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Adjuvant-induced mono-arthritis potentiates cerebral hemorrhage in the spontaneously hypertensive rats

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ABSTRACT

Aims: Patients with rheumatoid arthritis (RA), have a higher incidence of hypertension and stroke than the normal population. Currently there exists no animal model to study the pathogenic interactions of hemorrhagic stroke (HS) subsequent to chronic inflammation and hypertension. We have created and defined a hypertensive-mono-arthritic animal model who demonstrate gros signs of cerebral hemorrhage in presence of mono-arthritis.

Main methods: Spontaneously hypertensive rats (SHR) were fed either a high salt diet (4% NaCl; HSD) or Purina chow (RD) from weaning. Complete Freund's adjuvant (CFA) was injected into the left hind paw at 21–28 weeks (control groups received saline (SAL)). Degree of inflammation, joint swelling, weight and blood pressure were monitored for 21 days. Animals were then sacrificed and their brain and left hind paw evaluated.

Key findings: All groups were hypertensive throughout the experimental period (>180 mm Hg systolic), irrespective of diet. Both CFA groups produced significant local inflammatory response in their injected paw with associated joint degradation and cellular infiltrates. Systemic plasma TNF-α levels were significantly elevated in CFA groups, with significant increase in TNF-α at 7 and 14 days, compared to SAL groups. Cerebral hemorrhage was visualized in the CFA groups but not SAL controls, with a higher severity in HSD-CFA group.

Significance: The mono-arthritic hypertensive animals are capable of developing HS upon induction of inflammatory insult. The HSD appears to exacerbate the inflammatory response and influence degree of the hemorrhage. Our novel, multi-disease model may provide an appropriate platform to study the pathogenesis of HS among arthritic patients.

1. Introduction

Arthritis is a long term, physically debilitating disease that is the second most common chronic condition in Canada, with an annual cost of $33 billion in related health care costs [1]. Rheumatoid arthritis (RA) is one of the more severe forms of arthritis. As a chronically progressive inflammatory disease, it can attack one or more joints, leading to joint degradation and loss of mobility. In addition to the destruction of joints and cartilage, the pathology of arthritic disease takes a progressive toll on many other tissues in the body as it produces diffuse inflammation in the lungs, pleura, pericardium, and sclera. The leading cause of death in RA patients is not due to the arthritis itself however, but due to cardiovascular (CV) complications and has an associated high mortality rate compared to the general population. [2,3].

The risk of ischemic heart disease and myocardial infarction has been extensively studied in RA, but more recent and fundamental studies indicate significant risk of stroke in autoimmune arthritis, with patients with RA having a 30% increase in stroke over age-matched controls [4,5]. Of all stroke subtypes, hemorrhagic stroke (HS) has the highest mortality rate, approaching 50% within the first month [6,7]. The risk of death from the first incidence of stroke has also been shown to be significantly higher for RA patients compared to non-arthritic subjects [2,8,9]. Traditional risk factors of cardiovascular disease (CVD), (hypertension, smoking, dyslipidemia, and insulin resistance) have been deemed more prevalent in the RA population [10]. There is also an increase the risk of myocardial infarction, CV morbidity, and CV mortality in the RA patients [11]. Literature suggests that approximately two-thirds of patients with primary cerebral hemorrhage have pre-existing or newly diagnosed hypertension [6,12]. The presence of hypertension is in itself a key risk factor for HS and may therefore be a key component in the likelihood of RA patients developing fatal HS.

A number of animal models have been extensively used for decades to study pathogenesis akin to RA and evaluate anti-arthritic agents, the most widely used being the adjuvant-induced arthritis (AIA) rat model using Lewis or Sprague Dawley strain rats [13–15]. Similarly, the stroke-prone spontaneously hypertensive rat (SHRsp) and Dahl salt sensitive rat is widely used to study HS where the onset of hemorrhage formation and mortality is accelerated with a specialized Japanese style high salt diet (at 4% NaCl) [16–18] However, there is currently no model that

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exemplifies the development of HS subsequent to chronic systemic inflammation induced by mono-arthritis and longstanding hypertension. No one has shown gros signs of hemorrhage (akin to the SHRsp models for example) in the brain with induction of mono-arthritis.

Our objective was to fill this knowledge gap by creating a hypertensive-arthritic animal model with the induction of adjuvant mono-arthritis in the stroke-resistant spontaneously hypertensive rat (SHR), a strain unique in that it normally develops spontaneous hypertension but does not spontaneously develop stroke. Also, there has been a recent surge in looking at the interaction of salt with autoimmunity, with the Th axis (activated in autoimmune disease) being activated by sodium chloride [19–23]. Therefore we also addressed the impact of high salt diet (HSD; 4% NaCl) on the severity of systemic inflammation and HS development in the model. We chose 4% NaCl to maintain the same diet as the positive control of HS as seen in the SHRsp model. We hypothesize that our animal model is capable of developing HS upon induction of mono-arthritis due to increasing systemic inflammation, and that severity of systemic inflammation, and consequent degree of hemorrhage, would be higher in hypertensive-arthritic animals on HSD. This novel, multi-disease model combines hypertension with systemic inflammation to convert a stroke resistant model into a stroke prone animal, reminiscent of the observed predisposition to HS among RA patients.

2. Materials and methods

2.1. Animals

All experimental procedures and animal breeding was carried out at Memorial University of Newfoundland Animal Care Facility and were in compliance with guidelines and recommendations set forth by the Animal Care ethics committee and the Canadian Council on Animal Care (Guide to Care and Use of Experimental Animals, vol.1, 2nd Ed.). In total, 53 male stroke resistant Spontaneously Hypertensive Rats (SHR; Original stock from Charles River Laboratories, Quebec, Canada) were included in the study. The animals were bred in-house and were housed two per cage in ventilated cages under standard light cycle (12 h light/dark), controlled temperature, and humidity conditions. Experimental design was implemented at 20–28 weeks of age. Ad libitum access to food and water was permitted.

2.2. Experimental design

Rats were divided into four experimental groups based on diet and treatment, and followed the experimental timeline outlined in Fig. 1. Briefly, SHR-high salt diet (HSD) groups were fed a Japanese-style high salt diet containing 4% NaCl (Zeigler Bros, Gardners, PA, USA) from weaning. SHR-regular diet (RD) groups were maintained on standard rat chow (Laboratory Rodent Diet 500I, Lab Diet, St. Louis, MO, USA; 0.58% NaCl). At 20–28 weeks of age, they were randomly divided into 4 groups based on treatment (Complete Freund's Adjuvant (CFA) model of mono-arthritis or saline (SAL) injected control) and diet (high salt, or standard rat chow), and labelled HSD-SAL ($n = 10$), HSD-CFA ($n = 14$); RD-SAL ($n = 11$), RD-CFA ($n = 10$).

2.3. Preparation of Complete Freund's Adjuvant

A suspension of *Mycobacterium butyricum* (10 mg/mL) in incomplete Freund's adjuvant (Sigma, USA; IFA) was prepared and used according to modified methods for induction of AIA, as commercial sources of CFA with *M. butyricum* were unsuitable for a robust arthritis induction. As well, studies have determined potency of CFA would increase if the *Mycobacterium* is ground and mixed into IFA for AIA induction [14,24,25]. AIA is used over Collagen Induced Arthritis (CIA; the more prevalent RA model) due to the availability of data on AIA induction in the SHR model for comparison purposes, the more rapid onset of the inflammatory response, the type of joint degradation and inflammatory response that it is capable of inducing [14,15,26,27]. It must be noted that the classical AIA use *Mycobacterium tuberculosis* rather than *M. butyricum* in the emulsion for CFA. However, a comparison of rats receiving CFA containing *M. tuberculosis* or *M. butyricum* indicated a higher induction of arthritis severity with CFA containing *M. butyricum* regardless of strain and sex, and is likely due to factors associated with both MHC and non-MHC (background) genes [24]. In order to insure success of disease induction, Heat-killed *M. butyricum* H37RA (Sigma, USA) were ground into smaller particles until fine, using an autoclaved, marble mortar and pestle in a sterile fume hood. IFA was added gradually and grinding continued until thoroughly mixed to make CFA.

2.4. Induction of adjuvant induced mono-arthritis (AIA)

Experimental arthritis was induced by intradermal injection of CFA (0.07 mL of 700 μg *M. butyricum*) into the plantar surface of the left

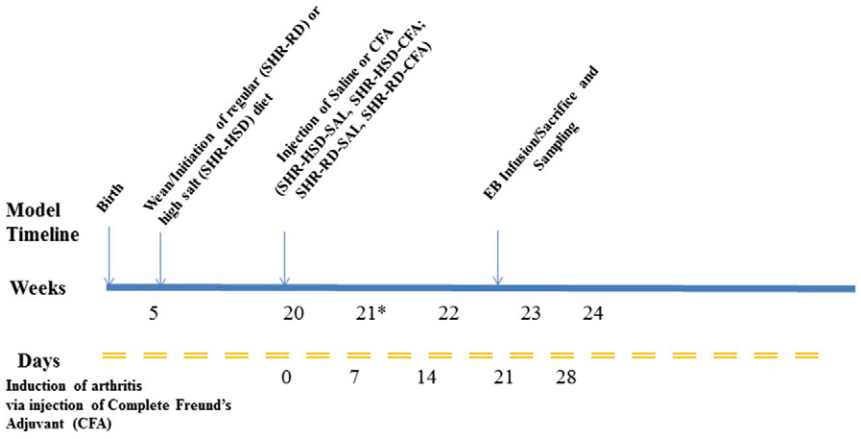

Fig. 1. Animal experimental timeline. Four experimental groups were followed over the course of 21–23 days, SHR-HSD-SAL, SHR-HSD-CFA; SHR-RD-SAL, SHR-RD-CFA. SHR-HSD groups were weaned and started on Japanese style high salt diet (4% NaCl) while SHR-RD groups were weaned and started on regular Purina (0.58% NaCl equivalent) diet at 5 weeks of age. At 21–28 weeks of age (experimental day 0), all groups received an intradermal injection in the left hind paw. Inflamed groups (CFA) received 0.07 mLs of CFA for the induction of adjuvant arthritis (AA) while control groups (SAL) received the same quantity of sterile saline (0.9%) solution. All groups were monitored for signs of inflammation and hypertension. * Note that although the figure chart notes the start of the experiment to be 21 weeks of age, the start age ranged between 21 and 28 weeks of age (5–7 months) as outlined in Fig. 1 description.