Pulmonary inflammation alters the lung disposition of lipophilic amine indicators

SAID H. AUDI,1,2 DAVID L. ROERIG,4,5 SUSAN B. AHLF,5 WIN LIN,2 AND CHRISTOPHER A. DAWSON1,2,3

Biomedical Engineering Department, Marquette University, Milwaukee 53201-1881; Departments of Pulmonary Medicine and Critical Care, Physiology, and Anesthesiology and Pharmacology/Toxicology, Medical College of Wisconsin, Milwaukee 53226; and Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin 53295

Audi, Said H., David L. Roerig, Susan B. Ahlf, Win Lin, and Christopher A. Dawson. Pulmonary inflammation alters the lung disposition of lipophilic amine indicators. J. Appl. Physiol. 87(5): 1831–1842, 1999.—Many lipophilic amine compounds are rapidly extracted from the blood on passage through the pulmonary circulation. The extent of their extraction in normal lungs depends on their physical-chemical properties, which affect their degree of ionization, lipophilicity, and propensity for interacting with blood and tissue constituents. The hypothesis of the present study was that changes in the tissue composition that occur during pulmonary inflammation would have a differential effect on the pulmonary extraction of lipophilic amines having different properties. If so, measurement of the extraction patterns for a group of lipophilic amines, having different physical-chemical properties, might provide a means for detecting and identifying lung tissue abnormalities. To evaluate this hypothesis, we measured the pulmonary extraction patterns for four lipophilic amines, [14C]diazepam, [3H]alfentanil, [14C]lidocaine, and [3H]codeine, along with two hydrophilic compounds, [3H]OH and [14C]phenylethylamine, after the bolus injection of these indicators into the pulmonary artery of isolated lungs from normal rabbits and from rabbits with pulmonary inflammation induced by an intravenous injection of complete Freund’s adjuvant. The pulmonary extraction patterns, parameterized using a previously developed mathematical model, were, in fact, differentially altered by the inflammatory response. For example, the tissue sequestration rate, kse (mL/s), per unit 3H OH accessible extravascular lung water volume significantly increased for diazepam and lidocaine, but not for codeine and alfentanil. The results are consistent with the above hypothesis and suggest the potential for using lipophilic amines as indicators for detection and quantification of changes in lung tissue composition associated with lung injury and disease.

change in lung tissue composition can be detected by a change in the extraction patterns of compounds having different physical-chemical properties. This implies their potential use for detecting and quantifying disease-related changes in lung tissue composition (1, 10, 12, 27, 36, 37, 39, 41, 43–47). The specific objective of the present study was to determine the impact of a pulmonary inflammatory response on the pulmonary extraction of four lipophilic amine test indicators: [14C]diazepam, [3H]alfentanil, [14C]lidocaine, and [3H]codeine. These compounds, which have a range of physical-chemical properties as reflected by differences in pKa, lipophilicity, and affinity to plasma proteins (14, 29, 44, 45) given in Table 1, were chosen because they are sufficiently and differentially extracted during a single pass through the pulmonary circulation of normal lungs for MID quantification (1, 3, 12, 45, 47). The studies were carried out on lungs isolated from normal rabbits and from rabbits treated by intravenous injection of complete Freund’s adjuvant (CFA), which produces a well-characterized inflammatory response in the rabbit lung dominated by a significant increase in macrophages, histiocytes, and granulomatous (7, 13, 38, 47). On the basis of these and other characteristics, this animal model has been considered useful for elucidating mechanisms that may be relevant to human interstitial lung diseases (13). The MID data were parameterized using a previously developed kinetic model and methodology (1, 47). The results indicate that the pulmonary extraction patterns for the chosen group of lipophilic amines and the resulting parameters were differentially altered by the inflammatory response, supporting the hypothesis that the extraction patterns of lipophilic amines may provide a useful signature of lung tissue composition (1, 12, 27, 37, 41, 43, 47).

Glossary

A-aDO2 Difference between alveolar and arterial PO2
C0(t) Venous effluent concentration of test indicator at time t
CFA Complete Freund’s adjuvant
Cf(t) Venous effluent concentration of 3H OH at time t after bolus injection
Cn(t) Venous effluent concentration of vascular reference indicator FITC-Dex at time t after bolus injection
C′t(t) Tubing outflow curve
F Flow
FITC-Dex Fluorescein isothiocyanate-labeled 40,000-mol wt dextran

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org
is arterial PO2.

EXPERIMENTAL METHODS

The studies were performed on 25 New Zealand White rabbits of either gender (2.70 ± 0.29 (SD) kg). Sixteen of the rabbits (2.56 ± 0.22 kg) were each given a 1-ml ear vein injection of CFA (8.5 ml Bayol F, 1.5 ml Aalarcel, and 5 mg Mycobacterium butyricum) (7, 13, 38, 47). The remaining nine rabbits (2.79 ± 0.27 kg) served as controls. Vehicle control experiments were not performed, because the objective was to produce changes in lung tissue composition and not to study the CFA-induced inflammatory response per se. At 3–98 days after the CFA or no treatment, the rabbits were anesthetized and the MID studies described below were carried out on the lungs. Just before the administration of anesthesia in 17 of the 25 rabbits studied, a 1-ml blood sample was obtained from an ear artery for arterial blood gas analysis and for the estimation of the difference between the alveolar and arterial PO2: A-aDO2 analysis and for the estimation of the difference between the inspired PO2 and PaCO2.

At 3–98 days after the CFA or no treatment, the rabbits were anesthetized and the MID studies described below were carried out on the lungs. Just before the administration of anesthesia in 17 of the 25 rabbits studied, a 1-ml blood sample was obtained from an ear artery for arterial blood gas analysis and for the estimation of the difference between the alveolar and arterial PO2: A-aDO2 analysis and for the estimation of the difference between the inspired PO2 and PaCO2.

Bolus Composition

The 1.0-ml bolus of the perfusate solution contained 2.5 mg of fluorescein isothiocyanate-labeled 40,000-mol wt dextran (FITC-Dex) and 0.5 µCi of 3H or 0.1 µCi of 14C of one or more of [14C]diazepam, [3H]alfentanil, [14C]lidocaine, [3H]codeine, [3H]codeine, [3H]OH, or [14C]phenylethylamine (PEA). The latter two hydrophilic indicators were included as test indicators to trace the lung disposition of the bolus through the pulmonary capillary network. The perfusate was equilibrated with the respiratory gas mixture, which maintained the pH at 7.39 ± 0.06 (SD) at 37°C. Before each of the bolus injections described below, the ventilator was stopped at end expiration for the duration of the sampling period.

To produce a bolus injection, a solenoid-operated injection loop (1, 3, 47) was situated in the inflow tubing so that a 1.0-ml bolus could be introduced into the inflow stream without changing the flow or pressure. Just before injection, the venous outflow was directed into the sample tubes of a modified (1, 3, 47) Gilson Escargot fraction collector. One hundred 2-ml samples were collected with a sampling interval of 0.6 s.

Table 1. Descriptors of properties of lipophilic amine compounds thought to be important in determining their lung disposition

<table>
<thead>
<tr>
<th>Test Indicator</th>
<th>pKs</th>
<th>Octanol/Water Partition Coeff</th>
<th>Kp</th>
<th>Mol Wt, g/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>3.4</td>
<td>261</td>
<td>2.80</td>
<td>285</td>
</tr>
<tr>
<td>Alfentanil</td>
<td>6.5</td>
<td>126</td>
<td>0.95</td>
<td>419</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>7.9</td>
<td>21</td>
<td>0.77</td>
<td>234</td>
</tr>
<tr>
<td>Codeine</td>
<td>8.2</td>
<td>2.2</td>
<td>0.04</td>
<td>299</td>
</tr>
</tbody>
</table>

Kp, BSA equilibrium dissociation constant per g BSA per 100 ml perfusate; mol wt, molecular weight. Data are from Refs. 3, 6, 12, 25, and 45.
tubes collected before the emergence of the indicators. These samples served as internal standards for the calculation of indicator concentrations. For lungs from CFA-treated and normal animals, the fractions of the injected FITC-Dex and \(^{3}H\)OH recovered in the collected samples, calculated on the basis of the internal standards, were 95.8 ± 3.9 and 98.8 ± 3.4\% (SD), respectively. The fractions of the injected lipophilic amines recovered in the collected samples from normal lungs were 99.2 ± 2.0\% (SD) for alfentanil, 94.4 ± 2.3\% for diazepam, 91.5 ± 3.8\% for lidocaine, and 88.8 ± 3.2\% for codeine. These fractions were generally lower in lungs from CFA-treated animals, as can be seen in Fig. 1, where the fractions of the injected amines remaining in the lung at the end of the 60-s sampling period are plotted vs. time after CFA treatment.

Caspase-3 Assay

After each experiment, the lungs were weighed and then lyophilized to determine the ratio of wet to dry weight. The lyophilized lungs were then ground to a powder with a glass mortar and pestle. The dried lung powder was used to assay for caspase-3 activity, as an index of lung inflammation (18), with use of a kit developed by Pharmingen (San Diego, CA) as follows. The dried lung powder (200 mg) was homogenized with 4.0 ml of the lysis buffer for 2 min with a Bio-Homogenizer (Biospec Products, Barthesville, OK) at its highest speed. Homogenates were kept at 4°C or frozen until assayed. The lysate buffer consisted of 10 mM Tris, 10 mM \(\text{NaH}_{2}\text{PO}_{4}/\text{Na}_{2}\text{HPO}_{4}\), 130 mM \(\text{NaCl}\), 10 mM sodium pyrophosphate, and 1% Triton X-100 at pH 7.5. The assay buffer was pH 7.5 PBS. For each assay, 100 \(\mu\)l of appropriately diluted homogenate were mixed with 900 \(\mu\)l of PBS and 10 \(\mu\l\) [10 (\(\mu\g\))] of the fluorogenic tetrapeptide caspase substrate \(\text{N}-\text{acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin}\) with and without 10 \(\mu\l\) (1 \(\mu\g\)) of the caspase-3 inhibitor \(\text{N}-\text{acetyl-Asp-Glu-Val-Asp-CHO}\). The reaction mixtures were incubated at 37°C for 1 h, then the fluorescence was measured on a spectrofluorometer (model 650-10S, Perkin-Elmer) with use of a 1 x 1 cm cuvette. Reaction mixtures without the substrate served as negative controls. All lung homogenates were assayed in duplicate, and the coefficient of variation was 9.4\%. Caspase-3 activity is expressed as the difference in fluorescence units (in mV) between reaction mixtures with and without the inhibitor.

Histological Studies

After perfusion, one lung from a normal rabbit and one lung from a rabbit 14 days after CFA treatment were fixed in 10% neutral Formalin, and 5-µm-thick sections were stained with standard hematoxylin-eosin.

EXPERIMENTAL RESULTS

Values of the three indexes of lung inflammation, namely, the in vivo A-\(\text{aDO}_{2}\), lung wet weight, and caspase-3 activity over the time course of the inflammatory response, are given in Fig. 2. By these measures, the injury phase of the inflammatory response peaked at \(\approx 1-2\) wk after CFA treatment, then the inflamed lungs entered a recovery phase characterized by the return of these indexes toward normal values. Nine CFA-treated rabbits were studied within the 1- to 2-wk period after CFA treatment. In what follows, the parameter values from this group will be averaged and referred to as being obtained from the “peak response.”

Figure 3 shows histological sections of normal lungs and lungs treated with CFA for 14 days. Histological sections of the CFA-treated lungs show diffuse interstitial infiltrate primarily composed of epithelioid histiocytes with fewer lymphocytes, plasma cells, and eosinophils and, rarely, neutrophils. Some of the histiocytes are multinucleated, forming giant cell granulomatous patterns. Alveolar spaces contain many macrophages and some inflammatory cells. There is no evidence of vasculitis. The lung wet weight and microscopic changes due to the inflammatory response observed in the present study are consistent with those reported in previous studies of this model of pulmonary inflammation (7, 13, 38, 47).

The CFA-treated lung wet-to-dry weight ratios [5.27 ± 0.33 (SD)] were equal to or lower than those for normal lungs (5.79 ± 0.1), which is consistent with lung wet weight changes due to changes in cellular composition in this model of pulmonary inflammation (7, 13, 38, 47) rather than to edema.

The pulmonary arterial-venous pressure difference was significantly (P < 0.001) higher in peak-response lungs (10.6 ± 1.9 cmH\(_{2}\)O) than in normal lungs (6.1 ± 0.7 cmH\(_{2}\)O) with the same perfusate flow of 3.33 ml/s. The pulmonary arterial-venous pressure difference returned toward the normal value during the recovery phase of the inflammatory response.

The in vivo arterial blood pH and \(\text{PCO}_{2}\) were 7.46 ± 0.05 and 31.4 ± 2.2 (SD) Torr, respectively, for the peak-response rabbits compared with 7.40 ± 0.09 and 26.3 ± 5.0 Torr, respectively, for the normal rabbits, but the differences were not statistically significant.

MID Results

Concentration vs. time data. Figure 4 exemplifies the venous effluent concentration vs. time curves for FITC-Dex, \(\text{[^{14}C]}\)diazepam, \(\text{[^{3}H]}\)alfentanil, \(\text{[^{14}C]}\)lidocaine, \(\text{[^{3}H]}\)codeine, \(\text{[^{3}H]}\)OH, and \(\text{[^{14}C]}\)PEA from normal and CFA-treated lungs at different stages of the inflammation.
The patterns of the test indicator curves relative to those for the vascular reference indicator and to each other changed over the time course of the inflammatory response. For example, with diazepam there was a substantial reduction in its recovery and a leftward shift in the peak of its outflow concentration curve relative to that for the FITC-Dex. Comparison of lidocaine and codeine concentration curves (Fig. 4B) reveals that CFA treatment had a greater effect on the extraction of lidocaine than of codeine. The differences can be appreciated by noting the reversal of the order of the relative magnitudes of their respective concentration curves.

During the injury phase of the inflammatory response, there was a progressive reduction in the peak and a prolongation in the $^3$HOH concentration curve relative to that from normal lungs, reflecting the increase in the lung water volume (Fig. 4C). The effects of the inflammatory response on the PEA curve were relatively small, although the tail of the PEA concentration vs. time outflow curve tended to be depressed during the injury phase. All these changes in the concentration curves had nearly disappeared by 98 days after CFA treatment.

Residue curves. Figure 4 shows that the FITC-Dex outflow concentration curve also changed over the time course of the inflammatory response. This is the result of increased vascular transit time heterogeneity in CFA-treated lungs expressed by the relative dispersion (RD$_v$) of vascular transit times (Table 2), where RD$_v$ is the square root of the second central moment (variance) of lung vascular transit times divided by the lung vascular mean transit time (see Eq. 4). Thus it is useful to separate the effects of CFA treatment common to reference and test indicators from those unique to the test indicators by transforming the venous effluent concentration data of the vascular [CR($t$)] and test [CD($t$)] indicators in Fig. 4 into the residue curves.

Fig. 2. Lung wet weight, alveolar-arterial $P_{O2}$ difference ($\lambda-aDO_2$), and caspase-3 activity per gram of lung dry weight vs. time after CFA treatment.

Fig. 3. Tissue sections from normal lung (A and B) and lung treated for 14 days with CFA (C and D). Tissue sections were stained with hematoxylin-eosin. Original magnification $\times 40$ for A and C and $\times 100$ for B and D.
shown in Fig. 5 by use of the following relationship

\[
\text{Res}(t) = F \int_0^t [C_R(t) - C_P(t)] \, dt
\]

(1)

where, for a given test indicator, \(\text{Res}(t)\) is interpreted as the fraction of the injected test indicator that is in the lung tissue at a given time after the bolus injection.

The differences in the CFA-induced changes in the extraction patterns of the four lipophilic amines described above with respect to Fig. 4 are emphasized by this transformation, such that one can more readily appreciate the CFA-induced changes in the pattern formed by the combination of the four lipophilic amines.

To produce a concise parameterization of these patterns for statistical analysis and potentially for use in pattern recognition algorithms, we used the following analysis developed previously (1, 47).

**DATA ANALYSIS**

FITC-Dex and \(^3\)HOH

The mean transit times (\(t\)) and the second (\(\sigma^2\)) and third (\(m^3\)) central moments of the outflow curves of FITC-Dex \([C_R(t)]\) and \(^3\)HOH \([C_F(t)]\) and \(C_T(t)\) were obtained by fitting each to a shifted random walk function, the functional form of which can be specified by its first three moments (1, 2, 47).

The vascular volume \((Q_V)\) and the perfused extravascular water volume \((Q_W)\) were estimated from

\[
Q_V = F \times (t_R - t_T)
\]

(2)

\[
Q_W = F \times (t_F - t_R)
\]

(3)

where \(F\) is flow and \(t_R, t_F,\) and \(t_T\) are the mean transit times of \(C_R(t), C_F(t),\) and \(C_T(t),\) respectively.

The vascular relative dispersion \((R_{DV})\) was estimated from the moments of \(C_R(t)\) and \(C_T(t)\) from

\[
R_{DV} = \sqrt{\frac{\sigma^2_R - \sigma^2_T}{t_R - t_T}}
\]

(4)

where \(\sigma^2_R\) and \(\sigma^2_T\) are the second central moments of \(C_R(t)\) and \(C_T(t),\) respectively.

Lipophilic Amine Compounds

For each of the four lipophilic amine test indicators studied, parameter estimation was carried out using a

---

**Table 2. MID parameter values for lungs from normal and peak-response rabbits**

<table>
<thead>
<tr>
<th>Condition</th>
<th>FITC-Dex</th>
<th>3HOH</th>
<th>[^{14}\text{C}]\text{PEA}</th>
<th>[^{14}\text{C}]\text{PEA}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Q_V, \text{ml})</td>
<td>(Q_W, \text{ml})</td>
<td>(PS, \text{ml/s})</td>
<td>(V_r, \text{ml})</td>
</tr>
<tr>
<td>Normal</td>
<td>9.07 ± 0.46</td>
<td>6.17 ± 0.23</td>
<td>10.98 ± 0.24</td>
<td>5.74 ± 0.32</td>
</tr>
<tr>
<td>Peak response</td>
<td>10.19 ± 0.37</td>
<td>22.69 ± 2.61</td>
<td>12.02 ± 1.84</td>
<td>10.40 ± 0.98</td>
</tr>
</tbody>
</table>

Values are means ± SE of 9 lungs, except for phenylethylamine (PEA) peak response, wherein only 7 lungs were studied. Peak response is group of rabbits treated with Freund’s complete adjuvant (CFA) studied within 1–2 wk after treatment. \(Q_V, R_{DV},\) and \(Q_W,\) vascular volume, vascular relative dispersion, and perfused extravascular water volume accessible to \(^3\)HOH, respectively; MID, multiple-indicator dilution; FITC-Dex, fluorescein isothiocyanate-labeled dextran; \(V_r,\) virtual volume, which includes flow-limited volume accessible to PEA \((Q_F)\) and effects of rapidly equilibrating associations of PEA within \(Q_F.\) Statistically significant difference between normal and peak response values was calculated using Student’s \(t\)-test: *\(P < 0.05; \dagger P < 0.001.\)
previously described model (1, 47). Briefly, each model capillary element is composed of a capillary volume \( Q_c \) and a tissue volume \( Q_{ti} \). The model assumes that equilibration between the free and protein-bound test indicator (lipophilic amine) in \( Q_c \) (1, 3, 12, 47) is rapid and that the free form of the test indicator is the species having diffusional access to \( Q_{ti} \). Within \( Q_{ti} \), multiple classes of amine-tissue associations are represented with different dissociation rate constants (1, 47), which, along with the amine associations within \( Q_c \), are represented by the following parameters: \( Q_R \) (ml), \( Q_S \) (ml), mean sojourn time (\( \bar{T} \), s), and sequestration rate (\( k_{seq} \), ml/s) (1, 47). Each of these parameters can involve several kinetic parameters that are not separately identifiable (1, 47). Physically, \( Q_R \) and \( Q_S \) represent the capacities of two classes of amine-tissue associations referred to as rapidly and slowly equilibrating classes, respectively. The rapidly (relative to the capillary mean transit time) equilibrating amine associations within \( Q_{ti} \) and \( Q_c \), which are not mathematically distinguishable from each other, are represented by \( Q_R \). The slowly equilibrating amine-tissue associations within \( Q_{ti} \) are quantified by \( Q_S \) and by \( \bar{T} \), which is the mean time the lipophilic amine molecules associated with the slowly equilibrating class of associations spend in the lung tissue before returning to the capillary (1, 47). A third class of amine-tissue associations with dissociation rate constants that are so small that there is virtually no return to the perfusate within the MID sampling period is described by \( k_{seq} \).

\[ [^{14}C] \text{PEA} \]

The model used to interpret the uptake of PEA by the pulmonary endothelial cells was developed previously (47). The model assumes that PEA has access to a flow-limited volume \( Q_F \), within which it can participate in rapidly equilibrating associations with the tissue, or it can be transported into the endothelial cells via a linear unidirectional transport mechanism having a permeability-surface area product (PS) (47). The model parameters are then PS (ml/s) and \( V_F \) (ml), a virtual volume, which includes \( Q_F \) and the effects of the rapidly equilibrating associations of PEA within \( Q_F \).

PEA is metabolized within the pulmonary endothelial cells to phenylethylacetic acid (PAA) (16, 47). Thus, as time progresses during bolus passage, a fraction of the \( ^{14}C \) injected as \( [^{14}C] \text{PEA} \) returns to the perfusate as \( [^{14}C] \text{PAA} \). Previously (47), we found that, after the bolus injection of \( [^{14}C] \text{PEA} \) into the pulmonary artery of isolated perfused normal or CFA-treated lungs at the flow used in the present study, \( [^{14}C] \text{PAA} \) was not detectable in the \( ^{14}C \) outflow curve until samples collected after the peak of the FITC-Dex concentration vs. time curve. Thus, as described previously (47), the kinetic model parameters descriptive of PEA-tissue associations, namely, PS and \( V_F \), were obtained by fitting the PEA model to the \( [^{14}C] \text{PEA} \) concentration curve, only up to the peak of the FITC-Dex concentration curve.

Numerical aspects of parameter estimation were carried out as previously described using \( ^3\text{HOH} \) concentration data to obtain the function \( h_{\xi}(t) \), which accounts for the effects of the capillary transit time distribution on the indicator extraction pattern (2, 47). Model fits to the data are exemplified in Fig. 4. The overall coefficients of variation for the model fits were on average 8.0 \( \pm \) 0.5, 5.9 \( \pm \) 0.3, 9.9 \( \pm \) 0.6, 10.1 \( \pm \) 0.4, and 3.0 \( \pm \) 0.4\% (SE) for diazepam, alfentanil, lidocaine, codeine, and PEA, respectively.

**MODEL RESULTS**

Normal vs. Peak Response

The calculated MID parameters for the four lipophilic amine test indicators studied and the other MID parameters estimated from normal and peak-response lungs are given in Tables 2 and 3.

The estimated values of \( Q_V \) and the PS for PEA from peak-response lungs were not different from those estimated from normal lungs. However, RDV, the extravascular water volume accessible to \( ^3\text{HOH} \) \( Q_W \), and the virtual volume accessible to PEA \( V_F \) were higher in peak-response than in normal lungs. The ratio of...
extravascular water volume accessible to $^{1}{}$H$_{2}$O ($Q_{W}$) to the gravimetrically measured lung water volume decreased from $0.79 \pm 0.02$ (SE) in normal lungs to $0.42 \pm 0.03$ in peak-response lungs. For the four lipophilic amine test indicators, the differential effects of CFA treatment on the extraction patterns are revealed in the parameter patterns shown in Table 3. $Q_{R}$, $Q_{S}$, and $k_{seq}$ are extensive parameters reflecting changes in the total number of sites of interactions between the lipophilic amine test indicators and the lung tissue (1, 47). This number might be affected by a change in the lung tissue mass, a change in the fraction of the lung tissue mass accessible to the test indicators via the perfused vascular volume, and/or a change in tissue composition, i.e., in the number of sites per unit mass. The PEA $P_{S}$ and $Q_{W}$ are indexes of the perfused vascular surface area and extravascular volume accessible via the perfuse, respectively (47). Thus the normalization of $Q_{R}$, $Q_{S}$, and $k_{seq}$ to $P_{S}$ or $Q_{W}$ can provide additional information as to whether parameter changes shown in Table 3 simply reflect changes in lung tissue mass, changes in the fraction of the mass that is being perfused, or changes in the lung tissue composition. Because the $P_{S}$ was hardly affected by the CFA treatment, normalization to $P_{S}$ is not reported. However, normalization to $Q_{W}$, which increased substantially during the inflammatory response, is provided in Table 4.

Changes Over the Time Course of the Inflammatory Response

In addition to the 1- to 2-wk studies, lungs were studied at different times after CFA treatment to confirm the inflammation-recovery cycle typically observed in the rabbit CFA model (7, 13, 38). It also provided a gradation in the response, allowing for correlations between measured indexes of inflammation and the model parameters.

Table 3. MID parameter values for lipophilic amines in lungs from normal and peak-response rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Diazepam</th>
<th>Alfentanil</th>
<th>Lidocaine</th>
<th>Codeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{R}, ml$</td>
<td>Normal: 12.04 ± 0.75, Peak response: 16.29 ± 0.83*</td>
<td>Normal: 2.89 ± 0.16, Peak response: 3.63 ± 0.08*</td>
<td>Normal: 5.29 ± 0.30, Peak response: 9.32 ± 0.31†</td>
<td>Normal: 6.14 ± 0.31, Peak response: 7.11 ± 0.32*</td>
</tr>
<tr>
<td>$Q_{S}, ml$</td>
<td>Normal: 9.07 ± 1.09, Peak response: 46.08 ± 4.92†</td>
<td>Normal: 10.42 ± 0.75, Peak response: 14.53 ± 1.09, 44.15 ± 3.01†</td>
<td>Normal: 5.48 ± 0.57, Peak response: 37.50 ± 3.02†</td>
<td>Normal: 7.80 ± 0.50, Peak response: 25.20 ± 1.77†</td>
</tr>
<tr>
<td>$k_{seq}, ml/10^{-1}$</td>
<td>Normal: 2.85 ± 0.61, Peak response: 35.50 ± 4.82†</td>
<td>Normal: 1.15 ± 0.12, Peak response: 5.12 ± 0.53†</td>
<td>Normal: 4.80 ± 0.27, Peak response: 7.42 ± 0.31†</td>
<td>Normal: 3.35 ± 0.20, Peak response: 4.26 ± 0.19</td>
</tr>
<tr>
<td>$\overline{V}, s$</td>
<td>Normal: 5.88 ± 0.46, Peak response: 6.91 ± 1.67</td>
<td>Normal: 3.76 ± 0.33, Peak response: 5.57 ± 0.30*</td>
<td>Normal: 4.03 ± 0.27, Peak response: 8.25 ± 0.31†</td>
<td>Normal: 3.35 ± 0.20, Peak response: 4.26 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE of 9 lungs, except for alfentanil peak response, wherein only 6 lungs were studied. Statistically significant difference between normal and peak-response values was calculated using Student’s t-test: *P < 0.05; †P < 0.001.

For the lipophilic amines studied, the estimated values of the MID parameters reflected the differences in the extraction patterns of the various amines at the different stages of the inflammatory response. For instance, Fig. 6 shows that the estimated values of $Q_{S}$, the virtual volume reflective of the slowly equilibrating amine-tissue interactions, were highly correlated with lung wet weight for diazepam, but not for codeine. In addition, the hysteresis in the plot of $k_{seq}$ for lidocaine vs. lung wet weight (Fig. 7) reveals that, for a given lung wet weight, $k_{seq}$ for lidocaine during the injury phase was lower than that during the recovery phase of the inflammatory response. This result suggests that $k_{seq}$ for lidocaine is not simply a measure of lung wet weight and that other more specific aspects of lung composition may be responsible for the changes in the pulmonary disposition of lidocaine at the different stages of the inflammatory response. For each of the four lipophilic amine compounds studied, measures of correlation between the estimated values of the MID parameters and lung wet weight over the time course of the inflammatory response are given in Table 4.

To determine whether the changes in the parameters for a given lipophilic amine and among the different lipophilic amines studied at the different stages of the inflammatory response were significant, each individual parameter was transformed into a z score, i.e., (individual parameter − normal group mean)/standard deviation of normal group parameter (47, 49), also referred to as the normal deviate (49). This allows for a statistical evaluation of the probability that a particular parameter value for a given individual lung falls within the distribution of that parameter in the normal lungs (49). In other words, the probability that an individual parameter belongs to the control distribution and, hence, is not significantly different from that in normal lungs, is <1% when its z score exceeds 3 (49). The changes in the extraction

Table 4. Estimated values of parameters for lipophilic amines normalized to $Q_{W}$

<table>
<thead>
<tr>
<th>Normalized Parameters</th>
<th>Diazepam</th>
<th>Alfentanil</th>
<th>Lidocaine</th>
<th>Codeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_{R}/Q_{W}$</td>
<td>Normal: 1.95 ± 0.10, Peak response: 0.76 ± 0.06†</td>
<td>Normal: 0.47 ± 0.02, Peak response: 0.19 ± 0.02†</td>
<td>Normal: 0.86 ± 0.05, Peak response: 0.45 ± 0.05†</td>
<td>Normal: 0.99 ± 0.04, Peak response: 0.35 ± 0.04†</td>
</tr>
<tr>
<td>$Q_{S}/Q_{W}$</td>
<td>Normal: 1.50 ± 0.20, Peak response: 2.07 ± 0.13*</td>
<td>Normal: 0.45 ± 0.02, Peak response: 0.52 ± 0.05</td>
<td>Normal: 2.34 ± 0.14, Peak response: 2.14 ± 0.27</td>
<td>Normal: 2.43 ± 0.13, Peak response: 0.64 ± 0.07†</td>
</tr>
<tr>
<td>$k_{seq}/Q_{W}, s^{-1}/10^{-2}$</td>
<td>Normal: 4.60 ± 0.98, Peak response: 15.70 ± 1.75†</td>
<td>Normal: 1.90 ± 0.22, Peak response: 2.52 ± 0.28</td>
<td>Normal: 8.80 ± 0.81, Peak response: 17.40 ± 1.45†</td>
<td>Normal: 12.70 ± 0.58, Peak response: 11.70 ± 0.89</td>
</tr>
</tbody>
</table>

Values are means ± SE of 9 lungs, except for alfentanil peak response, wherein only 6 lungs were studied. Statistically significant difference between normal and peak-response values was calculated using Student’s t-test: *P < 0.05; †P < 0.001.
patterns of the lipophilic amines at the different stages of the inflammatory response exemplified in Figs. 4 and 5 are reflected in the calculated \( z \) scores of the parameters shown in Fig. 8. To put the individual values in perspective, \( z \) scores of \( \pm 3 \) are designated by the horizontal dashed lines. From Fig. 8 it is clear that certain parameters and different parameters for different test indicators track the time course of the inflammatory response better than others. The \( z \) scores for \( k_{\text{seq}} \) for all four lipophilic amines studied, \( Q_S \) for diazepam, alfentanil, and lidocaine, and \( Q_R \) for lidocaine were consistently \( >3 \) from 3 to 28 days after CFA treatment. \( Q_S \) for codeine, \( Q_R \) for diazepam, alfentanil, and codeine, and \( \Psi \) for all four lipophilic amines were not discriminating parameters. All lungs from 3 to 48 days after CFA treatment were identifiable as such by \( z \) scores exceeding \( 0.3 \) for at least five of the eight discriminating parameters. In other words, for these lungs the...
The extent of the pulmonary uptake of a lipophilic amine compound is determined by its physical-chemical properties. The objective of this study was to determine whether a change in lung tissue composition resulting from an inflammatory response would have differential effects on the extraction patterns for a group of lipophilic amine compounds having different physical-chemical properties. The affirmative result is a necessary condition in support of the hypothesis that the extraction patterns, expressed quantitatively in terms of the MID model parameter vector (a point in N-dimensional space, where N is the number of parameters), can distinguish among lung phenotypes. To put this concept in perspective, in the past the MID method has been applied to the lungs primarily to measure extravascular lung water, capillary permeability, and metabolic functions of the luminal endothelial surface (8–12, 16, 17, 19–22, 35, 36, 39). These applications have been shown to be useful experimental tools (8–12, 16, 17, 19–22, 35, 36, 39) with some clinical utility (11, 20, 39), and they have motivated development of the theoretical basis for MID data analysis (1–5, 19–22, 35, 47). However, except for lung water, the MID method has had little application for probing beyond the pulmonary endothelial surface. With appropriate indicators, the MID method has the potential for providing information about the lung tissue composition and intracellular function that has not been previously exploited. However, one problem has been that because the primary function of lung perfusion is to serve the gas exchange requirements of the body, lung perfusion is in tremendous excess relative to lung cell requirements for typical substrates of intermediary metabolism. Therefore, there is little expectation that a reference indicator-test indicator difference will be detectable for most endogenous hydrophilic substrates and products. One approach to this problem for MID studies of the physiological and pathophysiological status of the lungs has been to use substrates for certain metabolic functions that occur on the endothelial surface (10, 11, 16, 35, 36). Similar to the gas exchange functions of the lungs, these functions are apparently directed at controlling arterial blood composition rather than lung cell function, and, therefore, to be effective, they occur at rates consistent with the high rate of pulmonary blood flow. This has made MID measurement of their reaction kinetics feasible (10, 11, 16, 35, 36). Measurement of these functions can be useful if the endothelium is a focus of a particular lung injury (10, 11, 16, 35, 36), although even then these functions may not be tightly coupled to those intracellular functions of the endothelial cell that are of key importance to the viability of the endothelial cells themselves (11). Thus the probes that have been used have had limited access to the many aspects of lung cell function and tissue composition that may be altered by lung injury and disease.

To take greater advantage of the MID potential, the present approach attempts to exploit a key strategy of pharmacotherapeutics, wherein a large fraction of therapeutically important molecules (23). The reasons for this include the fact that compounds in this class have a high propensity for associations with biomacromolecules and intracellular organelles (1, 10, 14, 15, 26, 28–32, 37, 41, 42–48). Sometimes these interactions mimic or antagonize the action of some endogenous ligand, usually of an ostensibly very different chemical form. Another reason for the predominance of lipophilic amines in pharmacology is that, in contradistinction to the majority of pathogen substrates and ligands that do not readily pass through capillary or cell membranes, lipophilic amines have relatively free access to extravascular and intracellular binding sites (1, 3, 12, 47). Thus these sites of action can be manipulated from the bloodstream by use of lipophilic amines. However, cell-permeant compounds with affinities for various macromolecules and organelles are not limited to those of known pharmacological application, and their propensity for a broad range of biomacromolecular affinities is also the basis for their use as molecular probes in vitro cell biology (23).

The present study is based on the concept that one might take advantage of the differential binding of lipophilic amines to macromolecules and partitioning among subcellular organelles to distinguish among lung tissue phenotypes. There is no requirement that the test indicators have known macromolecular association sites as long as a differential pattern of associations can be detected and correlated with some other measure of the organ status. On the other hand, it is presumed that identification of association sites will result in increased specificity, and identification of such associations could be important for understanding mechanisms of disease as well. In the context of the present study, the large increases in the diazepam and lidocaine sequestration per unit H2O accessible extravascular lung water volume shown in Table 4 might suggest investigation into the possibility that this inflammatory response involves increased peripheral or mitochondrial benzodiazepine receptors for which diazepam and lidocaine have an affinity (33, 46).

An interesting feature of the CFA-induced inflammatory response is that the lungs almost completely recover by ~24 wk after the initial injury without residual fibrosis (7). The latter may suggest the relative importance of apoptosis vs. necrosis in the resolution of the inflammatory reaction. The caspase-3 measurements indicate that apoptosis was, in fact, a prominent feature of this inflammatory response (Fig. 2). Given the role of peripheral benzodiazepine receptors in the induction of apoptosis (24), this may provide additional motivation for evaluating the contribution of peripheral benzodiazepine receptors to the changes in the pulmonary disposition of diazepam and lidocaine in inflamed lungs (46).
macromolecules (14, 29, 44, 45). For the compounds used in this study, the importance of affinity to plasma protein in determining pulmonary uptake was demonstrated previously (1,3). It is reiterated by the results of the present study, wherein the uptake of the more lipophilic but highly plasma protein-bound alfentanil was less than the uptake of the less lipophilic but less plasma protein-bound codeine.

The extensive first-pass uptake of chemically and pharmacologically diverse lipophilic amine drugs by the lungs has been documented in studies primarily directed at understanding the role of the lungs in the pharmacokinetics of these drugs (14, 15, 27–32, 44, 45, 47). These studies have led to some generalizations about the processes involved in the lung uptake of lipophilic amines. This uptake is apparently via simple diffusion of the free, nonionized form of the amine from the plasma into the lung tissue followed by association with intracellular macromolecules and partitioning among membranes and within subcellular organelles (1, 31, 32, 44, 47). These associations can be very rapidly equilibrating (relative to capillary transit time), such that tissue diffusion and binding equilibration occur virtually instantaneously between contiguous vascular and accessible extravascular spaces at each point along the length of a perfused capillary (1, 3, 12, 47). Other associations are slowly equilibrating with respect to the capillary transit time (1, 15, 29, 31, 32, 44–48). These generalizations are the basis of the kinetic model for the pulmonary disposition of lipophilic amines used to parameterize the MID data in this study (1, 47).

Lipophilic amines have been used previously as MID test indicators to probe intact lung function. Those studies have not pursued the concept in depth, but they are consistent with the hypothesis that the pulmonary uptake of lipophilic amines is dependent on the condition of the lungs. For example, Jorfeldt et al. (27) reported a decrease in pulmonary extraction of lidocaine in patients with “pulmonary insufficiency” compared with a healthy group. Dargent et al. (10) and Morel et al. (39) found that the pulmonary extraction of radiolabeled propranolol was decreased after coronary bypass and in patients at risk for developing acute respiratory distress syndrome. Decreased pulmonary propranolol uptake has also been observed in human pulmonary emphysema (41). The increased iodobenzylpropanediamine observed in the lungs of smokers has been attributed to elevation of alveolar macrophages (43). Experimental studies have demonstrated how the use of indicators with a range of physical-chemical properties can increase the information content of the MID data. For example, Merker and Gillis (36) used propranolol to separate the effects of changing surface area and endothelial cell metabolism on endothelial serotonin uptake in injured lungs. Harris et al. (21) demonstrated how the ratio of PS values for hydrophilic and amphipathic indicators could be used to distinguish changes in capillary permeability from changes in perfused surface area. It has been suggested that measurement of the uptake of labeled lipophilic amines by nuclear medicine residue detection methods may have diagnostic utility (42). The inflammation-induced changes in lung persistence of the amines used in the present study are consistent with that suggestion.

Previously, we found that the parameter vector obtained with a group of indicators ($[^{14}C]$diazepam, $[^{3}H]$OH, and $[^{13}C]$PEA, along with a vascular reference indicator) having different physical-chemical properties could provide a signature with specificity for several experimentally induced variations in lung tissue composition (47). The present study extends those observations by demonstrating the differential change in extraction patterns for a group of lipophilic amines in a model of pulmonary inflammation. It seems clear that the pattern changes reflect differential changes in the tissue sites of association of these compounds. A question to be addressed in future research is to what extent the pattern changes might distinguish among disease models resulting in qualitatively and/or quantitatively different lung cellular and macromolecular compositions.

The MID method will of course only detect the presence of sites of associations to which the test indicator has access via the flowing perfusate. That access can be affected by alterations in the capillary perfusion, and part of the increased tissue mass that occurred in this model of inflammation was not accessible to $[^{3}H]$OH; i.e., the fraction of lung water detected ($Q_{W}$(lung wet − dry weight)) was lower in the inflamed lungs than in the normal lungs and, therefore, presumably inaccessible to the other test indicators as well. The MID parameters $Q_{R}$, $Q_{S}$, and $k_{po}$ are extensive parameters (similar to mass and heat) reflecting the amount of accessible sites of association. However, it is also useful to know whether there is a change in the relative amounts per unit of accessible tissue. Ratios of these extensive parameters obtained for different indicators having extraction patterns differentially affected by the changes in lung composition are intensive parameters (similar to concentration and temperature) that reflect the relative amounts of the perfused tissue as exemplified by the normalization to the $[^{3}H]$OH-accessible extravascular lung water volume ($Q_{W}$) in Table 4. Thus these ratios reflect the changes in perfused tissue composition independently of changes in the amount of accessible tissue.

In conclusion, this study demonstrates that the pulmonary disposition of these lipophilic amine indicators depends on the composition of the lung tissue. The results are encouraging with respect to the potential use of this or another combination of lipophilic amine compounds as indicators in the MID method for detecting and quantifying changes in lung tissue properties associated with lung disease or injury.

This study was supported by the Whitaker Foundation, the Department of Veterans Affairs, the Falk Trust, and National Heart, Lung, and Blood Institute Grant HL-24349.

Address for reprint requests and other correspondence: S. H. Audi, Research Service 151, Zablocki VA Medical Center, 5000 W. National Ave., Milwaukee, WI 53295-1000 (E-mail: audis@wms.csd.mil.edu). Received 22 February 1999; accepted in final form 8 July 1999.
REFERENCES


