Fatty Acid Methyl Esters Provide Neuroprotection After Ischemia

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Introduction

Fatty acids can provide neuroprotection from cerebral ischemia. Palmitic methyl ester (PAME) is a 16-C fatty acid with vasodilatory properties while, stearic acid methyl ester (SAME), an 18-C fatty acid, is simultaneously released from the autonomic ganglion and currently has no known function and has not been well explored. Since many neuro and/or vasoactive modulators have intrinsic neuroprotective properties, we investigated the possible neuroprotective effects of exogenous PAME/SAME in global (6mins of asphyxial cardiac arrest, ACA) and focal (middle cerebral artery occlusion with 2 minutes (mins) of reperfusion, MCAO) cerebral ischemia models. Since PAME (a potent vasodilator) is simultaneously released with SAME, we hypothesized that PAME/SAME can confer neuroprotection in rat models of focal/global cerebral ischemia.

Methods

Adult male Sprague-Dawley rats (250-350g) were fasted overnight before surgery and subsequently intubated and anesthesized. ACA and MCAO models were used to mimic ischemic conditions. For global ischemia, ACA (6mins) was performed 30mins after PAME/SAME treatment (0.02mg/kg, IV). Histopathology of hippocampal CA1 neurons was assessed 7 days after ACA. For focal ischemia, PAME or SAME was administered following reperfusion after 2hrs of MCAO. 2,3,5-triphenyltetrazolium (TTC) staining of the brain was performed 24hrs after MCAO and the infarct volume was quantified.

Schematic diagram of the experimental design.

(A) PAME or SAME(0.02 mg/kg, bolus, IV) was administered 30 mins before. 7 days after ACA and return of spontaneous circulation (ROSC), whole brain sections were stained with hemotoxylin and eosin. Normal neurons of the CA1 region of the rat hippocampus were manually counted on both hemispheres of the brain.

(B) MCAO (2 hrs) was performed on the rat. 2 mins after reperfusion, PAME or SAME (0.02 mg/kg, IV) was administered. After 24 hrs of recovery, the rats were sacrificed, brain slices stained with TTC, and brain infarct volumes were measured.

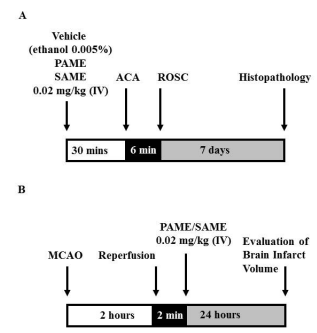


Table 1. Asphyxial cardiac arrest physiological parameters

Groups	Sham (n= 7)	No drug + ACA (n = 9)	Ethanol + ACA (0.005%a) (n = 3)	SAME + ACA (0.02 mg/kg) (n = 6)	PAME + ACA (0.02 mg/kg) (n = 7)
Body weight (g)	298.1 ± 16.2	317.8 ± 15.0	315.0 ± 10.1	315.6 ± 10.1	305.2 ± 10.5
pH	7.46 ± 0.01	7.45 ± 0.01	7.46 ± 0.04	7.49 ± 0.02	7.47 ± 0.01
P_{CO_2} (mm Hg)	37.2 ± 1.0	35.8 ± 1.2	36.3 ± 1.5	36.5 ± 2.2	34.6 ± 1.1
P_{O_2} (mm Hg)	144.1 ± 7.4	129.6 ± 8.3	128.9 ± 10.6	131.3 ± 5.1	141.9 ± 11.5
MABP (mm Hg)	118.6 ± 3.2	112.1 ± 6.5	113.7 ± 1.2	109.0 ± 6.5	104.0 ± 1.5
Glucose (mg/dL)	142 ± 13	150 ± 20	137 ± 19	131.0 ± 10.2	117 ± 17.9

Table 1. Physiological parameters such as body weight, pH, PCO_2, PO_2, MABP, and blood glucose were measured to ensure consistency of the animals within and between groups. Values are mean ± S.E.M. ACA, asphyxial cardiac arrest; MABP, mean arterial blood pressure.

Table 2. Middle cerebral artery occlusion physiological parameters

Groups	DMSO (0.01 %a) (n = 7)	SAME (0.02 mg/kg) (n = 6)	PAME (0.02 mg/kg) (n = 6)
Body weight (g)	300.1 ± 8.3	300.8 ± 5.1	306.2 ± 2.8
pH	7.40 ± 0.02	7.43 ± 0.01	7.43 ± 0.02
P_{CO_2} (mm Hg)	37.1 ± 1.3	38.4 ± 1.48	40.5 ± 0.69
P_{O_2} (mm Hg)	122.9 ± 5.8	137.4 ± 4.4	120.4 ± 5.8
MABP (mm Hg)	123.6 ± 7.7	139.0 ± 5.1	142.0 ± 1.2
Glucose (mg/dL)	111 ± 7	112 ± 2	132 ± 19

Table 2. Physiological parameters such as body weight, pH, PCO_2, PO_2, MABP, and blood glucose were measured to ensure consistency of the animals within and between groups. Values are mean ± S.E.M. MCAO, middle cerebral artery occlusion; MABP, mean arterial blood pressure.

Rats pretreated with PAME (0.02 mg/kg) and SAME (0.02 mg/kg) provide neuroprotection in the CA1 region of the rat hippocampus 7 days after ACA.

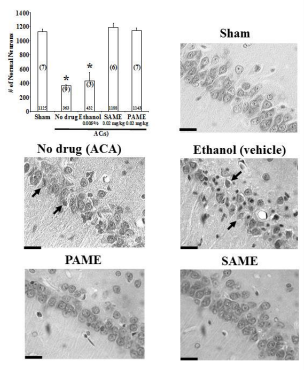
Rats were pretreated with PAME and SAME (IV bolus 30 mins before ACA).

Sham (no ischemia), no drug (ACA only), and ethanol (vehicle control) groups were performed as internal controls.

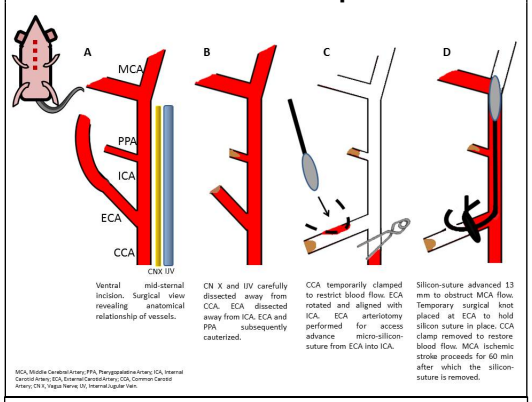
Neurons from the CA1 region of the hippocampus were counted and expressed in the bar graph shown here. Numerical values in the bar graph represent the number of normal neurons counted. Numbers in parentheses indicate the number of animals used per group.

Representative images of the CA1 region of the hippocampus are also shown here. Arrows represent typical neuronal cell death in the CA1 region of the hippocampus.

Short horizontal solid bars represent 30 µm in length in the field of view of each representative image ($n = 3$-9, * $p \leq 0.05$).



MCAO Technique



Post-treatment with PAME or SAME after 2 hours of MCAO decreases total rat brain infarct volume.

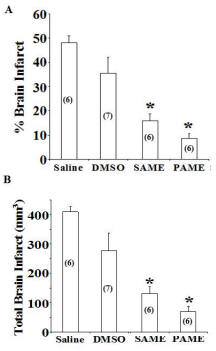
Representative images are shown corresponding to their relative drug treatment (n = 6-7, * $p \leq 0.05$).

TTC-treated brain slices were scanned and analyzed (Image J software) at the ipsilateral hemisphere.

Post-treatment with PAME or SAME after 2 hours of MCAO decreases total rat brain infarct volume.

Post-treatment with PAME (0.02 mg/kg, IV) or SAME (0.02 mg/kg, IV) decreased ipsilateral hemisphere volume as compared to dimethyl-sulfoxide [DMSO; partial vehicle for PAME or SAME].

The percent of brain infarct volume is depicted in white and tabulated in panel (**A**) and the total brain infarct volume (expressed in mm^3) is presented in (**B**).



Results

PAME (1143+/-39.3 normal neurons) or SAME (1188+/-57.8 normal neurons) pretreatment 30mins before ACA (6min) conferred neuroprotection in the CA1 region of the hippocampus 7 days after ACA as compared to no drug treatment (ACA only) or vehicle (0.005% ethanol). Additionally, SAME (15.97+/2.76%) and PAME (8.63+/-2.03%)-treated groups conferred reduction in percentage of brain infarct volume after MCAO as compared to controls.

Conclusions

PAME and SAME provide robust neuroprotection in both paradigms of ischemia. With proper timing and dosage, administration of PAME/SAME may one day prove to be an effective therapy against cerebral ischemia for at-risk patients.

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