Combination of Vepoloxamer and tPA extends the therapeutic window of stroke

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INTRODUCTION

The utilization of tissue plasminogen activator (tPA) for stroke treatment is hampered by its narrow therapeutic window, hemorrhagic complications, and marginal efficacy. Therefore, there is a compelling need to develop therapies for acute stroke which rapidly reestablish cerebral blood flow (CBF) in the ischemic cerebral microvascular bed, preserve vascular integrity, and minimize brain hemorrhage and parenchymal cell death, thus leading to restoration of the normal function of the neurovascular unit. Vepoloxamer is a amphipathic polymer that has shown potent hemorheologic, cytoprotective, and anti-inflammatory effects in both experimental models and clinical studies. It is currently under investigation in a global phase III clinical trial for patients with sickle cell disease and a phase II study in patients with acute limb ischemia. The current study evaluated the neuroprotective effect of the combination of with delayed tPA treatment in a rat model of embolic stroke.

MATERIALS AND METHODS

Experimental protocols:

Male Wistar rats (350-400g) were subjected to embolic middle cerebral artery occlusion (MCAO). After confirming successful stroke with a 5 point neurological scale (Longa scale) at 30 min after MCAO, ischemic rats were randomly divided into the following groups:

1.Vepoloxamer alone (Mast Therapeutic, n=10) at a dose of 300 mg/kg was administered (IV) 3.5h after MCAO, followed by a second dose of 2,000 mg/kg at 9.5h (IP).

2.tPA alone (n=10) at a dose of 10mg/kg was given (IV) 4h after MCAO.

3.combination of vepoloxamer with tPA (n=10).

4.saline (n=10).

Neurological functional outcome:

Neurological deficits were measured with adhesive removal test and modified Neurological severity scores (mNSS) at 1 and 7 d after stroke onset.

Histopathologic studies:

Animals were sacrificed 7d after stroke and brain coronal sections stained with hematoxylin and eosin were used for evaluation of ischemic lesion and gross hemorrhage.

Immunohistochemistry:

To examine microvascular fibrin deposition, immunostaining was performed with antibodies against fibrin/fibrinogen and endothelial barrier antigen (EBA).

RESULTS

Vepoloxamer alone and in combination with tPA improve neurological outcome

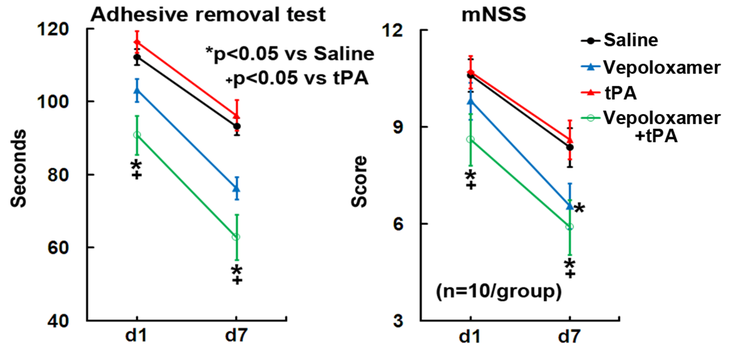


Fig. 1. Neurological outcome. Treatments with Vepoloxamer alone and in combination with tPA reduce neurological functional deficits compared with rats treated with saline and tPA monotherapy.

Vepoloxamer alone and in combination with tPA reduce lesion volume

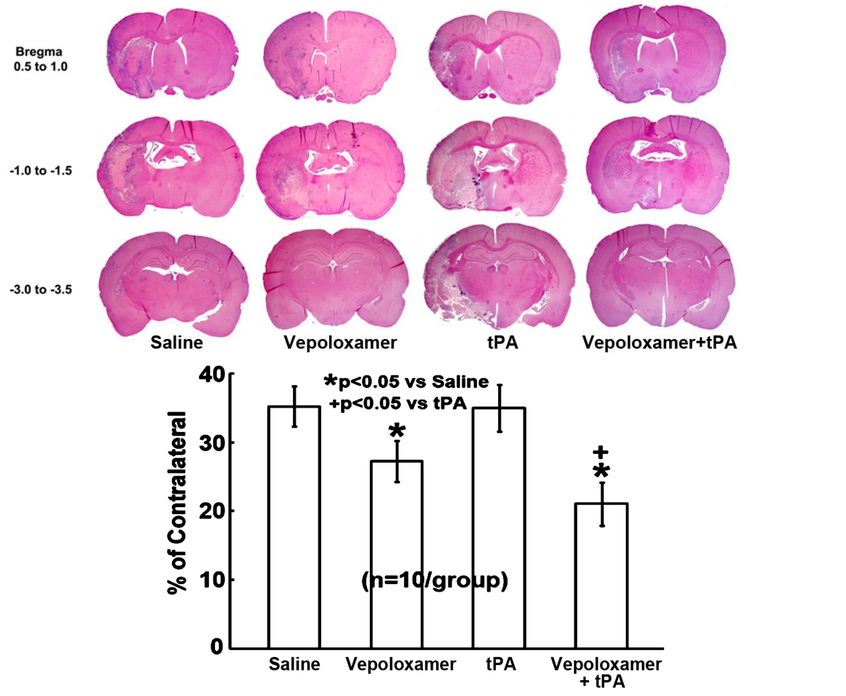
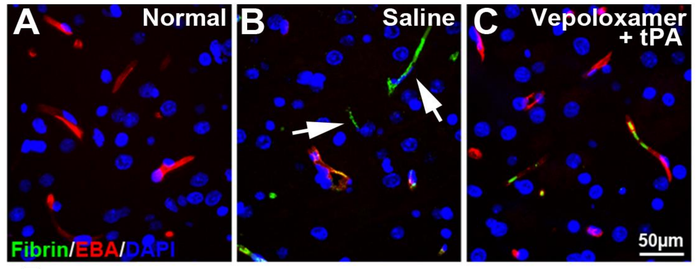


Fig. 2. Lesion volume. Panels are H&E stained coronal sections obtained from representative rats treated with saline, Vepoloxamer alone, tPA alone, and the combination of Vepoloxamer and tPA. Bar graph shows that treatments with Vepoloxamer alone and in combination with tPA significantly reduced lesion volume compared to ischemic rats treated with saline and tPA monotherapy.

Combination treatment with Vepoloxamer and tPA does not increase hemorrhage

Gross hemorrhage: Vepoloxamer monotherapy and the combination treatment with Vepoloxamer and tPA did not increase the incidence of gross hemorrhage (10% in the Vepoloxamer alone group, 20% in the combination group) compared to monotherapy with tPA (30%) and saline (10%). No significant differences were detected among the groups.

Combination treatment with Vepoloxamer and tPA reduces intravascular deposition



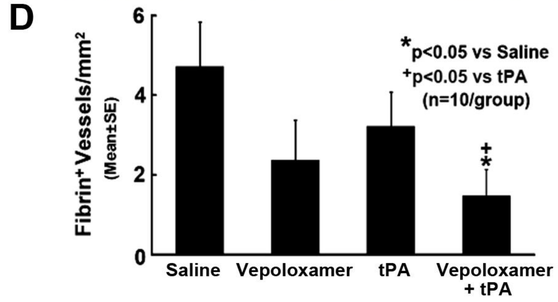


Fig. 3. Microvascular fibrin deposition. Double immunofluorescent staining (**A-C**) shows fibrin (green) and EBA+ blood vessels (red) from a representative normal rat (A) and ischemic rats treated with saline (**B**) and the combination of Vepoloxamer and tPA (**C**). Blood vessels with fibrin deposition lost EBA immunoreactivity (B, arrows), suggesting intravascular fibrin deposition induces vascular disruption. Quantitative analysis (D) shows the combination treatment with Vepoloxamer and tPA significantly reduces fibrin deposition compared with rats treated with saline and tPA monotherapy.

CONCLUSIONS

We demonstrated that treatment of acute stroke with Vepoloxamer significantly reduced infarct volume and neurological functional deficits. Vepoloxamer enhances the thrombolytic effect of tPA without increasing the incidence of hemorrhagic transformation when tPA is administered 4h after embolic stroke.

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