Influence of vehicle resistance on transdermal iontophoretic delivery of acetylcholine and sodium nitroprusside in humans

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Khan, Faisal, David J. Newton, Emily C. Smyth, and Jill J. F. Belch. Influence of vehicle resistance on transdermal iontophoretic delivery of acetylcholine and sodium nitroprusside in humans. J Appl Physiol 97: 883–887, 2004. First published April 30, 2004; 10.1152/japplphysiol.00373.2004.—Iontophoresis is a valuable method of noninvasive drug delivery for assessment of skin microvascular function, but it is important to consider and minimize its potential nonspecific electrical effects on blood flow. The use of sodium chloride (NaCl) instead of water as the iontophoresis vehicle has been reported to reduce these effects because it has a lower electrical resistance. However, this argument may not be valid when an agonist is added to the vehicle because its resistance will be changed. The aim of our study was to determine whether there is a difference in resistance between water and NaCl when used as vehicles for iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). Four cumulative doses of each drug, dissolved in either water or NaCl, were delivered via iontophoresis to the forearm skin of 14 healthy volunteers. We measured the resulting blood flow responses by using laser-Doppler imaging and the voltage across the electrodes for each delivery as an index of resistance. For ACh and SNP, there were no significant differences between the voltages measured when either water or NaCl was used as the vehicle. However, the blood flow responses to both agonists were significantly lower with NaCl (ACh: 25% lower, \( P < 0.001 \); SNP: 15% lower, \( P = 0.019 \)). The use of NaCl is therefore unlikely to decrease any nonspecific electrical effects, and it may in fact reduce the effective dose of drug delivered. Deionized water is a better iontophoresis vehicle for the assessment of microvascular function in skin when using ACh and SNP.

METHODS

We recruited 14 healthy participants into the study, which had been approved by the Tayside Committee on Medical Research Ethics and which was conducted according to the principles outlined in the Declaration of Helsinki. They were all nonsmokers aged 20–50 yr, with no history of cardiovascular disease and taking no medication. There were 11 women and 3 men, and each gave written, informed consent to participate in the study. They were instructed not to consume any food or any caffeine-containing drinks in the 2 h before their visit. All experiments were conducted in the early afternoon, in a laboratory at an environmental temperature of 22°C, and the participants were seated with their arms supported at heart level.

We prepared measurement sites on the volar surface of the forearm by removing surface keratinocytes with adhesive tape and cleaning the area with an alcohol swab. The iontophoresis chamber (Moor Instruments, Axminster, UK) is a Perspex ring of diameter 20 mm with a wire electrode encircling its inner surface, and this was fixed to the skin with adhesive tape. The hydrochloride salt of ACh (Sigma-Aldrich, Poole, UK) was dissolved in deionized water (Steri-Amp Water for Injection BP, Steripak, Runcorn, UK) to a concentration of 10 g/l, and 2 ml of this solution were used to fill the chamber. The positive lead of a current source was connected to the chamber, and the negative lead was attached to a conductive hydrogel pad on the skin with adhesive tape. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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to monitor the voltage across them throughout the experiment. This signal was recorded continuously on computer, and the mean voltage applied during each iontophoretic delivery was calculated. This was subsequently used as an index of the electrical resistance because, according to Ohm’s law, if current is constant, then resistance is linearly related to voltage.

We assessed cutaneous microvascular perfusion at the delivery site by using laser-Doppler imaging (moorLDI, Moor Instruments), which produces a color image representing the distribution of skin blood flow (6). This relative measure of production is called the laser-Doppler flux. A baseline image was recorded at the start. After each iontophoretic delivery, the solution was removed and the chamber dried, and then three images were recorded at 30-s intervals as the response developed. This was repeated for each dose of ACh. The laser head was positioned 50 cm from the skin surface, and the scan region, encompassing the iontophoresis chamber, was ∼8 × 8 cm. We used a spatial resolution of ∼1 mm per pixel and a scan speed of 4 ms per pixel. Our laboratory has demonstrated previously an intra- and intersubject variability for these measurements (10) ranging between 15 and 30%.

The experiment was repeated with SNP in water (Sigma-Aldrich; also 10 g/l), and, on a different occasion, twice more with each drug in a 0.5% solution of NaCl. The leads were reversed for SNP because it is negatively charged in solution, and five images were recorded at 45-s intervals after each dose because SNP takes longer to have an effect on blood flow. On a third occasion, we repeated the measurements with water alone or with NaCl alone in the chamber. Each set of measurements was performed at a different, randomly selected site on either arm, and the participants’ visits were separated by ∼1 wk. We collected ACh response data from all 14 participants, SNP data from 10, and measurements on vehicle alone from 6 people.

The laser-Doppler images were analyzed by using dedicated software (Moor Instruments), and we calculated the median laser-Doppler flux over the iontophoresis delivery site for each one. For the response to each dose, the mean of the two highest consecutive flux values was taken, and this was divided by the baseline measurement to give a ratio representing the change in flow.

The Shapiro-Wilk test indicated that most of our data followed an approximately normal distribution. We therefore used a univariate analysis of variance model and post hoc t-tests to determine the statistical significance of differences in the blood flow response and in the applied voltage between the two drugs and between the two vehicles used. We calculated Pearson’s correlation coefficients to test the strength of relationships between blood flow responses and voltage applied. Because of the lower number of measurements made on responses to vehicle alone, these data were analyzed by using non-parametric Mann-Whitney U-tests. All statistical analyses were performed using SPSS software (SPSS, Chicago, IL), and significance was acknowledged if the probability of a type I error was <5% (i.e., P < 0.05).

**RESULTS**

Iontophoresis of both ACh and SNP produced dose-dependent increases in blood flow (ACh: P < 0.001, Fig. 1A; SNP: P < 0.001, Fig. 2A), but the responses were significantly lower with a NaCl vehicle than with water (ACh: 25% lower on average, P < 0.001; SNP: 15% lower, P = 0.019).

However, for both drugs, there was no significant difference between the voltage applied when either water or NaCl were used as the vehicle (ACh: P = 0.706, Fig. 1B; SNP: P = 0.387, Fig. 2B). There was a significant drop in voltage with increasing dose (ACh: by 20%, SNP: by 30%; P < 0.001 for both drugs).

We found significant correlations between blood flow responses and the voltage applied for the first three doses of ACh in NaCl and for all four doses of SNP in NaCl (Table 1). The only significant correlation for any drug delivered in water was for SNP at 4 mC (R = −0.708, P = 0.022).

Iontophoresis of either vehicle alone produced no significant change in blood flow when the active electrode was connected as the anode (Fig. 3A). When it was connected as the cathode, iontophoresis of water alone produced a relatively small increase in flow (by 36%, compared with almost 500% for the SNP response) only at the highest dose (P = 0.016; Fig. 4A).

A significantly higher voltage was measured during iontophoresis of water alone than during iontophoresis of NaCl alone, at both electrodes (anode: P = 0.004, 0.004, 0.017, and 0.009 for each successive dose, Fig. 3B; cathode: P = 0.057, 0.036, 0.036, and 0.036, Fig. 4B).

The addition of SNP to water significantly reduced the voltage applied during iontophoresis (by 40–50%, P = 0.043 for the 3 highest doses), but it had no effect when added to NaCl. The addition of ACh reduced the voltage applied to water (by an average of 20%) and increased the voltage applied to NaCl (by an average of 25%), but these were not statistically significant.
**DISCUSSION**

Iontophoresis has become a valuable method of noninvasive drug delivery for the assessment of microvascular function, but there remain several questions about the influence of nonspecific electrical effects on blood flow. This so-called galvanic response is believed to be related to the voltage required to establish the iontophoretic current. The resistance of the drug in solution might therefore be an important factor, and investigators have attempted to limit this by using NaCl instead of water as the vehicle (1, 2). However, we have shown in this study that the choice of vehicle does not affect the voltage applied and therefore does not affect the resistance of the solution (Figs. 1B and 2B). This was true for both ACh and SNP at a concentration of 10 g/l; i.e., it holds for anodal and cathodal delivery.

Previous studies have found that NaCl alone has a lower resistance than water (2), and we found the same (Figs. 3B and 4B). However, when an agonist is added to the vehicle, the electrical characteristics of the resulting solution will clearly be different. We found that the resistance of water was reduced by the addition of an agonist to a value comparable to that of NaCl containing the same drug. In contrast with our results, however, Ferrell et al. (7) reported that ACh and SNP in water had a higher resistance than the same drugs in NaCl. It is difficult to compare these results with ours because, although they used the same iontophoresis system, this group used much lower currents than those used in the present study (5–20 μA) and delivered ACh and SNP simultaneously.

**Fig. 2.** A: relative change in skin blood flow to iontophoresis of 4 cumulative doses of sodium nitroprusside in water and NaCl vehicles. Responses were smaller for NaCl (P = 0.019). B: corresponding average voltage applied during each iontophoretic delivery. Values are means ± SD for 10 subjects.

**Table 1.** Correlations between voltage applied and skin blood flow response for the iontophoresis of 4 cumulative doses of ACh and SNP in sodium chloride vehicle

<table>
<thead>
<tr>
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<th>1 mC</th>
<th>2 mC</th>
<th>4 mC</th>
<th>8 mC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh R</td>
<td>-0.740</td>
<td>-0.715</td>
<td>-0.714</td>
<td>-0.288</td>
</tr>
<tr>
<td>P</td>
<td>0.004</td>
<td>0.006</td>
<td>0.006</td>
<td>0.340</td>
</tr>
<tr>
<td>SNP R</td>
<td>-0.810</td>
<td>-0.710</td>
<td>-0.775</td>
<td>-0.828</td>
</tr>
<tr>
<td>P</td>
<td>0.008</td>
<td>0.032</td>
<td>0.014</td>
<td>0.006</td>
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ACh, acetylcholine; SNP, sodium nitroprusside; R, Pearson’s correlation coefficient.

**Fig. 3.** A: relative change in skin blood flow to iontophoresis of 4 cumulative doses of water and NaCl vehicles alone delivered at the anode. B: corresponding average voltage applied during each iontophoretic delivery. Values are medians ± 75th and 25th percentiles for 6 subjects. Voltages were smaller for NaCl (*P = 0.004, **P = 0.017, and ***P = 0.009).
NaCl is that higher blood flow responses are achieved when water is used as the vehicle (2), and this has been presumed to be caused by the nonspecific effects of iontophoretic delivery. We also found that higher responses were measured using water (Figs. 1A and 2A). However, an alternative explanation for this may be that, when NaCl is used as the vehicle, these ions compete with those of the drug, so reducing the effective dose delivered. We have no direct evidence of this, but it is probably more likely to be this than a galvanic effect, given that we found no difference in resistance between the solutions. Ionic competition has also been suggested as an explanation for the greater variability in ACh and SNP responses measured with NaCl (2). However, another study found that 5.8% NaCl (but not 0.5 or 0.9%) reduced the blood flow responses, suggesting that there might only be a competition effect at higher NaCl concentrations (7).

When we iontophoresed the vehicles alone, the only significant change in flow we measured was a very small increase in response to the largest dose of water, administered via the cathode (Fig. 4A). Although we contend that this measurement of the galvanic response is not a valid assessment of the nonspecific effects that might occur during normal iontophoresis, also suggested by Ferrell et al. (7), it does indicate that the protocol we used is effective at limiting these effects. This is in marked contrast to other investigators who saw blood flow increases of up to sixfold, particularly with the cathode (1, 2, 8, 9). We used a lower current and/or a larger area of iontophoresis than many of these previous studies, and this will have reduced the charge density applied (which equals current divided by delivery area2) by up to a factor of 8. This has been observed in a study in which using a smaller iontophoresis chamber was found to triple the magnitude of the galvanic response (7). These are probably more important factors than choice of vehicle in reducing nonspecific blood flow changes.

Other investigators have reported using NaCl concentrations up to 5 mol/l (~15%), so perhaps the 0.5% we used was not high enough to lower the resistance sufficiently. In fact, we measured a similar, if not lower, voltage (7–10 V) across the electrodes with 0.5% NaCl alone compared with that (11 V) reported by Asberg et al. (2), who used 5 mol/l NaCl concentration is therefore unlikely to have been a significant factor in our results. Some authors have, in fact, suggested that higher NaCl concentrations lead to more ionic competition between the agonist and vehicle (7). Another influence on the resistance of an iontophoresis circuit is the electrical properties of the skin. A significant inverse relationship has been reported between the voltage applied during iontophoresis (i.e., the resistance) with a NaCl vehicle and the resulting blood flow response (11). The authors suggested that a lower resistance might be due to a greater availability of low-resistance pathways in the skin, such as sweat ducts and hair follicles. These are more highly vascularized, and drug delivery to these areas might therefore be more effective than delivery via high-resistance pathways further away from blood vessels. Our results do not support this hypothesis, however. Although we also found an inverse relationship between resistance and flow, it was only observed with the NaCl vehicle, not with water. This suggests that the explanation has more to do with the vehicle, its interaction with the drug being delivered, and the physical characteristics of the skin.

The electrical resistance of the skin is a function of the size and number of potential ionic pathways available. Na+ and Cl– are much smaller than ACh or SNP, and they will have a greater charge per surface area of the particle. They may therefore be favored when a current is formed, particularly when the skin resistance is higher. A consequently less effective delivery of ACh or SNP would result in a lower blood flow response. Conversely, lower skin resistance might mean fairer competition, more effective drug delivery, and a higher response. This would only be the case with an ionic vehicle such as NaCl. With water, there would be no competition, and therefore no such effect, as we have found. Furthermore, we measured lower blood flow responses using a NaCl vehicle than with water, which also suggests ionic competition. We have no evidence for this hypothesis, however, and there may be other credible explanations.

In summary, using NaCl as the vehicle for iontophoretic delivery of ACh or SNP does not lower the resistance of the skin.

Fig. 4. A: relative change in skin blood flow to iontophoresis of 4 cumulative doses of water and NaCl vehicles alone delivered at the cathode. Response was significant only at 8 mC dose of water (*P = 0.016). B: corresponding average voltage applied during each iontophoretic delivery. Voltages were significantly smaller for the 3 highest doses of NaCl (**P = 0.036). Values are medians ± 75th and 25th percentiles for 6 subjects.
solution and is therefore unlikely to decrease any nonspecific electrical effects. In fact, NaCl might actually compete with the agonist in establishing a current and so reduce the effective dose delivered. We therefore conclude that, at least with the size of current we are using and for these relatively small molecules, deionized water is a better iontophoresis vehicle for the assessment of microvascular function in skin and that large area electrodes should be used.

REFERENCES