Vascular Pharmacology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Vascular Pharmacology

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

# Endothelium-derived contraction in a model of rheumatoid arthritis is mediated via angiotensin II type 1 receptors

Kayleigh Hamilton[a], Lynette Dunning[a], William R. Ferrell[b], John C. Lockhart[a], Andrew MacKenzie[a,*]

[a] Centre of Musculoskeletal Science, Institute for Biomedical and Environmental Health Research, School of Science & Sport, University of the West of Scotland, Paisley, UK
[b] Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow, UK

ARTICLE INFO

*Keywords:*
Endothelium-derived constricting factor
Angiotensin II type 1 receptor
Rheumatoid arthritis
Losartan
Inflammation

ABSTRACT

A role for endothelium-derived constricting factors (EDCF), and the angiotensin II type 1 receptor (AT1R) pathway, in the vascular impairment found in the rat Freund's complete adjuvant (FCA)-model of rheumatoid arthritis (RA) was examined. FCA arthritis was induced in rats ± losartan. Vehicle-treated rats served as controls. Knee-joint swelling and red blood cell (RBC) aggregation were measured as indicators of inflammation and endothelium reactivity assessed by response to acetylcholine (ACh) on aortic rings. Results show that knee-joint swelling and RBC aggregation were elevated in the FCA + vehicle group and restored to control levels in the FCA + losartan-treated animals. ACh-induced relaxation of aortic rings taken from FCA + vehicle animals was significantly impaired compared to vehicle-controls and this vasoreactivity was restored to control levels in the FCA + losartan-treated group. Further examination of aorta from the FCA + vehicle animals revealed an EDCF that was reliant on cyclooxygenase-2 (but not cyclooxygenase-1), generation of superoxide anion generation (but not hydrogen peroxide) and activation of thromboxane-prostanoid receptor. Losartan administration in vivo or ex vivo (to aortic rings) prevented the generation of the EDCF. In summary, this is the first evidence of an EDCF in a model of RA and identifies this mechanism as potentially significant in the cardiovascular disorder associated with the disease.

## 1. Introduction

Rheumatoid arthritis (RA) is a chronic, progressive and disabling disease characterized by both articular and extra-articular indicators, including a high cardiovascular mortality beyond that found in the general population [1,2]. Indeed vascular incident is around 4-times more common in people with RA meaning the condition is comparable to type 2 diabetes as an independent risk factor for the development of atherosclerosis and cardiovascular disease [3–5]. The causes of RA and atherosclerosis are not fully understood and likely to be multifactorial in nature but both diseases are recognized to have a strong inflammatory component with suggestion that inflammatory pathways may be common to both disorders [6–8]. Of these, the renin-angiotensin system, and angiotensin II (Ang II) in particular, has received recent attention [9]. While most often recognized for its vasoconstrictor action, Ang II is now understood to be a significant mediator of inflammation through stimulation of the Ang II-type 1 receptor (AT1R) [10,11]. Ang II and AT1R stimulation have been reported to be pro-inflammatory through mechanisms including the generation of reactive oxygen species (ROS); activation of nuclear factor kappa B; promotion of cytokines like tumor necrosis factor and interleukin-6; elevation of adhesion molecules and stimulation of leukocyte proliferation and migration [12–15].

Within the context of RA, we have demonstrated that blockade of the AT1R with the antagonist losartan reduces knee joint swelling (a cardinal sign of RA) by more than half in a Freund's complete adjuvant (FCA)-model of arthritis in rats [16,17]. Moreover losartan has been reported to ameliorate pain and edema found in both mouse and rat models of RA [18]. Further support for a role of AT1R in the physical manifestation of arthritis was shown through demonstration that another AT1R receptor blocker (olmesartan) reduced the arthritis score and joint destruction found in a collagen-induced model of RA in mice [19]. Within human RA, AT1R expression is elevated in chondrocytes [20] and both we and others have reported raised synovial expression

*Abbreviations:* ACh, acetylcholine; Ang II, angiotensin II; AT1R, angiotensin II type 1 receptor; COX, cyclooxygenase; EC, endothelial cell; EDCF, endothelium-derived constricting factors; FCA, Freund's complete adjuvant; KJD, knee joint diameter; NO, nitric oxide; ROS, reactive oxygen species; RBC, red blood cell; RA, rheumatoid arthritis; SOD, superoxide dismutase; TP, thromboxane-prostanoid; VSMC, vascular smooth muscle cells

* Corresponding author.
*E-mail address:* andrew.mackenzie@uws.ac.uk (A. MacKenzie).

https://doi.org/10.1016/j.vph.2017.11.001
Received 7 September 2017; Received in revised form 2 November 2017; Accepted 4 November 2017

of the receptor [16,21]. Moreover, both the finding that plasma renin activity is elevated in normotensive patients with RA [22], and that inhibition of angiotensin-converting enzyme improved vascular reactivity in patients [23], suggest that renin-angiotensin system involvement in RA is systemic and not localized to inflamed joints.

Vascular endothelial cell (EC) dysfunction represents the earliest stage of atherosclerosis and is central to the pathogenesis found in a variety of cardiovascular disorders [24–26]. EC dysfunction is also a hallmark of human RA, as well as numerous animal models of the disorder [27,28], and is thought to be fundamental to the elevated cardiovascular risk of the disease [8,29,30]. At a functional level, impaired EC activity is typically defined by a reduction in the ability of the endothelium to mediate the relaxation of neighboring vascular smooth muscle cells (VSMC). Impaired EC in RA has classically been characterized by reduced nitric oxide (NO) bioavailability; however, our group was the first to demonstrate impaired EC activity mediated by the reduced influence of endothelium-derived hyperpolarizing factor [17]. In that study, conducted in isolated saphenous arteries of the FCA rat model, we demonstrated that the impaired endothelium-derived hyperpolarizing factor activity and EC function was restored by prophylactic treatment with losartan. As such we highlighted that that disordered endothelium in a model of RA involves mechanisms beyond that of impaired NO activity and also provided further support for the role of AT1R activation in EC dysfunction.

Importantly, impaired EC activity is not just defined by a reduction in endothelium-derived relaxing factors but also but also by elevation in endothelium-derived contracting factors (EDCF). Such EDCF-mediated contractions are augmented in arteries from both human and animal models of aging, hypertension, type 2 diabetes mellitus and atherosclerosis [31–34] and are considered to be central to the vascular pathophysiology and subsequent cardiovascular events associated with these disorders. EDCF is not mediated by a universal cellular pathway but rather several mechanisms are reported to contribute the contractile response and the nature of the EDCF can vary in different disorders. Frequently reported pathways underlying EDCF include that of generation of ROS (such as superoxide anion and hydrogen peroxide); enhanced cyclooxygenase (COX) activity and stimulation of thromboxane-prostanoid (TP) receptors on VSMC [35].

In this study we provide the first evidence of an EDCF in a model of RA and demonstrate that the impaired EC response is altered in an AT1R-senstive manner. Furthermore, we show AT1R blockade also provides protection against non-vascular inflammatory parameters induced by the model. As such we highlight that renin-angiotensin system and AT1R activation is likely to be key to the development of the clinical manifestations of RA and is identified as a means of therapeutic intervention to reduce, slow or even prevent the consequences of the disease.

## 2. Materials and methods

### 2.1. Chemicals and solution

All drugs and reagents were purchased from Sigma-Aldrich (Poole, Dorset, UK) with the exception of L655 (L665,240; selective TP receptor antagonist, Tocris Bioscience, Abingdon, UK). Drugs were prepared in distilled water and frozen in stock solutions with the exception indomethacin (10 mM stock) which was dissolved in $Na_2CO_3$ (0.4 mg $ml^{-1}$). Control studies demonstrated that the concentration of solvents was without influence on vascular reactivity. Serial dilutions were made in isotonic saline.

### 2.2. Animals

A total of 43 adult male Sprague Dawley rats (bred in-house, weighing 380–420 g) were used in the experiments described here. Animals were housed in a centralized animal facility in standard cages, with food and water available ad libitum. The rats were maintained in a thermoneutral environment, with a 12-hour light/dark cycle. All procedures were performed in accordance with UK Home Office regulations described in compliance with the ARRIVE guidelines for reporting of experiments involving animals [36,37].

### 2.3. Experimental groups and induction of inflammation

FCA-mediated inflammation, as a model of chronic monoarthritis, was induced by intra-articular (200 µl × 2) injection of FCA, supplemented with 10 mg $ml^{-1}$ of heat-killed *Mycobacterium tuberculosis*, into one (the left) knee, with the animal under general anesthesia ($O_2$/$N_2O$/2% halothane), as described previously [17,38].

In one group, losartan (15 mg $kg^{-1}$), an AT1R antagonist, was dissolved in saline and administered subcutaneously 1 h prior to FCA induction and maintenance doses administered every 48 h thereafter. This regime has been shown to substantially reduce knee joint swelling in rats with adjuvant arthritis [16,17]. Control animals (i.e. receiving neither FCA nor losartan) were administered with a saline vehicle.

Rats were euthanized with carbon dioxide and dislocation of the neck 21 days following the induction of FCA inflammation ± losartan or vehicle control.

### 2.4. Assessment of inflammation

Joint swelling, a key characteristic of inflammation, was assessed by measurement of knee joint diameter (KJD, using modified Vernier calipers) at set time points over the 21 days and expressed as the percentage of the pre-induction diameter. Repeated measurements of normal knees on 5 successive days are reproducible with a coefficient of variation of ≤ 2%.

RBC aggregation in whole blood is associated with inflammatory conditions [39] and is used in the clinical evaluation of RA [40]. Immediately following cull, whole blood (~2 ml) was collected via cardiac puncture and placed in a heparin-containing tube. The sample was then analyzed photometrically at room temperature using a Myrenne MA1 cone-plate aggregometer (Myrenne, Roetgen, Germany). After a period of high shear rate disaggregation, the aggregation index was determined from the change in light intensity over a 5 s period at a zero shear rate.

### 2.5. Vessels for the study

The thoracic aorta was carefully removed from the animal, placed in ice-cold Krebs' solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11 gassed with 95% $O_2$/5% $CO_2$, pH 7.4; cleaned of adhering connective tissue and sliced in to 2 mm length ring preparations. In some rings the EC were removed mechanically by gentle abrasion of the lumen with the tip of small forceps. The rings were mounted for isometric tension recording in 10 ml tissue baths (maintained at 37 °C and gassed with 95% $O_2$/5% $CO_2$), force was measured by TBM4 bridge amplifiers (World Precision Instruments) and data collected via Data-Trax2 acquisition system. The aortic preparations were stretched to a 1 g resting tension and allowed to equilibrate for 60 min adjusting the tension as necessary. Following equilibration, tissues were exposed twice to KCl (80 mM).

#### 2.5.1. Characterization of vascular responses

Following sub-maximal (~70% maximal) pre-contraction of the vessels with phenylephrine, responses to acetylcholine (ACh, 1 nM–30 µM) were measured. In some experiments the role of NO in endothelium-dependent relaxation was examined using the NO synthase inhibitor $N_\omega$-Nitro-L-arginine methyl ester hydrochloride (L-NAME; 100 µM).