LETTER TO THE EDITOR

Nicotinamide Postpones Stroke in Stroke-Prone Spontaneously Hypertensive Rats

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Stroke is the second most common cause of death and a major cause of disability worldwide [1]. Approximately, 80% of the cases are ischemic. Many stroke survivors require lifelong assistance. Prevention is considered the most effective strategy to curb stroke [2]. The improved control of risk factors could reduce the morbidity of stroke, such as reducing blood pressure and blood lipid. Besides these, finding some endogenous and safe medicines are also important.

Nicotinamide (NAM) is a precursor of nicotinamide adenine dinucleotide (NAD⁺) and is used to generate ATP in the mitochondrial electron-transport chain. NAM could readily enter the brain [3]. Recently, NAM has been reported to offer neuroprotection on brain [4–6]. Whether it postpones stroke for lifelong treatment is unclear.

In this study, we examined the preventive effect of NAM against ischemic stroke in stroke-prone spontaneously hypertensive rats (SHRSP), a commonly used animal model for spontaneous stroke. To test the potential effective dosage, six groups of SHRSP were received NAM (25–500 mg/kg/day) or vehicle for 4 weeks, and then were subjected to middle cerebral artery occlusion (MCAO). As shown in Figure 1(A), NAM (50, 100, and 200 mg/kg/day) significantly reduced infarction volume. Then, we administrated 80 SHRSP with NAM (50, 100, and 200 mg/kg/day) or vehicle via animal chow for their entire lifespan. Compared with the control group, long-term treatment with NAM did not affect the body weight (Figure 1B). Kaplan–Meier analysis of the survival is shown in Figure 1(C). Pretreatment with NAM (50, 100, and 200 mg/kg) significantly delayed the onset of stroke (log-rank testing, $P < 0.01$). These data demonstrated that NAM (50–200 mg/kg), when administrated to SHRSP before the ischemic event, could delay the onset of stroke and reduce infarction volume.

Increased levels of reactive oxygen species (ROS) are a feature in ischemic stroke and exaggerate the ischemic damage [7,8]. In this

study, ROS levels in infarct penumbra of cerebral cortex of male SHRSP (220–250 g) were assessed using rhodamine 123. Compared to sham operation, MCAO increased ROS level by 46% (Figure 2A). Such an increase was significantly attenuated by NAM (100 mg/kg, i.p.).

Nicotinamide adenine dinucleotide phosphate oxidase (NOX), an important ROS-generating enzyme, is a major source of ROS [9]. As a multisubunit complex, gp91phox is the catalytic subunit of NOX and requires subunit p22phox. Superoxide dismutase (SOD), including Cu/Zn-SOD and Mn-SOD, is one of ROS-scavenging enzymes. In our study, MCAO upregulated gp91phox and p22phox subunits, decreased SOD activity, and increased the MDA level in infarct penumbra of cerebral cortex of male SHRSP (220–250 g) (Figure 2B–D). These changes were reversed by NAM pretreatment (100 mg/kg, i.p.). The results suggested that NAM plays antioxidative action, possibly via reducing ROS formation and promoting ROS elimination.

In addition, MCAO produced a significant decrease in mitochondrial membrane potential (Figure 2E). Consistent with a previous study [4], NAM pretreatment alleviated the MCAO-induced loss of mitochondrial membrane potential in the infarct penumbra.

Taken together, NAM could prevent/delay the occurrence of ischemic stroke in SHRSP, possibly via reducing ROS level and maintaining mitochondrial membrane potential.

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Figure 1 Nicotinamide (NAM) decreased the infarction size induced by MCAO and postponed stroke death in male SHRSP. **(A)** NAM pretreated (50, 100, and 200 mg/kg/day) reduced infarction size in SHRSP induced by MCAO. The upper shows representative rostrocaudal series of TTC-stained coronal sections 24 after MCAO. Data are expressed as mean $\pm$ SD ($n = 9-11$ per group). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t -test. $*P < 0.05$, $**P < 0.01$. **(B)** Lifelong treatment of NAM (50, 100, and 200 mg/kg/day) did not change the body weight of SHRSP. **(C)** Lifelong treatment of NAM (50, 100, and 200 mg/kg/day) postponed stroke death of SHRSP. Kaplan–Meier survival curves by these 4 groups.

Figure 2 The antioxidative effect of nicotinamide (NAM, 100 mg/kg) in the infarct penumbra induced by MCAO in SHRSP. NAM (100 mg/kg, i.p.) decreased the level of ROS **(A)**, downregulated the expression of NAD(P)H oxidase subunit gp91phox and p22phox expression **(B)**, increased SODs activity **(C)**, reduced the MDA levels **(D)** and increased mitochondrial membrane potential **(E)** in the infarct penumbra induced by MCAO in male SHRSP. In **(B)**, GAPDH level served as loading control. Data are expressed as mean $\pm$ SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t -test, $n = 7-9$ in each group. $*P < 0.05$, $**P < 0.01$.

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