Neuroprotective effects of geneticin (G418) via apoptosis in perinatal hypoxic–ischemic brain injury

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Purpose: Some antibiotics were known to exert neuroprotective effects in the animal model of hypoxic–ischemic (H–I) brain injury, but the mechanism is still unclear. A recent study reported that geneticin (G418), an aminoglycoside antibiotic, increased survival of human breast cancer cells by suppressing apoptosis. We investigated the neuroprotective effects of systemically administrated geneticin via anti-apoptosis following the H–I brain injury

Methods: Seven-day-old Sprague–Dawley rat pups were subjected to unilateral (left) common carotid artery occlusion followed by 2.5 hours of hypoxic exposure and the cortical cell culture of rat brain was done under a hypoxic incubator. Apoptosis was measured in the injured hemispheres 7 days after H–I insult and in the injured cells from hypoxic chamber using morphologic analysis by Terminal dUTP Nick-end Labeling (TUNEL) assay and immunohistochemistry for caspase-3, and cytologic analysis by western blot and real time PCR for bax, bcl-2, and caspase-3.

Results: The gross appearance and hematoxylin and eosin stain revealed increased brain volume in the geneticin–treated animal model of perinatal H–I brain injury. The TUNEL assay revealed decreased apoptotic cells after administration of geneticin in the cell culture model of anoxia. Immunohistochemistry showed decreased caspase-3 expression in geneticin–treated cortical cell culture. Western blot and real–time PCR showed decreased caspase-3 expression and decreased ratio of Bax/Bcl-2 expression in geneticin–treated animal model.

Conclusion: Geneticin appears to exert a neuroprotective effect against perinatal H–I brain injury at least via anti-apoptosis. However, more experiments are needed in order to demonstrate the usefulness of geneticin as a preventive and rescue treatment for H–I brain injuries of neonatal brain.

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Key Words: Hypoxic–ischemic brain injury, Anti–apoptosis, Perinatal, Geneticin, G418

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Introduction

Hypoxic–ischemic (H–I) brain injury during the perinatal period remains the single major cause of acute mortality and chronic disability in newborn infants, leading to permanent neurodevelopmental sequelaes. The incidence of asphyxia at birth is estimated to be 0.2–0.4% in full-term newborn infants and approaches 60% in preterm infants. About 20–50% of infants suffering from H–I encephalopathy expire during the neonatal period, and 25–30% of the survivors exhibit permanent neurodevelopmental abnormalities such as mental retardation, learning disability, epilepsy, and cerebral palsy.

H–I brain injury can also result in attention deficit disorders and minimal brain disorder syndromes, and may in addition form the basis for psychiatric and neurodegenerative diseases later in life. H–I brain injury triggers biochemical events such as energy failure, membrane depolarization,
brain edema, increased neurotransmitter release, increased intracellular Ca
t+, increased production of oxygen-free radicals, increased lipid peroxidation, and decreased cerebral blood flow, leading to brain dysfunction and neuronal death. Neuronal death begins during the H-I insult and extends into the recovery period following resuscitation. It causes immediate neuronal loss during the insult itself (primary neuronal cell death), after which other mechanisms become operative either immediately after the injury (reactive cell death or reperfusion injury) or hours or days later (delayed neuronal cell death). The injured brain tissue undergoes selective neuronal necrosis or infarction and the immediate area surrounding the infarct consists of neurons with either necrosis or apoptosis.

The penumbral area appears most amenable to reversal of cellular injury through therapeutic intervention which includes prophylactic therapy during or immediately after the H-I insult and rescue therapy during delayed neuronal death. Although there are experimental therapeutic interventions that may ameliorate perinatal H-I brain injury, currently there is no clinical treatment for this disorder. The inhibition of molecular pathways leading to apoptosis may provide a novel therapeutic opportunity for treatment of perinatal H-I brain injury.

Cell death from perinatal H-I brain injury may result not only the result of classic necrosis but also may involve apoptosis. While necrosis is the dominant mechanism in severe ischemic insults, the prevention of apoptosis may potentially expand the therapeutic window for the treatment of perinatal H-I brain injury. The degradation of cells by apoptosis depends on the activation of caspases, and caspase-3, a widely studied caspase, plays an important role in neuronal cell death after an H-I insult. In addition, the Bcl-2 family of proteins also involves in apoptosis. Pro-apoptotic proteins such as Bax, Bid, and Bak promote cell death, whereas anti-apoptotic proteins such as Bcl-2 and Bcl-xL may enhance cell survival. Recently several antibiotics such as tetracycline derivatives, doxycycline and minocycline, and ceftriaxone have been shown to be neuroprotective in animal models of H-I brain injury. Especially the administration of minocycline either before or after an H-I insult substantially blocked tissue damage by preventing the formation of activated caspase-3. A recent study represent that genetin, an antibiotic of aminoglycosides, made human breast cancer cells survive by suppressing apoptosis, even more resistant to hypoxia.

We investigated a neuroprotective effect of genetin through anti-apoptosis using in vitro cell culture model of anoxia and in vivo animal model of perinatal H-I brain injury. Apoptosis was identified using morphologic analysis by TUNEL assay and immunohistochemistry for caspase-3, and cytologic analysis by Western blot and real-time PCR for bax, bcl-2, and caspase-3.

**Materials and Methods**

1. **Animal model**

This study was performed in accordance with the approved animal use guidelines of the Catholic University of Daegu. The protocol for the newborn animal model of H-I brain injury was based on a modification of the Levine preparation described by Rice et al. Sprague–Dawley rat pups at postnatal day 7 (P7, day of birth is postnatal day 1) were used to test perinatal H-I brain injury. Eighteen pups were anesthetized with ketamine and underwent permanent unilateral (left) common carotid artery ligation. Following a 1-hour recovery and feeding period, the pups underwent a 2.5-hour period of anoxia (92% N2, 8% O2) in an airtight container. The container was submerged in a 37 °C water bath to maintain a constant thermal environment during this period. Following hypoxia, the pups were returned to their dam. The pups received an intraperitoneal injection of 0.1 μg/kg of genetin in the right lower quadrant 30 minutes both before and after placement in the hypoxic chamber. The pups were sacrificed 7 days after H-I insult and brain tissues were immediately removed. The ipsilateral (injured) hemispheres of the animal were separated and prepared, and tissue homogenates were stored at -70°C pending further processing.

2. **Embryonic cortical neuronal cell culture**

The cortical cell culture procedure for rat brain based on the Brewer method was employed. Sprague–Dawley rats pregnant for 18 days were anesthetized with ether for 5 minutes at room temperature and the uteruses were removed. The brains of fetal pups were dissected and brain cortical tissues were digested for 5 minutes at 37°C in Hank’s balanced salt solution (HBSS) (Gibco BRL, USA) containing 0.25% trypsin. They were rinsed five times with 5 mL of HBSS containing 1 mM sodium pyruvate and 10 mM HEPES (pH 7.4). The cells were removed in 1 mL Hank’s solution.
pipetted 6–7 times with a small-bore Pasteur pipette, and dispersed. After cell counting, they were inoculated into Neurobasal plating media supplemented with B27 (Gibco BRL, USA) (100 mL Neurobasal, 2 mL B27 supplement, 0.25 mL glutamate I, 0.1 mL 25 mM glutamate, 0.1 mL 25 mM 2-mercaptoethanol), and cells were plated at a density of about $2 \times 10^5$ cells/mm$^2$ in 8 well chamber slides. They were cultured in CO2 chamber, and 1/5 of the total volume of the culture solutions were replaced every three days with feeding Neurobasal media (Gibco BRL, USA) (100 mL Neurobasal medium, 2 mL B27 supplement, 0.25 mL glutamate I). The cultured cells were divided two groups as the normoxia group and the anoxia group. The normoxia group was cultured in 5% CO2 incubators and the anoxia group was cultured in 1% O2 incubators (94% N2, 5% CO2) for 2–3 days with cell counting. After the determination was made of sufficient cellular injury, cells were divided into genetic–treated (10 μg/mL) and control groups.

3. TUNEL stain

Cultured cells on 8 well chamber slides were rinsed with phosphate buffered solution (PBS) and fixed with 4% paraformaldehyde in PBS (pH 7.4) for 1 hour at room temperature. Afterward, cells were treated with permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 2 minutes on ice and rinsed twice with PBS for 2 minutes, then were mixed with labeling solution and enzyme solution from the in situ cell death detection kit (TUNEL kit, Roche, Germany) at 50 μL per sample, and incubated for 1 hour at 37°C. After rinsing three times with PBS for 2 minutes, cells were treated with DAPI (4,6-diamidino-2-phenylindole) (Sigma, USA) (target concentration 1 g/mL) for 10 minutes at room temperature and rinsed with PBS again. The cells were plated with antifading solution and observed using Zeiss Axioshot microscope attached fluorescent viewing device. The TUNEL–stained cells were visualized as green-colored cells using the F09 filter set (BP450–490, FT510, LP 520), and the DAPI–stained cells were seen as blue-colored cells using the F02 filter set (BP365, FT 385, LP 420). A triple band pass filter (ex: 400/455/570, beam splitter: 410/505/585, em: 460/530/610) for simultaneous detection of DAPI–stained and TUNEL–stained cells in the same view was used to photograph the cells. The TUNEL–stained cells were detected as cells with marked condensation of chromatin compared to DAPI stain, or as cells with apoptotic bodies as seen with an optic microscope. Cell counts were done at 600 × magnification at 10 different sites in each chamber. TUNEL–stained cells only were counted as apoptotic, and the total cells count was calculated as the total number of TUNEL–stained cells plus DAPI–stained cells.

4. Hematoxylin and eosin (H & E) stain

Histologic studies were performed 7 days after H-I insult. After intracardiac perfusion with saline, the brains were removed, fixed in 4% paraformaldehyde, embedded in paraffin, and prepared for light microscopy. Four μm sections were mounted on glass cover slides. Sections were deparaffinized in xylene for 30 minutes and serially treated with 100% (5 minutes), 96% (10 minutes), and 70% (10 minutes) ethanol. Slides were stained with hematoxylin, rinsed for a few seconds with water to remove excess stain, placed in 1% eosin and then rinsed briefly in water to remove the acid, and washed through the following series: 70%, 95%, and 100% ethanol at 5 minutes each. Finally, the slides were transferred to xylene for clearing, mounted, and covered with cover slips.

5. Immunohistochemistry

Immunohistochemistry was performed 7 days after H-I insult. After intracardiac perfusion with saline, the brains were removed, fixed in 4% paraformaldehyde, and embedded in paraffin. Eight-micrometer-thick sections were cut from the bregma and mounted on coated slides. Sections were deparaffinized, rehydrated, washed in tris buffered saline (TBS), and microwaved two times for 2 minutes in citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked by treatment with 3% H2O2. Sections were permeabilized with 0.3% Triton X-100 (Sigma Chemical Co., USA), blocked in 10% goat serum for 60 minutes at room temperature, and incubated with a primary antibody against caspase-3 (Cell signaling, USA) at 4°C overnight. Sections were next washed twice in TBS for 10 minutes, followed by 1-hour incubation with the biotinylated secondary antibody (Santa Cruz Biotechnology, USA). After two 10 minute TBS washes, the sections were incubated with the avidin–biotin complex (Vectastain Elite: Vector Laboratories, Burlingame, CA, USA) in TBS for 1 hour. The signal was visualized by incubating in 0.06% 3,3’-diaminobenzidine (DAB) (Sigma Chemical Co.) and 0.006% H2O2 in 0.1 M Tris–HCl (pH 7.5). Sections were dehydrated, counterstained with Neutral Red, mounted with Depex (Serva), and covered with cover slips.
6. Western Blot

Samples of brain tissue were homogenized and total protein was extracted using a protein lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid [EDTA], 0.5% Nonidet P-40, 100 mM phenylmethylsulfonyl fluoride [PMSF], 1 mg/mL leupeptin, 1 mg/mL aprotinin, and 1 M 1,4-dithio-DL-threitol [DTT]). Equivalent aliquots of proteins were boiled in loading buffer (100 mM Tris–HCl [pH 6.8], 200 mM DTT, 20% glycerol, 4% sodium dodecyl sulfate (SDS), and 0.2% bromophenol blue) for 5 minutes. Samples contained in equal amounts of protein (40 μg) were subjected to a 12% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and proteins were then electrotransferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in TBS–T buffer (20 mM Tris–HCl [pH 7.5], 150 mM NaCl, and 0.1% Tween 20) containing 5% nonfat milk for 1 hour at room temperature. Proteins were visualized following incubation with specific primary antibodies of Bcl-2 (Santa Cruz Biotechnology, USA), Bax (Cell signaling, USA) and caspase 3 (Cell signaling, USA) at 4°C overnight. After four washes in a TBS–T buffer, the membranes were incubated with horseradish peroxidase–conjugated second antibodies (Santa Cruz Biotechnology, USA) for 1 hour at room temperature. Immunoreactivity was detected using enhanced chemiluminescence (ECL). The bands in the exposed films were analyzed by densitometer (Multi-gauze software).

7. RNA extraction and Real Time-PCR

Total RNA was extracted with TRIzol reagent (Invitrogen corporation, Carlsbad, CA). Briefly, total tissue was homogenized in 1 ml of TRIzol reagent and total RNA was separated from DNA and proteins by extracting with chloroform and precipitating using isopropanol. The precipitate was washed twice in 75% ethanol, air-dried, and re-dissolved in diethylpyrocarbonate (DEPC)-treated distilled water. The amount and purity of extracted RNA was quantitated with a spectrophotometry (Backman). The RNA was then stored at −70°C pending further processing. For reverse transcription, 1 μg total RNA was reverse transcribed for 1 hour at 37°C in a reaction mixture containing 20 U RNase inhibitor (Promega, Madison, WI), 1 mM dNTP (TaKaRa), 0.5 ng Oligo-(dT) 15 primer (Promega, Madison, WI), 1X RT buffer and 200 U M-MLV reverse transcriptase (Promega, Madison, WI). The reaction mixture was then heated at 95°C for 5 minutes to stop the reaction. The cDNA was then stored at −20°C pending further processing.

Real-time PCR was performed in 48 well PCR plates (MJ mini gradient thermal cycler) using the FINNZYMES DyNaMo SYBR green qPCR kit. Amplification conditions were the same for all apoptotic mRNAs assayed: 95°C for 15 minutes, followed by 40 cycles of 95°C for 45 seconds, 57°C for 45 seconds, and 72°C for 45 seconds. Real-time PCR data were analysed with LightCycler software (BIORAD, USA).

The primers used were as follows: caspase-3 sense 5′-AAT TCA AGG GAC GGG TCA TG-3′ caspase-3 antisense 5′-GCT TGT GCG CGT ACA GTT TC-3′; Bcl-2 sense 5′-TTG ACG CTC TCC ACA CAC ATG-3′ Bcl-2 antisense 5′-GGT GGA GGA ACT CTT CAG GGA-3′; Bax sense 5′-TGC TGA TGG CAA CTT CAA CT-3′ Bax antisense 5′-ATG ATG GTT CTG ATC AGC TCG-3′.

8. Statistical analysis

Data were analyzed using the SPSS version 12 statistical analysis package. Examined data were assessed using the T-test, GLM (general lineal model), and ANOVA. In each test, the data are expressed as the mean SD and P=0.05 was accepted as statistically significant.

Results

1. Decreased counts of TUNEL–stained apoptotic cells in genetin-treated cultures of cortical cell culture from rat brain

Apoptotic ratio is defined as the ratio of TUNEL–stained cells to the total cells (TUNEL–stained cells plus DAPI–stained cells). Genetin-treated groups in normoxia (B) and anoxia (D) appeared to contain fewer TUNEL–stained cells than were present in control groups in normoxia (A) and anoxia (C) (Fig. 1).

In normoxia, the mean apoptotic ratios of the control and genetin-treated groups were 0.76 (0.16) and 0.62 (0.27), respectively, which is a statistically significant difference between the two groups (P<0.01). In anoxia, the mean apoptotic ratios of the control and genetin–treated groups were 0.84 (0.08) and 0.30 (0.20), respectively, also with a statistically significant difference between the two groups (P<0.01). In the control group, the mean apoptotic ratio of normoxia was 0.76 (0.16) and was less than the ratio for
anoxia of 0.84 (0.08), but the difference between the two groups in this case was not statistically significant ($P>0.05$). In the genetin-treated group, the mean apoptotic ratio for normoxia was 0.62 (0.27) and was less than the ratio for anoxia of 0.30 (0.20), and the difference between the two groups was statistically significant ($P<0.01$) (Fig. 2). Therefore, the TUNEL assay showed decreased ratios of TUNEL-stained apoptotic cells in genetin-treated cultures in the in vitro cell culture model of anoxia.

2. Increased brain volume by gross appearance and H&E stain in the genetin-treated animal model for perinatal HI brain injury

The gross appearance together with H&E staining of 7-day-old rat brain after HI insult revealed that brain volume was more greatly decreased in anoxia (B) than in normoxia (A), and that rat brain was better protected in animals treated with genetin before an HI insult (C) than in animals treated after an HI insult (D) (Fig. 3, 4).

**Fig. 1.** TUNEL (Terminal dUTP Nick-end Labeling) assay control–stained with DAPI (4,6-diamidino-2-phenylindole) revealed green-colored cells with TUNEL-positive stain and blue-colored cells with DAPI stain (×800). (A) for control group in normoxia, (B) for genetin-treated group in normoxia, (C) for control group in hypoxia, (D) for genetin–treated group in hypoxia.

**Fig. 2.** Box analysis revealed control group in normoxia, genetin–treated group in normoxia, control group in hypoxia, and genetin–treated group in hypoxia with TUNEL assay. $P<0.01$.

**Fig. 3.** Gross appearance of 7-day-old rat brains revealed: (A) for control group in normoxia, (B) for control group in hypoxia, (C) for genetin–treated group hypoxia, (D) for genetin–treated group after hypoxia.
3. Decreased expression of Bax by immunohistochemistry in geneticin-treated cortical cells

Immunohistochemistry for caspase-3 seven days after H-I insult revealed that apoptotic cells were more greatly increased in anoxia (B) than in normoxia (A), and more decreased in cells treated with geneticin before an H-I insult (C) than after an H-I insult (D) (Fig. 5).

![Fig. 4](image-url) Hematoxylin and eosin (H&E) stain in coronal sections of 7-day-old rat brains revealed: (A) for control group in normoxia, (B) for control group in hypoxia only, (C) for geneticin-treated group before hypoxia, (D) for geneticin-treated group after hypoxia.

![Fig. 5](image-url) Light microscopic view of immunoreactivity for caspase-3 expressions in rat brains revealed: (A) for control group in normoxia, (B) for control group in hypoxia, (C) for geneticin-treated group before hypoxia, (D) for geneticin-treated group after hypoxia.
4. Decreased ratio of Bax/Bcl-2 expression and caspase-3 expression detected by western blotting in the geneticin-treated animal model

The ratio of Bax/Bcl-2 expression and the expression of caspase-3, which are indicators of the level of apoptosis, were more increased in anoxia than in normoxia and more decreased before administration of geneticin than after administration of geneticin (Fig 6, 7).

Fig. 6. Western blotting revealed: (A) for Bax, (B) for Bcl-2, (C) for caspase-3, (D) for actin.

Fig. 7. Histogram of Western blotting revealed: (A) for Bcl-2, (B) for Bax, (C) for Bax/Bcl-2 ratio, (D) for caspase-3.

Fig. 8. Histogram of real-time PCR revealed: (A) for Bcl-2, (B) for Bax, (C) for Bax/Bcl-2 ratio, (D) for caspase-3. (C, Control; H, Hypoxia; B, before treatment; A, after treatment)
5. Decreased ratio of Bax/Bcl-2 expression and caspase-3 expression using real-time PCR in the genenicin-treated animal model

The ratio of Bax/Bcl-2 expression and the expression of caspase-3 were more increased in anoxia than in normoxia, and more decreased before administration of genenicin than after administration of genenicin (Fig. 8).

Discussions

The antibiotic genenicin, also known as G-418 sulfate, is a water-soluble aminoglycosides. It is structurally related to gentamicin but has inhibitory activity against a much wider variety of prokaryotic and eukaryotic organisms, including protozoa and helminths. It is used most extensively in transfection experiments to select eukaryotic cells that have acquired neomycin resistance genes, although its mechanism of action is still elusive. It is not normally used as a standard antibiotic due to its toxicity. It is known to be toxic to bacteria, yeast, protozoa, helminthes, and mammalian cells. A recent study reported that genenicin increased survival of human breast cancer cells by suppressing apoptosis, and that it also made these cells more resistant to hypoxia. Some antibiotics such as the tetracycline derivative, doxycycline and the cephalosporin antibiotic, exhibited neuroprotective effects through anti-apoptosis in an animal model of neonatal hypoxia-ischemia. Therefore, we investigated the neuroprotective effect of genenicin through anti-apoptosis using an in vitro cell culture model of hypoxia and an in vivo animal model of perinatal H-I brain injury. Apoptosis was identified through morphologic analysis by TUNEL assay and by immunohistochemistry for caspase-3, and through cytologic analysis by Western blotting and real time PCR for bax, bcl-2, and caspase-3.

In the present study, the TUNEL assay revealed decreased TUNEL-staining of apoptotic cells after administration of genenicin in the in vitro cell culture model of hypoxia. Gross appearance and H & E staining of 7-day-old rat brain after H-I insult revealed greater brain volume for genenicin-treated animals than for non-treated animals in the genenicin-treated animal model of perinatal H-I brain injury. Immunohistochemistry revealed decreased expression of caspase-3 in genenicin-treated cortical cells from rat brain. Western blot analysis and real-time PCR revealed a decreased ratio of Bax/Bcl-2 expression and expression of caspase-3 in gene-
osomal cleavage, and cytologic analyses by FACS (fluorescence-activated cell sorter), western blot, and real time PCR of proteins involved in the apoptotic machinery. In the present study, we employed morphologic analyses by TUNEL assay and immunohistochemistry for caspase-3, together with cytologic analyses by western blot and real-time PCR for bax, bcl-2, and caspase-3.

Neurodevelopmental outcome following perinatal H-I brain injury depends substantially on the nature and extent of neuronal destruction and the degree of energy failure. H-I injury may be partially prevented by intervention with neuroprotective agents. Currently known neuroprotective agents and factors include oxygen-free radical inhibitors and scavengers, monosialogangliosides, calcium channel blockers, glucocorticosteroids, phenobarbital, hyperglycemia, carbon dioxide, hypoxic preconditioning, excitatory amino acid antagonists, inhibitors of nitric oxide production, growth factors, hypothermia, inhibitors of caspase activity, edaravone, ceramide, magnesium, several antibiotics, and apoptosis inhibitors.

As current neuroprotective strategies are very complicated, it is important to find animal models to help in understanding of the mechanisms leading to cell death after perinatal H-I brain injury and in studying the potential therapies and treatments. The most widely used animal model of perinatal H-I brain injury is a modification of the Levine preparation described by Rice et al. which utilizes a combination of ischemia produced by unilateral occlusion of carotid artery together with hypoxia achieved by the inhalation of 8% oxygen/balanced nitrogen at constant temperature (37°C) in Sprague–Dawley rats at postnatal 7 day. This model of H-I brain injury results in a reproducible pattern of hemispheric injury that is ipsilateral but not contralateral to the carotid ligation. There are prominent features of both apoptosis and necrosis when this model is performed in neonatal rats. The immature rat model has proved especially useful for numerous studies of perinatal H-I brain injury. The 7-day-old rat was originally chosen for study because the neurological events at postnatal day 1 are comparable to those in human fetus between the 32nd to 34th weeks of gestation, i.e., cerebral cortical neuronal layering is complete, the germinal matrix is involuting, and white matter has as yet undergone little myelination. Hence, H-I brain injury in 7-day-old rats can be considered as analogous to perinatal asphyxia in the full-term infants.

Tetracyclines inhibited microglial activation and protected hippocampal neurons against global brain ischemia in gerbils, even when administered after the H-I insult. Doxycycline was used in a model of focal CNS reperfusion injury in order to determine its efficacy for preserving neurological function, and it attenuated post-ischemic decreases in in vivo leukocyte counts and inhibited in vitro leukocyte adhesion. Doxycycline has also been suggested to play a role in reducing clinical CNS reperfusion injury, and has been shown to reduce cleaved caspase-3 and microglial activation in an animal model of neonatal hypoxia–ischemia. Tetracycline derivatives and ceftriaxone, a cephalosporin antibiotic, have been reported to protect neurons against apoptotic death by ionizing radiation in primary cortical cultures. Arvin et al. showed that minocycline administered either immediately before or immediately after H-I insult substantially blocks tissue damage in a rodent model of neonatal H-I brain injury, and they suggested that minocycline treatment prevents both the formation of activated caspase-3, a known effector of apoptosis, and the appearance of a calpain–cleaved substrate that is a marker of excitotoxic/necrotic cell death. Even in normoxia– and hypoxia– induced cortical cell cultures of rat brain, minocycline was found to be neuroprotective via an anti-apoptotic mechanism. Geneticin is an amidoglycoside antibiotic and has also shown neuroprotective properties. Previously we found that geneticin exerts a protective effect against hypoxic injury in rat kidney (unpublished).

The present study demonstrated that geneticin treatment results in decreased numbers of apoptotic cells, greater brain volume, a decreased ratio of Bax/Bcl-2 expression, and decreased expression of caspase-3 in the context of perinatal H-I brain injury.

In conclusion, geneticin appears to exert neuroprotective effects through anti-apoptotic in both in vitro and in vivo experimental studies of perinatal H-I brain injury. However, more experiments are needed in order to demonstrate the usefulness of geneticin as a preventive and rescue treatment for H-I brain injuries of neonatal brain.

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한 글 요약

주산기 저산소성 허혈성 뇌손상에서 항고사를 동반한 G418의 신경보호 효과

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목적: 몇몇 약물항체가 저산소성 허혈성 뇌손상에서의 보호 효과를 가진 것으로 밝혀졌지만 아직까지 그 기전에 대한 잘 알려지지 않고 있다. 최근 아미노글루타민계열의 항고소성 G418(geneticin)이 항고소에 의한 뇌손상 중추세포의 죽음 억제에 관한 연구가 제안되어 G418는 저산소성 허혈성 뇌손상에서 세포 고사를 억제함으로서의 보호 효과를 나타내는 지를 알아보고자 하였다.

방 법: 일산화 탄소를 이용한 액체상피성의 뇌손상 모델을 사용하여 G418를 주사하여 뇌의 생존률을 측정하였다.

결과: TUNEL 분석과 caspase-3에 대한 면역조직화학검사를 하였고, 생존 7일 뒤에 얻은 생존 약제의 대조군과 대비군의 생존율에 차이가 있는지 확인하였다. G418를 주사한 군에서 퇴비류 이후 활성화된 H&E 염색과 caspase-3에 대해 Western blot과 real-time PCR로 분석하였다.

결론: 주산기 저산소성 허혈성 뇌손상에서 G418는 성인 조직의 고사를 억제함으로서의 보호 효과를 나타내었다.

References