LOOKING AHEAD

NEU2000, AN NR2B-SELECTIVE, MODERATE NMDA RECEPTOR ANTAGONIST AND POTENT SPIN TRAPPING MOLECULE FOR STROKE

Targeting both NMDA receptors and free radicals may provide enhanced neuroprotection against hypoxic-ischemic injury.

by Sung Ig Cho, Ui Jin Park, Jun-Mo Chung and Byoung Joo Gwag

Stroke is a cerebrovascular injury caused by the interruption of blood flow to the brain due to thrombosis, embolic particles or blood vessel bursts. Stroke is the leading cause of serious, long-term disability in adults and the second leading cause of death in the U.S. and Europe (1). Rates of stroke mortality and burden are more affected in low-income countries including eastern Europe, northern Asia and central Africa (2). Ischemic stroke, a major type of stroke caused by thrombosis and arterial embolism, can cause neuronal death rapidly in core areas, which is accompanied by secondary death in the ischemic penumbra subsequent to activation of multiple death pathways. Tissue plasminogen activator, a thrombolytic agent approved in 1996 by the U.S. Food & Drug Administration, is used to dissolve the clot and to improve neurological outcome for acute ischemic stroke within 3 hours after the onset of symptoms (3).

Mechanisms and interventional therapies for ischemic neuronal death have been extensively investigated to establish a novel neuroprotection therapy with a greater therapeutic time window than that of thrombolytic agents (4). Preclinical studies have provided extensive evidence that excess activation of *N*-methyl-D-aspartate (NMDA) receptors mediates rapidly evolving neuronal death following ischemic insults. However, NMDA receptor antagonists that confer substantial neuroprotection in animal models of stroke have failed to show beneficial effects in clinical trials for stroke. Free radicals mediate an additional route of neuronal cell death after ischemia and reperfusion. Several antioxidants have advanced to clinical trials including edaravone, a hydroxyl radical scavenger that has shown beneficial effects in patients with transient ischemia and which was approved as a neuroprotective drug in Japan and China.

NMDA receptor antagonists and antioxidants are expected to confer neuroprotection in human stroke if drug safety and the therapeutic time window are justified. As NMDA receptor activation and free radicals are expected to contribute to neuronal death through distinct signaling pathways at different points of time after hypoxic ischemic injury, it is conceivable to reason that targeting both NMDA receptors and free radicals may provide enhanced neuroprotection against hypoxic-ischemic injury and increase the therapeutic time window, if safety is compromised.

THE ROLE OF NMDA RECEPTORS IN ISCHEMIC NEURONAL DEATH

The excitatory neurotransmitter glutamate is released and accumulates in ischemic brain areas deprived of glucose and oxygen (Fig. 1). Abnormal accumulation of glutamate triggers influx and overload of Ca^{2+} primarily through excess activation of the NMDA receptors. This increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) in neurons then triggers neuronal injury by activating cytotoxic proteins such as nitric oxide synthase, calpain and endonuclease and by impairing mito-

SUMMARY

Excess activation of ionotropic glutamate receptors, primarily *N*-methyl-D-aspartate (NMDA) receptors and free radicals, evoke nerve cell death following hypoxic-ischemic brain injury in various animal models. However, clinical trials in stroke patients using NMDA receptor antagonists have failed to show efficacy primarily due to the limited therapeutic time window for neuroprotection and a narrow therapeutic index. In comparison, antioxidants prolonged the time window for neuroprotection in animal models of ischemic stroke and showed greater therapeutic potential in clinical trials for ischemic stroke. Excess activation of NMDA receptors and free radicals mediate the two separate pathways of nerve cell death in stroke and a safe and multifunctional drug that can block both routes in the brain will likely provide a better therapeutic outcome in patients with stroke. Derivatives of the lead structures of sulfasalazine and aspirin have led to the discovery of a new molecule, Neu2000, that has demonstrated excellent neuroprotection against NMDA- and free radical-induced cell death. Neu2000 is an NR2B-selective, moderate NMDA receptor antagonist with potent cell-permeable, spin trapping antioxidant action even at nanomolar concentrations. Nonclinical and human phase I studies demonstrated that Neu2000 can be translated to treat patients with stroke with better efficacy and therapeutic time window.

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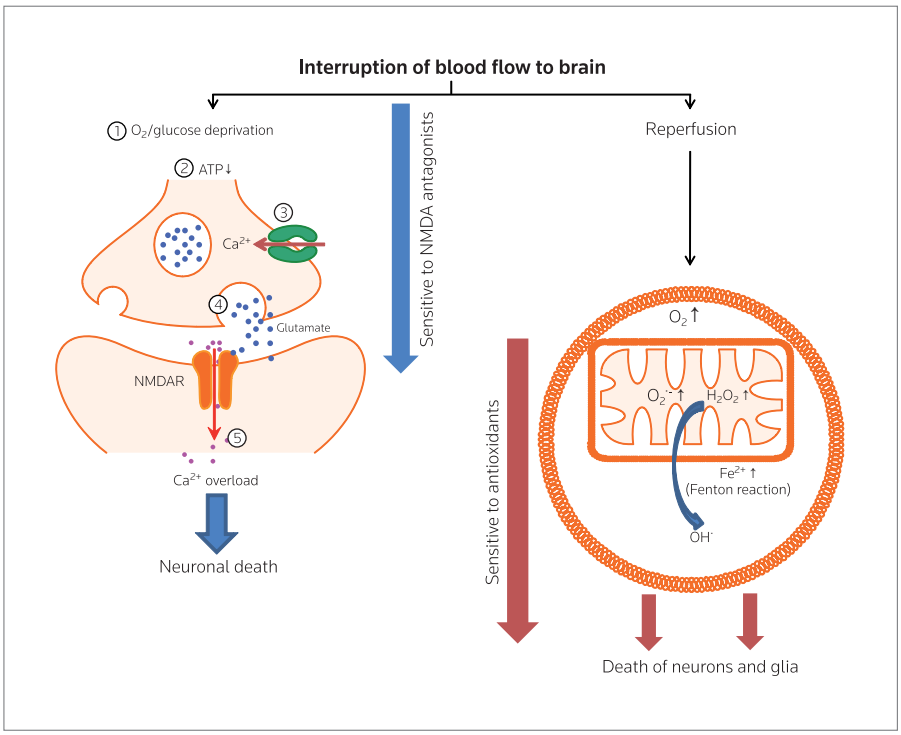

Figure 1. Role of NMDA receptors and free radicals in ischemic injury. Interrupted blood flow to the brain causes deprivation of oxygen and glucose (①) that results in loss of ATP (②), leading to membrane depolarization, Ca^{2+} entry into the presynaptic terminal (③) and glutamate release from the synaptic vesicles into the synaptic cleft (④). Accumulated glutamate causes influx and accumulation of Ca^{2+} through excess activation of postsynaptic NMDA receptors (NMDAR) (⑤). Sustained Ca^{2+} overload for a few minutes can induce neuronal death. Following reperfusion of reoxygenation, cells are exposed to excess oxygen molecules that can be converted to superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in mitochondria. $O_2^{\cdot-}$ and H_2O_2 can be further converted to hydroxyl radicals (•OH) through the Fenton reaction and the Haber–Weiss reaction catalyzed by ferrous and ferric ions. Such toxic free radicals mediate slowly evolving secondary nerve cell death following ischemic injury.

chondria and endoplasmic reticulum. Neurons overloaded with Ca^{2+} for even a few minutes can undergo neuronal death, suggesting that NMDA receptors mediate rapid and fulminant neuronal death following ischemic injury (5, 6). Furthermore, NMDA receptor antagonists prevent Ca^{2+} entry and overload in cultured neurons deprived of glucose and oxygen and markedly attenuate neuronal death induced by oxygen-glucose deprivation and in animals subjected to transient cerebral ischemia (7, 8).

In contrast to the neuroprotective effects verified in animal models of stroke, NMDA receptor antagonists have not improved neurological outcomes in stroke patients with transient ischemic injury. For example, potent NMDA receptor antagonists such as selfotel, aptiganel and eliprodil that act in competitive, noncompetitive and NMDA receptor subtype 2B (NR2B)-selective manners, respectively, showed no efficacy in phase III trials for acute stroke therapy (9). The lack of beneficial effects of NMDA receptor antagonists in clinical trials is largely attributable to the narrow therapeutic time window and unwanted side effects such as neuronal vacuolization and necrosis primarily in the posterior cingulate and retrosplenial cortex (10-12). Moreover, administration of potent NMDA receptor antagonists can produce psychiatric adverse effects in humans as shown with selfotel, which caused marked psychiatric and neurological side effects in human stroke patients at a subtherapeutic plasma level of 21 µg/mL. In animal models of transient focal cerebral ischemia, 40 µg/mL selfotel was neuroprotective when administered within 30 minutes after reperfusion (13).

ROLE OF FREE RADICALS IN ISCHEMIC NEURONAL DEATH

Ischemic injury produces free radicals through multiple pathways. Activation of NMDA receptors causes the accumulation of $[Ca^{2+}]_i$ that triggers production of superoxide and nitric oxide via the activation of xanthine oxidase, nitric oxide synthase and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (14-16). Reoxygenation or reperfusion can give rise to superoxide and H_2O_2 by the oxidation of accumulated hypoxanthine and the uncoupling of electron flow from mitochondria electron transport chain (Fig. 1) (4). During reperfusion, transient metal ions such as ferrous and ferric ions are uptaken and accumulate in vulnerable cells, which results in •OH production by the Fenton and the Haber–Weiss reactions (17-20). The therapeutic potential of antioxidants has been well documented in animal models of stroke with a longer therapeutic time window than NMDA receptor antagonists (21). Four antioxidants have advanced to clinical trials for acute ischemia. First, tirilazad, a lipid peroxidation inhibitor, failed to show significant beneficial effects in six randomized, controlled trials in patients with acute ischemic stroke. An additional phase III study using a higher dosage of tirilazad was stopped with questions emerging in Europe regarding its safety (22). Secondly, the administration of ebselen, a glutathione peroxidase mimic, to patients with acute ischemic stroke within 24 hours after stroke onset, improved outcome for 1 month but not after 3 months, in Japan (23, 24). The modified Mathew Scale and modified Barthel Index scores were also improved in stroke patients subjected to early treatment with ebselen. In Japan, a placebo-controlled, double-blind, randomized, multicenter trial of ebselen has been initiated for patients with acute cortical infarction but further clinical outcomes have not yet been reported. Thirdly, the phase III study of edaravone, a free radical scavenger, was performed with 250 patients with acute ischemic stroke in Japan, which showed a significant improvement in functional outcome within 3 months, as evaluated by the modified Rankin scale (25). Edaravone was approved for the treatment of acute stroke in Japan (2001) and China (2007). Fourthly, NXY-059 (disufenton sodium), a nitrone-based free radical-trapping agent, was subjected to a randomized, double-blind, placebo-controlled trial in 1,722 patients

within 6 hours after onset of acute ischemic stroke (SAINT I trial) (26). NXY-059 was shown to significantly improve the primary outcome (reduced disability at 90 days) without improving neurological functions as measured by the National Institutes of Health Stroke Scale (NIHSS) score. Efficacy of NXY-059, however, was not verified in the SAINT II trial in 3,195 patients even for the primary outcome (27).

Mixed clinical outcomes with antioxidants appear to largely stem from the translational mismatch between animal studies and clinical trials. For example, tirilazad was shown to protect against transient focal cerebral ischemia but was not beneficial in animal models of transient forebrain ischemia and permanent focal cerebral ischemia (28-30). In addition, neither therapeutic time window nor reproducibility of tirilazad was investigated in animal models of stroke. The therapeutic potential of NXY-059 as an antioxidant for ischemic stroke might be limited due to its extremely low cell and blood–brain barrier permeability (31). In a meta-analysis of studies of NXY-059 in animal models of transient and permanent brain ischemia, efficacy of NXY-059 was variable, ranging from 0% (no beneficial effect) to 60%, in reducing infarct volume, and also for the neuroprotective time window (32). Although clinical trials were not supported by such confounding efficacy in preclinical studies, NXY-059 nevertheless moved to SAINT I and II, phase III clinical trials in 5,028 acute stroke patients and again failed to show any beneficial effects (33, 34). Finally, edaravone has been prescribed to treat acute ischemic stroke regionally in Japan and China but its global application is limited due to unwanted side effects such as acute renal failure, fulminant hepatitis and caution in the elderly (35-38).

DISCOVERY OF NEU2000, A DUAL NEUROPROTECTANT TARGETING BOTH NMDA RECEPTORS AND FREE RADICALS

Acetylsalicylic acid (aspirin) is widely used to prevent recurrent ischemic stroke; its therapeutic effect is related to its role as an irreversible cyclooxygenase inhibitor, inhibiting the conversion of arachidonic acid to thromboxane A_2 and thereby inhibiting platelet aggregation (39). Recently, aspirin and salicylic acid were shown to prevent NMDA receptor-mediated excitotoxicity by blocking nuclear factor-κB and *c*-Jun N-terminal kinase (40, 41). Aspirin can protect against ischemic neuronal death by inhibiting voltage-gated Ca^{2+} channels, p44/42 mitogen-activated protein kinase and glutamate release (42-44). However, the therapeutic potential of aspirin in the brain may be limited given that high doses (as much as 10 mM) of the drug are needed to prevent NMDA and Zn^{2+} neurotoxicity in primary cortical cell cultures. The anti-inflammatory drug sulfasalazine, a conjugate of 5-aminosalicylic acid and sulfapyridine, inhibits NMDA neurotoxicity more potently than aspirin and in addition offers neuroprotection against free radical injury (45-46). We propose that the dual pharmacological actions of these salicylates against NMDA receptor-mediated excitotoxicity and free radical neurotoxicity offer the potential for improved neural protection after ischemic brain injury and that development of chemical derivatives of these compounds with greater potency, safety and effectiveness might offer a novel, successful therapy for stroke compared to a monotherapy targeting either NMDA receptor or free radicals.

We discovered a group of synthetic derivatives from the lead structure of sulfasalazine that prevented NMDA-induced neuronal death in cortical cell cultures more potently than aspirin and sulfasalazine (Fig. 2) (47). Among the derivatives, 2-hydroxy-5-(2,3,5,6-tetrafluoro-4-trifluoromethyl-benzylamino)-benzoic acid, named Neu2000, showed additional nerve cell protection against oxidative stress even at nanomolar concentrations. Moreover, Neu2000, a moderate NMDA receptor antagonist, did not produce neuronal cell apoptosis, one of the major side effects of potent NMDA receptor antagonists. Based upon its efficacy as a dual neuroprotective drug and safety, Neu2000 was chosen as a final drug candidate for the treatment of stroke, trauma and neurological diseases linked to NMDA receptor activation and oxidative stress.

MECHANISMS OF NEUROPROTECTIVE ACTION OF NEU2000

Neu2000 blocks free radical injury as a potent cell-permeable spin trapping molecule

Efficacy and potency of Neu2000 were examined and compared with those of other

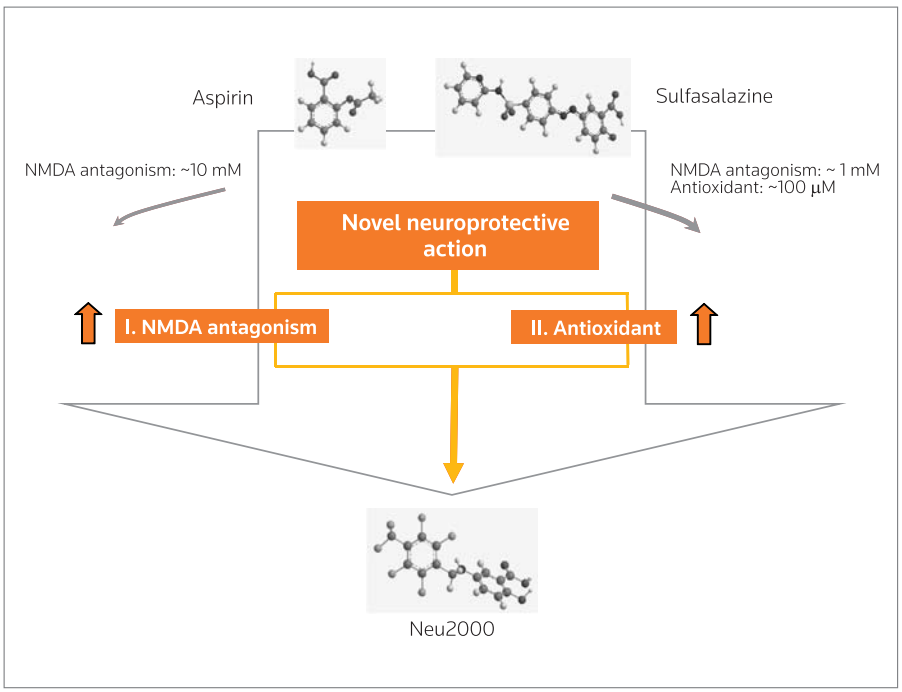

Figure 2. Discovery of Neu2000. Based upon novel pharmacological findings that aspirin is a very weak NMDA receptor antagonist and sulfasalazine acts as an antioxidant as well as a weak NMDA receptor antagonist, Neu2000 was developed as a moderate NMDA receptor antagonist and potent antioxidant that improved potency (against NMDA and free radicals) and safety of aspirin and sulfasalazine.

antioxidants in cortical cell cultures exposed to Fe^{2+}, a catalyst of the Fenton reaction producing hydroxyl radicals that was expected to mediate free radical production and cytotoxicity following ischemic brain injury (47) (Fig. 3). Cultured cortical neurons underwent widespread death 24 hours after exposure to 50 μM Fe^{2+}. Concurrent administration of Neu2000 attenuated Fe^{2+}-induced neuronal death (IC_{50} = 0.11 μM). Neu2000 completely blocked Fe^{2+} neurotoxicity even at 0.3 μM. Trolox, a water-soluble form of α-tocopherol, showed similar efficacy (100% blockade) but lower potency (IC_{50} = 3.34 μM) against Fe^{2+} injury compared with Neu2000. Edaravone partially reduced Fe^{2+}-induced neuronal death at a dose of 3 μM but increased Fe^{2+} injury at higher doses, suggesting a potential side effect of the free radical scavenger. NXY-059 attenuated Fe^{2+} neurotoxicity by up to 32% at a dose of 0.3 μM but did not show further neuroprotection at doses of 1–300 μM. The low efficacy of NXY-059 may be attributable to its polar, highly water-soluble character, very low cell permeability and two orders of magnitude less potency than vitamin E in inhibiting free radical-mediated peroxidative reactions in a cell-free assay system (31, 48, 49). In addition, Neu2000 blocked the degeneration of neurons and glia in cortical cell cultures exposed to DL-buthionine-[*S,R*]-sulfoximine, a glutathione-depleting agent, and sodium nitroprusside, a nitric oxide donor (IC_{50} = 0.1 μM) (47).

Neu2000 reacted with 2,2-diphenyl-1-picrylhydrazyl, a nitrogen-centered stable free radical, implying that Neu2000 is a free radical scavenger. The spectroscopic technique of electron spin resonance demonstrated that Neu2000 reduced hydroxyl radical adducts of 5,5′-dimethylpyrroline 1-*N*-oxide (DMPO) in a concentration-dependent manner and up to 80–90% at 0.1 μM in a cell-free in vitro assay (Fig. 3), suggesting that Neu2000 is a potent spin trapping molecule.

Neu2000 is a reversible, uncompetitive NR2B-specific antagonist with a good safety profile

Neu2000 inhibited 300 μM NMDA-induced currents at IC_{50} of 35 μM (47, 50). As illustrated upon comparison with other NMDA receptor antagonists (Fig. 4), Neu2000 is a reversible and uncompetitive NMDA receptor-gating modifier with fast binding kinetics. This NR2B-specific antagonist is sensitive to ifenprodil, an NR2B receptor antagonist, but not to NVP-AAM077, an NR2A receptor antagonist. Uncompetitive antagonists with activity-dependent block are generally expected to allow a stronger inhibition of NMDA receptors in neurological diseases such as stroke and epilepsy that cause excess accumulation of glutamate at the synaptic cleft, while showing minimal inhibition of normal synaptic transmission through NMDA receptors. Accordingly, open channel blockers with use-dependency showed marked protection against ischemic insults in animal models. However, once bound to the open channel of the activated receptor, open channel blockers with uncompetitive nature can be trapped within the closed channel and lead to a prolonged blockade of NMDA receptors, making them clinically undesirable. Gating modifiers, such as ifenprodil and Neu2000, would be beneficial for clinical application, as their agonist-dependent nature comes from preferential binding to an agonist-associated closed state of the receptor, not from being trapped within channels. In addition, the off-rate of Neu2000 with a use-dependent

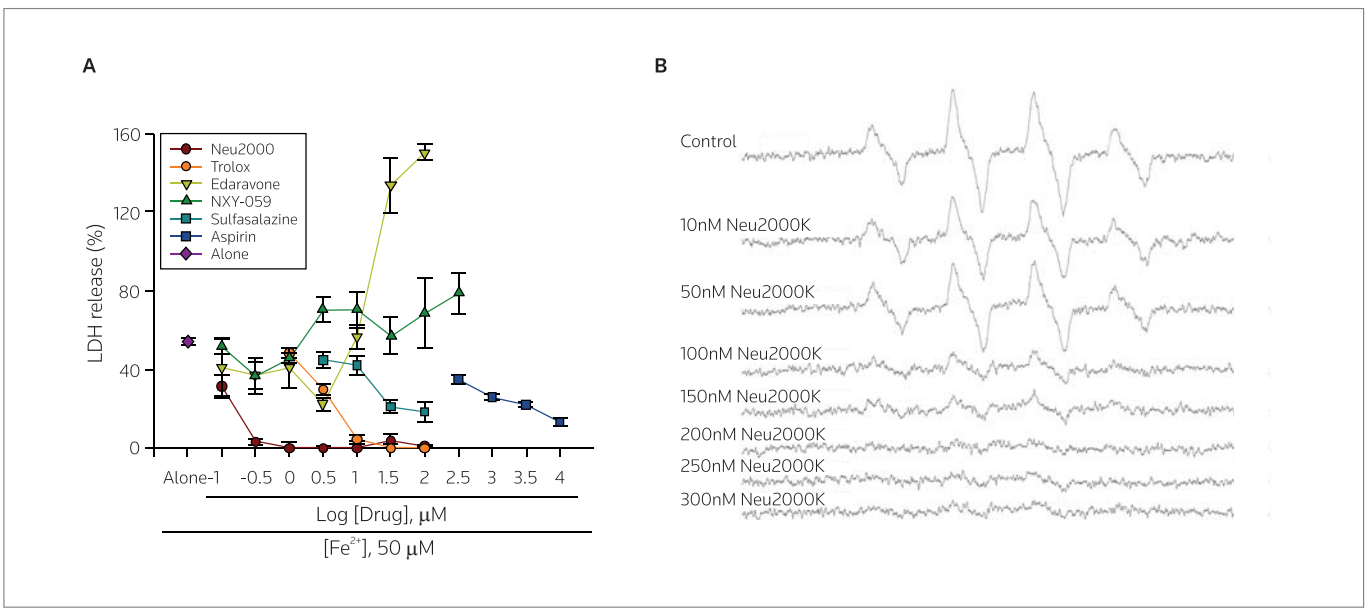

Figure 3. Better efficacy and potency of Neu2000 than other antioxidants. A) Fe^{2+}-induced neuronal death assay: Mouse cortical cell cultures (11 to 14 days in vitro) were continuously exposed to 50 μM Fe^{2+}, alone or with indicated doses of Neu2000, trolox, edaravone, NXY-059, sulfasalazine and aspirin. Neuronal death was analyzed 24 hours later by measurement of lactate dehydrogenase (LDH) released into the bathing medium and scaled to near complete neuronal death (= 100%) following exposure to 500 μM NMDA, mean ± SEM (n = 8 cultures for each condition). B) Potent spin trapping of Neu2000 against hydroxyl radicals. Neu2000 (as its potassium salt, Neu2000K) reduced the control signal intensity of hydroxyl radical adducts of 5,5′-dimethylpyrroline-1-*N*-oxide in electron spin resonance in a concentration-dependent manner and up to 80–90% at 0.1 μM in a cell-free in vitro assay.

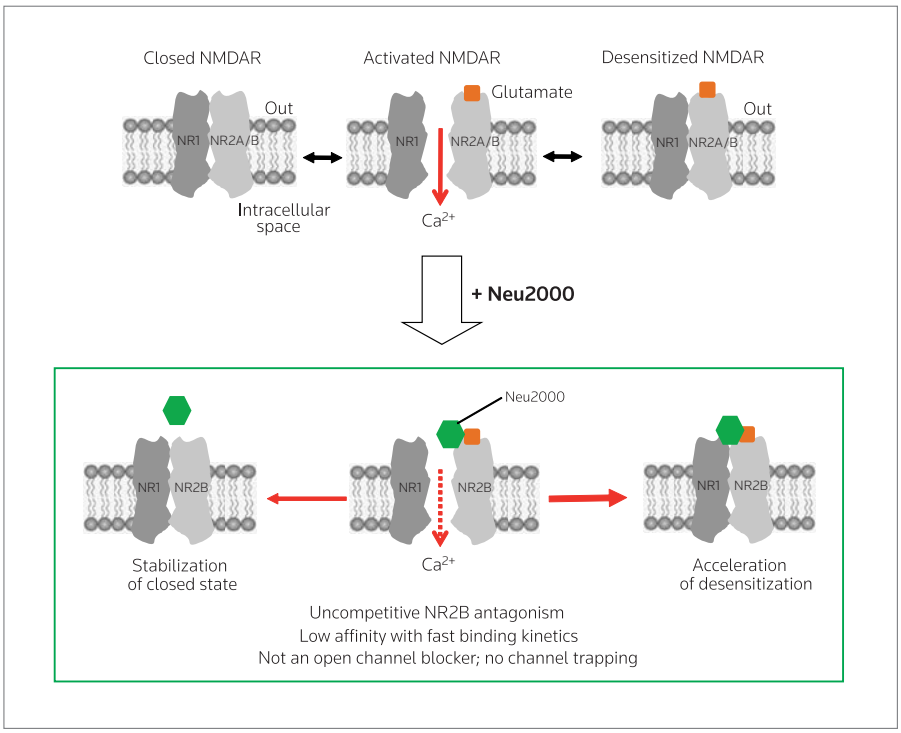

Figure 4. Mechanisms of NMDA receptor antagonism by Neu2000. Binding of glutamate to an NMDA receptor (NMDAR), consisting of NR1 and NR2A/B, at the resting closed state causes Ca^{2+} entry through the activation of NMDAR. The activated NMDAR can return to the resting state or reversibly change to a desensitized state which also does not allow Ca^{2+} entry. Neu2000 is a moderate reversible uncompetitive NR2B subtype-specific antagonist with very fast binding kinetics that can inhibit Ca^{2+} entry mainly by accelerating the desensitization of NMDAR and stabilizing the closed state of NMDAR. In contrast to open channel blockers that show uncompetitive antagonism, Neu2000 is not trapped within a receptor channel. In addition, Neu2000 exerts its blockade in a voltage-independent manner. These unique actions of Neu2000 might contribute to more efficient neuroprotection with better safety profiles compared with other NMDA antagonists in ischemic stroke conditions accompanying high extracellular Ca^{2+} concentration and excessively prolonged depolarization.

block is so surprisingly fast that it might have a greater safety for therapeutic application than high affinity blockers with slow block and unblock of NMDA receptors (51, 52). It is noteworthy that the unblocking rate of Neu2000 is about eight times faster than that of memantine, a moderate affinity NMDA receptor antagonist approved for the treatment of moderate to severe Alzheimer's disease (53). Open channel blockers usually decrease their blockade as the cells become depolarized (54). Accordingly, Neu2000's lack of voltage dependency may give it an advantage over open channel blockers in inhibiting NMDA responses effectively at the prolonged depolarization induced by excessive glutamate. Also, the acceleration of Ca^{2+}-dependent desensitization by Neu2000 should be an important factor for protecting neurons against insults because the Ca^{2+}-dependent desensitization is a negative feedback mechanism to prevent the undesirable effects of excessive activation. Moreover, it is highly probable that the specificity of Neu2000 for NR2B alleviates its undesirable side effects, as glutamate spilled over in a synapse during ischemic insult possibly recruits extrasynaptic NR2B-NMDA receptors (55, 56). In support of this, ischemic injury was shown to recruit death-associated protein kinase 1 into the NR2B complex at extrasynaptic sites (57), resulting in neurotoxic Ca^{2+} overload through excess activation of extrasynaptic NMDA receptors (57-59). In contrast, synaptic NMDA receptor activation was likely to enhance neuronal survival by preventing apoptosis through maintenance of neuroprotective levels of Ca^{2+} (58).

EFFICACY OF NEU2000 IN ANIMAL MODELS OF ISCHEMIA

A number of neuroprotectants including NMDA receptor antagonists and antioxidants have demonstrated substantial efficacy in animal models of stroke but have failed to show beneficial effects in clinical trials in stroke. In 1999, the Stroke Therapy Academic Industry Roundtable (STAIR) recommendations were published to overcome translational barriers from preclinical studies to human clinical trials (60, 61). The neuroprotective effects of Neu2000 have been examined turning to rodent models of transient and permanent focal cerebral ischemia to fulfill the initial STAIR recommendations with the exception of efficacy studies in gyrencephalic species with convulsions in the cerebral cortex.

Efficacy against transient occlusion of middle cerebral artery

Dose-response experiments of Neu2000 were performed in rats subjected to 60-minute occlusion of middle cerebral artery (47). Intravenous administration of Neu2000 (0.5–20 mg/kg) 5 minutes after reperfusion significantly and dose-dependently reduced the infarct volume. The maximal effects were observed at doses of 2.5 to 5 mg/kg, with a maximal reduction in the infarct volume of 66%. The maximal neuroprotective effects of Neu2000 were much higher than those of either MK-801, an NMDA receptor antagonist, or trolox, an antioxidant. The therapeutic time window was then determined using a dose of 5 mg/kg in 60-minute transient middle cerebral artery occlusion (tMCAO) models. The sooner Neu2000 was administered after reperfusion, the more the infarct volume 24 hours after 60-minute tMCAO was reduced. The neuroprotective effects of Neu2000 were significant even when it was delivered 8 hours after reperfusion. In contrast, NMDA receptor antagonists and antioxidants such as NXY-059, ebselen and edaravone significantly protected against tMCAO before reperfusion and 3 hours after reperfusion, respectively (48, 62-64). Taken together, these findings indicate that Neu2000 has better efficacy and a therapeutic time window than a monotherapy targeting either NMDA receptors or free radicals in rodent models of tMCAO.

Randomized and blind experiments of Neu2000 were carried out in the intralumi-

nal thread occlusion model of middle cerebral artery for 90 minutes to study if administration of Neu2000, 30 minutes after reperfusion would improve neurological functions over 4 weeks after 90-minute tMCAO. Neu2000 ameliorated neurological severity score, motor coordination and hemineglect behavior within 1 day after 90-minute tMCAO. In animals treated with Neu2000, such neurological functions were further improved 4 weeks later (47). Furthermore, Neu2000 was shown to attenuate white matter damage 3 days after 90-minute tMCAO.

Efficacy against permanent occlusion of middle cerebral artery

Dose-response experiments revealed maximal neuroprotective effects of Neu2000 at a dose of 30 mg/kg i.v. in rat permanent occlusion of middle cerebral artery (pMCAO) models. A single bolus injection of 30 mg/kg Neu2000 significantly reduced infarct volume 24 hours following pMCAO when delivered within 4 hours after occlusion (65). We have observed that pMCAO produces biphasic patterns of free radical production in the core and penumbra (66). The single injection of Neu2000 prevented the initial free radical production at 4 hours after occlusion but did not attenuate the delayed free radical production 24 hours after occlusion. Dual injections of 30 mg/kg Neu2000 at 2 and 16 hours following pMCAO prevented the initial and delayed free radical production in the core and penumbra, which resulted in significant attenuation of infarct volume. The neuroprotective effects of Neu2000 against pMCAO were reproduced in a double-blinded manner by independent laboratories.

Efficacy against transient forebrain ischemia and hemorrhagic stroke

Single administration of Neu2000 markedly attenuated the delayed degeneration of the hippocampal CA1 neurons evolving 3 days after 10-minute forebrain ischemia in adult rats (67). The neuroprotective effects of Neu2000 were manifest when administered 1 day after transient forebrain ischemia, suggesting Neu2000 can be applied to prevent brain injury occurring in cardiac arrest patients. In an animal model of hemorrhagic stroke, the hematoma volume and cell death were significantly reduced after administration of Neu2000 (5 mg/kg i.v.) at 30 minutes after induction of intracerebral hemorrhage (68).

STUDY OF NEU2000 IN HUMANS

A phase I, double-blinded, randomized, placebo-controlled ascending single i.v. dose study has been completed assessing the safety, tolerance and pharmacokinetics of Neu2000 in 95 healthy young and elderly volunteers in the United States (69). Neu2000 had a good safety profile without any serious adverse events up to 6000 mg, with a mean maximum plasma concentration (C_{max}) of 372 µg/mL and an area under the concentration (AUC; mean ± S.D.) of 3252 ± 1538 µg.h/mL (unpublished data). Based on the robust human safety profile of Neu2000 and AUC-based therapeutic index reaching about 35 (AUC at Neu2000 6000-mg dose in human/AUC at maximal effective dose in pMCAO animal models) to about 800 (AUC at Neu2000 6000-mg dose in human/AUC of maximal effective dose in tMCAO animal models), a phase II proof-of-concept trial of Neu2000 has been prepared for patients with stroke.

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DISCLOSURES

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