A Dual Role of the NF-κB Pathway in Neonatal Hypoxic-Ischemic Brain Damage

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Background and Purpose—NF-κB is a transcription factor that regulates inflammatory and apoptotic pathways. We described previously that intraperitoneal administration of the NF-κB inhibitor TAT-NBD at 0 and 3 hours after neonatal hypoxia-ischemia (HI) markedly reduced brain damage. We hypothesize that timing and duration of NF-κB inhibition will be a major factor in determining outcome.

Methods—HI was induced in P7 rats by unilateral carotid artery occlusion and hypoxia. In vivo TAT-NBD effects were determined on cerebral damage, NF-κB activity, cytokine expression, and pro- and antiapoptotic molecules. In vitro effects of TAT-NBD were determined using primary neurons and cell lines.

Results—HI induced 2 peaks of cerebral NF-κB activity at 3 to 6 and 24 hours after HI. Neuroprotective 0/3-hour TAT-NBD treatment only inhibited early NF-κB activity. However, inhibition of both early and late NF-κB activity by 0/6/12-hour TAT-NBD or only late NF-κB activity by 18/21-hour TAT-NBD aggrivated damage. 0/6/12-hour TAT-NBD did not prevent HI-induced upregulation of cytokines at 24 hours after HI. Protective 0/3-hour TAT-NBD treatment prevented nuclear accumulation of p53 at 24 hours after HI. Nuclear p53 was not reduced after 0/6/12-hour TAT-NBD. Prolonged TAT-NBD increased the proapoptotic factor PUMA and reduced the antiapoptotic factors Bcl-2 and Bcl-xL. Also in neuronal cultures prolonged TAT-NBD exposure overruled protective short-term TAT-NBD treatment.

Conclusions—Early NF-κB activation contributes to neonatal HI brain damage. Late NF-κB provides endogenous neuroprotection and upregulates antiapoptotic molecules. Inhibition of early NF-κB activity is neuroprotective only when late NF-κB activity is maintained. Moreover, cerebral cytokine production can occur independently of NF-κB.

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Key Words: nuclear factor-κB ■ inflammation ■ neonatal ■ neuroprotection ■ apoptosis ■ ischemia

Hypoxia-ischemia (HI) during the perinatal period is a common cause of brain injury in human newborns leading to mortality or life-long impairments. Effective therapeutic intervention strategies targeting HI are still lacking at present. The transcription factor Nuclear Factor kappa B (NF-κB) regulates expression of multiple genes involved in inflammation, apoptotic cell-death, and cell survival. Several studies have demonstrated that NF-κB is activated during development of brain injury, but the role of NF-κB in HI brain damage is complex and conflicting results have been obtained when exploring HI-NF-κB inhibition as a potential neuroprotective therapy. NF-κB can promote cell survival through upregulation of expression of antiapoptotic factors including Bcl-xL and Bcl-2. Bcl-2 and Bcl-xL bind to Bax and Bak thereby preventing Bax/Bak pore formation in the mitochondrial membrane. On the other hand, NF-κB can activate the proapoptotic machinery, eg, via upregulation of the tumor suppressor p53 and its downstream target PUMA.

Furthermore, NF-κB activation has been described to promote brain damage via induction of proinflammatory cytokines. Reciprocal interaction between pro- and antiapoptotic signals regulated by NF-κB and its role in cytokine production therefore complicate prediction of the effect of NF-κB inhibition on brain damage.

In a recent study using P7 rats, we showed that the selective NF-κB inhibitor NEMO Binding Domain peptide coupled to the protein transduction sequence of HIV-TAT (TAT-NBD) to facilitate cerebral uptake, was rapidly and transiently detected in the brain where it inhibited HI-induced cerebral NF-κB activity when administered intraperitoneally (i.p.) early after HI. The NBD peptide functions by disrupting the association of the IKK regulatory subunit NEMO with the catalytic subunits of the IKK complex (IKKα and IKKβ) required for NF-κB activation. Moreover, i.p. TAT-NBD treatment within 6 hours after HI had potent neuroprotective effects (up to 80% reduction in brain damage). Prevention...
of early mitochondrial p53 translocation seemed to be key to the neuroprotective effect of early TAT-NBD treatment. Surprisingly, upregulation of the cytokines TNF-α, IL-1β, IL-4, and IL-10 at 3 hours after HI was not inhibited by NF-κB inhibition by 0/3-hour TAT-NBD treatment. In an earlier study in P12 rats, however, we showed that prolonged treatment with NBD peptide not coupled to a TAT sequence (i.p. injection at 0/6/12 hours after HI) did not protect, but rather aggravated, neonatal HI brain damage.13 The marked difference between the strong protective effect of 0/3-hour TAT-NBD treatment and the aggravating effect of 0/6/12-hour NBD treatment prompted us to further investigate the possible contribution of coupling of the TAT sequence to NBD. In addition, we further investigated the kinetics of NF-κB activation and the mechanism of action of TAT-NBD in neonatal HI brain damage. We hypothesize that NF-κB may have protective and deleterious effects during HI brain damage and that timing of NF-κB inhibition will determine outcome.

Materials and Methods

Animals

The animal care committee of the University Medical Center Utrecht approved all experiments. At postnatal day 7 (P7), Wistar rat pups of both genders underwent occlusion of the right common carotid artery under isoflurane anesthesia. After recovery for 1 to 3 hours, pups underwent hypoxia by breathing 8% O₂ in N₂ for 120 minutes. Sham controls underwent anesthesia and incision only. All analyses were performed in a blinded set-up.

TAT-NBD (ygrkkrrqrr-TALDWSLQTE; with the TAT sequence in lower case) or mutant TAT-NBD (TAT-NBDmut; ygrkkrrqrr-TALDASALQTE) (W.M. Keck facility, Yale University, New Haven, Ct) was dissolved in DMSO (40 mg/mL), diluted in PBS, and administered i.p. at 20 mg/kg.11–13 Rats were terminated by 300 mg/kg pentobarbital and perfused with 4% paraformaldehyde in PBS or were decapitated.

Histology

Coronal paraffin sections (8 μm) were cut at ~3.30 mm from bregma and stained with hematoxylin-eosin. Both hemispheres were outlined on full section images and the ratio of ipsi- and contralateral areas was calculated.14 Deparaffinized sections ~3.30 mm from bregma ("hippocampal level") were incubated with mouse–anti-MAP2 (Sigma-Aldrich) or mouse–anti-CD68(ED-1)-FITC (Serotec) followed by peroxidase-labeled secondary antibodies and revealed using Vectastain ABC kit (Vector) and diaminobenzamidine. MAP2 loss was quantified as described above.

Proteins

Mitochondrial, nuclear, and cytosolic fractions of each hemisphere were prepared as described,14 and quality of fractionation was confirmed.15 Proteins were separated by SDS-PAGE, transferred to Hybond-C membranes (Amersham), and revealed using mouse-anticytochrome-c (BD Biosciences Pharmingen), rabbit-anticleaved caspase-3, mouse-antip53, rabbit-anti-PUMA, rabbit-anti-Bcl-2, rabbit-anti-Bcl-2 (all Cell Signaling), goat-anti-β-actin, mouse-antihistone-H1 (both Santa Cruz Biotechnology); donkey-antirabbit-peroxidase (Amersham), goat-antimouse-peroxidase (Jackson Immunoresearch), or donkey-antigoat-peroxidase (Santa Cruz Biotechnology) followed by enhanced chemiluminescence (Amerham) and analyzed with a GS-700 Imaging Densitometer (Bio-Rad).

Electromobility shift assays (EMSA) on nuclear extract with cold DNA probe eliminated the signal, showing specificity of the EMSA.

Quantitative Real-Time RT-PCR

Total RNA was isolated with TRIzol (Invitrogen). cDNA was synthesized with SuperScript Reverse Transcriptase (Invitrogen). The PCR reaction was performed with iQ5 Real-Time PCR Detection System (Bio-Rad) using primers for IL-1β, TNF-α, IL-10, IL-4, and IL-1RA; for sequences see Nijboer et al.11 To confirm appropriate amplification, size of PCR products was verified on gel. Data were normalized for expression of β-actin and GAPDH.

Neuronal Cultures

SK-N-MC or SH-SY-5Y human neuronal cells (ATCC) were plated in 96-well plates in DMEM/F12 (1:1) medium supplemented with 10% fetal calf serum, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.5 μg/mL Fungizone, and 0.1 mmol/L nonessential amino acids and grown for 48 hours before the experimental protocol.

Cortical tissue was isolated from E17 Sprague Dawley embryos, dissected in HBSS without Ca/Mg, supplemented with 10 mmol/L Heps and 25 mmol/L D-glucose, and dissociated using papain (Sigma-Aldrich). Primary cortical neurons were grown in Neurobasal medium containing B27 supplement, antibiotics as stated above and 25 mmol/L glutamate, and plated in poly-d-lysine coated 96-well plates. After 4 DIV, half of the medium was replaced with Neurobasal without glutamate. After 10 DIV, >95% of the cells was NeuN-positive and experiments were performed (all media used: Gibco).

SK-N-MC or SH-SY-5Y neuronal cells were incubated for 24 hours with 100 μmol/L H₂O₂ or 100 mmol/L staurosporine (Sigma-Aldrich) and primary cortical neurons with 50 or 100 μmol/L glutamate. Different concentrations of TAT-NBD or TAT-NBDmut peptide (10 to 100 μmol/L) were added for 24 hours. 0.5 mg/mL MTT (Sigma-Aldrich) was added for 4 hours. MTT crystals were dissolved in DMSO, and absorbance was measured at 550 nm.

Statistical Analysis

Data are presented as mean and SEM and were analyzed by 1-way ANOVA with Bonferroni post-tests. Gaussian distribution for all parameters was confirmed by using the D’Agostino and Pearson omnibus normality test.

Results

Kinetics of HI-Induced Cerebral NF-κB Activity

Detailed analysis of the kinetics of neonatal HI-induced NF-κB activation in the brain showed that NF-κB activity was high at 0.5 to 6 hours and returned to baseline levels at 12 hours after HI. Furthermore, we observed a distinct second peak of NF-κB activation 24 hours after the insult (Figure 1A). Treatment with TAT-NBD at 0/3 hours after HI resulted in inhibition of early NF-κB activation as determined at 3 hours after HI.13 However, here we show that this early short-term treatment did not affect the second peak of NF-κB activity at 24 hours after HI (Figure 1B).

To inhibit both the early and the second late-phase NF-κB peak, we treated animals with TAT-NBD at 0, 6, and 12 hours (0/6/12 hours) after HI. To inhibit only the second late-phase peak of NF-κB activity, TAT-NBD was administered at 18 and 21 hours (18/21 hours) after the insult. 0/6/12-hour TAT-NBD treatment inhibited both early and late NF-κB activity, and late TAT-NBD treatment (18/21 hour) inhibited only the second peak of NF-κB activity (Figure 1B).

Inhibition of NF-κB activity was specific for the NBD sequence because treatment with TAT-NBDmut, which is incapable of blocking the association of NEMO with IKKα or IKKβ,12 did not have any effect on NF-κB activity (Figure 1B).
Timing of Inhibition of NF-κB Activity: Effect on HI-Induced Brain Damage

In contrast to the marked neuroprotective effect of early NF-κB inhibition by 0/3-hour TAT-NBD, prolonged NF-κB inhibition by 0/6/12-hour TAT-NBD aggravated damage at 6 weeks after the insult (Figure 2A).

Prolonged inhibition of NF-κB also completely abolished the protective effect of 0/3-hour treatment on MAP2 loss determined at 48 hours after the insult (Figure 2B and 2C).

Inhibition of only late-phase NF-κB activity by 18/21-hour TAT-NBD resulted in exacerbation of MAP2 loss (Figure 2B and 2C). Treatment with TAT-NBD at 0/3/6/12 hours resulted in the same amount of MAP2 loss as treatment at 0/6/12 hours (Figure 2B), showing that prolonged treatment rather than omitting administration at 3 hours after HI was responsible for abolishing the neuroprotective effect of early NF-κB inhibition. Treatment with TAT-NBDmut did not have any effect (Figure 2). Moreover, prolonged or late treatment...
with TAT-NBD did not induce damage in the contralateral hemisphere. Collectively these data suggest that toxic or nonspecific effects of the TAT-NBD peptide are not responsible for overruling the protective effect of early transient treatment and that late NF-κB activity is required for the protective effect of early treatment.

Cytokine Expression and Microglia Activation
It has often been suggested that inhibition of NF-κB activity may contribute to prevention of brain damage via inhibition of the cytokine response. However, as shown in Figure 3A through 3E, HI-induced increase in expression of the proinflammatory cytokines IL-1β and TNF-α and the antiinflammatory...
cytokines IL-4, IL-10, and IL-1-RA at 24 hours after HI was not prevented by 0/6/12-hour TAT-NBD treatment, even though NF-H9260B activity was completely inhibited by this treatment (Figure 1B). Conversely, neuroprotection by 0/3-hour TAT-NBD treatment prevented upregulation of cytokines at 24 hours after the insult even though NF-H9260B activity at this time point was maintained (Figures 3A through 3E and 1B).

Consistent with the cytokine data, microglia activation as determined by CD68 staining at 24 hours after HI, was not reduced by 0/6/12-hour TAT-NBD treatment. Microglia activation was completely prevented by 0/3-hour TAT-NBD (Figure 3F).

**Effect of Prolonged NF-κB Inhibition on Pro- and Antiapoptotic Molecules**

Recently we described that neuroprotection after 0/3-hour TAT-NBD treatment was associated with a complete prevention of the HI-induced increase in cytosolic cytochrome c and in active caspase-3 that occurs at 24 hours after HI.11 The data in Figure 4A and 4B show that after 0/6/12-hour or 18/21-hour TAT-NBD treatment, the HI-induced increase in cleaved caspase-3 and cytosolic cytochrome c at 24 hours was no longer prevented, but was similar to that in vehicle-treated animals.

The data in Figure 5 show that HI induced a time-dependent increase in expression and nuclear localization of the proapoptotic factor p53 (starting at 3 hours after HI; Figure 5A and 5B). In addition, PUMA, a downstream proapoptotic target of p53, increased from 6 hours after the insult with increasing expression at 12 and 24 hours after HI (Figure 5C). Both 0/3-hour and 0/6/12-hour TAT-NBD treatment prevented the HI-induced increase in cytosolic p53 at 24 hours after the insult (Figure 5D). However, only early short-term NF-κB inhibition completely prevented the HI-induced increase in nuclear p53 at 24 hours after HI (Figure 5E). In contrast, the HI-induced increase in nuclear p53 was maintained after prolonged inhibition of NF-κB (Figure 5E). Consistent with the effect of NF-κB inhibition on nuclear localization of p53, PUMA expression at 24 hours after HI was low after 0/3-hour TAT-NBD (Figure 5F).

However, PUMA expression was significantly higher after prolonged treatment than in vehicle-treated animals (Figure 5F; *P<0.01 vs vehicle- or TAT-NBDmut-treated animals (n=9 animals/group)).

Next, we determined the kinetics of HI-induced changes in expression of the antiapoptotic factors Bcl-2 and Bcl-xL. HI induced a decrease in mitochondrial levels of Bcl-2 and Bcl-xL starting at 12 hours after the insult (Figure 5G and 5H). Prolonged TAT-NBD treatment (0/6/12 hours) led to a significant further reduction in expression of Bcl-2 and Bcl-xL (*P<0.01 versus vehicle-treated). In contrast, 0/3-hour TAT-NBD treatment markedly increased Bcl-2 and Bcl-xL at 24 hours after HI (*P<0.001 versus vehicle-treated; Figure 5I and 5J). TAT-NBDmut did not have any effect.

**In Vitro Effects of Prolonged TAT-NBD Treatment on Neuronal Cell Death**

In our previous study, we showed that 6-hour treatment with TAT-NBD protected neuronal cells in culture against cytotoxicity.11 Here we examined the effect of 24-hour exposure of neuronal cells to TAT-NBD or TAT-NBDmut in a dose range that protects against cell-death when present in the cultures for 6 hours. SK-N-MC and SH-SY-5Y human neuronal cell lines were exposed to 100 μmol/L hydrogen peroxide (H2O2) as a model for oxidative stress or 100 nmol/L staurosporine as a general inducer of apoptotic cell death. Primary cortical neuronal cultures were exposed to glutamate (50 and 100 μmol/L) as a model for oxidative stress or 100 nmol/L staurosporine as a general inducer of apoptotic cell death. Primary cortical neuronal cultures were exposed to glutamate (50 and 100 μmol/L). The presence of TAT-NBD but not TAT-NBDmut during 24 hours dose-dependently increased neuronal death in vitro in response to either H2O2 or staurosporine in SK-N-MC and SH-SY-5Y neuronal cells (Figure 6A and 6B, 6C and 6D) and to glutamate in primary neuronal cells (Figure 6E and 6F; *P<0.001 versus stimulated cells without TAT-NBD). In control cultures the addition of TAT-NBD or TAT-NBDmut alone had no effect on cell survival.

**Discussion**

In this study we demonstrate for the first time that neonatal HI brain injury induced biphasic activation of NF-κB with an
early peak of NF-κB activity (0.5 to 6 hours) followed by a second late-phase peak at 24 hours after HI in P7 rats. Moreover, we present novel insights into the opposite roles of early and late-phase NF-κB activity in cerebral damage, expression of pro- and antiapoptotic molecules, and the cytokine response in a model of neonatal HI brain damage.

The strong neuroprotection observed in our previous study after inhibition of only early NF-κB activity by 0/3-hour TAT-NBD was completely lost after prolonged treatment with TAT-NBD which inhibits both early and late-phase NF-κB activity. Moreover, after 18/21-hour TAT-NBD which only prevents late-phase NF-κB activity, brain damage was aggravated. Thus, inhibition of early NF-κB activity was neuroprotective only when the second peak of NF-κB activity was intact, suggesting that early inhibition of NF-κB rescues the brain from neurodegenerative processes via a mechanism that relies on late NF-κB activity to promote neuronal survival.

Interestingly, Duckworth et al described increased brain damage after MCAO in adult mice lacking the p50 subunit of NF-κB, which is in line with the more pronounced damage we observe with prolonged inhibition of NF-κB.15 In apparent contrast, it has been shown that permanent deletion of neuronal IKK2 is neuroprotective in an adult mouse stroke model.16,17 In these animals, there was no evidence for aggravation of injury as one would predict based on the aggravation of damage we observed after prolonged inhibition of the IKK/NF-κB pathway by TAT-NBD. It is conceivable, however, that activation of IKK1 compensates for the IKK2 deficiency by inducing protective NF-κB activity later after the insult. Indeed, some residual NF-κB activity was shown after stimulation of IKK2-KO neuronal cells in vitro with TNF-α.16 An alternative possibility would be that the immature brain depends more on the protective effect of late NF-κB activity than the adult brain, because apoptotic cell death is known to play a more pronounced role in neonatal than in adult cerebral cell death.9,18

How can prolonged inhibition of the NF-κB pathway overrule the strong neuroprotective effect of early short-term NF-κB inhibition? NF-κB stimulates the expression of the antiapoptotic molecules Bcl-2 and Bcl-xL, proteins known to protect the outer mitochondrial membrane by counteracting proapoptotic members of the Bcl-2 family.4,5 We showed that HI decreased expression level of Bcl-2 and Bcl-x rather late, from 12 hours after HI with a further decrease at 24 hours. Interestingly, neuroprotective 0/3-hour TAT-NBD treatment was associated with pronounced upregulation of Bcl-2 and Bcl-xL at 24 hours after HI. In contrast, prolonged NF-κB inhibition by 0/6/12-hour TAT-NBD reduced expression of these antiapoptotic factors even further. These data suggest that late-phase NF-κB activity is required for upregulation of these antiapoptotic factors that are required to propagate neuroprotection initiated by early NF-κB inhibition.

In the present study, we show increased cytosolic p53 and nuclear p53 accumulation starting at 3 hours and further increasing up to 24 hours after HI. Importantly, p53 is under transcriptional control of NF-κB19 and consistent with NF-κB-dependent upregulation, prolonged inhibition of NF-κB activity decreased cytosolic p53 expression at 24 hours after HI. Notably, nuclear accumulation of p53 at 24 hours after HI was not reduced by prolonged TAT-NBD, despite a marked reduction in cytosolic p53. These data indicate that increased nuclear p53 can occur independently of increased cytosolic levels, and that late NF-κB activity is necessary for preventing nuclear accumulation of p53. Nuclear p53 transcriptionally regulates other proapoptotic target genes including PUMA.20 The observed increase in PUMA expression was even higher after prolonged inhibition of NF-κB compared to vehicle-treated animals, despite equal levels of nuclear p53. One possible explanation may be that nuclear NF-κB competes with p53 for cofactor p300, which enhances transcriptional activity of p53. When NF-κB is not activated, it is conceivable that more p300 is available for interaction with p53 and thus reduced NF-κB activation may favor transcriptional activity of p53 leading to higher expression of PUMA.20

Pro- and antiinflammatory cytokines are expressed in the neonatal brain after HI and can contribute to damage.21,22 It is commonly accepted that inhibition of NF-κB will prevent proinflammatory cytokine production thereby contributing to neuroprotection.9,21,23 In contrast, however, we observed that prolonged inhibition of NF-κB activity did not inhibit cytokine production nor microglia activation at 24 hours after HI, although at this time point expression of p53, another NF-κB target, was clearly inhibited. On the other hand, after 0/3-hour TAT-NBD, cytokine production and microglial activation were not observed at 24 hours. The fact that late cytokine production and microglial activation did not occur after early (protective) TAT-NBD treatment suggests that late cytokine production and microglia activation are secondary to neuronal damage, ie, are part of a reactive process induced by the initial brain damage.

In our previous study, we already described that early cytokine production at 3 hours after HI was not prevented after inhibition of early NF-κB activity by 0/3-hour TAT-NBD.11 From our most recent data, we have indications that early cytokine production is not detrimental in our neonatal HI model and may even contribute to neuroprotection. Collectively, our data show that in the neonatal brain, HI-induced pro- and antiinflammatory cytokine production and microglia activation are not completely dependent on...
Figure 6. Prolonged TAT-NBD dose-dependently increases neuronal cell death in vitro. The human neuronal cell lines (SK-N-MC and SH-SY-5Y) were exposed to 100 μmol/L H₂O₂ or 100 nmol/L staurosporine and 10, 50, or 100 μmol/L TAT-NBD for 24 hours (SK-N-MC A-B; SH-SY-5Y C-D). Primary cortical neuronal cultures were exposed to 50 or 100 μmol/L glutamate and 10, 50, or 100 μmol/L TAT-NBD for 24 hours (E-F). Cell survival was determined using MTT. Addition of TAT-NBD for 24 hours dose-dependently increased neuronal death. MUT: addition of 100 μmol/L TAT-NBDmut. **P<0.01, ***P<0.001 vs stimulated cells without TAT-NBD. TAT-NBD or TAT-NBDmut treatment as such had no effect on neuronal survival. Data are from 3 independent experiments performed in quadruplicate.
NF-κB activity and may involve additional transcription factors. We propose that in the developing brain other transcription factors than NF-κB may contribute to HI-induced cytokine production or that the developing brain can switch to the use of other transcription factors such as AP-1, when NF-κB is inhibited.

This study provides novel insights in the dual role of NF-κB during HI brain damage and sheds a new light on the complex controversial results obtained in the past when studying inhibition of NF-κB as a potential therapeutic intervention in models of cerebral damage. Interestingly, Zhang et al showed that selective inhibition of NF-κB in neurons but not in glia reduced infarct size at 48 hours after MCAO, indicating that NF-κB activation in neurons contributes to the ischemic damage.24 In line with this observation, our in vitro studies show that the protective effects of transient TAT-NBD treatment and the detrimental effects of prolonged TAT-NBD treatment can be mimicked by using neuronal cells in vitro, indicating that inhibition of neuronal NF-κB can be sufficient to explain the observed effects in vivo. Nevertheless, it remains to be determined whether the effects of early or late NF-κB inhibition involve different cell populations.

The results presented here demonstrate that there are 2 distinct phases of NF-κB activity after neonatal cerebral HI. The ultimate effect of NF-κB inhibition on HI-induced brain damage will be determined by a balance between prevention of early proapoptotic processes on the one hand and maintenance of late upregulation of antiapoptotic factors on the other hand. We propose that the final decision to live or die is made relatively late after HI; crucial protective NF-κB activity after neonatal cerebral HI.

We have shown previously that peripheral administration of the TAT-NBD peptide early and transiently after HI has a marked neuroprotective effect with a therapeutic window of at least 6 hours in P7 rats. However, here we show that initiation of treatment at 18 hours after HI aggravated brain damage. Thus, TAT-NBD treatment should be confined to an early administration after a hypoxic-ischemic insult to preserve protective NF-κB-dependent mechanisms that occur in a later phase. Clinical applications of TAT-NBD should be restricted to cases of well-documented acute perinatal asphyxia in which detailed records on timing of the insult are available. The incidence of acute complications with detailed records, eg, uterine rupture, placental abruption, or umbilical cord compression, is more than 50% of the cases of neonatal encephalopathy.25 Our animal studies suggest that when timed properly, TAT-NBD treatment may represent a very effective strategy to combat neonatal HI brain damage.

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Disclosures
None.

References


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