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## TARGETING NEONATAL ISCHEMIC BRAIN INJURY WITH A SELECTIVE CASPASE-2 INHIBITOR

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We have designed and developed a new selective and irreversible caspase-2 inhibitor ( $IC_{50} = 65 \pm 5$  nM), called TRP601. Similarly to caspase-2 specific siRNA, this inhibitor efficiently blocks acute *in vitro* cell death of embryonic primary cortical neurons subjected to serum-deprivation<sup>1</sup>. A single-dose of TRP601 (5 mg/kg; i.p.) was administered to 7-day-old rat pups before permanent occlusion of the middle cerebral artery and subsequent transient unilateral carotid ligation. TRP601 induced a highly significant reduction (74 %;  $p < 0.001$ ) of infarct volume ( $V = 5.7 \pm 2.3$  %,  $n = 12$ ,) compared to control ( $V = 22.1 \pm 1.4$  %,  $n = 17$ ). TRP601 also produces significant reductions in cortical infarct volume when administered (i.p.) one hour after the ischemic onset (i.e. at reperfusion) at doses between 1  $\mu$ g/kg and 10 mg/kg, with an optimal dose of 1 mg/kg ( $V = 9.29 \pm 1.66$  %,  $n = 18$ ). In this experimental setting, TRP601 also provides neurological benefit on general behavioral profiles. To determine the therapeutic window of TRP601, we designed a large protocol ( $n = 250$  rat pups, 2 independent experimenters) with mixed litters in each group. When injected up to 6 hours post-ischemia at 1 mg/kg (i.p.), TRP601-induced reduction of infarct volume remained highly significant ( $V = 15.63 \pm 0.92$  %,  $n = 48$ ). Additional experiments indicate that TRP601 crosses the blood-brain barrier, reaches the brain and inhibits initiator caspase-2, cytochrome c release, downstream caspases, and apoptosis *in vivo*. TRP601 also confers neuroprotection against hypoxia-ischemia in newborn rats and drug-induced NMDA-receptor dependent excitotoxicity in newborn mice. TRP601 has no side detectable side-effects on blood flow, blood pressure, cardiac function, blood gases, temperature and body weight. Acute toxicity studies performed with single doses in 7-day-old rat pups indicate low toxicity of TRP601 ( $DL_{50}$  i.v. = 60 mg/kg;  $DL_{30}$  i.p. > 200 mg/kg). Histological analysis indicate no influence of TRP601 on forebrain, middle brain and hindbrain maturation at 21 days of age, and no alterations of liver and spleen haematopoiesis. Subchronic administration in newborn Beagles revealed no toxicity of TRP601 after repeated i.v. injections at 15 mg/kg. Hence, TRP601 may be a potent drug candidate for the treatment of perinatal brain ischemia.

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<sup>1</sup> Chauvier D, Lecoœur H, Langonne A, Borgne-Sanchez A, Mariani J, Martinou JC, Rebouillat D, Jacotot E. Upstream control of apoptosis by caspase-2 in serum-deprived primary neurons. *Apoptosis*. 2005 10(6):1243-59