Pulmonary First-Pass and Steady-State Metabolism of Phenols

Paul A. Dickinson[1,2] and Glyn Taylor[1]

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Purpose. To study (A) the effect of the administration route (i.v. and i.t.) on pre- and post-absorptive metabolism of phenol and 1-naphthol by the lung and (B) pulmonary extraction of phenol at steady-state.
Methods. Phenols were administered intra-arterially, intravenously and intratracheally and the pre- and post-absorptive metabolism assessed by comparing the AUCs in arterial blood. Phenol was infused to steady-state and the pulmonary extraction assessed by measuring jugular vein and carotid artery blood concentrations.
Results. In contrast to previously published data (e.g., Mistry and Houston, *Drug Metab. Dispos.* 13:740–745 (1985)) we could not detect pulmonary first-pass metabolism of the phenols; reasons for this disparity are discussed. An apparent negative pulmonary extraction of phenol at steady-state was observed, probably as a consequence of extraction by organs which are in series, and not parallel, with the lungs (*e.g.* liver, kidneys and GIT).
Conclusions. (A) Phenol and 1-naphthol do not undergo pulmonary first-pass metabolism. (B) Traditional methods of assessing organ extraction and clearance at steady-state cannot be utilised when metabolising organs are in series.

KEY WORDS: phenol; 1-naphthol; UDPGT; pulmonary; first-pass; steady-state extraction.

INTRODUCTION

For over a decade it has been recognised that the lung, due to its metabolic activity, can have a major impact on the bioavailability of an administered drug (1–3). Most studies have only considered post-absorptive first-pass metabolism; that which affects vascular (intra-venous) administration of a compound. Whereas the investigation of pre-absorptive pulmonary metabolism; that which occurs after airway delivery is more pertinent when considering pulmonary drug delivery. Additionally few studies have examined a congeneric series of compounds so that the physicochemical characteristics of a xenobiotic determining the extent of pulmonary first-pass metabolism can be elucidated.

Phenolic compounds have previously been shown to undergo extensive first-pass pulmonary metabolism (1, 2, 4), this is in contrast to *in vitro* studies of phenol metabolism, performed by our laboratory, which predicted little first-pass metabolism would occur (5). This study was performed to assess the importance of administration route on pulmonary first-pass metabolism and to investigate *in vitro/in vivo* correlations and physicochemical properties affecting pulmonary metabolism. This study also compared two methods for determining pulmonary drug extraction of phenol.

Of the two methods used to determine pulmonary metabolism, the first involves the administration of the compound under investigation intravenously and to the aorta via the carotid arch (1,6). Since the intravenous dose traverses the lungs before reaching the arterial circulation a comparison of the area under the blood concentration-time curves (AUCs) after administration via the two routes then provides the fraction of the intravenous dose avoiding pulmonary post-absorptive first-pass metabolism. The second method involves the administration of the compound as a bolus injection or infusion to steady-state and then sampling from the blood vessels entering and leaving the lungs. In the case of the bolus injection, comparison of the AUCs for both vessels allows determination of pulmonary first-pass metabolism (2), whilst after infusion to steady-state, comparison of the blood concentration in each vessel allows quantification of pulmonary drug extraction (7).

MATERIALS AND METHODS

All reagents were analytical grade, except acetonitrile which was HPLC grade. β-glucuronidase (Type HP-2S, EC 3.2.1.1.31) was obtained from Sigma (Poole, U.K.). The research adhered to the "Code of Practice for the Housing and care of Animals Used in Scientific Procedures." (HMSO publication 1989; HC107) and UFAW Guidelines.

Determination of Pulmonary First-Pass Metabolism by Administration via the Carotid Artery, Jugular Vein, and Intratracheal Instillation

Male Wistar rats (241–330 g) were anaesthetised by intraperitoneal administration of fentanyl citrate, droperidol and midazolam (0.26 mg kg^{-1}, 2.80 mg kg^{-1}, and 4.16 mg kg^{-1} respectively). Anaesthesia was maintained by the intra-peritoneal administration of fentanyl citrate and droperidol (0.087 mg kg^{-1} and 0.933 mg kg^{-1} respectively) every 30 min. A tracheotomy was then performed and the left femoral artery in all rats was cannulated; the right jugular vein and left carotid artery were cannulated where necessary.

Phenol and 1-naphthol were administered intratracheally as a 50% v/v ethanolic solution (7.5 mg ml^{-1}) and a 35% v/v ethanolic solution (11.5 mg ml^{-1}) respectively. The intra-arterial and intravenous doses of phenol and 1-naphthol were administered as isotonic saline solutions.

The intratracheal dose volumes were 200 μl kg^{-1}, administered by instillation from a Hamilton glass syringe with a blunt needle inserted into the trachea to the point of the tracheal bifurcation. The dose was instilled over a 20 sec period. Intravenous and intra-arterial doses comprised of 10 ml kg^{-1} volumes administered as 10 minute zero-order infusions, using a syringe pump (Model 700, IVAC, Harrow, UK). Separate groups, each of five rats, were used for each dosing route. Doses of 15.9 μmol kg^{-1} (equivalent to 1.5 mg kg^{-1} phenol and 2.3 mg kg^{-1} 1-naphthol) were administered.

250 μl blood samples were collected in microcentrifugation vials from the femoral artery at selected times up to 30 min. The samples were stored at 4–8°C until analysis.

[1] Welsh School of Pharmacy, University of Wales, Cathays Park, Cardiff, CF1 3XF, U.K.
[2] To whom correspondence should be addressed.

Notation: AUC, arterial area under the blood concentration-time curve (subscripts define route of administration); $E_{P.p.}$, pre-absorptive pulmonary first-pass extraction ratio; $E_{P.s.}$, post-absorptive pulmonary first-pass extraction ratio.

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Determination of Phenol Pulmonary Metabolism by Administration via the Penile Vein with Carotid Artery and Jugular Vein Sampling

Phenol was administered as an isotonic saline solution. The rats were anaesthetised as above and the trachea, left carotid artery, right jugular vein and penile vein cannulated. Phenol was administered via the penile vein as a zero-order infusion (132 μg min^{-1} kg^{-1}, 0.4 ml min^{-1} kg^{-1}) to 6 rats (260–315 g). In 3 rats after steady-state had been achieved the hepatic portal vein was clamped for approximately 10 min.

250 μl blood samples were taken from the left carotid artery and right jugular vein consecutively. The vessel from which the first sample was taken for each pair of samples was alternated. The samples were taken at time points up to 80 min. The samples were stored at 4–8°C until analysis.

Determination of Blood Concentrations

A HPLC-fluorescence method was utilised for the analysis of phenol in blood. The high pressure liquid chromatography system used has been described previously (8) and employed a C_{18} spherisorb S5ODS column (Hichrom, Reading, U.K.) and LS-5 luminescence spectrophotometer (Perkin-Elmer, Beconfield, U.K.), with flow-through cell. Slit widths were set to 10 nm. The excitation and emission wavelengths were 266 and 301 nm, respectively. The eluent was 300 ml of acetonitrile, water to 1000 ml and trifluoroacetic acid 1 ml, the flow rate was 1.5 ml min^{-1}.

Phenol was extracted from 200 μl of blood with added internal standard (4-methylphenol) using dichloromethane. The solvent was then added to tetraoctylammonium bromide (4.95 mg) and evaporated. The residue was reconstituted with 200 μl of 50% v/v acetonitrile solution and 100 μl subjected to HPLC analysis.

In four of the experiments, in which rats were infused with phenol to steady-state, phenyl β-D-glucuronide blood concentrations were determined. After extraction of phenol the remaining aqueous layer was treated with β-glucuronidase and then assayed for phenol as described above.

For the determination of 1-naphthol in blood the high pressure liquid chromatography system used was the same as above. The excitation and emission wavelengths were 231 and 364 nm; each with 10 nm slit widths. The mobile phase was acetonitrile 450 ml, water to 1000 ml and trifluoroacetic acid 1 ml, the flow rate was 1.5 ml min^{-1}.

To 250 μl of blood, 250μl of internal standard solution (4-chloro-1-naphthol, 2.1 μg ml^{-1} in acetonitrile) was added. The mixture was then centrifuged and 100 μl of the supernatant subjected to HPLC analysis.

Pharmacokinetic Analysis

The 10 min infusion and intratracheal data were fitted to a relevant pharmacokinetic model using a non-linear least square regression program (*Minim*, R. D. Purves, University of Otago, New Zealand). The area under the blood concentration-time curves (AUCs) were determined using the trapezoidal rule and extrapolation to infinity using the terminal rate constant. The post-absorptive pulmonary first-pass extraction ratio ($E_{P.s.}$) is given by:-

$$E_{P.s.} = 1 - \frac{AUC_{i.v.}}{AUC_{i.a.}}$$

The pre-absorptive pulmonary first-pass extraction ratio ($E_{P.p.}$) is given by:-

$$E_{P.p.} = 1 - \frac{AUC_{i.t.}}{AUC_{i.a.}}$$

Where $AUC_{i.v.}$, $AUC_{i.t.}$, and $AUC_{i.a.}$ are the arterial AUCs after intravenous, intratracheal and intra-arterial administration. Clearance and extraction ratios at steady-state were calculated using standard techniques (9).

RESULTS

Determination of Pulmonary First-Pass Metabolism by Administration via the Carotid Artery, Jugular Vein, and Intratracheal Instillation

The blood concentration-time profiles for phenol and 1-naphthol after 10 min infusion and intratracheal instillation are shown in Figs. 1 and 2 respectively. After intravascular administration of both phenols the decline in blood concentration was biexponential with very rapid initial half-lives and terminal half-lives of approximately 4 to 5 min for phenol and and 2 to 3 min 1-naphthol. The total body clearances of phenol and 1-naphthol were 141 ± 40 and 209 ± 47 ml min^{-1} kg^{-1} after intra-arterial administration. After i.t instillation phenol and 1-naphthol were rapidly and extensively absorbed with peak levels occurring within 1 min. The phenol absorption half-life was less than 25 sec with the phenol blood concentrations then declining in a biexponential manner similar to those after i.a and i.v administration. 1-naphthol's absorption half-life was approximately 17 sec with the blood concentration then declining biexponentially with half-lives of 20 sec and 4 min. The elimination half-lives after i.t. instillation of 1-naphthol were significantly longer than those after i.a. and i.v. administration ($p < 0.05$, 1-way ANOVA, Student-Newman-Keuls Test).

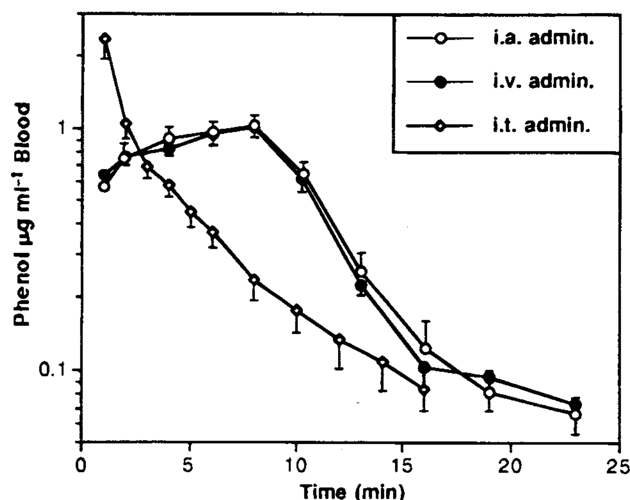

Fig. 1. Phenol geometric mean blood concentration-time profiles (n = 5 +/− s.e.m.) after i.a. and i.v. 10 min infusion and i.t. instillation of 1.5 mg kg^{-1} phenol.

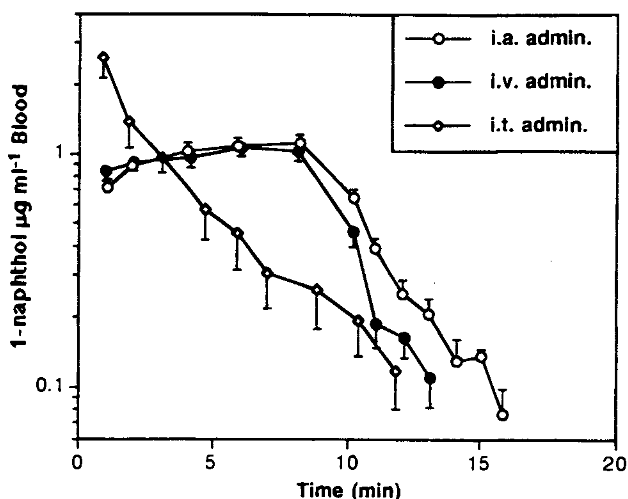

Fig. 2. 1-naphthol geometric mean blood concentration-time profiles (n = 8 (i.a and i.v.) n = 5 (i.t.) +/− s.e.m.) after i.a. and i.v. 10 min infusion and i.t. instillation of 2.3 mg kg^{-1} 1-naphthol.

The AUCs and first-pass extraction ratios after the 10 min infusion and intratracheal instillation of phenol and 1-naphthol are given in Table I. Comparison of the phenol AUCs indicates a post-absorptive first-pass metabolism (after i.v. administration) of 2% and a pre-absorptive first-pass metabolism (after i.t. instillation) of 10%, however neither the $AUC_{i.v.}$ or $AUC_{i.t.}$ were significantly different to the AUC found after i.a. administration (1-way ANOVA). Comparison of the 1-naphthol AUCs gave a post-absorptive first-pass extraction of 10% and a pre-absorptive first-pass extraction of 4%, however the $AUC_{i.v.}$ and $AUC_{i.t.}$ again, were not significantly different to the $AUC_{i.a.}$ (1-way ANOVA).

Determination of Phenol Pulmonary Metabolism by Administration via the Penile Vein with Carotid Artery and Jugular Vein Sampling

A representative example of the concentration-time profiles in the right jugular vein and left carotid artery is shown in Fig. 3. In all instances the intra-arterial blood concentrations of phenol exceeded the intravenous blood levels. At steady-state, prior to any portal vein clamping, the pulmonary extraction ratio was significantly negative at −0.26 ($p < 0.05$, t-test). When the hepatic portal vein was clamped the phenol blood concentration increased and the pulmonary extraction ratio became more negative for each animal (not shown). In the experiments in which the phenyl β-D-glucuronide levels were determined, the pulmonary extraction ratio of −0.007 was not significantly different from zero (t-test). The total body clearance determined from arterial steady-state concentrations was 152 ± 36 ml min^{-1} kg^{-1}.

Table I. AUCs and Pulmonary Extraction Ratios After Infusion and Intratracheal Instillation of Phenol and 1-naphthol

Administration Route	AUC (μg min ml^{-1})	Pulmonary Extraction Ratio
	Phenol	
i.a.	11.222 ± 2.756[a]	
i.v.	10.939 ± 1.416[a]	0.025
i.t.	10.046 ± 3.211[a]	0.105
	1-Naphthol	
i.a.	11.388 ± 2.076[b]	
i.v.	10.207 ± 3.351[b]	0.104
i.t.	10.941 ± 3.211[a]	0.039

[a] n = 5 ± s.d.
[b] n = 8 ± s.d.

DISCUSSION

Determination of Pulmonary First-Pass Metabolism by Administration via the Carotid Artery, Jugular Vein, and Intratracheal Instillation

Both phenol and 1-naphthol clearances exceed hepatic blood flow (68.6 ml min^{-1} kg^{-1}, (10)) suggesting extrahepatic metabolism is occurring, indeed 1-naphthol clearance approaches total cardiac output (324 ml min^{-1} kg^{-1}, (10)) suggesting some pulmonary clearance. Despite these high clearances the AUCs after intra-venous and intratracheal administration for both compounds are not significantly different from those after intra-arterial administration suggesting pulmonary first-pass metabolism is not occurring. These conclusions differ markedly from the first-pass pulmonary extraction ratios reported previously for phenol and 1-naphthol of 62% and 44% respectively (1, 2, 4).

Phenol's distribution and elimination half-lives are in agreement with those previously reported by Cassidy and Houston (4) who found values of 1 and 5 min respectively after i.a. administration, however they were unable to show a distribution phase after i.v. administration. Phenol's total body clearance is approximately twice that reported by Cassidy and Houston (4).

The results after 1-naphthol administration differ from those reported by Mistry and Houston (2) who found a distribu-

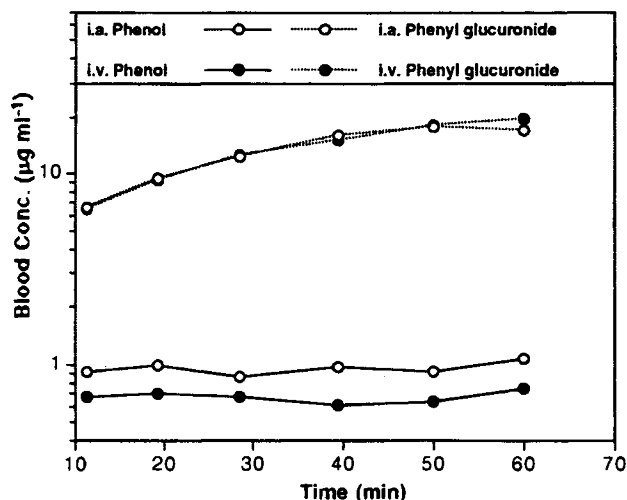

Fig. 3. Phenol and phenyl β-D-glucuronide blood concentration-time profiles, in the left carotid artery and right jugular vein, during penile vein infusion of phenol 132 μg min^{-1} kg^{-1}.

tion half-life of 2-3 min and an elimination half-life of 81 min. The absence of a long elimination half-life in our studies may have occurred because we could only measure 1-naphthol concentration until approximately six min after the cessation of the infusion, due to the constraints of administering the same molar dose as phenol and sensitivity of the assay. The terminal half-life found after i.t. instillation was significantly longer than after i.a. or i.v. administration probably because absorption rate limited pharmacokinetics apply to the i.t. administration of 1-naphthol. The rapid 1-naphthol blood peak observed is probably explained by the presence of 2 absorption processes, as reported for other compounds (11).

The reason why Houston and co-workers were able to demonstrate post-absorptive pulmonary first-pass metabolism whereas this laboratory could not, is unclear. The most obvious difference between the two sets of experiments is that Houston and co-workers used Sprague-Dawley rats as opposed to male Wistar rats, it is possible that the two strains have different metabolic profiles.

The urethane anaesthetic used by Houston and co-workers has been shown to decrease cardiac output by 46% (10) when compared to an anaesthetic regimen similar to ours. Standard theory suggests this reduction in cardiac output may cause an increase in the pulmonary extraction ratio and explain why the total body clearance values quoted by Houston and co-workers are approximately half those quoted here. There is some evidence in the literature that in the rat midazolam is hydroxylated to phenolic metabolites which are then glucuronidated and / or sulphated (12). Thus it is conceivable that our use of midazolam for induction may have reduced the pulmonary extraction ratios of phenol and 1-naphthol by competition for the enzyme. Although it seems unlikely that a major reduction in pulmonary extraction would have occurred considering the concentration of midazolam perfusing the lung at the time of phenol (and 1-naphthol) administration.

Determination of Phenol Pulmonary Metabolism by Administration via the Penile Vein with Carotid Artery and Jugular Vein Sampling

A negative pulmonary extraction ratio has been reported for salicylamide when administered as an intravenous infusion in dogs (7), this observation was attributed to experimental error. A negative extraction ratio suggests the lungs are not metabolising phenol but are rather producing it; possibly as a consequence of pulmonary β-glucuronidase activity which hydrolyses previously conjugated phenol. At steady-state, arterial-venous concentration differences are not due to partitioning into the organ across which the samples are taken (13). β-glucuronidase activity has been demonstrated in chickens *in vivo* (14) and in rat lungs *in vitro* (15). However the pulmonary extraction ratio of phenyl β-D-glucuronide, during phenol infusion, was slightly negative (-0.007) but not significantly different to zero. It is recognised that the β-glucuronidase used in the assay possessed some sulphatase activity and so even though the concentration measured is referred to as phenyl β-D-glucuronide it is a composite value reflecting phenyl β-D-glucuronide and phenyl sulphate concentration.

Another possible explanation for the negative extraction by the lungs is that, because the lungs are in series (and not parallel) with the other extracting organs (*e.g.* liver, gastro-intestinal tract, and kidneys) the pulmonary extraction is not measured but a value which represents the difference between pulmonary extraction ratio and extraction by the other organs. Initially it was expected that clamping the portal vein would substantially reduce hepatic and gastro-intestinal tract phenol clearance and so pulmonary extraction would be more easily measured: and not cause the apparent pulmonary extraction ratio to become more negative as observed. This decrease in apparent pulmonary extraction and total body clearance, in the absence of further knowledge of the effect, on blood flow throughout the body, of portal vein clamping, is difficult to explain: it was possibly a consequence of the non-physiological condition experienced by the animals during clamping. The results reported here suggest that the traditional method of determining organ extraction by sampling from either side of the organ cannot be used for investigating pulmonary extraction or extraction across other organs when pulmonary extraction is present.

We have previously characterised the enzyme kinetics for glucuronidation of phenols in lung and liver microsomes and used this data to predict pulmonary extraction ratios (5). These predicted extraction ratios were very small for both phenol and 1-naphthol suggesting the 'well-stirred' model used for the prediction is valid. The *in vitro* data suggested that 1-naphthol should be extracted more extensively than phenol which was apparently the case for intravenous administration but not intratracheal instillation. The *in vitro* data also suggested that 1-naphthol should exhibit a higher clearance than phenol which is in agreement with the data published here. Of the phenols studied *in vitro* 4-chloro-1-naphthol was the only one which had a predicted extraction ratio large enough to be measured *in vivo* and so investigation of that compound would have provided more accurate answers on the predictive nature of the model and physicochemical characteristics affecting pulmonary drug metabolism; unfortunately it proved impossible to develop an assay of sufficient sensitivity.

In summary this work demonstrated that phenol and 1-naphthol do not undergo pre-absorptive or post-absorptive metabolism in the Wistar rat which is in agreement with *in vitro* studies previously performed in our laboratory. The steady-state infusions highlight a major difficulty associated with the investigation of pulmonary drug metabolism: that is differentiating pulmonary effects from those of other organs as the lungs are in series and not parallel with the other organs.

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