The neuroprotective effect of mycophenolic acid via anti-apoptosis in perinatal hypoxic-ischemic brain injury

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Purpose : Mycophenolic acid (MPA), the active metabolite of mycophenolate mofetil (MMF), is a potent inhibitor of inosine-monophosphate dehydrogenase (IMPDH), a new immunosuppressive drug used. It was reported that MPA protected neurons after excitotoxic injury, induced apoptosis in microglial cells. However, the effects of MPA on hypoxic-ischemic (HI) brain injury has not been yet evaluated. Therefore, we examined whether MPA could be neuroprotective in perinatal HI brain injury using Rice-Vannucci model (*in vivo*) and in rat brain cortical cell culture induced by hypoxia (*in vitro*).

Methods : Cortical cells were cultured using a 18-day-pregnant Sprague-Dawley (SD) rats and incubated in 1% O_2 incubator for hypoxia. MPA (10 μg/mL) before or after a HI insult was treated. Seven-day-old SD rat pups were subjected to left carotid occlusion followed by 2 hours of hypoxic exposure (8% O_2). MPA (10 mg/kg) before or after a HI insult were administrated intraperitoneally. Apoptosis was measured using western blot and real-time PCR for Bcl-2, Bax, caspase-3.

Results : H&E stain revealed increased brain volume in the MPA-treated group *in vivo* animal model of neonatal HI brain injury. Western blot and real-time PCR showed the expression of caspase-3 and Bax/Bcl-2 were decreased in the MPA-treated group In in vitro and in vivo model of perinatal HI brain injury,

Conclusion : These results may suggest that the administration of MPA before HI insult could significantly protect against perinatal HI brain injury via anti-apoptotic mechanisms, which offers the possibility of MPA application for the treatment of neonatal HI encephalopathy. **(Korean J Pediatr 2007;50:1-)**

Key Words : Mycophenolic acid, Hypoxia-Ischemia, Apoptosis, Caspase-3, Bax, Bcl-2

Introduction

Hypoxic-ischemic (HI) brain injury in the perinatal period is a major cause of morbidity and mortality in infants and children[1]. Perinatal hypoxic-ischemic encephalopathy (HIE) often leads to permanent neurological dysfunction including cognitive impairment, learning disabilities, seizure disorders, and cerebral palsy. Multiple pathogenic mechanisms have been implicated in the neuronal cell death that occurs within a few hours to a few days following the perinatal HI brain injury[2]. These events include the following: a decrease of cerebral blood flow; energy failure; membrane depolarization; brain edema; an increase of neurotransmitter release; the inhibition of neurotransmitter uptake; an increase of intracellular Ca^{2+}; the production of

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oxygen-free radicals; lipid peroxidation; immune/inflammatory activation[3]; and glutamate and nitric oxide (NO) neurotoxicity. Programmed cell death, or apoptosis, also plays a key role in delayed neuronal cell death after HI injury, particularly in the developing brain.

A variety of therapeutic strategies are employed to protect against the pathologic mechanisms of HI injury. These include the following: hypothermia[4]; antagonists of excitotoxic glutamate receptors[5]; calcium channel blockers; anti-inflammatory agents; free radical scavengers; NOS inhibitors; growth and neurotropic factors; and inhibitors of the caspase pathway[6] and of stress kinases[7]. Pharmacological agents which mediate one or more of these processes may provide neuroprotection for patients against this condition. Furthermore, study of neuroprotective agents may provide important information about treatment for perinatal HI brain injury. The neonatal rat HI model and the cortical cell culture model of rat embryos have been well characterized and used extensively to search for neuroprotective agents[8].

Mycophenolate mofetil (MMF) is clinically used as an immunosuppressant following solid organ transplantation. MMF is the 2-morpholino-ethyl ester of mycophenolic acid (MPA), which is a potent inhibitor of inosine-monophosphate dehydrogenase (IMPDH), the critical enzyme in the de novo biosynthesis of purines. MPA emerged in clinical trials in the early 1990s as a new immunosuppressive agent with a different mechanism of action from other drugs[9]. Recently, it has been tested as a treatment for several types of vasculitis including Takayasu's arteritis[10], polyarteritis nodosa, ANCA-associated vasculitis[11], Wegener's granulomatosis[12], lupus nephritis[13], and skin vasculitis[14]. MPA has been shown to induce apoptosis in microglial cells and in T cells[15], and definitively exerts antiproliferative effects and possibly induces microglial apoptosis[16].

It is possible that the improved neuronal survival seen after treatment with MPA may occur independently from the inhibition of microglial activation. Although immunosuppressive and anti-inflammatory properties are currently under evaluation in several autoimmune disorders, the effect of MPA on brain neuroprotection is still not fully understood.

The aim of this study was to determine whether MPA is anti-apoptotic, as measured by Bcl-2, Bax and caspase-3 antibodies in an animal model of neonatal HI brain injury (*in vivo*) and in a culture model of hypoxia (*in vitro*).

Materials and Methods

1. Animal protocol

The neonatal rat HI procedure was performed as described by Rice et al.[17]. We chose 7-day-old (day of birth is postnatal day 1) Sprague-Dawley (SD) rat pups weighing between 12 and 16 g because there are no differences between male and female rat with regard to neonatal HI brain injury[8], and because HI brain injury in 7-day-old rats is similar to perinatal asphyxia in full-term infants. The rat pups were anesthetized with ether. The left common carotid artery was exposed, isolated, and permanently doubly ligated with 5-0 surgical silk. The incision then was sutured and the animal was allowed to recover in a warm environment. The whole surgical procedure was completed in less than 5 minutes. After a recovery of 1 hour, the pups were placed in plastic chambers, which were submerged in a 37℃ water bath under an atmosphere of 8% O_2 and 92% N2 for 2.5 hours. Following this hypoxic exposure, the pups were returned to their dams.

2. Administration of drugs

MPA were purchased from Sigma (St. Louis, MO, USA). MPA in doses of 10 mg/kg was dissolved in 100 μL of 50% methanol per gram of body weight and administered by intraperitoneal (IP) injection at 30 minutes either prior to or following hypoxic exposure. The animals were divided into four groups. In group 1 (HI/MPA "before"), pups were injected with 10 mg/kg of MPA 30 minutes before HI insult (a dose determined to be optimal in preliminary studies). Hypoxic exposure was started after 1 hour of recovery. In group 2 (HI/MPA "after"), pups were injected at same dose 30 mintues after HI insult. Group 3 (HI only) animals were subjected to HI without MPA treatment, and group 4 (normoxia group) animals were not exposed to hypoxia. Pups from each litter were randomly assigned to different normoxia groups and drug treatment groups.

3. Embryonic cortical neuronal cell culture

Culture of cortical neuronal cells from rat embryos was performed on the Brewer[18] method. SD rats pregnant for 18 days were anesthetized with ether for 5 minutes at room temperature and the uteruses were removed. The fetal pups were washed in 100% ethanol and Hanks' balanced salt

solution (HBSS) (GibcoBRL, USA). The brains of the fetal pups were dissected at 37℃ in HBSS containing 1 mM sodium pyruvate and 10 mM HEPES (pH 7.4), after which the cortical tissue were added to 2 mL trypsin and incubated at 37℃ in a water bath for 1 minute. Trypsin was removed by rinsing five times with 5 mL HBSS. The cells were re-suspended in 1 mL HBSS and were dispersed by passing 6-7 times through a small-bore Pasteur pipette. The cells were centrifuged at 1,000 rpm at 25℃ for 5 minutes and pellets were washed with HBSS (without phenol red).

Cells were counted, re-suspended in plating neurobasal medium (GibcoBRL) (100 mL neurobasal medium with 2 mL B27 supplement, 0.25 mL glutamax I, 0.1 mL 25 mM glutamate, and 0.1 mL 25 mM 2-mercaptoethanol), and plated at approximately 2×10^6 cells/mm^2 in each dish. Cells were cultured in CO_2 chamber with 1/5 of the culture solutions replaced every three days with feeding neurobasal medium (GibcoBRL) (100 mL neurobasal medium with 2 mL B27 supplement and 0.25 mL glutamax I).

The cultured cells were divided three groups; a normoxia group, a hypoxia group, and an MPA-treated (10 μg/mL) group. The normoxia group was placed in 5% O_2 incubators (90% N_2, 5% CO_2), and the hypoxia and MPA-treated groups (before a HI insult) were placed in 1% O_2 incubators (94% N_2, 5% CO_2) for 18 hours. After verifying the desired amount of cellular injury in the hypoxia group, the MPA-treated group (after a HI insult) was further divided into two groups. This produced four final groups; normoxia, hypoxia, MPA-treated group before HI insult and MPA-treated after HI insult.

4. Brain extraction and protein isolation

Pups were sacrificed at both 6 hours and 7 days after HI insult. Left cerebral hemispheres from rat brains were immediately removed, frozen on liquid nitrogen, and stored at -70℃ until used. Frozen tissues were homogenized in protein lysis buffer containing complete protease inhibitor cocktail tablets (Roche Applied Science, Mannheim, Germany), 1 M Tris-HCl (pH 8.0), 5 M NaCl, 10% Nonidet P-40 and 1 M 1,4-dithio-DL-threitol (DTT). After incubation for 20 minutes on ice, the samples were centrifuged at 12,000 rpm at 4℃ for 30 minutes and the supernatants were transferred to new tubes. Total protein was measured with a Bio-Rad Bradford kit (Bio-rad Laboratories, Hercules, CA, USA).

5. Western blotting

Equal amounts of protein (40 μg) were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after denaturing in SDS gel-loading buffer (100 mM Tris-HCl pH 6.8, 200 mM DTT, 20% glycerol, 4% SDS and 0.2% bromophenol blue) in boiling water for 10 minutes. After electrophoresis, proteins were electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) at a constant voltage of 10 V for 30 minutes. After transfer, the membrane was washed twice with 1x Tris-buffered saline (TBS) plus 0.1% Tween-20 (TBST, pH 7.4) and preincubated with a blocking buffer (5% nonfat dry milk in TBST) at room temperature for 1 hour. The membrane then was incubated with rabbit polyclonal Bax and caspase-3 primary antibodies (Cell Signaling Technology, Beverly, MA, USA), and mouse monoclonal Bcl-2 primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:1,000 dilutions in TBST at 4℃ overnight. After washing, blots were incubated with secondary anti-rabbit or anti-mouse antibody conjugated with horseradish peroxidase at 1:2,000 dilution at room temperature for 1 hour. Finally, the membrane was washed and developed with by Enhanced Chemiluminoscence (ECL) Plus Western Blotting Detection System (Amersham Biosciences, Piscataway, NJ, USA).

6. Semiquantification of the western blots

The intensities of the western blot bands were measured using a densitometer (Multi Gauge Software, Fuji Photofilm) and were calculated as the ratio of the signal intensity in the ischemic hemisphere to the intensity in the contralateral hemisphere.

7. RNA extraction and real-time PCR

Total RNA was extracted from tissue with TRIzol reagent (Invitrogen corporation, Carlsbad, CA, USA). Briefly, total tissue was homogenized in 1 mL of TRIzol reagent. Total RNA was separated from DNA and proteins by adding chloroform and was precipitated using isopropanol. The precipitate was washed twice in 75% ethanol, air-dried, and re-diluted in diethylpyrocarbonate (DEPC)-treated distilled water. The amounts and purity of extracted RNA was quantitated by spectrophotometry (Beckman, USA), and the RNA was then stored at -70℃ until further processing. For real-time PCR, total RNA (1 μg) was reverse transcribed

for 1 hour at 37℃ in a reaction mixture containing 20 U RNase inhibitor (Promega, Madison, WI, USA), 1 mM dNTP (TaKaRa, Otsu, Japan), 0.5 ng oligo- (dT) 15 primer (Promega), 1x RT buffer and 200 U M-MLV reverse transcriptase (Promega). The reaction mixture was then incubated at 95℃ for 5 minutes to stop the reaction. The cDNA was stored at -20℃ until further processing.

Real-time PCR was performed in 48-well PCR plates (Mini OpticonTM Real-Time PCR System, Bio-Rad, USA) using the Finnzymes DyNAmo SYBR green qPCR kit (Finnzymes, Beverly, MA, USA). Amplification conditions are shown in Table 1. The thermal profile was 95℃ for 15 minutes, followed by 40 cycles of denaturation, annealing, and extension as indicated in Table 1. real-time PCR data were analysed with Light Cycler software (Bio-rad). Each sample was run in triplicate.

Table 1. Primer Pairs and Annealing Temperatures for Real-time PCR

Name	Primer Sequence (5′-3′)	Annealing
Bcl-2	F:TTGACGCTCTCCACACACATG R:GGTGGAGGAACTCTTCAGGGA	57℃
Bax	F:TGCTGATGGCAACTTCAACT R:ATGATGGTTCTGATCAGCTCG	55℃
caspase-3	F:AATTCAAGGGACGGGTCATG R:GCTTGTGCGCGTACAGTTTC	56℃

8. Statistics

The data were presented as mean standard error. The statistical differences between means were determined using Student's t-test; $P<0.05$ was considered significant.

Results

1. Imaging of cultured cortical neuronal cells from rat embryos with treatment of MPA

The cortical neuronal cells were observed under microscopy with a high magnification (×400). Cells in the normoxia group (A) appeared normal, while cells in the hypoxia group (B) showed damage. Cells treated with MPA before hypoxic exposure (C) had a similar appearance to those in the normoxia group, and the cells treated with MPA after hypoxic exposure (D) were less numerous than in the group treated with MPA before hypoxic exposure (Fig. 1).

2. The expression of Bcl-2, Bax and caspase-3 in cultured cortical neuronal cells (in vitro) from 18-day-old rat embryos, as indicated by western blotting

The expression of Bcl-2, an anti-apoptotic marker, was

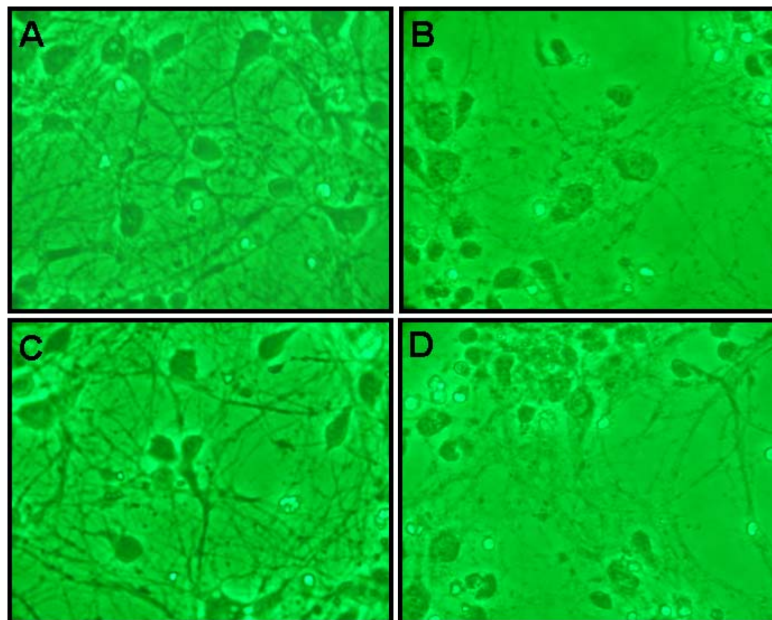

Fig. 1. High magnification (×400) photomicrographs of cultured embryonic cortical neuronal cells from 18-day-old rats. A, normoxia group; B, hypoxia group; C, mycophenolic acid (MPA) group treated before a hypoxic insult; D, MPA group treated after a hypoxic insult.

decreased in the hypoxia group compared to the normoxia group, and was greater in the group treated with MPA before hypoxic exposure than in the group treated with MPA after hypoxic exposure (Fig. 2A). However, expression of Bax and caspase-3, pro-apoptotic marker, was increased in the hypoxia group compared to the normoxia group, and was decreased in the groups treated with MPA both before and after hypoxic exposure (Fig. 2B, 2C). The ratio of Bax/Bcl-2 expression was greater in the hypoxia group than in the normoxia group and decreased in both MPA-treated groups (Fig. 2D).

3. The expression of Bcl-2, Bax and caspase-3 by real-time PCR from cultured cortical neuronal cells (*in vitro*) from 18-day-old rat embryos

The expression of the apoptotic marker Bcl-2 was decreased in the hypoxia group compared to the normoxia group, and was greater in the MPA group treated before hypoxic exposure than in the MPA group treated after hypoxic exposure (Fig. 3A). In contrast, the expression of the pro-apoptotic markers Bax and caspase-3 was greater in the hypoxia group than in the normoxia group, and were less in both MPA-treated groups (Fig. 3B, 3C). The ratio of Bax/Bcl-2 expression was greater in the hypoxia group than in the normoxia group, whereas it was decreased in the MPA-treated groups (Fig. 3D).

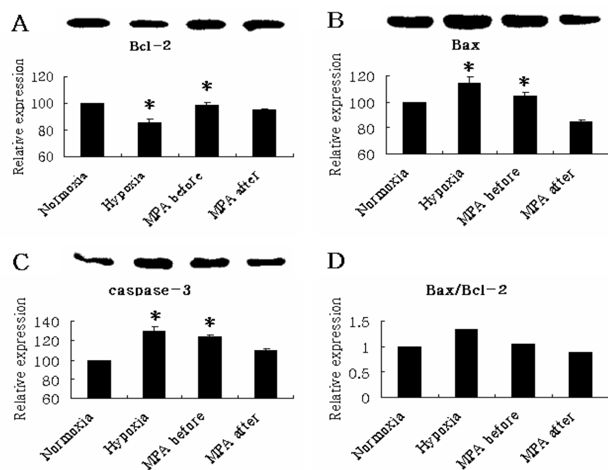

Fig. 2. Western blotting of Bcl-2 (A), Bax (B) and caspase-3 (C) from cultured cortical neuronal cells from 18-day-old rat embryos (in vitro). The ratio of Bax/Bcl-2 (D) expression is also shown. mycophenolic acid (MPA) was administered at 10 μg/mL. Data are presented as the ratios of band intensities for normoxia, hypoxia, MPA "before" and MPA "after" groups compared to those in the contralateral group. MPA before: MPA group treated before HI insult; MPA after: MPA group treated after HI insult. *P<0.05 compared with Normoxia.

4. The expression of Bcl-2, Bax and caspase-3 by western blot in the neonatal HI brain injury of rats (*in vivo*)

The expression of Bcl-2 was less in the hypoxia group than in the normoxia group, and was greater in the MPA group treated before HI insult than in the MPA group treated after HI insult (Fig. 4A). In contrast, the expression of Bax was greater in the hypoxia group than in the normoxia group and was decreased in both MPA-treated groups (Fig. 4B). There was no difference in the expression of caspase-3 between the normoxia group and the hypoxia group at 6 hours after HI insult (Fig. 4C), but the expression of caspase-3 in the hypoxia group was increased at 7 days after HI insult (Fig. 4D). The ratio of Bax/Bcl-2 expression was greater in the hypoxia group than in the normoxia group and was decreased in the groups treated with MPA both before and after hypoxic exposure (Fig. 4E).

5. The expression of Bcl-2, Bax and caspase-3 by real-time PCR in the neonatal HI brain injury of rats (*in vivo*)

The expression of Bcl-2 was less in the hypoxia group than in the normoxia group and was greater in the group treated with MPA before HI insult than in the group treated after HI insult (Fig. 5A). However, the expression of Bax was greater in the hypoxia group than in the nor-

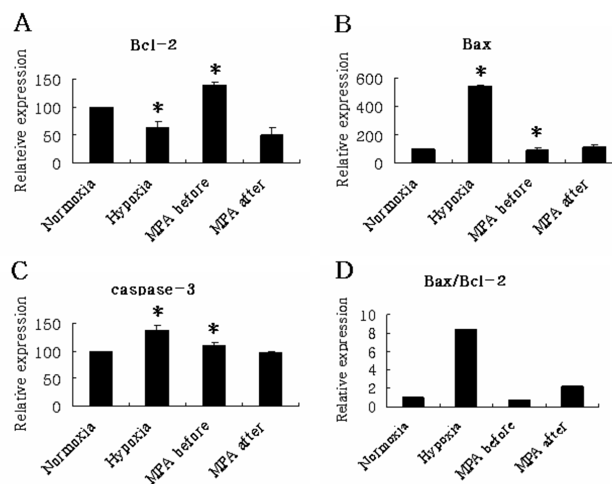

Fig. 3. Real-time PCR was performed for Bcl-2 (A), Bax (B) and caspase-3 (C) proteins from cultured cortical neuronal cells from 18-day-old rat embryos (in vitro). The ratio of Bax/Bcl-2 (D) expression is also shown. mycophenolic acid (MPA) was administered at 10 μg/mL. MPA before: MPA group treated before HI insult; MPA after: MPA group treated after HI insult. *P<0.05 compared with Normoxia.

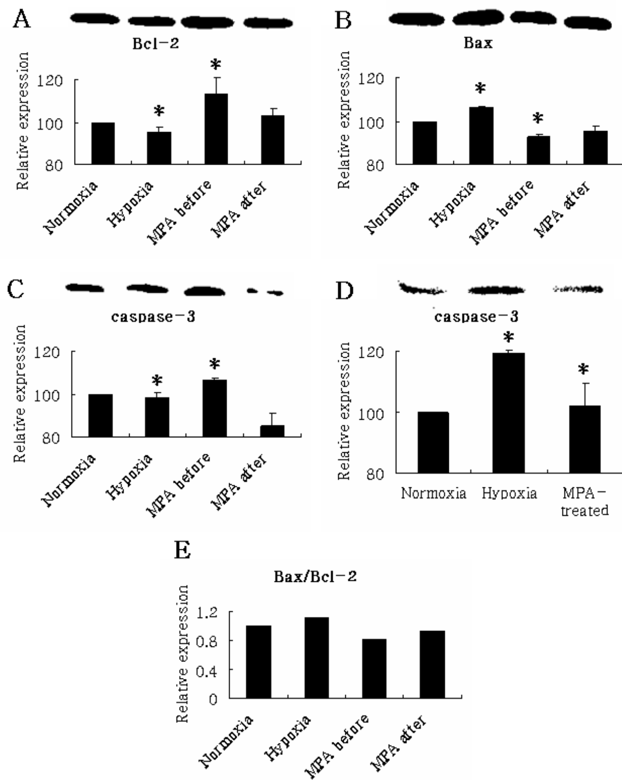

Fig. 4. Western blotting of Bcl-2 (A), Bax (B) and caspase-3 (C) proteins (*in vivo*) at 6 hours after HI injury. The expression of caspase-3 at 7 days after HI (D) and the ratio of Bax/Bcl-2 (E) expression are also shown. mycophenolic acid (MPA) was administered at 10 mg/kg. MPA before: MPA group treated before HI insult; MPA after: MPA group treated after HI insult. $^{*}P<0.05$ compared with Normoxia.

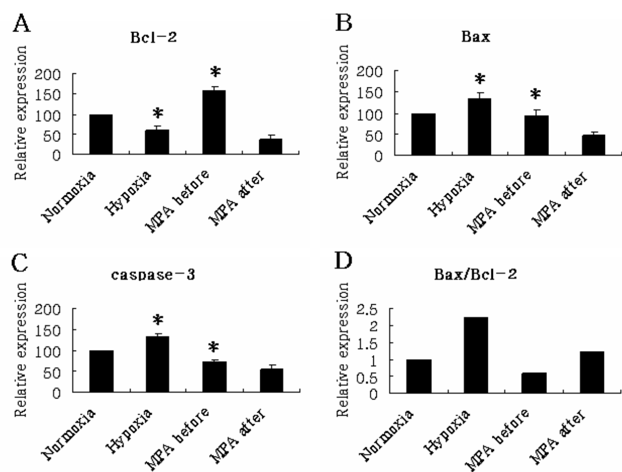

Fig. 5. Real-time PCR for Bcl-2 (A), Bax (B) and caspase-3 (C) at 6 hours after HI injury (*in vivo*). The ratio of Bax/Bcl-2 (D) expression is shown. mycophenolic acid (MPA) was administered at 10 mg/kg. MPA before: MPA group treated before HI insult; MPA after: MPA group treated after HI insult. $^{*}P<0.05$ compared with Normoxia.

moxia group and was decreased in the both MPA-treated groups (Fig. 5B, 5C). The ratio of Bax/Bcl-2 expression was greater in the hypoxia group than in the normoxia group and was decreased both MPA-treated groups (Fig. 5D).

Discussion

MPA, a product of a Penicillium fungus, was originally isolated in 1896 and has been shown to have anti-neoplastic, anti-viral, anti-fungal, and immunosuppressive activity[19, 20]. MPA is not a nucleoside. It is a potent, non-competitive, reversible inhibitor of eukaryotic but not prokaryotic IMPDH[21]. MPA has been recently introduced as an immunosuppressive drug used in heart transplantation, a procedure during which the organ is sequentially exposed to ischemia and reoxygenation[22]. It is now widely accepted that MPA is a more potent inhibitor of the proliferation of lymphocytes than of other cell types. It inhibits the glycosylation, expression and function of adhesion molecules[23].

Observations from several laboratories showing that MPA can inhibit chronic allograft rejection in various experimental animal models. The activity of MPA in experimental animal models of kidney disease, autoimmune uveoretinitis, experimental autoimmune encephalomyelitis, and adjuvant arthritis suggest possible new clinical applications. MPA has long been known to have antimicrobial effects, and recent evidence indicates that its activities against *Pneumocystis carinii*, hepatitis C virus (HCV), and human immunodeficiency virus (HIV) may be clinically useful[15].

Although the principal mode of action of MPA on lymphocytes is cytostatic, it can also induce apoptosis of polyclonally activated human T-lymphocytes and of human T-lymphocytic cell lines[24]. The induction of apoptosis by MPA may be selective for lymphocytes and monocyte-macrophage lineage cells. In patients treated with MPA, the number of apoptotic cells in renal tubular epithelium was found to be less than in those receiving azathioprine, cyclosporin A and steroids[25].

We studied a neuroprotective effect of MPA using an *in vitro* culture model of hypoxia and an *in vivo* animal model of neonatal HI brain injury. Apoptosis was assayed by cytologic analysis with western blotting and real-time PCR for Bcl-2, Bax and caspase-3.

The present study revealed that in cells cultured from rat embryonic cortical neurons, cells treated with MPA,

whether treated before or after hypoxic exposure, were morphologically better preserved than those in the hypoxia group. Apoptosis contributes significantly to cerebral damage in the perinatal period. Infants who die following intrauterine insults have a significant number of cells in the brain that exhibit the morphologic characteristics of apoptosis[26]. Apoptosis as a result of HI is morphologically different from developmental apoptosis; many hybrid necrotic-apoptotic phenotypes are seen. From a biochemical perspective, by contrast, apoptotic processes are involved in HI. Indeed, key factors in apoptosis, such as caspase-3[6], AFAF-1[27], Bcl-2[28], and Bax[29], are upregulated in the immature as compared to the adult brain and could be expected to play prominent roles in pathological situations. From the results of many studies including those described above, it is evident that Bcl-2 and Bcl-xl are anti-apoptotic in nature, whereas Bax and caspase-3 are pro-apoptotic. Caspase-3, a widely studied caspase, plays an effector role in neuronal cell death both during normal brain development and after HI insult.

The expression of Bcl-2, Bax and caspase-3 by western blot and real-time PCR in the culture of cortical neuronal cells from 18-day-old rat embryos (*in vitro*) and in neonatal HI brain injury in rats (*in vivo*) revealed that the expression of the anti-apoptotic factor Bcl-2 was less in the hypoxia group than in the normoxia group, whereas it was greater in the MPA group treated before HI insult than in the MPA group treated after HI insult. However, the expression of Bax and caspase-3, pro-apoptotic factor, was greater in the hypoxia group than in the normoxia group, whereas it was decreased in the MPA groups treated before and after hypoxic exposure. The ratio of Bax/Bcl-2 expression was greater in the hypoxia group than in the normoxia group, but was less in the MPA-treated groups. These results suggest that MPA might exert a neuroprotective effect via anti-apoptosis.

The current results suggest that MPA may exert neuroprotective effects against neonatal HI brain injury at least via an anti-apoptotic mechanism.

In conclusion, the administration of MPA before HI injury can significantly protect against perinatal HI brain injury via anti-apoptotic mechanisms, as indicated by the decreased expression of Bcl-2 and increased expression of Bax and caspase-3 seen in both *in vivo* and *in vitro* experimental studies of perinatal HI brain injury. However, more experiments are needed to test the usefulness of MPA as a preventative measure and treatment for perinatal HI brain injuries. Future investigations in this field will continue to unravel the distinct mechanisms of injury-induced cell death in the immature brain, especially at early developmental times, and lead to a thorough evaluation of the developmental and behavioral sequelae that follow injury.

한 글 요 약

주산기 저산소성 허혈성 뇌손상에서 항세포자멸사를 통한 mycophenolic acid의 신경보호 효과

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목 적 : Mycophenolate mofetil (MMF)의 활성 대사산물인 (MPA는 IMPDH의 잠재적인 반응 억제제로써 새로운 면역치료제로 사용되고 있다. 이러한 MPA는 신경계에서 흥분독성 손상 후 뇌세포를 보호하고, 미세아교세포에서는 세포사멸사(apoptosis)를 유도하지만, 저산소성 허혈성 뇌질환에서 MPA의 효과는 아직 알려지지 않아, 본 연구에서 Rice-Vannucci 모델을 이용한 신생 백서의 저산소성 허혈성 뇌 손상과 저산소 상태의 태아 백서 뇌세포 배양에서 MPA의 뇌보호 효과를 알아보고자 실험하였다.

방 법 : 생후 7일된 백서의 좌측 총 경동맥을 결찰한 후 저산소 (8% O_2) 상태에서 2시간 노출하여, 저산소성 허혈성 뇌 손상을 유발하고 뇌 손상 전후에 MPA(10 mg/kg)를 투여하여 대조군과 비교하였다. 또한, 재태기간 18일된 태아 백서의 대뇌피질 세포를 배양하여 1% O_2 배양기에서 저산소 상태로 세포손상을 유도하여 저산소군, 손상 전후 MPA 투여군(10 μg/mL)으로 나누어 정상산소군과 비교하였다. 세포사멸사와의 관련을 알아보기 위해서 Bcl-2, Bax, caspase-3 항체로 western blotting하였고 Bcl-2, Bax, caspase-3 primer를 이용하여 real-time PCR을 하였다.

결 과 : 형태학적으로 H&E 염색상 MPA를 투여한 군에서 뇌보호 효과를 보였다. Western blotting과 real-time PCR을 이용한 저산소성 허혈성 뇌손상 동물 모델뿐만 아니라 저산소 상태로 태아 백서 뇌세포 배양 실험에서도 MPA 투여한 경우 caspase-3의 발현과 Bax/Bcl-2의 비율이 감소함을 보였다.

결 론 : 본 연구에서 MPA가 anti-apoptosis 작용을 통하여 주산기 저산소성 허혈성 뇌 손상에 뇌보호 역할을 하는 것을 알 수 있었고 향후 신생아 저산소성 허혈성 뇌병증의 치료에 임상적 적용이 가능하리라 생각된다

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