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MRI of combination treatment of embolic stroke in rat with rtPA and atorvastatin

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Abstract

To test the hypothesis that combination treatment of embolic stroke with rtPA and statins improves the efficacy of thrombolytic therapy in rats. Rats subjected to embolic MCA occlusion (MCAo) were randomized into control ($n=10$) and treatment ($n=9$) groups. Four hours after MCAo, a combination of rtPA and atorvastatin (treatment) or saline (control) was administered. MRI measurements were performed on all animals at 2 h, 24 h and 48 h after MCAo. The patency of cerebral microvessels was examined using fluorescent microscopy. MRI images showed complete blockage of the right MCA and a reduction of CBF in the territory supplied by the MCA 2 h after MCAo for all animals. By 48 h after stroke, MRI showed that the decreased lesion size, elevated CBF and increased incidence of recanalization were found in treated rats compared with the control rats. The combination treatment significantly increased microvascular patency (16.3 ± 5.5% vs. 12.4 ± 3.5%, of field-of-view) and reduced the infarct volume (23.1 ± 9.6% vs. 38.8 ± 13.3%, of hemisphere). These data demonstrate that the co-administration of rtPA and atorvastatin 4 h after ischemia is efficacious and is reflected by the MRI indices of recanalization of the MCA, reduction of secondary microvascular perfusion deficits and reduction of the ischemic lesion.

Keywords: Embolic stroke; Middle cerebral artery occlusion (MCAo); Thrombolysis; Recombinant tissue plasminogen activator (rtPA); Atorvastatin; Magnetic resonance imaging (MRI)

1. Introduction

Acute thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) is a successful treatment for ischemic stroke when administered within 3 h of stroke onset [1]. However, treatment with rtPA is given to only approximately 5% of stroke patients, primarily because of the narrow 3-h therapeutic window [2]. Monotherapy with rtPA or atorvastatin at 4 h after stroke onset fails to produce therapeutic benefit, such as reduction of the cerebral infarction volume [3,4], or increases the risk of developing hemorrhage [3–6]. Therefore, it would be desirable to improve the efficacy and safety, as well as to increase the therapeutic window for thrombolytic therapy of stroke.

Statins, or 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, reduce the incidence of stroke and have beneficial microvascular effects beyond their primary targeted purpose of reducing serum cholesterol [7]. Statins reduce hemostasis via reducing platelet activation and increasing fibrinolysis. Statins regulate key steps of the coagulation and fibrinolytic cascades, including tissue factor (TF), tissue plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1) and thrombin [8]. Furthermore, by inhibiting matrix metalloproteinase-9 (MMP9) activity [9] and possessing anti-inflammatory effects [10], statins promote cerebral microvascular integrity. Statins have a broad safety profile and are widely employed in humans

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[11]. Thus, statins are potential agents for co-treatment of stroke with rtPA.

Atorvastatin (Lipitor®, Pfizer) is a second-generation inhibitor of HMG-CoA reductase, clinically employed for lowering plasma cholesterol [12]. In the present study, using magnetic resonance imaging (MRI), we investigated the effects of combination treatment of atorvastatin and rtPA including the measurements of apparent diffusion coefficient of water (ADC_w), cerebral blood flow (CBF), magnetic resonance angiography (MRA), relaxation time constants of T_1 and T_2, as well as fluorescence microscopy and histology.

2. Materials and methods

All studies were performed in accordance with institutional guidelines for animal research under a protocol approved by the IACUC of Henry Ford Hospital.

2.1. Animal model and experimental groups

Male Wistar rats ($N=19$, 300 to 400 g) subjected to embolic stroke [13] were randomized into two groups, one group ($n=9$) receiving combination treatment of rtPA and atorvastatin, and the other receiving saline ($n=10$) at 4 h after embolic middle cerebral artery (MCA) occlusion (MCAo). The method of embolic stroke induction has been previously described [13]. Briefly, an aged white clot, which was prepared 24 h before ischemia, was slowly injected into the internal carotid artery to block the MCA. This model of embolic stroke provides a reproducible infarct volume localized to the territory supplied by the MCA [13]. Treatment of embolic stroke with rtPA or atorvastatin alone as a monotherapy at 4 h after MCAo provides no therapeutic benefit [4]. Therefore, the combination of rtPA and atorvastatin treatment was compared to a saline control population. rtPA (Genentech, San Francisco, CA) was injected intravenously at a total dose of 10 mg/kg with 10% bolus at 4 h after ischemia and the remainder at a continuous infusion over a 30-min interval using a syringe infusion pump (Harvard Apparatus, South Natick, MA). Atorvastatin was given subcutaneously at a dose of 20 mg/kg at 4 h after embolic MCAo and was followed by a second dose of 20 mg/kg 24 h after the first dose. The dose of rtPA received by rats in this experiment is 10 times higher than that used in clinic, because the sensitivity of the rat to rtPA is 10 times lower than in the human. We selected a dose of 20 mg/kg for atorvastatin treatment. Although this dose is much higher than the dose usually used in clinic (80 mg/day), our previous study has demonstrated that this dose is effective in extending the therapeutic window of rtPA [4]. The rats in the control group received saline 4 h after embolic MCAo. All animals were sacrificed 48 h after MCA occlusion after the final MRI measurements.

2.2. Magnetic resonance imaging

MRI measurements were performed using a 7-T, 20-cm bore superconducting magnet (Magnex Scientific, Abingdon, U.K.) interfaced to a Bruker console (Bruker Company, Billerica, USA), with a 12-cm bore actively shielded gradient coil set capable of producing a magnetic field gradient up to 200 mT/m. A birdcage radio-frequency (RF) coil was used as the transmitter and a surface coil as the receiver. Stereotaxic ear bars were utilized to minimize movement during the imaging procedure. During MRI measurements, the anesthesia was maintained using a gas mixture of N_2O (69%), O_2 (30%) and halothane (0.75–1.00%). Rectal temperature was kept at 37 °C ± 1.0 °C using a feedback controlled water bath. The right femoral artery and vein were cannulated with a PE-50 catheter for monitoring of blood pressure and gases (pH, $[PO_2]$, $[PCO_2]$), and drug administration, respectively.

A tri-pilot scan imaging sequence was used for reproducible positioning of the animal in the magnet at each MRI session. A complete set of MR images were obtained prior to ischemia, repeated after the onset of embolization and at 24 h, as well as 48 h after embolization for all animals. The interval between the surgical induction of embolic stroke and the initiation of MRI scans was approximately 1 h. A complete set of all sequences, including DWI, T1WI, Look-Locker (L-L) T_1 measurements, multiecho T_2 measurement, PWI and MRA, required approximately 2 h of scan time. MRI acute data were collected immediately after embolization of rats and designated as “02 h” data set because the median time of this data set is approximately 2 h after MCAo. The follow-up MRI scans at 24 and 48 h after MCAo were, accordingly, designated as “24 h” and “48 h” data sets, respectively.

Measurement of MRA employed a 3-D gradient echo imaging sequence with calculated gradient trims [14]. This sequence comprises the first-order flow compensation achieved via gradient-moment nulling in the frequency-encoding, phase-encoding and slice directions. Thus, the effect of the flow compensation is maximized whenever imaging materials possess linear flow characteristics. The acquisition matrix was set as $256 \times 192 \times 64$ for fitting the field-of-view (FOV) $32 \times 32 \times 16$ mm^3 in Coronal-Head-Foot orientation. Repetition time (TR) and echo time (TE) were 50 ms and 4.5 ms, respectively. The 500-ms Gaussian RF pulse generated a flip angle of approximately 40°.

The arterial spin tagging (AST) technique [15] was used for quantifying blood flow in cerebral tissue. The adiabatic inversion of arterial water protons [16] was accomplished via an axial gradient of ±0.3 kHz/mm and a continuous wave (CW) RF power of approximately 0.3 kHz at a frequency offset of ±6 kHz. A hyperbolic secant shaped pulse of 1-s duration was employed as an adiabatic fast passage RF pulse, followed by a spin echo imaging sequence with TR/TE = 1000 ms/20 ms. The labeled slice was 2 cm distal from the imaging slice with 1 mm thickness.