Cutaneous Responses to Endothelin-1 and Histamine in Patients with Vibration White Finger

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Vibration white finger (VWF) is the episodic blanching of the fingers that occurs in response to cold in those who work with hand-held vibrating tools. Clinically the condition differs from primary Raynaud's phenomenon as persistent pain and paresthesia are common in the hands and arms and occur independently of the "white attacks." We have previously reported a decrease in protein gene product 9.5 and calcitonin gene-related peptide-immunoreactive nerve fibers in the digital skin of individuals with VWF. In this study, we have sought to determine whether this deficit of immunoreactive sensory-motor nerves has a functional counterpart in vivo. Histamine produces a rapid wheal and flare response following intradermal injection, whereas endothelin-1 (ET-1) produces a central area of pallor with a surrounding neurogenic flare. In contrast, calcitonin gene-related peptide produces a non-neurogenic erythema. In this study, histamine and ET-1 were injected into the dorsum of the middle phalanx and the local neurovascular response was assessed by measuring the area of the visible flare or pallor. Basal finger blood flow was also measured by laser Doppler flowmetry in each of the digits prior to intradermal injection. The experiments were performed at 21°C and 4°C. Patients with VWF and asymptomatic vibration-exposed workers had significantly lower resting skin blood flow at both 21°C and 4°C than heavy manual workers with no vibration exposure. The size of the histamine- and ET-1-induced flares at both 21°C and 4°C was significantly smaller in patients with VWF when compared with the asymptomatic vibration-exposed workers and heavy manual workers. The size of the ET-1-induced pallor was smaller in patients with VWF when compared with the heavy manual workers at both 21°C and 4°C. In contrast, the area of erythema induced by intradermal injection of calcitonin gene-related peptide at both 21°C and 4°C was of a similar size in patients with VWF and in heavy manual workers. These results indicate that the neuronal deficit identified by immunohistochemistry in the digital skin of patients with VWF has a functional counterpart in vivo and is evident as a reduced ability to propagate an axon-reflex vasodilator response when challenged with histamine and ET-1. Furthermore, these results enable patients with VWF to be differentiated from both asymptomatic vibration-exposed workers, in whom the histamine- and ET-1-induced flares are normal, and those with primary Raynaud's disease, in whom the ET-1 flare is reduced and the histamine-induced flare is normal. Key word: skin blood flow. J Invest Dermatol 110:127–131, 1998

It is now well recognized that the long-term use of hand-held vibrating tools can result in episodic vasospasm and blanching of the fingers. Neurologic symptoms of pain and paresthesia occur independently of the attacks of vasospasm (Bremner and Taylor, 1982). Hand-arm vibration syndrome/vibration white finger (VWF) has been recognized as an occupational disease since early this century (Hamilton, 1918), but the pathophysiology of the disease is poorly understood. In the United Kingdom, VWF has been a financially compensatable industrial disorder (i.e., a "prescribed" disease) since the mid-1980s (Social Security, 1985). At present there is no objective diagnostic test and claims are awarded on clinical grounds alone.

We have previously reported that patients with VWF have a significant reduction in the number of sensory-motor nerves containing the vasodilator calcitonin gene-related peptide (CGRP) and also a reduction in the number of protein gene product 9.5 (PGP 9.5) immunopositive nerve fibers in their digital skin (Goldsmith et al., 1991; Dowd et al., 1995). The reduction in the number of PGP 9.5 immunopositive nerve fibers is greater than can be accounted for solely by the reduction in the number of CGRP immunopositive nerve fibers, indicating that these individuals have a more widespread neuronal loss than has been described in patients with other primary Raynaud's phenomenon or Raynaud's phenomenon secondary to connective tissue disease (Terenghi et al., 1991; Dowd et al., 1995).

Axon-reflex vasodilatation is an important mechanism in the local response when challenged with histamine and ET-1. Furthermore, these results enable patients with VWF to be differentiated from both asymptomatic vibration-exposed workers, in whom the histamine- and ET-1-induced flares are normal, and those with primary Raynaud's disease, in whom the ET-1 flare is reduced and the histamine-induced flare is normal. Key word: skin blood flow. J Invest Dermatol 110:127–131, 1998

1 Two terms are recommended in a recent report written by a working party of the Faculty of Occupational Medicine at the Royal College of Physicians (United Kingdom). They are "hand-arm vibration syndrome" and "vibration-induced white finger." We have opted to use the term vibration white finger, first because all the subjects used in the studies suffer with episodes of blanching in the fingers and second because the term is commonly adopted and universally understood.
control of blood flow in skin and in cutaneous responses to changes in environmental temperature. Capsaicin-sensitive unmyelinated “C” fibers and thinly myelinated “Aδ” fibers are involved in axon-reflex vasodilation (Janco et al, 1967; Lynn, 1988; Janig and Lisney, 1989) and a proportion of these nerves contain CGRP (Dalsgaard et al, 1989; Lawson, 1992). Sensory-motor fibers form a network of branches: one branch is associated with polymodal nociceptors and the other branch forms a neuro-effector junction with target cells such as blood vessels and mast cells (Cleland and Folkow, 1953). A decrease in the number of functionally active sensory-motor fibers and the degree of branching may contribute significantly to the reduced ability to mount a vasodilator response in the face of increasing vasoconstriction in situations such as cold or emotional provocation, or trauma induced by physical agents such as vibration.

Axon-reflex vasodilatation can be assessed in vivo using intradermal injection compounds such as endothelin-1 (ET-1) and histamine and measuring the area of the flare generated. Intradermal injection of histamine induces a classic wheal and flare response; the “triple response” of Sir Thomas Lewis (Lewis, 1927). The flare response is maximal within 2 min of injection and is inhibited by pretreatment of the skin with capsaicin (Bernstein et al, 1981) or by local anesthetic (Lewis, 1927). We have already established that the response to histamine in patients with primary Raynaud’s phenomenon is similar to that in age- and sex-matched controls at both 21°C and 4°C (Bunker et al, 1991a, b).

When injected intradermally into normal skin ET-1 produces an intense vasoconstrictor response that is evident as an area of pallor in the immediate vicinity of the injection site. ET-1 also induces a flare that spreads for several square centimetres around the area of pallor (Bunker et al, 1992). This flare is blocked by pretreatment of the skin with capsaicin and by local anesthetic (Bunker et al, 1992), indicating that it too is neurogenic. In patients with primary Raynaud’s phenomenon, the ET-1-induced flare is reduced at 21°C and more markedly so at 4°C. In contrast the ET-1-induced pallor is not significantly different in patients with primary Raynaud’s phenomenon (Bunker et al, 1996).

CGRP is a potent vasodilator and intradermal injection into human skin produces an intense and long-lasting erythema (Piotrowski and Foreman, 1986). The CGRP-induced erythema is not neurogenically mediated because neither local anesthetic nor capsaicin pretreatment block the vasodilator response. Instead, CGRP acts directly on the blood vessels of the cutaneous microvasculature to induce vasodilation partly by stimulating release of nitric oxide from the endothelium (Goldsmith et al, 1996; Bull et al, 1996).

In this study, we have sought to determine whether the neuronal deficit that has been identified by immunohistochemistry in the digital skin of patients with VWF has a functional counterpart in vivo. Resting skin blood flow has been measured in each of the digits and the axon-reflex vasodilator responses to intradermal injections of ET-1 and histamine have been compared in patients with VWF, in vibration-exposed workers (VEW) who have been exposed to vibration for a long time, and in heavy manual workers (HMW) who have not worked with vibrating tools. The ability of the blood vessels to vasococontract and vasodilate in response to direct stimulation has been investigated by measuring the area of the ET-1-induced pallor and the CGRP-induced erythema, respectively.

**MATERIALS AND METHODS**

**Subjects**  Local Ethical Committee approval was obtained for this study. Twenty-one men with VWF, 20 VEW, and 13 HMW were recruited for this study (Table I) and all gave written, informed consent. All subjects were nonsmokers.

VWF was diagnosed if the patients had episodes of digital ischemia occurring in response to cold or emotional stimuli and characteristic sequential color changes (white, blue, red) in the affected parts. In addition, the patients had to have experienced pain and/or paresthesia in their fingers and hands that occurred independently from the vasopastic episodes. All patients had to have worked with hand-held vibrating machinery of the type that is recognized to cause the disease (Social Security, 1985), but not to have had any symptoms of peripheral vasospastic disease or neurologic symptoms prior to exposure to hand-held vibrating tools. The patients had to have negative appropriate screening investigations for associated diseases such as systemic sclerosis, systemic lupus erythematosus, and rheumatoid arthritis according to the criteria of the Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee (1980) and had not to be taking any drugs. The patients were assessed as to the severity of their vascular and neurologic symptoms and signs and were staged clinically according to the Stockholm (Genne et al, 1987) and Griffin scales (Griffin, 1990).

**Experimental protocol** All subjects were initially equilibrated in the laboratory at an ambient temperature of 21°C. Resting skin blood flow was measured in each digit using a laser Doppler flowmeter (Perimed, Stockholm, Sweden). Intradermal injections of 25 µl containing 750 pmol histamine (Sigma, Poole, U.K.) and 63 pmol human synthetic ET-1 (Novabochem, Nottingham, U.K.) were made into the dorsal digital skin over the middle phalanx of one hand. The injections were made randomly and the investigators were unaware of the contents of each syringe. After completion of the experiments at 21°C, the patients with VWF and the HMW, wearing normal outdoor clothing, were introduced into an environmental chamber set at 4–5°C and the experiments repeated on the fingers of the contralateral hand. HMW and nine patients with VWF also received an intradermal injection of 25 µl containing 12.5 pmol human synthetic CGRP (Peninsula Labs, St Helens, U.K.) at both 21°C and 4°C. All of the agonists were prepared in endotoxin-free sterile saline.

The neurovascular responses were measured by planimetry as previously described (Bunker et al, 1991a). The erythema, flare, and pallor size were measured along the longitudinal axis of the finger in millimetres and at a right angle to the longitudinal axis and the area calculated. Measurements were made at 2 and 10 min after injection of ET-1 and histamine and at 10 and 20 min after injection of CGRP.

**Statistical analysis** Experimental results are expressed as the mean ± SEM. Differences between the groups were analyzed using either the Mann–Whitney U test or the unpaired Student’s t test (two group analysis) or analysis of variance with the confidence limits set at 95% (three group analysis). A p ≤ 0.05 was considered to be statistically significant.

Regression analysis was used to compare the size of the ET-1 and histamine flares, the CGRP-induced erythema, and the ET-1-induced pallor with the clinical scores obtained using the Stockholm and Griffin scales.

**RESULTS**

**Long-term vibration exposure reduces resting digital skin blood flow** The resting digital skin blood flow was significantly reduced (p < 0.05) in patients with VWF and in asymptomatic VEW at both 21°C (Fig 1a) and 4°C (Fig 1b), in comparison with HMW. In each group, there was no significant difference in the resting digital skin blood flow at 4°C compared with that at 21°C.

The size of the histamine-induced flare is reduced in patients with VWF At 21°C (Fig 2a), the size of the histamine-induced flare in the patients with VWF was significantly smaller (p < 0.05) at 2 min after injection, when compared with either the VEW or HMW, but was only significantly smaller (p < 0.05) at 10 min post-injection when compared with the VEW. Similarly, at 4°C (Fig 2b) the histamine-induced flare in patients with VWF was significantly smaller (p < 0.05) at 2 min but not at 10 min after injection, when compared with the HMW.

The size of the ET-1-induced flare is reduced in patients with VWF Intradermal injection of ET-1 induced a flare response that surrounded the central area of pallor. At 21°C (Fig 3a), the size of the ET-1-induced flare in patients with VWF was significantly smaller.

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**Table I. Details of groups investigated**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VWF</th>
<th>VEW</th>
<th>HMW</th>
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<tr>
<td>Number</td>
<td>21</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Mean age (y)</td>
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<td>43.3</td>
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<td>26–62</td>
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<tr>
<td>Mean vibration exposure (µ)</td>
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<td>19</td>
<td>–</td>
</tr>
<tr>
<td>Range (µ)</td>
<td>9–41</td>
<td>5–34</td>
<td>–</td>
</tr>
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</table>
Figure 1. Long-term vibration exposure reduces resting digital skin blood flow. Resting digital skin blood flow in patients with VWF (n = 20), in VEW (n = 19), and in HMW (n = 13) at (a) 21°C and (b) 4°C. The results are expressed as the mean ± SEM; *p < 0.05 relative to HMW. LDF, laser Doppler flowmetry.

Figure 2. The histamine-induced flare is reduced in VWF. The area of the histamine-induced flare in patients with VWF (n = 20), in VEW (n = 19), and in HMW (n = 13) was measured at 2 min and 10 min after injection at (a) 21°C and (b) 4°C. The results are expressed as the mean ± SEM; *p < 0.05 relative to HMW and VEW; p < 0.05 relative to VEW alone.

Figure 3. The ET-1-induced flare is reduced in VWF. The area of the ET-1-induced flare in patients with VWF (n = 20), in VEW (n = 19), and in HMW (n = 13) was measured at 2 min and 10 min after injection at (a) 21°C and (b) 4°C. The results are expressed as the mean ± SEM; *p < 0.05 relative to VEW and HMW; p < 0.05 relative to VEW alone; p < 0.05 relative to 2 min.

Figure 4. The ET-1-induced pallor is increased in patients with VWF. Intradermal injection of ET-1 induced an area of pallor immediately around the injection site. In all groups at 21°C (Fig 4a) and 4°C (Fig 4b) the area of pallor was larger at 10 min than at 2 min after injection. This increase in size was statistically significant (p < 0.05) in the HMW at 21°C and 4°C and in the patients with VWF at 4°C. At 2 min after injection the size of the ET-1-induced pallor at both 21°C (Fig 5a) and 4°C (Fig 5b) was significantly larger (p < 0.05) in patients with VWF in comparison with the HMW.

DISCUSSION

The results of this study have demonstrated that patients with VWF have a reduced ability to propagate an axon-reflex vasodilatation response when challenged with either histamine or ET-1, indicating that the neuronal deficit identified by immunohistochemical studies in
with VWF (Goldsmith et al, 1994) compared with that identified in patients with primary Raynaud’s phenomenon (Bunker et al, 1990; Terenghi et al, 1991).

Patients with VWF and also VEW exhibited significantly lower resting skin blood flow than the HMW as measured by laser Doppler flowmetry. Lower resting skin blood flow has previously been demonstrated in Raynaud’s phenomenon (Bunker et al, 1996, and refs therein); however, in patients with VWF the data have been contradictory with decreased (Olsen et al, 1989) and unchanged resting skin blood flow (Allen et al, 1992) being reported. Olsen et al (1989) also reported normal resting skin blood flow in asymptomatic VEW. Acute vibration of individual digits decreases blood flow in vibration-exposed digits in normal individuals and in both the vibration-exposed and adjacent nonexposed digits in patients with VWF (Kent et al, 1991), indicating that acute exposure to vibration induces local vasoconstriction whereas long-term exposure to vibration results in increased centrally mediated vasoconstrictor tone. The results of this study have shown that the resting digital skin blood flow is reduced in asymptomatic VEW, suggesting that these individuals have incurred subclinical neuronal damage that has selectively affected the centrally mediated sympathetic tone without impairing their ability to mount a local axon-reflex vasodilator response. This, in turn, may explain why these workers do not suffer from attacks of digital vasospasm.

Hand-transmitted vibration does not impair the ability of the cutaneous microvasculature to respond to vasoactive stimuli because the size of neither the CGRP-induced erythema nor the ET-1-induced pallor are reduced. The erythema generated in response to intradermal injection of CGRP is of a similar size in patients with VWF and HMW. Furthermore, the size of the ET-1-induced pallor is significantly larger in patients with VWF than in HMW at 2 min after injection and of comparable size 10 min after injection. The rapid onset of the vasoconstrictor response to ET-1 is a reflection of the damage to the sensory-motor neuronal network and the reduced ability to generate an axon-reflex vasodilator response.

The lack of correlation between the size of the ET-1- and histamine-induced flares and the clinical score is not unexpected. The clinical scores are based on subjective assessment of the area prone to be affected by vibration-induced damage. In contrast, the size of the flare is an objective indicator of sensory-motor neuronal function at a particular anatomical site.

VWF is an occupationally induced disease for which there is currently no objective test. The results of the in vivo pharmacologic studies presented here and the immunohistochemical analysis presented previously (Goldsmith et al, 1994) enable patients with VWF to be differentiated from asymptomatic VEW. In vivo pharmacologic testing either alone or in combination with the immunohistochemical analysis of the peripheral innervation may therefore provide the basis for a objective diagnostic test for VWF.

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REFERENCES


Figure 4. The ET-1-induced pallor is increased in VWF. The area of the ET-1-induced pallor in patients with VWF (n = 20), in VE (n = 19), and in HMW (n = 13) was measured at 2 min and 10 min after injection at (a) 21°C and (b) 4°C. The results are expressed as the mean ± SEM; *p < 0.05 relative to HMW; **p < 0.05 relative to 2 min.

Figure 5. The CGRP-induced erythema is unchanged in VWF. The area of the CGRP-induced erythema in patients with VWF (n = 9) and in HMW (n = 9) was measured at 10 min and 20 min after injection at (a) 21°C and (b) 4°C. The results are expressed as the mean ± SEM.

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Lynn B: Neurogenic inflammation. Skin Pharmacol 1:217–224, 1988
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