Comparison of Pulmonary Accumulation of Pyrilamine and Pyrilamine N-oxide

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Abstract—Our previous studies have indicated that a phenothiazine drug, chlorpromazine, and a tricyclic antidepressant, imipramine, are metabolized by the isolated perfused rat lungs via N-oxidation from whence their N-oxides are released into the circulation. This work was undertaken to compare the pulmonary accumulation of another pneumophilic tertiary amine drug, pyrilamine, with that of its N-oxide. Approximately 10-fold greater accumulation of pyrilamine than that of its N-oxide was observed in the mouse lung after a single pass perfusion with 40 nM of the drug for a 3 min period. The largest difference between accumulation of pyrilamine and its N-oxide was noted in the lung among the various tissue slices tested, suggesting the tissue specificity of affinity.

Many tertiary amine drugs are metabolized by N-oxidation in mammals (1). Ziegler's group (2) has presented evidence that the formation of many N-oxides may be catalyzed by the microsomal flavin-containing monoxygenase enzyme (E.C. 1.14.14.8). The metabolites formed by the action of this enzyme on these substrates are often salt-like compounds which are considerably more polar than their parent tertiary amines. Marked species differences in the rate of formation of N-oxides have been observed which may, at least partly, relate to differences in the intrinsic activity of the flavin-containing monoxygenase enzyme. Much interest has been focused on various nonrespiratory functions of the lung and the role that these may have in the pulmonary toxicity produced by drugs, herbicides and environmental pollutants (3–5). Representative groups of basic, lipophilic drugs which are avidly accumulated by the lung tissue include antihistamines, antidepressants, β-adrenergic antagonists and analgesics. We have reported previously that while a phenothiazine drug, chlorpromazine, and a tricyclic antidepressant, imipramine, were not metabolized by isolated perfused rabbit lungs, they were metabolized by perfused rat lungs via N-oxidation from whence their N-oxides were released into the circulation because of low affinity for the lung tissue (6–8). In the present communication, a direct comparison between affinities for the lung tissue of an antihistaminic, pyrilamine, and its N-oxide has been made using the synthetic, radiolabeled metabolite and the isolated, ventilated, perfused mouse lung preparations.

3H-Pyrilamine (pyridinyl-5-labeled, 26 mCi/mmol) was purchased from Radiochemical Centre, Amersham. 3H-Pyrilamine was diluted with the desired amounts of unlabeled pyrilamine maleate (Sigma Chemical Co.) to achieve designated concentrations of the drug. Labeled or unlabeled pyrilamine N-oxide was synthesized by the reaction of pyrilamine with H2O2 in 2.5% ammonia ethanol solution. The purity of both labeled drugs was better than 97% as assessed by thin layer chromatography. Male ICR mice weighing about 35 g were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and were given sodium heparin (2000 U/kg, i.v.) to prevent blood coagulation in the lung. The lungs were surgically removed and prepared for perfusion as described previously (6–8). The perfusion medium consisted of Krebs Ringer bicarbonate buffer to which 4.5% bovine serum albumin (Fraction V, Seikagaku Kogyo Co.)
and 5 mM glucose were added. The pH of the medium was adjusted to 7.4 with 1 N NaOH after saturation with a 95:5 mixture of O₂:CO₂. The lungs were ventilated and perfused via the pulmonary artery in the chamber warmed at 37°C. After the lungs were equilibrated with drug-free medium at the constant flow rate of 1.66 ml/min, the perfusion was switched to the medium containing 40 μM of ³H-pyrilamine or ³H-pyrilamine N-oxide. The effluent was collected at 15-sec intervals with a fraction collector for a 3 min time period.

For slice experiments, shortly after the brain, lung, liver and kidneys were removed from the animals, the tissue slices were prepared by cutting free hand with a razor blade. Approximately 100 mg of tissue slices were incubated with 3 ml of Krebs Ringer bicarbonate buffer (pH 7.4) containing 10 μM of ³H-pyrilamine or ³H-pyrilamine N-oxide at 37°C for 60 min. At the end of incubation, the slices were removed, blotted on a filter paper and homogenized with 1 ml of distilled water. Radioactivity in the perfusate and tissue homogenates was determined by the addition of scintillation cocktail (4 ml) to samples (0.2 ml) in counting vials and counting in a liquid scintillation spectrometer. The estimations of the drug and its metabolites in tissue, perfusate and incubation medium were determined by essentially the same analytical procedures as described for chlorpromazine metabolites (7).

The rate of pulmonary accumulation of pyrilamine and pyrilamine N-oxide is shown in Fig. 1. The rate decreased exponentially with time for both drugs, indicating that the accumulation was approaching equilibrium. However, the rate of accumulation of pyrilamine N-oxide was rapidly reduced as compared to pyrilamine and reached nearly zero at around 1.5 min. The total amounts of drug accumulated, calculated by integrating the rates from zero to 3 min, are 510.3±27.3 nmol/g for pyrilamine and 57.1±2.8 nmol/g for pyrilamine N-oxide. No metabolites were detected in the effluent and lung tissue after single pass perfusion with both drugs. In order to evaluate the tissue specificity of affinity, the slices of the lung, kidneys, liver and brain were incubated with pyrilamine or its N-oxide for 60 min (Fig. 2). Accumulation of pyrilamine was in the rank order lung>kidney=liver=brain, with the tissue/medium ratios of 30 for lung and nearly 10 for other tissues. In contrast, accumulation of pyrilamine N-oxide was more or less constant for each tissue, with the tissue/medium ratio remaining roughly 2. The accumulation of pyrilamine in lung slices has been suggested to result from the uptake partly due to the active transport process and subsequent binding to the tissue components (9). Antihistamines such as chlorcyclizine and diphenhydramine are known to be highly concentrated in the pulmonary tissue (3). However, no information is available on the pharmacokinetics of pyrilamine (10). Very recently we have found that isolated perfused mouse and rat lungs metabolize pyrilamine via N-oxidation and release its metabolite N-oxide into the recirculating perfusate as in the case with chlorpromazine and imipramine (Y. Ohmiya et al., unpublished observations). The striking differences between tertiary amines and their corresponding N-oxides with
regard to distribution and excretion have been well documented (1). These differences are caused by physicochemical differences between these types of compound, e.g., the marked increased polarity of N-oxides as compared to their parent tertiary amines as speculated by loss of affinity for lung tissue (7, 8). In the present study with lung perfusion and slices, direct evidence for large differences between affinities of a basic tertiary amine drug and its N-oxide for pulmonary tissue has been provided.

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**Fig. 2.** Accumulation of pyrilamine and pyrilamine N-oxide by mouse tissue slices. Approximately 100 mg of tissue slices were incubated with 3 ml of Krebs Ringer bicarbonate buffer (pH 7.4) containing 10 μM of pyrilamine (P) or pyrilamine N-oxide (P-NO) for 60 min. Results are means±S.E.M. for four incubations, expressed as nmol/100 mg tissue.

**References**


