Retroviral expression of human arginine decarboxylase reduces oxidative stress injury in mouse cortical astrocytes

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Abstract

Background: In physiologic and pathologic conditions of the central nervous system (CNS), astrocytes are a double-edged sword. They not only support neuronal homeostasis but also contribute to increases in neuronal demise. A large body of experimental evidence has shown that impaired astrocytes play crucial roles in the pathologic process of cerebral ischemia; therefore, astrocytes may represent a breakthrough target for neuroprotective therapeutic strategies. Agmatine, an endogenous polyamine catalyzed from L-arginine by arginine decarboxylase (ADC), is a neuromodulator and it protects neurons/glia against various injuries.

Results: In this investigation, agmatine-producing mouse cortical astrocytes were developed through transduction of the human ADC gene. Cells were exposed to oxygen-glucose deprivation (OGD) and restored to a normoxic glucose-supplied condition. Intracellular levels of agmatine were measured by high performance liquid chromatography. Cell viability was evaluated by Hoechst/propidium iodide nuclear staining and lactate dehydrogenase assay. Expression of inducible nitric oxide synthase (iNOS) and matrix metalloproteinase s (MMPs) were assessed by a reverse transcription polymerase chain reaction, Western immunoblots, and immunofluorescence. We confirmed that ADC gene-expressed astrocytes produce a great amount of agmatine. These cells were highly resistant to not only OGD but also restoration, which mimicked ischemia-reperfusion injury in vivo. The neuroprotective effects of ADC seemed to be related to its ability to attenuate expression of iNOS and MMPs.

Conclusion: Our findings imply that astrocytes can be reinforced against oxidative stress by endogenous agmatine production through ADC gene transduction. The results of this study provide new insights that may lead to novel therapeutic approaches to reduce cerebral ischemic injuries.

Keywords: Agmatine, Arginine decarboxylase, Astrocyte, Neuroprotection, Oxidative stress

Background

Astrocytes comprise the structural architecture of the brain and support neurons to maintain homeostasis [1,2]. Physiologically, they stabilize the extracellular environment of neurons: they control ion/water distribution, regulate neurotransmitter recycling, release growth factors, and scavenge reactive oxygen species (ROS). Under pathologic conditions, when noxious stress exceeds limitations, astrocytes work in the opposite direction [1,2]. Water accumulation induces brain edema, failure of neurotransmitter control, which causes glutamate excitotoxicity, and releases ROS/neurotoxic factors that contribute to further neuronal damage. In cerebral injuries, astrocytes are the main culprit of neurodegeneration and scar formation [2,3]. Meanwhile, they also contribute to neurite outgrowth and neurogenesis [4]. Astrocytes have recently garnered attention as a breakthrough target in development of new therapeutic strategies for patients suffering from neuronal damage [2].

Agmatine, an endogenous polyamine derived from L-arginine by arginine decarboxylase (ADC), is naturally found in the mammalian central nervous system (CNS) and acts as a multifunctional neuromodulator [5-7]. It is packed into synaptic vesicles and released from synaptosomes by neuronal depolarization [8,9]. Agmatine can stimulate α2-adrenergic and imidazoline receptors [10,11],

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block the N-methyl-D-aspartic acid (NMDA) receptor and voltage-gated calcium channel [12,13], and inhibit inducible/neuronal nitric oxide synthases (iNOS/nNOS) [14,15]. Through these kinds of intracellular signaling, agmatine shows various antineurotoxic, anticonvulsant, antipsychotic, antidepressant, anxiolytic, and antinoceptive neuroprotective effects [7,16]. Regarding ischemic insults, exogenous agmatine significantly reduces the infarct size and brain edema caused by ischemia-reperfusion injury in rodents [17-20]. Agmatine also rescues glial cells, as well as cortical neurons, from oxidative stress in vitro and in vivo [17,21,22].

In this study, a recombinant retroviral vector containing the human ADC (hADC) gene was introduced into mouse cortical astrocytes to maximize the astrocyte-protective effect of agmatine. Cells overexpressing hADC show increased production of agmatine and greater resistance against oxidative stress [23-25]. Here, we transduced primary cultured cortical astrocytes with hADC-expressing retroviral vector and assessed whether it induces synthesis of endogenous agmatine and attenuates oxidative stress injuries.

Methods

Cell culture

Primary cortical astrocyte cultures were prepared from postnatal 1–3 days old Crl:CD1 mouse pups (Samtako, Osan, Korea) [21,26]. Our protocol was approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine and all animals were treated in accordance with their guidelines. Animals were etherized and decapitated on ice-cold ethanol. Cortical hemispheres were dissected free of the skull and meninges, and then treated with 0.09% trypsin for 20 min at 37°C. The cells were mechanically dissociated into single cells and collected as a suspension. The cells were grown in minimum essential medium (MEM; Life Technologies, Grand Island, NY) containing 10% fetal bovine serum (FBS) and 10% equine serum (Thermo Fisher Scientific, Waltham, MA). The cultures were incubated at 37°C in a 5% CO2 incubator and the culture media was replaced every 3 days. One week after the first seeding, the other cells including microglia were removed by vigorous shaking. The highly-purified astrocytes (passage 3), at least 3 weeks after the first seeding, were used for experiments at about 80% confluence (Additional file 1).

Construction and infection of recombinant retroviral vector containing the hADC gene

A recombinant retroviral vector containing the hADC gene was constructed as described previously (Figure 1) [23-25]. Briefly, the full-length cDNA of hADC (GenBank accession no. AY325129) was amplified by a polymerase chain reaction (PCR) and ligated to the retroviral expression vector pLXSN (Clontech Laboratories, Mountain View, CA) that also contained a neomycin resistance gene. They were then amplified in E.coli DH5α and identified by restriction analysis. The hADC-expressing pLXSN vector was transfected into the retroviral packaging cell line PT67 using Lipofectamine 2000 (Sigma-Aldrich, St. Louis, MO). A stable clone selection was carried out by adding G418 (200 μg/mL; Sigma-Aldrich) to the culture medium of Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies) supplemented with 10% FBS. High-titer clones were selected, and virus-containing supernatant was filtered through a 0.45-mm polysulfonic filter. Primary cultured mouse cortical astrocytes were infected with hADC pLXSN. The cells were incubated with the virus-containing media for 24 hrs and then maintained in normal culture medium for a week before being used for the experiments.

Oxygen-glucose deprivation and restoration

For oxygen-glucose deprivation (OGD), cultures were transferred to a closed anaerobic chamber (Forma Scientific Co., Seoul, Korea) in which the oxygen level (less than 0.1% O2) and temperature (37°C) were controlled automatically [21,27]. After being washed three times with deoxygenated, glucose-free balanced salt solution (BSS5.5) at pH 7.4, cells were kept in the anaerobic chamber for 4 hours. Cultures were then restored with glucose at a concentration of 5.5 mM (BSS5.5) and recovered to normoxic conditions (37°C, 5% CO2) for up to 10 hours.

High performance liquid chromatography analysis

The concentration of agmatine was measured by high performance liquid chromatography (HPLC) [23,24]. Harvested cells were homogenized in phosphate buffer containing 10% trichloroacetic acid and centrifuged at 12,000 rpm for 10 min. The collected supernatant was derivatized with o-phthalaldehyde and loaded onto the HPLC column (5 mm) connected with a fluorescence detector. Recovery of agmatine was calculated from an added external standard. The content of L-Arginine and putrescine was also measured using same method.

Hoechst and propidium iodide nuclear staining

Cell death was characterized by double nuclear staining with Hoechst 33258 and propidium iodide (PI) [23,24]. Cells were stained with Hoechst 33258 dye (5 μg/mL; Sigma-Aldrich) for 30 min at 37°C and consecutively stained with PI (5 μg/mL; Sigma-Aldrich) just before observing it with a fluorescence microscope. Living cells showed Hoechst-positive blue fluorescence and nonviable cells had PI-positive red fluorescence.
Lactate dehydrogenase activity

Cell viability was quantified by measuring the amounts of lactate dehydrogenase (LDH) released from injured cells into the medium [27-30]. LDH released (% cytotoxicity) was calculated by dividing the value at the experimental time point by the maximum value × 100. Maximum LDH release values were obtained after freezing each culture at −70°C overnight and then rapidly thawing them, thus inducing nearly complete cell damage.

Reverse transcription polymerase chain reaction

Gene expression was assessed by reverse transcription PCR (RT-PCR) [23,24]. Total RNA was extracted from cells with Trizol (Life Technologies) and quantified by measuring the absorbance at 260 nm. cDNA synthesis from mRNA and normalization of the samples were carried out by RT-PCR. PCR amplification of iNOS was performed at 94°C for 30 s, 57.5°C for 30 s, and 72°C for 30 cycles. Amplification for MMP-2 was performed at 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s for 35 cycles. Amplification for MMP-9 was performed at 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s for 30 cycles. Specific primer sequences used are as follows: iNOS, sense 5'-TTT GAT GTG CTG CCT CTG GT-3' and antisense 5'-CAT TCT GCT TCT GGA AAC TAA GG-3'; MMP-2, sense 5'-GAG TTG GCA GTGCAA TAC CT-3' and antisense, 5'-GCC GTG CCC TCT GGA TGT GT-3'; MMP-9, sense 5'-TTA CCA GGG CCA GCC GAC TTT TG-3' and antisense 5'-CGT CGT CGT CGA AAT GGG CAT C-3'; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), sense 5'-ATG TCG TGG AGT CTA CTG GT-3' and antisense 5'-TGG CAT GGA CTG TGG TG-3'. PCR products were separated by electrophoresis in a 1.5% agarose gel stained with ethidium bromide.

Western immunoblots

Protein levels were determined by Western immunoblots [23,24,31]. Protein was extracted from whole cell lysates, and their concentration was calculated with the BCA protein assay (Thermo Fisher Scientific). Equal amounts of protein (40 μg) from each sample were resolved on a 10% sodium dodecyl sulfate-polyacrylamide gel. The separated proteins were then electrotransferred onto Immobilon-NC membranes (Millipore, Billerica, MA) and incubated overnight with primary antibodies against iNOS (1:1000 dilution), MMP-2 (1:2000 dilution), MMP-9...
(1:2000 dilution), or β-actin (1:2000 dilution). The primary antibody against iNOS was purchased from Millipore (Billerica, MA) and the others were obtained from Santa Cruz Biotechnology (Dallas, TX). Immunoreactive bands were detected with horseradish peroxidase-conjugated secondary antibody and visualized using an enhanced chemiluminescent protein detection kit (Thermo Fisher Scientific).

**Immunofluorescence staining**

Protein expression was also confirmed by immunofluorescence [23,31,32]. Cells were fixed with 90% ethanol for 30 min on ice, and then treated with 1.6% hydrogen peroxide and 0.025% triton. They were reacted with specific antibodies (1:500 dilution) for iNOS (Millipore), MMP-2 (Santa Cruz Biotechnology), MMP-9 (Santa Cruz Biotechnology), and glial fibrillary acidic protein (GFAP; Abcam, Cambridge, MA). Subsequently, the cells were exposed to the corresponding fluorescent secondary antibodies (Life Technologies) and their nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Finally, four random fields were imaged under a fluorescence microscope. Immunoreactive cells with fluorescence were manually counted at 200× magnification.

**Statistical analysis**

Data are presented at the mean of ± SD of at least three different experiments performed from separate cell preparations, with at least triplicate determination performed for each experiment. Kruskal-Wallis test and Mann–Whitney test were used in order to examine statistical significance (MedCalc Statistical Software version 12.7.8.; MedCalc Software bvba, Ostend, Belgium). P-values less than 0.05 were considered statistically significant.

**Results**

**Transduction of mouse cortical astrocytes with recombinant retroviral vector containing the human arginine decarboxylase (hADC) gene**

The vector map of hADC pLXSN is shown in Figure 1A. It includes the Col E1 origin of replication and E.coli Amp' gene for propagation and antibiotic selection. The 5’ viral LTR contains promoter/enhancer sequences that control expression of the gene of interest in the multiple cloning site. The SV40 early promoter (PSV40) controls expression of the neomycin resistance gene (Neo'), which allows for antibiotic selection in eukaryotic cells. PSV40, was genetically confirmed by restriction analysis (Figure 1B). In astrocytes, as well as PT67 cells, infection of the hADC pLXSN vector effectively induced hADC transcription (Figure 1C). Expression of hADC was validated by immunocytochemistry in hADC pLXSN-infected astrocytes (Figure 1D).

**Endogenous agmatine production from human arginine decarboxylase (hADC)-overexpressed astrocytes**

Intracellular levels of agmatine were measured by HPLC (Figure 2). Endogenous agmatine concentration was markedly greater for astrocytes overexpressing hADC compared to that of control astrocytes (11.28-fold, p < 0.001). The amount of L-arginine and putrescine (precursor and metabolite of agmatine, respectively) showed similar tendency to that of agmatine. Meanwhile, OGD itself did not have a significant influence on the level of these amines.

**Astrocyte-protective effect of human arginine decarboxylase (hADC) transduction against oxygen-glucose deprivation (OGD)**

OGD damaged naive astrocytes and the majority of cells had PI-labeled nuclei (Figure 3B). However, astrocytes overexpressing hADC (hADC-astrocytes) were much less damaged by OGD, and the proportion of PI-positive cells were considerably lower (Figure 3D). hADC transduction itself did not have any significant effects on cell viability (Figure 3C), as determined by Hoechst 33258 and PI double nuclear staining.

**Neuroprotective effect of human arginine decarboxylase (hADC) transduction on oxygen-glucose deprivation (OGD) and restoration injury**

To mimic the ischemia-reperfusion injury in vivo, cells were restored to normoxic glucose-supplied conditions after 4 hrs of OGD (Figure 4). Cytotoxicity was measured by LDH leakage from the injured cells. Cytotoxicity gradually increased in a time-dependent manner for up to 10 hrs during restoration. Even in hADC-astrocytes, the...
restoration from OGD caused cytotoxicity in a time-dependent manner; the extent of damage was much less than that in control astrocytes.

Suppression of inducible nitric oxide synthase (iNOS) and matrix metalloproteinases (MMPs) in human arginine decarboxylase (hADC)-overexpressing astrocytes

Expression of iNOS and MMP-2/9 was assessed by RT-PCR (Figure 5A) and Western immunoblots (Figure 5B). OGD significantly increased levels of iNOS and MMP-2/9; however, hADC transduction attenuated the OGD effects. Introduction of the hADC gene itself did not change glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and β-actin levels, which served as internal controls for RT-PCR and Western immunoblots, respectively.

Inhibited expression of inducible nitric oxide synthase (iNOS) and matrix metalloproteinases (MMPs) in human arginine decarboxylase (hADC)-transduced astrocytes

Naive astrocytes seldom expressed iNOS and MMP-2/9 (Figure 6A, D, G). After 4 hrs of OGD, control astrocytes exhibited expression of iNOS and MMP-2/9 (Figure 6B, E, H); however, hADC-astrocytes did not show immuno-reactivity to either iNOS or MMP-2/9 (Figure 6C, F, I).
Discussion
Since Reis and colleagues first discovered agmatine and ADC activity in the mammalian brain [5], agmatine has been widely studied. It is known to have various biological actions through multiple molecular targets, exhibiting affinity not only to $\alpha_2$-adrenoceptors and $I_1$- and $I_2$-imidazoline receptors but also to $\beta$-adrenoceptors and 5-HT$_3$ serotonin, nicotinic cholinergic NMDA, $D_2$-dopamine, $\kappa$-opioid, and adenosine A$_1$ receptors [10-12]. Agmatine also inhibits iNOS/nNOS and monoamine oxidase [10,14,15]. As well, agmatine reduces heart rate and blood pressure [10], stimulates insulin and $\beta$-endorphin release, leading to increased cellular glucose uptake [33,34], and accelerates glomerular ultrafiltration [35]. Agmatine protects systemic organs, especially against oxidative stress. Additionally, it shows cardioprotective and renoprotective effects on ischemic insult [36,37], and it directly protects mitochondria from ROS [38,39]. In the CNS, agmatine shows neuroprotective effects on various neuronal injuries: reduces the infarct size and edema after cerebral ischemia [17,18,40], attenuates brain damage and reactive gliosis caused by trauma [41,42], and also decreases traumatic/ischemic spinal cord injuries [43,44]. Agmatine also decreases neuropathic pain and convulsive events [45,46], attenuates opioid dependence and alcohol withdrawal anxiety [47,48], and it improves motor and cognitive functions in Parkinson’s and Alzheimer’s disease, respectively [49,50]. Even in the eyes, agmatine lowers intraocular

Figure 4 Neuroprotective effect of human arginine decarboxylase (hADC) transduction against oxygen-glucose deprivation (OGD) and restoration injury. Astrocytes restored to normoxic glucose-supplied conditions after 4 hrs OGD. Cytotoxicity was assessed by the lactate dehydrogenase (LDH) assay. Data are expressed as mean of ± SD of the three different experiments performed from separate cell preparations, with triplicate determinations performed in each experiment. Asterisks indicate a $p < 0.001$.

Figure 5 Inducible nitric oxide synthase (iNOS) and matrix metalloproteinases (MMPs) expression in human arginine decarboxylase (hADC)-introduced astrocytes after oxygen-glucose deprivation (OGD). The mRNA levels were determined using reverse transcription polymerase chain reaction (RT-PCR) (A) and protein expression was evaluated using Western immunoblots (B). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and $\beta$-actin were used as internal controls, respectively. Asterisks indicate a $p < 0.05$ when the samples were compared to no treatment control. Double asterisk indicate a $p < 0.05$ that two samples were significantly different which was confirmed by a post-hoc analysis.
pressure and protects retinal ganglion cells [51,52]. Taken together, agmatine seems to be a miracle cure rather than a specific drug.

In routine clinical settings, exogenous drug administration may be the most straightforward therapeutic strategy. However, exogenous drug administration is not always the best approach for real patients. Taken orally, agmatine is readily distributed throughout the body [53,54] and can even cross the blood–brain barrier [55,56]. Moreover, its reported half-life in the body is about 2 hrs [57]. The various functions of agmatine have pros and cons. In addition to neuronal effects, agmatine also has cardiovascular, endocrinal, renal, gastric, and growth effects [10]. Therefore, taken orally, agmatine may cause unwanted systemic responses. Thus, it is important to focus on the action of the drug at the site of injury and extend its duration of action. In this regard, in the ophthalmologic field, a topical agmatine ophthalmic solution has been formulated to preserve the optic nerves against glaucomatous damage [51]. Agmatine eye drops effectively lower intraocular pressure and protect retinal ganglion cells from chronic ocular pressure injuries. Along the same lines, investigators have attempted to develop a distinctive method for increasing endogenous agmatine production via a recombinant retroviral vector system containing the hADC gene. The hADC gene can be effectively delivered into mouse fibroblast NIH3T3 cells [23], primary mouse cortical neural stem cells [24], and human bone marrow mesenchymal stem cells [25]. Different types of transformed cells synthesize agmatine when they are exposed to hydrogen peroxide, resulting in resistance against oxidative stress. Irrespective of the cell type in an oxidative stressed environment, intracellular agmatine concentration is boosted by about 2 to 5-fold in hADC-overexpressing cells compared to no treatment control cells, as determined by HPLC [23-25]. While in normal culture conditions, agmatine synthesis is significantly increased in cortical neural stem cells [24] but not fibroblasts or mesenchymal stem cells [23,25]. It is not yet clear why the activity of an introduced hADC gene differs according to cell type, especially in non-stressful conditions; nonetheless, it is assumed that agmatine controls the expression of the introduced hADC gene. If normal cells use little agmatine, the transformed cells are presumed not to utilize the introduced ADC without harmful stimuli. However, if normal cells actively use agmatine, the transformed cells are presumed to utilize the introduced ADC. When cells are attacked, all the cell types seem to use the introduced ADC.

In this investigation, we focused on cortical astrocytes, which have recently came to the forefront due to their suspected roles in many CNS injuries, including cerebral

![Figure 6 Immunofluorescence staining of human arginine decarboxylase (hADC)-introduced astrocytes after oxygen-glucose deprivation (OGD). (A, D, G) No treatment control astrocytes; (B, E, H) astrocytes after 4 hrs OGD; (C, F, I) hADC-astrocytes after 4 hrs OGD. Cells reacted to inducible nitric oxide synthase (iNOS) (A-C), matrix metalloproteinase (MMP) 2 (D-F), and MMP 9 (G-I) showed green fluorescence. Cells were counter-stained with astrocyte marker glial fibrillary acidic protein (GFAP; red fluorescence) and nuclear marker 4',6-diamidino-2-phenylindole (DAPI; blue fluorescence). Scale bar = 50 μm.](http://www.biomedcentral.com/1471-2202/15/99)
ischemia [2,3]. Our results showed that intracellular levels of agmatine, as well as its precursor arginine and byproduct putrescine, are definitively increased (about 11-fold compared to the control, p < 0.001) in normal and oxidative stressed conditions upon hADC gene transduction, as measured by HPLC. Simultaneuously, cell viability was determined by Hoechst / PI double nuclear staining and the LDH assay. hADC-overexpressing cells remained viable and healthy under oxidative stress conditions induced by OGD for 4 hrs. This astrocyte-rescuing property was gradually potentiatted as restoration time proceeded for up to 10 hours. In addition, the neuroprotective effect of endogenous agmatine seemed to be related to iNOS intracellular signaling and the activity of MMPs, as assessed by RT-PCR, Western immunoblots, and immunofluorescence. Our present and previous findings are similar to those of other groups in regards to iNOS [15,58-60] and MMPs [61,62], respectively. As MMPs are upregulated by ischemic insult and degrade the basement membrane of brain microvessels [63], their suppression via ADC gene transduction may reduce ischemic injuries. Our retroviral system to deliver the hADC gene to target cells cannot be directly applied to non-dividing cells. Accordingly, another vector system using the Lentivirus is currently under development. It may be directly applied to non-dividing CNS neurons, specifically through in vivo transduction.

### Conclusion

In summary, our findings imply that endogenous agmatine production through ADC gene transduction can protect astrocytes against oxidative stress. The results of this study provide new insights that may lead to novel therapeutic approaches to reduce oxidative stress-related diseases including cerebral ischemia.

### Additional file

**Additional file 1: Immunofluorescence staining of primary cultured astrocytes.** Cells reacted to anti-glia fibrillary acidic protein (GFAP) (A, red fluorescence), and anti-CD11b (B, green fluorescence) antibodies. Nuclei were counterstained with Hoechst 33258 (C, blue fluorescence). Panel D is a merged image. Scale bar = 200 μm.

### Abbreviations

ADC: Arginine decarboxylase; ANOVA: One-way analysis of variance; BSS: Balanced salt solution; CNS: Central nervous system; DAPI: 4',6-diamidino-2-phenylindole; DMEM: Dulbecco’s modified Eagle’s medium; FBS: Fetal bovine serum; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GFAP: Glial fibrillary acidic protein; hADC: Human arginine decarboxylase; HPLC: High performance liquid chromatography; iNOS: Inducible nitric oxide synthase; LDH: Lactate dehydrogenase; MEM: Minimum essential medium; MMP: Matrix metalloproteinase; NMDA: N-methyl-D-aspartic acid; nNOS: Neuronal nitric oxide synthase; OGD: Oxygen-glucose deprivation; PCR: Polymerase chain reaction; PI: Propidium iodide; ROS: Reactive oxygen species; RT-PCR: Reverse transcription polymerase chain reaction.

### Competing interests

The authors declare that they have no competing interests.

### Authors’ contributions

LIE, HS, and SMR designed the experiments. HS, SMR, and YK carried out the molecular studies and Lee JE supervised the whole process. HS and SMR wrote the bulk of the manuscript and LWT and PKA revised the manuscript. LIE has given final approval of the version to be published. All authors read and approved the final manuscript.

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### References

1. Henrika MT, Rodrigue JJ, Verkhratsky A: Neurogia in neurodegeneration. Brain Res Rev 2010, 63:189–211.
2. Barreto G, White RE, Ouyang Y, Xu L, Giffard RG: Astrocytes: targets for neuroprotection in stroke. Cent Neurol Sys Agents Med Chem 2011, 11:164–173.
3. Nederaggiard M, Dinagla U: Role of glial cells in cerebral ischemia. Glu 2005, 50:281–286.
4. Chen Y, Swanson RA: Astrocytes and brain injury. J Cereb Blood Flow Metab 2003, 23:137–149.
5. Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ: Agmatine: an endogenous clonidine-displacing substance in the brain. Science 1994, 263:966–969.
6. Reis DJ, Regunathan S: Agmatine: an endogenous ligand at imidazoline receptors is a novel neurotransmitter. Ann N Y Acad Sci 1999, 881:65–88.
7. Halaris A, Piletz J: Agmatine: metabolic pathway and spectrum of activity in brain. CNS Drugs 2007, 21:885–900.
8. Reis DJ, Yang XC, Milner TA: Agmatine containing axon terminals in rat hippocampus form synapses on pyramidal cells. Neurosci Lett 1998, 250:185–188.
9. Goracke-Potable CJ, Nguyen HO, Stone LS, Fairbanks CA: Release of tritiated agmatine from spinal syncytomas. Neuroreport 2006, 17:13–17.
10. Raasch W, Schäfer U, Chun J, Dominiak P: Biological significance of agmatine, an endogenous ligand at imidazoline binding sites. Br J Pharmacol 2001, 133:755–780.
11. Sugiuira T, Kobuchi S, Tsutuki H, Takaoka M, Fujii T, Hayashi K, Matsumura Y: Preventive mechanisms of agmatine against ischemic acute kidney injury in rats. Eur J Pharmacol 2009, 603:108–113.
12. Yang XC, Reis DJ: Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J Pharmacol Exp Ther 1999, 288:544–549.
13. Wang G, Gobatgyuk OS, Dayanithi G, Ouyang W, Wang J, Milner TA, Regunathan S, Reis DJ: Evidence for endogenous agmatine in hypothalamo-neurohypophysial tract and its modulation on vasopressin release and Ca2+ channels. Brain Res 2002, 932:25–36.
14. Auguet M, Vossat I, Marin JG, Chabrier PE: Selective inhibition of inducible nitric oxide synthase by agmatine. Jpn J Pharmacol 1995, 69:285–287.
15. Demady DR, Jammongkol S, Vuletich JL, Bender AT, Osawa Y: Agmatine enhances the NADPH oxidase activity of neuronal NO synthase and leads to oxidative inactivation of the enzyme. Mol Pharmacol 2001, 59:24–29.
16. Uzbay TI: The pharmacological importance of agmatine in the brain. Neurosci Biobehav Res 2012, 365:502–519.
17. Kim JH, Yenari MA, Giffard RG, Cho SW, Park KA, Lee JE: Agmatine reduces infarct area in a mouse model of transient focal cerebral ischemia and
protects cultured neurons from ischemia-like injury. Exp Neurol 2004, 189:122–130.

18. Kim JH, Lee YW, Park KA, Lee WT, Lee JE. Agmatine attenuates brain edema through reducing the expression of aquaporin-1 after cerebral ischemia. J Cereb Blood Flow Metab 2010, 30:943–949.

19. Utahimejirō D, Kim JH, Kim JS, Lee WT, Park KA, Barbatarra G, Tundreveldt S, Angalabirbar D, Lee JE. Recovered changes in the spleen by agmatine treatment after transient cerebral ischemia. Anat Cell Biol 2010, 43:44–53.

20. Wang CC, Chio CC, Chang CH, Kuo JR, Chang CP. Beneficial effect of agmatine on brain apoptosis, astrogliosis, and edema after rat transient cerebral ischemia. BMC Pharmacol 2010, 10:11.

21. Lee WT, Hong S, Yoon SH, Kim JH, Park KA, Seong GJ, Lee JE. Neuroprotective effects of agmatine on oxygen-glucose deprived primary-cultured astrocytes and nuclear translocation of nuclear factor-kappa B. Brain Res 2009, 1281:64–70.

22. Ahn SK, Hong S, Park YM, Lee WT, Park KA, Lee JE. Effects of agmatine on hypoxic microglia and activity of nitric oxide synthesis. Brain Res 2011, 1373:48–56.

23. Moon SU, Kwon KH, Kim JH, Bokara K, Park KA, Lee WT, Lee JE. Recombinant hexamidine arginine decarboxylase (hisADC) induced endogenous arginine decarboxylase synthesis during stress. Mol Cell Biochem 2010, 345:53–60.

24. Bokara K, Kwon KH, Nho Y, Lee WT, Park KA, Lee JE. Retroviral expression of arginine decarboxylase attenuates oxidative burden in mouse cortical neural stem cells. Stem Cells Dev 2011, 20:527–534.

25. Seo SK, Yang W, Park YM, Lee WT, Park KA, Lee JE. Overexpression of human arginine decarboxylase rescues human mesenchymal stem cells against H2O2 toxicity through cell survival protein activation. J Korean Med Sci 2013, 28:366–373.

26. Lee JE, Kim CT, Gaccia AJ, Giffard RG. The E6 and E7 genes of human papilloma virus type-16 protect primary astrocyte cultures from injury. Brain Res 1995, 705:10–16.

27. Hong S, Lee JE, Kim CY, Seong GJ. Agmatine protects retinal ganglion cells from hypoxia-induced apoptosis in transformed rat retinal ganglion cell line. BMC Neurosci 2007, 8:81.

28. Wroblewski F, Ladue JS. Arginine pathways and the synthesis of nitric oxide in the brain. Pharmacol Rev 2003, 55:365–391.

29. Condello S, Calabrò E, Caccamo D, Currò M, Ferlazzo N, Satriano J, Magazù S, Pizzo DP, Ientile R, Colombatto S, Toninello A, Satriano J. Neuroprotective effects of agmatine on oxygen-glucose deprived primary-cultured cerebral ischemia. Exp Biol Med 2007, 232:1009–1017.

30. Chen H, Zhang N, Zhang Y, Chen J. Effects of agmatine on oxygen-glucose deprived neural stem cells. Stem Cells Dev 2010, 19:890–897.

31. Kilkenny D, Browning DJ, Cuthill IC, Emerson M, Altman DG. Reporting guidelines: the ‘Figure 1’ flowchart. Nat Methods 2010, 7:365–367.

32. van Wimersma Greidanus TB, de Boer WI, de Jong T, Killeen AR, van Leeuwen MA, van der Tweel I, Spronk PE, van der Meer NJM, Kruip LJ. Agmatine attenuates neuropathic pain in rats: possible mediation of nitric oxide and norepinephrine activity in the brainstem and cerebellum. Life Sci 2003, 73:413–428.

33. Su RB, Wei XL, Zheng JQ, Liu Y, Lu XQ, Li J. Anticonvulsive effect of agmatine in mice. Pharmacol Biochem Behav 2004, 77:345–349.

34. Naidu S, Agmatine: biological role and therapeutic potentials in neurodegeneration. J Neural Transm 2010, 117:1317–1328.

35. Takeda BG, Katagale NR, Patel MR, Shelker GP, Uggale RR, Choppa CT. Agmatine, an endogenous imidazoline receptor ligand modulates ethanol anxiolysis and withdrawal anxiety in rats. Eur J Pharmacol 2010, 637:89–101.

36. Westing H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. Prog Neurobiol 2001, 64:365–391.

37. Condello S, Calabrò E, Caccamo D, Currò M, Ferlazzo N, Satriano J, Magazù S, Lenti R. Protective effects of agmatine on rotenone-induced damage of human SH-SY5Y neuroblastoma cells: different impact on the survival of normal and cancer cells in culture. BMC Pharmacol 2010, 10:18.

38. Sato T, Kageyama H, Konishi Y, Tsubura Y, Ishioka K, Yamasaki Y, Nakamura Y, Asai Y, Kitamura W, Yamashita T, et al. Protective effects of agmatine on cardiac ischemia/reperfusion-induced renal injury in rats. J Cardiovasc Pharmacol Ther 2001, 6:37–45.

39. Battaglia V, Grancara S, Satirano J, Saccosso S, Agostinelli E, Toninello A. Agmatine prevents the Ca2+-dependent induction of permeability transition in rat brain mitochondria. Amino Acids 2010, 38:431–437.

40. Huang YC, Tseng WS, Wang CC, Cheng BC, Chang YK, Chen HH, Lin PC, Huang TY, Chuang TJ, Lin JW, Chang CP. Neuroprotective effect of agmatine in rats with transient cerebral ischemia using MR imaging and histopathological evaluation. Magn Reson Imaging. 2013, 31:174–181.

41. Sengul G, Talcí E, Malcok UA, Akar A, Erdogan F, Kadioglu HH, Aydin H. A preliminary histopathological study of the effect of agmatine on diffuse brain injury in rats. J Clin Neurosci 2008, 15:1125–1129.

42. Kuo JR, Lo CJ, Chang CP, Lin KC, Lin MT, Chio CC. Agmatine-promoted angiogenesis, neurogenesis, and inhibition of gliosis-reduced traumatic brain injury in rats. J Tissue 2011, 71:587–593.

43. Glad CM, Glad VH. Accelerated functional recovery and neuroprotection by agmatine after spinal cord ischemia in rats. Neurosci Lett 2000, 296:97–100.

44. Yu CG, Marcello AE, Fairbanks CA, Wilcox GC, Yezierski RP. Agmatine improves locomotor function and reduces tissue damage following spinal cord injury. Neuroreport 2000, 11:3265–3269.

45. Oral A, Delen Y, Ulker S, Soykan N. Agmatine attenuates neuropathic pain in rats: possible mediation of nitric oxide and noradrenergic activity in the brainstem and cerebellum. Life Sci 2003, 73:413–428.

46. Su RB, Wei XL, Zheng JQ, Liu Y, Lu XQ, Li J. Anticonvulsive effect of agmatine in mice. Pharmacol Biochem Behav 2004, 77:345–349.

47. Reguñahiam S. Agmatine: biological role and therapeutic potentials in neurodegeneration. J Neural Transm 2010, 117:1317–1328.

48. Takeda BG, Katagale NR, Patel MR, Shelker GP, Uggale RR, Choppa CT. Agmatine, an endogenous imidazoline receptor ligand modulates ethanol anxiolysis and withdrawal anxiety in rats. Eur J Pharmacol 2010, 637:89–101.

49. Westing H. Arginine metabolism and the synthesis of nitric oxide in the nervous system. Prog Neurobiol 2001, 64:365–391.

50. Condello S, Calabrò E, Caccamo D, Currò M, Ferlazzo N, Satriano J, Magazù S, Lenti R. Protective effects of agmatine on rotenone-induced damage of human SH-SY5Y neuroblastoma cells: different impact on the survival of normal and cancer cells in culture. BMC Pharmacol 2010, 10:18.

51. Sato T, Kageyama H, Konishi Y, Tsubura Y, Ishioka K, Yamasaki Y, Asai Y, Kitamura W, Yamashita T, et al. Protective effects of agmatine on cardiac ischemia/reperfusion-induced renal injury in rats. J Cardiovasc Pharmacol Ther 2001, 6:37–45.

52. Molderings GJ, Heinen A, Menzel S, Lubbecke F, Homann J, Gothert M. Effects of agmatine on macrophages and astrocytes. BMC Pharmacol 2010, 10:18.

53. Molderings GJ, Heinen A, Menzel S, Lubbecke F, Homann J, Gothert M. Effects of agmatine on macrophages and astrocytes. BMC Pharmacol 2010, 10:18.
62. Jung HJ, Yang MZ, Kwon KH, Yenari MA, Choi YJ, Lee WT, Park KA, Lee JE: Endogenous agmatine inhibits cerebral vascular matrix metalloproteinases expression by regulating activating transcription factor 3 and endothelial nitric oxide synthesis. Curr Neurovasc Res 2010, 7:201–212.

63. Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ: Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab 1999, 19:624–633.

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