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Further Evidence of Extensive Pulmonary First-pass Ester Hydrolysis after Airways Administration in Rats

PAUL A. DICKINSON, PETER C. SEVILLE AND GLYN TAYLOR

The Welsh School of Pharmacy, Cardiff University, Cathays Park, Cardiff CF10 3XF, UK

Abstract

The aim of this study was to synthesize an ester with low lipophilicity, and to use this ester to further investigate pre-absorptive pulmonary first-pass metabolism.

Hexanoic acid phenethyl ester was synthesized by reacting 2-phenylethanol with hexanoyl chloride. Pre-absorptive first-pass metabolism was assessed by comparing the areas under the blood concentration–time curves after intra-arterial administration of the hexanoic acid phenethyl ester with those after intratracheal instillation. Hexanoic acid phenethyl ester experienced extensive first-pass metabolism (53% of the absorbed dose) before or during absorption. This and earlier data suggests that the extent of this first-pass extraction is dependent on the physicochemical properties of the ester and in particular whether a compound experiences diffusion-limited absorption.

Pre-absorptive pulmonary first-pass metabolism of compounds whose absorption is diffusion-rate limited may be extensive even when pulmonary enzyme expression is low. This has consequences for the systemic delivery of drugs and in particular esters via the lungs.

Using a series of biphenylacetic acid esters we recently demonstrated extensive pre-absorptive pulmonary first-pass metabolism (after airways administration) but little post-absorptive first-pass metabolism (after intra-vascular presentation) (Dickinson & Taylor 1998). The in-vitro enzyme kinetics predicted the extent of post-absorptive first-pass metabolism, however they did not predict the pre-absorptive first-pass metabolism. The pre-absorptive metabolism was substantially greater than predicted by the in-vitro enzyme kinetics and in the inverse rank order for the esters investigated (Dickinson & Taylor 1998). Reasons for this lack of correlation between the in-vitro enzyme kinetics and pre-absorptive first-pass metabolism, include the possibility that the esters may have encountered the esterases at a site where blood flow was much less than that of the whole organ (i.e. the well-stirred model did not apply), or that a distribution-limited rather than a perfusion-limited kinetic model applied. That is, the esters investigated were all highly lipophilic and therefore may have exhibited poor permeability across the pulmonary mucosa, their absorption, therefore, being diffusion-limited rather than perfusion-limited.

Correspondence: P. A. Dickinson, The Welsh School of Pharmacy, Cardiff University, Cathays Park, Cardiff CF10 3XF, UK.
E-Mail: dickinsonpa@cardiff.ac.uk

The aim of this study was to investigate the pre-absorptive pulmonary first-pass metabolism of a less lipophilic ester to elucidate the reasons for the observed pre-absorptive pulmonary first-pass metabolism. An ester of 2-phenylethanol was chosen for investigation as 2-phenylethanol has a similar structure to some commonly used β-agonists.

Materials and Methods

All reagents were analytical grade, except acetonitrile which was HPLC grade. 2-Phenylethanol and Pluronic F-68 were obtained from Sigma (Poole, UK). Acetonitrile, triethylamine, petroleum ether, ethyl acetate and trifluoroacetic acid were purchased from Fisher Chemicals (Loughborough, UK). Hexanoyl chloride, octanoyl chloride and dichloromethane were obtained from Aldrich Chemical Company (Poole, UK). Magnesium sulphate was purchased from Acros Organics (Geel, Belgium). 1-(*p*-Tolyl) ethanol was from Fluka Chemika (Buchs, Switzerland). The research

adhered to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures (HMSO publication HC107) and the Universities Federation for Animal Welfare Guidelines.

Synthesis of 2-phenylethanol–hexanoic acid ester model prodrug

Dry dichloromethane (60 mL) was added to 2-phenylethanol (5·0 g, 40·9 mmol) and triethylamine (5·6 mL, 40·9 mmol). The solution was stirred rapidly over ice (0°C). A solution of hexanoyl chloride (5·7 mL, 40·9 mmol) in dry dichloromethane (30 mL) was slowly added dropwise and then the reaction was maintained at 0–5°C with stirring under nitrogen for 3·5 h. The reactants were concentrated under reduced pressure. Ethyl acetate (100 mL) was added to dilute the residue (precipitation of triethylammonium chloride occurs). The ethyl acetate layer was washed with water (4 × 50 mL). The combined aqueous layers were washed with ethyl acetate (3 × 50 mL). The majority of the product was found in the initial ethyl acetate layer, which was dried over anhydrous magnesium sulphate and concentrated under reduced pressure. This yielded a yellow liquid. The progress of the reaction was monitored by thin-layer chromatography, using petroleum ether–ethyl acetate (12 : 1 v/v) as the mobile phase, giving a spot at RF 0·64 that stained green on heating with vanillin dip.

Determination of hexanoic acid phenethyl ester pre-absorptive pulmonary first-pass metabolism

Male Wistar rats, 271–330 g, were anaesthetized by intra-peritoneal administration of 0·26 mg kg^{-1} fentanyl citrate, 8·3 mg kg^{-1} fluanisone (Hypnorm; Janssen Animal Health, High Wycombe, UK) and 4·16 mg kg^{-1} midazolam (Hypnovel; Roche Product Ltd, Welwyn Garden City, UK). Anaesthesia was maintained by the intra-peritoneal administration of 0·087 mg kg^{-1} fentanyl citrate and 2·8 mg kg^{-1} fluanisone every 30 min. In all rats the trachea was cannulated with polythene tubing (2·08 mm o.d.). The femoral artery and, where necessary, the left carotid artery were cannulated with polythene tubing.

Hexanoic acid phenethyl ester was administered intratracheally neat. The intra-arterial doses of hexanoic acid phenethyl ester were administered as 0·5% w/v hexanoic acid phenethyl ester, 5% w/v Pluronic F-68 normal saline (94·5% v/v) oil in water microemulsions. The intratracheal doses (177·4 mg kg^{-1} and 182·9 μL kg^{-1}) were administered by instillation from a glass syringe with a blunt needle inserted into the trachea to the point of the tracheal bifurcation. The dose was then instilled over a 20-s period during which time the rat was inclined at an angle of 35° to ensure retention and distribution of the dose within the lung. Intra-arterial doses (44·4 mg kg^{-1} and 8·9 mL kg^{-1}) were administered as 10-min zero-order infusions using a syringe pump, via the carotid artery. Separate groups of four rats each were used for each dosing route.

Blood samples (250 μL) were collected from the femoral artery, at selected times up to 90 min after dosing, and immediately added to micro-centrifugation vials containing internal standard in acetonitrile (375 μL) and stored at 4–8°C until analysis.

After intratracheal dosing the lungs were removed at the end of the experiment to determine the amount of hexanoic acid phenethyl ester and 2-phenylethanol remaining.

Assay of hexanoic acid phenethyl ester and 2-phenylethanol

2-Phenylethanol and 2-phenylethanol prodrug concentrations were determined by isocratic reverse-phase HPLC using a Fluofix column at ambient temperature, with detection at 213 nm; Promis II Injection System (Spark Holland, Emmen, Netherlands); Constametric 3000 solvent delivery system (Laboratory Data Control, Stone, UK); Spectrasystem UV1000 detector (Thermoquest, Withenshaw, UK); Berthold Version 1·65 integration software (Herts, UK). 2-Phenylethanol was assayed using a mobile phase consisting of acetonitrile–0·1% trifluoroacetic acid (20 : 80 v/v) at a flow rate of 1 mL min^{-1}. 2-Phenylethanol eluted with a retention time of approximately 10·5 min. 1-(*p*-Tolyl) ethanol, used as an internal standard, eluted with a retention time of approximately 19·5 min. Hexanoic acid phenethyl ester was assayed using acetonitrile–0·1% trifluoroacetic acid (60 : 40 v/v) as the mobile phase at a flow rate of 1 mL min^{-1}. Hexanoic acid phenethyl ester eluted with a retention time of approximately 9 min. Octanoic acid phenethyl ester, used as an internal standard, eluted with a retention time of approximately 13 min.

Pharmacokinetic analysis

A non-linear least-squares regression program (Minim; R. D. Purves, University of Otago, New Zealand) was used to analyse the intra-arterial 10-min infusion and intratracheal data. The area under the blood concentration–time curve (AUC) was

determined using the trapezoidal rule and extrapolation to infinity using the terminal rate constant.

The dose fraction escaping pre-absorptive (presystemic) first-pass metabolism ($f_{P.p.}$) was calculated from (Dickinson & Taylor 1998):

$$f_{P.p.} = AUC_{i.t.}/(AUC_{i.a.} \times f_a) \quad (1)$$

Where $AUC_{i.a.}$ and $AUC_{i.t.}$ are the hexanoic acid phenethyl ester AUC after intra-arterial and intratracheal hexanoic acid phenethyl ester administration; f_a is the fraction of the dose absorbed in any form (i.e. as intact hexanoic acid phenethyl ester or metabolized to 2-phenylethanol) after intratracheal administration and calculated from:

$$f_a = PE\ AUC_{i.t.}/PE\ AUC_{i.a.} \quad (2)$$

Where PE $AUC_{i.t.}$ and PE $AUC_{i.a.}$ are the 2-phenylethanol metabolite AUC after intratracheal and intra-arterial administration of hexanoic acid phenethyl ester. The pulmonary first-pass extraction ratio ($E_{P.p.}$) was determined from:

$$E_{P.p.} = 1 - f_{P.p.} \quad (3)$$

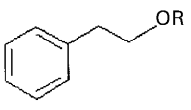
Results and Discussion

Synthesis of hexanoic acid phenethyl ester

The synthesis gave a yield of 7·67 g equivalent to 85% hexanoic acid phenethyl ester (Figure 1). Analysis, found: δ_H ($CDCl_3$) 0·95 (3H, m, CH_3); 1·35 (4H, m, $^{6'}CH_2$ and $^{7'}CH_2$); 1·66 (2H, quintet, J 7·3 Hz, $^{5'}CH_2$); 2·35 (2H, t, J 7·5 Hz, $^{4'}CH_2$); 3·00 (2H, t, J 7·0 Hz, $^{1'}CH_2$); 4·36 (2H, t, J 7·1 Hz, $^{2'}CH_2$); 7·29 (3H, m, Ph*H*); 7·37 (2H, m, Ph*H*) for hexanoic acid phenethyl ester. The product was greater than 95% pure by HPLC and exhibited a retention time that would be predicted for hexanoic acid phenethyl ester.

Determination of hexanoic acid phenethyl ester pre-absorptive pulmonary first-pass metabolism

Table 1 gives the AUC of hexanoic acid phenethyl ester and the metabolite, 2-phenylethanol, after administration of hexanoic acid phenethyl ester. The geometric mean concentration–time curves of hexanoic acid phenethyl ester and 2-phenylethanol after intra-arterial and intratracheal administration of hexanoic acid phenethyl ester are shown in Figure 2.



2-Phenylethanol: R = H
Hexanoic acid phenethyl ester: R = CO-CH_2-CH_2-CH_2-CH_2-CH_3

Figure 1. Structure of 2-phenylethanol and hexanoic acid phenethyl ester.

Table 1. Hexanoic acid phenethyl ester and 2-phenylethanol areas under the blood concentration–time curve (AUC) after administration of hexanoic acid phenethyl ester.

Administration route	Hexanoic acid phenethyl ester $AUC_{0-\infty}$ (mg min mL^{-1})	2-Phenylethanol AUC_{0-60} (mg min mL^{-1})
Intra-arterial	0·12 ± 0·04	0·51 ± 0·20
Intratracheal	0·04 ± 0·01	0·27 ± 0·05

Data are mean ± s.e.m., n = 4. All values are normalized to the intra-arterial dose.

After intra-arterial administration of hexanoic acid phenethyl ester the exponential decline in hexanoic acid phenethyl ester blood concentration was very rapid, with a half-life of less than 2 min. The total body clearance of hexanoic acid phenethyl ester after intra-arterial administration was 27·9 ± 7·1 L h^{-1} kg^{-1} (mean ± s.e.m., n = 4) and the volume of distribution was 1·2 ± 0·8 L kg^{-1} (mean ± s.e.m., n = 4).

After intratracheal instillation, hexanoic acid phenethyl ester had low bioavailability. The post-peak hexanoic acid phenethyl ester blood concentrations declined mono-exponentially, with a half-life of 27·0 ± 8·9 min (mean ± s.e.m., n = 4),

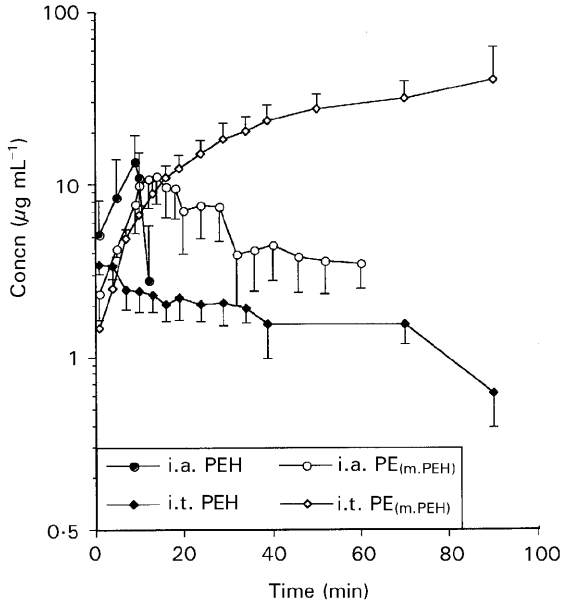


Figure 2. Hexanoic acid phenethyl ester (PEH) and 2-phenylethanol (PE) blood concentration–time profiles after intra-arterial 10-min infusion (●, ○, respectively) and intratracheal instillation (◆, ◇, respectively) of 44·4 mg kg^{-1} and 177·4 mg kg^{-1} hexanoic acid phenethyl ester, respectively. Data are the geometric mean ± s.e.m., n = 4.

which is significantly longer than the terminal half-life after intra-arterial administration of hexanoic acid phenethyl ester. This indicates that the pharmacokinetics of intratracheally administered hexanoic acid phenethyl ester are absorption rate-limited.

The AUC of a metabolite after administration of the parent compound may be used as an indicator of the dose of the parent compound absorbed or administered (Houston 1982). Using this technique and the 2-phenylethanol AUC_{0-60}, the fraction of hexanoic acid phenethyl ester absorbed (f_a) after intratracheal instillation was 0·54 (Eqn. 2), suggesting that approximately 50% of the intratracheal dose of hexanoic acid phenethyl ester reaches the circulation in some form. However, hexanoic acid phenethyl ester is subject to absorption rate-limited pharmacokinetics and therefore f_a is probably underestimated in this instance, as at 60 min hexanoic acid phenethyl ester was still being absorbed/hydrolysed after intratracheal administration. After intra-arterial administration practically all the hexanoic acid phenethyl ester would be eliminated by 60 min (approx. equiv. 30 terminal half-lives). This means that the PE $AUC_{0-60,i.a.}$ accounts for a greater proportion of PE $AUC_{0-\infty,i.a.}$ than PE $AUC_{0-60,i.t.}$ does for PE $AUC_{0-\infty,i.t.}$, therefore underestimating f_a. If f_a is underestimated then $E_{P.p.}$ will also be underestimated.

In an attempt to conclusively quantify f_a, the rat lungs were removed at the end of the experiment and analysed for hexanoic acid phenethyl ester and 2-phenylethanol. It was found that $30 \pm 6\%$ (mean $\pm$ s.e.m., $n = 4$) of the administered dose remained in the lungs as hexanoic acid phenethyl ester, while $11 \pm 1\%$ (mean $\pm$ s.e.m., $n = 4$) remained in the lungs as 2-phenylethanol. This indicates that f_a was 0·59. This is in close agreement with the f_a (0·54) derived from the 2-phenylethanol AUC_{0-60} data, suggesting this method produces a good estimate of the fraction of dose absorbed.

Comparison of the hexanoic acid phenethyl ester $AUC_{i.t.}$ with $AUC_{i.a.}$ indicates a pre-absorptive pulmonary first-pass extraction of 46 or 53% (Eqn. 1) for $f_a = 0{\cdot}54$ and 0·59, respectively. That pre-absorptive first-pass metabolism occurred was strongly indicated by the fact that 11% of the administered dose remained in the lungs (equiv. 27% of the material in the lung) as 2-phenylethanol.

An extraction ratio of 0·53 is less than that reported previously for aliphatic esters of biphenylacetic acid (Dickinson & Taylor 1998). The extraction ratio for biphenylacetic acid esters ranged from 0·83 to 0·99 and was in the same rank order as the C log P (computed log octanol–water partition coefficient) of the biphenylacetic acid esters (6·353 to 9·527). It is probable that the lower C log P of hexanoic acid phenethyl ester (4·24) explains the less extensive extraction measured and suggests that the ester physicochemical properties, and in particular ester lipophilicity, determines the extent of pre-absorptive first-pass metabolism. This can be rationalized by applying a diffusion-limited model to the in-vivo data. That is, the diffusion of the drug into the blood stream and thus residence time in the lung tissue controls the extent of metabolism. The maximum diffusion rate can be expected to occur at a log P of approximately 2 (Taylor 1990); as the log P increases above this value the partitioning rate of the compound from the lipophilic lung tissue into the relatively hydrophilic blood will slow and metabolism increase.

If diffusion-limited, rather than perfusion-limited, metabolism is occurring this has implications for the systemic delivery of drugs, in particular biotechnological drugs (e.g. peptides and proteins) via the lungs. The lungs are known to express a wide range of enzymes capable of metabolizing biotechnological drugs (Wall & Lanutti 1993; Forbes et al 1997). The absorption of biotechnological drugs from the lungs is undoubtedly diffusion-limited; that is, the alveolar epithelium presents a substantial barrier to pulmonary absorption even for small peptides (Evans et al 1998). This suggests that even though peptidase expression in the lungs is relatively low, extensive first-pass metabolism of biotechnological drugs is likely to occur. Although many studies have determined the bioavailable fraction after airway administration of biotechnological drugs (Patton 1996), the relative importance of incomplete absorption versus first-pass metabolism has not been investigated. This is probably because of the need to assay parent biological and metabolite concentrations in blood if first-pass metabolism is to be determined (Dickinson & Taylor 1998).

One other explanation for the extensive pre-absorptive pulmonary first-pass metabolism observed for the esters is the presence of *p*-glycoprotein transporters at the epithelial membrane (Abulrob et al 1999). If substrates for *p*-glycoprotein, these esters will be continually effluxed back into the pulmonary airways leading to the potential for several first-pass metabolism episodes to take place before the esters can enter the systemic circulation.

In summary a model ester with low lipophilicity has been synthesized and a blood assay developed. The pre-absorptive pulmonary first-pass metabolism of the ester was approximately 50%. This,

together with previously published data, suggests that pre-absorptive pulmonary first-pass metabolism for compounds whose absorption is diffusion-rate limited may be extensive even when pulmonary enzyme expression is low.

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