Iontophoresis with Laser Doppler Assessment of Blood Flow Response.

Introduction

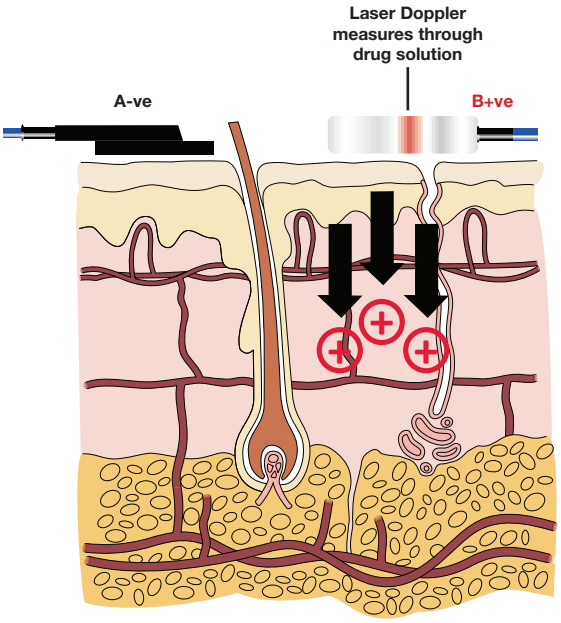
Penetration of healthy skin by drugs in solution is normally very limited due to the excellent barrier function of the stratum corneum (the most superficial layer of the skin).

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 exclusively for testing skin blood flow responses (using laser Doppler monitoring/ imaging or laser speckle contrast imaging) due to vasoactive drugs iontophoresed (using the Moor iontophoresis controller, moorVMS-ION).

Use of iontophoresis as a test modality for investigating microvascular mechanisms is an on-going area of research with a range of protocols described.

The Iontophoresis Technique



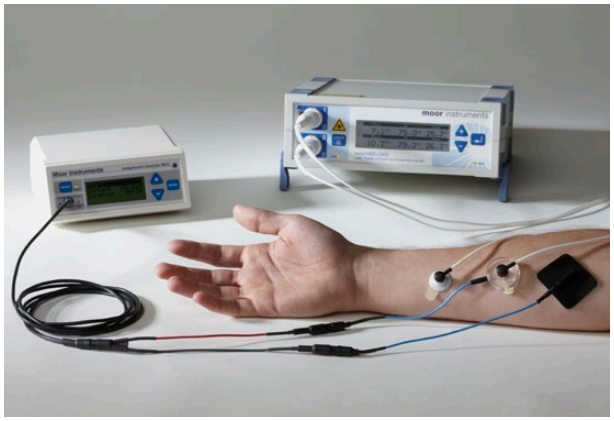
A. Negatively charged reference electrode.
B. ION chamber containing **positively** charged drug ions and the laser Doppler probe.

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; i.e. A and B are reversed.

For direct electrical current to pass through the skin, two electrodes are required: one positive (the anode) and one 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.

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

Clinical Research Applications



Typical setup using a Moor Instruments moorVMS-LDF2 and MIC2; one LD probe in the ion chamber, the second LD probe at a control site.

Typical setup using a Moor Instruments moorFLPI-2 and moorVMS-ION.

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

Endothelial function has been assessed by iontophoresis of acetylcholine (Ach); reproducibility for this technique has been assessed by Kubli et al, 2000. Areas of study include:

Alzheimer's Disease; as a predictive tool for response to cholinesterase inhibitors (Connelly et al, 2019).

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

Diabetes; responses are reduced in diabetic neuropathy, with Charcot arthropathy and with neuropathy and vascular disease (Veves et al, 1998). Iontophresis of treprostinil increased flow in the malleolus area of diabetic patients (Hellmann et al, 2015).

Diet; Mediterranean diet was found to improve endothelial function in older people (Klonizakis et al, 2014) and has also been observed with beetroot juice supplementation in patients with Raynaud's Phenomenon (Shepherd et al, 2020); a decline was found following weight gain, over 3 years, in people with diabetes (Casanova et al, 2020).

Ethnicity; differences have been studied by Pienar et al, 2014.

Exercise; increased response in athletes before exercise but controls were similar post exercise (Kvernmo et al, 1998; also, see Klonizakis et al, 2014, regarding Mediterranean diet).

Heart failure; attenuated response in transplant recipients (Andreassen et al, 1998) and role of proinflammatory cytokines (Holm et al, 2000).

Menopause; a lower response in post-menopausal women than for pre-menopausal and post-menopausal women taking estrogen replacement therapy (Arora et al, 1998).

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

et al, 2003).

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

Skin temperature; its effect on the assessment of endothelial function, with iontophoresis of Ach, has been studied by Abraham et al, 2013.

Stroke; survivors with high serum urate had a reduced response to Ach (Khan et al, 2008).

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

Peripheral Autonomic Neuropathy has been 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 et al, 2002); Linagliptin increased axon reflex related flare to Ach, used as a marker of neurovascular function (Baltzis et al, 2016).

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

Complex Regional Pain Syndrome (referred to as Reflex Sympathetic Dystrophy); axon reflex vasodilation to iontophoresis of acetylcholine was impaired in the painful limb compared with the non-affected limb (Westerman et al, 1992).

Local Anaesthesia has been found to reduce the axon reflex flare to acetylcholine (Caselli et al, 2003); iontophoresed norepinepherine caused vasoconstriction (Beed et al, 2009).

Technical considerations

There are a number of confounding factors to the application of the iontophoresis technique and interpretation of results.

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. Practical ways to reduce skin resistance, and vehicle influence, are described by Khan et al, 2004.

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 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 et al, 1999). However, use of saline as a drug vehicle is contentious (see Ramsay et al, 2002 and Khan et al, 2004). Use of topical anaesthetic has also been used to eliminate Galvanic effect (Morris et al, 1996). This has also been shown to reduce axon reflex flare (Caselli et al, 2002).

Drug Dose and Concentration

The drug dose delivered by iontophoresis is proportional to the charge (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.

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

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

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

Dose calculations

Note: a single dose can be programmed on the moorVMS-ION when used in stand-alone mode; single or multiple doses can be applied when moorVMS-PC PC software controls the moorVMS-ION.
The current entered on the moorVMS-ION or PC software at set-up 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 = 50μA and duration = 60 sec, mass of drug delivered is not greater than 5.66μg

(Compare this with the mass of drug in an ION1 ion chamber containing a 1% solution of Ach; it is about 2130μg.)

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, the patients' state of relaxation and the duration of acclimatisation to test conditions. Recent tea, coffee, meal, cigarettes, medication or other stimuli can also affect blood flow responses.

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.

Drug concentration effect has been 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, the indication for use of moorVMS-ION.

Advantages of Iontophoresis

Local stimulation of skin avoids systemic drug effects

- Non-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 gel pads 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.

Poor contact between the passive electrode and the skin could cause skin burn and should be avoided. This condition is indicated on the moorVMS-ION with a 'HIGH' resistance and/or low current warning.

Contra-indications

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

- Do not use chambers, electrodes or connecting leads that appear damaged.
- Do not use the moorVMS-ION on patients who have electrically sensitive support systems fitted (e.g. pace-makers).
- Do not apply chambers or 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.
- Do not apply chambers or electrodes over or across the right and left temporal regions of the head.
- Do not use the Iontophoresis Controller for tests in the orbital region (eyes).
- Do not apply chambers or electrodes over damaged skin or in any position where current could flow through damaged skin.
- Do not apply iontophoresis chambers or electrodes to areas of skin where ultrasound measurements have been recently performed.

The moorVMS-ION is not suitable for use with flammable anaesthetics.
The moorVMS-ION is not suitable for use in an oxygen rich environment.
The moorVMS-ION is intended for clinical research use only. It is not intended to deliver drugs for the treatment of illness or disease.

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NB: this list is intended to illustrate the range of research conducted with use of the iontophoresis technique and potential clinical significance of its use.

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