Transdermal Iontophoresis of Tacrine *in Vivo*

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INTRODUCTION

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine) is a reversible cholinesterase inhibitor used perorally for Alzheimer′s disease (1). Oral administration of tacrine is associated with a low bioavailability (due to the gut-wall first-pass effect), a short elimination half-life (2), a reversible, dose-dependent hepatotoxicity, and peripheral cholinenergic side effects (3). The potential advantages of transdermal delivery of tacrine are: minimized first-pass metabolism, reduced incidence of gastrointestinal side effects and hepatotoxicity associated with peroral administration, and provision of fairly constant blood levels for an extended period of time. It has been postulated that the constant levels of tacrine in the brain may maximize its effects on memory enhancement (3).

Passive permeation through the skin (*stratum corneum*) is especially difficult to compounds which are hydrophilic, very lipophilic, of high molecular weight or charged (4). Iontophoresis improves the transport of ions into and through the skin by the application of an external electric field across the skin. Clinically significant doses of, e.g., lidocaine, apomorphine and fentanyl, have been iontophoresed across the human skin *in vivo* (5–7). When the device controls the transdermal drug flux instead of the skin, delivery of the drug is more reproduciple. One way to control the transdermal drug delivery is the use of ion-exchange fiber(s) and iontophoresis (8,9). Binding of charged molecules into the ion-exchange material has been presented as a method to store active molecules until they are released from the ion-exchange groups by mobile co-ions. The improved transdermal drug permeation is then accomplished by iontophoresis (8,10).

The aim of this short-term preclinical study was to determine, whether therapeutically relevant plasma concentrations of tacrine could be achieved using iontophoretic transdermal drug delivery in healthy human volunteers. We wanted to compare the *in vivo* transdermal delivery of tacrine from a commercial Iogel®-electrode to a novel ion-exchange fiber formulation. Reduction of interindividual variation in the plasma levels of tacrine was attempted by the ion-exchange approach. Adverse reactions on the skin (irritation/erythema, swelling/drying), caused by tacrine and/or electric current, were evaluated by visual observations and by asking the volunteers about their personal sensations/symptoms. Hepatotoxicity caused by long-term tacrine use is common during oral tacrine therapy (1,3). Therefore, serum alanine aminotransferase (ALT) level of the volunteers was measured before and after the tests. Finally, we compared *in vitro* tacrine permeation to the *in vivo* results.

SUBJECTS, MATERIALS, AND METHODS

Subjects

Ten healthy adult volunteers (5 males and 5 females) were included in the Test I and Test II. The age of the study subjects ranged from 19–52 years, and the body weight of the subjects was 50–86 kg. All the study subjects signed an informed consent, and they were given information about tacrine and the protocol of the experiments. The studies were approved by the ethical committee of the Helsinki University Hospital and Finland′s National Agency for Medicines. A physician (R.S.) supervised the experiments and followed the well-being of the volunteers. The blood samples were taken by a registered nurse.

Iontophoretic Delivery of Tacrine

All the experiments were performed using a battery operated (9V) constant current source Phoresor® II Auto (Iomed Inc., Salt Lake City, Utah). In the first experiment (Test I) the electrodes were commercial Iogel®-electrodes (Salt Lake City, Utah) (7). In the second test (Test II) the heart of a custom-built transdermal device was ion-exchange fiber, wherein tacrine was attached (Fig. 1).

The ion-exchange fiber used was Smopex®-102 [poly-(ethylene-g-acrylic acid)] fiber with -COOH ion-exchange groups (Smoptech Ltd., Turku, Finland). Tacrine(-HCl) and HEPES were obtained from Sigma (St. Louis, Missouri). Ion-selective Nafion® membrane was purchased from ElectroCell AB (Täby, Sweden) and Durapore® porous membrane from Millipore (Ireland). Silver-silver chloride electrodes were used for current delivery (Fig. 1). Next to the anode and cathode electrodes was 1.5 M NaCl solution to maintain proper current delivery. The preparation of ion-exchange fiber discs (that contained the tacrine) has been described in detail previously (8,9). Physiologic NaCl solution in the fiber compartment ensured a predetermined drug release for tacrine permeation. The area of this device on the skin was 10 cm^2, the same area as with the Iogel®-electrode in Test I. The total amount of free tacrine in each experiment was adjusted to 64 mg.

A constant current of 0.4 mA/cm^2 was applied for 3 h on the ventral forearm of the volunteers. For the next 1 h passive tacrine flux was measured. The current/voltage was monitored throughout the experiments by RTO3800G multimeter. To prevent painful sensations on the skin, the current was

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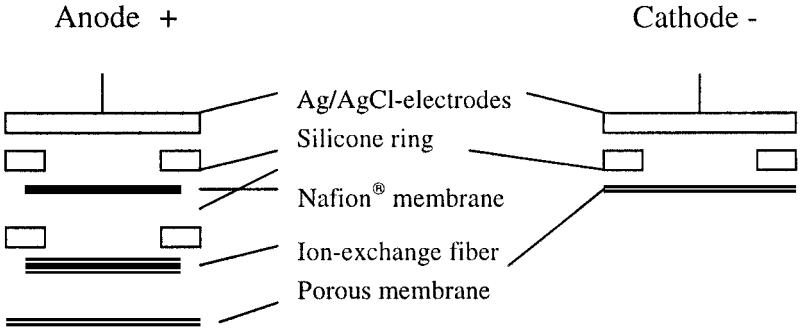

Fig. 1. The structure of the ion-exchange fiber device in the Test II.

gradually increased from 0.1 to 0.4 mA/cm^2 during the first five minutes of the Tests I and II. The position of the electrodes was changed three times during the 3-h experiment.

Assay of Serum Tacrine Concentrations

Venous blood samples of 10 ml were withdrawn from the volunteers at 30, 60, 90, 120, 150, 180, and 240 min. The withdrawn blood samples were centrifugated 30 min after the sampling, and plasma was separated. The plasma samples were kept in a freezer until analysis. Tacrine was extracted from the plasma in an exctracting tube Chrompack Varian® (Bond Elut-C_{18}, Varian Inc., Harbor City, California), (11). The extracting tube was regenerated by 1 ml of MeOH and 1ml of purified water. One ml of the plasma sample was placed in the tube, whereafter the tube was washed with 1.5 ml of purified water. Tacrine was eluted using a 6 ml solution of 22% acetonitrile, 1% triethylamine, and 77% deionized water at pH 6.5. The extracted tacrine solution was evaporated with air and dissolved in 200 μL of HEPES-buffered saline at pH 7.4. The linear concentration range for tacrine extraction was 5–250 ng/ml with a precision of ±4.2% (SD). Drug concentrations were analyzed by HPLC (Beckman Instruments Inc., San Ramon, California), using the method described in detail previous (8).

Safety Evaluation

The study subjects did not have a disease of the liver or a skin damage at the sites of transdermal application. Alanine aminotransferase (ALT) level of the test subjects was determined before and after the experiments. The value had to be ≤50 U/l before the subject was accepted for the tests. Adverse effects of tacrine and iontophoresis on the skin were evaluated visually (erythema, swelling/drying), and by asking the volunteers about their sensations/symptoms during the tests and up to one week after the tests were finished. We also measured the possible irritating effect of tacrine (no current) and iontophoresis (0.1–0.4 mA/cm^2; no tacrine), on the skin of 5 volunteers.

Drug Permeation across the Human Skin *in Vitro*

These studies were performed across the excised human *epidermis* (Helsinki University Hospital) in Franz-type diffusion cells (Laborexin Inc., Helsinki, Finland). The test formulations were placed in the donor compartment, and HEPES-buffered physiological NaCl was placed in the receiver compartment (area 2.41 cm^2). Samples (200 μL) were collected from the receiver compartment and replaced by fresh buffer at 30, 60, 90, 120, 150, 180 (current off), and 240 min.

The *in vitro* steady-state fluxes of tacrine (J_{ss}, μg/min/cm^2) were calculated by linear regression of the permeation curves. The predicted *in vivo* plasma levels were calculated by equation $C_{ss} = J_{ss}A/CL$ (12). The total clearance of tacrine from the plasma, CL, is 150l/h. The area of the devices (A) was related to 10 cm^2. Clinically relevant plasma level at steady state, C_{ss}, is 5–30 ng/ml after oral tacrine intake (1,8).

Data Analyses

Possible statistical differences between the plasma levels in the Tests I and II were determined using a paired *t* test. The standard deviations (a measure of biologic variation) were proportioned to the average values, and tested for a possible difference by the paired *t* test as well. Statistically significant level was set as $P \leq 0.05$.

RESULTS AND DISCUSSION

Transdermal Delivery of Tacrine *in Vivo*

Figure 2 shows the plasma concentrations of tacrine during (0–3h) and 1 h after the application of current. The plasma concentrations of tacrine during the Tests I and II were 21.3 ± 5.9 and 14.9 ± 2.6 ng/ml, respectively. The clinically significant plasma level of orally administered tacrine is 5–30 ng/ml (1). In Test I, all the individual plasma concentrations determined were above 5 ng/ml. In Test II, two study subjects had a few samples lower than the therapeutic tacrine level. In Test II, the average plasma concentrations were slightly lower than in the experiments using the Iogel®-electrodes ($P < 0.05$).

Typically, the tacrine plasma profile of a volunteer plateaued already after the first 30 min of current delivery. During the 1 h passive tacrine permeation (180–240 min), the plasma concentrations of tacrine decreased only slightly (Fig. 2). This may be due to the formation of tacrine reservoir in the skin during the drug delivery. Tacrine is a rather lipophilic drug with a logP octanol/water 3.3, (8), which may well be bound into the lipophilic skin structures (*stratum corneum*) and to be released slowly from there into the systemic circulation.

The standard deviations during the iontophoresis in Test II were slightly smaller than using the gel system (Test I), but the difference was not statistically significant ($P > 0.05$). Thus, the aim to reduce the biologic variation of tacrine delivery by the ion-exchange fiber (8) was not accomplished in this study. Subject variations were, however, quite small in both the gel-

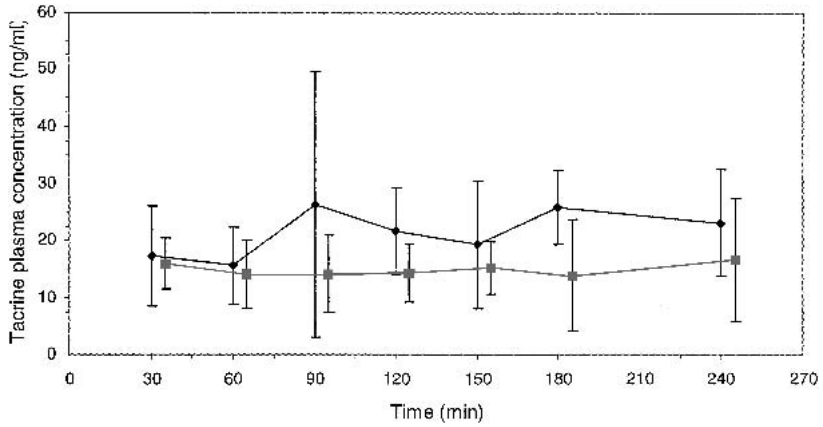

Fig. 2. Plasma concentrations of tacrine as a function of time. Test I, Iogel®-electrodes (N = 10) (♦) and Test II, ion-exchange fiber device (N = 9) (■). Average ± SD To distinguish the Tests I and II, the curve of the latter has been transferred for 5 min.

and ion-exchange formulations. The high plasma concentration (about 90 ng/ml) of one volunteer in Test I at 90 min time-point caused the great standard deviation at this point (Fig. 2). Therefore, both the systems might well be utilized in controlling the transdermal tacrine delivery with clinically relevant early plasma profiles.

Safety of the Iontophoretic Tacrine Delivery

Table I lists the adverse reactions on the skin by the visual observations and by the personal comments of the volunteers during the iontophoretic tacrine delivery. One volunteer interrupted the Test II due to painfull blood sampling (no relation to skin irritation). Application of tacrine solution (64 mg) in a cotton cloth on the skin did not result in any visible/sensitization reactions in 4 h. In contrast, the skin of all the study subjects was clearly erythematous by the iontophoretic current delivery (Table I). The irritation of the skin was directly related to the iontophoretic current density (0.1–0.4 mA/cm^2) and the duration of application. Typically, the maximum constant current density used is 0.5 mA/cm^2, to avoid inconvenient pain or prolonged skin irritation *in vivo* (13). The observed side effects caused by the iontophoresis in these experiments did not differ significantly from the side effects observed previously (6,7,14). Because all the plasma concentrations determined were higher than the smallest therapeutic tacrine concentration, a lower or intermittent current density might be used and still reach clinically relevant medication transdermally.

As indicated in Table II, no difference in the adverse skin effects in Tests I and II could be detected. Slightly pinching sensation was felt by all the study subjects at the first minutes of current passage at the site of application and at nearby regions. A sensation was felt every time the current density was increased or the position of the device was changed. The subjects that had a light skin reported a stronger and longer lasting erythema. Although tacrine itself did not increase the erythema at the concentration used, drying of the skin was observed on several study subjects (Table I). Tacrine, an anticholinergic drug, caused also sensations of coldness on the skin and on the fingertips of 50% of the study subjects.

Transdermal delivery of tacrine had no effect on the alanine aminotransferase (=ALT) levels of the volunteers in these short tests. All the ALT-values of the test subjects stayed under the normal range (≤50 U/l). To determine the possible effects of long-term transdermal delivery of tacrine on the liver function, one obviously needs longer lasting (iontophoretic) experiments. Elucidation of other putative therapeutical benefits/disadvantages of transdermal tacrine delivery also requires at least a 24-h study design.

In Vitro/*in Vivo* Correlation of Tacrine Permeation

Tacrine was delivered from the gel (Test I) and from the ion-exchange fiber (Test II) for 3 h by iontophoresis and for 1 h passively *in vitro*. At the beginning there was a short lag time (30 min) in the tacrine flux. Thereafter, the flux was constant until the current was turned off. After current ter-

Table I. Adverse Side Effects following Transdermal Iontophoresis of Tacrine

Adverse effect	Test I	Test II	Tacrine	Iontophoresis
Pinching	10	9	0	5
Erythema	7	7	0	3
Strong erythema	3	2	0	2
Drying of the skin	5	5	1	2
Coldness on the skin and fingertips	5	5	0	0

Note: Number of volunteers in Test I = 10 and II = 9. Control measurements (N = 5) included tacrine in solution (no iontophoresis) or iontophoretic current (no tacrine).

Table II. Steady State Concentration (C_{SS}, ng/ml) of Tacrine in Human Plasma Based on the *in Vitro* (Calculated) and *in Vivo* Experiments

Tacrine formulation	Steady state drug concentration (ng/ml)	
	In vitro	*In vivo*
Test I Iogel® formulation	22.4 ± 5.3	21.3 ± 5.9
Test II Ion-exchange fiber formulation (Smopex®-102)	0.43 ± 0.19	14.9 ± 2.6

Note: The iontophoretic current density was 0.4 mA/cm^2 for 3 h.

mination the *in vitro* transdermal tacrine flux returned rapidly to a passive level. The flux values for the tacrine permeation were 5.61 ± 1.31 μg/min/cm^2 (Test I) and 0.11 ± 0.049 μg/min/cm^2 (Test II). Based on these flux values, the predicted *in vivo* plasma levels would be 22.4 ± 5.3 ng/ml and 0.43 ± 0.19 ng/ml using the gel and the ion-exchange formulations, respectively.

The correlation between the *in vitro* and *in vivo* data was very good in the case of gel formulation. However, with the ion-exchange fiber formulation, the *in vitro* data predicts a significantly smaller flux than the actual drug delivery *in vivo* was (Table II). Phipps and Gyory (15) also observed that the drug concentration *in vivo* was generally higher than the concentration *in vitro*, while Van der Geest *et al.* (6) observed that the *in vitro* permeation of apomorphine was greater than the *in vivo* delivery. The correlation seems to be, therefore, highly dependent on the experimental conditions, the individual skin source, and the drug in question. To find out the reason(s) for the poor correlation between the *in vivo* and *in vitro* studies involving the ion-exchange fiber formulation (Test II), further studies are needed.

CONCLUSIONS

This short-term study demonstrates that transdermal iontophoresis can be used to deliver clinically significant doses of tacrine in humans. By the combination of iontophoresis and ion-exchange fibers, one may obtain a controlled and constant drug delivery comparable to a commercially available system. The side effects of tacrine delivery during this short study were minimal, mainly transient skin irritation occurred due to iontophoresis. The *in vitro* and *in vivo* correlation of tacrine permeation was dependent on the experimental conditions and device structure. Our preliminary results suggest that transdermal iontophoretic delivery is a potential way to administer tacrine.

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