Lauroylethanolamide and linoleoylethanolamide improve functional outcome in a model of stroke

Alexander Zabaneh, R. Scott Duncan, Puja Garg, Simon Kaja, Peter Koulen

Vision Research Center and Departments of Ophthalmology and Basic Medical Science, University of Missouri – Kansas City, School of Medicine, Kansas City, MO

Background

•Stroke is the third most common cause of death in the United States (1).

•N-acylethanolamines (NAEs) are endogenous lipid signaling molecules that are involved in numerous physiological functions in mammals, many of which mediated by central nervous system processes, including neurotransmission, reproduction, inflammation, analgesia, appetite and cytoprotection. (2)

•Recently, NAEs have gained recognition as neuroprotective compounds that have therapeutic potential for ischemic injury and stroke (3).

Objective

We here aimed at determining the neuroprotective potential of NAE 12:0 (lauroylethanolamide) and NAE 18:2 (linoleoylethanolamide, LEA) *in vivo*, using a rat model for stroke.

Methods

•Middle cerebral artery occlusion (MCAO) - MCA occlusion and reperfusion was performed as described by us previously (3), using an intraluminal filament model.
•Male Sprague Dawley rats (300-325 g; Harlan Laboratories, Indianapolis, IN) were anesthetized with Ketamine (60 mg/kg) and Xylazine (10 mg/kg).

•For MCAO, the left common carotid artery, left internal carotid artery (ICA) and the left external carotid artery were exposed, and a 3-0 monofilament nylon suture (Ethilon; Ethicon Inc., Sommerville, N.J., USA) was introduced into the ICA lumen through a puncture.
•After 90 minutes, the suture was withdrawn. Reperfusion period was 24 hr post MCAO.
•Rats either received vehicle (ethyl alcohol) treatment or were dosed with either 10 mg/kg or 20 mg/kg LEA 6 hours and 30 minutes before MCAO, administered intra-peritoneally.

•Measurement of cerebral infarct volume - 2,3,5-Triphenyl-tetrazolium chloride (TTC) staining of mitochondrial and cellular viability was performed as described by us previously (3).
•Infarction volume was calculated with a previously described method (3) to compensate for brain swelling in the ischemic hemisphere using Image J software.

•Neurological evaluation was performed at 24 hours of reperfusion after MCAO, modified from [4]. Using six criteria of neuromuscular function assigning a score based on the severity of the phenotype, the maximum score using our modified version of the test is 7.0.

•Statistical data analysis - Data are expressed as the mean ± s.e.m. Statistical significance was determined by analysis of variance (ANOVA) with post-hoc Student-Newman-Keuls multiple comparison test. A *P* value of less than 0.05 was considered significant.

Results

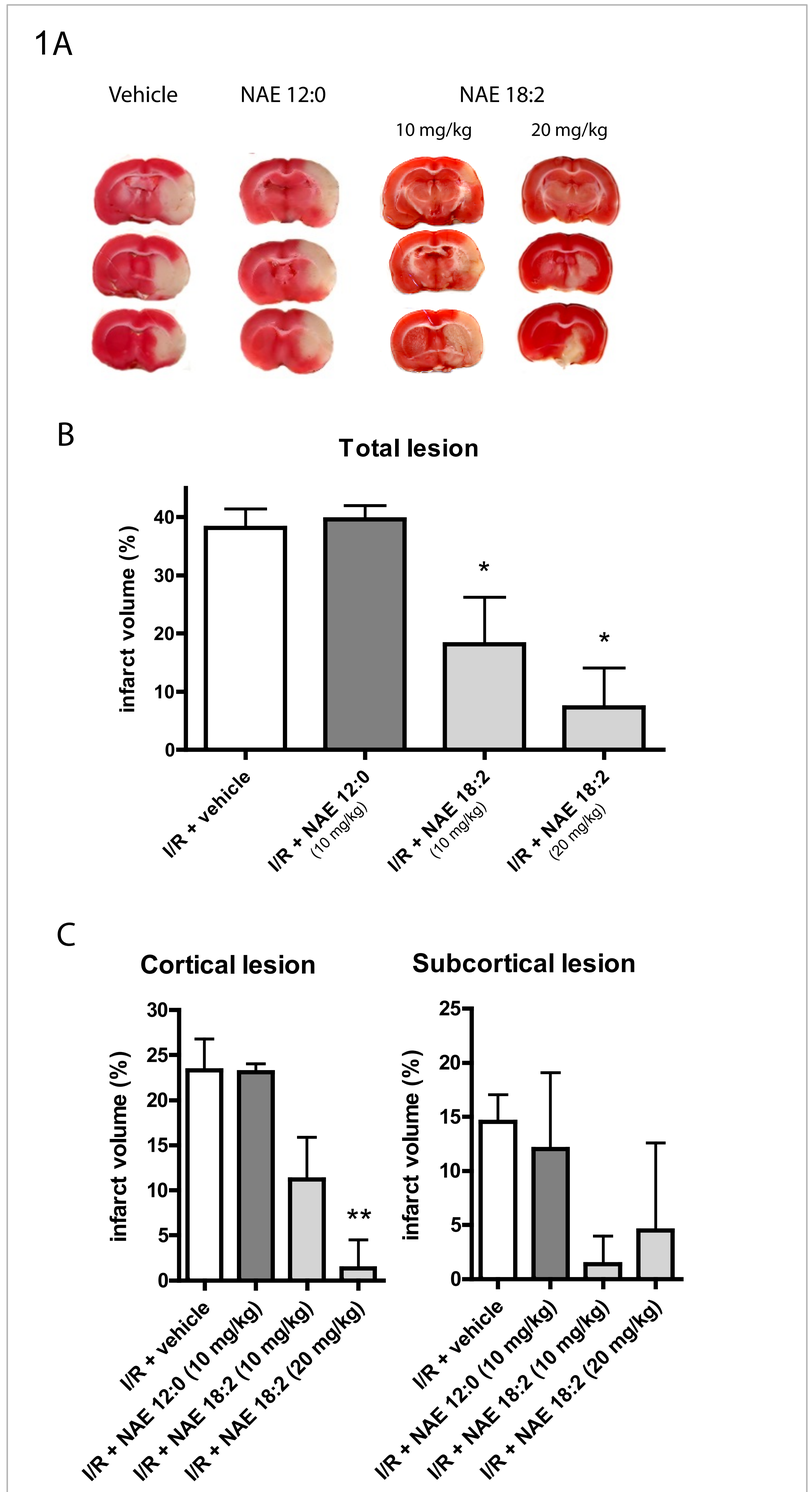

Figure 1: A, Comparison of ischemic regions of the brain as determined by TTC staining. **B**, Infarct volume of the total lesion as a percentage.
C, comparison of infarct volume based on brain subregion. * *P*<0.05, ** *P*<0.01.

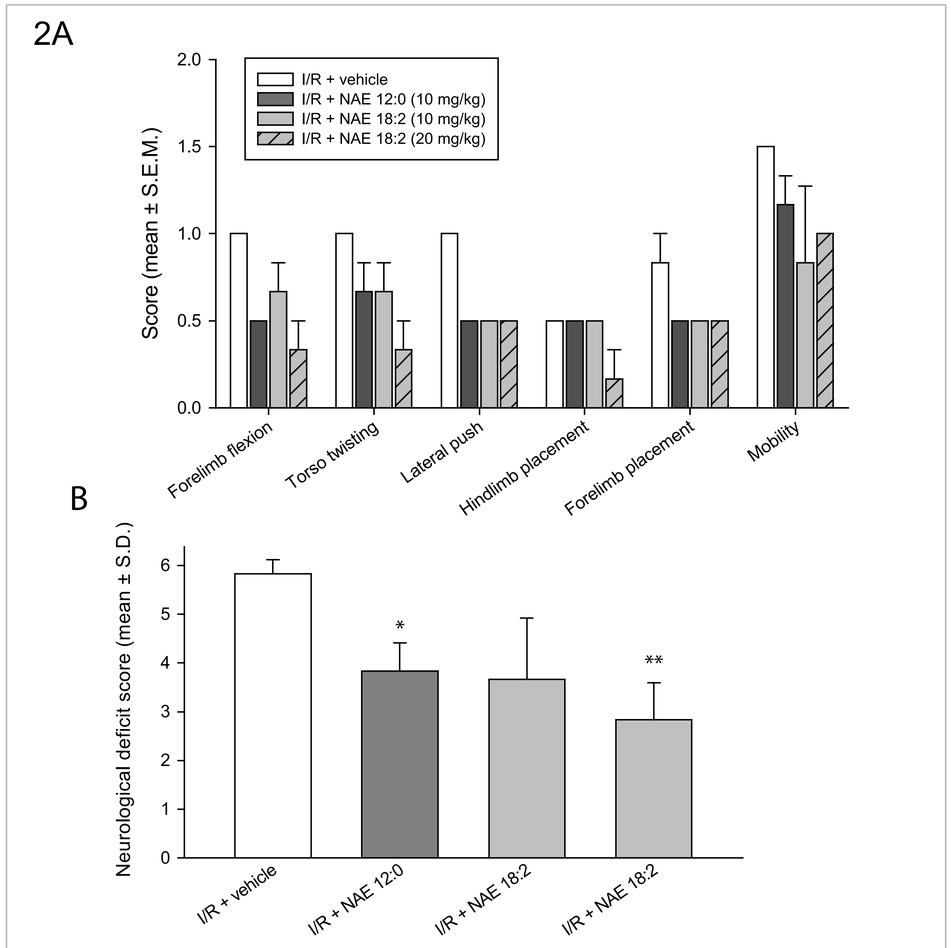

Figure 2: A, Neurological deficit scores for each treatment group by individual behavioral test.
B, Combined neurological deficit scores for each treatment group. * *P*<0.05, ** *P*<0.01.

Conclusions

•Administration of NAE 18:2 prior to MCAO reduces lesion size following 24 hr reperfusion and improves functional outcome after ischemia/ reperfusion injury.
•NAE 12:0 does not reduce lesion size, but significantly improves functional deficits.
•As these NAEs do not act on cannabinoid receptors (CB1 or CB2), their mechanism of action is like intracellular, similar to that described by us for the related NAE 16:0 [3].
•NAEs prove useful candidates for neuroprotection in stroke that could complement existing thrombolytic and surgical approaches in stroke therapy and might be useful as neuroprotectants in both acute and chronic neurodegenerative diseases.

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