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Laboratory studies

Study of cerebral aneurysms in a modified rat model: From real-time imaging to histological analysis

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ABSTRACT

Cerebral aneurysm (CA) is a life-threatening condition with a pathogenesis that remains unclear. Previous hypotheses have primarily been based on *in vitro* examinations of animal models. Therefore, we attempted to observe CA in living rats and to establish a multi-level evaluation system. The rat model was produced by deoxycorticosterone-acetate (DOCA; Sigma Aldrich, St. Louis, MO, USA) induced hypertension and a single injection of elastase into the basal cistern. The animals were assessed 35 days later. At the endpoint, we induced well-developed CA in 41.7% of the surviving rats. Using synchrotron radiation angiography (SRA), we observed the experimental aneurysms and their surrounding arteries dynamically in the living model. Further anatomical and histological analyses demonstrated the typical degenerative changes of the mural structure and a major infiltration of macrophages into the aneurysmal wall. In conclusion, we visualised well-developed experimental CA in living rats using SRA and demonstrated the associated degenerative histological changes and macrophage involvement; thus, we have provided an effective model for the study of dynamic multi-level changes associated with CA in a rat model.

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1. Introduction

Cerebral aneurysm (CA) is a life-threatening vascular condition, but its pathogenetic mechanisms remain unclear. Mechanistic studies mainly rely on effective animal models that are similar to human CA at the micro and macro-levels.

The current animal CA models for mechanistic study primarily consist of the classical hemodynamic CA model originally developed by Hashimoto [1–3] and a recently introduced model that is induced by hypertension and the injection of elastase into the cistern [4,5]. The conclusions using these models have mainly been drawn from *in vitro* pathological and molecular biological observations [3,6,7]. Few *in vivo* morphological studies using the commonly employed murine models are available. Our colleagues once attempted to visualize CA in rats with a novel imaging system that utilises synchrotron radiation and has extremely high spatial resolution [8]. However, the effectiveness of inducing well-developed aneurysms was low, and this study lacked corresponding histopathological evidence.

In the present study, which is based on a more efficient rat model and synchrotron radiation angiography (SRA), we attempted to establish a multi-level study model in which real-time imaging and sequential pathological examinations are used to comprehensively analyse the CA.

2. Materials and methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the School of Biomedical Engineering, Shanghai Jiao Tong University, China. Twenty-seven 8-week-old male Sprague–Dawley rats (Sippr-BK, Shanghai, China) were enrolled in this study. Twenty-two were experimental rats, and five were controls.

All the experimental rats underwent a 35 day treatment process that is illustrated in Figure 1A. The animals were anesthetised with ketamine (100 mg/kg) and xylazine (10 mg/kg) via intraperitoneal injection during all surgical procedures. On Day 1, the animals underwent unilateral nephrectomy. Seven days later, we began to administer bolus injections of deoxycorticosterone-acetate (DOCA; Sigma Aldrich, St. Louis, MO, USA, 40 mg/kg) subcutaneously once a week and to feed the rats with hypertonic saline (1% NaCl and 0.2% KCl) continuously. On Day 20, a single stereotaxic injection of elastase (MP Biomedicals, Santa Ana, CA, USA, 50 mg/ml, diluted to 17.5 units/ml by phosphate buffered saline into the right basal cistern was performed. A burr hole was drilled

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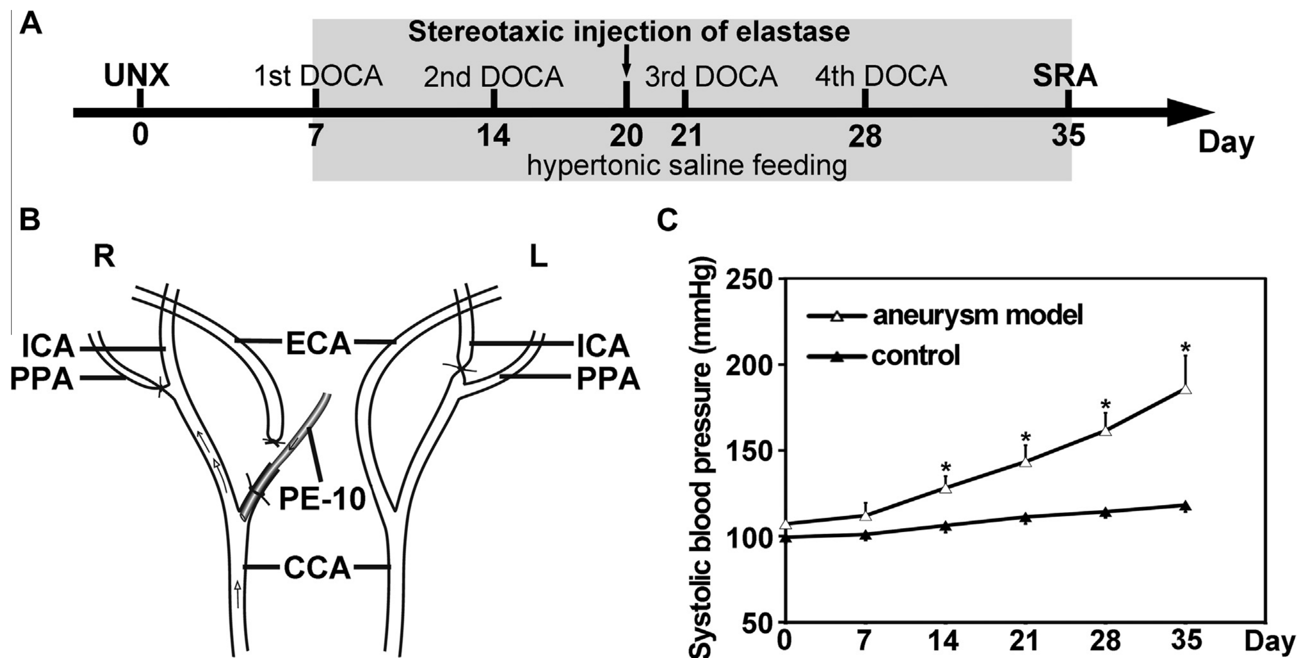

Fig. 1. (A) Flowchart of the 35 day procedure. (B) Schematic diagram of surgical preparation for synchrotron radiation angiography. The solid arrows represent the contrast agent, and the hollow arrows represent physiological blood flow. (C) The systolic blood pressures of the model and control rats. $^*p < 0.01$. CCA = common carotid artery, DOCA = deoxycorticosterone-acetate (Sigma Aldrich, St. Louis, MO, USA), ECA = external carotid artery, ICA = internal carotid artery, PE-10 = polyethylene-10 tube, PPA = pterygopalatine artery, SRA = synchrotron radiation angiography, UNX = unilateral nephrectomy.

6.3 mm posterior to the bregma and 1.8–2.0 mm to the right of the midline. A 10 μl Hamilton syringe (Hamilton, Shanghai, China) was slowly inserted into the brain through the hole and advanced 1 cm ventral to the dorsal skull surface. Elastase solution (8 μl) was continuously injected into the cistern for 10 minutes using a stereotactic frame (WPI, Sarasota, FL, USA). The control rats were fed normally and raised without any treatment. We measured the systolic blood pressure (BP) of all the rats on Day 0 and once per week thereafter with an automatic sphygmomanometer (Softron, Tokyo, Japan) using the tail cuff technique.

On Day 35, SRA was performed on all of the living rats at Beamline 13W in the Shanghai Synchrotron Radiation Facility. After anesthesia, a midline incision was made on the neck. A polyethylene-10 tube was cannulated in a retrograde fashion into the right external carotid artery through a sidewall puncture until its end reached the bifurcation of the right common carotid artery. The ipsilateral pterygopalatine artery and contralateral internal carotid artery were ligated (Fig. 1B). The rat was then placed perpendicular to the X-ray beam on its left side. A contrast agent (350 mg/ml, Omnipaque, GE, Shanghai, China) was injected at a rate of 8 ml/minute for a total volume of 180 μl. These procedures have been described in detail previously [8]. Subtraction between the images taken before and after the injection was performed with Matlab software (Mathworks, Natick, MA, USA). The images of each layer were stitched together using Photoshop (Adobe Systems Inc. San Jose, CA, USA).

After SRA, the rats were deeply anesthetised and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde and at last with 2 ml bromophenol blue saline solution. The whole brain was then removed. The Circle of Willis and its major branches were assessed by two independent investigators in a blinded manner. Pictures were taken under a stereomicroscope (Leica, Wetzlar, Germany).

The aneurysmal vessel along with the neighbouring brain tissue were harvested and cut into a small cube. Serial cross-sections (5 μm in thickness) were obtained and stained with hematoxylin and eosin for structural examination and localization and then stained with Weigert–Van Gieson stain for examination of connective tissue. Pictures were taken under a microscope (Leica).

The localized aneurysmal vessel sections were then subjected to immunohistochemical staining. The primary antibody for marking macrophages was mouse polyclonal anti-rat CD68 (1:300, AbD Serotec, Martinsried, Germany). After blocking, the sections were first incubated with primary antibody, then with the appropriate biotinylated secondary antibodies (VECTASTAIN ABC Kit, Vector Laboratories, Burlingame, CA, USA) and a complex of avidin-biotin-horseradish peroxidase (Vector Laboratories). Immunoreactivity was visualized by incubating the sections with 0.05% 3,3′-diaminobenzidine (DAB, Vector Laboratories). The nuclei were counterstained with hematoxylin.

The data are presented as mean ± standard deviation. Statistical analyses were performed with Student's unpaired t-tests. Statistical significance was set at $p < 0.05$.

3. Results

Based on the previous literature [4], we defined an aneurysm as a localized outward bulging of a vascular wall that was observed by both SRA and microscopic observation, with a diameter greater than 1.5 times that of the parent vessel.

Twelve rats in the model group and all of the control rats survived to the endpoint. Three rats died shortly after the injection of elastase, three died from later subarachnoid hemorrhage (confirmed by autopsy), and four died due to unknown reasons. Thus, the procedure-related death rate was 31.8% (7/22). At the endpoint, the average systolic BP of the model group was 185 ± 19 mmHg, which was significantly higher than that of the control group (118 ± 4 mmHg, $p < 0.01$; Fig. 1C). After 7 weeks, five rats in the model group developed intracranial aneurysms. The incidence of aneurysm was 41.7% among the surviving rats. No aneurysms occurred in the cerebral vessels of the control rats.

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