Innovation

An effective method for skin blood flow measurement using local heat combined with electrical stimulation

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Electrical stimulation (ES) is a modality used to increase skin blood flow (SBF) and to aid in wound healing. A greater SBF in non wounded skin is induced if ES is used in a warm environment compared to a thermoneutral environment, where ES is usually applied. Therefore, in this paper, a method to investigate the effect of local heating and ES on the SBF is developed. A total of 33 males (18–40 years) were divided into group G (n = 15) who received the ES during a global heating protocol and group L (n = 18) who received ES during a local heating protocol. In the global heating protocol, ES (30 Hz, 250 μs) was applied for 15 min on the subject’s thigh in thermoneutral (25 ± 0.5°C) and warm (35 ± 0.5°C) environments. In the local heating protocol, ES was applied for 15 minutes at 25°C, 35°C and 40°C local skin temperatures. A laser Doppler imager measured the SBF in both protocols pre, during, and post ES. The results of the experiment showed the significant differences in the SBFs were found at pre, during, and post ES in a thermoneutral environment or when the skin was locally cooled to 25°C. The SBFs were significantly increased during and post ES after global heating or during local heating at 35°C and 40°C. There were no significant differences in SBFs between the warm environment and at 35°C of local heating. However, the SBF response to ES was the highest at 40°C of local heating. Thus, ES during local heating of the skin, as well as during global heating is an effective method to increase SBF.

Keywords: Electrical stimulation; Wounds; Thermoregulation; Cutaneous blood flow; Laser Doppler imager

1. Introduction

Electrical stimulation (ES) is a physical therapy modality which has been used to increase skin blood flow [1–3], especially as an aid for the healing of wounds [2,4,5]. A meta-analysis study found a 40% increase in the healing of wounds using different currents and electrical waveforms compared to conventional methods of wound healing [4]. For example, high voltage galvanic electrical stimulation [6], monophasic pulsed current [7,6], and AC biphasic electrical current have all been shown to increase the healing of wounds [5]. Increasing skin blood flow by ES is a proposed mechanism for wound healing, as well as several other mechanisms, such as inhibiting bacterial growth [8], increasing the white blood cell count, and improving O2 perfusion [9,10].

Recently, we have found that in a thermoneutral environment, electrical stimulation induces only a small
increase in vasodilatation in non wounded skin. Stimulation on wounded skin, however, induces a larger increase in skin blood flow [5]. We also found that by increasing the temperature of the room by 10°C, the SBF was significantly increased in response to ES (unpublished observation, 2006). Similar results have been found in animal studies. When the sympathetic nerve of the inflamed skin of rats was stimulated, a small electrical current was needed to induce vasodilatation, whereas in the same rat model, a very high current was needed to induce the same vasodilatation in normal skin [11]. These findings suggest that the SBF response to electrical stimulation is affected by the local environment of the skin, and the interaction between local and neural control of SBF. In heat stress, a 0.5°C increase in body temperature was enough to increase the SBF to all limbs [12]. This increase was caused by the release of vascular tone by the sympathetic nervous system [13,14] or the activation of the vasodilator system [15], which in turn enhances the SBF in response to ES. This may not be the case during local heating, which is controlled by the integrity of the skin blood vessels and sensory nerves. Local heating induces a biphasic skin blood flow response. The initial phase is a rapid increase in blood flow [13] mediated by the sensory nerve neurotransmitters, such as calcitonin gene related peptide (CGRP) and substance P (SP) [16]. The second phase is a slow and prolonged increase in SBF, which is mediated by nitric oxide (NO) [16,17]. This biphasic skin response to local heating may affect the SBF response to ES. Nothing has been done to investigate the effect of local heating and ES on SBF, which may be a helpful method to improve wound healing. Further, local heating is a less costly and an easier procedure to be employed to increase healing of wounds in a therapeutic setting. We hypothesized that local skin heating will enhance the response of skin blood flow to electrical stimulation.

2. Subjects

A convenience sample of 33 young healthy males between the ages of 18 and 40 years participated in this study. Subjects were free of skin circulation disorders, diabetes, hypertension, cardiovascular disease, and were non-smokers. All subjects had their most recent meal at least 2.5–3 hours prior to the experiment. Each subject received a full explanation of the purposes and the procedures of the study, and then signed an Informed Consent Document approved by the Institutional Review Board of Loma Linda University. Participants were divided into two groups: group G (n = 15) who received the ES in the global heating protocol, and group L (n = 18) who received the ES in the local heating protocol. Age, height, weight, and body mass index (BMI) were not significantly different between the two groups (table 1).

3. Methods

3.1. Core temperature

Body temperature was measured by a tympanic infrared thermometer (Braun, ThermoScan IRT 4520T, Germany). The thermometer measured the infrared heat generated by the eardrum and the surrounding tissues. The temperatures were measured in Fahrenheit and later converted to Celsius.

3.2. Skin temperature

Skin temperature was monitored during the experiment using temperature sensors (Yellow Spring Instruments, Yellow Spring, OH, USA) taped securely to the subject’s skin. The output of the temperature sensors was transduced by a temperature transducer module (Biopac Inc., Goleta, CA, USA), and the data recorded were digitized with a 16 bit A/D Converter at 200 samples/second on a Biopac MP 100 system (SKT100C, Biopac Inc.) using Acqknowledge software version 3.8.1. The temperature transducers were calibrated at the beginning and periodically during the experiment.

3.3. Electrical stimulator

Electrical stimulation was provided by a current controlled electrical stimulator (Challenge 8000, Tustin, CA, USA). The stimulator provided a biphasic square wave output, which was current balanced. The pulse width was 250 μs at a frequency of 30 Hz and a maximum current of 20 mA. The current was delivered through two carbonized rubber

<table>
<thead>
<tr>
<th>Age (Mean ± SD)</th>
<th>Height (Mean ± SD)</th>
<th>Weight (Mean ± SD)</th>
<th>BMI (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group G</td>
<td>32.1 ± 8.3</td>
<td>177.5 ± 9.3</td>
<td>81.4 ± 16.6</td>
</tr>
<tr>
<td>Group L</td>
<td>36.6 ± 12.2</td>
<td>180.1 ± 18.2</td>
<td>82.7 ± 17.7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.20</td>
<td>0.59</td>
<td>0.82</td>
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electrodes (Pin electrodes, Medi-Stim, Inc., Wabasha, MN, USA, 5 × 10 cm) with electrode gel applied (Spectra 360 Electrode gel, Parker laboratories, Fairfield, NJ, USA) to maximize electrical conductivity.

3.4. Skin blood flow

Skin blood flow was measured by a laser Doppler imager (Moore Instruments Oxford, UK). Laser Doppler imaging is a non-invasive procedure that works by illuminating the tissues with a low power laser light. Part of the laser light scatters within the tissues and some will scatter back with altered frequency proportional to the red blood cell velocity (Doppler principle). The back-scattered light from moving red blood cell and static tissues is photodetected by a scanner head. The photodetected light is processed and converted into electrical signals which undergo further signal processing that provides the measurement of the blood flow. The output reading is expressed in arbitrary perfusion unit ‘flux’, which represents the blood flow as the product of the numbers of moving blood cells (volume) multiplied by their average velocity. The perfusion unit is standardized by measuring a flux of standard comprising polystyrene latex spheres undergoing Brownian motion in water [18].

The laser was warmed for 30 minutes prior to flow measurements to increase stability and was calibrated by the factory just prior to these experiments. Also, the laser was periodically calibrated using a standardized suspension provided by the factory. The device sat on a stand 30–35 cm above the subject’s right thigh and the distance between the laser and the skin was always kept constant at 30 cm, while the subject was lying supine.

3.5. Local heating

In the local heating protocol, the skin temperature was controlled by a Peltier junction (TE Technology, Inc., Traverse City, MI, USA) 40 × 40 mm, height 4.0 mm and with a hole of 7.9 mm diameter in the centre. Two heat sinks with small electrical fans were mounted on the opposite side of the Peltier to prevent overheating (figure 1). The target temperature was controlled by the integration of the input of the power supply and the temperature reading by a feedback controller using skin temperature as an input.

4. Procedures

4.1. Global heating protocol

Subjects in group G completed the two parts of the first protocol. The first part was to assess the response of SBF to ES in a thermoneutral environment (25 ± 0.05°C, relative humidity 32 ± 3%), and the second in a warm environment (35 ± 0.5°C, relative humidity 42 ± 3%). The subjects were asked to lie supine and rest for 15 minutes. The right thigh was cleaned with alcohol to remove surface lipids and improve electrical conductivity. Two carbonized rubber electrodes were moisturized with conductivity gel and taped on the anterior aspect of the right thigh 8 ± 1 cm above the knee joint and 11 ± 2 cm apart from each other. The electrical current was applied for 15 minutes at intensities ranging from 1 to 15 mA, just below the threshold of muscle contraction. SBF was measured in each of three 15 minute periods: pre-ES (acclimatization), during, and post-ES (after ES is ceased) using LDI. An area of 48 cm² (183 × 66 pixels) about 1 cm below the proximal rubber electrode was scanned at a rate of 10 ms/pixel every 2.5 minutes. The SBF was determined as the average of blood flow over this area. Markers placed on the skin allowed repeat measurements in this same area of interest under different experimental conditions. Only one experiment was conducted per day per subject and each experiment lasted approximately 60 minutes. Tympanic temperature was measured every 5 minutes, while skin temperature was monitored continuously during the experiment. A skin temperature probe was placed on the subject’s thigh between the electrodes and below the scanned area.

4.2. Local heating protocol

The subjects in group L completed the three parts of the second protocol. The first part was to assess the response of SBF to ES at a local temperature of 25 ± 0.05°C, and at 35 ± 0.05°C and 40 ± 0.05°C for the second and third parts respectively. The experiment took place at room temperature (25 ± 0.5°C) and at 32 ± 3% relative humidity. The preparation procedures were the same as described above for the first protocol, except a thermocouple Peltier system was placed between the two electrodes, and secured with two straps to control the skin temperature at the target level. The electrical current was applied for 15 minutes. The
SBF was measured continuously using a single point scan in the centre of the Peltier for three 15-minute periods: pre-ES (acclimatization), during, and post-ES (after ES ceased) using LDI. One or two part(s) of this protocol was/were conducted per day per subject and each experiment lasted approximately 45 minutes. If the subject finished the first part, and agreed to finish the second part, the second part was conducted on the contralateral thigh. Otherwise, the subject had to return to the lab once or twice until the three parts were conducted. A tympanic temperature was measured every 5 min, while skin temperature was monitored continuously during the experiment. A temperature probe was placed on the ipsilateral thigh between the Peltier device and the subject’s skin.

5. Results

5.1. Core temperature

The tympanic temperatures for group G and L in the thermoneutral environment were the same (mean ± SD, 36.5 ± 0.3°C). The tympanic temperature of group G in the warm environment (36.8 ± 0.3°C) was higher \((p = 0.001)\) than the tympanic temperature in the thermoneutral environment (36.5 ± 0.3°C).

5.2. Skin temperature

The thigh skin temperature (36.8 ± 0.60°C) of group G in the warm environment was higher \((p < 0.01)\) than the skin temperature in the thermoneutral environment (33.7 ± 0.9°C).

5.3. Global heating

When the subjects in group G received electrical stimulation in the thermoneutral environment, there was no significant difference in SBF between pre (129.3 ± 47.7) and both during (135.6 ± 50.8, \(p = 0.12\)) and post ES (132.0 ± 50.8, \(p = 1.0\)) (figure 2). When the subjects were exposed to the warm environment, the SBFs were significantly higher during (198.5 ± 83.1, \(p = 0.003\)) and post ES (198.7 ± 87.5, \(p = 0.003\)) compared to pre ES (172.3 ± 73.4). No significant difference was found between during, and post ES \((p = 1.0)\). In the comparison between the SBF in the two environments, repeated measures ANOVA with Bonferroni multiple comparisons showed a higher response of the SBF during stimulation in the warm environment \((p = 0.007)\) compared to the SBF in the thermoneutral environment.

5.4. Local heating

When ES was applied at a skin temperature of 25°C, there was no significant difference \((p > 0.05)\) in SBF between pre (91.7 ± 52.0), during (91.4 ± 57.2), and post ES (82.7 ± 43.8) (figure 3). When the skin was locally heated to 35°C, SBFs were significantly different during (199.6 ± 103.8, \(p = 0.03\)) and post ES (229.6 ± 128.0, \(p = 0.006\)) compared to pre ES (164.9 ± 88.3). Also, the SBF was significantly higher post ES \((p = 0.01)\) than during ES. Likewise, during 40°C of local heating, the SBFs were significantly higher during (617.1 ± 433.8, \(p = 0.001\)) and post ES (639.9 ± 441.0, \(p = 0.001\)) compared to pre ES (471.2 ± 321.8). No significant difference \((p = 0.60)\) was found between during and post ES (figure 3). We used repeated measures ANOVA with Bonferroni multiple comparisons to compare the SBF pre, during, and post ES among the three local temperatures. We found that the SBFs were significantly higher with a skin local temperature of 35°C \((p < 0.001)\), and 40°C \((p < 0.001)\) compared to the SBF of local temperature at 25°C. Furthermore, the SBF was significantly higher at 40°C than the SBF at 35°C of local heating \((p = 0.001)\).
5.5. Comparison of global and local heating

We used repeated measures ANOVA with Bonferroni multiple comparisons to compare the SBF response to ES in thermoneutral and warm environments with the SBF during local heating.

5.6. Thermoneutral environment

The SBF in the thermoneutral environment was significantly higher ($p = 0.01$) than the SBF with a temperature of 25°C of local cooling (figure 3 (a)). The SBFs with skin local temperature at 35°C and 40°C, however, were significantly higher ($p = 0.03$, $p = 0.001$ respectively) than the SBF in the thermoneutral environment (figures 4(a) and 4(b)).

5.7. Warm environment

The SBF in the warm environment was significantly higher ($p = 0.001$) than the SBF at 25°C local cooling (figure 4 (a)). No significant difference was found between the SBF in the warm environment and the SBF clamped at 35°C ($p = 0.80$) (figure 4(b)). The SBF at 40°C of local heating, however, was significantly higher ($p = 0.001$) than the SBF in the warm environment (figure 4(c)).

6. Discussion

In the present investigation, we examined the effect of different ambient and local temperatures on the SBF response to ES. In the thermoneutral environment, the vascular tone dominates the control of the SBF [19], and electrical stimulation could not overcome this resting vascular tone. Moreover, when the skin was locally cooled to 25°C, there was a further vasoconstriction via axon reflex, which makes it harder, if not impossible, for ES to increase the SBF. Yet, other studies show that ES increases the SBF in the thermoneutral environment. These studies, however, generally use higher currents (40–80 mA) than the current used in this investigation (5–20 mA) [20,21]. Other studies also used small electrodes applied on a small area of the skin, which dramatically increased the current density over the stimulated area [22,23]. In this study, we used large electrodes over a large area, thereby keeping current density low.

Electrical stimulation was capable of increasing the SBF in a warm environment or on locally heated skin at 35°C or 40°C. This poses the question of why local or global heating elicits such a response. During global heating, the increase in SBF response to ES is potentiated if there is a corresponding central release of vascular tone by the sympathetic nervous system due to global heating [12,14]. This may increase the sensitivity of skin to noxious stimuli, such as ES. Moreover, heating may induce the body to release vasodilators, such as vasoactive intestinal peptide (VIP) [24] and nitric oxide (NO) [17,25]. On the other hand, electrical stimulation is believed to induce vasodilatation by releasing vasodilators such as VIP [1], NO [26], and also neuropeptides, such as calcitonin gene-related peptide (CGRP) and substance P, from the afferent nociceptors C-fibres in skin [22,23]. Thus, if global heating induces VIP and NO release, and in turn these cause active vasodilatation, a further vasodilatation will occur when ES induces additional VIP and NO release by the endothelial cells. Even if ES induces either VIP or NO, the established relation between them allows the assumption that one incites the other. Nitric oxide causes the release VIP in human skin [27], and VIP and NO have a synergistic effect in gastric smooth muscle in rats [28]. Also, a similar relation has been established between NO and substance P, but not with CGRP. Nitric oxide induces and enhances the effect of substance P in rats, and in human skin [29,30]. Thus, if ES induces substance P and the NO is present from either global heating or ES, the substance P vasodilatory effect will be enhanced. Similarly, local heating initially and temporarily induces substance P and CGRP [16,13], but...
after a few minutes, NO dominates and maintains the prolonged skin vasodilatation [17,16]. Thus, with NO already induced by local heating, the effect of substance P and NO will be enhanced with further release of NO by ES. This leads us to suggest that NO may be the neurotransmitter that plays a major role in the response of SBF to ES in local and global heating.

When the skin was locally heated to 35°C, the SBF continued to rise post ES (figure 4(b)), while the SBF reached a plateau during global heating. In global heating, sweating caused by the thermoregulatory response prevents skin temperature from a further increase, and, in fact, cools the skin due to evaporative cooling. But local heating of the skin forces the skin to warm and sweating cannot cool down the skin, because of the lack of sympathetic nerve stimulation. Furthermore, when the skin was heated locally to 40°C, the SBF significantly increased about three times more than the SBF during global and local heating to 35°C, and the maximum capacity of vasodilatation was reached during and post ES. Therefore, local heating is more effective, because to get the same sympathetic outflow and the same amount of NO to be released during global heating would necessitate considerable increases in core temperature. To achieve the same effect as local heating to 40°C, we may need to warm the room to more than 40°C (figure 4(c)).

It was encouraging to find that when skin was globally or locally heated to about 35°C, the same increase in SBF was obtained, while a three-fold increase in SBF response occurred when the skin was locally heated to 40°C. In our labs, we found that heating the room to 32°C increased the SBF and increased wound healing compared to the control group in the thermoneutral environment [31]. We expect the same result if we locally heated the skin to 35°C, or maybe a better result if it is heated to 40°C. Local heating is more practical in a medical setting than global heating, in that it is a less costly, versatile, and a more specific method that may be employed for wound healing.

7. Conclusion

Previously, we found that using ES in a warm environment is a helpful method to induce SBF and enhance healing of the wounds. The use of ES with local heating, however, induced the same or better SBF. This led us to assume that the combination of local heating and ES is potentially an effective method to enhance wound healing. Therefore, further studies are needed to confirm this assumption.

7.1. Key points

Electrical stimulation is an efficient modality to increase SBF, especially if applied in a warm environment. Electrical stimulation with local heating as well is a better and more efficient method to increase SBF than global heating.

Electrical stimulation with local heating is easier and a less costly procedure than applying ES in a warm environment.

Electrical stimulation and local heating may have the potential to induce wound healing.

Declaration of interest: None of the authors of this work has any financial associations or relationship with any commercial company that may benefit from this work.

References


A method for skin blood flow measurement


