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Role of neuroinflammation in hypertension-induced brain amyloid pathology

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Abstract

Hypertension and sporadic Alzheimer's disease (AD) have been associated but clear pathophysiological links have not yet been demonstrated. Hypertension and AD share inflammation as a pathophysiological trait. Thus, we explored if modulating neuroinflammation could influence hypertension-induced β-amyloid (Aβ) deposition.

Possible interactions among hypertension, inflammation and Aβ-deposition were studied in hypertensive mice with transverse aortic coarctation (TAC). Given that brain Aβ deposits are detectable as early as 4 weeks after TAC, brain pathology was analyzed in 3-week TAC mice, before Aβ deposition, and at a later time (8-week TAC mice).

Microglial activation and interleukin (IL)-1β upregulation were already found in 3-week TAC mice. At a later time, along with evident Aβ deposition, microglia was still activated. Finally, immune system stimulation (LPS) or inhibition (ibuprofen), strategies described to positively or negatively modulate neuroinflammation, differently affected Aβ deposition.

We demonstrate that hypertension per se triggers neuroinflammation before Aβ deposition. The finding that only immune system activation, but not its inhibition, strongly reduced amyloid burden suggests that stimulating inflammation in the appropriate time window may represent a promising strategy to limit vascular-triggered AD-pathology.

Keywords: Alzheimer's disease; Cerebral hemodynamics; Glial cells; Hypertension; Inflammation

1. Introduction

Several epidemiological studies show a clear association between late-life dementia/sporadic Alzheimer's disease (AD) and a wide range of vascular risk factors (de La Torre, 2003; Fotuhi et al., 2009; Skoog and Gustafson, 2006; Zlokovic, 2008). Among them, hypertension seems to play a prominent role. In addition to its well-known peripheral outcomes, hypertension exerts detrimental effects on the brain (Hainsworth and Markus, 2008; Iadecola and Davisson, 2008), and recent experimental studies using antihypertensive therapies further support the association between hypertension and cerebral amyloid pathology (Tsukuda et al., 2009; Wang et al., 2007).

Recent evidence suggests that inflammation may be the bridge connecting hypertension and target organ damage (Li and Chen, 2005). Inflammation in the brain parenchyma can occur as a local process that can be triggered and sustained by activated glial cells, in the absence of immune cells

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recruited from the periphery, and it is thought to contribute to pathogenesis of several diseases (Minghetti, 2005; Wyss-Coray, 2006).

Hemodynamic changes due to hypertension can be expected to activate neurovascular unit signaling, eventually leading to activation of microglia, the brain resident macrophages, and to neuroinflammation. Thus, hypertension could influence onset/progression of late-life dementia/sporadic AD by promoting neuroinflammatory processes.

To investigate the possible link among hypertension, inflammation and amyloid pathology, we focused on a particular model of hypertension, obtained by transverse aortic coarctation (TAC), in which pressure overload impact is selectively driven to the cerebral circulation. In previous studies, we demonstrated a significant Aβ deposition in hippocampus and cortex of TAC mice, as early as 4 weeks after the induction of hypertension (Gentile et al., 2009). In the present study we aimed at exploring the role of neuroinflammation in the hypertension-driven amyloid-related pathology by monitoring the process of microglial activation and the expression of typical pro-inflammatory and anti-inflammatory/immunoregulatory genes, at an early time preceding Aβ deposition (3 weeks after TAC), and at a later time (8 weeks after TAC), characterized by overt Aβ deposition. Finally, to clarify the role of neuroinflammation in hypertension-induced amyloid pathology, one of the main traits of the disease, we explored the effects of two strategies aimed at modulating inflammatory responses: the first one stimulating immune system with a peripheral LPS challenge (Perry et al., 2007; Yong and Rivest, 2009); the second one inhibiting it through a chronic, long-term treatment with the nonsteroidal anti-inflammatory drug (NSAIDs) ibuprofen.

2. Methods

2.1. Animals and surgery

All experiments were conducted in conformity with European Communities Council Directive. C57Bl/6J Male mice 12–15 week old were used and kept under 12-hour light-dark cycle at 22–25 °C. Standard chow and water were provided ad libitum. TAC-induced hypertension was obtained as previously described (Brancaccio et al., 2003). Mice anesthetized with ketamine/xylazine were placed on thermal beds and respiration was assisted with a volume-cycled ventilator (Basile, Milan, Italy) connected to an 18-gauge cannula inserted into the trachea. Transverse aortic coarctation was performed between truncus anonymous and left carotid, with a 7.0 nylon suture ligature placed around the aorta. Sham mice underwent the same surgical procedures without realizing aortic stenosis.

A systolic trans-stenotic gradient was measured by echo Doppler (VeVo 770, Visualsonics, Inc., Canada), positioning the probe on the aortic banding and only animals with a gradient ranging from 70 to 90 mmHg were selected for further analyses.

2.2. Cerebral blood perfusion

Mice anesthetized as above were placed in prone position, ECG monitored and body temperature was maintained at 37 °C with a heating pad. A T-shaped skin incision was performed for skull exposition. An acoustic window for imaging was obtained by bilateral parietal craniotomy starting from the bregma (4 mm along the interparietal suture; 2.5 mm laterally towards the temporal bone).

A microbubbles suspension of 60 μL (MicroMarker non-targeted Contrast Agent, Visualsonics, Inc., Canada) was injected with a catheter in jugular vein. Imaging was acquired through the acoustic window by a 30 MHz probe equipped ultrasound device (VeVo 770, Visualsonics, Inc., Canada). A measure of cerebral blood perfusion was obtained by plotting the Contrast Region time versus Intensity and fitting the resulting curve by a Visualsonics dedicated software. Relative cerebral blood velocity was the slope of the curve.

2.3. Dissection of brain areas for RNA and metabolites extraction

After decapitation, brain areas were dissected, frozen on dry ice and stored at −80 °C. TRIzol reagent (Invitrogen, Eugene, OR) was used for RNA extraction according to manufacturer's instructions. A detailed procedure for 15-F_{2t}-IsopP extraction has been described elsewhere (Minghetti et al., 2000).

2.4. Reverse transcription and quantitative PCR

RNA was transcribed into cDNA using the Superscript III kit (Invitrogen, Eugene, OR) according to manufacturer's instructions. Real-time PCR was performed with Sybr green PCR master mix, following manufacturer's instructions, using an ABI Prism 7,500 Sequence Detection System (Applied Biosystems, Inc, Foster City, CA). Primer sequences are indicated in Table 1. Samples were run in triplicate and gene expression levels were determined using the Relative Quantification (−ΔΔCt) Study of Applied Biosystems 7500 System SDS Software.

2.5. 15-F_{2t}-IsoP measurement

15-F_{2t}-IsoP levels were measured in duplicate for each tissue extract by a high sensitivity EIA kit (Cayman Chemical, Ann Arbor, MI). Results are expressed as pg/mg of total proteins (measured by the BCA Protein Assay kit; Pierce, Rockford, IL) in pellets obtained after the extraction procedure.

2.6. Immunohistochemistry, histology and image analysis

After an overdose of sodium penthobarbital mice were transcardially perfused with saline followed by formaldehyde. Brains were removed, postfixed overnight and sectioned (30 μm) on a cryostat. Primary antibodies used were rabbit anti-Iba-1 (1:1000, Wako Chemicals, GmbH, Neuss, Germany); rat