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ORIGINAL ARTICLE

Pharmacokinetics of amitriptyline in rabbit skin and plasma following iontophoretic administrations

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Abstract

Background: Amitriptyline (AMT) is a tricyclic antidepressant with demonstrated local analgesic effects in human skin. *Aim*: We investigated the feasibility and mechanisms of iontophoretic delivery of AMT to rabbit dermis and plasma. *Method*: Two microdialysis probes were inserted into the upper dorsal shaved skin of tranquilized rabbits. After 1 hour, an iontophoresis cartridge was placed on top of one probe. The cartridge consisted of a stainless steel electrode covered with a pad that was filled with a 4.3 % AMT glycerin/water (50:50). Iontophoresis was performed at 100, 200, or 300 μA/cm^2 constant-current density for 60 minutes. Dialysate samples were collected every 8 minutes for 3 hours and analyzed for AMT via a validated high-performance liquid chromatographic assay. Retrodialysis was performed at the other site. Blood samples were collected serially for 4 hours. *Results*: In vivo retrodialysis recovery was 89 ± 2%. AMT skin exposure increased non-proportionally with current density: AUCs were 19 ± 7, 119 ± 56, and 615 ± 302 mg/L/min for the 100, 200, and 300 μA/cm^2, respectively, and C_{max} were 107 ± 15, 1070 ± 537, and 5870 ± 1289 μg/L. In vivo plasma concentrations were always below LLOQ (0.1 μg/mL). *Conclusion*: AMT can be administered by iontophoresis and produces significant skin concentrations and negligible plasma levels.

Key words: *Amitriptyline; anesthetic; iontophoresis; local analgesia; microdialysis*

Introduction

Amitriptyline (AMT) is a tricyclic antidepressant that is typically prescribed for major depressive disorders[1]. AMT is also used off-label for chronic and neuropathic pain syndromes. The mechanism of pain relief is still under investigation[2–4]; however, it appears located at the peripheral level. Indeed, it has been shown that AMT exhibits local anesthetic/analgesic properties. For example, AMT showed potent local anesthetic properties in a rat sciatic-nerve-block model as well as in rats and sheep spinal-block models[5]. It has been proved that AMT produces longer local anesthetic effect than the long-acting local anesthetic bupivacaine when injected at rat sciatic notch for sciatic nerve blockage[5] as well as a longer effect over bupivacaine in producing cutaneous infiltration blockade after subcutaneous injection[6]. Further testing has shown that AMT is more potent than lidocaine in providing cutaneous analgesia when applied transdermally with an occlusive dressing in rats[7]. Studies in human volunteers confirmed that AMT has local anesthetic and analgesic properties also in human skin with differential effect on different pain-fiber structures[8]. These studies suggest that the local administration of AMT to skin may alleviate neuropathy symptoms without the side effects produced by the high doses necessary to achieve the results systemically.

The primary objective of this project was to examine the feasibility of AMT delivery through iontophoresis for either the induction of local skin anesthesia/analgesia or as an alternative route to systemic administration for the treatment of depression. Iontophoresis[9] is a delivery technique that uses a mild current to force ionized molecules into the skin. Iontophoresis was selected over other methods of skin delivery systems because of the fast onset of delivery that is desirable in a pain relief system. Indeed, the small molecular weight and the fact that AMT is ionized at physiological pH (pK_a 9.45)[10]

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make AMT a suitable candidate for electrical delivery. Experiments were conducted in vivo in a rabbit model using intradermis microdialysis[11] as the sampling technique in the skin and conventional blood sampling to assess systemic availability. Intradermis microdialysis is the ideal tool to perform such a study because the terminal nerve fibers that mediate AMT anesthesia are mostly located in the dermis. The results of these studies will help us to understand the disposition of AMT in skin following iontophoresis and to plan future efficacy studies.

Material and methods

Chemicals

AMT hydrochloride, imipramine hydrochloride, nortriptyline hydrochloride, and triethylamine were purchased from Sigma (St. Louis, MO, USA); lactated Ringer's injection, USP, was from Hospira, Inc. (Lake Forest, IL, USA); dichloromethane was from EM Science (Gibbstown, NJ, USA); methanol, acetonitrile, high-performance liquid chromatography (HPLC) grade water, and phosphoric acid were from J. T. Baker (Philipsburg, NJ, USA); sodium phosphate, monobasic, monohydrate (granular), and sodium phosphate dibasic anhydrous (granular) were from Mallinckrodt Baker, Inc. (Paris, KY, USA). All other reagents were of analytical or HPLC grade.

Amitriptyline HPLC analysis

AMT was quantified using HPLC in dialysates and plasma samples. The HPLC apparatus consisted of L-6200A Intelligent Pump (Hitachi Ltd., Tokyo, Japan), 717 plus Autosampler (Waters, Milford, MA, USA), a reversed-phase C_{18} column (Synergi 4μ Hydro-RP 81A, 150 × 4.60 mm, 4 μm; Phenomenex, Inc., Torrance, CA, USA), L-4250 UV-VIS Detector (Hitachi Ltd.). Perkin Elmer software TotalChrom® Chromatography Data System 6.1 was used for peak integration. AMT concentrations were determined according to a method adapted from Zarghi et al.[12] The isocratic mobile phase consisted of 0.05 M phosphate buffer (pH 5.1), acetonitrile, and triethylamine in the proportion (60:40:0.1) for microdialysis samples and (70:30:0.1) for plasma samples. Detection wavelength was 239 nm. Flow rate and the injection volume were 1.0 mL/min and 10 μL, respectively, for microdialysis samples whereas they were 1.2 mL/min and 25 μL for plasma samples. Samples in the HPLC-autosampler were kept at 10°C. Validation of the analytical method in Ringer's showed that peak eight increased linearly with concentrations in the range 5–10,000 ng/mL. Accuracy, expressed as % error (%E), was always below 8%, and precision (CV%) was below 12% with an exception of the lowest concentration (5 ng/mL; LLOQ) for which it was 17%. Validation in plasma gave an LLOQ of 100 ng/mL and a linearity range of 10–0.1 μg/mL. Selectivity was tested versus blank samples of skin dialysates and plasma. Also, the major metabolite, nortriptyline, did not interfere with the AMT peak.

Plasma samples

A new plasma extraction method was developed during the course of this project. Hundred microliters of plasma was added with the internal standard solution (imipramine) and AMT. Proteins were precipitated by adding 400 μL of acetonitrile and thoroughly mixed by vortexing for 1 minute. After 5-minute centrifugation at 93.52 × *g*, 350 μL of upper phase was transferred to a clean Eppendorf vial and added with 1 mL of dichloromethane. The tube was gently shaken and then centrifuged at 9352 × *g* for 5 minutes; the upper layer was discarded and the lower layer was evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was re-dissolved in 50 μL of methanol, and a 25-μL aliquot was injected onto the HPLC column. Recovery of AMT from spiked plasma was 80 ± 4% (n = 15) compared with that from Ringer's solution.

Microdialysis equipment

The Microdialysis equipment consisted of a CMA/102 microdialysis pump (CMA/Microdialysis AB, Stockholm, Sweden), a CMA/142 auto sample collector (CMA/Microdialysis AB), 1.0 mL EXMIRE micro syringe type I (ITO Corporation, Fuji, Japan), and custom-made linear probes that were made in our laboratory according to the method described by Stagni et al.[13] Probes had 1 cm dialysis membrane made of a tubular polyacrylonitrile membrane with a molecular weight cutoff of 50 kDa (AN69 HF, Hospal-Gambro, Inc., Lakewood, CO, USA).

In vitro microdialysis method validation

The suitability of the 'retrodialysis' method[14] for calculation of probe recovery in vivo was tested in vitro, at 35°C to mimic the skin temperature, as described previously[15]. The flow rate was set at 2 μL/min and dialysate samples were collected every 8 minutes for 2 hours.

For calculation of retrodialysis recovery, the probe was perfused with AMT in lactated Ringer's solution of 10,000, 250, or 150 ng/mL ($C_{perfusate}$). The inner chamber of glass cells was filled with blank lactated Ringer's solution. At the end of the experiments, the solution in the inner chamber was sampled thrice and analyzed using HPLC. Relative recovery (RR) was calculated as

$$RR = \frac{C_{perfusate} - C_{dialysate}}{C_{perfusate}} \quad (1)$$

where $C_{perfusate}$ is the concentration of the perfusing solution and $C_{dialysate}$ is the concentration of the dialysate solution.

For calculation of extraction recovery, the probe was perfused with blank lactate Ringer's solution and the inner chamber was filled with the following concentration of AMT solution: 10,000, 250, or 150 ng/mL. RR was calculated as

$$RR = \frac{C_{dialysate}}{C_{bulk}} \quad (2)$$

where C_{bulk} is the concentration in the glass cell and $C_{dialysate}$ is the concentration of the dialysate solution.

Binding studies were performed to assess possible binding of AMT to the probe materials and glass apparatus. It was found that AMT binds to the glass apparatus; thus a correction factor was experimentally determined and the bulk concentrations were corrected by this factor. Recovery was 0.67 ± 0.07 (n = 10) and 0.64 ± 0.06 (n = 7) from retrodialysis and extraction, respectively.

Iontophoresis

In vitro

The stability of AMT to electrical current was studied in vitro. A stainless steel electrode identical to that used in vivo but without the pad was immersed in a beaker containing 50 mL of an AMT solution 20 μg/mL in distilled water. Electrical current was applied for 3 hours at 100, 200, or 300 μA/cm^2 current density. For each experiment a control experiment was carried out in identical conditions but without electrical current. Samples from the solution were taken every 15 minutes for 90 minutes and every 30 minutes thereafter. Samples were analyzed using HPLC for AMT content within 12 hours.

In vivo

The iontophoretic delivery system consisted of (i) a plastic cartridge (donated by Transport Pharmaceutics, Inc, Framingham, MA, USA) with a steel electrode with a constant surface area of 2.0 cm^2 covered with a nonwoven polypropylene pad of 3.14 cm^2 in which the drug formulation was placed, (ii) a dispersive electrode (from the Iomed TransQ® kit; IOMED, Inc., Salt Lake City, UT, USA), and (iii) a constant current source (Phoresor II, model no. PM 700; IOMED, Inc.). Iontophoresis applications were done at a current density of 100, 200, or 300 μA/cm^2 on each of three rabbits. Each iontophoresis pad was completely wetted by 0.3 mL of AMT solution (43 mg in 1 mL of 50:50 water/glycerin) making sure that no portion of pad was dry. The pad was put on the skin exactly on the top of the microdialysis probe without giving any pressure to avoid leaking from the pad or blocking of the probe. Particular care was taken to assure that the inlet and the outlet of the microdialysis probes were always outside the drug cartridge. The inlet and the outlet of the microdialysis probes were sealed with cyanoacrylate glue (Pacer® Technology, Rancho, Cucamonga, CA, USA). AMT is positively charged in these conditions, so the positive electrode was attached to the drug patch and negative electrode or anode was attached to the dispersive pad. After 1 hour, the patch was removed from the skin and patch site was cleaned with alcohol swab without applying any pressure. At the end of the experiments the surface pH of the cloth pad was measured with a surface electrode (Orion® Ross; Thermo Scientific, Waltham, MA, USA).

In vivo experiments

The iontophoresis experiments were performed on three healthy and pathogen-free New Zealand albino female rabbits. The rabbits were kept in hygienic laboratory environment (22 ± 1°C, relative humidity 40–60%) and were given normal rabbit chow to eat and tap water to drink. All experimental conditions and procedures on rabbits were approved by IACUC of the Long Island University. Rabbit's age ranged from 3 to 6 months and weight was about 2–3.5 kg. The day before the experimental day, rabbit's dorsal skin was shaved with an electrical animal hair clipper. On the experimental day, the rabbit was tranquilized and two microdialysis probes were inserted according to the procedure described by Stagni et al.[13] One probe was used for calibration (retrodialysis) and it was perfused with a 1-μg/mL AMT solution for 2 hours and then with lactated Ringer's solution for 4 hours. The other probe was placed under the iontophoresis patch and was perfused with lactated Ringer's solution for 6 hours. Flow rate was 2 μL/min. Dialysis samples were collected every 8 minutes. Recovery factor was calculated using Equation (1). Blood samples were collected serially from the auricular artery at 0, 20, 40, 60, 80, 100, 120, 140, 170, and 200 minutes. The plasma was immediately separated, frozen, and stored at –20°C until assayed.

Data analysis

Drug concentrations in the skin interstitial fluid were obtained by dividing the concentration in the dialysate by the recovery factor (RR). For pharmacokinetic analysis, time was corrected to account for the time necessary for the solution to travel from the microdialysis membrane to the sample's collector.

Means, SDs, CV%, *t*-tests, analysis of variance (ANOVA), and linear regression were performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA) or with Microsoft Excel 2007 (Microsoft Corp., Seattle, WA, USA). The noncompartmental pharmacokinetic analysis was performed using WinNonlin 5.2 (Pharsight Inc., Mountain View, CA, USA). The following parameters were estimated: AUC_{last}, C_{max}, elimination half-life, and T_{max}. The limited number of time points available for declining concentrations did not always allow a clear determination of the log-linear component of elimination (λ_z). Therefore, AUC_{last} was used rather than AUC_{inf} as that could be affected by the estimation of λ_z. Pharmacokinetic parameters were summarized as mean ± SE. One-way ANOVA was done on the log-transformed AUCs and C_{max}. The Scheffé post hoc procedure for multiple comparisons was applied to detect differences between means. The significance level was 0.05.

Results

Rabbits tolerated the iontophoresis procedure well and no skin irritation such as rash, erythema, or edema was observed during or after the experiments. No changes in rabbit's eating and drinking habits, feces, and urination were observed for 2–3 days after experiments. In vivo retrodialysis RR was 89 ± 2% (mean ± SD, n = 7). There were no significant differences in microdialysis recovery across experiments ($P > 0.05$), which indicates that recovery was consistent and reproducible. Dialysate concentrations collected at the iontophoresis sites were corrected by the corresponding recovery to obtain dermis interstitial fluid concentrations. When recovery from the same experiment was not available, the average of available recoveries was used.

Because of the large variability in data, more than the initial 3 × 3 crossover-design experiments planned were done. A total of 14 experiments were completed: seven experiments were conducted for 200 μA/cm^2 compared with three for 100 μA/cm^2 and four for 300 μA/cm^2 current density. The additional experiments for the middle value of current density were done to make sure to detect the correct relationship between current density and AUC or C_{max}. Figure 1 shows the average dermis concentration sorted by current density. The log-scale was used because of the wide ranges of concentrations observed. Table 1 reports the pharmacokinetic parameters obtained by noncompartmental pharmacokinetic analysis.

ANOVA was performed on the log-transformed AUC, and C_{max}, because the distribution of these parameters is skewed to the right and consistent with a log-normal distribution as that of most pharmacokinetic parameters. ANOVA showed that C_{max} and AUC were clearly dependent on the current density applied ($P > 0.001$) but not in a linear way (Figure 2). Indeed, there was no statistically significant difference ($P > 0.05$) following the 100- and 200-μA/cm^2 skin exposures. However, both the 100- and the 200-μA/cm^2 exposures were significantly different from the 300 μA/cm^2 ($P > 0.001$ and 0.008, respectively). An exponential equation fitted the data with a correlation coefficient larger than 0.99 (Figure 2). T_{max} was always independent of current density.

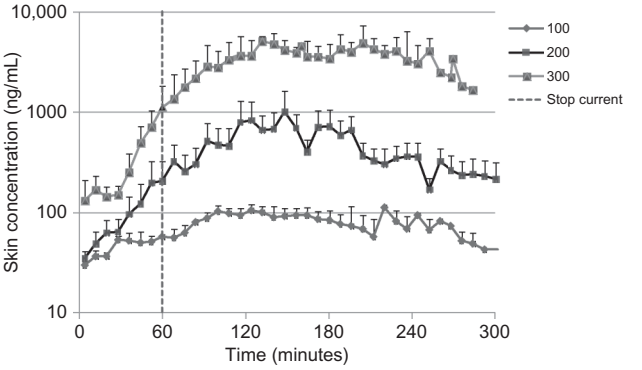

Figure 1. Dermis concentrations of amitriptyline following iontophoresis of 100, 200, and 300 μA/cm^2. Data are reported as mean + SE.

Table 1. Pharmacokinetic parameters estimated in skin following iontophoresis.

	Current density		
Parameter	100 μA/cm^2 (n = 3)	200 μA/cm^2 (n = 7)	300 μA/cm^2 (n = 4)
AUC-last (mg/L/min)	19 ± 7	119 ± 56	615 ± 302
C_{max} (μg/L)	107 ± 15	1070 ± 537	5870 ± 1289
T_{max} (minutes)	143 ± 12	165 ± 15	132 ± 8.6
$t_{1/2}$ lambda-z (minutes)	93 ± 40	80 ± 19	75 ± 46

ANOVA performed on the in vitro iontophoresis data showed that after 1 hour, AMT underwent a statistically significant degradation ($P < 0.01$) with current compared with the noncurrent experiments. However, the extent of degradation was minimal: –2.3 ± 1.4%, –2.9 ± 0.3%, and –2.7 ± 0.8% for the 100, 200, and 300 μA/cm^2, respectively, and was independent of current density. At the end of the 3 hours of electrical current delivery, the effect of the current density was still statistically insignificant and degradation was –3.7 ± 3.0%, –4.7 ± 1.9%, and –6.4 ± 4.7% for the 100, 200, and 300 μA/cm^2, respectively.

Passive delivery of AMT from the iontophoretic patch to the skin was determined. For these experiments no current was applied. AMT concentration in dialysate was always below the limit of detention, showing negligible passive diffusion of AMT into the skin. AMT formulation was not optimized for passive delivery as AMT was simply dissolved in water/glycerin solution (50:50) in which AMT is still in ionized form. This could probably explain the lack of delivery. The pH

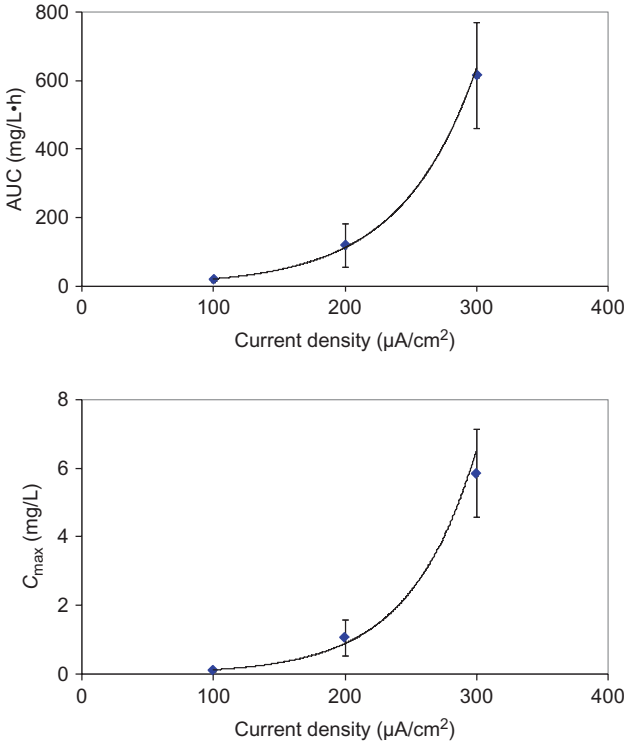

Figure 2. Average area under the dermis concentration curve (AUC) and C_{max} of AMT versus current densities. Error bars represent SE.

of drug patches was measured after each experiment, and it was observed that pH decreased (−1.9 units ±0.44) compared with the initial pH following iontophoresis. However, it remained unchanged after passive delivery (pH: 5.43 ± 0.03). The decrease in pH is probably due to the production of H^+ ions resulting from the electrolysis of water at the stainless steel surface of the iontophoretic steel electrode.

To characterize the elimination from skin independently from iontophoresis, in five experiments, the in vivo retrodialysis was followed by a washout period in which plain lactated ringer's solution was passed throughout the probe to evaluate the elimination phase of AMT from dermis interstitial fluid. The dermis concentrations declined always mono-exponentially with an average half-life of 69 ± 16 minutes (mean ± SE, $n = 5$). This value is not statistically different ($P > 0.05$) from the half-lives measured from the iontophoresis studies (71 ± 10 minutes, $n = 14$).

AMT concentration was always below the LLOQ in the plasma samples collected.

Discussion

The goal of this project was to characterize the pharmacokinetics of AMT in dermis interstitial fluid and in plasma resulting from iontophoretic delivery at three increasing current densities. Delivery of drugs through the skin is meant either to treat local diseases or to produce effective plasma concentrations as an alternative route to the oral or intravenous administrations. Local sampling in skin was obtained by microdialysis, a technique that allows the collection of unbound molecules present in the extracellular fluid with a minimal disruption of the physiological function of the target tissue[16]. Comparison of the microdialysis recovery from the studies performed in vitro with those performed in vivo shows that AMT has different diffusion behavior compared with other drugs studied in specific tissues by microdialysis. It is usually observed that the recovery in vivo is less than that in vitro because of the resistance of the surrounding tissues[17]. However, AMT exhibited the opposite behavior with an average in vitro recovery of 65% compared with an average in vivo recovery of 89%; showing that the drug has a high affinity for the surrounding dermal tissue. Indeed, AMT is a highly lipophilic molecule with a log *P* (octanol/water, pH 7.4) of 3.0, and it is classified as a high-permeability drug[10]. Despite its lipophilicity, AMT is easily recovered by microdialysis with a simple hydrophilic perfusion solution, because AMT is mostly ionized at physiological pH (99% at pH 7.4 as calculated by the Henderson–Hasselbalch equation).

Following iontophoresis, AMT appears in the dermis slowly and the higher concentrations are observed at 150 minutes (T_{max}), that is, 70 minutes after the discontinuation of current delivery, removal of the patch, and cleaning of the surface of the skin. It is possible that a large amount of AMT delivers immediately to the upper layers of the skin where it is stored at first and then it moves slowly to the lower part of the dermis where the concentration is measured. For all the treatments the maximum dermis concentration was reached approximately at the same time (Table 1), showing that the time to reach plateau is independent of dose and it depends only on the elimination half-life. Figure 1 also shows a prolonged plateau and that the concentration starts to decline only in the last hour of the experiments. A steady state is usually observed when the rate of input equals the rate of elimination. This means that 1 hour of iontophoresis may produce a sustained delivery for an additional 2–3 hours. The elimination phase cannot be well characterized because of the lack of data during the elimination phase. A longer sampling period would have caused too much stress to the rabbit, and it was not considered necessary.

AMT skin exposure (AUC and C_{max}) increased exponentially with current density (Figure 2).

These findings may be explained by the fact that AMT is highly lipophilic; consequently it penetrates easily into cells, can be stored up in fat, and can also bind to the proteins and the other structures in the skin. Therefore only a little concentration of AMT is free in

the interstitial fluid and detectable by microdialysis at lower doses. The increasing amount of AMT delivered to the dermis caused by the increasing current density may saturate both the skin proteins' binding sites and the lipophilic sites causing a buildup of AMT concentration in the interstitial fluid. However, the possibility that at higher current densities the skin barrier becomes more permeable to AMT may not be ruled out. Degradation of AMT by the electrical current is negligible as demonstrated by the in vitro studies.

In plasma samples, AMT concentration was always below the limit of detection (100 ng/mL), showing that negligible systemic delivery occurred with the iontophoretic area used.

The results of this study demonstrate that iontophoresis can be an alternative route of administration for delivery of AMT as a local analgesic with minimum local and systemic side effect, but it may not be a promising technique for systemic delivery.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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