Double Indicator Dilution Technique (DIDT)

In isolated rat lungs perfused with a 3% BSA solution, 5 ml of Ringer’s solution containing 0.16 mg/ml of 70 kDa Texas red dextran (TRD) were instilled into the trachea at time $t_{-10}$ (10 minutes before connecting the lungs to the perfusion system). Subsequent mixing of the BALF promoted the homogeneous delivery of TRD to the distal air space. Simultaneously, Na$^+$ fluorescein (NaF; 0.02 mg/ml) with a molecular weight of 376 Da was added to the perfusate. After 10 min ($t_0$) and 70 min ($t_{60}$), samples of 0.5 ml of the alveolar instillate and 1 ml of the perfusate were collected and stored for fluorescence spectroscopic analysis. Drugs were added to the alveolar instillate prior to instillation. Samples were diluted by adding 40 $\mu$l of sample in 940 $\mu$l of ddH$_2$O in cuvettes and then analyzed for fluorescent intensities at excitation wavelengths of 494 nm for NaF and 595 nm for TRD, respectively.

We will assume all the TRD stays in the lungs throughout a given experiment. Based on the law of conservation of mass the number of moles of TRD in the solution to be instilled into the lungs will be equal to the number of moles in the fluid in the lungs.

After instillation the volume of fluid in the lungs is the sum of the volume that was instilled $V_{IF}$ and the volume of the epithelial lining fluid (ELF) $V_{ELF}$. Then we have:

$$[\text{TRD}]_{IF}^A \cdot V_{IF}^A = [\text{TRD}]_{0}^A \cdot (V_{IF}^A + V_{ELF})$$

where

$[\text{TRD}]_{IF}^A$ is the TRD fluorescence intensity of the alveolar instillate and $[\text{TRD}]_{0}^A$ is the TRD fluorescence intensity of the bronchoalveolar lavage fluid (BALF) at $t_0$.

Isolating for $V_{ELF}$ yields:
\[ V_{ELF} = \frac{[TRD]^A_{IF}}{[TRD]^A_0} \times V_{IF}^A - V_{IF}^A \]  \hspace{1cm} [1]

The total fluid volume in the alveolar space at \( t_0 \) \( V_0^A \) was calculated as
\[ V_0^A = V_{IF}^A + V_{ELF} - V_{sample}^A \]  \hspace{1cm} [2]

\( V_{sample}^A \) is the volume of BALF collected at \( t_0 \) i.e. 0.5 ml

The total volume of fluid in the alveolar space at \( t_{60} \) was calculated from the fluorescence intensities of TRD in the alveolar samples collected at \( t_0 \) and \( t_{60} \). As previously mentioned, we assumed that the number of moles of TRD in the alveolar space remains unchanged throughout the experiment. Then
\[ [TRD]^A_{0} \times V_0^A = [TRD]^A_{60} \times V_{60}^A \]

Isolating for \( V_{60}^A \) yields:
\[ V_{60}^A = \frac{[TRD]^A_{60}}{[TRD]^A_{0}} \times V_0^A \]  \hspace{1cm} [3]

The difference between \( V_{60}^A \) and \( V_0^A \) reflects the net fluid shift into the alveolar space. Net fluid shift is the sum of fluid fluxes into the alveolar space \( V_{I}^A \) and out of the alveolar space \( V_{R}^A \). Thus assuming constant fluid fluxes (or flux rates), into and out of the alveolar space:
\[ V_{60}^A = V_0^A + (\dot{V}_{I}^A - \dot{V}_{R}^A) \times 60 \text{min} \]  \hspace{1cm} [4]

Substitute \( V_{60}^A \) from [3] into \( V_{60}^A \) in [4] to yield
\[ V_0^A + (\dot{V}_{I}^A - \dot{V}_{R}^A) \times 60 \text{min} = V_0^A \times \frac{[TRD]^A_{60}}{[TRD]^A_{0}} \]  \hspace{1cm} [5]

After solving for \( \dot{V}_{R}^A \):
\[ V_R^A = V_I^A + \frac{V_0^A}{60} \times (1 - \frac{[\text{TRD}^A_{60}]}{[\text{TRD}^A_{60}]}) \]  

[6]

Next, we estimated \( V_I^A \) from the amount of NaF in the alveolar space at t\(_{60} \). The low molecular weight trace NaF exchanges rapidly between the alveolar space and the capillary space by solvent drag and hence, reflects fluid fluxes into and out of the alveolar space. The total amount of NaF in the alveolar space at t\(_{60} \), \( \text{NaF}^A_{60} \), can be calculated as

\[ \text{NaF}^A_{60} = [\text{NaF}]^A_{60} \times V^A_{60} \]  

[7]

where \([\text{NaF}]^A_{60}\) represents the NaF fluorescence intensity of the BALF sample at t\(_{60} \).

Substitution of \( V^A_{60} \) by [4] yields:

\[ \text{NaF}^A_{60} = [\text{NaF}]^A_{60} \times (V^A_0 + (V^A_I - V^A_R) \times 60\text{min}) \]  

[8]

\( \text{NaF}^A_{60} \) is the cumulative result of convective NaF influx and removal, into and out of the alveolar space respectively. We assumed that alveolar fluid influx is completely derived from the vascular compartment. Thus the total convective influx of NaF equals \( V_I^A \) (alveolar fluid influx) multiplied by \([\text{NaF}]^P_t\) (the NaF fluorescence intensity of the perfusate at time t). Similarly, the convective removal of NaF equals the product of \( V_R^A \) and \([\text{NaF}]^A_t\) (the NaF fluorescence intensity of the fluid in the alveolar space at time t).

Due to the large perfusion volume of 50 ml, \([\text{NaF}]\) in the perfusate remained constant throughout the experiment. Thus, \([\text{NaF}]\) in the perfusate will be given as \([\text{NaF}]^P_0\) in the equations that follow. \([\text{NaF}]\) in the alveolar space, changes over time but \([\text{NaF}]^A_0 = 0\) since the NaF is initially dissolved only in the perfusate and not in the fluid in the alveolar space.

\([\text{NaF}]^A_t\) will approach a maximum value at infinity ([NaF]\(_\infty^A\)) which will become equal to \([\text{NaF}]^P_0\) assuming an eventual concentration balance.
The number of moles of NaF will remain constant throughout the experiment meaning the amount of NaF initially in the perfusate will equal the amount of NaF in both the perfusate and alveolar space as time approaches infinity. That is

\[ [\text{NaF}]_0^P \times V_0^P = [\text{NaF}]_{\infty}^P \times (V_0^P + V_0^A) \]

Where \( V_0^P \) is the volume of the perfusate at \( t_0 \) and \( (V_0^P + V_0^A) \) is the total fluid volume in both compartments, which remains constant throughout experiments assuming there is no leak.

Isolating for \([\text{NaF}]_{\infty}^A\) yields:

\[ [\text{NaF}]_{\infty}^A = [\text{NaF}]_0^P \times \left(\frac{V_0^P}{V_0^P + V_0^A}\right) \quad [9] \]

Assuming the rate of increase of alveolar NaF concentration \([\text{NaF}]_t^A/\text{dt}\) is proportional to the difference between the concentration at infinity and at time \( t \) we have

\[ \frac{[\text{NaF}]_t^A}{\text{dt}} = b([\text{NaF}]_{\infty}^A - [\text{NaF}]_t^A) \quad \text{[Where b is the rate constant]} \quad [10] \]

The concentration of \([\text{NaF}]_t^A\) follows an exponential curve described by:

\[ [\text{NaF}]_t^A = [\text{NaF}]_{\infty}^A \times (1 - e^{-bt}) \quad [11] \]

\([\text{NaF}]_{60}^A\) is known since it is measured in DIDT protocol. Isolating for \( b \) and plugging in \( t = 60 \) yields

\[ b = \frac{\ln(\frac{1}{1 - \frac{[\text{NaF}]_{60}^A}{[\text{NaF}]_{\infty}^A}})}{60} \quad [12] \]

The mean alveolar concentration of NaF over the 60 minute experimental interval can be calculated as

\[ [\text{NaF}]_{\text{mean}}^A = \int_0^{60} [\text{NaF}]_t^A \text{dt}/60\text{min} \]
Solving for $[\text{NaF}]_\text{mean}^A$ yields

$$[\text{NaF}]_\text{mean}^A = [\text{NaF}]_0^A * (1 + \frac{e^{-60b}}{60})$$  \[13\]

As described above, $\text{NaF}_{60}^A$ is the sum of convective NaF influx and removal, hence

$$\text{NaF}_{60}^A = ([\text{NaF}]_0^P * \dot{V}_I^A - [\text{NaF}]_\text{mean}^A * \dot{V}_R^A) * 60\text{min}$$  \[14\]

Substituting $\text{NaF}_{60}^A$ from [14] into [8] yields

$$\dot{V}_I^A = \frac{[\text{NaF}]_0^A * \dot{V}_0^A * 60\text{min} - \dot{V}_R^A ([\text{NaF}]_60^A - [\text{NaF}]_\text{mean}^A)}{[\text{NaF}]_0^P - [\text{NaF}]_{60}^A}$$  \[15\]

Finally, substitution of $\dot{V}_I^A$ in [6] by [15] and isolating for $\dot{V}_R^A$ yields

$$\dot{V}_R^A = \frac{[\text{NaF}]_0^A * \dot{V}_0^A * 60\text{min} + \dot{V}_0^A * 60\text{min} * (1 - [\text{TRD}]_0^A * [\text{TRD}]_{60}^A) * ([\text{NaF}]_0^P - [\text{NaF}]_{60}^A)}{[\text{NaF}]_0^P - [\text{NaF}]_\text{mean}^A}$$  \[16\]

Substituting $\dot{V}_R^A$ from [16] into [4] and isolating for $\dot{V}_I^A$ yields

$$\dot{V}_I^A = \frac{[\text{NaF}]_{60}^A * \dot{V}_0^A * 60\text{min} + \dot{V}_0^A * 60\text{min} * (1 - [\text{TRD}]_0^A * [\text{TRD}]_{60}^A) * ([\text{NaF}]_0^P - [\text{NaF}]_{60}^A)}{[\text{NaF}]_0^P - [\text{NaF}]_\text{mean}^A} + 60\text{min}$$  \[17\]

Alveolar fluid reabsorption $\dot{V}_R^A$ and alveolar fluid influx $\dot{V}_I^A$ have therefore been isolated and can be calculated based on the volume and fluorescence measurements made in the double-indicator dilution technique.
REFERENCES
