Ramprasath, V.R. & Jones, P.J. (2010). Anti-atherogenic effects of resveratrol. Eur J Clin Nutr 64 (7): 660-668.

Reiter, C.E., Kim, J.A. & Quon, M.J. (2010). Green tea polyphenol epigallocatechin gallate reduces endothelin-1 expression and secretion in vascular endothelial cells: roles for AMP-activated protein kinase, Akt, and FOXO1. Endocrinology 151 (1): 103-114.

Rothstein, J.D., Bristol, L.A., Hosler, B., Brown, R.H. Jr. & Kuncl R.W. (1994). Chronic inhibition of superoxide dismutase produces apoptotic death of spinal neurons. Proc Natl Acad Sci USA 91 (10): 4155-4159.

Schurr, A., Payne, R.S., Miller, J.J. & Rigor, B.M. (1997). Glia are the main source of lactate utilized by neurons for recovery of function posthypoxia. Brain Res 774 (1-2): 221-224.

Schilder, Y.D., Heiss, E.H., Schachner, D., Ziegler, J., Reznicek, G., Sorescu, D. & Dirsch, V.M. (2009). NADPH oxidases 1 and 4 mediate cellular senescence induced by resveratrol in human endothelial cells. Free Radic Biol Med 46 (12): 1598-1606.

Shigematsu, S., Ishida, S., Hara, M., Takahashi, N., Yoshimatsu, H., Sakata, T. & Korthuis R.J. (2003) Resveratrol, a red wine constituent polyphenol, prevents superoxide-dependent inflammatory responses induced by ischemia/reperfusion, platelet-activating factor, or oxidants. Free Radic Biol Med 34 (7): 810-817.

Sies, H. Polyphenols and health: update and perspectives. (2010). Arch Biochem Biophys 501 (1): 2-5.

Sinha K, Chaudhary G, Gupta YK. (2002). Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. Life Sci 71 (6): 655-665.

Spanier ,G., Xu, H., Xia, N., Tobias, S., Deng, S., Wojnowski, L., Forstermann, U. & Li, H. (2009). Resveratrol reduces endothelial oxidative stress by modulating the gene expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1) and NADPH oxidase subunit (Nox4). J Physiol Pharmacol 60 (Suppl 4): 111-116.

Tagami, M., Nara, Y., Kubota, A., Sunaga, T., Maezawa, H., Fujino, H. & Yamori, Y. (1987). Ultrastructural characteristics of occluded perforating arteries in stroke-prone spontaneously hypertensive rats. Stroke 18 (4): 733-740.

Tagami, M., Yamagata, K., Ikeda, K., Nara, Y., Fujino, H., Kubota, A., Numano, F, & Yamori, Y. (1998). Vitamin E prevents apoptosis in cortical neurons during hypoxia and oxygen reperfusion. Lab Invest 78 (11): 1415-1429.

Tagami, M., Yamagata, K., Ikeda, K., Fujino, H., Nara, Y., Nakagawa, K., Kubota, A., Numano, F. & Yamori, Y. (1999). Genetic vulnerability of cortical neurons isolated from stroke-prone spontaneously hypertensive rats in hypoxia and oxygen reperfusion. Hypertens Res 22 (1): 23-29.

Thompson-Gorman, S.L. & Zweier J.L. (1990). Evaluation of the role of xanthine oxidase in myocardial reperfusion injury. J Biol Chem 265 (12): 6656-6663.

Ungvari, Z., Labinskyy, N., Mukhopadhyay, P., Pinto, J.T., Bagi, Z., Ballabh, P., Zhang, C., Pacher, P. & Csiszar, A. (2009). Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. Am J Physiol Heart Circ Physiol 297 (5): H1876-H1881.

Vita, J.A. (2005). Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr 81 (Suppl1): 292S-297S.

Wang, H.J., Lo, W.Y., Lu, T.L. & Huang, H. (2010). (-)-Epigallocatechin-3-gallate decreases thrombin/paclitaxel-induced endothelial tissue factor expression via the inhibition of c-Jun terminal NH2 kinase phosphorylation. Biochem Biophys Res Commun 391 (1): 716-721.

Wolfram, S. (2007). Effects of green tea and EGCG on cardiovascular and metabolic health. J Am Coll Nutr 26 (4): 373S-388S.

Xu, Q., Hao, X., Yang, Q. & Si, L. (2009). Resveratrol prevents hyperglycemia-induced endothelial dysfunction via activation of adenosine monophosphate-activated protein kinase. Biochem Biophys Res Commun 388 (2): 389-394.

Yang, D., Liu, J., Tian, C., Zeng, Y., Zheng, Y.H., Fang, Q. & Li, H.H. (2010). Epigallocatechin gallate inhibits angiotensin II-induced endothelial barrier dysfunction via inhibition of the p38 MAPK/HSP27 pathway. Acta Pharmacol Sin 31 (10): 1401-1406.

Yamagata, K., Nara, Y., Tagami, M. & Yamori, Y. (1995). Demonstration of hereditarily accelerated proliferation in astrocytes derived from spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 22 (9): 605-609.

Yamagata, K., Tagami, M., Ikeda, K., Noguchi, T., Yamori, Y. & Nara, Y. (2000a). Reduced production of lactate during hypoxia and reoxygenation in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Neurosci Lett 296 (2-3): 113-116.

Yamagata, K., Tagami, M., Ikeda, K., Yamori, Y. & Nara, Y. (2000b). Altered gene expressions during hypoxia and reoxygenation in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. Neurosci Lett 284 (3): 131-134.

Yamagata, K., Tagami, M., Torii, Y., Takenaga, F., Tsumagari, S., Itoh, S., Yamori, Y. & Nara, Y. (2003). Sphingosine 1-phosphate induces the production of glial cell line-derived neurotrophic factor and cellular proliferation in astrocytes. Glia 41 (2): 199-206.

Yamagata, K., Tagami, M., Ikeda, K., Tsumagari, S., Yamori, Y. & Nara, Y. (2002). Differential regulation of glial cell line-derived neurotrophic factor (GDNF) mRNA expression during hypoxia and reoxygenation in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Glia 37(1): 1-7.

Yamagata, K., Ichinose, S. & Tagami, M. (2004). Amlodipine and carvedilol prevent cytotoxicity in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. Hypertens Res 27(4): 271-282.

Yamagata, K., Shoji, Y., Terashima, T. & Yokogoshi, H. (2006). Glutamate reduces secretion of l-serine in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Neuroscience 143 (3): 729-737.

Yamagata, K., Hakata, K., Maeda, A., Mochizuki, C., Matsufuji, H., Chino, M. & Yamori Y. (2007a). Adenosine induces expression of glial cell line-derived neurotrophic factor (GDNF) in primary rat astrocytes. Neurosci Res 59 (4): 467-474.

Yamagata, K., Tagami, M., Nara, Y., Fujino, H., Kubota, A., Numano, F., Kato, T. & Yamori Y. (1997b). Faulty induction of blood-brain barrier functions by astrocytes isolated from stroke-prone spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 24 (9-10): 686-691.

Yamagata, K., Tagami, M. & Yamori, Y. (2008). Nitric oxide reduces astrocytic lactate production and induces neuronal vulnerability in stroke-prone spontaneously hypertensive rats. Glia 56 (4): 387-393.

Yamagata, K. Ichinose, S., Tagawa, C. & Tagami, M. (2009). Vitamin E regulates SMase activity, GSH levels, and inhibits neuronal death in stroke-prone spontaneously hypertensive rats during hypoxia and reoxygenation. J Exp Stroke Transl Med 1 (2): 41-48.

Yamagata, K., Kitazawa, T., Shinoda, M., Tagawa, C., Chino, M. & Matsufuji. H. (2010a). Stroke status evoked adhesion molecule genetic alterations in astrocytes isolated from stroke-prone spontaneously hypertensive rats and the apigenin inhibition of their expression. Stroke Res Treat pii:386389. 1-11.

Yamagata, K., Miyashita, A., Matsufuji, H. & Chino, M. (2010b). Dietary flavonoid apigenin Inhibits high glucose and tumor necrosis factor alpha-induced adhesion molecule expression in human endothelial cells. J Nutr Biochem 21 (2): 116-124.

Yamagata, K., Tagami, M., & Yamori, Y. (2010c). Neuronal vulnerability of stroke-prone spontaneously hypertensive rats to ischemia and its prevention with antioxidants such as vitamin E. Neuroscience 170 (1): 1-7.

Yamagata, K., Miyashita, A., Chino, M. & Matsufuji, H. (2011). Apigenin inhibits tumor necrosis factor alpha plus high glucose-induced LOX-1 expression in human endothelial cells. Microvasc Res 81 (1): 60-67.

Yamori, Y. (1974). Importance of genetic factors in hypertensive cerebrovascular lesions: an evidence obtained by successive selective breeding of stroke-prone and-resistant SHR. Jpn Circ J 38 (12): 1095-1100.

Yamori, Y., Igawa, T., Kanbe, T., Kihara, M., Nara, Y. & Horie, R. Clin Sci (Lond) (1981). Mechanisms of structural vascular changes in genetic hypertension: analyses on cultured vascular smooth muscle cells from spontaneously hypertensive rats. 61 Suppl (7): 121s-123s.

Yang, E. & Korsmeyer, S.J. (1996). Molecular thanatopsis: a discourse on the BCL2 family and cell death. Blood 88 (2): 386-401.

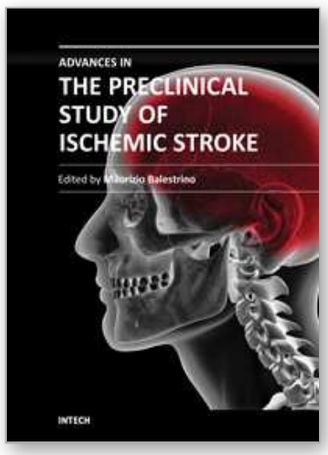
Zhang, Y.H., Park, Y.S., Kim. T.J., Fang, L.H., Ahn, H.Y., Hong, J.T., Kim, Y., Lee, C.K. & Yun, Y.P. (2000). Endothelium-dependent vasorelaxant and antiproliferative effects of apigenin. Gen Pharmacol 35 (6): 341-347.

Zheng, Y., Lim, E.J., Wang, L., Smart, E.J., Toborek, M. & Hennig, B. (2009). Role of caveolin-1 in EGCG-mediated protection against linoleic-acid-induced endothelial cell activation. J Nutr Biochem 20 (3): 202-209.

Zheng, Y., Toborek, M. & Hennig, B. (2010). Epigallocatechin gallate-mediated protection against tumor necrosis factor-α-induced monocyte chemoattractant protein-1 expression is heme oxygenase-1 dependent. Metabolism 59 (10): 1528-1535.

Zou, Y., & Chiou, G.C. (2006). Apigenin inhibits laser-induced choroidal neovascularization and regulates endothelial cell function. J Ocul Pharmacol Ther 22 (6): 425-430.

Zou, J.G., Wang, Z.R., Huang, Y.Z., Cao, K.J. & Wu, J.M. (2003). Effect of red wine and wine polyphenol resveratrol on endothelial function in hypercholesterolemic rabbits. Int J Mol Med 11 (3): 317-320.



Advances in the Preclinical Study of Ischemic Stroke
Edited by Dr. Maurizio Balestrino

ISBN 978-953-51-0290-8
Hard cover, 530 pages
Publisher InTech
Published online 16, March, 2012
Published in print edition March, 2012

This book reports innovations in the preclinical study of stroke, including - novel tools and findings in animal models of stroke, - novel biochemical mechanisms through which ischemic damage may be both generated and limited, - novel pathways to neuroprotection. Although hypothermia has been so far the sole "neuroprotection" treatment that has survived the translation from preclinical to clinical studies, progress in both preclinical studies and in the design of clinical trials will hopefully provide more and better treatments for ischemic stroke. This book aims at providing the preclinical scientist with innovative knowledge and tools to investigate novel mechanisms of, and treatments for, ischemic brain damage.

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University Campus STeP Ri
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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