In vivo assessment of extrahepatic conjugative metabolism in first pass effects using the model compound phenol

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It has been known for a number of years that the liver is not the only site where drugs and other xenobiotics undergo conjugation reactions (Hartiala 1973; Dutton & Burchell 1978; Hook & Bend 1978). In vitro studies have shown that a number of extrahepatic tissues including the intestinal mucosa and lung contain significant glucuronyl transferase (Chhabra & Fouts 1974, 1976; Litterst et al 1975;) and sulphotransferase (Bostrom 1965; Hook & Bend 1978). However, little attempt has been made to quantify the role of extrahepatic metabolism in the in vivo disposition of drugs and xenobiotics.

We have evaluated the relative contribution of the intestinal mucosa, liver and lung in the in vivo disposition of phenol in rat. This compound was selected for investigation because it is essentially completely biotransformed to phenyl glucuronide and phenyl sulphate and very little oxidative metabolism occurs (Capel et al 1972; Weitering et al 1979). Blood concentration-time profiles for phenol and its metabolites have been obtained following administration of phenol by a number of different routes. First pass considerations (Rowland 1973; Gibaldi & Perrier 1974) have been used to assess the relative conjugative ability of hepatic, intestinal and pulmonary enzymes.

When administered intra-arterially (i.a.) a compound is immediately distributed throughout the body and may be regarded as being 100% available. The area under the blood concentration-time curve between zero and infinity ($AUC_{i.a.}$) is governed by the dose administered (D) and the systemic clearance of the drug (CL).

$$AUC_{i.a.} = \frac{D}{CL} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

In contrast when the same dose is given orally, the area under the blood concentration-time curve between zero and infinity ($AUC_{p.o.}$) is also influenced by a third term—the fraction of the dose reaching the systemic circulation ($F_{p.o.}$).

$$AUC_{p.o.} = \frac{F_{p.o.}D}{CL} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Combining equations (1) and (2) gives the well known relationship used to assess oral availability (Dost 1958).

$$F_{p.o.} = \frac{AUC_{p.o.}}{AUC_{i.a.}} \quad .. \quad .. \quad .. \quad .. \quad (3)$$

As depicted diagrammatically in Fig. 1, there are three potential sites for biotransformation across which an orally absorbed drug must pass before reaching the systemic circulation—the gastrointestinal mucosa, liver and lung. These eliminating organs are anatomically arranged in series, therefore $F_{p.o.}$ is a hybrid parameter which is equal to the product of the fractions of dose escaping metabolism by the gastrointestinal mucosa (f_G), liver (f_H) and lung (f_L).

$$F_{p.o.} = f_G.f_H.f_L \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Similarly if a compound is given by injection into the hepatic portal vein (Fig. 1) its availability ($F_{h.p.v.}$) will be dependent upon the fractions of the dose traversing the liver and lung intact.

$$F_{h.p.v.} = f_H.f_L \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

This may be calculated by comparing the area under the blood concentration-time curves for hepatic portal administration ($AUC_{h.p.v.}$) with that from i.a. administration.

$$F_{h.p.v.} = \frac{AUC_{h.p.}}{AUC_{i.a.}} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

In addition, if a compound is administered by injection into a vein just proximal to the heart (see Fig. 1), then direct assessment of the fraction of the dose escaping metabolism by the lung (f_L) can be made by comparing the area under blood concentration-time curve ($AUC_{i.v.}$) resulting from this route of administration with $AUC_{i.a.}$.

$$f_L = \frac{AUC_{i.v.}}{AUC_{i.a.}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

Thus by administration of a compound by the four routes depicted in Fig. 1 (ia, iv, hpv and po) any first pass effect which is evident after oral absorption may be factored into three components (f_L, f_H and f_G). f_L may be calculated using equation (7) f_H and f_G using equations (8) and (9).

$$f_H = \frac{F_{h.p.v.}}{f_L} = \frac{AUC_{h.p.v.}}{AUC_{i.v.}} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

$$f_G = \frac{F_{p.o.}}{F_{h.p.v}} = \frac{AUC_{p.o.}}{AUC_{h.p.v}} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

Male Sprague-Dawley rats of mean weight 266 g (s.d. 38) were anaesthetized with urethane (1·3 mg kg^{-1}) and cannula (PE 50, Intramedic) placed in the left carotid artery. This cannula was used for drug administration in the intra-arterial studies. The rats receiving intravenous phenol were fitted with a second cannula in the left jugular vein for drug administration. A third set of rats received the phenol dose via a butterfly

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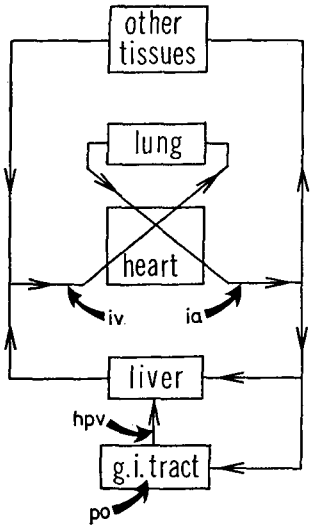

FIG. 1. Schematic representation of the anatomical positions of the sites of metabolism and the routes of administration studied.

infusion set (27G, Abbott Labs) into the hepatic portal vein. A fourth set of rats were dosed orally by direct injection of phenol into the duodenal lumen.

[U-^{14}C]Phenol (1·5 mg kg^{-1}; 10 μCi kg^{-1}) was administered in aqueous solution (0·4 ml) and the cannula flushed with an equal volume of heparinized 0·9% NaCl (saline) to ensure the entire dose was administered. Blood samples (100 μl) were removed from the carotid artery cannula at −5, 2, 5, 8, 12, 16, 22, 30, 60, 90, and 120 min. Each sample was diluted with heparinized saline (0·3 ml, 500 units ml^{-1}) and extracted with Scintillation Fluid 1 (5 ml toluene containing 0·5% 2,5-diphenyloxazole and 0·05% 1,4-di-2-(5-phenyloxazolyl)-benzene). Four ml of the organic layer was counted on Packard Tri-carb Scintillation Counter (Model 2405) using an external standard quench correction method. The remainder of the organic phase was discarded. The aqueous layer was then vortexed and a 150 μl sample removed, oxidized (perchloric acid), bleached (hydrogen peroxide) and counted in Scintillation Fluid 2 (toluene: Triton X-100, 2:1 containing 0·4% 2,5-diphenyloxazole and 0·01% 1,4-di-2-(5-phenyloxazolyl)-benzene).

It was established that the extraction procedure with Scintillation Fluid 1 had an efficiency of 94·5% for phenol. Thin layer chromatography using the method of Weitering et al (1979) demonstrated that the procedure was specific for phenol with no extraction of phenyl conjugates. The radioactivity in each blood sample was quantified as parent drug (Scintillation Fluid 1) and conjugates (Scintillation Fluid 2) with appropriate correction factors.

Representative blood-concentration-time curves for phenol after administration by each of the 4 routes are

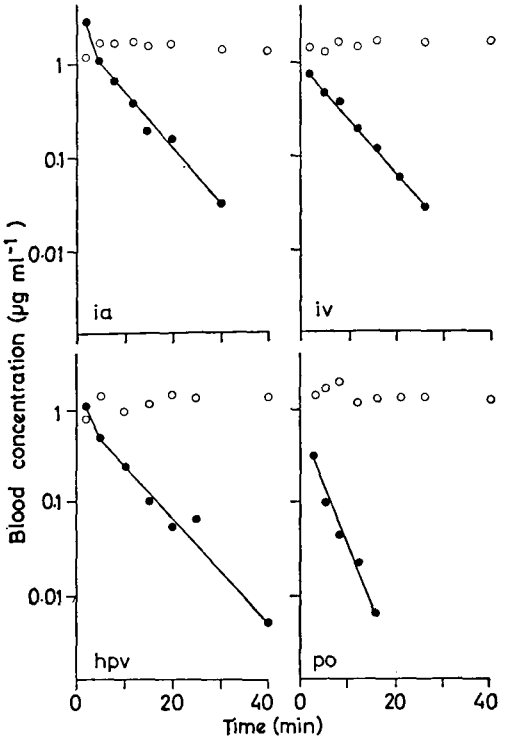

FIG. 2. Typical blood concentration-time profiles for intact phenol (●) and phenyl conjugates (○) in rats receiving phenol by i.a., i.v., h.p.v. or p.o. administration.

shown in Fig. 2. Similar results were obtained for each of the i.a. (n = 7), i.v. (n = 7), h.p.v. (n = 6) and p.o. (n = 5) animals.

Following intra-arterial administration phenol disposition is extremely rapid. As shown in Fig. 2 blood concentrations decline in a biexponential manner with half-lives of approximately 1 and 5 min for each phase. Total body clearance is 64·0 ml min^{-1} kg^{-1} (s.d. 16·2) and the volume of distribution (calculated by the AUC method) is 474·4 ml kg^{-1} (s.d. 42·1).

In the i.a. set of animals, administration of the phenol dose and blood sampling was carried out via the same cannula. Therefore it was necessary to determine whether phenol would bind to the cannula and hence contaminate subsequent samples. Under the conditions of the experiment, the flushing procedure reduced radioactivity retention on the cannula to negligible amounts. In addition separate studies where the i.a. dose was administered via a butterfly infusion set instead of via the carotid cannula, gave similar blood concentration-time curves for phenol and metabolites to the standard i.a. method. Thus the phenol concentrations achieved from i.a. administration are not artifactually high.

Blood concentrations of phenol following intra-arterial administration are much higher than from administration via the other routes (see Fig. 2). One

Table 1. Area under blood concentration-time curves for phenol (AUC) and phenyl conjugates (AUC_m) following administration of phenol by a number of different routes.

Route	AUC[a] (μg ml^{-1} min kg)	AUC_m[b] (μg phenol equiv. ml^{-1} min kg)
i.a. (n = 7)	6·13 (1·83)[c]	39·74 (4·64)
i.v. (n = 7)	2·53 (0·94)	49·48 (19·12)
h.p.v. (n = 6)	2·22 (0·72)	41·66 (12·20)
p.o. (n = 5)	0·18 (0·12)	46·25 (22·92)
Variance due to error[d]	1·33	32·25
Variance due to routes[d]	38·24	5·18
Statistical significance of difference between routes[e]	$P < 0·01$	N.S.

[a] AUC from zero to infinity calculated by the trapezoidal rule.
[b] AUC from zero to 120 min calculated by the trapezoidal rule.
[c] Standard deviation.
[d] By one way analysis of variance.
[e] By F test.

way analysis of variance demonstrates a statistically significant difference in the AUCs for the 4 routes (Table 1). Further analysis by decomposition of the sum of squares (Netter & Wasserman 1974) was carried out to compare the mean AUCs for each route; i.a. is significantly different ($P < 0·01$) from the three other routes, p.o. is significantly different ($P < 0·05$) from i.v. and h.p.v. but there is no significant difference between i.v. and h.p.v.

Also shown in Table 1 are the AUCs between zero and 120 min for the conjugates. The AUC for a terminal metabolite (AUC_m) is independent of route of administration providing absorption is complete. It is governed by dose administered (D), fraction of the dose metabolized by that pathway (f_m) and the clearance of the metabolite (CL_m) (Pang & Gillette 1978).

$$AUC_m = \frac{f_m . D}{CL_m} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (10)$$

An F test shows there is no statistical difference between AUC_m for the 4 routes. Therefore the same phenol dose entered the body irrespective of route of administration. Reductions in phenol AUC are not due to incomplete absorption from the intestinal tract or exhalation of intact compound by the lung.

The results of this investigation show that phenol undergoes a very large first pass effect when administered orally. Only 3% of the dose appears as the parent compound in the systemic circulation. It would appear that the intestine, liver and lung contribute to this presystemic biotransformation. Application of equations (7), (8) and (9) allow the factoring out of the contribution of each tissue. The major contribution is due to the intestinal enzymes ($f_G = 0·08$) but a pronounced pulmonary effect is also apparent ($f_L = 0·38$). In contrast the role of the hepatic enzymes is minimal ($f_H = 0·94$). This latter observation supports the claim by Powell et al (1974) that the detoxication function of the liver has been over-emphasized in the case of phenolic compounds. Certainly the anatomical position of the intestinal and pulmonary conjugative enzymes is ideal to protect the body against accidently ingested or inhaled phenolic compounds. The observations reported here would suggest that these two extrahepatic tissues are suitably equipped for this role.

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