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PHENOL CONJUGATION BY LUNG *IN VIVO*

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Considerable attention has been focused in recent years on the role of extrahepatic biotransformation in the disposition and toxicity of drugs and other xenobiotics. Many studies have been carried out which compare the *in vitro* activity of the liver with that of other tissues, in particular the intestine, kidney and lung (for example - 1,2,3). Due to the methods involved in the enzyme isolation, semi-purification and incubation under optimal conditions, these studies provide only rough guides to the potential activity of the tissues *in vivo*. The importance of the intestine and kidney as sites for conjugation of xenobiotics has been confirmed under *in vivo* conditions (for example - 4,5). We have studied the ability of the lung to carry out conjugation reactions using a direct *in vivo* procedure.

The model compound selected for investigation was (U - ^{14}C)-phenol. In the rat this compound is essentially completely biotransformed to two conjugate species - phenyl glucuronide and phenyl sulphate (6). Urinary and biliary excretion studies have shown that within two hours of phenol administration these two conjugates account for greater than ninety percent of the dose (7). *In vitro* studies suggest that lung can form glucuronides readily however using isolated perfused lung preparations only minimal glucuronyl transferase activity has been observed (8).

We have taken advantage of the anatomical position of the lung to evaluate the importance of pulmonary glucuronyl transferase and sulphotransferase *in vivo*. Sprague-Dawley rats (male, mean weight 260g, anaesthetised with urethane) were surgically prepared with cannulae in the left jugular vein and left carotid artery. (U-^{14}C)-Phenol (1.5 mg/kg; 10 μCi/kg) was administered intravascularly via one of these two cannulae. When given intravenously (IV) the compound enters the right atrium in a comparatively

undiluted form and it must cross the lung prior to reaching the general arterial system for distribution throughout the body. In contrast when given intra-arterially (IA) the compound is immediately available for tissue distribution. By comparison of the phenol blood concentration-time profiles following administration by both routes, it is possible to distinguish between systemic biotransformation (which presumably occurs mainly in the liver) and biotransformation which occurs during a single pass across the

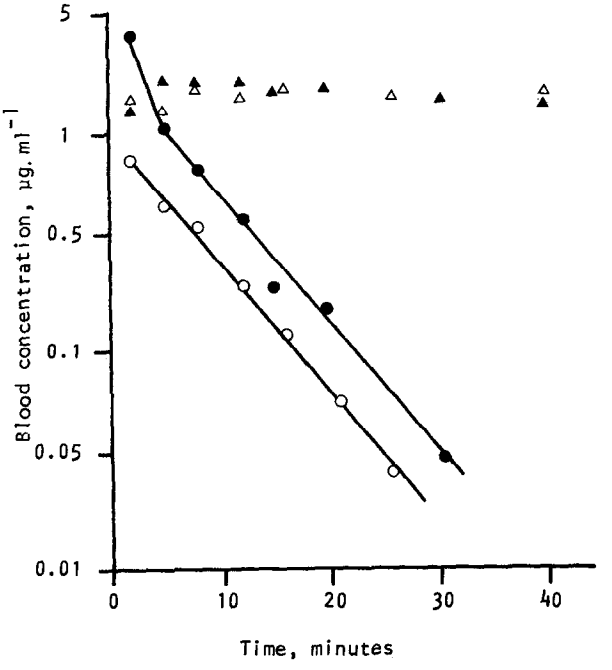

FIGURE 1. Blood concentration-time profiles for intact phenol and phenol metabolites in a typical set of animals receiving phenol by intra-arterial (●,▲) or intravenous (○,△) administration. Blood samples (100 µl, collected at -5, 2, 5, 8, 12, 16, 22, 30, 60, 90 and 120 minutes) were diluted with heparinised saline, extracted with Scintillation Fluid 1 [5 mls, Toluene containing 0.5% 2,5-diphenyloxazole and 0.05% 1,4-di-2-(5-phenyl-oxazolyl)-benzene] and 4 mls of the organic layer counted. The remainder of the organic phase was discarded and the aqueous layer (150 µl aliquot) removed, oxidised, bleached 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 was specific for phenol with an efficiency of 94.5%. The radioactivity in each blood sample was quantified as parent drug (Scintillation Fluid 1) and conjugates (Scintillation Fluid 2) with appropriate correction factors.

lung. Similar pharmacokinetic techniques have been used to assess presystemic biotransformation by intestinal mucosa and liver after oral administration of drugs (9, 10).

A typical set of phenol blood concentration-time profiles is shown in the Figure 1. Similar results were obtained for each of the IA (n = 7) and IV (n = 7) rats. Calculation of the area under the blood concentration-time curve between zero and infinity gave values of 6.13 ± 1.83 and 2.35 ± 0.94 $\mu g.ml^{-1}$. minute. kg (significantly different $p < 0.01$ by t test) for intra-arterial and intravenous administration respectively. Thus approximately 60 percent of the phenol dose is extracted by the lung on the first pass following IV administration.

The data is presented as a semi-logarithmic plot to stress the rapidity of phenol disposition. After IA administration an initial rapid distribution phase is observed. Presumably this phase is not apparent after IV due to a dampening effect of the pulmonary first pass. Terminal half-lives for phenol disposition from the blood are not statistically different (by t test) for IA (5.0 ± 0.9 minutes) and IV (5.2 ± 0.7 minutes) administration. Since these latter phases extend over a time period which exceeds 5 half-lives it is felt that this is the true terminal phase which reflects the rate of biotransformation. Consistent with this is the rapid attainment of high metabolite blood concentrations (see Figure 1) and the complete excretion of phenol conjugates from the body within 2 hours (7).

The differences in the area under the blood concentration-time curves for the two routes are not due to differences in dose of phenol entering the body. Since the same phenol dose is administered, the extent of metabolism is irrespective of route of administration. Hence as shown in Figure 1, there is no difference between the total phenol conjugate concentrations achieved by either route. The area under the metabolite blood concentration-time curves between zero and 120 minutes are 39.74 ± 4.64 and 49.48 ± 19.12 µg phenol equivalents. ml^{-1}. minute. kg (not significantly different by t test) for IA and IV administration respectively. Similarly the differences in blood concentration of phenol are not due to exhalation of intact compound.

Further studies have been carried out administering phenol at different doses. At a lower dose (0.4 mg/kg) the areas under the phenol blood concentration-time curve between zero and infinity are 1.32 ± 0.31 and 0.65 ± 0.14 $\mu g.ml^{-1}$. minute. kg (significantly different $p < 0.01$ by t test) for IA and IV respectively and at a higher dose (4.5 mg/kg) 14.11 ± 4.71 and 9.76 ± 0.75 (not significantly different) for IA and

IV respectively. These results indicate that the pulmonary first pass is linear in the dose range 0.4 - 1.5 mg/kg but becomes saturated at higher doses.

The lungs occupy a unique position in the body as a protective organ. They could act very efficiently as a site of systemic biotransformation since 100 percent of cardiac output crosses this organ. In contrast, cardiac output to the liver is only 25 percent. In terms of presystemic biotransformation, the lung is the primary site by which atmospheric xenobiotics enter the body. Also the lung is the third in a series of 3 potential biotransformation sites (the others being the intestinal mucosa and liver) which orally ingested xenobiotics must cross prior to entering the general circulation.

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