

Understanding Phenylephrine Metabolism, Pharmacokinetics, Bioavailability and Activity

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Phenylephrine (PE) is a synthetic, selective, sympathomimetic agent that, when dosed intravenously, has been demonstrated to have potent vasoconstrictor properties¹. PE appears to have little effect on the beta receptors of the heart. When given intravenously it slows the heart rate but increases the stroke output thereby causing a rise in systolic and diastolic pressures¹.

PE is also used in oral, ophthalmic and intranasal dosage forms. It is used as a decongestant in many over-the-counter (OTC) and prescription products where constriction of blood vessels in the nasal mucosa is intended to relieve nasal congestion. Contrary to the intravenously administered drug the orally administered drug has not demonstrated any effect on blood pressure and heart rate except in the case where the dose is much greater than the current OTC Monograph levels of 10 mg (therapeutic changes seen at 250 mg with a single oral dose compared to the intravenous dose)².

There is sparse data for PE in relation to its absorption, metabolism, distribution and excretion from an oral dosage form. Studies were conducted in the 1970s, '80s and early 1990s with a small number of subjects and used bio-analytical techniques which lacked the sensitivity of today's methods and instrumentation. It is important to note that PE from an oral dose undergoes pre-systemic conjugation in the small intestine to produce PE-glucuronide and PE-sulfate. We have investigated the activity of these conjugates and have shown that, in assays in which parent-PE (never conjugated) molecule has measurable activity, these conjugates are not pharmacologically active.

In the course of drug development a sponsor is required to demonstrate bioavailability by an analysis of the blood levels of the active drug³. In at least one previous application - the approval of the bitartrate salt - the sponsor characterized the pharmacokinetic profile of PE with reference to total-PE. They did this in part by using an assay that combined the contributions from parent (never conjugated) PE and the PE conjugates in plasma after the PE was released from the conjugate by enzymatic cleavage in the test tube.

Based on our observations, we submit that the valid approach to characterizing the bio-pharmaceutics of PE drug products is by measuring the parent-PE profile. We have conducted pharmacokinetic studies of a single oral dose of 10 mg PE and measured total-PE and parent-PE. The exposure to parent-PE is orders of magnitude less than total-PE. The parent-PE peaks within 30 minutes and decays rapidly compared to total-PE which has a much broader profile.

We have also studied the regional absorption of PE and have concluded that there are opportunities to optimize the drug delivery of PE by, for example, improving the availability of systemically available parent-PE.

Our overall conclusion is that the appropriate moiety to measure in plasma is parent-PE and we have discovered that this moiety when administered orally in conventional oral dosage forms appears in plasma at a fraction of the levels of total-PE. Its time course in plasma from a 10 mg dose given orally is short-lived. These findings may go some way in helping to interpret the small, transient efficacy of PE seen in some clinical efficacy studies.

¹ Phenylephrine hydrochloride injection USP, 1% Physician Labeling

² Keys A. and Violante A. (1942) The cardio-circulatory effects in man of neo-synepherine. *J. Clin. Invest.* 21 1-12.

³ FDA Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations (March 2003)

Metabolism and Pharmacokinetics

PE undergoes extensive pre-systemic metabolism, with a majority of the metabolism taking place within the enterocytes of the gastrointestinal tract. The metabolic fate of PE is summarized in Figure 1. PE is metabolized by Phase I and Phase II enzyme systems, mainly monoamine oxidase and sulfotransferase, respectively. The ratios of the metabolites differ depending on the route of administration.

Ibrahim and coworkers measured the metabolism of PE after oral and inhalation administration using a gas chromatographic/mass spectrometric ion monitoring method with deuterated internal standards⁴. After oral administration of a dose equivalent to approximately 24 mg of PE to 3 healthy human volunteers, four main metabolites were excreted in urine, reported as percent of dose

- (1) unconjugated m-hydroxymandelic acid (30%)
- (2) sulfate conjugate of m-hydroxyphenylglycol,
- (3) sulfate conjugate of PE (47%)
- (4) glucuronide conjugate of PE (12%).

The amounts of the same metabolites after inhalation of PE were 24, 6, 56 and 5%, respectively.

Approximately 1 mg of ³H-Phenylephrine free base was administered as an intravenous (iv) infusion over 12.5 to 20 minutes (mean 0.84 mg ± standard deviation 0.17 mg) and as an oral solution (0.99 ± 0.15 mg) to a small number of adult volunteers (N = 4 and 10, respectively). Total ³H-activity was measured in the urine and serum. The serum was separated using chromatographic techniques to quantitate parent ³H-phenylephrine, conjugated ³H-phenylephrine and ³H-m-hydroxymandelic acid. The cumulative urinary excretion of ³H-activity, ³H-phenylephrine, conjugated ³H-phenylephrine and ³H-m-hydroxymandelic acid after iv administration were reported as percent of dose: 86.3, 16.0, 8.3 and 56.9 respectively; and after oral administration – 79.5, 2.6, 45.7 and 24.2, respectively. The urinary recovery data showed that the administered dose of PE is well absorbed and approximately 80 percent of the dose was recovered.

An in-depth investigation of the pharmacokinetics of PE and its metabolites was reported in 1993 by Gumbhir⁵. After oral administration of Comhist® tablets containing 10 or 20 mg of PE, Gumbhir reported concentrations of parent-PE in plasma were below the limit of quantitation of 2 ng/ml and the concentrations of m-hydroxymandelic acid were not detectable for the 10 mg dose. m-Hydroxymandelic acid is not extensively conjugated, whereas m-hydroxyphenylglycol is extensively conjugated. The plasma concentrations of phenylephrine conjugates were the highest, followed by m-hydroxymandelic acid, m-hydroxyphenylglycol conjugates and m-hydroxyphenylglycol.

⁴ Ibrahim, K.E. et al. (1983) The Mammalian Metabolism of R-(-)-m-Synephrine. *Journal of Pharmacy and Pharmacology* 35, 144-147

⁵ Gumbhir, K. (1993) An Investigation of Pharmacokinetics of Phenylephrine and its Metabolites in Humans. In *Pharmaceutical Sciences*, pp. 216, University of Missouri-Kansas City

The pharmacokinetics of PE and its major metabolites were studied by Hengstmann and Goronzy⁶. Approximately 1 mg of ³H-Phenylephrine free base was administered as an intravenous (iv) infusion over 12.5 to 20 minutes (mean 0.84 mg ± standard deviation 0.17 mg) and as an oral solution (0.99 ± 0.15 mg) to a small number of adult volunteers (N = 4 and 10, respectively). Upon intravenous administration, phenylephrine rapidly distributes into the peripheral tissue, which yields a very low plasma concentration. Its distribution volume during steady state (V_{ss}) ranged from 184 to 543 liters, indicating most of the drug was distributed in the peripheral tissue or organs (Figure 2). The calculated oral phenylephrine absolute bioavailability was reported as 38% relative to intravenous dosing but the validity of this value is questionable. The biphasic distribution showed that the drug partitioned into the peripheral tissue or organs upon administration, accounting for extremely low plasma concentration observed for parent phenylephrine relative to its major metabolites. The biphasic distribution of unchanged PE was confirmed by Gumbhir⁵.

Bioavailability

Developing a sensitive and specific bioanalytical method to measure the extremely low plasma or urine concentrations of parent-PE after oral administration of doses within the range mandated in the Over-the-Counter Nasal Decongestant Drug Products Monograph (21CFR Part 341)⁷ has been challenging.

Several authors have tried to develop bioanalytical methods that are sensitive and specific enough to measure parent PE in plasma, and have failed^{5, 8}. Instead of measuring parent-PE, there are a few examples in the literature that study the bioavailability of PE by measuring total PE in plasma and urine. Total PE is derived after treating the plasma with either acid or enzyme (β -glucuronidase and sulfatase) to hydrolyze conjugated PE metabolites. Thus, after hydrolysis is complete, total-PE comprises previously conjugated PE and unconjugated parent-PE.

An example of the use of total-PE as a measure of bioavailability is a comparison of two salts of PE, hydrochloride and bitartrate. Bayer Healthcare's submitted a Citizen Petition to include the PE bitartrate salt as a generally recognized as safe and effective oral nasal decongestant active ingredient in the Cough, Cold, Allergy, Bronchodilator and Anti-asthmatic Drug Products for Over-the-Counter Human Use Final Monograph⁷.

⁶ Hengstmann, J.H. and Goronzy, J. (1982) Pharmacokinetics of ³H-Phenylephrine in Man. *European Journal of Clinical Pharmacology* 21, 335-341

⁷ FDA. (2004) Proposed Rule - Docket No. 1976N-0052N/CP18. Cold, Cough, Allergy, Bronchodilator, and Antiasthmatic Drug Products for Over-the-Counter Human Use; Proposed Amendment of Monograph for Over-the-Counter Nasal Decongestant Drug Products. *Federal Register* 69 (211), 63482-63488

⁸ Stockis, A. et al. (1995) Relative Bioavailability of Carbinoxamine and Phenylephrine from a Retard Capsule after Single and Repeated Dose Administration in Healthy Subjects. *Arzneim.-Forsch./Drug Res.* 45 (9), 1009-1012

Bayer compared an effervescent product containing the bitartrate salt against the same product with the bitartrate salt replaced by the hydrochloride salt; both products were immediate-release products of PE. Total PE was measured in plasma and urine. The results showed comparative bioavailability of the two salts. The FDA determined that the pharmacokinetic study was acceptable in lieu of a clinical trial because of similarity in the bioavailability of the two effervescent tablets.

Using up-to-date LC-MS-MS analytical technology, Schering-Plough Healthcare Products has determined that the concentration of total PE in the plasma is approximately 100-fold greater than parent-PE, enabling greater sensitivity (higher value for lower limit of quantitation) with readily available analytical instrumentation.

A bioavailability study has been conducted by Schering-Plough Healthcare Products (SPHCP). A validated bioanalytical method for parent PE was developed for SPHCP with a lower limit of quantitation of <0.05 ng/ml. Unlike bioavailability results reported in the literature which report only total-PE, the amount of parent-PE compared with the total PE was much smaller, by approximately 100-fold as described above (Figure 3). If the activity of PE is directly related to the concentration of parent-PE in the plasma and the conjugates of PE have no activity, as confirmed below, it may help to explain why there might be a transient efficacy of 10 mg immediate release PE in clinical efficacy studies.

Activity of the Conjugates

Schering-Plough Research Institute (SPRI) conducted studies to determine the affinity and functional activity of m-hydroxymandelic acid, PE sulfate conjugate and PE glucuronide conjugate at the human recombinant α_1 -adrenoreceptors (α_{1a} and α_{1b} subtypes) and α_2 -adrenoreceptors (α_{2a} , α_{2b} and α_{2c} subtypes). Affinity of the metabolites was determined by receptor binding assays. Functional activity of the metabolites was assessed using an [35 S]-GTP γ S binding exchange assay for the α_2 receptor subtypes and a cell-based calcium flux response for the α_1 receptor subtypes.. The conjugated metabolites were synthesized and purified by SPRI. The [35 S]-GTP γ S binding exchange assay and the calcium flux assay are considered sensitive assays of α_1 and α_2 adrenoreceptor activity because they utilize cells overexpressing the recombinant human adrenoreceptors. The metabolite activities were compared to the activity to parent (never-metabolized) PE⁹.

3-hydroxymandelic acid, obtained from a reliable commercial source, had no activity at the highest concentration evaluated in the α_1 or α_2 assays assessing agonist activity. In addition, 3-hydroxymandelic acid had no affinity for the α_1 or α_2 receptor subtypes at the highest concentration evaluated. Thus, 3-hydroxymandelic acid is an inactive metabolite of PE. PE sulfate had no affinity for the α_1 or α_2 receptor subtypes at the highest concentration evaluated. Accounting for the very low level of PE contamination in the samples studied (< 0.1% PE), PE sulfate had no activity in the α_2 subtype [35 S]-GTP γ S assays and the calcium flux assays at the highest concentration evaluated. PE glucuronide was pharmacologically inactive in the α_1 and α_2 subtype receptor binding assays as well as in the assays measuring functional activity of the α_1 and α_2 receptors. PE glucuronide had no binding affinity for the α_{1a} or α_{1b} receptors nor did it activate binding of [35 S]-GTP γ S to the α_2 receptor subtypes. Accounting for the very low level of PE contamination in the samples studied (~ 0.28% PE), no activity was observed in the α_{1a} or α_{1b} calcium and α_2 receptor binding assays.

In summary, none of the conjugates tested demonstrated any activity in these test systems.

⁹ Umland, S.P. and Shah, H.R. (2007) Evaluation of the Affinity and Activity of Phenylephrine Metabolites, 3-hydroxymandelic acid (SCH 1382498), Phenylephrine sulfate (SCH 1382852) and Phenylephrine glucuronide (SCH 1399841) in Human Recombinant α_1 and α_2 Adrenoreceptor Binding and Activity Assays (Schering-Plough Research Institute Study Report # 50143)

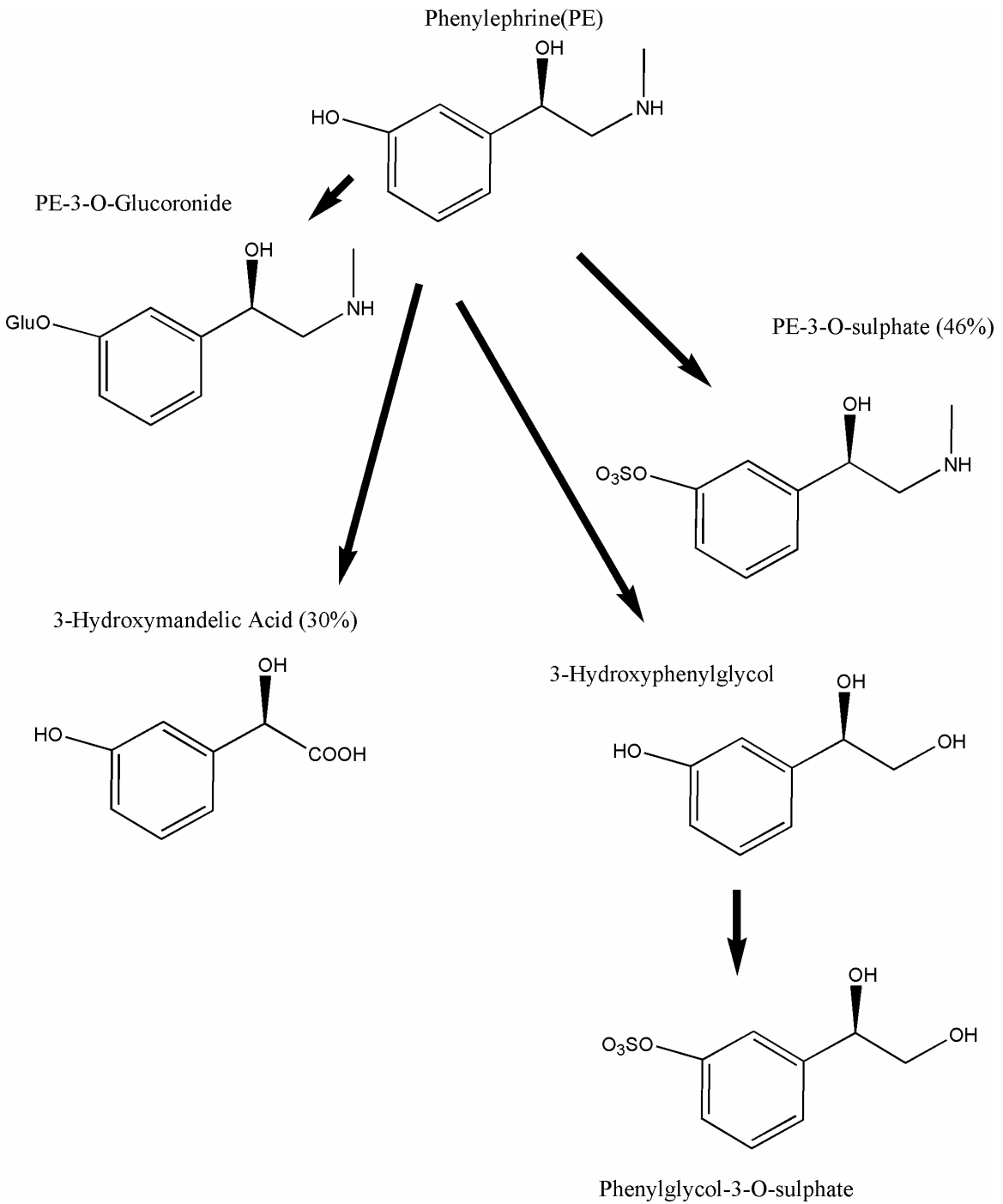


Figure 1 Phase I and Phase II metabolites of Phenylephrine in humans. The percentage values in the schematic refer to the percent of a 24 mg oral dose reported by Ibrahim⁴.

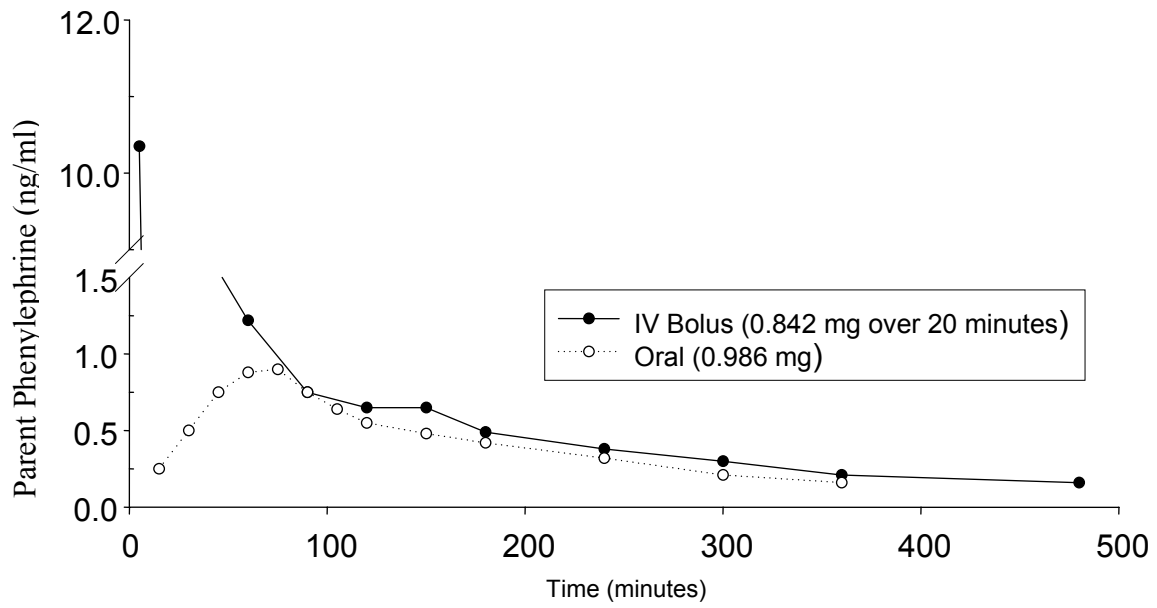


Figure 2 ³H-Phenylephrine serum concentration (ng/ml) versus time after intravenous (IV) and oral administration of ³H-Phenylephrine free base solution⁶.

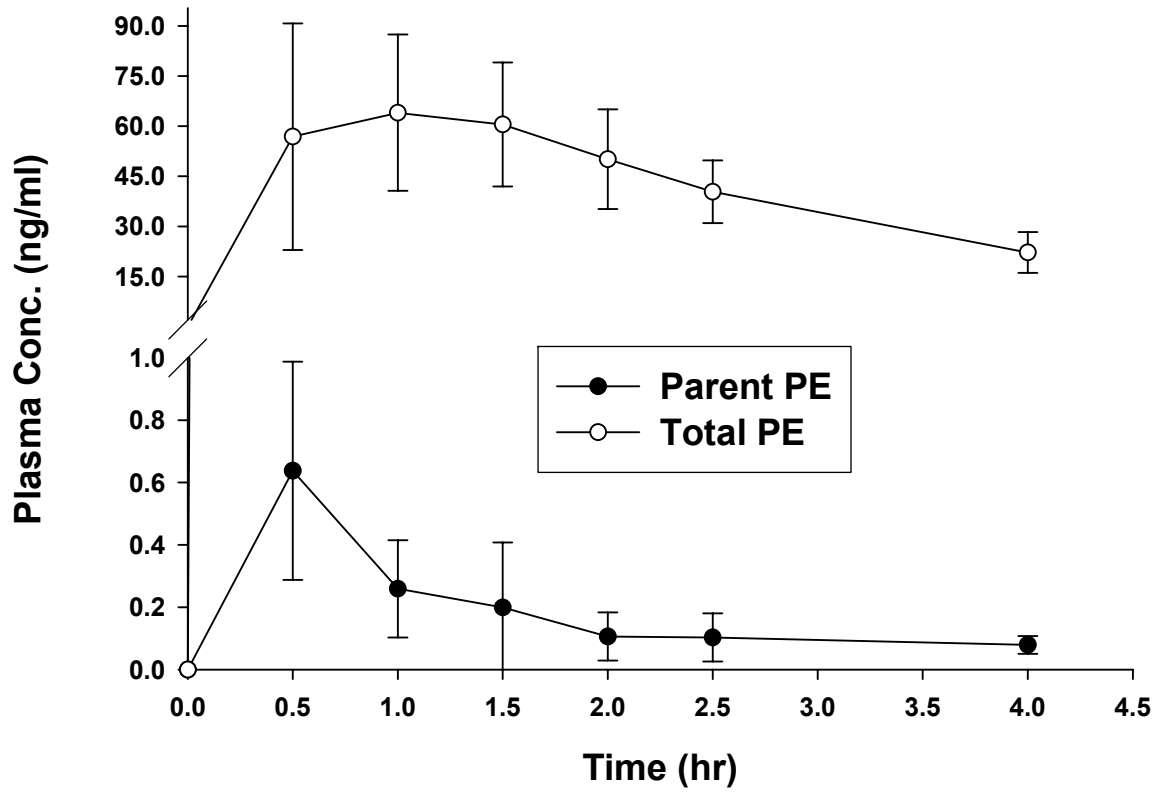


Figure 3: Plasma concentrations of parent PE and total PE (ng/ml) verses time for a 10 mg single dose of a Phenylephrine tablet (Schering-Plough Study CL2005-07, 2005).