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Role of cyclooxygenase in the vascular response to locally delivered acetylcholine in Caucasian and African descent individuals

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ABSTRACT

Introduction: Individuals of African descent (AFD) are more susceptible to non-freezing cold injury (NFCI) compared with Caucasian individuals (CAU). Vasodilatation to acetylcholine (ACh) is lower in AFD compared with CAU in the non-glabrous foot and finger skin sites; the reason for this is unknown. Prostanoids are responsible, in part, for the vasodilator response to ACh, however it is not known whether the contribution differs between ethnicities.
Methods: 12 CAU and 12 AFD males received iontophoresis of ACh (1 w/v%) on non-glabrous foot and finger skin sites following placebo and then aspirin (600 mg, single blinded). Aspirin was utilised to inhibit prostanoid production by inhibiting the cyclooxygenase (COX) enzyme. Laser Doppler flowmetry was utilised to measure changes in skin blood flow.
Results: Not all participants could receive iontophoresis charge due to high skin resistance; these participants were therefore excluded from the analyses.
Foot: ACh elicited greater maximal vasodilatation in CAU than AFD following placebo ($P = 0.003$) and COX inhibition (COXib) ($P < 0.001$). COXib did not affect blood flow responses in AFD, but caused a reduction in the area under the curve for CAU ($P = 0.031$).
Finger: ACh elicited a greater maximal vasodilatation in CAU than AFD following placebo ($P = 0.013$) and COXib ($P = 0.001$). COXib tended to reduce the area under the curve in AFD ($P = 0.053$), but did not affect CAU.
Conclusions: CAU have a greater endothelial reactivity than AFD in both foot and finger skin sites irrespective of COXib. It is concluded that the lower ACh-induced vasodilatation in AFD is not due to a compromised COX pathway.

1. Introduction

Non-freezing cold injury (NFCI) is a preventable clinical injury that affects the peripheral skin sites (particularly fingers and toes) of individuals who experience prolonged exposure to local cold tissue temperatures (0 °C to 20 °C) (Ungley and Blackwood, 1942). Symptoms of this injury may last for many years and often include pain, numbness and hyperhidrosis which, combined with cold hypersensitivity of the injured limb, can lead to increased susceptibility to further cold injuries (Golden et al., 2013; Ungley et al., 1945). This type of injury is a concern for those involved in outdoor work (e.g. agriculture or forestry work, military) or recreational activities (e.g. skiing, mountaineering) that take place in cold conditions which may also elicit freezing cold injuries (Hashmi et al., 1998; Mäkinen et al., 2009; Morrison et al., 2015).

Individuals of black African descent (AFD) are more susceptible than Caucasian (CAU) individuals to NFCI (Burgess and Macfarlane, 2009; DeGroot et al., 2003). The reason for this is not known but it is thought that sustained skin blood flow in the extremities in low environmental temperatures can prevent local cold injuries (Daanen and van der Struijs, 2005; Lewis, 1941; Wilson and Goldman, 1970). During hand immersion in cold water (8 °C) for 30 min and subsequent rewarming of dry skin in 30 °C air, AFD experienced greater finger vasoconstriction and slower rewarming compared with CAU (Maley et al., 2014) indicating AFD received a greater "dose of cold". We investigated whether this was due to alterations in the control of the microcirculation of the extremities and demonstrated that endothelial-dependent (ACh), but not -independent (SNP), vasodilatation was significantly attenuated in AFD compared with CAU in non-glabrous finger and toe skin sites (Maley et al., 2015).

Local application of acetylcholine (ACh) increases prostanoid and nitric oxide production eliciting vasodilatation (Holowatz et al., 2005;

Abbreviations: ACh, acetylcholine; AFD, African descent; AUC, area under the curve; CAU, Caucasian; COXib, cyclooxygenase inhibition; ED50, half-maximal effective dose; IQR, interquartile range; LDU, laser Doppler units; MAP, mean arterial pressure; Mdn, median; NFCI, non-freezing cold injury; SD, standard deviation; TXA_2, thromboxane A_2.

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Kellogg et al., 2005). Prostanoids are produced from arachidonic acid, released from the cell membrane, metabolised by the enzyme cyclooxygenase (COX) (Vane et al., 1998) to produce prostaglandin H_2 which is further metabolised by various synthase enzymes to produce various prostanoids (Félétou, 2011; Hamberg et al., 1975; Moncada et al., 1976; Moncada and Vane, 1979). The vascular wall synthesises each of these prostanoids, the most abundant being prostacyclin (PGI_2), whilst platelets are the main source of thromboxane A_2 (TXA_2) (Dubois et al., 1998; Félétou, 2011; Majed and Khalil, 2012; Moncada and Vane, 1978; Tang and Vanhoutte, 2008). In young healthy individuals TXA_2 and PGI_2 elicit vasoconstriction and vasodilatation, respectively (Félétou, 2011; Majed and Khalil, 2012).

Blocking COX inhibits all vasodilator and vasoconstrictor prostanoid production (Roth et al., 1975; Vane, 1971). The net action of COX inhibition (COXib) varies between populations. In young, healthy individuals, COXib attenuates the vasodilator response to ACh in the forearm circulation assessed with laser Doppler flowmetry (Holowatz et al., 2005; Kellogg et al., 2005; Noon et al., 1998). However, the role of COX in response to ACh appears compromised in certain populations. Normotensive aged (>60 years) and hypertensive individuals (>46 years) exhibit similar endothelial dysfunction in response to ACh, with COXib (indomethacin) restoring the vasodilator response as assessed by plethysmography (Taddei et al., 1997b). This vasodilator restoration was due to an increase in nitric oxide bioavailability (Taddei et al., 1997a). More recently, in-vitro studies performed on human small arteries noted the antioxidant, ascorbic acid, and a non-selective COX inhibitor (indomethacin) augmented the vasodilator response to ACh in hypertensive samples, although their actions were not additive (Virdis et al., 2013). Collectively, this body of research provides evidence that the mechanism of endothelial dysfunction in aged and hypertensive individuals is due, in part, to COX activity diminishing the vasodilator response to endothelial-dependent vasodilators through reductions in nitric oxide bioavailability. Whether the endothelial dysfunction in AFD observed previously (Maley et al., 2015) is caused by a differing contribution of the COX pathway between ethnic groups is not known. Given that AFD experience greater levels of oxidative stress (Feairheller et al., 2011; Kalinowski et al., 2004), and COX increases reactive oxygen species (Kukreja et al., 1986; Virdis et al., 2013) as well as producing TXA_2, it is possible that the COX pathway may contribute to the attenuated ACh-induced vasodilatation compared with CAU.

Therefore, the aim of the present study was to establish the contribution of COX to ACh-induced vasodilatation in both CAU and AFD. As we have previously observed an attenuated ACh-induced vasodilator response in AFD compared to CAU, it was hypothesised that AFD would experience a lower vasodilator response to ACh compared with CAU, and COXib would augment endothelial reactivity in AFD.

2. Methods

2.1. Participants

This study was given a favourable ethical opinion from the University of Portsmouth Science Faculty Ethics Committee. The participants were made aware of the purpose, procedures and risks of the study prior to giving their informed written consent. 12 CAU and 12 AFD male volunteers participated in the study. All CAU were born in the UK. Eight AFD were born in the UK whilst four were born in Africa (Zimbabwe, Ghana, Kenya and Uganda) and had resided in the UK for an average of 11 years with a minimum of seven years. CAU and AFD were of similar age (mean [SD], 22 [4] years and 20 [2] years, $P = 0.069$), height (mean [SD], 178.2 [6.9] cm and 176.0 [7.9] cm, $P = 0.790$) and body mass (mean [SD], 73.1 [12.3] kg and 74.1 [12.8] kg, $P = 0.583$).

In attempt to reduce heterogeneity female participants were not included in the present study as the menstrual cycle is known to effect vasodilator capacity and thermoregulation (Charkoudian and Stachenfeld, 2015; Hashimoto et al., 1995), therefore the results of the present study should only be applied to young healthy male participants.

2.2. Experimental procedures and measurements

Participants attended the laboratory on one occasion where they received iontophoresis of ACh. The technique of iontophoresis has been described previously (Morris and Shore, 1996; Roustit et al., 2014). Briefly, iontophoresis is a non-invasive method of transdermal drug delivery which transfers charged molecules using a low-intensity electric current into and through the skin to a depth of approximately 2 mm to 4 mm (Anderson et al., 2003). Iontophoresis was performed using both an anode and cathode connected to a battery powered iontophoresis controller (MIC2, Moor Instruments, UK). The iontophoresis chamber, which is a small Perspex ring (MIC-ION1R-P1, Moor Instruments, UK) with an inner diameter of 9.5 mm, was filled with approximately 0.2 mL of ACh (1 w/v% [55.05 mM], Sigma-Aldrich, UK), diluted in water for injection. A laser Doppler probe (VP1T/7, Moor Instruments, UK), utilised to measure skin temperature and skin blood flow, was placed into the Perspex ring and connected to a laser Doppler flowmetry monitor (moorVMS-LDF, Moor Instruments, UK). Laser Doppler and iontophoresis data were recorded using a data acquisition system and software (Powerlab and LabChart 7, AD Instruments, New Zealand).

On the day of testing participants were asked to consume 150 mL of diluted orange squash immediately prior to entering a temperature controlled chamber set at a dry bulb temperature of 23.2 (0.8) °C. All participants rested for 30 min in a supine position to allow skin temperature and skin blood flow to stabilise. Participants were supine throughout the experiment and each skin site was cleaned with deionised water prior to iontophoresis. Iontophoresis of ACh was delivered to either the right medial or right lateral dorsal foot first using the anode, with the cathode placed proximally within 5 cm to 10 cm. Secondly, iontophoresis was applied to the third or fourth non-glabrous finger skin site (medial phalanx) on the right hand (Fig. 1). Following this, participants were then asked to consume 150 mL of diluted orange squash which contained dissolved aspirin tablets to the total of 600 mg of aspirin (acetylsalicylic acid) (Boots Company, UK). Participants were blinded to the order of placebo and aspirin. Aspirin irreversibly inhibits COX by acetylation of the active site of COX (Vane, 1971; Vane and Botting, 2003) with this dose of aspirin shown to inhibit 86% of bradykinin-induced production of PGI_2 and 99% inhibition of TXA_2 production by platelets at 30 min (Heavey et al., 1985).

Thirty minutes after aspirin treatment, iontophoresis began on the foot at a skin site that had not been used (medial or lateral). Following this, iontophoresis was applied to the second finger skin site (third or fourth). The reason for not using the same skin site was that during pilot experiments the vasodilator response to iontophoresis of ACh was much longer lasting than 30 min, thus using the same skin site would influence subsequent skin blood flow results; this has been reported previously (Brocx and Drummond, 2009). The order of participants' skin sites tested (lateral vs. medial dorsal foot, third vs. fourth finger) was counter-balanced between participants. Repeatability studies on six participants demonstrated that the responses to ACh did not differ between sites (medial vs lateral foot; middle vs fourth finger) and over time (two dose ACh response curves following placebo).

The iontophoresis protocol employed in the present study is the same as previously used (Maley et al., 2015) which consisted of six pulses of 25 μA (0.5 mC) followed by one pulse of 50 μA (1 mC) and one of 100 μA (2 mC) applied for 20 s separated by 60 s intervals in which no current was applied. On completion of the protocol, and

Placebo	Foot Site 1	Finger Site 1	Aspirin (600 mg)	Foot Site 2	Finger Site 2

Fig. 1. Schematic of the experimental procedure.