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Pharmacokinetics of physostigmine in man following a single application of a transdermal system

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1 The pharmacokinetics of physostigmine were investigated in a three-way cross-over design in six healthy, male volunteers comparing a physostigmine transdermal system (PTS), an oral solution and an i.v. infusion.

2 A single application of the patch over 24 h produced detectable plasma drug concentrations after a mean lag-time of 4 h. Thereafter, the drug was absorbed continuously from the PTS and putative therapeutic plasma concentrations were measured over approximately 18 h.

3 A mean absolute bioavailability of 36% was determined for the transdermal system and 3% for the oral solution. In comparison with the oral solution, interindividual variability of pharmacokinetics was less with the PTS.

4 The mean amount of physostigmine released from the transdermal system after 24 h was 5.7 mg. Because of extensive metabolism, only 2.2 mg of physostigmine were detected systemically.

5 After removing the PTS, the mean apparent half-life of elimination was 4.9 h, compared with 0.5 h for the i.v. infusion. This indicates continued drug absorption from a skin depot.

6 Physostigmine was well tolerated by the volunteers. With the PTS, a mild erythema was observed at the area of application, disappearing within a few hours.

Keywords physostigmine pharmacokinetics transdermal system healthy volunteers

Introduction

In contrast to other carbamate anticholinesterases, physostigmine is a tertiary amine with lipophilic properties and crosses the blood-brain barrier. Thus, physostigmine is also able to inhibit acetylcholinesterase in the brain. Physostigmine is used to treat diseases associated with central cholinergic deficiency and its application in Alzheimer's disease is of particular current interest [1]. Beneficial effects of physostigmine in Alzheimer's disease have been reported with oral formulations [2, 3] and i.v. infusions [4, 5]. Although the efficacy of intravenous physostigmine was confirmed by the US Department of Health and Human Services [6], its practical use in chronic therapy is restricted due to the problems of i.v. administration.

The pharmacokinetic properties of physostigmine, including a high-first-pass metabolism after oral administration and a short half-life of elimination, also restrict its therapeutic use [6]. To gain any benefit following oral administration, physostigmine has to be given up to eight times a day in doses of 1 mg as the salicylate [7]. However, the disadvantages of intravenous infusion and oral administration of the drug may be avoided by topical application. Therefore, a transdermal system was developed, releasing physostigmine *in vitro* continuously over 24 h.

In this study, we investigated the pharmacokinetics of physostigmine following a single administration of the transdermal system in comparison with an oral solution and an intravenous infusion. The aims were to obtain information about the tolerability of physostigmine, its relative and absolute bioavailability and the apparent half-life of elimination.

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Methods

Study design, subjects and medications

The study was performed according to a three-way cross-over design in six healthy male volunteers. Their mean (± s.d.) age was 29.3 ± 6.3 years, height was 179.5 ± 4.5 cm and weight was 76.0 ± 10 kg. All subjects gave written informed consent. The study protocol and consent form were approved by the *Unabhängige Ethikkommission Schwaben* in Ulm/ Germany. The volunteers received three different treatments in random order: a Physostigmine Transdermal System (PTS), an intravenous infusion and an oral solution.

The PTS contained 30 mg physostigmine base and had a surface area of 30.2 cm^2 (Lohmann Therapie Systeme GmbH, Co & KG, Batch No.: 8/28055/1). The *in vitro* release profile of the drug from the system is shown in Figure 4. The PTS was applied to the right shoulder for 24 h.

To prepare the infusion and the oral solution, Anticholium ampoules (Köhler, Alsbach/Germany) were used, containing 2 mg physostigmine salicylate in 5 ml. The rate of infusion was calculated to achieve plasma drug concentrations similar to average concentrations following oral administration of physostigmine [8]. Thus, 495 μg physostigmine salicylate in a volume of 720 ml were infused over 6 h. The oral solution contained 2 mg physostigmine salicylate in 200 ml water.

Blood samples (10 ml) were drawn into lithium-heparinised containers by venous puncture or through an indwelling catheter. To maintain the stability of physostigmine until assay, the blood was transferred immediately into tubes containing pyridostigmine bromide. Blood was sampled before dosage and after administration of the oral solution at 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 140, 160, 180, 210 and 240 min. Sampling times for the PTS were: 0 (predose) and 1, 2, 3, 4, 6, 8, 12, 18, 24, 24.5, 25, 26, 28, 30 and 36 h after the application. For the intravenous infusion, blood was taken before administration and at 5, 10, 30, 45, 60, 120, 180, 240, 300, 360, 362.5, 365, 367.5, 370, 375, 380, 390, 405, 420 and 450 min after onset of infusion. Plasma was obtained immediately, deep-frozen and kept at −35° C until analysis.

Vital signs (systolic and diastolic blood pressure, heart rate) and 12-lead ECG were recorded before the start of the treatments and after 2 h following oral administration and transdermal application. During the i.v. infusion, additional measurements were made at 0.5, 1, 2, 3, 4, 5, 6 and 8 h (vital signs) and 1, 2 and 8 h (12-lead ECG). Local skin reactions were assessed and photographed immediately after removal of the PTS and 20 min later.

Drug assay

Plasma physostigmine concentrations were measured by h.p.l.c. The internal standard, 10 ng *N,N*-dimethylcarbamoyleseroline, was added to 4 ml plasma, which was then made alkaline with 1 ml ammonium hydroxide solution (3.5% v/v) and extracted with 10 ml methyl *t*-butyl ether. The organic phase was removed, concentrated to approximately 1 ml, and then extracted with 0.2 ml hydrochloric acid (0.01 M). A 150 μl aliquot of the aqueous phase was injected onto a Nucleosil 100 C 18 5μ analytical column. The eluent was 0.02% (v/v) phosphoric acid : acetonitrile (75 : 25; pH 4.0) containing 2.5 g l^{-1} heptanesulphonic acid. At a flow rate of 1 ml min^{-1} and a column temperature of 45° C typical retention times were 4.9 min for physostigmine and 10.2 min for the internal standard. The fluorescence detector was set at 255 nm excitation and 345 nm emission.

For a sample volume of 4 ml plasma, the lower limit of quantitation (LLQ) was 25 pg ml^{-1}. At this level, accuracy and precision were 9.6% and 11.9% (n = 9). Linearity was shown over the range of 25 to 1000 pg ml^{-1}. The recovery was 94% with a coefficient of variation of 10.4% (n = 7).

Data analysis

Plasma physostigmine concentration data obtained after the three administrations were analysed using a non-compartmental approach with TOPFIT 2.0 [9]. AUC values were estimated using the linear trapezoidal rule. The residual area was determined by dividing the value of the last concentration by the terminal rate constant. Values of C_{max} and t_{max} were noted directly from the data. Clearance (CL) was calculated from the intravenous dose divided by AUC. The apparent volume of distribution (V_z) was calculated by dividing the clearance (CL) by the terminal rate constant. The mean residence time (MRT) was determined by dividing the area under first moment curve (AUMC) by the AUC.

The cumulative fraction of the available drug absorbed during PTS application was determined by the Wagner-Nelson method [10]. The amount of physostigmine released from the patch was calculated by subtracting the residual amount after application from the initial amount. The amount of physostigmine absorbed systemically was calculated by multiplying the clearance following i.v. administration by the AUC observed after application of the patch.

Results

The three formulations of physostigmine were well tolerated by the volunteers. Only mild drug-related systemic effects were observed (two headache, one diarrhoea). Local effects of the patch were either absent or mild in nature (one intermittent itching, four erythema, disappearing within a few hours). Patch adhesion problems were reported for four subjects (one completely separated from the skin after 20 h, three partly separated).

Average plasma physostigmine concentrations after the three modes of administration are shown in Figure 1 and pharmacokinetic parameters are listed in

Table 1. Figure 2 shows the individual plasma drug concentrations following PTS application. After the oral solution, mean peak plasma drug concentrations of 161 pg ml^{-1} (range: 58 to 421 pg ml^{-1}) were reached within 0.6 h (range: 0.3 to 0.8 h) and concentrations declined subsequently with a half-life of 0.3 h (range: 0.2 to 0.4 h). During the i.v. infusion, a mean peak concentration of 194 pg ml^{-1} was observed (range: 120 to 279 pg ml^{-1}). Mean clearance was 5.7 l min^{-1} and mean elimination half-life was 0.5 h. After application of the PTS, plasma physostigmine concentrations were not detected for about 4 h. Thereafter, they increased to a mean peak value of 341 pg ml^{-1} (range: 159 to 680 pg ml^{-1}) at 14 h (range: 6 to 24.5 h). Concentrations were sustained over almost the entire application period of 24 h. Following the removal of the patch, plasma physostigmine concentrations declined with a mean apparent half-life of elimination of 4.9 h (range: 3.0 to 6.9 h).

The mean amount of physostigmine released from the patch over 24 h was 5.7 mg (range 3.3 to 7.5 mg). Based upon the patch area of 30.2 cm^2, a mean drug release rate of 7.9 μg h^{-1} per cm^2 (range: 4.6 to 10.4 μg h^{-1} per cm^2) was calculated. Figure 3 compares the mean and individual absolute bioavailabilities after the oral solution (3%) and the PTS (36%—based on the amount of physostigmine released from the patch). Mean systemically available physostigmine amounted to 2.2 mg. The mean cumulative absorption of the available fraction of physostigmine from the PTS is shown in Figure 4. Absorption was approximately linear from 8 h after administration and remained steady over 22 h. The mean amount of physostigmine absorbed from the skin after removal of the patch was about 20% of the fraction available (Figure 4).

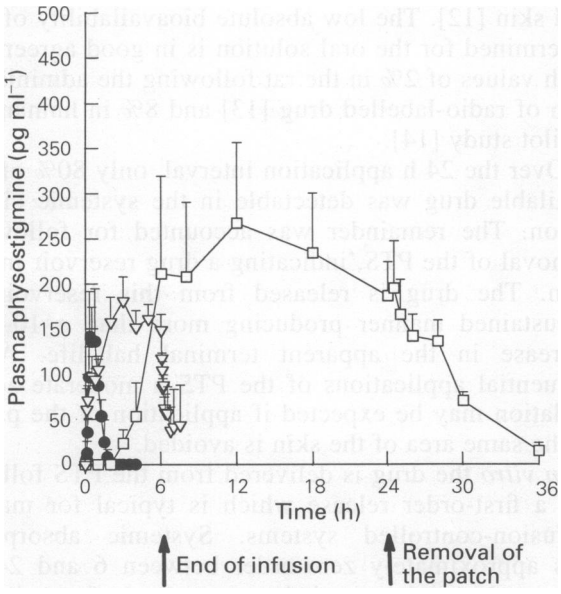

Figure 1 Mean (± s.e. mean) plasma physostigmine concentrations following the administration of the PTS (□), the oral solution (●) and an i.v. infusion (▽).

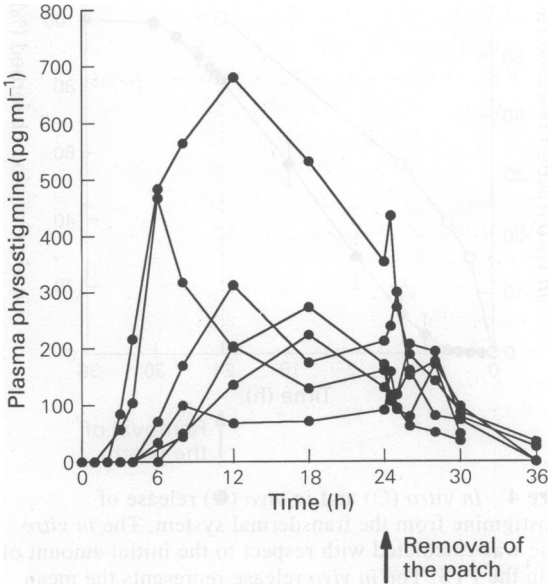

Figure 2 Individual plasma physostigmine concentrations following the administration of the PTS.

Table 1 Pharmacokinetic parameters of physostigmine after administration of the oral solution (dose: 2 mg physostigmine salicylate), the intravenous infusion (dose: 495 μg physostigmine salicylate in 6 h) and application of the PTS

	Intravenous infusion			*Oral solution*			*PTS*		
Parameters	*Mean*	*s.d.*	*Range*	*Mean*	*s.d.*	*Range*	*Mean*	*s.d.*	*Range*
C_{max} (pg ml^{-1})	194	55	120–279	161	137	58–421	341	199	159–680
t_{max} (h)	4.2	1.3	3.0–6.0	0.6	0.2	0.3–0.8	14.1	6.4	6.0–24.5
$t_{½}$ (h)	0.5	0.6	0.1–1.7	0.3	0.1	0.2–0.4	4.9	1.6	3.0–6.9
AUC (pg ml^{-1} h)	1018	310	751–1536	127	109	34–331	5718	3682	1958–12421
MRT (h)	3.6	0.5	3.0–4.5	0.8	0.0	0.8–0.9	19.1	2.7	15.6–22.6
CL (l min^{-1})	5.7	1.4	3.6–7.3						
V_z (l)	192	182	72–510						
t_{lag} (h)				0.2	0.1	0.0–0.3	4.0	1.8	2.0–6.0
F%				3.2	2.4	1.1–7.9	35.9	16.1	12.6–53.2
Amount released (mg/PTS)							5.7	1.8	3.3–7.5
Amount absorbed (mg/PTS)							2.2	1.3	0.4–3.9
Drug release rate (μg h^{-1} per cm^2)							7.9	2.5	4.6–10.4

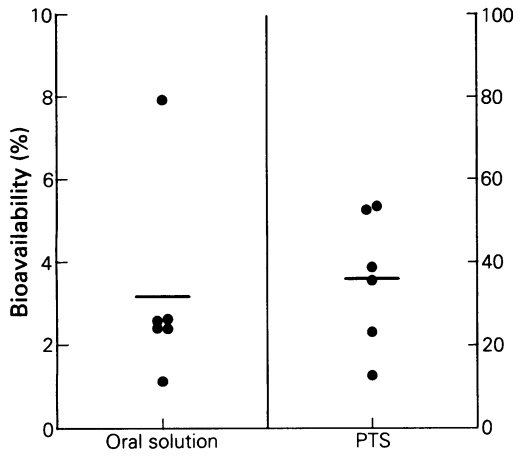

Figure 3 Mean and individual absolute bioavailabilities of physostigmine from the oral solution and the PTS.

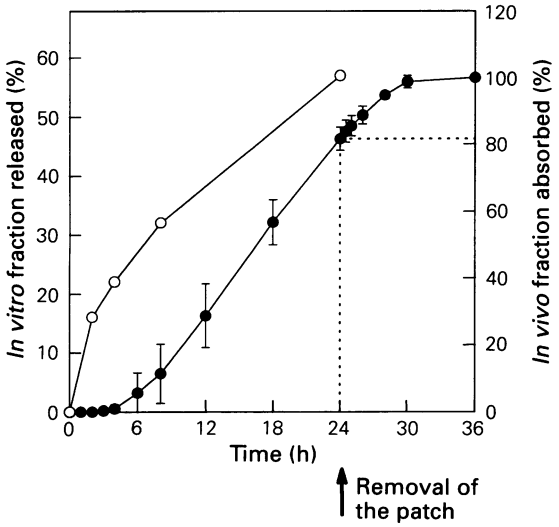

Figure 4 *In vitro* (○) and *in vivo* (●) release of physostigmine from the transdermal system. The *in vitro* release was calculated with respect to the initial amount of drug in the PTS. The *in vivo* release represents the mean fraction absorbed (± s.d.) calculated with respect to the amount delivered from the PTS during the period of application.

Discussion

A major finding of this study was that physostigmine is absorbed in man following cutaneous application of a transdermal system. Furthermore, the transdermal system used produced plasma physostigmine concentrations similar to those after the oral dosage of 2 mg physostigmine salicylate often used in clinical trials. This indicates that the peak plasma drug concentrations observed with the patch are in the therapeutic range. These concentrations were maintained after a single administration for about 18 h. Thus, compared with oral administration several times a day, once-a-day application of the patch provides an advantage and is a prerequisite for compliance with therapy, especially in an indication like Alzheimer's disease. The patch also has clear advantages over i.v. infusion, which is difficult to implement for chronic therapy.

Administration of the PTS decreased the inter-individual variability in absolute bioavailability by about 30% in comparison with the oral solution. The pharmacokinetic variability associated with the PTS might be decreased further if the adhesiveness of the patch is improved.

About 60% of the drug released from the patch was not detectable systemically. However, this loss is not surprising, considering that physostigmine is rapidly metabolised by non-specific esterases in blood [11] and skin [12]. The low absolute bioavailability of 3% determined for the oral solution is in good agreement with values of 2% in the rat following the administration of radio-labelled drug [13] and 8% in humans in a pilot study [14].

Over the 24 h application interval, only 80% of the available drug was detectable in the systemic circulation. The remainder was accounted for following removal of the PTS, indicating a drug reservoir in the skin. The drug is released from this reservoir in a sustained manner producing more than a 10-fold increase in the apparent terminal half-life. After sequential applications of the PTS a moderate accumulation may be expected if application of the patch to the same area of the skin is avoided.

In vitro the drug is delivered from the PTS following a first-order release which is typical for matrix diffusion-controlled systems. Systemic absorption was approximately zero-order between 6 and 24 h. These release characteristics are typical of membrane permeation-controlled drug delivery [15]. The observed differences in the *in vitro* and *in vivo* drug release characteristics and the higher *in vitro* delivery rate indicate that drug release from the PTS does not rate-limit drug absorption. This suggests that the absorption kinetics do not reflect the release of the drug from the transdermal system, but rather its permeation through the skin.

The results of this study indicate that the transdermal administration of physostigmine might be a feasible approach to resolve the problems of oral and intravenous administration of physostigmine in the treatment of Alzheimer's disease.

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