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Neu2000 as a novel neuroprotectant to treat stroke

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Stroke is a cerebrovascular injury that occurs when blood flow to brain is interrupted by local thrombosis, embolic particles, or rupture of blood vessels. Thrombolytic and anti-thrombotic therapy has been developed for timely reperfusion of the ischemic territory. The clot-dissolving drug, tissue plasminogen activator (tPA), was shown to be beneficial in ischemic stroke patients when it was administered within 3 hours after the onset of symptoms (NINDS t-PA Stroke Study Group. New Eng J Med, 333: 1581-1587, 1995). Besides the roles of the anti-platelet drug aspirin in secondary stroke prevention, a modest beneficial effect of aspirin has been reported in the Chinease Acute Stroke Trial (CAST), International Stroke Trial (IST) and Multicenter Acute Stroke Trial – Italy (MAST-I) with treatment starting within 48 hours of the onset of acute ischemic stroke (Lancet 346:1509-14, 1995; Lancet 349:1569-81, 1997; Lancet 349:1641-9, 1997).

As the reperfusion therapy shows limited effects only at narrow therapeutic windows, a new therapeutic strategy of neuroprotection has been extensively explored

over the last two decades. When the ischemic condition persists for a prolonged time, primary neuronal death appears rapidly in the core areas and is accompanied by the secondary death in the ischemic penumbra that slowly evolves subsequent to activation of multiple death pathways and thus has been targeted for therapeutic intervention.

The first line of interventional therapy stems from findings that excitotoxicity underlies a leading cause of neuronal death following hypoxic-ischemic insults (Choi, Stroke 21:III20-22, 1990). Accordingly, antagonists of ionotropic glutamate receptors have been developed, shown to reduce hypoxic-ischemic brain injury in various animal models, and applied for clinical trial of ischemic stroke with little therapeutic efficacy to date. Free radicals are produced primarily during a period of reperfusion and believed to contribute to delayed neuronal death (Siesjo et al., Cerebrovasc Brain Metab Rev. 1:165-211, 1989). Finally, several lines of evidence suggest that apoptosis or programmed cell death comprises a portion of ischemic neuronal death (Choi. Curr Opin Neurobiol. 6:667-72, 1996). It is conceivable to postulate that the therapeutic intervention of ischemic neuronal death likely involves appropriate combination of neuroprotective drugs designed to prevent excitotoxicity, oxidative stress, and apoptosis (Gwag et al., Excitotoxicity, Oxidative stress, and Apoptosis in Ischemic Neuronal Death, 4 : 79~112, CRC press, 2002).

In addition to therapeutic roles of aspirin as an anti-inflammatory, analgesic, antipyretic, and anti-thrombotic drug (Bednar & Gross,Stroke 30: 887 - 893, 19990), neuroprotective actions of aspirin have been demonstrated in animal models of acute and chronic neurodegenerative diseases such as hypoxic ischemia, Parkinson's disease, and amyotrophic lateral sclerosis (Aubin et al., J Neurochem. 71:1635-42, 1998; Barneoud & Curet, Exp Neurol 155:243-251. 1999; Castillo et al., Neurosci.Lett. 339:248.-50, 2003). Studies in vitro suggest that aspirin protects neurons from NMDA receptor-mediated excitotoxicity through mechanisms involving the blockade of NF-κB and c-Jun N-terminal kinase (Grilli et al., Science 274: 1383-1385, 1996; Ko et al., J.Neurochem. 71:1390-1395, 1998). Aspirin can act as an inhibitor of votage-gated Ca^{2+} channel, which attenuates Zn^{2+} influx into neurons and thus neurotoxicity (Kim et al., Neurobiol.Dis 8:774.-83, 2001). However, the therapeutic potential of aspirin is limited by remarkably high doses as much as 10 mM that are needed for the prevention of NMDA and Zn^{2+} neurotoxicity in cultured neurons. The anti-inflammatory drug sulfasalazine, a conjugate of 5-aminosalicylic acid and sulfapyridine, has been shown to act as a NMDA receptor antagonist and also protect neurons from free radical injury as a direct anti-oxidant (Ryu et al., J.Pharmacol.Exp.Ther. 305:48-56. 2003). Thus, the pharmacological actions of aspirin and sulfasalazine can be applied to prevent NMDA

receptor-mediated excitotoxicity and oxidative stress that comprise of two major routes of pathological neuronal death in ischemia and neurological diseases.

We have developed an NMDA receptor antagonist, referred to Neu2000 that is derived from sulfasalazine (Figure 1). The pharmacological actions of Neu2000 have been extended to a powerful antioxidant and anticoagulant (Figure 2). As a result, Neu2000 is an exciting drug candidate that possesses the ability to protect neuronal cells on multiple levels: preventing NMDA receptor-mediated neuronal cell death, oxidative stress, and zinc neurotoxicity. The diverse mechanistic action of Neu2000 clearly renders it far superior to any currently available drug. In animal models of focal cerebral ischemia, Neu2000 markedly prevented infarct volume following occlusion of middle cerebral artery even delivered 36 hr after reperfusion (Figure 3). Due to structural elements similar to those in aspirin, it is not surprising that Neu2000 shows the added benefit of anti-platelet action. The breadth of neuroprotective activity of Neu2000 renders it a powerful drug candidate for the treatment of stroke patients and related neurological diseases.

Figures

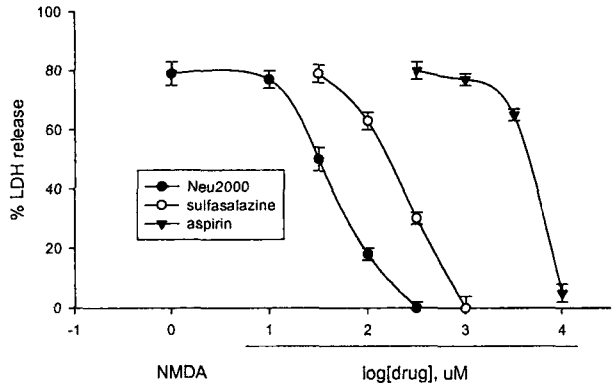

Figure 1. Dose-response curve of Neu2000 against NMDA neurotoxicity

Mouse cortical cell cultures (DIV 12 - 14) were exposed to 300 M NMDA for 10 min, alone or with inclusion of indicated doses of Neu2000, sulfasalazine, and aspirin. Neuronal death was analyzed 24 hr later by measuring levels of LDH released into the bathing medium (mean ± SEM; n = 9 - 12 culture wells per condition), scaled to mean LDH efflux values 24 hr after sham wash (=0 %) and continuous exposure to 500 M NMDA (=100 %) that causes near complete neuronal death.

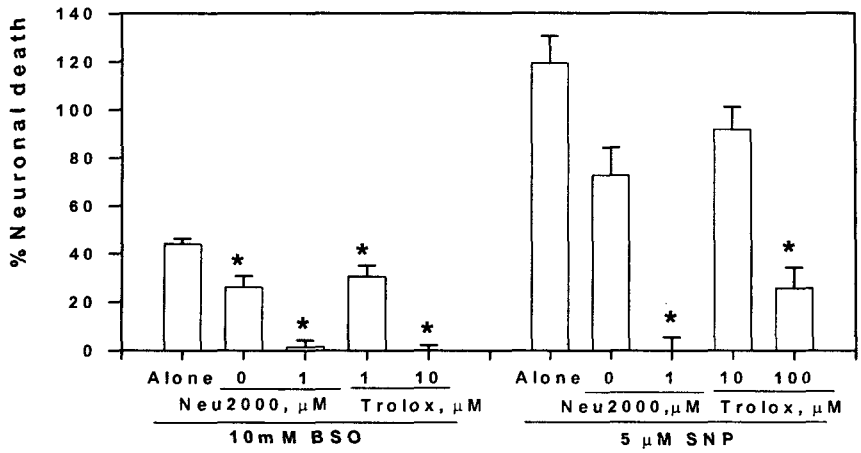

Figure 2. Blockade of free radical neurotoxicity by Neu2000

Cortical cell cultures (DIV 12 - 14) were exposed to 10 mM BSO or 5 μM SNP for 24 hr, alone or with indicated doses of Neu2000 or trolox. Neuronal death was analyzed 24 hr later by measuring LDH efflux into the bathing media (mean ± SEM; *n* = 4 – 32 culture wells per condition). *, Significant difference from relevant control (sham control) at $P < 0.05$ using ANOVA and Student-Newman-Keuls test.

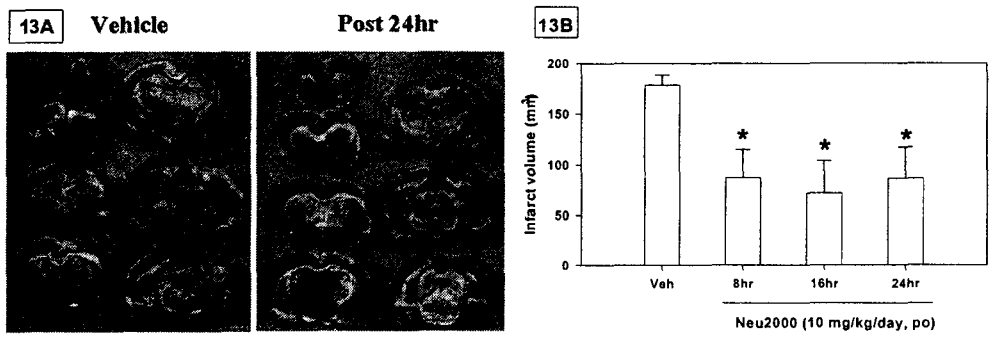

Figure 3. Wide therapeutic window of Neu2000 against delayed onset infarct 2

weeks after mild focal cerebral ischemia

A: Adult rats received occlusion of middle cerebral artery for 30 min, with daily administration of vehicle (veh, left panel) or Neu2000 (10 mg/kg, P.O., right panel) beginning from 24 hr after reperfusion. Note that delayed administration of Neu2000 attenuated cerebral infarct (stained with TTC).

B: Same as A except additional groups of animals delivering from 8 or 16 hr after reperfusion. Infarct volume was analyzed 2 weeks later after staining with TTC, mean ± SEM (n = 8 – 11 rats per each condition). *, Significant difference from the vehicle control at p<0.05 using ANOVA and Student-Neuman-Keuls' test.