Hypoxia-Inducible Factor and Vascular Endothelial Growth Factor Are Targets of Dietary Soy During Acute Stroke in Female Rats

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Dietary soy and soy isoflavones are neuroprotective in experimental cerebral ischemia. Because these isoflavones have estrogenic properties, we hypothesized that, like estrogens, they would inhibit acute vascular injury and the detrimental acute increase in hypoxia-induced vascular endothelial growth factor (VEGF) that leads to cerebral edema after stroke. Mature ovariectomized female Sprague Dawley rats were fed soy-free or soy-containing diets for 4 weeks followed by 90 minutes of transient middle cerebral artery occlusion. Similar to estrogens, dietary soy significantly reduced cerebral edema and vascular apoptosis 24 hours after stroke. Soy also inhibited the ischemia-induced increase in cortical VEGF and VEGF receptor (VEGFR)-2 protein expression observed 4 and 24 hours after stroke, although mRNA levels increased. The reduction in VEGF/VEGFR-2 was associated both with decreases in receptor phosphorylation and signaling to AKT and endothelial nitric oxide synthase. Furthermore degradation of the VEGFR-2 was increased with dietary soy. The primary ischemic stimulus for VEGF, hypoxia-inducible factor 1α (HIF1α), was similarly reduced by dietary soy 4 hours after transient middle cerebral artery occlusion in both the cortex and striatum. The inhibition of HIF1α activity was further confirmed by a significant decrease in the HIF1α-activated apoptotic mediator BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (Nip3-like protein X). These data suggest that soy isoflavones target events early in the ischemic cascade as part of their neuroprotective actions and counterbalance some of the detrimental effects of the endogenous response to cerebral injury. (Endocrinology 154: 1589–1597, 2013)

**V**ascular endothelial growth factor (VEGF) exhibits 2 opposing roles in the brain's response to cerebral ischemia. Although VEGF promotes angiogenesis and protects both the vasculature and neurons, these effects are counterbalanced by increased vascular permeability leading to edema (1). Thus, VEGF administration during the acute phase of cerebral ischemia leads to increased edema, increased infarct volume, poor neurological outcomes, and increased mortality (2). In contrast, delayed administration of VEGF (>24 h after ischemia) improves these measures (2–4) and enhances neurogenesis (5). In agreement with these findings, inhibition of VEGF before or during the acute phase of cerebral ischemia is protective (6–8), but inhibition of VEGF at later time points is detrimental (7, 9). Similarly, knockdown of hypoxia-inducible factor 1α (HIF1α) to decrease VEGF levels 1 hour, but not 3 hours, after stroke is associated with better outcomes (10). These data suggest that transient inhibition of endogenous VEGF signaling during the acute phase of stroke is beneficial.

Diets high in soy phytoestrogens or isolated phytoestrogen isoflavones are neuroprotective in experimental stroke (11–14). In rats, this neuroprotection is similar to that observed with low doses of estradiol and includes...
reduced infarct size, decreased caspase-dependent and caspase-independent neuronal apoptosis, increased B-cell lymphoma 2 (Bcl2) and Bcl-XL expression, and inhibition of oxidative stress (11–13). At low doses, soy phytoestrogens stimulate VEGF secretion in breast cancer cells via estrogen receptors (15). In contrast, higher doses of soy phytoestrogens (>10 μM) inhibit VEGF expression in neuroblastoma cells (16), ovarian cancer cells (17), and prostate cancer cells (18). There is also evidence that the soy isoflavone genistein can enhance endothelial barrier function in vitro (19) and in vivo (20). However, the role of soy phytoestrogens in the regulation of VEGF in the brain, particularly in response to injury, is not known.

In the present study, we sought to determine whether dietary soy altered VEGF signaling in the rat brain in response to cerebral ischemia and whether this was associated with beneficial or detrimental effects on the cerebral vasculature during the acute phase of ischemic injury.

Materials and Methods

Animals and treatments

The Medical College of Georgia or University of North Texas Health Science Center Institutional Animal Care and Use Committees approved all animal protocols. Adult female Sprague Dawley rats (200-225 g; Harlan, Indianapolis, Indiana) were maintained in a temperature-controlled room (22°C–25°C) with 12-hour light, 12-hour dark cycles. Rats were pair housed and had free access to laboratory chow and water. One week after arrival, animals were randomly assigned to either an isoflavone-free diet (IF) (Ziegler Brothers, Inc, Gardners, Pennsylvania) or a matched high-soy diet (Teklad 8640; Harlan Teklad, Madison, Wisconsin). The Teklad diet contains approximately 600-μg/g soy isoflavones and results in an average of 6 μM circulating total isoflavones, an amount equal to or greater than a typical Asian diet, which is high in soy (21). One week later, animals were bilaterally ovariectomized under isoflurane anesthesia.

Transient middle cerebral artery occlusion (tMCAO)

Four weeks after ovariectomy, rats underwent 90 minutes of tMCAO with an intraluminal suture. Rats were anesthetized with 5% isoflurane in 100% oxygen and maintained on 1.5–2.0% isoflurane during the procedure. Body temperature was maintained at 37 ± 0.5°C with a heating pad controlled by a rectal probe (Cambridge Electronic Devices, Cambridge, United Kingdom). A laser Doppler flow (LDF) probe (Perimed, North Royalton, Ohio) was attached to the left side of the dorsal surface of the skull 2 mm caudal and 6 mm lateral to bregma to monitor cerebral blood flow. The MCA was occluded with a sterile, silicone-coated 4.0 monofilament nylon suture introduced into the left external carotid artery and advanced cranially into the internal carotid artery until resistance was felt and a rapid drop in blood flow to the left cerebral hemisphere was observed. LDF was observed for 90 minutes before the suture was withdrawn and reperfusion monitored by LDF. Animals that did not undergo reperfusion were excluded from the study. Animals were allowed to recover for 4 or 24 hours from the time of stroke initiation with free access to food and water. Three animals in the IF group and 2 animals in the soy-fed group died during or shortly after tMCAO.

Determination of cerebral edema

Twenty-four hours after reperfusion for 90 minutes of tMCAO, rats were decapitated under deep anesthesia, and brains were removed. Olfactory bulbs, cerebellum, and brainstems were removed before weighing.Brains were then dried at 80°C for 48 hours and weighed again. Percent water content (edema) was calculated as ([wet-dry weight]/wet weight) × 100.

Tissue isolation

Rats were deeply anesthetized with urethane (1.7 g/kg ip) and decapitated with a guillotine. The brain was rapidly removed and placed in ice-cold sterile saline for 2 minutes. Two-millimeter coronal sections were made in a brain matrix (Braintree Scientific, Braintree, Massachusetts) starting at the frontal pole. Tissue samples were microdissected with a scalpel blade and processed immediately or frozen on dry ice for isolation of RNA. In stroke animals, alternate sections were stained with 2,3,5-triphenyl-2H-tetrazolium chloride to determine the infarct border, and tissue was dissected from penumbra regions bordering the infarct. Brains from a separate group of rats were rapidly frozen in TissueTek OCT (Andwin Scientific, Schaumburg, Illinois) medium and frozen at −80°C for later histochemical analysis.

RNA collection and real-time RT-PCR

RNA was isolated from the cerebral cortex using RNaseasy kits (QIAGEN, Valencia, California). RNA concentration was determined in triplicate using RiboGreen RNA-binding dye (Molecular Probes, Invitrogen, Carlsbad, California), and RNA was stored at −80°C until used. mRNA (200-500 ng) was reverse transcribed with oligo-dT using a commercial kit (OmniScript; QIAGEN). After reverse transcription, samples were diluted to 5 ng/μL, and aliquots were stored at −80°C. Real-time RT-PCR was performed on 25-ng equivalents in triplicate on an Applied Biosystems (AB) 7500 Sequence Detection System using AB TaqMan Gene Expression Assays for VEGF-A (Rn00582935_m1), VEGF receptor (VEGFR)-1 (Rn00570815_m1), and VEGFR-2 (Rn00564986_m1) (Life Technologies, Grand Island, New York). Gene products were amplified in multiplex reactions with a primer-limited glyceraldehyde-3-phosphate dehydrogenase (GAPDH) endogenous control Gene Expression Assay using AB TaqMan Gene Expression Assay for GAPDH with Amp UNG and Rox dye in 25 μL. Cycling conditions were the same for all gene products. No significant differences in GAPDH expression were detected between groups. Threshold amplification cycle number data from multiple plates were combined using AB Relative Quantitation software (SDS1.2) and the delta-delta Ct method.

Capillary density and vascular apoptosis

Total capillary volume was determined by immunofluorescent staining for the brain endothelial glucose transporter Glut1. Coronal sections (10 μm) corresponding to approximately 0, −2.0, and −4.0 mm relative to bregma were cut on a cryostat (n = 4/group). Frozen sections were fixed in 4% formaldehyde for 15 minutes and processed in parallel for Glut1 using a Shan-
don Cover plate system (Thermo Scientific, Pittsburgh, Pennsylvania). Glucose transporter 1 (Glut1) was detected with a rabbit polyclonal antibody (Chemicon AB1340; Chemicon, Temecula, California) at 1:200 and a Cy3 direct-tagged donkey antirabbit secondary antibody (Jackson Immunochemicals, West Grove, Pennsylvania) at 1:400. Slides were coverslipped with Krystalon (EMD Chemicals, Gibbstown, New Jersey), and digital 8-bit gray scale images were captured at ×200 using an Olympus BX60 microscope (Olympus, New York, New York) and an Optronics MagnaFire SP camera with an 800-ms exposure. Four regions of interest encompassing the dorsal to ventral cerebral cortex were collected from each section, totaling 12 images per rat. Images were analyzed with ImageJ (National Institutes of Health, Bethesda, Maryland). A median filter with a 2-pixel radius was applied to each 1280 × 1024 pixel image, areas of tissue folding or tearing were masked, and Glut1 area was determined by after binary conversion using the autothreshold function.

Perfused capillary density was determined by fluorescein isothiocyanate (FITC)-dextran. Rats in each group were anesthetized with isoflurane and fitted with an iv femoral catheter. FITC-dextran (50 mg in 1 mL; Sigma, St. Louis, Missouri) was infused over 1 minute and allowed to circulate for 30 minutes. Animals were decapitated, and brains were rapidly removed and submerged in 4% buffered formaldehyde for 48 hours. Coronal sections (100 μm) were made with a vibratome, and every sixth section was mounted onto slides and coverslipped with Krystalon. Total capillary length, as a measure of density, was determined using unbiased stereology and the “spaceballs” protocol (StereoInvestigator, MicroBrightfield, Wiliston, Vermont). Regions of interest were outlined on each slide with a Antibodies Used in the Experiments

Table 1. Antibodies Used in the Experiments

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Source</th>
<th>Molecular Weight (kDa)</th>
<th>Dilution</th>
<th>Incubation</th>
<th>Company</th>
<th>Catalog No.</th>
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<tr>
<td>Actin</td>
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<td>1:5000</td>
<td>Overnight</td>
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<tr>
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<td>Overnight</td>
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<tr>
<td>Flik1</td>
<td>Mouse</td>
<td>150, 200, 230</td>
<td>1:1000</td>
<td>Overnight</td>
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<td>sc-6251</td>
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<tr>
<td>Flik1 insert domain receptor/Flik-1</td>
<td>Rabbit</td>
<td>150, 200, 230 200–230</td>
<td>1:1000</td>
<td>Overnight</td>
<td>Santa Cruz Biotechnology, Inc Upstate (Lake Placid, New York)</td>
<td>sc-505 36-019</td>
</tr>
<tr>
<td>eNOS</td>
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<tr>
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<td>1:500</td>
<td>Overnight</td>
<td>Millipore (Bedford, Massachusetts)</td>
<td>05-1050</td>
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</table>

*Dependent on the molecular weight of the tyrosine phosphorylated protein being detected.

To estimate the extent of vascular apoptosis, frozen sections (10 μm) were processed from stroked animals as described above. Slide-mounted sections underwent processing for terminal deoxynucleotidyl transferase 2'-deoxyuridine, 5'-triphosphate nick end labeling (TUNEL) staining using a commercial kit (Fragel; Calbiochem, Temecula, California). The diaminobenzidine colorimetric reagent was replaced with Vector VIP (Vector Labs, Burlingame, California) to yield a purple reaction in TUNEL positive cells. After TUNEL staining, Glut-1 staining was performed using diaminobenzidine to label endothelial cells brown. Glut1 positive and Glut1:TUNEL dual labeled capillaries were quantified by unbiased stereology using StereoInvestigator. Vessels were identified by both morphology and positive Glut1 staining. Counting was limited to vessel profiles greater than 10 μm in length, thus eliminating fragments and vessels appearing perpendicular to the section.

**Immunoblotting and immunoprecipitation**

Protein was extracted using T-Per reagent (Pierce, Rockford, Illinois) supplemented with HALT Protease Inhibitor Cocktail (Pierce). Concentrations were determined using a BCA protein assay kit (Pierce), and samples were divided into aliquots to avoid multiple freeze-thaw cycles. Total protein (15-50 μg) from each sample was separated using precast 4%–20% polyacrylamide gels (Pierce Precise) under reducing conditions. Samples were transferred to nitrocellulose and blocked for 1 hour at room temperature using Odyssey Blocking Buffer (LiCor, Lincoln, Nebraska). Blots were subjected to immunoblotting in primary antibody overnight at 4°C followed by antirabbit or antimouse horseradish peroxidase-conjugated secondary antibod-
hours after tMCAO, as observed by a decrease in water content in the ischemic hemisphere (Figure 1). In addition, significantly fewer Glut1 positive cerebral vessels were TUNEL positive 24 hours after tMCAO in soy-fed rats compared with IF rats (Figure 1), suggesting a reduction in vascular injury. Four weeks of dietary soy in the absence of injury had no effect on vascular density determined by stereological estimation of total vessel length of FITC-dextran-stained vessels. Total vessel length in the cortex was $9.27 \times 10^6 \pm 1.78 \times 10^7 \mu m$ in soy-free rats and $9.72 \times 10^7 \pm 1.96 \times 10^7 \mu m$ in soy-fed rats. Similarly, in the hippocampus, total vessel length was $5.47 \times 10^6 \pm 1.55 \times 10^6 \mu m$ in soy-free rats and $4.61 \times 10^6 \pm 1.58 \times 10^6 \mu m$ in soy-fed rats. Total fluorescent intensity from Glut1-stained vessels in 4 regions of interest across the cortex and hippocampus also failed to reveal any difference in vascular density (soy free, 0.039 ± 0.003 pixels/area vs soy fed, 0.039 ± 0.001 pixels/area).

VEGF is a primary mediator of blood brain barrier leakage in the early stages of stroke injury. VEGF acts on VEGFR-1 (VEGFR-1/fms-like tyrosine kinase 1) to increase vascular permeability and on VEGFR-2 (VEGFR-2/fetal liver kinase 1 [Flk1]) to provide cytoprotection (1). Four hours after tMCAO, steady-state mRNA levels for VEGF decreased significantly in the ischemic hemisphere in both groups of rats and remained low in IF rats at 24 hours (Figure 2). In contrast, VEGF mRNA rose significantly above basal levels in soy-fed rats (Figure 2). VEGFR-1 mRNA levels in the cortex were significantly higher in soy-fed rats under basal conditions but fell to IF levels 4 hours after tMCAO (Figure 2). In both groups, VEGFR-1 mRNA levels rose 24 hours after tMCAO to reach soy-fed basal levels (Figure 2). VEGFR-2 mRNA levels dropped dramatically 4 hours after tMCAO in both groups and recovered to basal levels in soy-fed, but not IF, rats 24 hours after tMCAO (Figure 2).

In contrast to the drop in VEGF and VEGFR-2 mRNA levels 4 hours after tMCAO, cortical protein expression was increased in IF rats both 4 and 24 hours after tMCAO (Figure 3). However, in soy-fed rats, VEGF protein levels rose only modestly at 4 hours, and VEGFR-2 protein levels were unchanged after stroke (Figure 3). We were unable to consistently detect VEGFR-1 in ischemic cortex using several different antibodies, but VEGFR-2 protein levels in the ischemic hemisphere in-
increased significantly 4 and 24 hours after tMCAO only in IF rats (Figure 3). Because both VEGF and VEGFR-2 protein levels were lower in soy-fed rats, we next examined whether VEGFR-2 activity was similarly reduced. Cerebral cortical protein from IF and soy-fed rats collected 24 hours after tMCAO revealed reduced phosphorylation of VEGFR-2 in soy-fed, compared with IF, rats (Figure 4). This result was observed both with an antibody directed against phosphorylated VEGFR-2 and by immunoprecipitation with a phosphotyrosine antibody followed by immunoblotting with a VEGFR-2 antibody (Figure 4). Phosphorylation of 2 primary downstream mediators of VEGF/VEGFR-2 action, AKT and endothelial nitric oxide synthase (eNOS), was also reduced in soy-fed rats (Figure 4).

Because VEGFR-2 mRNA was increased in soy-fed rats 24 hours after tMCAO, we hypothesized that the reduction in VEGFR-2 protein could result from increased degradation. Immunoprecipitation revealed increased VEGFR-2 and phospho-VEGFR-2 ubiquitination in soy-fed rats after tMCAO, suggesting active degradation of the VEGFR (Figure 5).

During ischemia, HIF1α provides the major stimulus to increase VEGF expression (1). Like VEGF, HIF1α overexpression in response to reduced oxygen tension can exacerbate injury and lead to enhanced cell death in vitro and in vivo (22). We reasoned that a significant reduction in VEGF levels after stroke likely requires an inhibition of HIF1α signaling. Consistent with this notion, Western blotting revealed that HIF1α expression was significantly lower in the ischemic cortex of soy-fed rats than IF rats 4 hours after MCAO, whereas the constitutively expressed HIF1β was not significantly different (Figure 6). Further confirming the reduction in HIF1 signaling, we observed that soy-fed rats also showed a significantly lower expression of BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3L)/Nip3-like protein X (Nix), an autophagic/apoptotic mediator under the regulation of HIF1α (Figure 6) (23). In the striatum, at the core of the ischemic injury, HIF1α also increased in soy-free rats but decreased in soy-fed rats, but there were no significant differences in BNIP3L/Nix (Figure 6).

**Discussion**

The neuroprotective mechanisms of dietary isoflavones in stroke appear to involve several complementary actions, including those that reflect the estrogenic properties of these compounds. The present study extends previous findings in a rat model of transient focal cerebral ischemia to implicate inhibition of the HIF1α and VEGF pathways as targets of isoflavones in acute isch-
Changes in HIF1α and BNIP3L in the brain of soy-free (IF) and soy-fed (Soy) ovariectomized female rats 4 hours after tMCAO. (A) HIF1α, HIF1β, BNIP3L, and β-actin expression in the ischemic penumbra on a representative blot probed for each protein separately. (B) Mean ± SE of protein expression normalized to β-actin. Asterisks represent significant differences between groups (P < .05). (C) HIF1α expression in striatum of the contralateral (CL) and stroke (ST) hemispheres in IF and Soy rats. (D) Mean ± SE of protein expression for HIF1α normalized to β-actin. (E) Mean ± SE of protein expression for BNIP3L normalized to β-actin. Graphs represent data from 6 rats per group. Asterisks represent significant differences between groups (P < .05). Crosses represent a significant difference from the contralateral striatum (P < .05).

Previous studies demonstrate that, despite neuroprotective actions of VEGF, inhibition of VEGF signaling early in the ischemic process can be beneficial (6–8). However, long-term pretreatment or induction of VEGF can also improve outcomes from cerebral ischemia (24–26). Similar results are observed for HIF1α signaling, an early response to ischemia that lies upstream of VEGF. The estrogen-derived chemotherapeutic steroid 2-methoxyestadiol, which does not have estrogenic actions, potently inhibits HIF1α and VEGF and reduces ischemic injury when administered early in the ischemic process (22, 27), but later inhibition (22) or neuronal gene deletion of HIF1α (28) results in increased delayed injury. Although beneficial when intervention is initiated in the acute ischemic period, 2-methoxyestadiol is unsuitable chronic use in humans. Nevertheless, the aforementioned studies provide evidence that suppressing the brain’s endogenous initial response to ischemia can be beneficial.

Although both VEGF and VEGFR-2 protein levels were inhibited in soy-fed animals compared with soy-free animals as early as 4 hours after MCAO, mRNA levels were similar in both groups. These results implicate posttranscriptional mechanisms in the early stage of injury, and this was confirmed by VEGFR-2 ubiquitination. Indeed, the subsequent rise in VEGF and VEGFR-2 mRNA expression in soy-fed rats 24 hours after stroke is likely a compensatory mechanism for the loss of protein function that is not needed in the soy-free state.
strain combination used in these studies has been replicated. Strain differences may even be reflected in alterations in the VEGF signaling cascade. For example, male stroke-prone spontaneously hypertensive rats have lower basal levels of VEGF, VEGFR-2, and eNOS in the frontal cortex compared with Wistar-Kyoto rats (38). Future examination of such strain x sex differences may provide important clues as to the clinical usefulness of soy as a neuroprotectant. The neuroprotective actions of soy isoflavones share many similarities with estrogen, including reductions in oxidative stress (13), increased expression of Bcl2 and Bcl-XL (12), increased mitochondrial function (39), and reduced inflammation (40). Similar protective effects have been demonstrated in vitro in neurons (39, 41, 42) and brain microvascular endothelial cells (40). However, modulation of the initial hypoxic response to ischemia by soy isoflavones could provide a more global effect on cells and alter many of these downstream targets.

In peripheral tissues, such as the breast and uterus, estradiol stimulates HIF1α and VEGF (43). However, studies in the brain have revealed increases, decreases, or no effect of estrogen on VEGF (44–46). Because HIF1α is labile under normoxia, there is a paucity of data on the effects of estrogen under basal conditions. Soy and genistein both inhibit HIF1α and VEGF induction by radiation in prostate cancer cells (47). Similarly, genistein dose dependently inhibits hypoxic induction of HIF1α and VEGF in the retina (48). However, these effects are observed at phytoestrogen concentrations (10 μM to 100 μM) higher than those used in the present study and likely reflect the inhibition of receptor tyrosine kinases. In contrast, inhibition of hypoxia-induced HIF1α in a neuronal cell line is evident at nanomolar concentrations (Ma, Y., and D. Schreihofer unpublished results).

A more likely role for soy isoflavones on the regulation of HIF1α and VEGF involves protein:protein interactions between isoflavone-bound estrogen receptors (ER) and HIF1α. In transfected HEK293 cells, ERβ inhibits HIF1α-dependent transcription and decreases hypoxia-induced VEGF expression (49). In prostate cancer cells, ligand-bound ERβ also enhances HIF1α degradation (50). Direct interactions between ERα and HIF1α have been observed in transfected cells (51), suggesting that either ER can target HIF1α. Soy isoflavones display a dose preference for ERβ, but at low micromolar concentrations observed in soy-fed rats, soy isoflavones can activate both ERα and ERβ (52). Thus, the neuroprotective effects of soy might be expected to mimic mechanisms of low-dose estradiol. Indeed, the ER antagonist ICI182,780 blocks isoflavone-dependent neuroprotection in primary cortical neurons exposed to hypoxia or oxygen-glucose deprivation (42). Furthermore, the selective ERβ antagonist 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol inhibits the neuroprotective effects of genistein in global ischemia in Mongolian gerbils (53). Whether these protective effects are the result of cross talk between ERs and HIF1α remains to be determined.

The ability of a chronic dietary soy intervention to inhibit ischemic injury is promising for menopausal women who elect not to take traditional HT. Such a chronic intervention for neuroprotection may help to bypass one of the major barriers to stroke intervention: timing of treatment. Chronic estradiol shares this benefit in animal studies, but detrimental side effects in postmenopausal women reduce the potential for HT as a neuroprotective strategy. A potential confound of the mechanism hypothesized in the present study is that continued inhibition of the HIF1/VEGF pathway during recovery from ischemic injury could prove deleterious by reducing revascularization. Several studies confirm that delayed or sustained inhibition of this pathway can be detrimental. However, in normal rats fed high-soy diets before and throughout recovery, no detrimental effects are yet evident. Dietary equol, the primary circulating isoflavone in ovariectomized female Sprague Dawley rats fed soy, continued to show benefits in neurological function 7 days after tMCAO (13). Similarly, in male Long-Evans rats fed soy, neurological function was improved compared with casein-fed rats up to 8 weeks after MCAO (14).

The results of the present study provide evidence that the neuroprotective effects of dietary soy in focal cerebral ischemia may involve buffering of the endogenous brain response to hypoxic/ischemic injury. This inhibitory effect, although incomplete, lies upstream of several other pathways affected by ischemia and may be a primary mechanism for isoflavone neuroprotection. Indeed, the reduction in edema and vascular cell death suggests that the first target of isoflavones may be the vasculature. Future studies will be necessary to determine whether the inhibition of HIF1/VEGF is restricted to particular cell types and whether actions of isoflavones could complement protective strategies aimed at other intracellular targets.

Acknowledgments

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