Clinical Neurology and Neurosurgery 131 (2015) 11–17

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Safety of thrombolytic therapy with rt-PA and transcranial color Doppler ultrasound (TCCS) combined with microbubbles: A histopathologic study on rabbit brain tissues

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ARTICLE INFO

Article history:
Received 25 December 2014
Accepted 13 January 2015
Available online 21 January 2015

Keywords:
Transcranial color Doppler ultrasound
rt-PA
Thrombolytic therapy
Brain damage
Pathology

ABSTRACT

Objective: To evaluate effect of thrombolytic therapy with rt-PA (recombinant tissue plasminogen activator) and transcranial color Doppler ultrasound (TCCS) combined with microbubbles on histology of brain tissue.

Methods: New Zealand rabbits were subjected to TCCS based thrombolytic therapy, in 8 groups depending on dose of rt-PA, exposure duration of TCCS and presence of attenuation by skull bone window, 2 animals/group: (1) skull + 1/2 rt-PA + TCCS + MBs, 10 min, (2) skull + rt-PA + TCCS + MBs, 10 min, (3) skull + 1/2 rt-PA + TCCS + MBs, 20 min, (4) skull + rt-PA + TCCS + MBs, 20 min, (5) skull + 1/2 rt-PA + TCCS + MBs, 30 min, (6) skull + rt-PA + TCCS + MBs, 30 min, (7) 1/2 rt-PA + TCCS + MBs, 10 min, (8) 1/2 rt-PA + TCCS + MBs, 20 min. The brain tissues were harvested after therapies and submitted for microscopic, electronic microscope and immunohistochemical examination. The histological changes were scored.

Results: TCCS caused exposure duration dependent brain tissue damage. With attenuation by bone window, TCCS based therapies for 10–20 min caused minimal tissue damage. However, significant tissue damage was observed upon TCCS for 30 min in presence of skull bone window, presenting as hemorrhage, misdistribution of organelles, demyelination of nerve fibers, and thinning of basement membrane in blood–brain barrier, which was milder than that after 20 min of exposure to TCCS in absence of bone window. Dose of rt-PA did not affect brain histology in all groups.

Conclusion: Short treatment of brain tissue with TCCS through a bone window is relative safe. And skull bone window protected brain tissue from TCCS induced damage.

1. Introduction

Stroke is a leading cause of death and disability worldwide. Most strokes are due to blockage of an artery in the brain by a blood clot. Intravenous administration of recombinant tissue plasminogen activator (rt-PA), by inducing arterial recanalization, is the fastest and standard treatment to restore blood flow and improve recovery after stroke after acute ischemic stroke [1,2]. Yet several lines of clinical data have also shown that exposure to 2-MHz diagnostic transcranial ultrasound, for example transcranial color Doppler ultrasound (TCCS), can deliver mechanical pressure waves to the thrombus and expose more thrombus surface to circulating drug, thus augment arterial recanalization, which results in improved thrombolytic benefits in the setting of acute ischemic stroke [3–7]. In addition, researchers have demonstrated that combing microbubbles (MB) and rt-PA yields enhanced thrombolytic effects [8–11].

It is widely accepted that ultrasound at different frequencies and intensity can cause tissue damage to certain extent, and the safety of application of ultrasound in the brain is controversial. Currently, studies on ultrasound caused tissue injury focus on aspects of therapeutic ultrasound or TCD and the observations include pathological changes in tissue, thermal effects and impact of ultrasound on the blood–brain barrier caused [12–20]. In our previous

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http://dx.doi.org/10.1016/j.clineuro.2015.01.011
0303-8467/

studied, we tested efficacy of simultaneously delivering of rt-PA, TCCS and MB, with the latter two undergoing attenuation generated by adult skull, and we found that this combination had promising thrombolytic activity for thrombi in vivo and in vitro. To ensure the safety of the therapeutic modality in clinical setting, herein we further investigated pathological changes in rabbit brain tissues receiving extended exposure to ultrasound. In the present study, we simulated adult skull bone window by covering the frontal and parietal bone-removed area in the rabbit head with adult human skull piece. And safety of the combinatory therapy was evaluated with histopathologic studies on thrombolyrtic treatments on the rabbits with microbubble, varied exposure time to TCCS, different doses of rt-PA, in presence or absence of the simulated bone window.

2. Materials and methods

2.1. Animals and experimental groups

Sixteen healthy male adult New Zealand rabbits were purchased from Department of Laboratory Animal Science, Fudan University School of Medicine, with an average weight of 2.5 ± 0.25 kg.

The animals were randomly divided into 8 groups based on three conditions, i.e. duration of TCCS (10 min, 20 min, 30 min), with (referred to as skull) or without simulated bone window, and rt-PA dosage (1/2 of rt-PA full dose, or full dose): (1) skull + 1/2 rt-PA + TCCS + MBs, 10 min, (2) skull + rt-PA + TCCS + MBs, 10 min, (3) skull + 1/2 rt-PA + TCCS + MBs, 20 min, (4) skull + rt-PA + TCCS + MBs, 20 min, (5) skull + 1/2 rt-PA + TCCS + MBs, 30 min, (6) skull + rt-PA + TCCS + MBs, 30 min, (7) 1/2 rt-PA + TCCS + MBs, 10 min, and (8) 1/2 rt-PA + TCCS + MBs, 20 min.

3. Making simulated bone window and animal manipulation

The rabbits were anesthetized with 10% chloral hydrate by intraperitoneal injection. To make a simulated bone window, the head hairs was removed with 8% sodium sulfide and a 3 cm incision was made on the top center of the forehead skin. A hand drill was used for craniotomy to open the skull to the dura via a drilling point at 1 cm to ear base of the midpoint of the line between the eyes. The brain tissue was fully exposed after symmetrical removal of surrounding skull with double joint rongeur, while the dura was kept intact. A piece of scale part of human adult temporal bone was

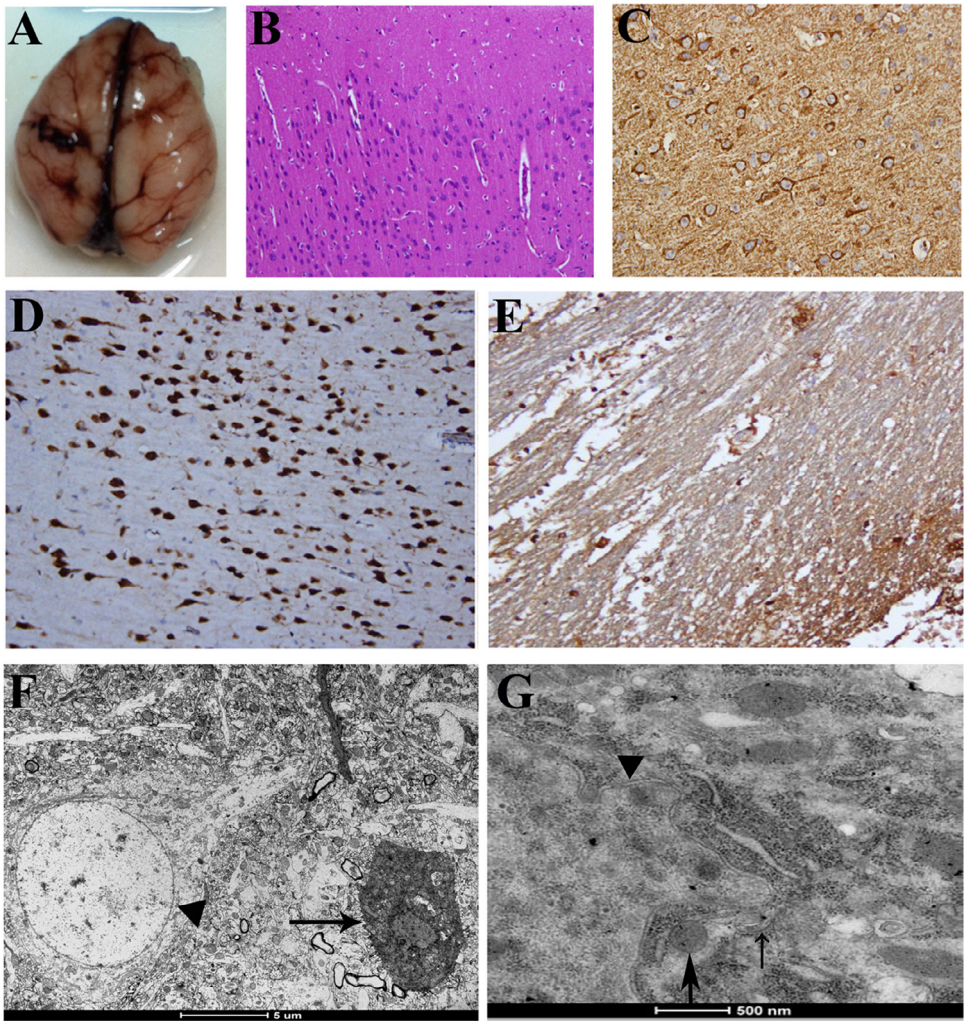

Fig. 1. Minimal brain damage after 10 min of exposure of the animals to TCCS through the skull bone window (groups 1 and 2). Grossly, the meningeal surface was intact, with no hemorrhage or brain edema (A). Normal white matter and gray matter could be visualized with orderly arranged neurons in normal morphology (B, 200×, HE staining), normal nerve fibers (C, 400×, NF immunohistochemical staining), preserved cell polarity (D, 200×, NeuN immunohistochemical staining), and normal myelin integrity (E, 400×, MBP immunohistochemical staining). Normal dark cells (→) and clear cells (▲) were present (F, bar = 5 μm). The normal dark neuronal cells were with normal double nuclear membrane (▲), and normal organelles such as mitochondria (bold ↑), rough endoplasmic reticulum (thin ↑) (G, bar = 500 nm).