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CONJUGATION OF PHENOL BY RAT LUNG

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Abstract—The metabolism of [U-^{14}C]phenol was studied in whole rat lung preparations and it was shown that [U-^{14}C]phenol was extensively metabolized. The fate of [U-^{14}C]phenol in isolated perfused rat lung preparations was the same when phenol was administered either intratracheally or systemically. [U-^{14}C]Phenyl sulphate and [U-^{14}C]phenyl glucuronide were the major metabolites thus demonstrating the presence of appropriate sulphotransferase and glucuronyl transferase activity in the whole lung. [U-^{14}C]Phenol was not metabolized by rat pulmonary macrophages.

The principal products of phenol metabolism in mammals, including man, are phenyl sulphate and phenyl glucuronide [1]. Orally administered [^{14}C]phenol was excreted in the urine of rabbits largely as these conjugates together with small amounts of quinol and catechol. More recently, in a comparative study [2] it was shown that the major metabolic products in the majority of species studied were phenyl glucuronide and phenyl sulphate, with small amounts of quinol glucuronide and quinol sulphate being formed in some cases.

The ability of organs, other than the liver, to metabolize phenol has been demonstrated in a number of studies. It was shown [3] that hepatectomized rats receiving [^{14}C]phenol intravenously excreted conjugated products in the urine. Moreover, phenol introduced into the gut lumen was conjugated in the gut wall before entering the blood. Isolated perfused small intestines of rats also produced conjugated metabolites. Conjugation of phenol with sulphate and glucuronic acid has also been demonstrated using isolated intestinal cells [4]. Collectively, these data suggest that phenolic substances entering the body via the gastro-intestinal tract are metabolized and detoxicated by the cells of the tract.

It is now recognized that just as the gut represents the first line of defence with respect to orally administered substances, so the lungs may act in a defensive capacity towards compounds entering the body via the respiratory tract. In this paper we investigate the metabolic capabilities of the rat lung with respect to phenol.

MATERIALS AND METHODS

Chemicals

[U-^{14}C]Phenol (368 μCi/mg) was purchased from the Radiochemical Centre, Amersham, Bucks. All other chemicals were obtained from BDH, Poole, Dorset.

Animals

Mature male MRC hooded rats (250–320 g body wt) were used throughout.

Preparation of whole lung incubates

In some experiments designed to investigate the metabolism of [U-^{14}C]phenol, whole lung incubates were used. Rats were killed by CO_2 asphyxiation. A cannula was inserted into the trachea, the thoracic cavity was opened and the trachea–lung preparation was removed. Each preparation was washed briefly in warm (37°) isotonic (0.9% w/v) saline. The lungs were slightly inflated with compressed air via the tracheal cannula and an aqueous solution (200–300 μl) of [U-^{14}C]phenol (5–10 μCi of ^{14}C) was introduced into the lungs via the trachea. The trachea was sutured with thread and the whole preparation was totally immersed in isotonic saline (25 ml) at 37° for 3 hr. The trachea–lung preparation was blotted to remove excess moisture and the trachea and bronchi were removed. The pulmonary tissue was then cut into small pieces with scissors and placed in 5 ml of isotonic saline. The tissue was minced using a Polytron blender (Northern Media Supplies, Hull) and then homogenized using a Potter–Elvehjem homogenizer with a Teflon pestle. After centrifuging (300 *g* for 10 min) the supernatant was analysed by descending paper chromatography in solvents A and B. The medium in which the trachea–lung preparation was immersed was freeze-dried; the residue was dissolved in water (2–5 ml) and the solution was subjected to chromatography.

Experiments with isolated perfused rat lungs

Isolated rat lungs were perfused as previously described [5]. The perfusate (110–125 ml) was heparinized homologous whole blood obtained from rats (under ether anaesthesia) by cannulation of the abdominal aorta. In these experiments, phenol metabolism was investigated by introducing [U-^{14}C]phenol (20 μCi in 200–250 μl) either intratracheally or systemically via the main perfusion-reservoir. When the intratracheal route was used, [U-^{14}C]phenol was introduced admixed with pulmonary surfactant prepared from 6–8 rats [6]. Lungs were perfused for 2–3 hr. Samples (0.6 ml) of perfusate were withdrawn from the reservoir at suitable time intervals and assayed for total radioactivity. Cells and plasma were separated by centrifuging, the plasma was removed and the cell pellet was homogenized in isotonic saline. All the radioactivity was extracted in 50% ethanol. The extracts and the plasma samples were assayed for radioactivity both quantitatively and qualitatively by chromatography.

Experiments with alveolar macrophages

Pulmonary alveolar macrophages were obtained by pulmonary lavage with isotonic saline [7]. Macrophage incubations were carried out in flat tissue culture flasks (Falcon Plastics, Los Angeles, CA, U.S.A.) providing a surface area of 25 cm^2.

Macrophages were suspended in a total volume of 5 ml of isotonic saline containing pulmonary surfactant, prepared from rats, to give a final concentration of approx 1.05×10^6 cells/ml. This suspension was incubated with [U-^{14}C]phenol (approx 2 μCi) at 37°. After 5 hr a sample of the incubate was subjected to chromatography in solvent 'A'.

Chromatography

Samples (20–50 μl) of solutions were applied to Whatman No. 1 paper and chromatograms were developed by descending irrigation (approx 16 hr) using the following solvent systems: A, butan-1-ol–acetic acid–water (3:1:1, v/v); B, propan-2-ol–ammonia solution (sp.g. 0.88)–water (20:1:2, v/v).

Determination of radioactivity

Samples of whole blood, plasma and lung homogenate were oxidized using a sample oxidizer (Oxymat model IN4101, Intertechnique, Plaisir, France) and $^{14}CO_2$ was collected using the following recommended scintillant: 2,5-diphenyloxazole (PPO), 37.5 g; 1,4-di-2(methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP), 2.7 g; ethanolamine, 600 ml; methanol, 1800 ml; toluene, 1600 ml (PPO and dimethyl POPOP were both purchased from Fisons Scientific Apparatus Ltd, Loughborough). The radioactive content of each vial was then determined by liquid scintillation counting in either a Packard 3375 or Intertechnique 4000 liquid scintillation counter. Efficiency of counting of oxidized samples was determined by oxidizing samples of known radioactive content each time the machine was used.

Radioactivity was detected on paper chromatograms using a Packard 7201 radiochromatogram scanner, and the peak size on the print-out was quantitated [8]. Alternatively, the chromatograms were cut laterally into 1-cm strips and the radioactive content of each strip was measured by liquid scintillation counting in PCS scintillation cocktail (Amersham/Searle, Arlington Heights, IL, U.S.A.)

Identification of major phenol metabolites in the circulating perfusate

In preliminary experiments, [U-^{14}C]phenol (20 μCi) was introduced intratracheally into the isolated perfused rat lung system. At the end of the perfusion, plasma samples were obtained by centrifuging and aliquots (20–50 μl) were subjected to paper chromatography in Solvent B. Radioactivity was located by scanning which revealed the presence of two radioactive components corresponding in mobility to phenyl sulphate (R_f 0.68) and phenyl glucuronide (R_f 0.24), respectively [9].

The identities of phenyl sulphate and phenyl glucuronide were confirmed as follows: plasma (approx 500 μl) was applied, as a streak, to Whatman No. 1 paper which was subjected to chromatography in Solvent B. The two major areas of radioactivity were located by scanning, and radioactivity was eluted from those areas with water (5 ml in each case). Each solution was freeze-dried and the residue was dissolved in water (100 μl). A solution (50 μl) containing the suspected [U-^{14}C]phenyl glucuronide was mixed with a solution (50 μl) of β-glucuronidase (approx 1000 U, bacterial type 1, Sigma Chemical Co.) in 0.1 M Na_2HPO_4–NaH_2PO_4 buffer, pH 7.0 and incubated at 37° for 24 hr. Samples (50 μl) of the incubate were subjected to chromatography in Solvent A, and radioactive areas were located on chromatograms by scanning. A single radioactive spot was present identical in mobility to [U-^{14}]phenol. A similar incubation containing 1,4-saccharolactone (0.5 mM) also contained a single radioactive spot but this corresponded in mobility to the suspected [U-^{14}C]phenyl glucuronide.

A solution (100 μl) containing the suspected [U-^{14}C]phenyl sulphate was incubated with 4 N HCl (100 μl) for 1 hr at 100°. A sample (40 μl) of the incubate subjected to chromatography in Solvent A was shown to contain a single radioactive component corresponding in mobility to [U-^{14}C]phenol. Further evidence for the identification was provided when the solution (100 μl) containing the metabolite was incubated with arylsulphatase (*Helix pomatia*, Boehringer, W. Germany) in 0.1 M sodium acetate–acetic acid buffer, pH 6.6 for 2 hr at 37°. An aliquot (40 μl) of the incubate was subjected to chromatography in Solvent A and scanning revealed the presence of only one radioactive component corresponding in mobility to [U-^{14}C]phenol.

The identity of [U-^{14}C]phenyl sulphate was confirmed by reverse isotope dilution analysis. Authentic phenyl sulphate was prepared [10] and 100 mg was dissolved in a solution (1 ml) containing suspected [U-^{14}C]phenyl sulphate (approx 3×10^5 dpm) and the whole was heated (75–80°). After cooling at room temperature, the solution was allowed to crystallize at 4°. The crystals were separated by filtering and washed with diethyl ether. A sample (approx 1 mg) of the crystals was dissolved in water (100 μl) and 50 μl was assayed for total radioactivity. The remaining 50 μl was diluted with water to a volume of 2.5 ml and the extinction was measured at 256 nm for calculation of specific activity. The crystallization procedure was repeated a further 3 times, the specific activity being determined after each crystallization. The specific activity of the crystals was constant over successive recrystallizations.

RESULTS

Metabolism of phenol in whole-lung incubates

The ability of the rat lung to metabolize phenol was shown in experiments in which various amounts (27.2 μg–2.7 mg) of [U-^{14}C]phenol (5–10 μCi) were introduced into whole-lung preparations via the trachea and incubated for 3 hr at 37°. After this time, the amount of radioactivity (expressed as a percentage of the total radioactivity added to the lungs) which was transferred to the surrounding medium ranged from 34–70% with a mean value of approx 53%. Chromatographic analysis of supernatants of the lung homogenates showed that at all doses the majority of the phenol was metabolized. However,

Table 1. Distribution of radioactivity between [U-^{14}C]phenol and its metabolites*

Amount of phenol administered	% Radioactivity present as			
	[U-^{14}C]phenol	[U-^{14}C]phenyl sulphate	[U-^{14}C]phenyl glucuronide	[U-^{14}C]quinol sulphate
27.2 μg (n = 6)†	16.8 ± 2.5	40.9 ± 8.0	37.8 ± 7.1	4.5 ± 2.3
272 μg (n = 2)	26.9	31.4	32.8	7.6
2.72 mg (n = 2)	34.9	31.4	28.6	5.2

* [U-^{14}C]Phenol was administered intratracheally at three different dose levels to whole rat lung preparations. The values given are expressed as percentage of the total radioactivity in the lung preparation. For experimental details see the text.

† Values given are means ± S.E.M.

the amounts of unchanged phenol were dependent on the administered dose (Table 1).

At the lowest dose (27.2 μg), most of the radioactivity (73–90%) was present as [U-^{14}C]phenyl sulphate and [U-^{14}C]phenyl glucuronide, although the relative proportions of these conjugates varied from one experiment to another. In some experiments, a further metabolite was present, the chromatographic behaviour of which was consistent with its identification as [U-^{14}C]quinol sulphate [9]. It represented a relatively minor proportion (4.3–13.6%) of the recovered radioactivity. The relative proportions of unchanged phenol and its metabolites were not significantly altered when lungs obtained from animals starved for either 24 or 48 hr were used in the experiments. In experiments with the higher (272 μg and 2.72 mg) doses of phenol the major metabolites were again [U-^{14}C]phenyl sulphate and [U-^{14}C]phenyl glucuronide with [U-^{14}C]quinol present as a minor metabolite.

Metabolism of phenol in the isolated perfused rat lung

In preliminary experiments, 20 μCi of [U-^{14}C]phenol in aqueous solution (200–250 μl) was introduced into the lungs via the trachea. This resulted in pulmonary damage, evident from the appearance of the lungs, which had a characteristic dark blotchy aspect, and from the ejection of fluid, containing blood, from the trachea. The flow-rate of blood through the lungs, which prior to the administration of the phenol was approx 30 ml/min, fell rapidly and ceased after 30–40 min. This situation was considerably improved by introducing the [U-^{14}C]phenol mixed with rat pulmonary surfactant. Using this protocol no visible signs of damage to the lungs were evident nor was fluid ejected from the trachea. The duration of these perfusion experiments was significantly increased, a satisfactory blood flow being maintained for 85–210 min.

When [U-^{14}C]phenol was administered intratracheally mixed with surfactant, radioactivity appeared rapidly in the perfusate, as shown in Fig. 1(a). The level of radioactivity in the blood reached a maximum after 25 min. In the plasma, the level of isotope reached a maximum after 10 min. At 10 min all the isotope was present in the plasma. Subsequently, there was a redistribution of radioactivity between the cells and the plasma, with the latter containing between 35 and 42% of the total radioactivity in the blood. At the end of the experiment, approx 92% of the radioactivity had been transferred into the perfusate. Of the remaining radioactivity, approx 6% was recovered in the lung lavage and the remainder (approx 3%) was associated with the lung tissue.

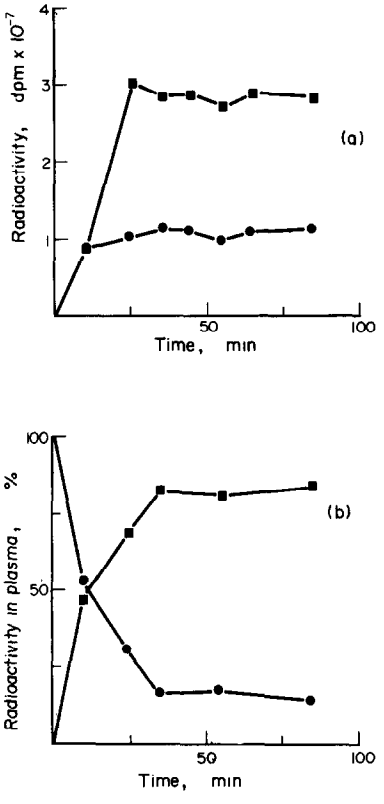

Fig. 1. (a) Time course of appearance of radioactivity in the perfusate in an experiment in which [U-^{14}C]phenol was administered intratracheally to an isolated perfused rat lung preparation. (■), whole blood, (●), plasma. (b) Time course of appearance of radioactivity in the plasma in an experiment in which [U-^{14}C]phenol was administered intratracheally to an isolated perfused rat lung preparation. The results are expressed as percentage of radioactivity present in the plasma as (●), [U-^{14}C]phenol, (■), [U-^{14}C] conjugates.

Chromatographic analysis of plasma samples at the end of the experiment showed that only 15% of the radioactivity was present as unchanged [U-^{14}C]phenol. [U-^{14}C]Phenyl sulphate and [U-^{14}C]phenyl glucuronide accounted for 72% and 13% of the radioactivity, respectively. Analysis of blood cell homogenates showed that the distribution of radioactivity between phenol and its metabolites was almost identical with that in the plasma. The time course for the disappearance of free phenol and the appearance of conjugated products in the plasma is shown in Fig. 1(b). After 10 min perfusion the amounts of phenol and phenol conjugated material were approximately equal and the progressive disappearance of phenol was accompanied by a concomitant increase in conjugated products.

When [U-^{14}C]phenol was added directly to the perfusate, the results obtained were substantially the same as those obtained when [U-^{14}C]phenol was introduced intratracheally. After 180 min perfusion, approx 10% of the radioactivity in the plasma was present as [U-^{14}C]phenol, approx 70% as [U-^{14}C]phenyl sulphate and approx 20% as [U-^{14}C] phenyl glucuronide.

Metabolism of phenol by rat alveolar macrophages

When alveolar macrophages were incubated with [U-^{14}C]phenol for 5 hr, metabolites of phenol could not be detected and all the radioactivity was present as unchanged [U-^{14}C]phenol.

DISCUSSION

The contribution of the lungs to the conjugation of foreign compounds *in vivo* is not known. Indeed, attempts to show the existence of some conjugating enzymes such as the glucuronyltransferases and the sulphotransferases have produced conflicting results. More recently, a clearer picture is emerging and it has been established that glucuronyltransferase activity is present in the lung [11–15]. The position regarding sulphotransferase activity is less clear but it seems likely that differences in the *in vitro* assay systems used to detect sulphoconjugation might be responsible for the conflicting results. Sulphotransferase activity towards *p*-nitrophenol could not be detected in subcellular fractions of rabbit lung [16], whereas Hook and Bend [17] showed sulphoconjugation of this substrate using PAPS itself rather than a PAPS generating system. In addition, the appearance of sulphoconjugates as minor metabolites of a range of substrates in experiments using subcellular fractions [18], cell culture [19] and isolated perfused lungs [20] demonstrates that the lungs might be a potential site of sulphoconjugation *in vivo*.

The present study has shown that in two different whole-lung preparations the sulphate and glucuronide conjugates are the major metabolites of [U-^{14}C]phenol. The metabolism of phenol by the lungs has already been suggested [21] although no indication was given as to the nature of the metabolic products. By contrast, phenol was not metabolized when microsomal preparations from rat, rabbit and hamster lung were incubated with [U-^{14}C]phenol [22]. However, these results can be explained if the sulphotransferase for phenol is located predominantly in the cytosol. On the other hand, the lack of phenylglucuronide formation by the microsomes is more difficult to explain because the present studies using rat lung consistently demonstrated the pulmonary formation of [U-^{14}C]phenylglucuronide. In rabbit lung, the results of Harper *et al.* [22] agree with those of Aitio [14] who showed that when 4-methylumbelliferone was used as substrate the glucuronic acid conjugate could not be detected. In preliminary work with [U-^{14}C]phenol and whole rabbit lung preparations, phenyl glucuronide was not detected and phenol was metabolized primarily to phenylsulphate and quinol sulphate (Powell, unpublished work). These findings suggest a basic difference between rat and rabbit lung in the conjugation of phenol. Recently it has been demonstrated [23] that major differences exist between man and rat in the metabolism of benzo(a)pyrene and 1-naphthol by short-term cultures of lung tissue. In human lung cultures these substrates were metabolized principally to sulphate conjugates with little or no production of glucuronides which were the major products in cultures of rat lung.

Collectively, the results reported here emphasize the importance of using whole-organ preparations when studying the metabolic capabilities of those organs. However, just as investigations using subcellular fractions have their limitations in terms of evaluating the significance of enzyme systems so also do whole organ studies. In particular, the contribution of pulmonary glucuronyltransferase and sulphotransferase activities towards the metabolism of phenol and related compounds in the whole animal will depend on the effectiveness of the lung in competing with other organs for the substrates. In this respect, it is unlikely that the lungs make a significant contribution to the metabolism of orally administered phenol because of the extensive first-pass metabolism in the gut wall [3]. However, the conjugation capacity of the lungs is well placed for the conjugation of inhaled phenolics as well as those transported to the lungs via the pulmonary artery.

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