moor instruments
laser Doppler blood flow assessment

Iontophoresis with Laser Doppler Assessment of Blood Flow Response, Issue 1.

Introduction

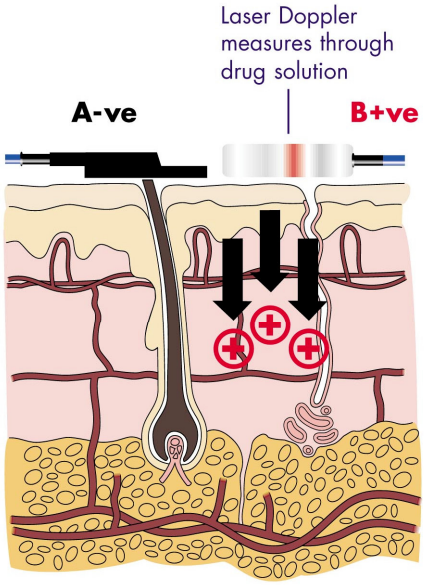
Penetration of healthy skin by drugs in solution is normally very limited due to the excellent barrier function of the stratum corneum (the most superficial layer of the skin).

This barrier can be overcome using iontophoresis: by applying an electrical potential (voltage) across the skin, drug ions become the charge carriers that convey the electrical current through the skin.

The equipment produced by Moor is primarily for testing skin blood flow responses (using laser Doppler monitoring or imaging) due to vasoactive drugs iontophoresed using the Moor iontophoresis controller (MIC2).

Use of iontophoresis as a test modality for investigating microvascular mechanisms is an on-going area of research. There are a number of confounding factors to the application of the technique and interpretation of results. Some of these will be reviewed with examples of applications.

The Iontophoresis Technique



A. Negatively charged reference electrode.

B. ION chamber containing **positively** charged drug ions and the laser Doppler probe.

The electric field at the ION chamber (electrode B) repels the drug ions which are forced through the surface of the skin. For negatively charged drugs, the ION chamber is attached to the negative terminal of the supply.

For direct electrical current to pass through the skin, two electrodes are required: one positive (the anode) and the other negative (the cathode). For iontophoresis of a positively charged drug ion (e.g. acetyl choline, Ach +) the drug solution (normally 1% w/v) is contained in an ion chamber which incorporates the positive platinum electrode (platinum is used to avoid polarisation/bubbles forming on the electrode).

The circuit is completed via the skin and flesh of the body to a second electrode. The second electrode may be another ion chamber, if an oppositely charged drug ion is to be used (e.g. sodium nitroprusside) or a passive electrode.

Moor Instruments Ltd Millwey Axminster Devon EX13 5HU UK
tel +44 (0)1297 35715 fax +44 (0)1297 35716
email sales@moor.co.uk website www.moor.co.uk
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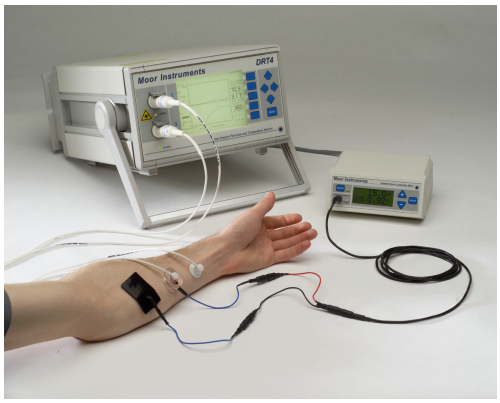
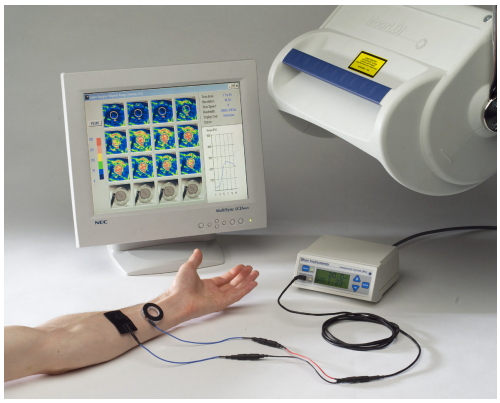
Applications



Typical setup using a Moor Instruments DRT4 and MIC2.



Typical setup using a Moor Instruments moorLDI2 and MIC2.

Iontophoresis and LD monitoring/imaging (as shown above) has been used in a wide range of applications to investigate disease mechanisms (see reference list). A small sample is briefly reviewed.

Endothelial function is assessed by iontophoresis of acetylcholine: areas of study include:

Chronic fatigue syndrome; increased response observed in CFS patients compared with controls (Spence VA et al, 2000; Khan F et al, 2004).

Diabetes; responses are reduced in diabetic neuropathy, with Charcot arthropathy and with neuropathy and vascular disease (Veves A et al, 1998)

Exercise; increased response in athletes before exercise but controls were similar post exercise (Kvernemo HD, 1998).

Heart failure; attenuated response in transplant recipients (Andreassen AK et al, 1998) and role of pro-inflammatory cytokines (Holm T et al, 2000);

Renal transplant; improved response was observed after statin therapy (Aasberg et al, 2001)

Rheumatic diseases; impaired responses have been observed in polyarteritis nodosa and Wegener's granulomatosis (Filer AD et al, 2003)

Smoking; impaired responses observed in older smokers (Pellaton C et al, 2002).

Smooth Muscle function is assessed by iontophoresis of sodium nitroprusside: responses were studied in many of the examples of endothelial function assessment, above.

Peripheral Autonomic Neuropathy can be assessed by indirect application of Acetylcholine chloride or histamine. In practice, it is assessed by monitoring or imaging from the area outside of the region iontophoresed or within a stimulated annulus. Areas of study include:

Small fibre neuropathies have been shown to produce a reduction in flare area but not maximal LD response to histamine (Bickel A et al, 2002).

Inhibition of Histamine Flare by iontophoresis of necrodomil sodium has been shown by Ahluwalia P et al, 2001).

RSD; axon reflex vasodilation to iontophoresis of acetylcholine was impaired in the painful limb compared with the non-affected limb (Westerman et al, 1992).

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Local Anaesthesia reduces the axon reflex flare to acetylcholine (Caselli A et al, 2003).

Skin Resistance

Skin normally presents the highest electrical resistance to current flow. The electrical resistance of underlying flesh and blood is much lower than skin. This does not impose any restriction on the positioning of the ion chamber(s) electrodes. However, for safety reasons the current path should not pass across the heart or the brain.

For practical reasons the ion chamber(s)/electrode should not be too close such that vasoactive responses at one site influence the other site. A separation of between 5 and 10cm is usually adequate.

"Galvanic Effect" and Skin Preparation

The non-specific response of skin blood flow to electricity has been termed the "galvanic effect". This has been shown to be stronger at the cathode than the anode (see Grossmann M et al 1995). It is observed when non-vasoactive ions are iontophoresed but can be present when active drugs are used.

To avoid the galvanic effect, lower currents are recommended (the same dose can be applied by extending iontophoresis duration). Currents lower than 50µA are recommended when small (~1cm^2) ion chambers are used. It may also help to reduce skin resistance by gently cleaning the skin with an alcohol wipe to remove natural oils. Flaky skin may also be removed by gently applying and removing adhesive strip: do not repeat this often as it can remove the stratum corneum and loss of this barrier function would impair the dose control obtained with iontophoresis.

Estimation of the Galvanic effect by iontophoresis of de-ionised water is not appropriate because the required voltage is higher than for an equivalent charge of drug ions (Asberg A et al, 1999). However, use of saline as a drug vehicle is contentious (see Ramsay ER et al, 2002 and Khan F et al, 2004). Use of topical anaesthetic has also been used to eliminate Galvanic effect (Morris SJ et al, 1996). This has also been shown to reduce axon reflex flare (Caselli A et al, 2002).

Drug Dose and Concentration

The drug dose delivered by iontophoresis is proportional to the change (Q) delivered:
i.e. the current (I) and its duration (t): $Q = I \times t$

In principle, the concentration of the drug solution does not matter so long as it is sufficient to contain enough ions for the dose intended. However, in practice a higher concentration of drug solution can produce a higher response for the same iontophoresis dose. This is thought to be due to the effect of competition with natural skin salts.

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Calculation of Iontophoresis Dose

The following calculation assumes that all of the electrical current is due to the passage of drug ions:

One univalent molecule passing into the tissue conveys one electron charge

1 amp for 1 sec conveys 1 coulomb of charge

1 electron charge = 1.6×10^{-19} coulombs

The number of molecules conveyed during iontophoresis = the dose (in coulombs)/ one electron charge

1 mol (gram molecular weight: 'M') contains $6.02 \times 10^{+23}$ molecules (Avogadro Constant, N)

Mass of one molecule = M/N

$$\text{Mass of drug iontophoresed} = \frac{\text{Dose in Coulombs}}{\text{Electron Charge}} \times \frac{M}{N}$$

$$\text{i.e. Dose (Mass)} = \frac{\text{Dose (coulombs)} \times \text{Molecular Weight}}{9.632 \times 10000}$$

Dose calculated from DRT4 / moorLDI settings

The current entered on the laser Doppler monitor at set-up and displayed on the Iontophoresis Controller is in units of micro Amps (1 µA = 10^{-6} A)

Time is entered in seconds and current in micro Amps, therefore:

$$\textbf{Mass of drug delivered (in µg)} = \frac{\textbf{Time x current x molecular wt}}{\textbf{96320}}$$

e.g. for acetyl choline chloride M = 181.66

If current = 100µA and duration = 60 sec

Mass of drug delivered is not greater than 11.3µg

(Compare this with the mass of drug in an ION1 ion chamber containing a 1% solution of ACh is about 2.13mg.)

External Influences on Skin Blood Flow

Microvascular responses during iontophoresis can also be influenced by other stimuli e.g. room temperature and humidity, ambient noise, movement and the patients' state of relaxation. Recent tea, coffee, meal, cigarettes, medication or other stimuli can also affect blood flow responses.

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Factors Affecting Iontophoresis

The effect of current and its duration have been described above: i.e. the ion dose delivered is proportional to current and time; however prolonged application can result in increased skin resistance due to polarisation of the skin. Pulsed or intermittent current application has been used to avoid this effect (Chien YW, 1989). This has not been observed during short-term tests.

Drug concentration has also be described above (see Drug Dose).

Other factors, such as pH, have also been shown to affect iontophoretic transport during longer, higher dose therapeutic applications. These have not been considered as confounding factors for the low doses used during iontophoresis as a test modality.

Advantages of Iontophoresis

Local stimulation of skin avoids systemic drug effects

- Minimally invasive i.e. no injections by needle so no blood flow response due to needle trauma.
- Easy to apply, though skills and care are required.
- Re-usable ion-chambers: drugs, adhesive discs and batteries are the only consumables.
- Controllable and verifiable dose delivery (within the constraints of competition from natural skin salts.
- Wide range of ionic drugs available.

Disadvantages of Iontophoresis

Occasional minor irritation and itching, erythema is normally short lasting.

Skin pigmentation can occur with longer application of some drugs (e.g. sodium nitroprusside can stain brown) but normally subsides after a few weeks.

Minor skin damage (including burns) can occur when higher currents are used. This has been observed in and around sweat ducts.

Contact sensitisation to some drugs has been reported.

Contra-indications

Iontophoresis should only be used on patients under the direction of a physician.

a) DO NOT apply electrodes to the chest or in any position where current could flow through the heart or lungs: i.e. do not attach electrodes to different limbs.
b) DO NOT apply electrodes over or across the right and left temporal regions of the head.
c) DO NOT apply electrodes in the orbital region (eyes).
d) DO NOT apply electrodes over damaged skin.
e) On patients with known skin allergies, use only under *direct* supervision of a physician.
f) DO NOT use iontophoresis on patients with electrically sensitive support systems (e.g. pace-makers).
g) DANGER - EXPLOSION HAZARD. Equipment not suitable for use in the presence of a flammable mixture with air or with oxygen or with nitrous oxide.

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References

A more extensive list of iontophoresis/laser Doppler references can also be downloaded.

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