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Effect of continuous vs pulsed iontophoresis of treprostinil on skin blood flow

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ABSTRACT

Introduction: Systemic sclerosis (SSc) is a rare disease affecting digital microcirculation, leading to finger ulcers and in some cases to amputation. Prostacyclin analogues can be used intravenously but their therapeutic effect is counterbalanced by potentially serious vasodilatation-induced side effects. Iontophoresis of treprostinil could be a promising local therapeutic alternative for SSc-related digital ulcers. Iontophoretic drug delivery is complex, and whether continuous or periodic current should be used remains debated. The objective of the present work is to compare the effect of continuous vs pulsed iontophoresis of treprostinil in rats.

Materials and methods: Treprostinil (0.64 mM and 0.064 mM) and NaCl were delivered by cathodal iontophoresis onto the hindquarters of anaesthetized rats. Three protocols delivering the same quantity of current were compared: one was continuous (100 μA during 20 min) and two were periodic (B: twenty 1-min cycles with 200 μA during 30 s followed by 30 s Off; and C: twenty 1-min cycles with 600 μA during 10 s followed by 50 s Off) (n = 8 for each protocol with each concentration). Skin blood flow was quantified using laser Doppler imaging and skin resistance was calculated with Ohm's law.

Results: All protocols induced a significant increase in skin blood flow. At the lower concentration (0.064 mM treprostinil) the pulsed 10/50 sequence significantly enhanced cutaneous blood flow (Table 1; Fig. 1B) compared to continuous iontophoresis or the 30/30 sequence. We noted that the pulsed iontophoresis of NaCl (10/50 sequence) induced a significant early increase in cutaneous blood flow in comparison with continuous iontophoresis. Skin resistance measures were negatively correlated with current intensity delivered.

Conclusion: In conclusion, pulsed iontophoresis of treprostinil with a 10 s/50 s (On/Off) protocol at 600 μA increases the efficacy of iontophoresis at 0.064 mM but not at a tenfold higher concentration. Pulsed iontophoresis could be used to optimize treprostinil iontophoresis, to provide similar efficacy with decreased costs, and should now be tested on humans.

1. Introduction

Systemic sclerosis (SSc) is a rare disease affecting digital microcirculation, leading to finger ulcers and in some cases to amputation (Herrick, 2000). The therapy of SSc-related ulcers is challenging. Prostacyclin analogues are used intravenously (Wigley et al., 1992), but their therapeutic effect is counterbalanced by potentially serious vasodilatation-induced side effects (e.g. severe headaches, flushing, tachycardia and hypotension). The topical administration of vasodilators by iontophoresis, a non-invasive current-driven drug delivery method, has been suggested as a treatment strategy for digital ulcers as it allows elevated concentrations to be reached in the target tissue (skin of the finger pad) while limiting systemic toxicity (Murray et al., 2005, 2008). Recent experiments in animals and in humans have established the proof-of-concept of iontophoretically-administered treprostinil (a prostacyclin analogue) as a treatment of SSc-related digital ulcers (Blaise et al., 2011, 2013; Roustit et al., 2014). However, we observed high variability in the response, which is partly attributable to the inter- and intra-individual variability of skin to the iontophoretic current.

In iontophoresis the choice of current intensity and duration of application are crucial. On the one hand, the efficiency of

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iontophoretic transport depends on the quantity of current delivered (i.e. product of intensity by time) (Kalia et al., 2004), but on the other hand it also depends on the effect of the current itself on the electrical properties of the skin (Chen and Chien, 1996; Chizmadzhev et al., 1998; Kanebako et al., 2002a, 2003, 2002b; Nair and Panchagnula, 2004). In particular, it is known that using a relatively high voltage during iontophoresis facilitates the transport of drugs by a mechanism of pore formation which decreases the skin's resistance (Glaser et al., 1988; Sims et al., 1992; Inada et al., 1994). However, elevated current density causes irritation and skin damage (Roustit et al., 2014). Moreover, prolonged continuous cathodal current may cause electrochemical polarization of the skin responsible for an increase in skin resistance and a reduced efficiency of transport. Attempts to find the ideal combination between duration and current intensity so as to optimize iontophoretic transport while limiting skin damage or polarization, has been the subject of number of studies. Pulsed current profiles (with variable On/Off ratios) have been proposed to allow skin depolarization, permitting the return to steady-state conditions and limiting irritation (Chen and Chien, 1996; Zakzewski et al., 1992; Okabe et al., 1986). Moreover, pulsed DC current has been shown to enhance the iontophoretic delivery of buserelin (Knoblauch and Moll, 1993) and arginine-vasopressin (Nair and Panchagnula, 2004) as compared with continuous direct current.

These studies suggest that applying a pulsed current could limit skin resistance, enhance iontophoretic transport and improve safety. The primary objective of this experimental study was to compare the effect of iontophoresis of treprostinil using pulsed vs continuous DC profiles on skin blood flow in rats. As a secondary objective we measured the changes in cutaneous resistance induced by each protocol.

2. Materials and methods

2.1. Animals

Twenty-four male Wistar rats (eight-weeks old, 275–290 g; CERJ, Le Genest-St-Isle, France) were housed in controlled conditions conforming to the current French legislation and fed with standard rat chow. The protocol was approved by the Rhône-Alpes Region Animal Ethics Committee. Rats were kept in a day/night cycle of 12 h/12 h with food and water at will. Thirty minutes before iontophoresis, rats were shaved as previously described (Blaise et al., 2011; Roustit et al., 2012; Kotzki et al., 2013).

2.2. Drug supply and preparation

Commercial treprostinil solution at 2.5 mg mL^{-1} (6.4 mM; MW 390.5 g mol^{-1}; pKa 4.5; pI 1.25) was used (Remodulin®, Bioprojet Pharma, Paris, France), and isotonic sodium chloride (NaCl 0.9%) (Aguettant, Lyon, France) was used as a control solution. Dilutions were performed with 0.9% NaCl to obtain 0.64 mM and 0.064 mM solutions. The pH of treprostinil solutions was controlled before iontophoresis using a microprocessor-based pH meter (pH 210, Hanna Instruments, Woonsocket, RI, USA) to ensure compatibility with cathodal iontophoretic delivery (pH > 5.5). No pH adjustment was required for any dilution.

2.3. Iontophoresis protocols and experimental procedure

Rats were anesthetized with sodium pentobarbital (50 mg kg^{-1} i.p) and were maintained in the prone position for the duration of the whole experiment, with the back uppermost. They were placed on a thermal pad to maintain their temperature at 37 °C (Homeothermic Blanket Control Unit 50-7053, Harvard apparatus, Boston, MA, USA). Three 1.2-cm^2 circular iontophoresis electrodes (PeriIont System, Perimed, Järfälla, Sweden) containing NaCl 0.9%, treprostinil 0.64 mM and 0.064 mM respectively were placed on the hairless skin of the back/hind legs. A passive electrode was placed on the back of the neck, as previously described (Blaise et al., 2011). Each iontophoresis electrode was connected to a constant current generator socket (PF 382b, PeriIont System, Perimed, Järfälla, Sweden) and the passive electrode was connected to another output. As treprostinil is negatively charged at cutaneous pH, a cathodal polarity was applied to the drug containing electrodes.

Three different iontophoresis protocols were assessed with 0.64 mM treprostinil, 0.064 mM treprostinil and NaCl 0.9% (Fig. 1). The continuous protocol of 100 μA during 20 min (Protocol C) has been described in a previous study (Blaise et al., 2011). Then, two protocols with different ratios of pulsed current were compared to this reference. The first consisted of twenty repeated 1-min cycles of 30 s at 200 μA (On) followed by 30 s without current (Off) (Protocol $P_{30/30}$). The second consisted of twenty cycles of 10 s at 600 μA (On) followed by 50 s without current (Off) (Protocol $P_{10/50}$). We adapted the current intensity so as to always deliver a total charge of 120 mC over 20 min for each protocol. Treprostinil dilutions and 0.9% NaCl were tested simultaneously on the same animal for each protocol ($n = 8$). Passive permeation of treprostinil was not performed since it has ever been published that there was no significant transdermal diffusion of treprostinil without current (Blaise et al., 2011).

During iontophoresis, we measured the voltage across the passive and active electrode containing NaCl every five minutes to calculate the skin's resistance using the Ohm's law (Blaise et al., 2011).

2.4. Skin blood flux measurement and data analysis

Skin blood flux was continuously recorded though the active (i.e. drug-containing) electrodes with laser Doppler imaging (LDI; PeriScan PIM 3, Perimed, Järfälla, Sweden). The laser was placed 17 cm above the skin, and its resolution was set to a 2 mm step length. LDI scans were made every minute.

For each protocol the iontophoretic current started to be delivered after a 5-min baseline (BL) recording. Skin blood flux was then recorded throughout the 20-min iontophoresis and for 40 min following the end of current delivery. Skin blood flux was recorded as arbitrary perfusion units (PU) and subsequently expressed as the percentage increase from baseline (%BL) to take into account inter-individual variations in baseline flux. Areas under curves were calculated for the duration of the whole experiment (AUC_{0-60}), and for the iontophoresis period only (AUC_{0-20}).

2.5. Statistical analysis

Continuous data are expressed as mean ± standard deviation. Data were analyzed with 1-way ANOVA for the primary objective, and with paired t tests for 2 × 2 comparisons. Repeated-measures ANOVA was used to assess current profile effects on skin resistance during the iontophoretic protocol (secondary objective). Mauchly's test of sphericity was used to assess equality of variance. As inequality of variance could not be excluded a Greenhouse–Geisser adjustment was used. We tested the effect of time, of protocol, as well as the interaction between time and protocol. Two-sided significance tests were used throughout. We considered p values <0.05 as significant, corrected by Bonferroni's method for multiple comparisons. Statistical analysis was performed with SPSS 13.0 for Windows (SPSS Inc., Chicago IL, USA).