J. Pharm. Pharmacol. 1995, 47: 206–212
Received April 6, 1994
Accepted October 26, 1994

Transdermal Delivery of Physostigmine. A Pretreatment Against Organophosphate Poisoning

J. JENNER, A. SALEEM* AND D. SWANSTON

Biology Division, Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wiltshire, UK

Abstract

Physostigmine absorption through isolated human skin and inhibition of plasma and red blood cell cholinesterases in guinea-pigs have been measured to assess the feasibility of its transdermal delivery as a pretreatment for organophosphate poisoning.

Penetration of radiolabelled physostigmine across human epidermis was measured in-vitro using glass diffusion cells and optimization of physostigmine delivery was achieved by changes in vehicle formulation and use of penetration enhancers.

Two-component vehicles consisting of propionic acid/isopropyl myristate (50:50) and propionic acid/oleic acid (50:50) produced the highest transdermal delivery of physostigmine. A comparison of formulations containing propionic acid alone with propionic acid plus oleic acid when applied to guinea-pigs, showed that inclusion of oleic acid allowed the amount of physostigmine and the size of the transdermal patch to be substantially reduced, whilst maintaining effective delivery rates. The formulation containing oleic acid was not irritant to guinea-pigs when applied to the skin for 48 h.

It is concluded that a mixture of propionic acid and oleic acid containing physostigmine is a good candidate for transdermal delivery of physostigmine as a pretreatment for organophosphate poisoning.

Carbamate cholinesterase inhibitors in combination with anticholinergic drugs have applications in the treatment of organophosphate poisoning (Leadbeater 1988). Pyridostigmine is the anti-cholinesterase presently used for pretreatment against organophosphate nerve agent poisoning. Being a quaternary carbamate, it is unable to penetrate the central nervous system (CNS) to any significant extent and, therefore, cannot protect against the central incapacitating effects of organophosphates. Physostigmine has been suggested as an alternative prophylactic against organophosphate poisoning because it is an unquaternized carbamate which does penetrate the CNS (Solana et al 1990). However, physostigmine has a short plasma half-life and a narrow therapeutic index which necessitates the use of a sustained release formulation. Presently, the lack of such a formulation is a major problem in the development of physostigmine for long-term administration.

The delivery of drugs by the transdermal route is an area of increasing interest and offers the advantage of allowing prolonged consistent input of drug into the blood. Unlike pyridostigmine, physostigmine is an uncharged species which can cross membranes, including the blood-brain barrier. This property makes physostigmine a candidate for transdermal delivery and recent work to find a treatment for Alzheimer's disease (Christie et al 1981) has identified transdermal formulations which deliver physostigmine at therapeutically useful doses.

The excellent barrier properties of the skin impose a major limitation on the drug with respect to required dosage. Consequently, before the development of a transdermal delivery system, it is necessary to determine whether the drug to be incorporated permeates human skin in the required therapeutic amount. Clinical studies indicate that an intravenous infusion rate of 400 $\mu g\,h^{-1}$ produces blood cholinesterase inhibitions of approximately 30% (R. Metcalf, personal communication) which does not cause overt signs of cholinesterase poisoning nor impair performance (Wetherell 1992). When delivering a drug transdermally, if skin permeability becomes the limiting factor, bioavailability can be increased by using a larger surface area. Practical considerations limit the size of a patch to about 20 cm^2 which would require a permeability rate of at least 20 $\mu g\,cm^{-2}\,h^{-1}$ to achieve 400 $\mu g\,h^{-1}$ delivery of physostigmine. Higher permeability rates would allow the quantity of physostigmine and the patch size to be reduced, minimizing the risk of physostigmine intoxication following injury at the patch site.

The current study optimizes a formulation for the delivery of physostigmine through the skin at delivery rates which will allow the use of patch sizes below 20 cm^2.

Materials and Methods

Chemicals

[^{3}H]Water and [^{3}H]physostigmine, >90% radiochemical purity, were obtained from Amersham International plc (Amersham, UK). Ethyl oleate, squalene, propylene glycol, propionic acid (all >99%) and oleyl alcohol (65%) were obtained from Aldrich Chemical Co. Ltd (Gillingham, UK). Isopropyl myristate (98%), *N*-hydroxyethyl lactamide (70% aqueous solution), physostigmine (free base) and purified acetylcholinesterase were obtained from Sigma Chemical Co. Ltd (Poole, UK). Emulsifier-Safe scintillation fluid and Soluene-350 were obtained from United

* Present address: Technology and Health Sciences Division, The Health and Safety Executive, Magdalene House, Stanley Precinct, Bootle, Merseyside, UK.

Correspondence: J. Jenner, Biology Division, Chemical and Biological Defence Establishment, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK.

Technologies, Packard (Illinois, USA). Oleic acid (>99%), 5,5′-dithiobis (2-nitrobenzoic acid), and acetylthiocholine were obtained from BDH Chemicals Ltd (Eastleigh, UK) and dipropylene glycol monomethyl ether was obtained from Dow Chemicals (Rotterdam).

Human abdominal skin was obtained post mortem and stored in sealed polythene bags at −20°C. When required, the tissue was thawed at room temperature (21°C) and the subcutaneous fat removed. Epidermal sheets were separated from full-thickness skin by immersion in water at 60°C for 45 s and gently peeling away the epidermis.

Guinea-pigs (Dunkin-Hartley) were purchased from Interfauna Ltd, and were kept in accordance with the Home Office Code of Practice for the Housing of Animals used in Scientific Procedures. For experiments measuring cholinesterase activity, female guinea-pigs, 700–1200 g, were used and for the irritancy studies male animals, 300–500 g, were used.

In-vitro studies

The solubility of physostigmine in various vehicles was determined by weighing 10–20 mg of physostigmine base into a 1-mL volumetric flask and adding an initial 25 μL volume of the chosen vehicle. If dissolution had not occurred after 30-min ultrasonic agitation, further 10–50-μL portions of vehicle were added and the ultrasonic agitation repeated until complete dissolution had occurred.

The partitioning of physostigmine between each vehicle and human epidermis was measured by equilibrating weighed pieces of human epidermis for 48 h at 30°C with 2% solutions of [^{3}H]physostigmine in candidate vehicles, with occasional shaking. The epidermal membrane was blotted dry between sheets of filter paper and solubilized in 1 mL Soluene-350. The radioactivity in the solubilized epidermis was measured before and after the addition of 25 μL standard [^{3}H]physostigmine solution to correct for quenching. The concentration of radioactivity in the tissue, assuming the density of the skin to be 1 g mL^{-1}, was then calculated.

The radioactivity in a 50-μL sample of the vehicle was measured and quenching corrected for by comparison with 10 μL [^{3}H]physostigmine added directly to scintillation fluid with 50 μL of the vehicle before equilibration with epidermal membranes. Background was determined and corrected for from the tissue, Soluene and scintillation-fluid systems. The partition coefficient (K_m) was calculated as follows:

$$K_m = \frac{\text{Concn in tissue}}{\text{Concn in solution}} = \frac{Ct}{Cs} \qquad (1)$$

Working formulations of physostigmine for diffusion studies, in isopropyl myristate, propylene glycol and propylene glycol/oleic acid (50/50) were prepared by adding excess physostigmine to 1 mL vehicle. The solutions were agitated using a vortex mixer and then allowed to stand. After five further mixings the excess physostigmine was allowed to settle. To these saturated solutions was added 5–10 μL [^{3}H]physostigmine immediately before use for percutaneous absorption studies.

All other formulations were prepared by adding vehicle to a weighed amount of physostigmine, and agitating in an ultrasonic bath until dissolution had occurred. [^{3}H]Physostigmine (5–10 μL) was added to enable estimation by liquid scintillation counting. All formulations were protected from light and stored at 4°C until required.

Percutaneous diffusion was measured in-vitro by mounting epidermal membranes in Franz-type diffusion cells having a cross-sectional area of 2·54 cm^2. The cells were maintained at 30°C in a water bath and the upper surface of the membrane exposed to donor solution. The under side of the membrane was bathed by 4–5 mL of continuously stirred receptor fluid.

Before each experiment the integrity of the membranes was assessed by measuring its permeability to tritiated water. Physiological saline was used as the receptor fluid and 1 mL saline containing tritiated water (0·2 mCi mL^{-1}) applied to the donor chamber. The receptor was sampled (25 μL) at hourly intervals for 6 h, after which, donor and receptor solutions were removed and the skin surfaces rinsed 2–3 times with distilled water. The chambers were then filled with distilled water and left overnight to desorb any residual activity from the membranes.

The following day any membrane with a permeability coefficient greater than $1{\cdot}5 \times 10^{-3}$ cm h^{-1} was considered damaged and discarded (Dugard et al 1984). The receptor chambers were filled with 50% aqueous ethanol and 200 μL candidate formulation containing labelled physostigmine (1 μCi) placed in the donor chambers. The movement of physostigmine across the membrane was followed by sampling the receptor solution (50 μL) over a period of 3 days.

Physostigmine was measured by liquid scintillation counting in 5 mL scintillation fluid, using a 1215 Rackbeta II liquid scintillation counter.

Calculations from in-vitro data

Steady-state flux, permeability coefficient and lag time were estimated from the line of best fit to the data after 24 h by linear regression according to the equation:

$$Jss = \frac{K_m \cdot D}{\delta} \cdot C = Kp \cdot C \qquad (2)$$

where Jss = steady-state flux, Kp = permeability coefficient, C = penetrant concentration (μg mL^{-1}) in the donor chamber, K_m = partition coefficient of the penetrant between the vehicle and the skin, D = diffusion coefficient of the penetrant in the epidermis, and δ = membrane thickness.

The intercept on the time axis is termed the lag time (τ) and is related to the diffusion coefficient, D by:

$$\tau = \frac{\delta^2}{6D} \qquad (3)$$

Where data did not justify the fitting of a straight line, as indicated by a regression value of less than 0·95, no values for Jss, Kp or τ are quoted.

As an indicator of damage to the membrane, the ratio of water permeability before and after application of test formulation was measured. This damage ratio (DR) was given by:

$$DR = \frac{\text{Water Kp after vehicle application}}{\text{Water Kp before vehicle application}} \qquad (4)$$

In-vivo studies

Formulations were applied to skin in a rubber cup taken from the plunger of a disposable syringe and referred to as the applicator. The dead space of the applicator was filled with silicon rubber to leave a volume of approximately 100 μL which was filled with candidate formulation. The cup was glued to an area of preclipped skin of the dorsal thoracic region of guinea-pigs approximately 2 cm below the base of the ears, with cyanoacrylate adhesive. SuperGlue Xtra formulated as a gel was found to be the most effective because it filled the area around the hair stubble and produced a more effective seal than similar liquid adhesives.

Applicators were applied after blood sampling on the first day and removed after blood sampling on the third day of the experiment. Animals were lightly anaesthetized with halothane to facilitate removal of the applicators, which could be removed by hand, and the application site cleaned with aqueous alcohol to remove any remaining medicament. The area of application was 1 cm^2 for physostigmine in propionic acid and 0·5 cm^2 for physostigmine in propionic and oleic acid mixture.

Blood was sampled by venepuncture of the ear vein, daily for 5 days and again on the seventh day, directly into sample tubes containing EDTA anticoagulant. Blood samples were kept on ice until assayed. Red blood cells (RBC) were separated by centrifugation (30 s at 14 000 rev min^{-1} in an Eppendorf 5415 centrifuge), plasma was reserved and the RBCs washed in ice-cold saline. Washed cells were resuspended to original volume in ice-cold saline. Haematocrits of the original blood and resuspended cells were measured using a Hawksley microhaematocrit system.

Acetylcholinesterase activity was assayed by the method of Ellman et al (1961). Assay mixtures contained either RBC or plasma (diluted 1 : 600 in the final assay mixture), 0·01 M 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and 1 mM acetylthiocholine in 0·1 M phosphate buffer. Assay volumes were made up to a final volume of 5 mL and divided between two cuvettes for spectrophotometric monitoring (Pye-Unicam SP 8-100). The reaction was started by addition of substrate and followed at 412 nm for 10 min (uninhibited) or 20 min (inhibited) and the initial reaction rate calculated from graphs of absorbance change against time. The assay was calibrated daily with cysteine standard solutions.

Blanks contained all reaction constituents except substrate. Spontaneous hydrolysis rates were measured separately and subtracted from enzyme catalysed rates. Corrections were made using haematocrit values so that enzyme activities could be expressed as amount product min^{-1} mL^{-1} original blood volume.

Enzyme activities on day one were used as baseline values for each individual and results expressed as percent inhibition of this value. A control group was bled each day for a week without treatment with physostigmine to show any effects of repeat bleeding.

Irritancy studies

Primary irritancy potential was measured on the skin of preclipped male guinea-pigs (300–500 g). Twenty five microlitres 25% (w/v) physostigmine in 1 : 1 propionic to oleic acids was applied for 48 h with occlusion. The site was washed with alcohol and water to remove any excess formulation and the application sites scored using the modified Draize Scoring system described by Fairhurst et al (1987). Scores were averaged as outlined by the EEC guideline L/257.

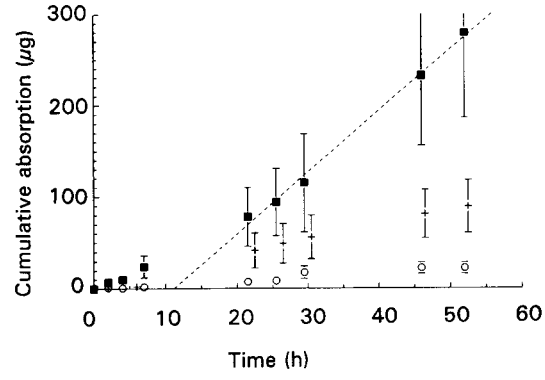

FIG. 1. Penetration of physostigmine across human epidermis from saturated propylene glycol (○), isopropyl myristate (+) and propylene glycol/oleic acid (10/90) (■). Points are mean ± s.d. of 3–5 experiments.

Results and Discussion

In-vitro studies

The accumulation of physostigmine in receptor fluid produced by each vehicle is shown in Figs 1–4. Delivery from all vehicles other than propylene glycol and isopropylmyristate produced delivery rates after 24 h which could be approximated to linearity, so Jss and τ were calculated from the lines of best fit (linear regression) and are presented in Table 1. Apart from the saturated isopropyl myristate and the 1 and 10% propionic acid/oleic acid formulations, penetration was less than 10% of applied dose which satisfies infinite dose conditions (i.e. a non-depleting donor source), ensuring achievement and maintenance of steady-state conditions. Physostigmine rapidly depleted from the isopropyl myristate formulation (Fig. 1). As a result only a transient steady state was observed with almost 50% of applied dose being absorbed by 50 h. The formulation giving

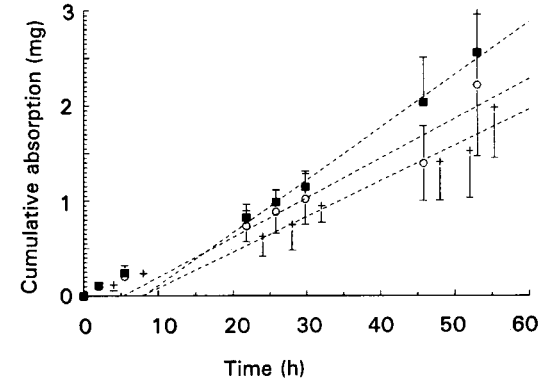

FIG. 2. Penetration of physostigmine across human epidermis from 50% physostigmine in propionic acid (+), 25% physostigmine in propionic acid/isopropyl myristate (50 : 50, v/v) (○) and 25% physostigmine in propionic acid/oleic acid (50 : 50 v/v) (■). Points are mean ± s.d. 3–5 experiments.

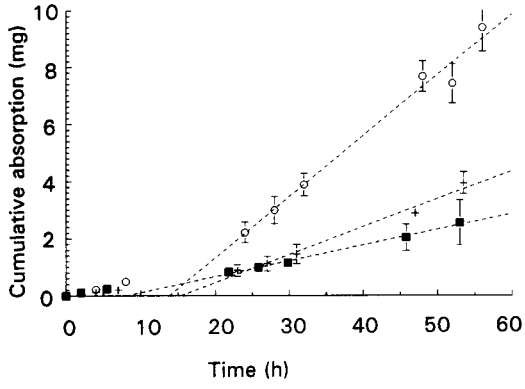

FIG. 3. Penetration of physostigmine across human epidermis from propionic acid/oleic acid (50:50, v/v). ■ 1, + 25 and ○ 10% physostigmine (w/v). Points are mean ± s.d. of 3–5 experiments.

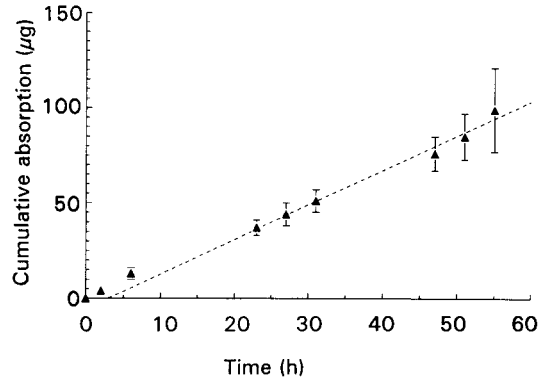

FIG. 4. Penetration of physostigmine across human epidermis from 10% solution of propionic acid/oleic acid (10:90). Points are mean ± s.d. of three experiments.

the highest delivery rate was a propionic acid : oleic acid (50 : 50) containing 25% (w/v) physostigmine.

Use of propionic acid as a cosolvent with isopropyl myristate and oleic acid increased the solubility and stability of physostigmine (Table 2). The two-component-based physostigmine formulations, 25% in propionic acid/isopropyl myristate and 25% in propionic acid/oleic acid, achieved higher transdermal fluxes (17 and 24 $\mu g\,cm^{-2}\,h^{-1}$, respectively) than the 50% propionic acid formulation (13 $\mu g\,cm^{-2}\,h^{-1}$, Table 1). Use of saturated solutions of physostigmine may increase fluxes from the two-component systems and it may be concluded that both two-component systems are capable of delivering therapeutic amounts of physostigmine through the skin from patches of 23 and 16 cm^2, respectively.

The increased fluxes observed from the two-component systems are probably due to a combination of partitioning and diffusivity effects. These vehicles have similar partition coefficients, K_m (Table 2), allowing favourable partitioning of physostigmine into the skin. Oleic acid and isopropyl myristate are thought to enhance penetration by interfering with the lipid layers of the stratum corneum and opening pathways for diffusion (Albery & Hadgraft 1979). This mechanism entails diffusion of the enhancer into the stratum corneum before enhancement is seen, which may account for the longer lag times from these vehicles. Oleic acid caused a greater enhancement of physostigmine penetration than isopropyl myristate. The mechanism of oleic acid enhancement of percutaneous absorption is thought to be by interference with the lipid layers of the stratum corneum. Oleic acid is a non-linear fatty acid, due to the presence of a *cis* double bond; its insertion into the lipid of the stratum corneum is thought to open up channels for diffusion and alter the fluidity of the lipid, and may cause greater fluidization than the saturated alkyl chain of isopropyl myristate (Barry 1987; Guy et al 1990).

Decreasing the physostigmine concentration in the propionic acid/oleic acid vehicle resulted in large increases in skin permeability (Fig. 3). These increases were, however, associated with increased water damage ratios (DR), as shown in Table 1. This indicates membrane damage was responsible for the increased permeability from the 1 and 10% formulations as compared with oleic acid (DR = 1·5). The DR value of 2·4 caused by the 25% formulation represents little damage to the barrier layer.

To produce a non-damaging formulation containing 10%

Table 1. Physostigmine concentrations (w/v), percentage saturations and diffusion parameters for penetration through human epidermis. Entries are mean ± s.d. of the number of determinations shown in parenthesis.

Vehicle	Physostigmine (% w/v)	Saturation (%)[a]	Steady-state flux ($\mu g\,cm^2\,h^{-1}$)	Lag time (h)	r	Damage ratio
Isopropyl myristate	0·2	17	—	—	—	—
Propylene glycol	2·0	26	—	—	—	—
Propylene glycol/oleic acid (50:50; v/v)	2·7	54	2·83 ± 0·70 (3)	8·5 ± 3·7	0·999 ± 0·001	—
Propionic acid	50	100	13·0 ± 3·93 (4)	5·48 ± 1·77	0·988 ± 0·009	—
Propionic acid/isopropyl myristate (50:50; v/v)	25	83	17·1 ± 6·22 (3)	4·73 ± 3·82	0·991 ± 0·004	—
Propionic acid/oleic acid (50:50; v/v)	25	50	24·1 ± 9·48 (4)	6 ± 6	0·994 ± 0·003	2·4 ± 0·2 (3)
Propionic acid/oleic acid (50:50; v/v)	10	22	91·8 ± 8·98 (3)	—	0·999 ± 0·004	8·2 ± 1·2 (3)
Propionic acid/oleic acid (50:50; v/v)	1	2	39·8 ± 7·50 (3)	—	0·992 ± 0·005	10·6 ± 2·3 (3)
Propionic acid/oleic acid (10:90; v/v)	10	100	0·83 ± 0·06 (4)	6·85 ± 2·73	0·997 ± 0·004	—

[a] % Saturation calculated using the solubilities shown in Table 2 as 100%.

Table 2. Solubility and partitioning data for physostigmine.

Vehicle	Solubility in vehicle ($mg\,mL^{-1}$)	Partition coefficient (vehicle/skin) (K_m)
Squalene	<10	45
Isopropyl myristate	12	11
Ethyl oleate	17	10
Propionic acid/isopropyl myristate (50:50)	400	1·6
Propionic acid/oleic acid (50:50)	500	1·2
Oleyl alcohol	60	0·6
Dipropylene glycol monomethyl ether	250	0·5
Oleic acid	55	0·4
N-Hydroxyethyl lactamide	67	0·3
Propylene glycol	77	—
Propylene glycol/oleic acid (50:50)	50	—
Propionic acid/oleic acid (10:90)	80	—

.Solubilities determined by sonication as described in Materials and Methods.

physostigmine, the ratio of propionic acid to oleic acid was reduced from 50:50 to 10:90 (Fig. 4). This vehicle was saturated with physostigmine and was expected to be non-irritant. Oleic acid still appeared to interact with the skin lipid as indicated by the long lag time, but the transdermal flux of physostigmine was very low. This may be due to the less favourable partition coefficient of physostigmine from oleic acid compared with that from 50:50 propionic acid/oleic acid. Increasing the content of oleic acid might, therefore, decrease the partitioning of physostigmine into the epidermis.

Concentration and partitioning are critical determinants of transdermal flux, as shown by the steady-state flux relationship (eqn 2). It follows that increases in partition coefficient into the skin and applied concentration will lead to higher flux rates. However, these two parameters are interdependent in that a vehicle with a high solubility for physostigmine will be less likely to allow physostigmine partitioning into skin and vice versa; squalene, isopropyl myristate and ethyl oleate have relatively low solubilities for physostigmine and high K_m values (Table 2). Conversely oleyl alcohol, dipropylene glycol monomethyl ether, *N*-hydroxyethyl lactamide and oleic acid have greater solubilities for physostigmine but lower K_m values. It is apparent that a compromise has to be sought between K_m and C when optimizing vehicle formulations. This has been achieved with the two-component systems tested here. Of the five vehicles evaluated, ethyl oleate in combination with propionic acid may prove to be the best vehicle for transdermal physostigmine delivery. It has a more favourable K_m than oleic acid and contains the *cis* double bond which is thought to be responsible for the enhancing effects of oleic acid.

The possible formation of a complex between physostigmine and propionic acid needs to be considered. At the surface pH of the skin (5·5) a large proportion of an applied dose of physostigmine will be ionized and will not readily diffuse across the stratum corneum. Diffusion of the molecular form would, however, cause a shift in the ionic equilibrium and allow the diffusion of the portion of an applied dose which was initially ionized. Formation of an uncharged complex between physostigmine and its vehicle would produce another species capable of diffusing across the membrane, eliminating the need for physostigmine to pass through in its molecular form.

Physostigmine must be chemically stable in each formulation during the period of diffusion measurement. Physostigmine is heat and light labile, producing degradation products by hydrolysis and subsequent oxidation, including rubreserine which is bright red. Of the formulations investigated in this study only the propylene glycol, propylene glycol/oleic acid and *N*-hydroxyethyl lactamide acquired a pink/red colouration (over 10–24 h). The precise extent of this breakdown was not determined. The saturated solutions of isopropyl myristate, propylene glycol, and propylene glycol/oleic acid used to obtain flux rates shown in Table 1 were prepared by vortex mixing. Subsequent analysis by UV spectroscopy and comparison with solutions prepared by sonication showed them to have the saturations shown in Table 1. The discrepancy was probably due to inadequate agitation from vortex mixing as opposed to sonication. Diffusion rates from these vehicles are too low to allow the use of a practical size of patch, but it is unlikely that chemical instability or the discrepancy in saturation is responsible for this. The difference in diffusion rates between these vehicles and those containing propionic acid is too large to be overcome by increasing the concentration to saturation.

In-vivo studies

The results of our in-vitro experiments led us to compare the inhibition of blood cholinesterase produced by 25% physostigmine in propionic and oleic acids with 50% physostigmine in propionic acid in guinea-pigs.

Physostigmine in propionic acid (50:50, w/v) produced an increasing inhibition of plasma and red cell cholinesterases until the removal of the transdermal applicator after 72 h (Fig. 5). The inhibition continued to increase over

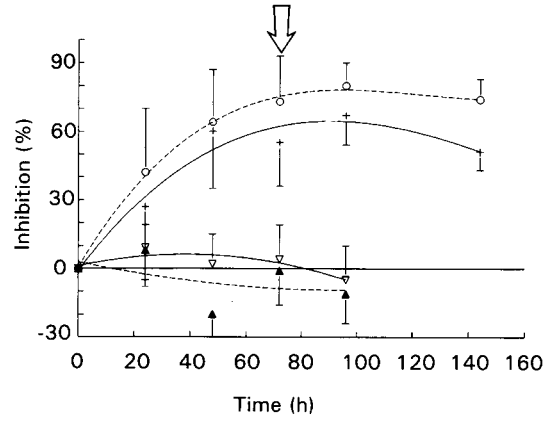

FIG. 5. Inhibition of blood cholinesterases by transdermal physostigmine in propionic acid (50:50, w/v). The applicator was applied at time zero and removed at 72 h (arrow). Plasma cholinesterase activities are shown by broken lines, red cell activities by solid lines and the triangles denote untreated controls. Points are mean ± s.d. of five animals. Area of application = 1 cm^2.

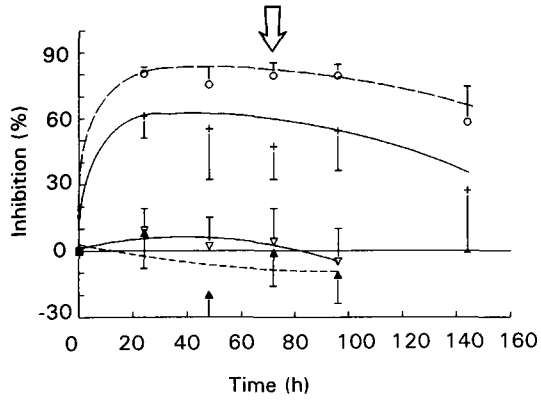

FIG. 6. Inhibition of blood cholinesterases by transdermal physostigmine (25%, w/v) in propionic and oleic acids (50:50, v/v). The applicator was applied at time zero and removed at 72 h (arrow). Plasma cholinesterase activities are shown by broken lines, red cell activities by solid lines and the triangles denote untreated controls. Points are mean ± s.d. of five animals. Area of application = 0·5 cm^2.

the following 24 h. Activities had started to recover 72 h after applicator removal, but plasma and RBC cholinesterases were still inhibited by greater than 50% at this time. One animal in this group died within 24 h treatment, all other animals showed no signs of cholinesterase poisoning. We were not able to reproduce the results of a previous study (Fischer et al 1986), where a constant 30% inhibition of guinea-pig whole blood cholinesterase was achieved from the physostigmine in propionic acid formulation between 5 h and 3 days from a 1-cm^2 area. In our hands, a constant level of inhibition was not produced from this formulation.

Physostigmine (25%, w/v) in propionic and oleic acids (50:50, v/v) produced 80% inhibition of plasma cholinesterase and 60% inhibition of RBC cholinesterase after 24 h (Fig. 6). These inhibitions were maintained until 24 h after applicator removal. Seventy two hours after removal of the applicator, inhibitions had fallen to 60% (plasma) and 30% (RBC). Two of the animals in this group died within 24 h application, the remainder showed no signs of poisoning for the duration of the experiment.

Since physostigmine is labile in guinea-pig blood with a plasma half-life of approximately 40–50 min (Lukey et al 1990), persistence of blood cholinesterase inhibition for 3 days after cessation of treatment indicates continued delivery of physostigmine into the blood from a depot. This depot effect could be clinically beneficial or disadvantageous depending upon circumstance. Loading the depot by short-term patch application would reduce the risk of irritancy or sensitization from repeated treatment. Conversely, the presence of a loaded depot would maintain dosing after removal of the patch, which might compromise other drug treatment.

The location of the depot is open to conjecture. Since depot effects are not seen when physostigmine is given intravenously the depot is probably in the skin. The stratum corneum is the most likely site. Drug which partitioned into this layer will slowly diffuse into the blood. However, the possibility that the depot resides elsewhere, for instance, in the upper layers of the dermis, cannot be excluded on current data.

Table 3. Primary irritancy of physostigmine (25%, w/v) in propionic and oleic acids (50:50, v/v).

Time after application (days)	Average scores		
	Erythema	Oedema	Desquamation
1	0·5	0	0
2	0·33	0	0
3	0	0	0
6	0	0	0·9
7	0	0	0·9

Average Draize scores are shown for groups of six animals against time after first application. An average score of 2 is required to classify a compound as irritant.

Inhibition of blood cholinesterase in this study is higher than required to protect against organophosphate poisoning (30%). Deaths in the early part of the treatment period were associated with animals which had succeeded in scratching medicament out of the applicator. It is probable that these animals ingested a bolus dose of physostigmine which, combined with the transdermal dose, proved fatal. Clearly, further experiments are required to relate area of application to inhibition. This would enable investigation of the depot effect at this dose level and 7-day treatment periods.

The inclusion of oleic acid allowed both the amount of physostigmine in the formulation and the patch size, to be halved. This accords with the results of our in-vitro experiments where oleic acid doubled the rate of percutaneous penetration in-vitro.

To be clinically useful, a transdermal preparation of physostigmine must be non-irritant. The 25% (w/v) physostigmine in propionic and oleic acids (50:50, v/v) was found to be non-irritant in the modified Draize test. Of the six animals treated, three showed mild erythema 24 h after application and two at 48 h. Six and seven days after treatment, five of the six animals showed mild diffuse scaling of the application site (Table 3). The average scores for erythema, oedema or desquamation did not exceed 2 at any time, which does not classify the formulation as irritant under EEC guideline L/257. Although propionic acid alone is irritant to the skin, a 50:50 mixture with physostigmine has been previously found to be non-irritant (Fischer et al 1986). However, it is not known at present whether this minor irritation would be associated with subjective sensations such as itching, or with sensitization after repeated application. Although propionic acid is irritant, physostigmine in propionic acid is likely to form a complex which reduces or eliminates irritancy potential in formulations containing high concentrations of physostigmine. This complex is clearly not stoichiometric because an equal mass of physostigmine will neutralize propionic acid which has a much lower molecular weight and, therefore, contains fewer molecules. At lower concentrations there may be sufficient free acid in the formulation to cause membrane damage.

In conclusion, we have shown that it is feasible, using saturated physostigmine solutions in propionic acid/isopropyl myristate (50:50, v/v) and propionic acid/oleic acid (50:50, v/v), to deliver physostigmine via the skin as a pretreatment against organophosphate poisoning. Inclusion

of oleic acid or isopropyl myristate in transdermal formulations will allow the use of a smaller patch (ca 16–20 cm^2) containing less physostigmine. This will reduce the risk of poisoning from mechanical damage to the patch and underlying skin. The formulation containing 25% physostigmine in a 50:50 mixture of oleic and propionic acids was non-irritant and produced sustained, constant inhibition of plasma and red cell cholinesterase over 3 days with evidence of a drug depot forming in the skin.

References

Albery, W. J., Hadgraft, J. (1979) Percutaneous absorption: in vivo experiments. J. Pharm. Pharmacol. 31: 140–147

Barry, B. W. (1987) Mode of action of penetration enhancers in human skin. J. Contr. Rel. 6: 85–97

Christie, J. E., Shering, A., Ferguson, J., Glen, A. I. M. (1981) Physostigmine arecoline: effects of intravenous infusions in Alzheimer presenile dementia. Br. J. Psychiatry 138: 46–50

Dugard, P. H., Walker, M., Mawdsley, S. J., Scott, R. C. (1984) Absorption of some glycol ethers through human skin in vitro. Environ. Health Perspect. 57: 193–197

EEC Guideline L/257 (1983) Classification, labelling and packaging of dangerous substances. Off. J. Eur. Community 26: 19

Ellman, G. L., Courtney, K. D., Andres Jr., V., Featherstone, R. M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7: 88–95

Fairhurst, S., Maxwell, S. A., Scawin, J. W., Swanston, D. W. (1987) Skin effects of trichothecenes and their amelioration by decontamination. Toxicology 46: 307–319

Fischer, A., Levy, D., Spiegelstein, M. Y., Grunwald, Y., Levy, A., Kishnir, M. (1986) UK Patent Application 2163347A

Guy, R. H., Mak, V. H. W., Kai, T., Bommannan, D., Potts, R. O. (1990) Percutaneous penetration enhancers: mode of action. In: Scott, R. C., Guy, R. H., Hadgraft, J. (eds) Predictions of Percutaneous Penetration. Billing and Sons Ltd, pp 213–223

Leadbeater, L. (1988) When all else fails [antidotes to chemical agents for troops]. Chem. Britain 24: 683–688

Lukey, B. J., Parrish, J. H., Marlow, D. D., Clarke, C. R., Sidell, F. R. (1990) Pharmacokinetics of physostigmine intramuscular administration to guinea-pigs. J. Pharm. Sci. 79: 796–798

Solana, R. P., Harris, L. W., Carter, W. H., Talbot, B. G., Carchman, R. A., Gennings, C. (1990) Evaluation of a two drug combination pretreatment against organophosphate exposure. Toxicol. Appl. Pharmacol. 102: 421–429

Wetherell, A. (1992) Effects of physostigmine on stimulus encoding in a memory-scanning task. Psychopharmacology 109: 198–202