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Laboratory studies

A small animal model for early cerebral aneurysm pathology

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ABSTRACT

Prior studies, using systemic hypertension and elastase infusion, have induced cerebral aneurysm (CA) formation in mice. However, the CAs induced were rapidly formed, relatively large, and often ruptured. These features are not completely representative of human CAs. We set out to develop a mouse model representative of the early pathological features of human CA. Twenty male C57/BL6 mice were placed in a stereotactic frame. Low dose elastase solution (2 μl/min) was manually injected into the right basal cistern. Human angiotensin II (0.11 μl/h) was infused subcutaneously. Mice were observed for 2–3 weeks prior to euthanasia. Early CA histopathological features including endothelial change (EC) and internal elastic lamina degeneration (IELD) were systematically sought at major cerebral arterial bifurcations. Brains were harvested from 11 of 15 mice, yielding 27 bifurcations. Sub-arachnoid haemorrhage (SAH) without CA formation was observed in one brain. Macroscopic CA without SAH was observed in another brain. Early CA features were observed in 8/11 (73%) brains. All bifurcations with IELD demonstrated EC: where EC was absent, IELD was also absent. EC severity appeared to correlate with IELD severity. EC and IELD were both severe within the CA. Using lower dose elastase solution than previously employed, we developed a model of early CA pathology. Our model demonstrated that the spectrum of known early CA pathology can be created at multiple bifurcations in mice, with EC severity appearing to correlate with IELD severity. This model permits the study of factors which potentially advance or retard the progression of CA formation.

1. Introduction

Aneurysmal subarachnoid haemorrhage (SAH) is a neurosurgical condition with mortality approaching 50% [1–6]. In most cases, however, human cerebral aneurysms (CAs) remain occult prior to rupture. Thus, whilst CAs are prevalent within 2–5% of the population, the incidence of SAH is as low as 8 in 100,000 patients per year [7]. Most studies investigating human CA pathophysiology have obtained specimens from either autopsy or surgery. However, such specimens have become rare due to increased neuroendovascular treatment, and to more stringent Human Research Ethics Committees' consenting requirements. Moreover, such specimens have been obtained at a single, and necessarily advanced, moment in time. This necessarily limits any delineation of the sequence of early aneurysm pathology which eventually culminates in macroscopic CA formation. In consequence, there is currently a pressing need for the development of a simple, and representative animal model of early CA pathology.

Relatively few animal models of CAs are currently available [7–12]. Such models typically exaggerate risk factors for human CA formation (i.e. increased vessel wall stress [8] and endothelial damage [9,10]). In agreement with Glyn [13], 'medial defects' are not considered a *conditio sine qua non* of CA formation in animal models. Whilst hypertension may, in principle, be induced by a number of methods (e.g. renovascular hypertension via unilateral nephrectomy) recent studies in mice have incorporated angiotensin II (A-II).

Endothelial damage is currently achieved in animal models by infusing elastase intra-arterially, or directly into the basal cisterns. Such use of elastase is considered to more accurately replicate the features of early CA pathology [14,15]. Early CA pathological features potentially include endothelial changes (EC) (i.e. endothelial cell loss, disruption or detachment; sub-intimal thickening/apical pad elevation; and inflammation and/or destruction/protrusion of vessel wall) [12] and internal elastic degeneration (IELD) [16]. IELD

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ultimately culminates in IEL rupture ('Reuterwall's tears'). The IEL is frequently absent in human CAs altogether [16]. Prior studies have not systematically and simultaneously studied both EC and IELD in cerebral artery bifurcations.

Notably, the CAs produced by recent models have been rapidly formed (2–6 weeks), relatively large (even giant) compared to their parent vessel, and have often ruptured [7–12,14,17–22]. This questions their representativeness to human CAs, most of which are small-medium in size, and which appear to develop over a more protracted period [1–6]. We therefore sought to develop a mouse model representative of early CA pathology. We used lower doses of elastase than previous investigations to ameliorate vessel wall injury, in an attempt to more closely mimic the presumed slower time-course of human CA development. Early CA pathological changes were systematically sought, including both EC and IELD.

2. Methods

Approval for animal study was obtained from the local ethics committee (Approval No. A1926). Experimental work was performed in accordance with the institutional and ethical guidelines of James Cook University, but also conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, USA). Mice were housed in an individually ventilated, tempera ture/humidity-controlled cage system (Aero IVC Green Line; Tecniplast) on a 12-h light/dark cycle, and maintained on normal laboratory chow and water *ad libitum*. 20 male C57/BL6 mice (Animal Resources Centre, Canning Vale, WA, Australia) aged 12–14 weeks, of mean weight 25.5 ± 0.4 g, were used. Under isoflurane general anaesthesia (GA) (2% isoflurane in 2 L/min oxygen) and secured in a stereotactic frame (Model 940 Linear Scale Digital Display, David Kopf instruments, Tujunga, CA, USA). A small right sided burr-hole was made 1.2 mm rostral and 0.7 mm lateral to the bregma (co-ordinates were obtained from the Mouse Brain Atlas and had been validated by a previously described model [17]). A 10 μl Hamilton blunt tipped microliter syringe (Model 701N, Hamilton Instruments, Nevada, LV, USA) was adjoined to a semi-automated repeating dispenser (Model PB600-1, Hamilton Instruments, Nevada, LV, USA) and advanced until contact with the skull base was achieved. It was then withdrawn 0.3 mm and either methylene blue dye (control group) or 1.0 u/ml elastase solution (experimental group) was manually infused into the basal cisterns (see below). The incision was then closed with sutures.

To induce chronic hypertension, A-II, (Sigma Aldrich, Castle Hill, NSW, Australia), dissolved in phosphate buffered saline, was continuously infused via an implanted Alzet micro-osmotic pump (model 1004, Durect Corporation, Cupertino, CA, USA) placed subcutaneously at a point 5 mm cephalad to the base of the tail immediately after closure of the cranial wound. The A-II dose was determined by use of a weight-based algorithm delivering 1000 ng/kg/min [14,21,23]. Two study groups were employed.

2.1. Sham operative controls

A dilute solution of methylene blue dye was infused into the right basal cistern of five mice at a rate of 2 μl/min using the stereotactic method described above.

2.2. Experimental group

Ten microliter of elastase solution (1.0 μ/ml) was injected manually into the right basal cistern of 15 mice, at a rate of 2 μl/min, using the stereotactic method described above.

Following the procedure, animals were recovered under a heat lamp, housed individually, and observed for a period of 2–3 weeks using a previously validated rodent neurological scoring system [13,24]. Blood pressure (BP) was measured by standard tail cuff manometry at three time points: Baseline (24 h pre-intervention); interim (8–10 days, post-procedure); and final at 24 h prior to euthanasia. Multiple individual systolic (sBP), diastolic (dBP) and mean (mBP) were recorded at each of the three stages, and an average value for each obtained. At the completion of the observation period, mice were placed under deep isoflurane anaesthesia (flow rate 4 L/min) until agonal breathing was observed. Each mouse was then euthanized using CO_2.

2.3. Histological assessment

After removal of the brain from the vault, and prior to fixation, macroscopic CA formation (saccular CA visible either to the naked eye, or under × 3.5 magnification), and any associated SAH, were sought by direct vision. Each brain was then fixed in 10% neutral buffered formalin, inked to maintain orientation, and subsequently sliced and processed using a Leica Peloris tissue processor. Following processing, brain slices were blocked in paraffin wax and cooled on an ice plate. Blocked paraffin brains were sectioned at eight microns on a Leica RM2235 Manual Rotary Microtome. Sections were floated out on a water bath at 35°C, collected on silanized-activated slides, and dried overnight. Total processing time was 87 hours. Each stage, excluding the wax stages, was performed at 35°C. All tissue was kept under a vacuum during processing.

Sections were stained with haematoxylin and eosin (H&E), elastic van Giesen (EVG) and silver stains. All sections containing cerebral arterial bifurcation points were identified and analysed by an experienced histopathologist (LK), blinded to the outcome of the index animal. Early CA pathological changes sought were defined as EC, IELD and any associated arterial wall inflammation/thickening. EC was defined after the classification proposed by Jamous et al. [12] (Table 1). IELD was defined after the classification proposed by Aoki et al. [16] (Table 2).

2.4. Statistical analysis

Inter-group comparisons were performed using analysis of variance. Statistical significance was assessed at $P < 0.05$.

3. Results

Mean operative time (i.e. from induction to completion of GA) for sham controls was 31.40 ± 3.98 min. Mean total procedural time (i.e. from induction of GA to observable recovery of spontaneous movements) was 45.40 ± 4.93 min. Mean total procedural time for the experimental cohort was 60.36 ± 7.58 min (mean operative time: 36.90 ± 3.0 min, mean recovery time: 14.30 ± 2.20 min).

Table 1
Histological categorisation of the vessel-wall endothelial change (EC) associated with early cerebral aneurysm (CA) formation. Modified after Jamous et al. [12]

EC–1		Endothelial cell loss or disruption
EC–2	A	EC–1 with endothelial cell detachment and/or sub-intimal thickening/apical pad elevation
	B	EC–2A with inflammation and destruction/protrusion of vessel wall
EC–3		Macroscopic saccular aneurysm formation