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ORIGINAL ARTICLE

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Effects of acupuncture on skin and muscle blood flow in healthy subjects

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Abstract In 14 healthy female subjects, the effects of needle stimulation (acupuncture) on skin and muscle blood flow were investigated using a non-invasive custom-designed probe and photoplethysmography (PPG). In randomised order, 2–7 days apart, three modes of needle stimulation were performed on the anterior aspect of the tibia: superficial insertion (SF), insertion into the anterior tibial muscle (Mu), and insertion into the muscle including manipulation of the needle in order to elicit a distinct sensation of distension, heaviness or numbness (DeQi). Before intervention, the subjects rested for 30 min. After the intervention, the needle was left in situ for 20 min. Blood flow recordings were performed intermittently from 10 min prior to the intervention to the end of the trial. In a fourth session, serving as control, corresponding measurements were performed without any needle stimulation. Area under curve was calculated for 5-min periods prior to and after stimulation, respectively, and for the remaining 15-min period after stimulation. Compared to the control situation, muscle blood flow increased following both Mu and DeQi for 20 min, with the latter being more pronounced for the initial 5 min. Skin blood flow increased for 5 min following DeQi. However, no increase was found following SF. The DeQi stimulation was preceded by higher visual analogue scale ratings of anxiety prior to stimulation, which might have influenced skin blood flow to some extent. The results indicate that the intensity of the needling is of importance, the DeQi stimulation resulting in the most pronounced increase in both skin and muscle blood flow.

Keywords Axon reflex · Needle stimulation · Non-invasive · Photoplethysmography (PPG) · Vasodilatation

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Introduction

In traditional Chinese medicine a characteristic needle sensation, DeQi, is a prerequisite for acupuncture to be effective in treating pain and disease (Cheng 1987). The DeQi sensation is induced by manually twirling the needle after insertion into muscle tissue, and is characterised by a sore, distending, heavy or numb feeling. The DeQi is suggested to relate to activation of A-delta and unmyelinated C-fibres from free nerve endings in the skin or from high-threshold ergoreceptors in muscle (Andersson and Lundeberg 1995). In superficial acupuncture (SF), the needles are inserted subcutaneously without manipulation (Baldry 1998). SF is more convenient to the patients, but may be less effective than the DeQi technique in the treatment of chronic pain by less afferent input to the dorsal horn of the spinal cord (Thomas and Lundeberg 1994).

Upon suprathreshold stimulation peripheral terminals of nociceptors release vasodilative substances, such as calcitonin gene-related neuropeptide (CGRP), to the tissue, leading to vasodilatation in small vessels and increased blood flow (Jänig and Lisney 1989; Kashiba and Ueda 1991; Häbler et al.1997; Sato et al. 2000). By activation of collaterals and dorsal horn circuits, nociceptive stimulation is suggested to innervate adjacent tissue antidromically and result in vasodilatation in skin and muscles (Willis 1999). These types of responses are consequences of axon or dorsal root reflexes (Willis 1999; Sato el al. 2000) and develop with a latency of

~15–20 s and outlast the time of stimulation (Häbler et al. 1997).

In addition, afferent input in somatic nerve fibres, like acupuncture, is suggested to have an effect on autonomic functions, such as the cardiovascular system (Andersson and Lundeberg 1995; Sato et al. 1997), and mental stress is suggested to affect radial artery haemodynamics and the pain-alleviating effects of acupuncture (Wiederström et al. 1998; Boutouyrie et al. 2001). At high levels of cutaneous sympathetic activity, but not at low levels under normal conditions, sympathetic vasoconstriction can suppress antidromic skin vasodilatation induced by the release of vasoactive substances (Häbler et al. 1997). The onset of sympathetic vasoconstriction is quick and its effect lasts only a few seconds after end of the noradrenaline secretion, as opposed to the slower onset and longer duration of vasodilatation induced antidromically by vasoactive substances from nociceptors (Ernst and Lee 1985; Sugiyama et al. 1995; Häbler et al. 1997).

In experimental studies by Jansen et al (1989a, 1989b) similar skin blood flow increase and healing of musculocutaneous flaps were shown after both electro-acupuncture and injections of CGRP. CGRP in the saliva and increased skin blood flow were also found in patients suffering from xerostomia, associated with Sjögren's syndrome, after acupuncture (Blom et al. 1993). The blood flow increases were suggested to relate to the release of vasodilatatory neuropeptides from afferent or parasympathetic efferent nerve endings, such as CGRP and vasoactive intestinal polypeptide (VIP), and possibly to interactions with sympathetic vasoconstriction neurones. These results indicate that acupuncture might have alleviating properties in certain ischaemic conditions by increasing blood flow to the tissue.

Photoplethysmography (PPG) is a non-invasive optical technique mostly used for measuring changes in skin blood flow (Challoner 1979; Kamal et al. 1989). A modified PPG technique might provide a non-invasive assessment of deeper blood flow, such as muscle tissue, by using specific wavelengths and probe geometry (Zhang et al. 2001a, 2001b). In the present study a custom-designed probe and PPG were used to assess blood flow changes in healthy subjects. The aim of the present study was to investigate the effects of different modes of stimulation, using needles, on blood flow in the anterior tibial muscle and overlying skin in healthy subjects. In addition, the psychological influence of the needling situation on blood flow changes was evaluated.

Methods

Fourteen healthy non-smoking women between 20 and 55 years [mean (SD): 38.0 (12.4) years] were recruited from the staff at the participating departments. Anthropometric data for the subjects were: height 169.9 (4.2) cm and body mass 67.0 (11.6) kg. Each subject participated in three randomly distributed intervention sessions, separated by 2–7 days, each at the same time of day. In a fourth session the subjects passed through an identical procedure without any intervention (control). The subjects were instructed not to eat or to drink coffee, chocolate drinks or tea, or to exercise within 2 h before the sessions. Those who had no previous experience of acupuncture were invited to experience the sensation of DeQi. All subjects were informed about the project and gave their informed consent. The ethical principles in the Declaration of Helsinki were followed and the local Ethics Committee approved the study.

Blood flow recording

A custom-designed optical probe was used (Fig. 1). It consists of six green light emitting diodes (LEDs) (560 nm) and three photodetectors (PDs) for monitoring blood flow at a depth of ˜1–2 mm, and three near-infrared LEDs (880 nm) and three PDs (same as above) for monitoring blood flow in deep tissue, i.e. part of the muscle belly. All optical components encircled a 17-mm-diameter hole for insertion of the needle, and the components were embedded in black-coloured silicon. The centre-to-centre distance between the green LEDs and the PDs was 3.5 mm, and the centre-to-centre distance between the near-infrared LEDs and the PDs was 12 or 22.5 mm depending on the geometry. The signals from each wavelength were processed in an amplifier and stored on a PC. PPG_{880} refers to muscle (deep) blood flow and PPG_{560} to skin blood flow.

Stimuli

Three modes of needle stimulation were used in each subject. The needle insertion site corresponded to acupuncture point ST 36 (Jenkins 1990) in the anterior aspect of the tibia. Superficial needle insertion (~2–3 mm) into the skin overlying ST 36 (SF), deep needle (~20 mm) insertion into the anterior tibial muscle (Mu), and deep needle insertion into the anterior tibial muscle, immediately followed by eliciting the sensation of DeQi (Fig. 2). The needles were inserted perpendicular to the skin using sterile stainless-steel acupuncture needles with the same dimension in all three sessions (Hwato 0.30 mm diameter, 30 mm long).

Procedure

All interventions were performed by one of the authors (M.S.) with clinical experience in acupuncture, in a quiet room with moderate light and temperature of 23 (1)°C. The subjects were supine with

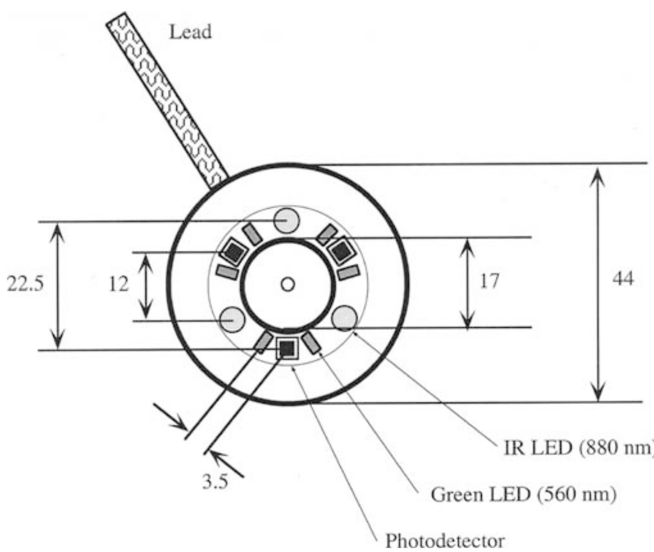

Fig. 1 Probe configuration showing the optical components and physical dimensions in mm

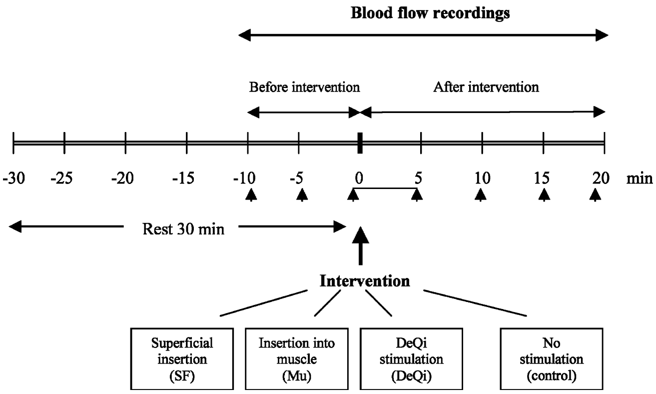

Fig. 2 Schematic protocol depicting the experimental design and procedure. An *upward-pointing filled arrowhead* indicates 30-s periods of blood flow recording. *Upward-pointing filled arrowheads joined by a solid line* indicate 360 s of continuous blood flow recordings, starting 60 s prior to the intervention

the circular probe attached to the skin overlying ST 36 in the right leg. Thirty minutes of rest was allowed for blood flow stabilisation. After the intervention the needle was left in situ for 20 min without any further manipulation. Blood flow recordings were performed for 30 s every 5th min, starting 10 min before the intervention and throughout the trial. From 1 min before to 5 min after the intervention, blood flow recordings were performed continuously (Fig. 2). The blood flow changes are expressed as mean percentages of the value obtained from the mean of the first 30 s immediately prior to the intervention.

After randomisation to one of the needle procedures the subject rated her degree of anxiousness about the trial on a visual analogue scale (VAS) with the left endpoint depicting "not at all" and the right endpoint "worst possible". Immediately after removal of the needle, the subject rated both pain intensity and discomfort experienced from the trial using the same VAS scale. No VAS ratings were performed at control, when the subjects were resting in a supine position without any intervention, but otherwise following the protocol.

Statistical methods

The statistical package Statview 5.1 (Abacus SAS Institute, North Carolina, USA) was used. Mean (1 SD) are presented in the figures. The area under the curve (AUC) (blood flow vs. time) was calculated for the 5-min period prior to stimulation (pre-5) and for the first 5-min period after stimulation (T:1) (Altman 1999). AUC was calculated for the last 15-min period after stimulation and divided by 3 in order to obtain mean AUC for the latter post-simulation period (T:2). Blood flow changes are expressed as mean percentages of the mean value obtained from the 60-s recording prior to the intervention (= baseline, denoted 0). The non-parametric Friedman's two way analysis of variance was used for statistical analysis, and if significant followed by the non-parametric Student-Newman-Keuls' (SNK) test in order to correct for multiple comparisons. The Wilcoxon signed rank test was used for testing changes during Pre-5, and Spearman's test was used for testing associations between variables. A p-value of < 0.05 (two-tailed) was considered significant.

Results

Changes at pre-5

No significant differences existed between −5 min and 0 min for any of the interventions, including control, in terns of skin or muscle blood flow. No differences existed between the pre-5 periods. This indicates a stable 5-min period prior to stimulation (Table 1, Figs. 3, 4).

Changes during control and within the different modes of stimulation

During control measurements, neither skin nor muscle blood flow changed significantly at T:1. However, at T:2 there was an increase in muscle blood flow (Figs. 3, 4). Within all three modes of needle stimulation a significant increase in muscle blood flow was found at both T:1 and T:2. Skin blood flow increased following *DeQi* and *Mu*, whereas no change was found following *SF*. In contrast to the control situation, no significant differences existed at either mode of needle stimulation between the two post-stimulation periods T:1 and T:2.

Changes between the different modes of needle stimulation and control

A significant increase, compared to control, was found in skin blood flow at T:1 following the *DeQi* stimulation, and in muscle blood flow at both T:1 and T:2 (Table 1). Following *Mu*, no significant increase was found in skin blood flow, whereas muscle blood flow increased at both T:1 and T:2. Neither at T:1 nor at T:2 were blood flow increases following *SF* significantly different from

Table 1 Relative blood flow changes at Pre-5, T:1 and T:2 for skin and muscle blood flow, respectively. (*DeQi* Insertion into the muscle eliciting the DeQi sensation, *Mu* insertion into the muscle, *SF* superficial needle insertion.) Pre-5 and T:1 denote 5-min period prior to and after stimulation, respectively. T:2 denotes 15-min period divided by 3 (mean area under curve) after stimulation

Time period	Skin blood flow (area under curve)		Muscle blood flow (area under curve)	
	Friedmans test p-value	Post hoc tests	Friedmans test p-value	Post hoc tests
Pre-5	0.056	NA	0.053	NA
T:1	0.003	DeQi > control, SF, Mu	0.001	DeQi > control, SF, Mu; Mu > control, SF
T:2	0.348	NA	0.007	DeQi > control, SF; Mu > control

NA = not applicable. > = significantly different compared to…

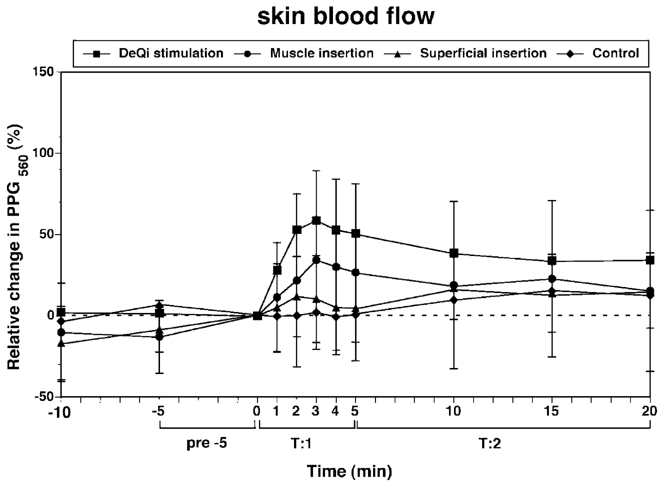

Fig. 3 Mean values for skin blood flow changes at control and the different interventions: *DeQi* stimulation, needle inserted into the muscle (*Mu*) and superficial needle insertion (*SF*). The blood flow values are expressed as the percentage changes in the mean (SD) 30 s prior to intervention (= baseline, denoted 0), and corresponding time point for control

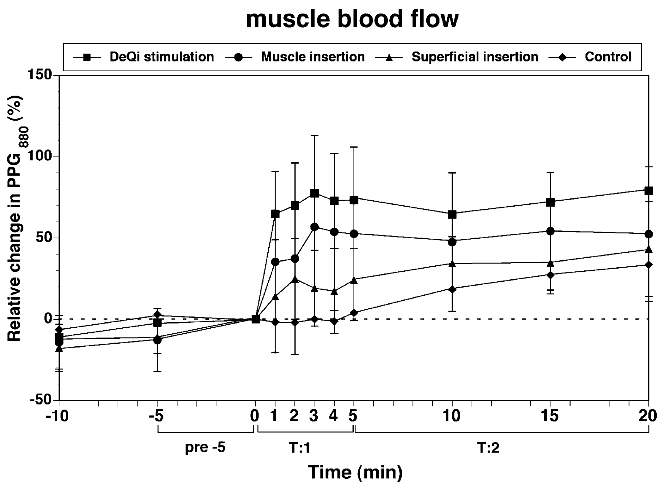

Fig. 4 Mean values for muscle blood flow changes at control and the different interventions: *DeQi* stimulation, needle inserted into the muscle (*Mu*) and superficial needle insertion (*SF*). The blood flow values are expressed as the percentage changes in the mean (SD) 30 s prior to intervention (= baseline, denoted 0), and corresponding time point for control

control values, although a non-significant transient increase in muscle blood flow was seen.

Differences between the three modes of needle stimulation

When comparing the different modes of needle stimulation, the *DeQi* stimulation and *Mu* were superior to *SF* in increasing muscle blood flow at T:1, whereas only *DeQi* was superior to *SF* at T:2. The *DeQi* stimulation was superior to both *SF* and *Mu* in increasing skin blood flow at T:1 (Table 1).

Psychological aspects

VAS estimations of anxiety before as well as pain intensity and discomfort after the needling differed between the stimulation techniques. All three qualities of psychological ratings were higher at the *DeQi* stimulation compared to both *Mu* and *SF*, whereas the ratings did not differ between *SF* and *Mu*. Anxiety prior to the *DeQi* stimulation was positively correlated with the subsequent skin blood flow increase at both T:1 ($r_s = 0.57$, $p < 0.05$) and T:2 ($r_s = 0.68$, $p < 0.05$).

Discussion

In healthy females, one needle stimulation into the anterior tibial muscle (ST 36) resulted in a muscle blood flow increase, the *DeQi* stimulation being superior to *Mu*. In addition, skin blood flow increased following the *DeQi* stimulation. In contrast, no significant skin or muscle blood flow increase was found at *SF*. The *DeQi* stimulation was related with more anxiety prior to the stimulation, as well as more pain and discomfort of the needling compared to *SF* and *Mu*. Furthermore, anxiety before the *DeQi* stimulation was correlated to the skin blood flow increase.

Methodological considerations

Inserting the needle into the muscle (*Mu*) at times provoked a DeQi sensation non-deliberately, making comparisons between the two modes of needle stimulation difficult. Therefore, in the following, *Mu* as a separate mode of stimulation will not be discussed.

The probe for measurements of deep (utilising 880 nm) blood flow, preferably from the muscle, was described by Zhang et al. (2001a). It was confirmed that the probe might extract information about circulatory conditions from the tibialis anterior muscle, even at distances of > 5 mm between the skin surface and the muscle fascia. However, the signal may contain information from the superficial vascular bed as well.

During the first 5 min, the *DeQi* stimulation increased both skin and muscle blood flow, and more so in muscle than in skin. In contrast, within *SF* no significant increase in skin blood flow existed, yet muscle blood flow increased. This indicates that the probe measures blood flow from the muscle tissue. The greater increase in muscle flow compared to skin blood flow may depend on some influence of skin blood flow on blood flow recorded from the deeper vascular bed (unpublished material). Optical radiation in the green wavelength range, here 560 nm, is absorbed in tissue and blood (e.g. haemoglobin). This makes it possible to utilise PPG_{560} for measuring skin blood flow (Giltvedt et al. 1984; Lindberg and Öberg 1991) and to determine flap ischaemia and differentiate between venous-compromised and arterial-compromised flaps (Futran et al. 2000).

During application of an optical probe to the skin surface, convection of heat will be hindered and radiation from the light source may be transferred to skin and deeper tissues. In both cases the temperature will rise, preferably followed by an increase in blood flow due to temperature regulation. During monitoring exceeding ~20 min, blood flow may vary. The increase in blood flow at control corresponds to an increased temperature of 4% (mean in 13 subjects, unpublished material). The conclusions of blood flow changes following needle stimulation are consistently compared to corresponding control values, or to another mode of stimulation; hence, the impact of warming will not substantially affect the results of stimuli-induced blood flow changes.

Blood flow changes following needle stimulation

The finding that the *DeQi* stimulation increased skin blood flow, whereas *SF* did not, is in accordance with data by Jansen et al. (1989a, 1989b) on skin blood flow change measured by laser Doppler flowmetry. Similarly, in patients suffering from xerostomia, blood flux in the skin overlying the parotid gland increased after manual acupuncture with *DeQ*i stimuli, as opposed to *SF* (Blom et al. 1993). In both studies, release of CGRP was suggested to be involved in the mechanisms of blood flow increase. In contrast to these studies, which included repeated DeQi stimuli, the present study deals with the effect of one single needle stimulation only, yet the results are similar. Recently, by using invasive laser Doppler flowmetry, muscle blood flow was shown to increase after electrical stimulation of dorsal roots and electroacupuncture-like stimulation to the hindpaw in anaesthetised rats (Noguchi et al 1999; Sato et al. 2000). Sato et al. (2000) concluded that antidromic vasodilatation in skeletal muscle for 3–15 min following 30 s of stimulation of group IV afferent fibres in dorsal roots is mediated by the release of CGRP from afferent nerve terminals. A similar result was found in the present study on human muscle blood flow by needle stimulation; however, measured by use of a non-invasive technique.

There was a relationship between blood flow increases and intensity of the stimuli, the *DeQ*i stimulation leading to the largest increases and also being rated as the most painful mode of needle stimulation. The noxious mode of stimulation and the long-lasting effects on blood flow indicate vasodilative substances, such as CGRP, from sensory nerve terminals being involved in the mechanisms (Jänig and Lisney 1989; Kashiba and Ueda 1991; Häbler et al.1997; Sato et al. 2000). The mostly non-painful *SF* stimulation may be too weak to induce the release of vasoactive substances, albeit a tendency to increase in muscle blood flow was found for a few minutes.

Interactions with sympathetic neurones of the needling must also be considered in the mechanisms of blood flow changes (Ernst and Lee 1985; Andersson and Lundeberg 1995; Sugiyama et al. 1995; Sato et al. 1997; Häbler et al. 1997; Sato et al. 2000). However, since effects of sympathetic neurones on blood flow are triggered quickly and are suggested to outlast the stimulation with a few seconds only, such effects are likely to be hidden by the more powerful effects of vasodilative substances induced by the needling (Ernst and Lee 1986; Sugiyama et al. 1995; Häbler et al. 1997). Moreover, the very initial blood flow changes may not be detectable because of signal artefacts produced by the needling. The importance of eliciting the *DeQi* sensation in acupuncture, as proposed in traditional Chinese medicine (Cheng 1987), is supported by the results of the present study on blood flow changes.

Anxiety prior to the intervention or a sense of familiarity to the situation may influence the effect of needling on blood flow differently (Wiederström et al. 1998; Boutouyrie et al. 2001). By inviting the subjects to an initial trial to experience the needle sensation, these different starting positions were partly compensated for. Nevertheless, the *DeQi* stimulation was preceded by higher VAS ratings of anxiety compared to *SF* and *Mu* which possibly might have influenced blood flow changes to some extent. A finding was the correlation between the skin blood flow increase and high VAS ratings of anxiety prior to the *DeQi* stimulation. However, no relationship was found between blood flow changes following stimulation and pain or discomfort, respectively.

Conclusion

Needle stimulation into the anterior tibial muscle in healthy female subjects increases both skin and muscle blood flow. Eliciting the *DeQi* sensation is superior to merely inserting the needle into the muscle. A superficially inserted needle does not increase either skin or muscle blood flow. The results indicate that the intensity of the needling is of importance: more intense stimulation resulting in more pronounced blood flow increases.

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