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IN-VITRO AND *IN-VIVO* EVALUATION OF GLYCERIN AS A SOLVENT FOR THE IONTOPHORETIC DELIVERY OF DIPHENHYDRAMINE HYDROCHLORIDE

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ABSTRACT: Diphenhydramine hydrochloride (DPH) is an antihistamine used topically to relieve skin itching and, at higher doses, DPH also has the capability to induce local skin anesthesia. The present study investigates the possibility to deliver DPH via iontophoresis using a stainless steel electrode. *In-vitro* iontophoresis was performed using 1% (w/v) DPH dissolved in glycerin (100%), deionized (DI) water (100%) and a mixture of both (50:50) through porcine ear skin and Franz cell assembly. The applied electrical current was 0, 100, 200, and 300 μA/cm^2 for 60 min. Of the solvents tested, the 100% glycerin provided a consistent increase in DPH skin concentrations with current density and no skin damage. Therefore, the 100% glycerin was selected for *in-vivo* iontophoresis in a rabbit model at the same three current densities for 60 min. DPH skin concentrations were measured in skin via microdialysis for 240 min. Plasma to skin distribution of DPH was studied following short IV-infusions of 6, 8 and 10 mg/Kg in rabbits. Skin and plasma samples were collected for 420 min. No skin damage was observed even at the higher current density. DPH skin concentrations were higher following iontophoresis than IV-infusion. Thus, iontophoresis of DPH in glycerin from a stainless steel patch may be a safe, effective, and inexpensive way to deliver high dose of DPH topically to the skin.

INTRODUCTION: Diphenhydramine (DPH, **Fig.1**) is a first-generation H_1 antihistamine receptor antagonist that is used topically to relieve skin itching due to insect bites, poison ivy, or other skin irritations [1].

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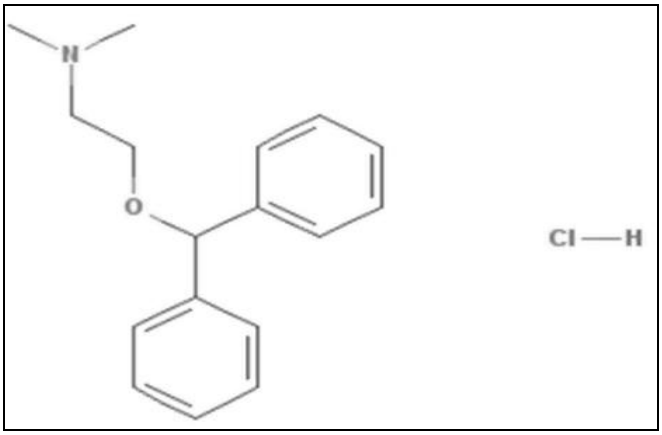

FIG. 1: 2D CHEMICAL STRUCTURE OF DIPHENHYDRAMINE HYDROCHLORIDE

Commercially available dermatological formulations of DPH include cream, lotion, gel, and spray [1] at a strength of 1 or 2% (w/v). Kuo et al [2] proposed that DPH has also the capability to induce local anesthesia. Pollack et al [3] suggested the use of DPH for local anesthesia in -caine sensitive patients. Recently, Davari et al [4] discussed different fundamental principles of oral procedures and highlighted the use of 1% DPH administration as a local anesthetic in patient with histories of allergy to commonly used local anesthetics. Although the minimum effective concentration necessary to induce local anesthesia is still unknown, it is anticipated that DPH acts as a local anesthetic at concentration in skin several orders of magnitude higher than those required to antagonize histamine [5]. Such high concentrations are usually achieved with subcutaneous injections that however, in some cases, can induce local necrosis [6]. For this reason, DPH injections are not currently recommended by the FDA as a local anesthetic [6].

Iontophoresis is an alternative to subcutaneous injection and can quickly deliver high concentration of charged molecules to the skin via the application of a mild electrical current [7]. Kotwal et al [8] performed *in-vitro* studies and concluded that DPH can be successfully delivered by iontophoresis, however, to the best of our knowledge, no *in-vivo* data actually confirms that DPH can be effectively delivered *in-vivo*, either to the skin or systemically, with iontophoresis. Hence, the goal of this study was to evaluate the possibility to deliver DPH to skin via a simple iontophoretic device for potential dermatological indications.

DPH is a small (MW=291.82 g/mol) basic molecule with a pKa of 8.98 [1]. Therefore, at neutral or acidic pH, DPH is mostly ionized and it can be successfully delivered by anodic iontophoresis. Iontophoresis is usually more efficient when the molecule is ionized rather than neutral [9]. Most iontophoresis vehicles generally include a large portion of water.

However, water undergoes electrolysis in the presence of electrical current and generate skin-damaging hydrogen or hydroxyl ions [10]. Several methods were proposed to overcome this problem, with the most common being the use of buffered electrodes, like silver/silver chloride [11]. However, these electrodes are expensive, and they must be properly calibrated to maintain the buffer capacity for the entire duration of the current application.

In our laboratory, we found that the use of glycerin as solvent allows usage of stainless steel electrodes with minimal skin damages [12]. In this research project, we first studied the effect of different percentage of glycerin on the i*n-vitro* iontophoresis of DPH then we performed *in-vivo* iontophoresis in a rabbit model.

MATERIALS AND METHODS:

Chemicals: All chemicals were of analytical grade quality. DPH (Lot - 096K0036), ethyl acetate, monosodium and disodium phosphate, sodium carbonate anhydrous and triethylamine (TEA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO); methanol, phosphoric acid, acetonitrile and glycerin were from Spectrum Chemical Mfg. Corp., (New Brunswick, NJ). Deionized water was obtained using Milli-Q system from Millipore Corporation (Billerica, MA); Veterinary lactated ringers (LR) injection USP was from Henry Schein Animal Health (Forth Worth, TX); acepromazine maleate injection (10 mg/ml) was from Boehringer Ingelheim Vetmedica Inc. (St. Joseph, MO); DPH USP IV injection 50 mg/ml (Lot – 091010) was from Bioniche Pharma (Lake Forest, IL).

Analytical Method: DPH in dialysate samples was quantified by reversed phase HPLC with a method adapted from Thompson et al [13]. The chromatographic system consisted of a Waters 717plus Autosampler (Waters Corp., Milford, MA), Schimadzu LC-20AT solvent delivery unit (Schimadzu Corp., Somerset, NJ), Hitachi L-4250 UV-VIS detector (Hitachi High Technologies America, Inc., Schaumburg, IL) and Perkin Elmer Nelson 900 Series interface (PerkinElmer Inc., Waltham, MA). Peak integration, data acquisition and chromatographic analysis were done with a Totalchrom workstation version 6.3.2 (PerkinElmer Inc., Waltham, MA). Analysis was performed using Waters Symmetry® C18 column (5 µm, 3.9 x 150 mm, Waters Corp., Milford, MA).

The microdialysis and plasma samples were both analyzed with the same HPLC method.

The optimized mobile phase composition was 65:35 (containing 0.2% TEA of total mobile phase composition) (v/v) of 50 mM phosphate buffer (set to pH-3 with phosphoric acid): acetonitrile. The mobile phase was freshly prepared every time, filtered through a 0.45µm Nylon membrane filter (Pall Life Sciences, Ann Arbor, MI) and degassed using a Aquasonic 50T sonicator (VWR International LLC, West Chester, PA). The isocratic flow rate was 0.8 mL/min and the detector wavelength was set at 230 nm. The plasma samples were extracted by liquid-liquid extraction method. The injection volume for skin and plasma samples were 10 and 30 µl respectively. The observed DPH retention time was 3.2±0.1 min. The HPLC assay was linear and validated within the concentration range of 0.05 to 10 µg/ml of DPH for microdialysis samples and 0.1 to 10µg/ml for plasma samples. Lower limit of quantification was 0.05µg/ml and 0.10µg/ml for microdialysis and plasma respectively. The plasma extraction procedure used was adapted from Kumar's method [14]. The frozen plasma samples were allowed to thaw at room temperature. 100 µl of plasma sample was transferred to a 1.5 ml micro-centrifugation tube and alkalinized by addition of 200 µl of saturated sodium carbonate solution.

Further, 0.5 ml of ethyl acetate was added to it and the tubes were capped. The samples were vortex mixed for 1 min and then centrifuged for 12 min at 10000 rpm. The upper organic layer was carefully pipetted out into a micro centrifugation tube and evaporated to dryness under a gentle stream of nitrogen at 28 ˚C (Pierce – 18780 Reactivap Evaporating unit). The residue was reconstituted by 100 µl of mobile phase and vortex mixed for 1 min.

Microdialysis: The microdialysis system consisted of a Fusion 400 touch syringe pump (Chemyx Inc., Stafford, TX), CMA/102 microdialysis pump and a CMA/142 microfraction collector (Harvard Apparatus, Holliston, MA). A Teflon tubing (Valco Instruments, Houston, TX) connected the syringes to the probes. Disposable linear probes were custom made as described by Stagni et al. [15]. They consisted of two 7 cm arms made of polyamide tubing (Vention Medical, Chattanooga, TN) connected by a 1 cm long semipermeable AN69 hollow fiber membrane made of polyacrylonitrile with a molecular weight cut-off of 50 kDa (Donation by Dr. Thierry Crost, Gambro Industries, France). All the connections were sealed with ethyl cyanoacrylate glue (Henkel Corp., Rocky Hill, CT).

Iontophoresis: The iontophoresis kit consisted of (i) constant electric power source - Phoresor II PM 700 (Iomed Inc., Salt Lake City, UT), (ii) TL2 twin lead connector (Iomed Inc., Salt Lake City, UT), (iii) a disposable stainless steel electrode with a constant surface area of 3.14 cm^2 covered with a non-woven polypropylene pad (delivery electrode) and (iv) a dispersive electrode (cathode) that for *in-vitro* experiments consisted in a 10 cm long silver chloride wire with 0.5 mm diameter (Sigma-Aldrich Inc., St. Louis, MO), whereas for *in-vivo* experiments was a IOMED (Iomed Inc., Salt Lake City, UT) patch with an adhesive base.

***In-vitro* microdialysis experiments**: *In-vitro* experiments were performed to confirm the capability of the microdialysis technique to recover and quantify DPH consistently. Relative recoveries: %-gain and %-loss were estimated as described by Lange et al [16] using the following equations:

$$\%\ \text{Relative recovery (\%gain)} = \frac{C(\text{bulk}) - C(\text{dialysate})}{C(\text{bulk})} \text{ X } 100$$

$$\%\ \text{Retrodialysis (\% loss)} = \frac{C\,(\text{perfusate}) - C(\text{dialysate})}{C(\text{perfusate})} \text{ X } 100$$

Glass cells were filled with 2 mL of 1, 5, and 10 µg/ml DPH standard solutions for gain studies or with pure LRS for recovery (loss) experiments. Solutions in cells were continuously stirred with a magnetic stirrer and maintained at 37±0.5 °C to mimic the skin temperature (VWR International LLC, West Chester, PA). The perfusate flow rate was 2 µL/min and dialysate samples were collected every 10 min for 240 min.

Preparation of 1% (w/v) DPH solution for iontophoresis experiments: The amount of DPH required to prepare 10 mg/ml was accurately weighed and dissolved in the following solvents by continuous stirring for 120 min using a magnetic stirrer system Corning stirring hot plate PC-220 (Corning Incorporated Life Sciences, Tewksbury, MA): 100% glycerin; 50:50 (v/v) mixture of glycerin: DI water; and 100% DI water.

***In-vitro* iontophoresis experiments:** The procedure for *in-vitro* iontophoresis was adapted from that described by Pawar et al [17] and Patel et al [18] using porcine ear skin membrane in amberized vertical Franz diffusion cell assembly (PermeGear Inc., Hellertown, PA) with stir bars (Corning Incorporated Life Sciences, Tewksbury, MA). A fresh full porcine ear was obtained from a local meat shop, stored at -20 °C and used within 3 days. On the day of experiment, the porcine ear was allowed to thaw at room temperature for 60 min, cleaned with Kimwipes (Kimberly-Clark Inc., Roswell, GA) and cut into sections of 3.5x3.5 cm^2. Full thickness skin (1.5±0.1 mm) was obtained by removing fat and other connective tissues using disposable surgical scalpels (Hamilton Bell Co., Inc., Montvale, NJ). Further, these prepared skin pieces were visually examined against light to look for any cuts or pinholes. The thickness of the prepared skin membrane was measured by digital caliper (VWR International LLC, West Chester, PA). The skin membrane was hydrated in LRS for 15 min before the experiments. The cells used were standard insulated diffusion cells with 11.28 mm orifice diameter, 1 cm^2 diffusion area and 8 ml receptor volume. Receptor compartment was filled with LRS, continuously stirred at 500 rpm, and maintained at 37±0.5˚C.

The prepared skin membrane was carefully mounted on the receptor cells with the stratum corneum facing the donor in such a way to avoid any air bubble formation at the interface of skin membrane and receptor. Anode was uniformly loaded with accurately measured 0.3 mL of 1% (w/v) DPH in each of the three solvents and mounted on the skin membrane. Particular care was taken to avoid any possible dry spots on the patch while loading the drug formulation. The pH of drug delivery anode was measured using Orion 8135BN flat surface pH electrode (Thermo Fisher Scientific, Bridgewater, NJ) on two different occasions during each *in-vitro* iontophoresis experiment: (i) Pre-iontophoresis drug loaded anode and (iii) Post-iontophoresis drug loaded anode. Cathode was dipped in the receptor solution that was stirred at 500 rpm with a magnetic stirrer.

Iontophoresis was performed at constant direct current of 0, 100, 200, and 300 μA/cm^2 applied for 60 min. A 100 μL sample was withdrawn from receptor every 15 min up to 240 min and replaced with 100 μL of fresh LRS. Samples were analyzed by reversed phase HPLC on the same day. At the end of the 60 min treatment, the iontophoresis patch was removed and skin membrane was visually observed for any damage due to iontophoresis procedures.

***In-vivo* experiments**

The Institutional Animal Care and Use Committee (IACUC) at Long Island University, Brooklyn, New York approved all animal procedures. Iontophoresis experiments were performed on three female, pathogen free, New Zealand albino rabbits that were housed under standard laboratory conditions. At the time of experiments, the animal's age ranged from 12 to 16 months and they weighed 4.1 – 4.8 kg. Iontophoresis and IV-infusion experiments were performed according to a randomized cross over design. After each experiment, the rabbits were allowed at least a week period of full recovery and drug washout before beginning the next study. The day before the *in-vivo* experiment, rabbit's dorsal skin was shaved using electrical animal hair clipper (Oster Turbo A5, Sunbeam Products, Inc. USA) and cleaned by alcohol swab.

On the day of experiment, they were tranquilized by intramuscular administration of 10 mg/ml of acepromazine maleate injection. At 30 min after the sedation, two microdialysis probes were implanted approximately 5 cm apart according to the technique described by Stagni et al [15] using a 25 G x 1.5 inch needle (Becton Dickinson & Co., Franklin Lakes, NJ) as a guide. One probe was used for retrodialysis and was perfused with 1, 5, or 10 μg/ml DPH standard solutions whereas the other probe was used for DPH recovery and hence it was perfused with only LRS.

The position of the probe was adjusted to assure that the semipermeable window was placed exactly at the center of the probe beneath the skin. Both probes were checked for leakage prior of the insertion. Skin was allowed to recover from the insertion trauma for about 45 min. Meanwhile probes were connected to the microdialysis pump and the micro-fraction sample collector with the Teflon tubing.

The procedure for *in-vivo* iontophoresis was adapted from that described by Mannem et al [12]. *In-vivo* passive studies were performed using 1% (w/v) DPH in 100% glycerin and 100% DI water. The *in-vivo* iontophoresis studies were performed using 1% (w/v) DPH in 100% glycerin (G). The electrode sites on the rabbit skin were prepared by briskly rubbing the alcohol prep to remove any contaminants and the areas were allowed to dry thoroughly.

The paper backing from the dispersive pad was removed and the polymer was wetted with water to form a slimy layer. The dispersive pad was then applied to the skin. The drug containing electrode was applied about 2 inches away from the dispersive pad on top of one the microdialysis probes. Both the pads were applied in such a way that the entire surface area of the electrode was in proper contact with the skin. Excess pressure was avoided on the drug delivery pad to prevent leaking of the medication. Current density of 0, 100, 200, and 300 $\mu A/cm^2$ was delivered to each rabbit for 60 min. Microdialysis samples were collected every 10 min for 240 min and analyzed on the same day. Retrodialysis samples were collected every 10 min for 120 min and were also analyzed on the same day.

The IV-infusion experiment procedures were adapted from Juluru et al [19] and consisted of IV administration, skin and blood sampling. Skin sampling procedure was similar to microdialysis as described for *in-vivo* iontophoresis experiments. Serial blood samples were collected by an indwelling catheter (Exel Safelet Cath 24G x 3.4 inch, Exelint International Co., Los Angeles, CA) that was introduced in the auricular artery of one of the rabbit's ear. The exposed artery and catheter were flushed with 100 units/ml heparinized solution.

A short 10 min DPH IV-infusion was administered in the peripheral vein of other ear. The precise amount 6, 8 and 10 mg/Kg DPH IV dose was administered over a 10 min infusion time, using a 3 ml plastic syringe (Becton Dickinson, Franklin Lakes, NJ), a Surflo 2.5 G x ¾ inch winged infusion set (VWR) and a Fusion 400 syringe pump.

Following the 10 min infusion, microdialysis samples were collected every 10 min for 420 min. About 0.5 ml of blood was collected in BD Vacutainer Plus plastic K_2 EDTA tubes (Becton Dickinson) at pre-dose (blank) and at 5, 10, 20, 30, 60, 90, 120, 180, 240, 300, 360 and 420 min post-dose. The plasma was separated by centrifugation of blood samples at 3500 rpm at 3 ˚C for 13 min. Plasma samples were stored at -20 ˚C until further extraction and analysis as described above. All experiments were performed in replicates of three at each current as well as IV dose.

Data analysis: For *in-vitro* iontophoresis experiments, the cumulative amount of DPH released was calculated based on amount of DPH in reservoir plus the amount discarded from each sample. A correction factor was used to account for the discarded drug lost by removing a 100 µL sample and replacing it with 100 µL of fresh LRS. Finally, cumulative amount released was estimated by adding the amount in the reservoir at a sampling time point plus the sum of all previously discarded samples. Cumulative amount released was plotted against the respective sampling time points, the slope of the linear portion of this plot was used to estimate Flux [20]. For all microdialysis involving experiments, dialysate data were plotted at the mid-point of the sampling time interval. By microdialysis sampling, the sample is collected over a time interval and not at a specific time point, hence mid-point of the sample collection time interval was used to perform data analysis.

All skin dialysate concentrations were corrected by the recovery factor determined from *in-vivo* retrodialysis, to estimate the actual peri-probe concentration of DPH in skin [21]. Statistical parameters as Mean, Standard deviations (SD) and Coefficient of variation (CV %) were calculated using Microsoft Excel 2010 (Microsoft Corporation, Seattle, WA). Linear regression with uniform weight or weight inverse method and the non-compartmental pharmacokinetic analysis were performed using Phoenix® WinNonlin 6.3 software (Pharsight, Certara USA, Inc., Princeton, NJ)

RESULTS:

Microdialysis: The estimated *in-vitro* %-gain and %-loss were 63.2±7.5% and 73.9±5.5% respectively. The closeness of the *in-vitro* %-gain and %-loss recoveries indicates that the retrodialysis method can be used to calculate the actual dermis concentration [19]. The *in-vivo* retrodialysis recovery was 61.3±4.4%. The recovery was consistent and reproducible at different concentrations of DPH in LRS. The smaller estimates obtained from *in-vivo* recovery may be due to the fact that *in-vivo* recovery is hindered by the tortuosity of the extracellular fluids.

***In-vitro* iontophoresis experiments:** **Table 1** reports the skin membrane thickness and the estimated permeability parameters (flux and lag time). Additionally **Table 1** also reports the dichotomous response of skin damage following iontophoresis. **Fig. 2** shows the DPH permeation profile for each solution across the porcine skin membrane for the four different treatments.

TABLE 1: *IN-VITRO* ESTIMATED PERMEATION PARAMETERS FOLLOWING IONTOPHORESIS OF 1% DPH (W/V) THROUGH PORCINE EAR SKIN MEMBRANE USING DIFFERENT SOLVENTS AT DIFFERENT CURRENT DENSITY; DATA ARE REPORTED AS MEAN ± SD (N=3).

Solvent	Anodic current for 60 min	Skin thickness (mm)	DPH Flux (µg/cm^2*min)	Lag time (min)	Skin burning observed (Yes/No)
100 % Glycerin	Passive	1.58±0.01	0.10±0.04	100.6±25.33	No
	0.3mA	1.43±0.01	0.12±0.06	64.88±39.21	No
	0.6mA	1.47±0.03	0.27±0.07	40.56±25.27	No
	0.9mA	1.48±0.15	0.70±0.32	35.55±25.23	No
50:50	Passive	1.44±0.08	0.99±0.22	49.54±8.02	No
Glycerin/Water	0.3mA	1.45±0.13	0.48±0.17	54.49±37.23	Yes
	0.6mA	1.42±0.13	0.49±0.33	61.91±32.06	Yes
	0.9mA	1.41±0.16	0.49±0.46	67.73±17.79	Yes
100 % Water	Passive	1.43±0.04	0.92±0.41	52.04±20.04	No
	0.3mA	1.46±0.07	0.75±0.65	50.01±47.61	Yes
	0.6mA	1.45±0.05	0.69±0.23	46.16±16.91	Yes
	0.9mA	1.42±0.08	0.37±0.08	38.7±18.65	Yes

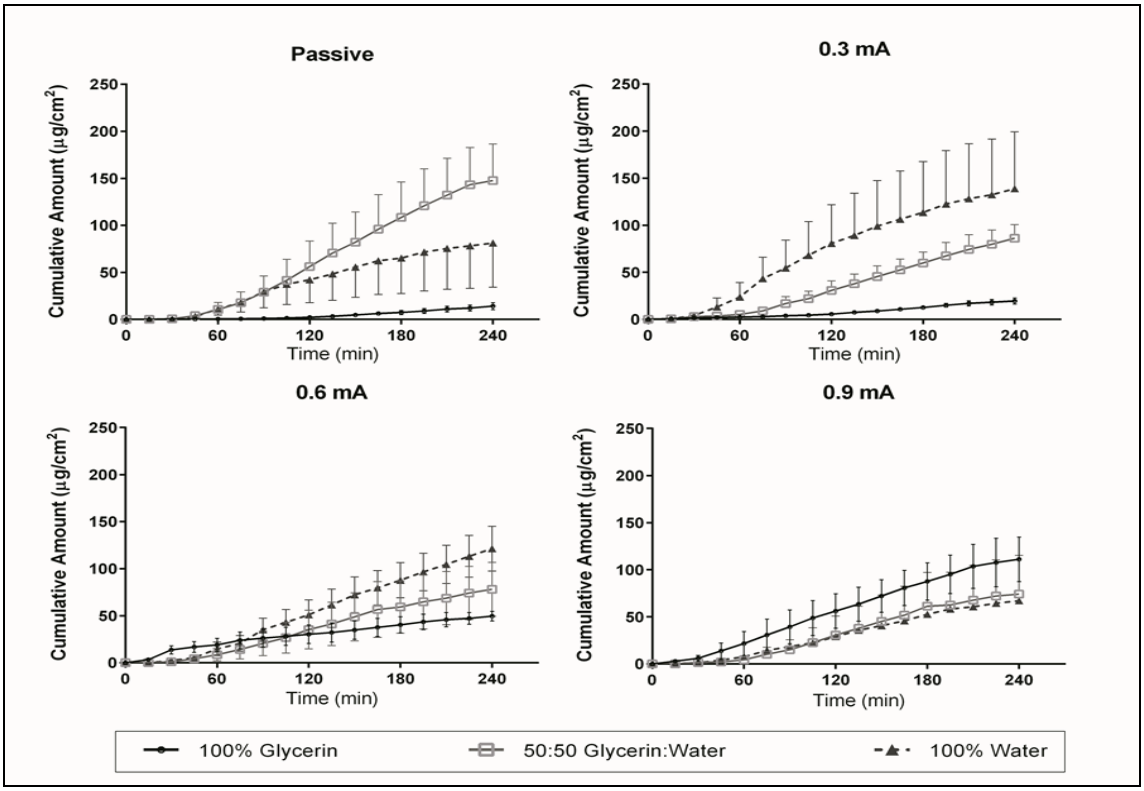

FIG. 2: *IN-VITRO* DPH PERMEATION PROFILES ACROSS PORCINE SKIN FOR EACH OF THE FOUR DIFFERENT CURRENT DENSITY APPLIED (0, 100, 200, AND 300 µA/cm^2).

Iontophoresis of DPH performed in 100% DI water caused remarkable skin damage even at the lowest current density, whereas no skin damage was observed in case of DPH in 100% glycerin even at the highest current density. The amount of DPH permeated from the 100% glycerin solution increased proportionally with the increase in current density, whereas no current related trend was observed for the DI-water containing solvents (**Table 1**). **Fig. 3** shows the difference in pH between before and after application of electrical current: the pH of the donor compartment increased during passive delivery whereas it decreased with application of positive current (**Fig. 3**). However, the change in pH was substantially lower when the solvent contained 100% glycerin.

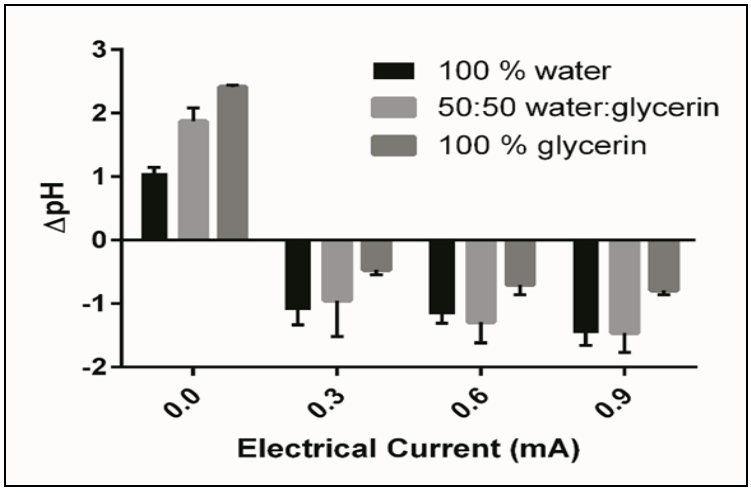

FIG. 3: DIFFERENCE IN THE pH MEASURED BEFORE AND AFTER THE APPLICATION OF 60 MINUTES ELECTRICAL CURRENT *IN-VITRO.*

In-vivo* experiments*: **Fig. 4** shows the pharmacokinetic profiles in skin resulting from DPH iontophoresis (Panel A) and IV-infusion administrations (Panel B), as well as in plasma from the IV-infusion (Panel C). The estimated *in-vivo* pharmacokinetic parameters are reported in **Table 2.** Following iontophoresis, DPH appeared in skin with a delay that decreased with increasing current density. The time to the peak (T_{max}) also decreased with current density, whereas exposure in skin increased proportionally to current density. Low levels of DPH were detectable with passive delivery (0 current density) when DPH was dissolved in glycerin, however no passive delivery was observed when DPH was dissolved in water, contradicting the *in-vitro* results. Following the 10 min IV-infusion, plasma exposure increased proportionally to the dose. DPH appeared immediately in skin and the skin exposure increased proportionally to the dose, however the time to the peak (T_{max}) increased.

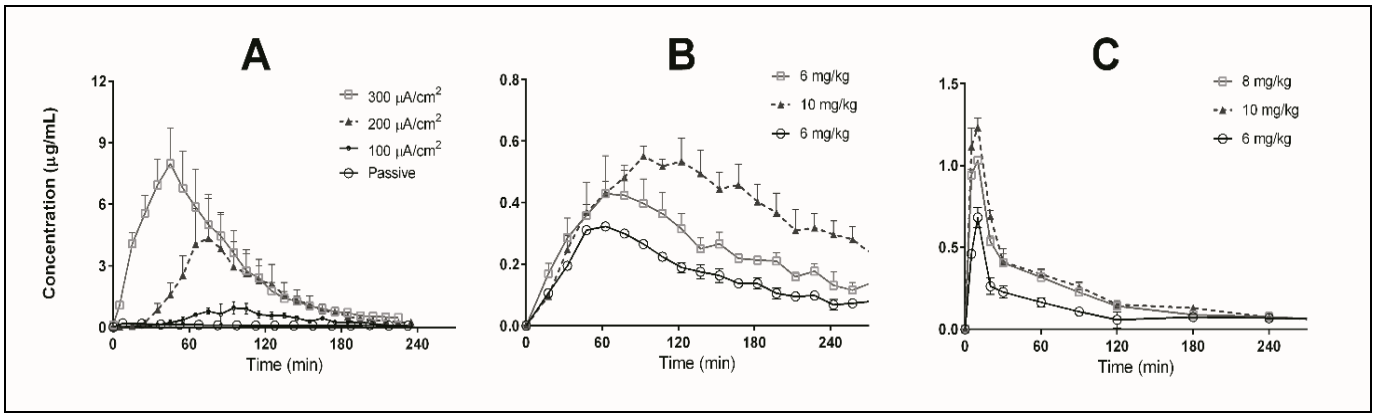

FIG. 4: PANELS A AND B: SKIN CONCENTRATION PROFILES RESULTING FROM THE IONTOPHORESIS AND IV-INFUSION ADMINISTRATIONS RESPECTIVELY. PANEL C: PLASMA LEVELS FROM THE IV-INFUSIONS. PANEL A ALSO SHOWS THE PASSIVE DELIVERY (0 CURRENT) DATA FROM DPH DISSOLVED IN GLYCERIN. PASSIVE DELIVERY FROM WATER SOLUTIONS WAS UNDETECTABLE.

TABLE 2: THE ESTIMATED *IN-VIVO* PHARMACOKINETIC PARAMETERS. DATA ARE REPORTED AS MEAN ± SD (N=3).

Parameter		AUC (µg*min/mL)		C_{max} (µg/mL)		T_{max} (min)		Half-life (min)	
		Skin	Plasma	Skin	Plasma	Skin	Plasma	Skin	Plasma
IV-infusion Ionto -phoresis	0.3mA	85.3±24	-	0.91±0.38	-	63 - 93	-	51.88±33.96	-
	0.6mA	350±249	-	4.37±3.38	-	66 - 76	-	37.63±7.11	-
	0.9mA	663±226	-	7.98±3.15	-	55	-	42.73±7.64	-
	6 mg/kg	52±2	53±3	0.3±0.01	0.70±0.06	54 - 66	10	120.5±25.2	218±70.1
	8 mg/kg	78±7	84±2	0.5±0.1	1.03±0.01	65 - 79	10	114.9±39.3	101±15.4
	10 mg/kg	129±15	97±4	0.6±0.05	1.20±0.06	91 - 123	10	176.0±28.1	97.2±0.09

DISCUSSION: The main purpose of this study was to investigate the effects of current on the iontophoretic delivery of DPH using Glycerin and DI-water as solvents and to understand the skin concentration as a function of time, which was effectively possible via cutaneous microdialysis technique.

The *in-vitro* results suggest that glycerin might be a better choice of solvent for iontophoresis of DPH as there was no skin damage observed and also the amount of DPH permeated through porcine skin was directly proportional to the current applied. The decrease in pH observed following the 60 min of electrical current application on the electrode containing water was twice the decrease observed for the 100 % glycerin solvent.

The water electrolysis and production of hydrogen ions may explain the brown spots observed on the skin following those experiments. This "burning" of the skin might have lead to protein coagulation and consequent increase in the barrier capacity of the skin which would explain the decrease in Flux (**Table 2**) observed in the iontophoresis experiments compared with the passive ones. Glycerin dissolves many salts and the resulting solutions are current conductors. The advantage of using glycerin over water is that unlike water glycerin does not release hydrogen ions through hydrolysis.

Glycerin avoids the occurrence of electrochemical burning and act as a skin protectant. Interestingly, passive *in-vitro* delivery of DPH from the 100% water and 50:50 glycerin/water solutions was much higher than from the 100 % glycerin (**Fig. 2**). However, these result was not confirmed *in-vivo*, where the DPH was never detected in skin dialysates when 100% water was the solvent. Conversely, low levels of DPH were detectable from passive delivery in 100% glycerin. These results suggests that in-vitro experiments may not always be a dependable predictor of in-vivo behaviour.

The *in-vivo* results demonstrate that DPH can be effectively delivered to rabbit skin by the iontophoresis technique with no skin damage when DPH is dissolved in Glycerin. Indeed, the current was well tolerated by the rabbits and no irritation or burning was observed at the application site even at the highest current density applied for 60 min. The exposure of DPH in the skin dialysate increased propotionally to the current applied, showing that the delivery of DPH can be finely controlled by changing current density.

The DPH skin exposures observed from iontophoretic delivery were of higher magnitude and more variable than those observed from the IV-infusions. Skin concentrations during iontophoresis are affected by several factors, such as the inherent characteristics of the skin site where the patch is applied, the transport of DPH to different depth, binding of drug to phospholipids of skin or other components of the skin as well as the position of the microdialysis probe membrane relative to the patch.

The skin concentration profiles changed considerably at the different current densities. An apparent plateau was reached for the administration of DPH through iontophoresis at lowest current density while on contrary, at the higher current densities the curves became sharper and comparatively narrow.

Conversely, the skin exposures resulting from the IV-infusion increased proportionally with the dose administered and the peak concentrations was reached at later times in spite of the fact that the length of the infusion was the same for all doses and the plasma peaks occurred at the end of the infusion for all the experiments (**Table 1**). Also the half-life is substantially longer in the IV-infusion experiments compared with iontophoresis, showing that distribution plasma to skin continues also at the lower plasma concentrations.

CONCLUSION: In conclusion, the results of this project proves that 100% glycerin can be effectively used as a solvent for the iontophoretic delivery of DPH using a stainless steel electrode. The use of cutaneous microdialysis technique to study DPH pharmacokinetics in skin showed to be a powerful tool to evaluate and compare the concentration–time profiles of DPH following iontophoresis at different current. These results show that electric current is the driving force for the increased penetration of DPH across rabbit skin and significant skin concentration of DPH can be achieved in a short time through iontophoresis. Further this project opens the door for more research to explore the skin concentration range at which DPH acts as a local anesthetic, promoting this molecule as an alternative therapy for patients allergic to lidocaine, prilocaine and similar local anesthetics.

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