6. Clinical Applications of Iontophoresis and Sonophoresis
I. Modified sweat chloride test in diagnosis of cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations of a gene located on the long arm of chromosome 7. The gene product is the 1480-amino-acid cystic fibrosis transmembrane conductance regulator (CFTR), a protein that normally regulates and participates in the transport of electrolytes across epithelial cell membranes and probably across intracellular membranes as well (Stern, 1997). The diagnosis of CF presents a formidable challenge to the physicians because of the great variability in the frequency and severity of clinical manifestations and complications. The diagnosis of CF is mainly based on phenotypic (clinical) features (Table) and in most cases, confirmed by demonstration of an elevated concentration of electrolytes in the sweat (Rosenstein, 1998). Pilocarpine iontophoresis sweat test developed by Gibson and Cook (1959) is the standard test used for screening of patients susceptible of CF. This standard sweat test and similar tests with slight modifications are the routine diagnostic tests in many Western countries for CF (Rosenstein, 1990).

Cystic fibrosis is reported to be extremely rare in blacks and orientals. Until now only 29 cases are reported from India (Prasad et al., 1990). The reported rarity of the disease in India and the concerns of a fellow pediatrician (at Kasturba Hospital, Manipal) regarding the presentation of cases susceptible of CF prompted us to undertake this work. We report here a modified pilocarpine iontophoresis sweat test (modified from Gibson and Cook, 1959) for screening of patients susceptible of CF. In all, 60 normal individuals and 120 patients (suspective of CF) were screened by the modified pilocarpine iontophoresis sweat test. In three cases, presence of CF was confirmed based on results of sweat chloride test and clinical features. The test is now routinely being employed in Kasturba Hospital, Manipal (South India) for screening of CF patients.

Materials and Methods

Pilocarpine nitrate was obtained as a gift sample from FDC Ltd., Mumbai. Filter paper disks (Whatman Std No.1, 3 cm in diameter), squares of plastic sheet 4 x 4 cm, water proof adhesive tape, an electronic analytical balance with LCD display (Sartorius, Model BP 61, sensitivity 0.001mg), disposable plastic jars with tight lids and double distilled Milli-Q® filtered ultrapure water were used. Chloride concentration in the sweat
was analysed by using MenaGent Chlorofix® kits (Menarini Diagnostics, Firenze). The mechanism of this reagent is based on reaction of chloride ions with mercuric thiocynate leading to formation of a colored compound which is then analysed at 460 nm. In few cases chloride was also analysed by flame photometry.

**Iontophoresis unit**

We used a specially designed pulsed direct current iontophoresis apparatus in which the source of energy was 220 volts AC transformed to a maximum of 20 volts DC with a variable current output (0 µA - 9000 µA). The device has a facility to increase or decrease the current, according to the needs and a digital read out device to monitor the current levels. The electrodes were made of aluminum foil or circular silver/silver chloride electrodes (2- 2.5-cm diameter).

**Patient selection criteria**

The 60 normal non-cystic control subjects were children in age group of 2-10 years and few between 15-25 years of age. Few healthy adults were also recruited from personnel groups in College, hospital, students and friends. The 120 patients suspicious of CF included were those referred to the Department of Paediatrics of the Kasturba Medical College and Hospital, Manipal. The patients were mostly from Tamil Nadu, Kerala and Karnataka states (South India), aged between 4 months to 15 years of age. The Paediatrics department of Kasturba Hospital had set certain indications for cystic fibrosis sweat testing based on clinical experience and literature reports (Table 23).

The screening test for CF by sweat test was conducted at Department of Paediatrics in consultation with the Department of Chest and Allergy, Kasturba Hospital, Manipal.

**Test Procedure**

The principle of the test is that iontophoresis with pilocarpine produces localised sweating in a small and limited area of the skin. The sweat is then collected with the help of ashless filter paper disks and analysed for chloride content after suitable dilution. The detailed procedure is as follows.

The iontophoresis was performed using the forearm of the patient. As pilocarpine is positively charged, anodal iontophoresis was performed. First, the forearm of the patient
was thoroughly cleaned with double distilled Milli-Q® filtered water using absorbent
cotton. Filter paper disk (or thin cotton gauze) was moistened with few drops of
pilocarpine solution (0.2%; if cotton gauze, 0.1%, 3-4 mL). This disk was placed on the
area cleaned with distilled water. Positive electrode was then placed over the disk and
secured in place using rubber bands or adhesive bandage. Negative electrode using cotton
gauze soaked in sodium bicarbonate solution (0.07 mol/L) was used as indifferent
electrode and was placed adjacent to positive electrode. When in place, the electrodes were
connected to the pulsed current iontophoresis unit (2.5 KHz, 50% on: off ratio). Slowly,
the current was raised to 2.5 - 4 mA and the iontophoresis was continued for a period of
5 - 10 minutes. Direct contact between the skin and the electrodes was avoided to reduce
the possibility of small burns or itching sensation arising out of iontophoresis. If the
patient complained of discomfort, the current was further reduced. A tickling sensation at
the site of electrode is a common finding and should be disregarded. After the test, the skin
may be somewhat reddish (in small children), but the color disappears within a few hours.
After the iontophoresis was completed, the electrodes were removed and the area of
iontophoresis was cleaned several times with double distilled water and dried completely
with cotton gauze (Drying the area completely is absolutely essential). The skin area must
not be touched with figures or contaminated in any other way. Then, a disk of filter paper
(or stacks of 2-3 disks) from a previously weighed bottle (plastic jars) was removed with
forceps and placed over the area that was iontophoresed. The filter paper disk was then
covered with a plastic square, the edges of which were carefully secured with four stripes
of adhesive tapes (Fig. 50). Iontophoresis of pilocarpine induces localised sweating and
sweat starts collecting in the filter paper disk. The collection period of 30-90 minutes is
required, however it may be extended as long as necessary. In general, the appearance of
droplets on the plastic sheet indicates that enough sweat has been accumulated. The
droplets must be included in the collection. The plastic square is then removed and the
moist filter paper quickly returned to the weighed plastic jar and again weighed to know
the exact weight of sweat collected.

Tests with less than 60 mg of sweat were discarded. The sweat thus collected was
analysed for the chloride content using MenaGent Chlorofix® kits by Autoanalyser
(Technicon, RA-2000). Double distilled Milli-Q filtered ultrapure water was used for
dilution of the samples. The chloride content of the filter paper disk was also measured
Fig. 50. Collection of sweat from forearm by pilocarpine iontophoresis. Iontophoresis unit used for pilocarpine delivery is also seen.

periodically (which served as control sample). Few samples were also analysed by flame photometry to check the obtained results with Chlorofix®. For each patient, the test was done in duplicate i.e. both on left and right arms. The average sweat chloride value was used.

Table 23. Indications for cystic fibrosis sweat testing

<table>
<thead>
<tr>
<th>Pulmonary and upper respiratory tract infections</th>
<th>Gastrointestinal indications</th>
<th>Metabolic and other indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Cough</td>
<td>Meconium ileus</td>
<td>Positive family history</td>
</tr>
<tr>
<td>Recurrent or chronic pneumonia</td>
<td>Meconium plug syndrome</td>
<td>Failure to thrive</td>
</tr>
<tr>
<td>Wheezing</td>
<td>Prolonged neonatal jaundice</td>
<td>Salty taste to skin</td>
</tr>
<tr>
<td>Hyperinflation</td>
<td>Stetorrhea</td>
<td>salt crystals on skin</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>Rectal prolapse</td>
<td>Salt depletion-syndrome</td>
</tr>
<tr>
<td>Retractions</td>
<td>Mucoïd impacted appendix</td>
<td>Metabolic alkalosis</td>
</tr>
<tr>
<td>Atelactasis (especially of the right upper lobe)</td>
<td>Late intestinal obstruction</td>
<td>Hypoprotrombinemia</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>Recurrent intussusception</td>
<td>Vitamin A deficiency (bulging</td>
</tr>
<tr>
<td></td>
<td>Cirrhosis</td>
<td>fontanelle is a key sign)</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>Portal hypertension</td>
<td>Azoospermia</td>
</tr>
<tr>
<td>Mucoid <em>Psuedomonas</em> infection</td>
<td>Recurrent pancreatitis</td>
<td>Absent vas deferens</td>
</tr>
<tr>
<td>Nasal polyps</td>
<td></td>
<td>Scrotal calcifications</td>
</tr>
<tr>
<td>Pansinusitis</td>
<td></td>
<td>Hypoproteinemia</td>
</tr>
<tr>
<td>Digital clubbing</td>
<td></td>
<td>Edema</td>
</tr>
</tbody>
</table>
Results

In the above tests, between 80 to 930 mg of sweat was obtained in each test. Test yielding sweat less than 60 mg was discarded. Preferably, a minimum of 100 mg or more of sweat was used for analysis. Results of sweat chloride analysis from normal individuals indicated a range of sweat chloride from 9 to 51 milliequivalents/liter with an average of 31 milliequivalents/liter. Results with Chlorofix reagent and flame photometry correlated well. Most of the patients included in the sweat chloride test showed normal sweat chloride levels. Only four patients showed marked increase in sweat chloride i.e. 70 mEq/lit, 110 mEq/lit, 132 mEq/lit and 120 mEq/lit. Repeated tests in these four patients showed almost similar sweat chloride tests. Cystic fibrosis was confirmed in three patients based on reports of repetitive sweat chloride analysis and clinical presentation of the patients (clinical signs such as pulmonary and/or gastrointestinal and laboratory tests) as discussed earlier. The borderline sweat test in the patient with 70-mEq/liter sweat chloride was repeated several times with almost same results at all times. The patient was a three-year-old boy with bronchitic asthma and atopic dermatitis. Supplementary clinical examination did not confirm the diagnosis of CF.

Other than CF, elevated sweat chloride levels are seen in a very few diseases, such as, adrenocortical insufficiency, certain types of ectodermal dysplasia, diabetes insipidus, fucosidosis, hypothyroidism, familial hypoparathyroidism, atopic dermatitis and malnutrition. In above three patients in whom the disease was confirmed, none of these conditions were observed.

Discussion

Cystic fibrosis (CF) is the most common life-limiting genetic disorder in whites, with an incidence of 1 in 3200 newborns in the United States (Rosentein, 1998). The disease is reported to be extremely rare in blacks and orientals (Prasad et al., 1990). Early diagnosis is highly desirable for a variety of reasons, including improved prognosis, avoidance of diagnostic and therapeutic misadventures and timely genetic counseling. There are various diagnostic tools available in developed countries for diagnosis of CF such as pilocarpine iontophoresis sweat test, Macroduct® and Sweat.Check™ analyser (Wescor, Inc. USA), CF Indicator (Medtronic, Inc.), Genotyping (DNA testing) and
immunoreactive trypsinogen (IRT) measurements (Rosenstein, 1998; Warwick et al, 1986; Wescor Information Booklet, UT, USA, 1997).

As the disease is reported to be rare in India, the diagnostic test (pilocarpine iontophoresis) is not the routine analytical tool available in many hospitals. There are very few reports available from Indian hospitals regarding the use of quantitative pilocarpine iontophoresis sweat test. The lack of expertise and non-availability of the suitable instruments and method of chloride analysis further complicates the prospects of sweat chloride test. The present study was initiated at behest of the interest shown by fellow pediatrician from local referral hospital (Kasturba Hospital, Manipal) because of the manifestation of symptoms of the disease in few cases. The results of screening test for CF confirmed the presence of disease in three patients. The finding is contrary to the general observation that CF is extremely rare in India. Therefore, much more such screening tests are required to establish the exact epidemiology of the disease in India.

In literature, various methods of sweat chloride analysis have been reported which includes use of flame photometry, chloride specific microelectrodes, coulometric titration, anion exchange chromatography and use of high performance liquid chromatography (Miller et al., 1985; Itano et al., 1985; Keevil and Wong, 1995). In the present study, Chlorofix reagent based on mercuric thiocyanate was used. The easy availability of the reagent and use of simple instrument such as spectrophotometer makes the method further simpler and acceptable. The results also correlated well with flame photometry results.

Conclusion

From the results of the study it can be concluded that the quantitative pilocarpine iontophoresis sweat test can be conducted easily. Maintenance of proper quality control protocols and collection of sufficient amount of sweat were found to be absolutely essential for reliable results. The simpler test becomes important particularly because of the lack of advanced means (Genotyping) available to general public in developing country like India. Such a screening procedure may help one to know the exact status of CF in India.

The bottom line however remains that negative sweat test does not completely ‘rule out’ CF.
II. Application of iontophoresis in pediatric dentistry: Extraction of deciduous teeth*

The extraction of deciduous tooth in children frequently requires periapical injection of local anaesthetic, lignocaine. Fear and pain associated with the insertion of needle makes the procedure unpleasant and traumatic experience for the patient. Injections even by experienced pedodontist may produce severe distress and lead to the development of 'needle phobia', especially if the child requires subsequent repeat extraction. The problem is not solved by use of fine needles or by patient consolation. Smaller children, particularly in age group of 4-15 pose real problems.

The use of lignocaine iontophoresis as the sole local anaesthetic for extraction of retained deciduous teeth has been suggested to avoid some of the problems associated with use of lignocaine injection (Gangarosa, 1974). In the present study, an attempt has been made to optimise the different parameters in lignocaine iontophoresis for rapid and maximal local anaesthesia with better patient compliance. The various parameters which have been optimised includes- drug concentration, current strength, duration of application, electrode size, distance between active and indifferent electrodes and use of natural permeation enhancer.

Materials and Methods

Lignocaine hydrochloride was a gift sample from Astra-IDL Ltd., Banglaore, d-limonene (Merck, Germany), sterile cotton gauze and sterilized dental equipment's. All solutions were made using Milli-Q filtered double distilled water.

Iontophoresis unit

We used a specially designed direct current iontophoresis apparatus built in-house in which the source of energy was 220 volts AC transformed to a maximum of 20 volts DC with a variable current output (0 μA - 9000 μA). The device has a facility to increase

*Part of the work presented in this section has been demonstrated as preconference course in National Conference of Indian Society of Pedodontics and Preventive Dentistry, Nov 13- 15, 1999. Amritsar. India.

or decrease the current, according to the needs and a digital read out device to monitor the current levels. In some cases, miniaturized portable battery operated iontophoresis unit was also used (See chapter 3).

The electrodes were specially designed and were made out of pure silver plates. The active clip like electrode (positive electrode) was designed in such a way that it facilitate easy placement of the electrode to gums without any additional support (Fig. 51a). Two types of indifferent electrodes were designed to examine the effect of distance between active and indifferent electrodes. In one study, the indifferent electrode was placed on the volar surface of forearm while in another it was fixed in the cheek, near to the active electrode. In the former case, a simple circular silver disk (coated with silver chloride) was used as indifferent electrode while in latter case specially designed cheek electrode (made of silver) was used (Fig 51b).

![CHEEK ELECTRODE CLIP ELECTRODE](image)

**Fig. 51.** Active clip electrode and indifferent cheek electrode.

**Patient selection criteria**

Forty healthy co-operative children were elected after obtaining prior consent from them as well as their guardians. Before starting the study, iontophoresis procedure was explained to all the patients and their guardians in their regional language. The patients comprised of 26 were boys and 14 girls with an average age of 9.1 (7- 13) year. The subjects were selected on the basis of following criteria,

1. Children with retained deciduous tooth or root stumps
2. Deciduous tooth requiring orthodontic extraction
Radiographs were taken to assess the root length prior to extractions and latter confirmed with a boley guage.

The subjects were divided randomly into groups depending upon the parameters examined (Table 24). In each group eight extractions were done to make a total of 48 extractions.

**Procedure of lignocaine iontophoresis**

For iontophoresis, solution of lignocaine hydrochloride was prepared in ultrapure water. In all solutions, adrenaline bitartrate was added to a final concentration of 1:50,000. The pH of the solution was adjusted to 5 to 5.5 with dilute sodium hydroxide.

Figure 52a shows the iontophoresis apparatus completely connected for use. The patient was prepared for extraction and the area of the tooth to be extracted was cleaned with sterile cotton. Small pieces of sterilized gauze were soaked in lignocaine solution and placed on the mucosal surface (bilaterally) of the tooth to be extracted. The active clip electrode (positive electrode) was placed over the gauze (Fig. 52b). The indifferent electrode was placed on the volar surface of forearm. The site of the indifferent electrode was dried and swabbed with spirit before application to remove surface oils and ensure good contact. In one group (group 5), the indifferent electrode was fixed to the cheek. The electrodes were connected to the iontophoresis unit and the current was then slowly increased to 4 to 6 mA. After the applying for 5 to 10 minutes, the current was slowly decreased to zero.

After the removal of electrodes, the induction of anaesthesia was examined by using a sickle probe. Visual analog scale (pain score by questionnaire) and measurement of specific activity for pain (eye squeeze, horizontal or vertical mouth stretch, eye blink, nose wrinkle etc) were used to denote child’s reaction for presence or absence of pain.
Fig. 52a. Iontophoresis device completely connected for use. The positive electrode (clip electrode) was attached at the area of tooth to be anaesthetised while negative electrode was placed on forearm.

Fig 52b. The clip electrode (positive electrode) placed over the area of the tooth to be anaesthetised.
Table 24. Various groups for iontophoresis

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug concentration (%)</th>
<th>Current strength (mA)</th>
<th>Time (Min.)</th>
<th>% Extraction without pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>87.50</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>87.50</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
<td>10*</td>
<td>100.00</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>100.00</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>100.00</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>4</td>
<td>5*</td>
<td>100.00</td>
</tr>
</tbody>
</table>

In all groups, the indifferent electrode was silver disk placed on the volar surface of forearm (except group 5) and the size of the active electrode was ≈ 1x1.5 cm (except group 3), see below.

*Effect of current strength and active electrode size (≈ 1.25 x 1.75 cm), with 1% limonene, indifferent electrode was cheek electrode, with limonene (1%).

Results

Most of the patients were comfortable during the iontophoresis procedure. Some patients felt slight tingling sensation which was absolutely bearable, but in some cases, the current was slightly reduced if the tingling sensation persisted. In about 96% of the cases, extraction of the tooth was satisfactory with no pain or discomfort to the patient. In about 4% of the cases (2 out of 48), sufficient level of anaesthesia was not achieved and the tooth extraction was then subsequently carried out using infiltration anaesthesia. The reason for the lack of anaesthesia in these two patients might be attributed to the observed longer root length (> 5 mm).

In all concentrations examined, duration of ten minutes of iontophoresis was found to be sufficient to achieve required degree of anaesthesia. With higher concentration, even 7-8 minutes duration was found to be sufficient for induction of anaesthesia. Bigger active electrodes (≈1.25 x 1.75 cm) were found to induce better anaesthetic effect. When the cheek electrode was used as indifferent electrode, induction of anaesthesia was found to be rapid. Addition of natural penetration enhancer d-limonene resulted in prompt induction of anaesthesia. With presence of d-limonene, 5 to 6 minutes of iontophoresis was found to be sufficient to achieve anaesthesia.
Discussion

The extraction of deciduous tooth by localised anaesthesia using lignocaine iontophoresis was found to be satisfactory. Patient acceptance of the procedure was high with no signs of anxiety or stress. The main advantages of this non-invasive means of anaesthesia are; avoidance of fear and anxiety caused by needle insertion, no tissue distortion, adequate local and minimal systemic concentration of drug, improved patient compliance and development of better doctor–patient relationship.

Iontophoresis has been reported to penetrate to an approximate depth of 1 cm (Tyle and Agrawala, 1989). Most of the retained deciduous teeth have root attachment less than 1 cm. In such cases, deep mucosal anaesthesia obtained by iontophoresis is adequate to extract the tooth. If the tooth has a bony attachment, local anaesthetics can be infiltrated into the iontophoretically anaesthetised areas surrounding the tooth even on the hard palate, without patient discomfort.

As the lignocaine is positively charged, anodal iontophoresis was performed. No extraneous ions (except adrenaline) were included so as to maximise the delivery of lignocaine. The adrenaline (vasoconstrictor) was included in the solution to prolong the duration of anaesthesia. As the adrenaline is also positively charged, combination of lidocaine and adrenaline iontophoresis was possible from anode. To keep the lidocaine in maximally ionised state for better iontophoretic delivery, solution pH was kept at 5.5 (Russo et al., 1980; Siddiqui et al., 1985).

The clip electrodes were fabricated so as to cover the mucosal surface around the tooth to be extracted. The clip electrode had a flexible bent which allowed proper placement of the electrode on the gums without patient discomfort. Bigger active electrodes resulted in better anaesthetic effect which might be due to increased surface area subsequently resulting in anaesthetic effect on larger portion of gums. The placement of indifferent electrode near to active electrode (i.e. use of indifferent cheek electrode) resulted in rapid anaesthetic effect. This might be due to decreased resistance to flow of ions by use of cheek electrode. With higher concentration of drug, anaesthetic effect was rapid.
Use of d-limonene in combination with lignocaine resulted in prompt induction of anaesthetic effect. D-limonene has been reported to enhance the iontophoretic delivery of drugs in vitro across skin (Bhatia and Singh, 1999). It has been reported to cause transient disruption of the skin barrier properties. On mucosal surface also d-limonene is expected to exhibit similar action. D-limonene is a natural penetration enhancer from citrus fruits. Essential oils in citrus fruits such as lemon or orange have been widely used as flavouring substances for perfumes, foodstuffs and medicines. The biological safety of these compounds has been well-documented (Kikuchi et al., 1992). With d-limonene, 5-6 minutes of duration was found to be sufficient to achieve desired anaesthesia.

Finally, the use of miniaturized portable battery powered iontophoresis unit (which was under development during the course of study and introduced only in latter few cases) was found to be more acceptable to both the patient as well to the dentist. This was particularly because of the miniaturized and less complicated look (with minimum wiring) of the device.

**Conclusion**

In the present study, lignocaine iontophoresis was found to be satisfactory for extraction of deciduous teeth in children. Construction of suitable electrodes and use of penetration enhancer was found to accelerate induction of anaesthesia. For realisation in clinics, further studies are however essential to improve patient compliance and further reduce the duration of current application and increase the depth of drug penetration.
III. Topical methotrexate delivered by iontophoresis in the treatment of recalcitrant psoriasis – a case report

Introduction

Palmoplantar psoriasis (PPP) is a disabling and disfiguring condition, in which the acral skin lesions cause considerable difficulties in every day life including employment problems and inability to work (Farber and Nall, 1992). Management of PPP is a test of skill for a dermatologists and test of endurance for the patient. A great variety of remedies have been used over the decades but PPP is notoriously refractory to conventional therapy. Lubricants, anthralin and topical steroids are the mainstay of treatment. In its severe form, systemic methotrexate is indicated with its associated risks. We report here a case of palmer psoriasis treated with iontophoresis of methotrexate.

Case report

A 46-year-old male presented with well-defined psoriatic plaques bilaterally on palms of 6 years duration. He had used several medications without success. We treated the lesion on the right palm with topical methotrexate delivered by iontophoresis while the left palm lesion served as a control and did not receive any treatment. The right palm lesion was selected for treatment because it was more severe than left palm lesion.

A locally fabricated portable iontophoresis device was used. The electrodes were made of aluminum foil supported by adhesive polyethylene sheet. As the methotrexate ions carry a negative electrical charge (Methotrexate disodium salt), cathodal iontophoresis was performed. Thin cotton gauze soaked in methotrexate disodium solution (10 mg/mL, total 4-6 mL) was placed over the lesion to be treated and was connected to the negative electrode of the instrument (aluminum foil). The positive electrode, using isotonic saline as the indifferent electrode, was placed on the adjacent

*Part of the work presented in this section has been presented and communicated for publication; see below;


area on forearm (Fig. 53). A direct current from the iontophoresis unit was then passed through the solution to deliver the drug to the affected lesion. The patient felt a mild electrical sensation at the moment when the electrical current from the iontophoresis unit started to pass into the skin, but the patient experienced no discomfort during therapy. For each treatment, the current strength was maintained at 10 - 15 mA (approximately 0.6 mA/cm² of electrode area) for 15 minutes. Iontophoresis was performed once in a week for a period of total four weeks.

The lesions were assessed on a severity scale of 0-4 with regards to erythema, scaling and induration and the sum total of scores in both the lesions was assessed at each visit. The lesion on right palm showed very good (> 75%) improvement at the end of four weeks whereas the left palm lesion remained same (Fig. 54). There were no side effects.

Discussion

Iontophoresis is the use of direct electrical current to increase the penetration of ionic drugs into the body for therapeutic purposes. With this technique, high concentrations of drugs are delivered to a limited area. It is a proven mode of drug delivery for antiviral and local anaesthetic agents, by means of which localised concentrations of drug can be obtained with negligible systemic levels. Iontophoresis of chemotherapeutic agents such as cisplatin and bleomycin have been used to treat epithelial neoplasms, including basal cell and squamous cell carcinoma (Chang et al., 1993; Tsuji 1991). Iontophoresis of antiviral agents such as Iodoxiuridine, Ara-AMP and acyclovir have been used to treat surface Herpes Simplex Virus (HSV) lesions (Ganarosa and Hill, 1995). In addition, vinblastin has been used in transdermal iontophoresis for the treatment of Kaposi's sarcoma (Smith et al., 1992). Iontophoresis has also been used to deliver medication to underlying joints and in pain therapy with neurotoxic medications, demonstrating that drugs are delivered through the superficial and adneal epithelium (Glass et al., 1980). However, there have been no reports of systemic side effects of medications after iontophoresis for such local applications.

Methotrexate is an antimetabolite that has been used systemically for the treatment of refractory palmoplantar psoriasis (Weinstein et al., 1989). The systemic administration of the drug is associated with the severe toxic effects of the drug (leukopenia,
Fig. 53. Iontophoresis of methotrexate to psoriatic palms. The negative electrode was connected to methotrexate containing gauze on palm.

Fig. 54. After treatment, the right palm lesions healed substantially, leaving partial scars. Left palm without iontophoresis treatment.
thrombocytopenia, anemia and gastrointestinal side effects). Therefore, topical methotrexate if effective in PPP is specially rewarding. Early trials of topical methotrexate therapy by Vonscott and Reinertson, (1959) and Nurse (1963) were unsuccessful despite the data that methotrexate acts directly on psoriatic plaques rather than systemically at a distant site (Weinstein et al., 1971). Stewart et al (1972) investigated the efficacy of topical methotrexate cream and compared it with intralesional methotrexate sodium injection. In case of topical application no clinical effect was detected and methotrexate was not found in skin or plasma. They concluded that the lack of cutaneous penetration of topically applied methotrexate might be attributed to the physical and chemical properties of the drug i.e. molecular size, ionisation at physiological pH and unfavorable lipid/water coefficient. Selection of proper vehicle and use of chemical penetration has been attempted to improve cutaneous penetration of methotrexate (Ball et al., 1982; Weinstein et al., 1989). The ionic structure of the methotrexate makes it a suitable candidate for iontophoretic therapy. Sweat glands and other shunt pathways offer paths of reduced resistance for iontophoresis; thus the drug is delivered more effectively down the cutaneous tissues.

Methotrexate iontophoresis has several advantages; a) Maximum local effects and no systemic side effects, b) Cosmetic results are equal to or better than those of other therapeutic modalities, c) It may be used in other surface areas in which the disease may be disfiguring or disabling, d) Treatment is on an outpatient basis with minimal physician contact necessary and e) There are no injections or discomfort for the patient.

Conclusion

Topical methotrexate delivered by iontophoresis is very effective for localised recalcitrant forms of psoriasis.
IV. Phonophoretic efficacy of diclofenac, ketorolac and nimesulide in soft tissue pain relief

Introduction

Ultrasound therapy is routinely used by physiotherapists for the treatment of a wide range of conditions, particularly in the management of soft tissue injuries. Topical pharmaceuticals containing anti-inflammatory drugs are also useful in the treatment of soft tissue injuries. It would, therefore seem rational to combine these two effective treatment modalities in the hope of obtaining a synergistic effect. Two important aspects exist regarding the concomitant use of topical drug application together with ultrasound in treatment of inflammatory conditions. First, drug application and ultrasound therapy can act independently to control symptoms and aid recovery and second, ultrasound energy may enhance the percutaneous absorption of the applied drug. The enhancement of percutaneous absorption of a drug by ultrasound leading to better therapeutic or clinical efficacy is termed as phonophoresis. In clinics phonophoresis is achieved by placing the formulation on the skin and massaging the area with the ultrasonic source.

Previous investigations have reported successful phonophoretic administration of a wide range of drugs including corticosteroids non-steroidal anti-inflammatory agents. Enhanced local anaesthesia has also been reported following treatment with phonophoretically administered local anaesthetic drugs (McElnay et al., 1985). Griffin et al. (1967) investigated the clinical effects of ultrasonically administered hydrocortisone, as compared to ultrasonically administered placebo in 102 arthritic patients. They reported that 68% of those patients receiving hydrocortisone in conjugation with ultrasound exhibited a marked decrease in pain and significant increase in range of motion while only 28% of those receiving placebo plus ultrasound showed similar improvement. Kleinkort and Wood (1975) conducted a retrospective study in 285 patients treated with ultrasonically driven hydrocortisone which was applied as a 10% (w/w) ointment or 1% (w/w) cream. They concluded that phonophoretic treatment with 10% hydrocortisone ointment provided an effective alternative for delivery of anti-inflammatory agents, thereby avoiding the discomfort of percutaneous injections.
The aim of the present study was to evaluate the phonophoretic efficacy of diclofenac sodium, ketorolac and nimesulide from a gel base in double-blind placebo controlled clinical trial. Diclofenac sodium, ketorolac and nimesulide are non-steroidal anti-inflammatory agents. These agents are shown to be active both orally and parenterally. The oral administration of these agents has been shown to be associated with severe gastrointestinal disturbances while parenteral administration is associated with “fear of needle”. Phonophoresis if effective, is really rewarding for these drugs. This is especially true for the management of acute localised inflammatory conditions such as sprains, strains, bruises, painful joints, sports injuries, low back pain, tired and stiff muscles.

The investigation was based on the hypothesis that if ultrasound did promote percutaneous absorption of these drugs, there would be a significant change in the pain threshold of the patients after treatment.

The topical application of diclofenac and nimesulide has already been shown to be useful for treatment of soft tissue injuries. To the best of our knowledge, topical application of ketorolac tromethamine, the potential of ketorolac tromethamine, a potent non-steroidal anti-inflammatory agent (NSAID) has not been explored for topical application.

**Materials**

Ketorolac tromethamine, nimesulide and diclofenac sodium were obtained as a gift samples from Torrent Pharmaceuticals Ltd., Ahmedabad. The drugs were incorporated in ultrasound transparent gel (Meditech™, Jayvee International, Pondichery) to get 2% w/w concentration. To obtain uniform dispersion, in case of nimesulide, the drug was first dissolved in a minimum amount of alcohol (2 mL) and then mixed with gel. The transmission of ultrasound energy through the gel preparation was measured using a Medisonics precision power meter (Medisonics, UK). Percentage transmission relative to deionised (Milli-Q® filtered) degassed water recorded for used frequency was 94%. This indicates that the gel provided a good coupling agent allowing good transmission of ultrasonic energy between the apparatus and the subjects skin.
Ultrasound equipment

A 1 MHz Ultrasound equipment (Sonodynator-834; Siemens, Germany) was used for the present study. The apparatus had facility for continuous as well as pulsed mode application. The ultrasound probe (head) was 4 cm² with maximum available intensity of 2.5 W/cm² for continuous mode.

Subjects

The study was conducted at the physiotherapy clinic of Kasturba Hospital, Manipal. The included subjects were those referred for physiotherapeutic intervention in pain relief. Only patients with specific acute soft tissue injury/pain were included. A total of 56 subjects, 20-40 years of age, either males and females participated in the study.

The patient exclusion criterion was; a) patients with associated multi-joint problems, b) patients on systemic and topical NSAID therapy, c) other indicated therapeutic modalities (electrotherapy etc.), d) infections, e) pains of mechanical dysfunction, and f) unreliability of patient.

Method

The study design was double blind randomised and placebo controlled. The patients were randomly divided into four groups of 14 patients each. The four groups were those treated with phonophoresis of diclofenac sodium, ketorolac tromethamine, nimesulide and placebo gels (control).

As discussed earlier, the patients were treated for soft tissue injury/pain of acute nature. The treatment site included the ankle, knee, lower back and shoulder. Each treatment group comprised of four cases each for shoulder, knee and lower back pain along with two cases for the ankle pain (total 14 subjects per group). After the application of gel over the treatment site, phonophoresis was performed by massaging the area with ultrasound head in a standardised circular motion. The intensity of the ultrasound was 1.4 Watts/cm² in continuous mode and the total duration of treatment for each subject was ten minutes. The ultrasound head and the method of treatment remained same for each subject in all four groups. Each subject was treated for a maximum of six days using above mentioned technique.
After the treatment, the patients were assessed for efficacy of phonophoresis on each day using visual analog scale for reduction in pain threshold levels. Before the start of treatment, the patients were asked to score the pain level on a scale of 0-10 (0- no pain, 10- very severe pain) (Table). The patients were then again interviewed the next day after treatment for pain score.

For convenience in analysis of data, the pain threshold levels were categorised into four groups viz. Very severe pain (Pain score 8 - 10), Severe pain (Pain score 5 - 8), Moderate pain (Pain score 2- 5) and No pain (0- 2). Only patients with very severe soft tissue injury/pain (pain score above 8) were included in all groups.

All the patients selected were explained the purpose of the pain score method (visual analog scale) and its relevance. The method of scoring was well explained to each subject. In addition to pain score, the patients were also assessed for specific activities like stair climbing, brisk walking, slow walking and overhead activities involving shoulder. Objective measurements such as range of motion testing, strength and speed and fluidity of movements were also assessed. Patients whose behavior (facial expressions and specific/objective measurements) appeared inconsistent with self-report were excluded from the study.

In the event, if the subject had no reduction in pain (at least by 50%), the treatment was discontinued and other therapeutic modalities were administered to the patient.

Results

The pain scores of the patients treated with phonophoresis of diclofenac sodium, ketorolac tromethamine, nimesulide and placebo gels are shown in Fig. 55 and Table 25.

Analysis of the results revealed that on first day after the treatment there was an extremely significant difference between the pain scores of ketorolac treatment group and control group. The difference between the ketorolac treatment group and nimesulide and diclofenac treatment group was also significant. This indicates that the phonophoresis of ketorolac is most effective in relieving the pain in first sitting.

Analysis of further data (day 2, 3 and 4) also indicated the effectiveness of ketorolac phonophoresis when compared with phonophoresis of nimesulide, diclofenac
Table 25. Pain scores of patients after phonophoresis with diclofenac, ketorolac, nimesulide and placebo gels.

<table>
<thead>
<tr>
<th>Days after Treatment</th>
<th>Control (Placebo)</th>
<th>Nimesulide</th>
<th>Diclofenac</th>
<th>Ketorolac</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.50 ± 1.50</td>
<td>8.00 ± 1.03</td>
<td>8.66 ± 1.69</td>
<td>9.12 ± 1.09</td>
</tr>
<tr>
<td>1</td>
<td>7.50 ± 1.04</td>
<td>6.85 ± 1.02</td>
<td>7.00 ± 1.30</td>
<td>4.75 ± 1.31*</td>
</tr>
<tr>
<td>2</td>
<td>6.50 ± 0.98</td>
<td>5.30 ± 1.20</td>
<td>7.00 ± 1.56</td>
<td>3.00 ± 1.69</td>
</tr>
<tr>
<td>3</td>
<td>5.30 ± 2.50</td>
<td>4.10 ± 2.50</td>
<td>5.13 ± 2.10</td>
<td>1.69 ± 1.50*</td>
</tr>
<tr>
<td>4</td>
<td>4.60 ± 2.40</td>
<td>3.20 ± 2.30</td>
<td>3.10 ± 1.96</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>3.86 ± 2.1</td>
<td>1.66 ± 1.66</td>
<td>2.33 ± 2.10</td>
<td>-</td>
</tr>
</tbody>
</table>

Each value is mean pain score of 14 subjects (± standard deviation)

Day 0: Day on which treatment was started.

* Significant difference ($P < 0.001$) compared to placebo & ($P <0.01$) compared to nimesulide and diclofenac group.

b Significant difference ($P < 0.001$) compared to placebo and diclofenac & ($P <0.01$) compared to nimesulide group.

c Significant difference ($P < 0.05$) compared to placebo and diclofenac group.

Fig. 55. Reduction in pain threshold after phonophoresis with diclofenac, nimesulide, ketorolac and placebo gels. Legends (x) ketorolac group; (■) nimesulide group; (▲) diclofenac group; (♦) control group.
sodium or placebo. Phonophoresis with nimesulide and diclofenac sodium did not bring about any significant change in pain score when compared with phonophoresis of placebo gel.

The pain score results were consistent with altered functional abilities of the patients. It was noted that the patients who were included in the ketorolac group reported better functional gains (assessed from specific activities/objective measurements) than those who were treated with nimesulide and diclofenac sodium group. No substantial functional gains were reported with nimesulide, diclofenac sodium and placebo groups. The subjects included in the ketorolac group reported substantial reduction of pain in the second sitting itself when compared to other three groups.

Since the study involved treatment at various joints as mentioned earlier, the correlation between the specific activity or particular objective parameter and pain score was not possible. Hence, the results and conclusions were based on total pain scores coupled with their correlation with overall (total) functional abilities of the patients.

Discussion

Acute soft tissue injuries/pain such as ligament sprains, tendinitis, trigger sprains, bursitis, muscle and joint pain, frozen shoulder etc. are the common painful conditions referred for physiotherapeutic intervention in pain relief. For treatment of such localised painful conditions, physiotherapists generally use modalities such as application of ultrasound, electrotherapy, heat, pressure or proper exercise. The use of ultrasound for relieving pain in above conditions is more common (Low and Reed, 1994; Bellary, 1998). The principle behind the use of ultrasound for resolving such painful conditions is its ability to cause localised tissue heating. Heating fibrous tissue structures such as joint capsules, ligaments, tendons and scar tissue can cause a temporary increase their extensibility, and hence a decrease in joint stiffness (Low and Reed, 1994). The advantage of using ultrasound to achieve this heating is due to the preferential heating of collagen tissue and to the effective penetration of this energy to deeply placed structures. Mild

*Ultrasound absorption by a particular tissue is directly proportional to its protein content. The protein content of fibrous tissues such as joints, ligaments is very high (because of collagen) and hence absorption of ultrasound by these structures is maximum leading to preferential heating over to other structures.*
heating can also have the effect of reducing pain and muscle spasm and promoting healing processes. Ultrasound can also affect nerve conduction in normal tissue nerve, affect the healing of damaged nerve and modify pain levels (Kitchen and Partridge, 1990).

Topical pharmaceuticals containing medicaments such as corticosteroids or non-steroidal anti-inflammatory agents are also used for the treatment of localised painful conditions. It has been reported in the literature that combination of ultrasound with topically applied pharmaceuticals (phonophoresis) may result in synergistic effect, particularly in pain control. Keeping this in mind, in the present study we have combined topical preparation of NSAID's with ultrasound therapy. Nimesulide, ketorolac tromethamine and diclofenac sodium were chosen because they are the most commonly used NSAID's.

For the present study, 1 MHz ultrasound equipment was used since the variable frequency equipment was not available. Moreover, 1 MHz frequency is commonly used in therapeutics. The choice of intensity (1.4 Watts/cm²), duration of ultrasound application (10 min) and concentration of medicaments (2% w/w) was empirical.

Ultrasound energy is rapidly attenuated in air, therefore to be effective, it must be transferred efficiently from the ultrasound transducer into the skin. The transmission characteristics of a number of topical proprietary preparations containing drugs suitable for use with ultrasound have recently been investigated (Benson and McElnay, 1994). Gel formulations were found to be most suitable coupling agents. Hence, in the present study, we chose ultrasound transparent gels as vehicles for formulation.

The results of the study indicated that phonophoresis of ketorolac was effective clinically in resolving pain as compared to phonophoresis of nimesulide, diclofenac and placebo gels. The observed effectiveness of ketorolac by phonophoresis over nimesulide and diclofenac might be attributed to the potency of ketorolac. Ketorolac is a very potent analgesic agent. Its therapeutic dose is only 40 mg orally per day (10 mg q.i.d.) and maximum 90 mg (30 mg t.i.d) by parenteral route (Litvak and McEvoy, 1990). In case of nimesulide, the therapeutic dose is 200 mg orally per day while in case diclofenac sodium,
it is 150 mg per day orally. It is clear that, to be effective therapeutically, the dosage required by ketorolac is least compared to nimesulide and diclofenac. Hence, in phonophoresis, if ultrasound aids in transdermal transport of ketorolac, nimesulide and diclofenac sodium, minimal enhancement will be required by ketorolac to be effective as topical NSAID.

Conclusion

From the results of the study, it can be concluded that phonophoresis of ketorolac is effective clinically in resolving soft tissue injury/pain as compared to phonophoresis of nimesulide and diclofenac. More clinical studies with larger sample size are however required to confirm the efficacy of this approach.