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Research Paper

"ChilDrive": A Technique of Combining Regional Cutaneous Hypothermia with Iontophoresis for the Delivery of Drugs to Synovial Fluid

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Purpose. Bioavailability of drugs in the synovial fluid when administered via transdermal route is highly limited due to the dermal clearance. The purpose of this project was to assess the efficiency of ChilDrive (CD) technique to improve the drug targeting to the synovial fluid. CD is a technique of transdermal delivery of drugs combining regional hypothermia and iontophoresis.
Methods. Diclofenac sodium and Prednisolone sodium phosphate were administered by transdermal route (Passive, Iontophoresis, Chil-Passive and ChilDrive) at the knee-joint region of hind limb in sprague dawley rats for 6 h. Intraarticular microdialysis was carried out to determine the time course of drug concentration in the synovial fluid. Drug levels in synovial fluid after intravenous and intraarticular administration were also determined.
Results. Iontophoretic delivery increased the $AUC_{0\text{-}t}$ (area under the curve) of drugs in the synovial fluid by 3-fold over passive delivery (0.86±0.04 and 2.0±0.06 μg.h/ml for diclofenac sodium and prednisolone sodium phosphate, respectively). CD resulted in an $AUC_{0\text{-}t}$ of 5.2±0.69 and 24.6±1.97 μg.h/ml for diclofenac sodium and prednisolone sodium phosphate which was ~6–12-fold higher than the passive and 2–4-fold higher than iontophoresis.
Conclusions. The results support our hypothesis that CD improves bioavailability of drugs to the synovial joints. CD could be developed as a potential noninvasive technique for treatment of arthritis.

KEY WORDS: intraarticular microdialysis; iontophoresis; synovial fluid; targeting; transdermal.

INTRODUCTION

The different types of joints present in the human body are classified into synovial, cartilaginous and fibrous joints based on the degree and type of movement. Synovial joints are the most freely moveable joints present in the knee, elbow, shoulder, hip, wrist and neck region of the human body (1,2). The presence of lubricating synovial fluid differentiates synovial joints from cartilaginous and fibrous joints. Arthritis is the most prevalent disorder affecting synovial joints and a major cause of disability among people of all ages. During arthritis, the synovial fluid secretion is affected due to the damage caused to the synovial membrane either due to body's own immune cells or due to break down of articular cartilage. This leads to thickening of the synovial membrane, eventually resulting in swollen joints and decreased movement between bones of the synovial joints. The major types of arthritis include rheumatoid arthritis, osteoarthritis, juvenile rheumatoid arthritis and infectious arthritis. Pain and inflammation are associated with arthritis (3). The most common treatment modalities for arthritic conditions involve oral NSAIDs, including cyclooxygenase (COX) 2 inhibitors and intraarticular injection of steroids. NSAIDs act by inhibiting the COX enzymes which are responsible for the conversion of arachidonic acid into prostaglandins, which is a major cause of inflammation (4). Corticosteroids suppress the inflammatory parameters, like erythrocyte sedimentation rate and C-reactive protein, resulting in decreased disease activity (5). Unfortunately most of the drugs are known to cause severe gastric irritation and other gastrointestinal disturbances following oral administration. In addition, the systemic side effects are unavoidable due to distribution of drugs (6,7). Moreover, the amount of drug reaching the synovial joint would be only a small fraction of the total administered dose. Although intraarticular injections provide maximum bioavailability in the synovial cavity, they are invasive in nature and therefore do not allow frequent administration (8,9). Harvey and Hunter suggested that the number of injections in a single joint should not be more than four in a single year (10).

The synovial fluid present in joints is an ultrafiltrate of plasma which is continuously absorbed and replenished by synovial lining of joint cavity, with a rapid turnover time of ~2 h (11,12). This is one of the main constraints in the treatment of arthritis as it results in rapid clearance of drug from the synovial cavity. This necessitates either frequent administration or continuous infusion of drugs into the synovial fluid to maintain effective level of drugs through the course of treatment. In an infusion-type drug delivery system, a steady state drug level could be maintained over prolonged durations. However, intraarticular infusion is

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practically not feasible unless the patient is hospitalized and bedridden.

Transdermal delivery of drugs is noninvasive and is associated with advantages such as steady state input of drugs, prolonged therapeutic activity, and minimized gastrointestinal and systemic side effects. However, passive delivery of drugs through skin is not capable of achieving required drug levels in the subdermal musculoskeletal tissues mainly due to the fact that the majority of the drug that penetrates into skin will be cleared by the dermal circulation (13,14). One of the approaches to enhance deeper penetration of drugs is likely to be increasing the rate of drug delivery higher than the rate of dermal clearance ($dQ/dt > R_{cl}$) (Fig. 1). The rate of drug delivery is known to increase when iontophoresis is applied across the skin due to the repulsive driving force on the drug ions. However, the rate of drug delivery by iontophoresis across skin is limited by the maximum current density that can be applied. The maximum current that is tolerable and safe is in the range of 0.4–0.5 mA/cm^2 (15,16).

The second approach would be to reduce the rate of dermal clearance in order to accumulate drug in the dermal tissue. Some research groups have attempted to enhance the delivery of drugs to subdermal tissues by reducing the rate of dermal clearance with the help of vasoconstrictors (17,18). Vasoconstrictors constrict the blood vessels and there-by reduce the blood perfusion rate (thus the drug clearance) in the tissue. Higaki *et al.* have shown that there was ~3.3–9.6-fold enhancement in the amount of antipyrine, salicylic acid and diclofenac reaching muscle followed by reducing blood flow upon topical administration of vasoconstrictor phenylephrine (18). But the use of vasoconstrictors over long durations is likely to cause severe undesirable systemic and cardiovascular effects. Therefore, use of a biophysical technique that reduces the regional blood perfusion would be safer than the vasoactive drugs.

Vuksanovic and coworkers studied the effect of temperature on the regional blood flow in skin and have shown that when the skin temperature is>31°C, the blood flow in the skin remains constant. However, a rapid decrease in blood-flow can be seen when skin temperature drops <31°C due to vasoconstriction (19). Unlike the vasoactive drugs, temperature induced vasoconstriction is a regional effect and hence regarded as relatively safe.

In this project, we assessed the effect of iontophoresis and iontophoresis combined with regional hypothermia (ChilDrive) for enhancing the bioavailability of drugs in the synovial compartment. Diclofenac sodium and prednisolone sodium phosphate were selected as model drugs to assess our hypothesis that regional hypothermia during iontophoretic transdermal delivery at the joints leads to increased bioavailability of drugs in the synovial fluid.

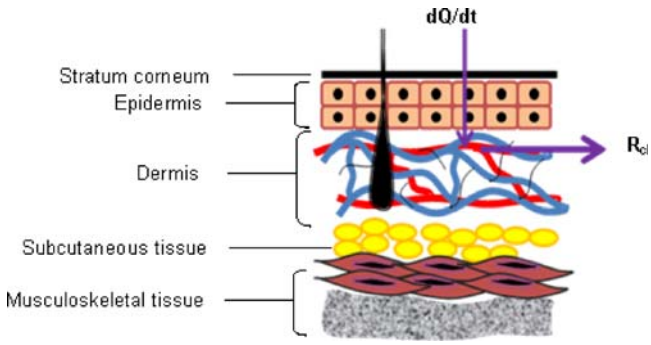

Fig. 1. Schematic representation of skin and subdermal tissues. dQ/dt represents rate of drug permeation across skin and R_{cl} represents rate of dermal clearance of drug. The amount of drug reaching the subdermal tissues could be increased by rendering R_{cl}<dQ/dt.

MATERIALS AND METHODS

Chemicals

Diclofenac Sodium, Prednisolone Sodium Phosphate were procured from Sigma-Aldrich Inc. (St. Louis, MO), Phosphate buffered saline (PBS, pH 7.4) premixed powder was obtained from EMD Chemicals (Gibbstown, NJ), and all other chemicals were obtained from Fischer scientific (Fairway, NJ).

Animals

All the experimental studies were performed on 24 male Sprague-Dawley rats (200–250 g) under ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia administered intraperitoneally. The rats were procured from Harlan (Indianapolis, IN) and maintained on a 12–12 h light/dark cycle in an animal facility with unlimited access to food and water. The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Mississippi (Protocol # 09–006).

Intraarticular Microdialysis

Intraarticular microdialysis was carried out to determine the amount of drug present in the synovial fluid. After anaesthetizing the rats, the hind limb was held in a fixed position and an introducer needle with split tubing was passed through the knee joint capsule, the needle was removed and a CMA 20 microdialysis probe of 4 mm length and 20 kDa molecular weight cut off (CMA, North Chlemsford, MA) was inserted through the split tubing. The split tubing was withdrawn leaving the probe implanted in the synovial cavity. The implantation of probe in the synovial cavity of rat is shown in Fig. 2. PBS was perfused for 30 min prior to drug administration for equilibration at flow rate of 2 µl/min. The proper placement of probe in the synovial cavity was confirmed by making an incision at the site of probe implantation after completion of studies.

The probe recovery was determined *in vivo* by using retrodialysis method. For this, the probe was first equilibrated by perfusing PBS at 2 µl/min for 30 min followed by drug solution of known concentration for 30 min. After equilibration, dialysate was collected for 1 h interval at 1, 2 and 3 h and the average recovery of three time points was considered. The *in vivo* recovery rate was calculated using the following formula:

$$\text{Recovery}(\%) = 100 - \left(\frac{\textit{Concentration of dialysate}}{\textit{Concentration of perfusate}} \times 100\right) \longrightarrow \quad (1)$$

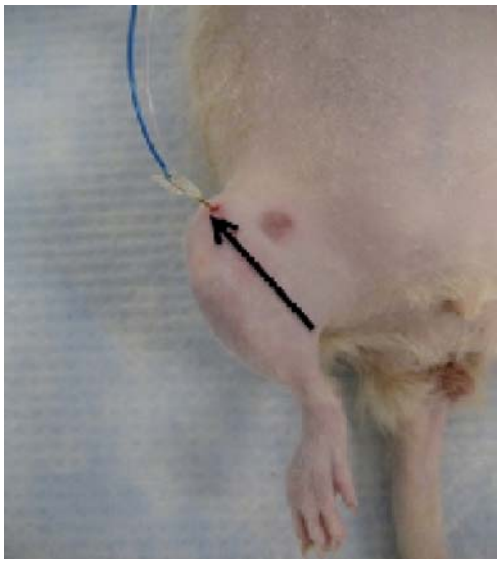

Fig. 2. Microdialysis probe implanted in the knee joint for carrying out synovial fluid microdialysis in hind limb of rat.

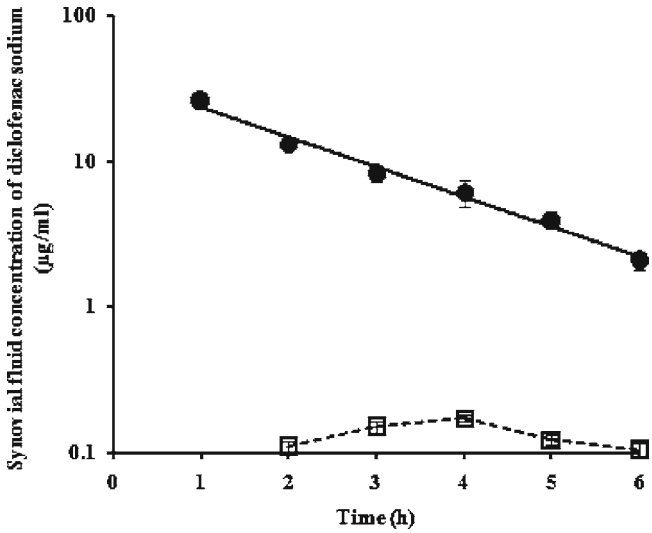

Fig. 4. The time course of diclofenac sodium in synovial fluid upon intraarticular (●) and intravenous (□) administration. All the data points are average of $n=3\pm$S.D.

Intravenous and Intraarticular Delivery of Drugs

Diclofenac sodium and prednisolone sodium phosphate were given by intravenous route through tail vein (4 mg/kg), and intraarticular microdialysis was carried out for 6 h to determine the time course of drug reaching the synovial fluid. Also, drug solutions were injected directly into the synovial cavity (50 μl) in separate set of rats, and intraarticular microdialysis was carried out for 6 h.

Transdermal Drug Delivery

Design of Iontophoretic Patch

The iontophoretic patch (Fig. 3) was fabricated using a woven adhesive backing membrane fitted with a snap-type Ag/AgCl electrode. One cm^2 foam pad was attached onto the backing membrane above the electrode. Drug solution (1%) made in PBS was filled in the foam pad just before the application of patch. A similar patch was made which was filled with PBS and acts as a counter electrode.

The patch containing drug solution was placed on the lateral side of the rat knee, and the counter electrode was placed on the opposite side for all the transdermal drug delivery studies. Intraarticular microdialysis was carried out for 6 h, and the dialysate was collected every hour to determine the amount of drug reaching the synovial fluid. In all the cases, the drug-containing patch was applied only after the equilibration of microdialysis probe in the synovial joint.

A) Passive drug delivery
Passive drug delivery studies were carried out for both the drugs by using the patches fabricated with

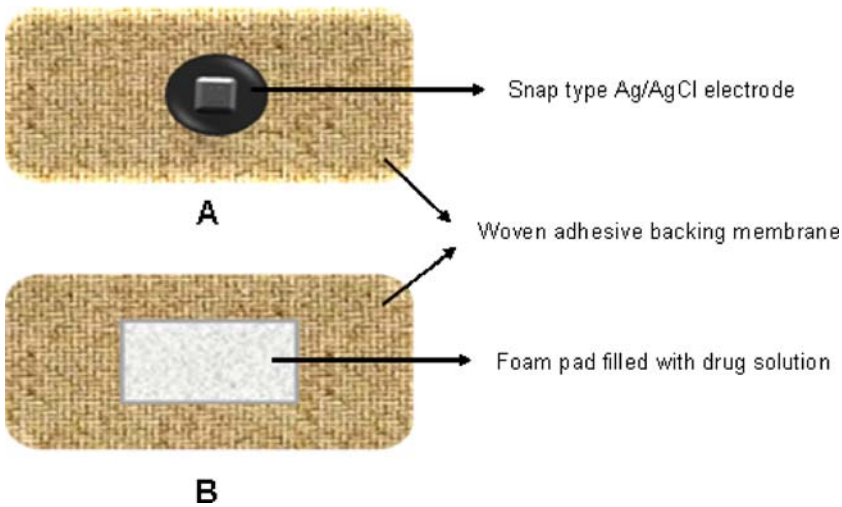

Fig. 3. Design of iontophoretic patch with 'A' displaying the dorsal view and 'B' displaying the ventral view.

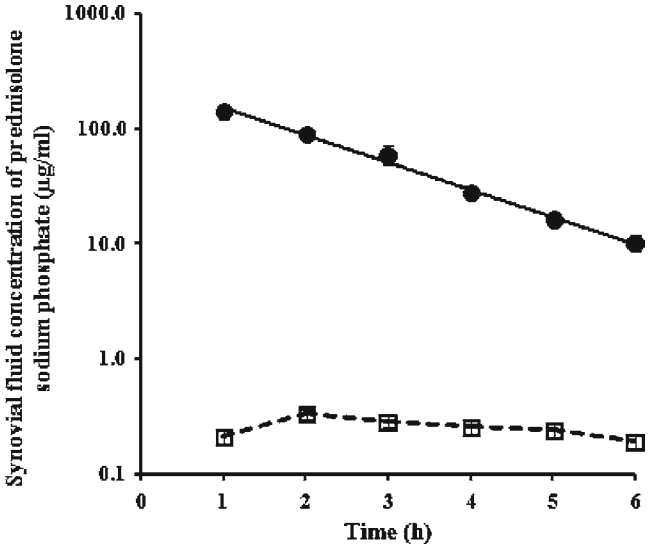

Fig. 5. The time course of prednisolone sodium phosphate in synovial fluid upon intraarticular (●) and intravenous (□) administration. All the data points are average of $n=3\pm$S.D.

electrodes to maintain similar experimental conditions compared to iontophoresis. In passive drug delivery, no current was applied.

B) Iontophoretic drug delivery
Iontophoresis was carried out using an IOMED Phoresor (Iomed, Inc, Salt Lake city, UT) by connecting the lead clips from the iontophoresis unit to the snap on the drug electrode and counter electrode. 0.5 mA/cm^2 was applied continuously for 6 h.

C) Chil-Passive drug delivery
Passive drug delivery carried out under regional hypothermia was termed as "chil-passive" drug delivery. Regional hypothermia was induced by placing the hind limb of the rats on a water pad circulated continuously with water at reduced temperature (15–20°C) for the entire period of study.

D) ChilDrive (CD) drug delivery
Iontophoresis carried out under regional hypothermia was termed as "ChilDrive" (CD). Regional hypothermia was induced according to method mentioned earlier. With the exception of regional hypothermia, all other experimental conditions were similar to iontophoretic drug delivery.

Analytical Methods

The amount of diclofenac sodium and prednisolone sodium phosphate present in microdialysis samples was analyzed by high-performance liquid chromatography. The HPLC system (Waters, MA) consisted of a chromatographic pump (Waters 1525), an autosampler (Waters 717 plus), a UV detector (Waters 2487) and a Symmetry® C18 column (4.6×150 mm).

Analysis of diclofenac sodium samples was carried out at a wavelength of 278 nm, and the mobile phase consisted of a mixture of acetonitrile and 0.01 M potassium dihydrogen phosphate (pH 6.3), (35:65 v/v) with flow rate of 1 ml/min (20). The linearity range was between 10–1,000 ng/ml ($R^2=0.99$).

Analysis of prednisolone sodium phosphate samples was carried out at a wavelength of 242 nm, and the mobile phase was prepared by mixing 250 ml of isopropanol with 2 ml H_3PO_4 and diluting with water to 900 ml. The pH of the solvent was adjusted to 3.0 with 1.0 M NaOH and then diluted to 1,000 ml with water, and the flow rate was set to 1 ml/min (21). The linearity range was between 10–1,000 ng/ml ($R^2=0.99$).

Statistical Analysis

The statistical analysis of $AUC_{0\text{-}t}$ data after transdermal, intravenous and intraarticular administration were determined by performing Unpaired *t*-test (GraphPad, Instat 3.0) to determine the level of significance between two groups. $P<0.05$ was considered as the level of significance.

RESULTS & DISCUSSION

In this study, microdialysis technique was employed as a tool to investigate the time course of drug concentration in the synovial fluid (22,23). The *in vivo* probe recovery was performed using retrodialysis method and was found to be 19.28±4.99% for diclofenac sodium and 23.01±5.48% for prednisolone sodium phosphate.

Intravenous and Intraarticular Delivery of Drugs

Drugs were administered intravenously to determine the fraction of the total dose reaching the synovial fluid. The time course of drug in the synovial fluid upon intravenous administration is shown in Figs. 4 and 5. The area under the curve ($AUC_{0\text{-}t}$) is a measure of the drug's bioavailability. The maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) indicate the rate of delivery of drug into the target tissue. $AUC_{0\text{-}t}$ was calculated using the trapezoid rule, and C_{max}, and T_{max} were determined from the concentration-time curves. The $AUC_{0\text{-}t}$ in the synovial fluid after intravenous administration was found to be 0.609±0.02 µg.h/ml for diclofenac and 1.445±0.17 µg.h/ml for prednisolone. The total $AUC_{0\text{-}t}$ in the synovial fluid by systemic administration was comparable with that of passive topical delivery of drugs (Tables I, II) and was significantly less than all the other modes of drug administration. From these results, it appears that the systemic delivery of drug for treating synovial disorders may not end in remarkable results unless

Table I. $AUC_{0\text{-}t}$ and C_{max} of Diclofenac Sodium in Synovial Fluid Following Transdermal (Passive, Chil-Passive, Iontophoresis, ChilDrive), Intravenous And Intraarticular Administration

	$AUC_{0\text{-}t}$ (µg.h/ml)	C_{max} (µg/ml)
Passive	0.864±0.042	0.188±0.022
Chil-Passive	1.336±0.105	0.287±0.024
Iontophoresis	2.683±0.152	0.644±0.036
ChilDrive	5.208±0.693	1.259±0.098
Intravenous	0.609±0.028	0.174±0.009
Intraarticular	77.829±5.422	25.974±1.340

Table II. $AUC_{0\text{-}t}$ and C_{max} of Prednisolone Sodium Phosphate in Synovial Fluid Following Transdermal (Passive, Chil-Passive, Iontophoresis, ChilDrive), Intravenous and Intraarticular Administration

	$AUC_{0\text{-}t}$ (μg.h/ml)	C_{max} (μg/ml)
Passive	2.003±0.067	0.468±0.106
Chil-Passive	3.958±0.303	0.863±0.055
Iontophoresis	6.364±0.486	1.724±0.423
ChilDrive	24.605±1.975	7.360±1.032
Intravenous	1.445±0.174	0.339±0.032
Intraarticular	479.946±45.040	141.890±20.179

the dose and frequency are high enough to maintain effective drug levels over long duration. The C_{max} and T_{max} in synovial fluid for diclofenac following intravenous administration were 0.174 μg/ml and 4 h. Similarly for prednisolone sodium phosphate, they were found to be 0.339 μg/ml and 2 h. Obviously, delivery of drugs directly into the synovial cavity resulted in significantly higher bioavailability than all the other modes of drug administration (Tables I, II). The concentration-time profile followed a mono-exponential disposition curve (Figs. 4, 5). The $AUC_{0\text{-}t}$ in synovial fluid was 77.82±5.42 and 479.94±45.04 μg.h/ml for diclofenac sodium and prednisolone sodium phosphate respectively. However, one should note that the drugs administered into the synovial cavity were eliminated rapidly as reflected by the elimination half-lives of drugs in the synovial fluid ($t_{1/2}$ was 1.55±0.02 h for diclofenac and 1.19±0.01 h for prednisolone respectively). The rapid elimination of drug could be attributed to the rapid turnover of synovial fluid and/or to the metabolism of drug in the synovial fluid. Relatively longer half-life of diclofenac in the synovial fluid is likely due to slightly higher protein binding (>98%) compared to prednisolone (65–91%) (24,25).

Passive Drug Delivery

Vuksanovic *et al.* have clearly demonstrated using the laser doppler studies that the blood perfusion rate in the skin tissue is decreased under hypothermic conditions (19). The decreased blood flow is likely to result in decreased rate of dermal clearance of drugs. Passive drug delivery studies were carried out at normal body temperature and at regional hypothermia condition (Chil-Passive) for both the drugs. The drug concentration-time profile in the synovial fluid was used to calculate the $AUC_{0\text{-}t}$. The total $AUC_{0\text{-}t}$ in synovial fluid after passive transdermal administration of drugs was almost doubled due to induction of regional hypothermia (Tables I, II).

Iontophoretic Drug Delivery

Application of iontophoresis (0.5 mA/cm^2) resulted in enhanced bioavailability of drugs into the synovial cavity (Figs. 6, 7). Iontophoresis-mediated delivery of drugs resulted in 3-fold ($P<0.01$) higher $AUC_{0\text{-}t}$ in synovial fluid compared to passive drug delivery (Tables I, II). These results are in agreement with previous reports in which other authors showed that iontophoresis aids in better treatment of joint disorders (26,27). Aiyejusunle *et al.* have reported that cathodal iontophoresis (0.1–0.3 mA/cm^2) of sodium

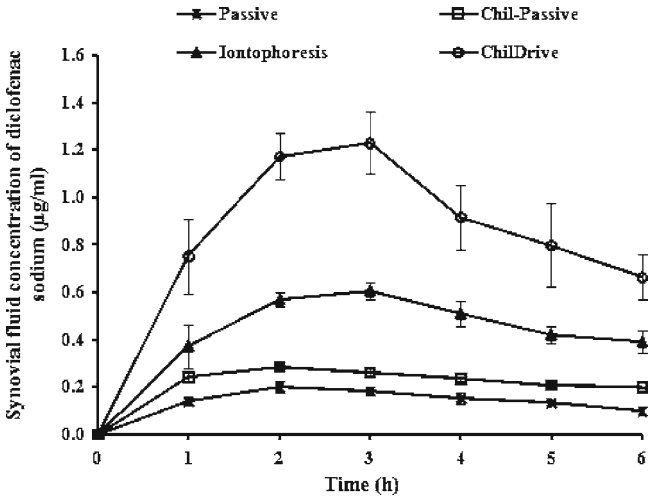

Fig. 6. The time course of diclofenac sodium in the synovial fluid following different modes of transdermal administration (Passive, Chil-Passive, Iontophoresis and ChilDrive). The data points represented are average of $n=3\pm$S.D.

salicylate resulted in reduced pain and functional disability (26). Bender *et al.* have shown that the concentration of etofenamate in the synovial fluid following iontophoresis was twice that of etofenamate levels in serum, and, hence, topical iontophoresis of etofenamate was suitable for alleviation of arthritis in superficially located joints (27).

In the case of CD, iontophoresis was carried out by placing the hind limb portion of the rat on a water pad through which water at 20°C was circulated, so that the temperature of skin was maintained in the range of ~20–25°C throughout the study. Reducing skin temperature has been widely used in treatment techniques like cryotherapy where in temperatures in the range of 7–20°C have been used. As hypothermia is induced regionally in the present studies, it is less likely to lead to any physiological changes in the whole body. Chesterton *et al.* reported that physiological changes, like local analgesia, reduced nerve conduction velocity and

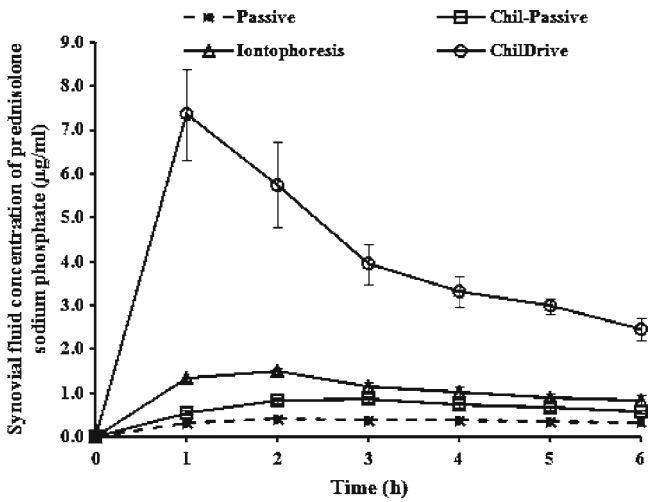

Fig. 7. Time course of prednisolone sodium phosphate in the synovial fluid following different modes of transdermal administration (Passive, Chil-Passive, Iontophoresis and ChilDrive). The data points represented are average of $n=3\pm$S.D.

reduced metabolic enzyme activity, are seen only at temperatures below 14°C (28). The $AUC_{0\text{-}t}$ of drug in the synovial fluid due to CD was ~2–4-fold ($P<0.01$) higher than iontophoresis in case of both the drugs. This is exactly the fold of enhancement observed between passive transdermal and chil-passive as well. Compared to passive drug delivery, the $AUC_{0\text{-}t}$ in the synovial fluid of drugs when administered by CD was ~6–12-fold higher ($P<0.01$).

Interestingly, all the synovial concentration-time profiles following transdermal drug administration showed a typical initial increase followed by decrease in drug concentration regardless of the mode of drug delivery. The depletion of donor compartment effect is less likely to be a factor responsible for this trend because the total amount of drug present in the patch (10 mg/ml) was quite high as compared to that absorbed. To reassess if the observed profile of drug concentration-time was due to any change in the membrane permeability of the microdialysis probe, the recovery of the probe was determined at different time points in the synovial joint by retrodialysis method. There was no significant change in the recovery even after 6 hours. It is likely that the change in the synovial fluid turnover rate due to insertion of the probe might have caused this kind of concentration-time profile.

These results make evident that the efficiency of drug targeting to synovial fluid by passive or active modes of transdermal drug administration could be significantly improved by inducing regional hypothermia. Translation of CD is believed to improve the success rate of transdermal therapy of joint disorders in humans. Minimized systemic distribution and specific targeting of drugs is likely to minimize the side effects as well. Sustained release intra-articular injections can prolong the residence time of drugs in the synovial fluid. However, in transdermal delivery, the treatment can be terminated in case of adverse effects by removing the formulation/patch system at any point of time.

CONCLUSION

CD can be an effective noninvasive treatment option for musculoskeletal disorders as it can overcome limitations of oral and parenteral administrations. Further studies need to be carried out in arthritic conditions since the disease state leads to changes in thickness of synovial membrane, secretion and viscosity of synovial fluid.

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