HIGHLIGHTED TOPIC | Physiological Imaging of the Lung

Visualizing lung function with positron emission tomography

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The purpose of this paper is to describe the technology of, applications for, and information obtained from positron emission tomography (PET) in the study of lung function. The range of applications of PET and the variety of information that has been obtained have expanded significantly in the past decade, and much of this new material has not been included in previous reviews (22, 23, 45, 53, 70).

PET has very high sensitivity, such that picomolar concentrations of labeled compounds can be readily detected in body tissues. This allows for the ability to image radiolabeled substances that target gene expression or cell surface receptors. Current technology allows for a resolution of ∼1 cm3 in the lungs, a size that would be expected to contain a few acini. The short half-lives of many of the positron-emitting compounds helps keep radiation exposure to subjects low. This, coupled with the desirable biological and chemical properties of many PET radionuclides, has made it an ideal technology to study lung function. A summary of methods and compounds used to study lung function is given in Table 1.

A positron, emitted from a decaying radionuclide, travels a few millimeters in tissue and then combines with an electron in few millimeters in tissue and then combines with an electron in a size that would be expected to contain a few acini. The short half-lives of many of the positron-emitting compounds helps keep radiation exposure to subjects low. This, coupled with the desirable biological and chemical properties of many PET radionuclides, has made it an ideal technology to study lung function. A summary of methods and compounds used to study lung function is given in Table 1.

A positron, emitted from a decaying radionuclide, travels a few millimeters in tissue and then combines with an electron in what is termed an “annihilation event,” yielding two γ-rays of equal energy (511 keV) at 180° from each other. These γ-rays then travel outward in opposite directions until they impact detectors opposite each other in a ring around the camera. The γ-rays are detected when they reach a scintillator material in the scanning device, creating a burst of light that can be detected. These electronically recorded coincidences eliminate the need for collimation, since the source of the γ-rays occurred somewhere along the path between the two detectors. However, the γ-rays are subject to a small amount of scatter, which contributes to noise. Another source of noise is random coincidences, where two γ-rays are detected simultaneously but whose source was different. The contribution to noise of these two effects is estimated to be <5% of total coincidences.

The γ-ray energy can be transferred to body tissues due to interactions with atoms along their path. Because of these interactions, the radioactivity of the source will be underestimated. An important advantage for PET is that this attenuation can be corrected for using an image of regional density called a transmission scan. This is performed by having a radioactive positron source (usually 68Ge/68Ga) rotate around the subject. A scan is also acquired without the subject in the camera, and the difference in counts between the two scans represents the reduction in counts due to tissue density. From the raw data, corrected for energy attenuation by body tissues, one can use reconstruction algorithms similar to those used for computed tomography (CT) scanning to generate axial slices or a three-dimensional image of the region of interest. The resulting image is called an emission scan to denote that the source of the radioactivity was within the body, as opposed to the transmission scan, where the source is outside the body.

The resolution of PET systems is dependent on the detector size, the detector coding process, annihilation photon acollinearity, positron range, and reconstruction algorithm. It is usually quantified by a term called full-width half-maximum, which is the width of an imaged point source at one-half of its maximum value. As PET resolution improves (mainly due to improvements in detector design), sensitivity can be degraded, since the sensitivity depends on the number of annihilation events per voxel and improved resolution means smaller voxel

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Table 1. Summary of functional PET methods

<table>
<thead>
<tr>
<th>Physiological Parameter</th>
<th>Compound and Method</th>
<th>Tracer Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas content</td>
<td>60Ge/68Ga rotating pin source (transmission scan)</td>
<td>270.8 days/67.6 min</td>
</tr>
<tr>
<td>VA</td>
<td>Bolus injection 15N-N2-saline</td>
<td>9.97 min</td>
</tr>
<tr>
<td></td>
<td>15N-N2 gas equilibration washout</td>
<td>9.97 min</td>
</tr>
<tr>
<td></td>
<td>111In gas equilibration washout</td>
<td>17.4 s</td>
</tr>
<tr>
<td></td>
<td>81mKr gas equilibration washout</td>
<td>13 s</td>
</tr>
<tr>
<td>Q</td>
<td>Bolus injection 15N-N2-saline</td>
<td>9.97 min</td>
</tr>
<tr>
<td></td>
<td>15N-Ga-transferrin injection</td>
<td>67.6 min</td>
</tr>
<tr>
<td></td>
<td>Bolus injection 15N-N2-saline</td>
<td>9.97 min</td>
</tr>
<tr>
<td></td>
<td>Continuous infusion 15N-N2-saline</td>
<td>9.97 min</td>
</tr>
<tr>
<td></td>
<td>Continuous infusion H215O</td>
<td>2.04 min</td>
</tr>
<tr>
<td>Blood volume</td>
<td>Inhalation of 13CO</td>
<td>20.4 min</td>
</tr>
<tr>
<td></td>
<td>Inhalation of C15O</td>
<td>2.04 min</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>13C methyl albumin injection</td>
<td>20.4 min</td>
</tr>
<tr>
<td></td>
<td>68Ga-transferrin injection</td>
<td>67.6 min</td>
</tr>
<tr>
<td></td>
<td>13C-methylalbumin injection</td>
<td>20.4 min</td>
</tr>
<tr>
<td>Inflammation</td>
<td>18F-fluorodeoxyglucose injection</td>
<td>110 min</td>
</tr>
<tr>
<td>Gene expression</td>
<td>Intrathoracal delivery of adenovirus carrying the mHSV1-tk gene, followed by intravenous administration of 9-(4-[18F]-fluoro-3-hydroxymethylbutyl)guanine</td>
<td>110 min</td>
</tr>
</tbody>
</table>

VA, alveolar ventilation; Q, perfusion; PTCER, pulmonary transcapillary escape rate; mHSV1-tk, mutant variants of the herpes simplex virus type I thymidine kinase.

To improve signal to noise, one can lengthen the image collection time, increase the dose of administered tracer, or filter the image. Therefore, there is a trade-off between resolution and sensitivity, such that spatial resolution may be killed to enable more accurate quantification, or vice versa.

MODELING AND VALIDATION

With PET, the tracer kinetics constitute a dynamic system that can be analyzed with a mathematical model. For example, Rhodes et al. (55) used the simple assumption of conservation of mass of the tracer at the alveolar-capillary interface (Fick principle) during the constant intravenous infusion of dissolved 13N-N2 to calculate regional effective (eff) alveolar ventilation-perfusion (VA/Q) in a volume element with the following formula:

\[ QCV = VA_{eff}/NCA + \lambda A QCA \] (1)

where C\(\bar{V}\) and CA are the mixed-venous and alveolar concentrations of tracer, respectively, and \(\lambda A\) is the blood-gas partition coefficient for nitrogen. This can be rearranged to yield:

\[ (VA/Q)_{eff}/NCA = C\bar{V}/CA - \lambda A \] (2)

The mixed-venous content is obtained by imaging the right heart during the infusion, and the alveolar concentration is obtained by imaging the lung, after the blood volume is subtracted from the image using a separate \(11\)C-labeled carbon monoxide inhalation (54). This model assumes the VA/Q is uniform within a voxel, mixed-venous concentration is supplied to all lung regions at the same concentration as the right heart, VA and Q are nonpulsatile, and the tracer is in equilibrium with the alveolus.

Using a modification of the continuous infusion technique, Venegas and colleagues developed the bolus intravenous injection technique first used in animals (39, 84) and then extended to humans (20, 44, 88). With this technique, the subject undergoes a brief 20- to 30-s apnea as the tracer bolus (usually \(\sim 30\) ml of \(13\)N-labeled \(N_2\)-saline with activity 30 mCi) is infused over 10 s. As the tracer arrives into the pulmonary capillaries, nearly all \(13\)N-N2 diffuses out into the aerated alveolar spaces due to nitrogen’s low blood-gas partition coefficient (\(\lambda N = 0.015\) at \(37^oC\)) and remains there until breathing resumes. The plateau of activity during the apnea is proportional to regional Q. When the subject resumes ventilation, the tracer washes out in proportion to regional-specific VA, or VA per unit volume. However, in lungs with atelectasis or edema, the injected \(13\)N-N2 is not retained in nonaerated units during breath hold, but is reabsorbed by shunting blood. In addition, single-compartment analysis of the tracer washout in these regions is not accurate, since one cannot separate clearance due to ventilation from clearance due to shunt.

Therefore, a three-compartment tracer kinetic model was developed to assess regional Q, regional shunt fraction, and regional-specific VA (13). The model was tested and found to provide regional parameters of pulmonary function in regions of lung, the size of which approaches the intrinsic resolution for PET images of \(13\)N-N2 in the lung (-7 mm for a multiring camera). This model was later refined (48) and compared with data obtained during PET imaging and found to accurately describe arterial \(13\)N-N2 kinetics.

The reliability of PET in accurately recovering parameters of interest depends on the number of counts. When capturing rapidly changing kinetics, one must use shorter collection times that necessarily lower the signal-to-noise ratio. One way to improve the reliability of parameter estimation is to combine neighboring voxels or low-pass filtering, but this lowers spatial resolution. Another method pioneered by Kimura et al. (32, 33) and improved by Layfield and Venegas (34) is that of principle component analysis, whereby voxels are grouped by their physiological behavior rather than their spatial proximity. If the number of discrete values is large enough, the reduction in precision due to grouping is not physiologically significant. This method was used to develop images of Q and VA from tracer kinetics data obtained with the bolus method of injected \(13\)N-N2-saline in a bronchoconstricted human subject. The tracer content (C) of each voxel was described by the sum of two exponential decays:

\[ \hat{C}(t) = A_1e^{-\alpha_1t} + A_2e^{-\alpha_2t} \] (3)

where \(A_1\) and \(A_2\) are amplitude parameters that are proportional to Q to fast and slow compartments, respectively; \(\alpha_1\) and \(\alpha_2\) describe the specific VA of the corresponding compartments; and \(t\) is time. Using this method, the spatial information of Q and VA was preserved, showing that apparent large uniform regions of hypoventilation during bronchoconstriction actually contained units with normal ventilation (Fig. 1). In a noise analysis, this method was found to yield parameters that deviated by <1% from noise-free data for up to 32-fold of expected noise levels (36).

For any new measurement technique, it is important to be able to validate the new measurements with established techniques. Richard et al. (60) found a good correlation between PET measurements of Q using a continuous intravenous infusion of \(H_215O\) with radiolabeled microspheres (\(r = 0.79, P < 0.001\)). The same group (62) showed that
PET measurements of $V/A$ using an equilibration washout of tracer amounts of $^{13}$N-$N_2$ gas compared favorably with ventilation estimated using changes in lung density from the transmission scans. Vidal Melo et al. (90) evaluated the ability of the $^{13}$N-$N_2$-saline bolus technique of $V/A/Q$ measurement to predict arterial blood gases in animal models of pulmonary embolism, acute lung injury, and bronchoconstriction (Fig. 2). From this analysis, it was clear that, to match the arterial blood-gas data, it was necessary to consider heterogeneity in $V/A/Q$ at a level smaller than the resolution of the PET images ($\sim 2.2 \text{ cm}^3$). This was done by analyzing the tracer washout and, depending on the behavior, treating the voxel as a one- or two-compartment washout. These results were supported by another study (38) of intravenous methacholine-induced bronchoconstriction in sheep, where, in addition to large, contiguous regions of hypventilation, there was a substantial subresolution $V/A/Q$ heterogeneity that had to be taken into consideration to account for bimodal $V/A/Q$ distributions. These studies emphasize that, although PET data obtained with the $^{13}$N-$N_2$ bolus technique is limited in spatial resolution, one can extract information from below the resolution of the camera because of the information contained in the kinetics of the PET tracer.

Rhodes et al. (54), using the $^{13}$N-$N_2$ continuous infusion technique in 12 subjects, found a vertical gradient in $V/A/Q$ ($-1.77\%$/cm). They measured a mean $V/A/Q$ of 0.8 and 0.76 in the right and left lungs, respectively. In some subjects, they also noted areas of very low $V/A/Q$ in the most dependent parts of the lung. These areas were associated with delayed tracer washout when the infusion was stopped that washed out quickly with increased tidal volume, suggesting that these were areas of regional airway closure. This group (5, 6) used equilibration washout of trace amounts of $^{19}$Ne corrected for blood volume using inhaled $^{11}$CO and the continuous infusion of $^{13}$N-$N_2$-saline to measure the gravitational gradients of blood volume, regional $V/A$, and $Q$. They found that variations in the vertical direction could explain 65% of the total variation in blood volume, alveolar volume, and $V/A$, and 61% of the variation in $Q$. The vertical gradient for $Q$ was 11%/cm. The relationship between alveolar volume and $V/A$ suggests that elastic properties of lung tissue explains $V/A$. They also found a relationship between blood volume and regional $Q$, suggesting that regional transit times are nearly equal in the gravitational direction.

![Fig. 1. Physiological parameters (perfusion parameters $A_f$ and $A_s$ on left, ventilation parameters $\alpha_f$ and $\alpha_s$ on right for fast and slow components, respectively) from a bronchoconstricted subject with asthma calculated by using the grouped parameter estimation method. These results show that bronchoconstriction is focal in nature, but, even within heavily constricted regions, well-ventilated lung units remain. Also apparent is the significant perfusion (yellow in bottom left image) to compartments with minimal ventilation (black in bottom right image). [Reproduced from (34) with permission from The Association of University Radiologists.]](image-url)
Musch et al. (44), using the $^{13}$N-$N_2$-saline bolus technique, studied the effect of the prone and supine positions on the topographical distribution of pulmonary VA and Q in healthy subjects breathing room air. They found that both VA and Q had vertical gradients that favored the dependent regions in both positions. The vertical gradients explained, on average, 24% of the Q heterogeneity and 8% of the VA heterogeneity. The squared coefficient of variation for Q was similar in both positions, whereas squared coefficient of variation for VA was lower in the prone position compared with supine. Perhaps most important in this study was the great variability among the six subjects in the response of Q to the change in position. All subjects demonstrated a redistribution of Q toward ventral, dependent lung on turning prone, but the magnitude of this redistribution varied substantially. This may mean that factors that make the vertical gradient of Q much more homogeneous in quadrupeds (such as the geometry of the vascular tree and/or the distribution of vasoactive compounds) may not be as important for bipeds. This also may explain discrepancies in the literature using other techniques (42, 46).

PET has been used to study the VA and Q changes after lung injury in both animal models (15, 43, 61, 65, 74, 75, 85, 92) and humans (73). Schuster et al. (73) found that, unlike some animal models of lung injury, in seven patients with acute lung injury/acute respiratory distress syndrome (ARDS), there was not a significant change in the Q distribution compared with healthy controls. This may be due to a blunting of hypoxic pulmonary vasoconstriction by substances such as endotoxin that are released during the initiation of lung injury (14, 15). However, this pattern of Q was also noted in a sheep model of smoke inhalation injury (92), where a progressive increase in shunt was noted in dependent regions up to 4 h after injury, suggesting pro-
ggressive alveolar derecruitmet or flooding in these zones. Attempts at alveolar recruitment with sustained high-pressure inflations may lead to an increase in shunt blood flow, if the inflation does not restore aeration to atelectatic regions (43). In lung injury characterized by substantial atelectasis, however, recruitment achieved by the prone position (with the abdomen unsupported) results in improved dorsal lung aeration and preservation of dorsal blood flow, resulting in reduced shunt and improved V_A/Q matching (65). In an oleic acid injury model of lung injury in pigs, there was a shift of Q to more ventral regions in the prone position (61), perhaps because this model is characterized by more alveolar flooding than derecruitment; thus the dorsal regions were less aerated. The addition of inhaled nitric oxide enhanced the ventral Q redistribution, regardless of position (prone vs. supine), but the addition of the vasoconstrictor alamatreine had no effect in either position.

Hypocapnic pneumoconstriction has been proposed as a homeostatic mechanism to match V_A and Q (80) and confirmed by planar positron imaging following pulmonary artery occlusion in dogs (81). Vidal Melo et al. (89) studied the effect of autologous blood clot (7-mm diameter × 7-mm length) embolism on Q and V_A in sheep using the 13N-N_2-saline bolus method and 13N-N_2 equilibration washout. They found that there was an increase in specific V_A in perfused areas after embolism, representing a shift in ventilation from zones affected by the clot likely secondary to hypocapnic pneumoconstriction. This study showed, for the first time, that autologous clots causing scattered subsegmental regions of absent Q resulted in a difference of ventilation between areas of clot and no clot of 100%.

Brudin et al. (4) compared regional V_A, Q, and V_A/Q measured in 10 subjects with chronic obstructive pulmonary disease (COPD) using inhaled 10^Ne and a continuous infusion of 13N_2. They reported marked differences in these variables, depending on the clinical type of COPD. For those with a low diffusing capacity for carbon monoxide (D_LCO), they had lower tissue density, lower peripheral vascular volume, lower blood flow per centimeter cubed thorax, and higher V_A/Q than patients with high D_LCO. For those patients with a high D_LCO, they had high tissue density, lower V_A, and higher Q per tissue volume. These findings support the notion that some patients with COPD have predominantly airway destruction vs. airway inflammation and edema.

Bronchoconstriction has been studied using PET in both animals (38, 87) and humans (20, 88) using the 13N-N_2-saline bolus infusion technique. A consistent finding in these studies with methacholine-induced bronchoconstriction is the formation of large-sized (subsegmental to segmental) regions of hypoventilation (ventilation defects) (Fig. 3). Associated with these ventilation defects is a substantial reduction in Q (Fig. 3), estimated to prevent a 33% increase in heterogeneity of the log SD of V_A/Q (20). Despite the large size of these regions, careful analysis of the washout within these ventilation defects shows that it consists of both normal and hypoventilating units (Fig. 1). Therefore, the formation of these defects cannot be attributed solely to large airway constriction (83). To interpret those findings, a computational model was formulated and used to test whether the clustering of severely constricted small airways could be explained with existing knowledge of lung structure and smooth muscle and airway physiology. The model incorporated the bistable behavior of airway constriction proposed by Anafi and Wilson (1) for a terminal bronchiolo into an airway tree structure and included the mechanical interaction of each airway with the surrounding lung tissues during breathing. The combination of local bistable behavior with short-range to long-range competing interactions in the model predicted patchy self-organized ventilation defects compatible with those seen in the PET images. Although ventilation in asthma was known to be heterogeneous, the reasons why had not previously been elucidated.

Fig. 3. PET images of perfusion and end-washout (End w/o) before (baseline) and after nebulized methacholine (bronchoconstriction) for a subject with mild asthma. Each row contains a selected slice of the lung from apex (top) to base (bottom) over a 10-cm span of lung. In each slice, the left lung is on the left, and the right lung is on the right. Each panel of images is mean-normalized with the color scale representing the 13N-N_2 activity. In the perfusion images, note the vertical gradient in perfusion from ventral to dorsal regions at baseline, but the marked heterogeneity of perfusion during bronchoconstriction. Almost all activity is washed out at baseline, but large areas of tracer retention are seen in the end w/o images during bronchoconstriction, and these areas correspond to areas of low perfusion. [Reproduced with permission from Harris et al. (20)].
ALVEOLAR-CAPILLARY PERMEABILITY

Reviews of acute lung injury often emphasize the nonbarrier functions of the pulmonary endothelium (49, 51, 91). Even so, barrier function per se is essential to preserving the most important purpose of the lungs: the adequate exchange of respiratory gases. Not surprisingly, then, measuring the severity of damage to the endothelial barrier is a common method of quantifying "acute lung injury" (51, 68).

One approach to evaluating barrier function—and one that can be applied to imaging methods—is to measure the rate at which protein moves across the endothelial barrier, from vascular to extravascular compartments. When PET imaging is used for this purpose, the goal is to measure the flux (i.e., the time-dependent behavior) of radiolabeled proteins across the pulmonary endothelium (40). This is quantified as the pulmonary transcapillary escape rate (PTCER) (40). Thus, as endothelial barrier function deteriorates, PTCER increases.

Both $^{68}$Ga-transferrin and $^{11}$C-methylalbumin have been used as the protein tracers for PET imaging of PTCER (77). The theory, validation, and limitations of this approach have been described elsewhere (16, 29, 40, 41, 68, 69, 77, 86). Furthermore, as PET imaging studies have repeatedly shown (7, 29–31, 72), measures of barrier function (such as PTCER) are a nonspecific index of lung injury, as an increase in PTCER has been measured by PET imaging in diverse settings, including acute pneumonia, active interstitial lung disease, the reimplantation response, and acute rejection after lung transplantation, in addition to ARDS. Thus such measures as PTCER are an index of functional not structural lung injury.

The value of an imaging approach to quantifying lung injury is illustrated by a study by Palazzo et al. (50), who used PET imaging to measure PTCER in an in vivo canine model of unilateral pulmonary ischemia-reperfusion injury. They found that PTCER increased on the ischemic side more than on the nonischemic side, but both sides of the lungs were increased compared with control lungs (i.e., with no ischemia on either side), suggesting that injury in one lung can lead to similar, but less severe, injury in the contralateral lung, a finding that has been observed in an analogous clinical setting (acute unilateral pneumonia, see below). Subsequently, in a series of studies, Hamvas et al. (16–18) used PET imaging to show that the lungs are remarkably resistant to ischemic tissue injury. In each of these studies, PTCER was used as the primary biomarker of lung injury, and the ability to make measurements regionally within the lungs over time was used to advantage in the study design.

Similar benefits can be found in PET studies of ARDS (7, 29, 67). In addition, these studies illustrate the value of being able to measure more than one variable with imaging, allowing region-specific correlations among these variables. For instance, Calandrino et al. (7) reported that, while PTCER and extravascular density (EVD) [a close correlate to extravascular lung water (EVLW)] were both elevated in ARDS patients, they correlated poorly with one another on a regional basis. [EVLW can also be measured directly with PET, as reviewed elsewhere (68, 70, 71, 78).] Furthermore, even as EVD returned to normal, PTCER remained elevated (although decreased relative to the baseline measurement). These results suggested that injury to lung tissue may be “subclinical” but, nevertheless, present, even after pulmonary edema has actually resolved.

In another study in this same patient population, Sandiford et al. (67) examined the regional distribution of PTCER and EVD more closely and found ventral-dorsal gradients for EVD in supine patients (similar to observations made by X-ray CT), but no ventral-dorsal gradient for PTCER (Fig. 4). Again, functional injury was present even in lung regions that appeared to be free of structural injury (measured as an increase in EVD).

The finding that the lungs of ARDS patients are more diffusely involved than what might otherwise be assumed from just structural imaging with X-ray CT could help explain why the lungs of ARDS patients are so vulnerable to ventilator-
induced lung injury during ARDS: radiographically “normal” lung (i.e., lung with a normal EVLW content) in nondependent lung regions may still be abnormal and, therefore, vulnerable to mechanical stresses caused by mechanical ventilation. Data from the PET imaging studies just cited suggest that nondependent portions of lungs in the ARDS patient are especially “at risk” because they demonstrate subclinical evidence of injury, which can be made manifest by inappropriate ventilator use.

INFLAMMATION

Despite the apparent importance of inflammation in the pathogenesis of such diverse conditions as acute pneumonia, ARDS, cystic fibrosis (CF), and chronic obstructive lung disease, among many others, no consensus exists about an acceptable biomarker of the inflammatory response for lung diseases. Molecular imaging methods (57), however, offer an exciting opportunity to develop and validate imaging biomarkers as adjuncts to diagnosis, tests of treatment efficacy, and treatment monitoring. Ideally, molecular imaging methods would be able to combine the attractiveness of noninvasive imaging with the specificity for the inflammatory response afforded by analysis of material obtained from the lower airways.

[18F]fluorodeoxyglucose ([18F]FDG), the most widely used PET tracer in clinical practice, is a glucose analog in which fluorine-18 is substituted for the hydroxyl group at the second carbon position (50a, 94). Entry into cells occurs via the same glucose transporter family of membrane transporters used by glucose, but, unlike glucose, FDG cannot be metabolized after it is phosphorylated by hexokinase. Thus FDG remains trapped after it is taken up by the cell. As fluorine-18-labeled FDG accumulates in tissue cells, the concentration of radioactivity builds, eventually reaching a point at which it can be detected and quantified by an appropriately calibrated PET camera. FDG-PET imaging has been used frequently in various studies to identify pulmonary inflammation, but its potential use as a quantitative biomarker of the inflammatory response is still under development.

Most studies involving the use of [18F]FDG suggest that increased uptake by the lungs is specific for neutrophilic infiltration. Animal studies evaluating [18F]FDG uptake in models of pneumonia, pancreatitis-induced lung injury, and sepsis-induced lung injury (19, 21, 24), as well as recent clinical studies (8, 9), not only show increased [18F]FDG uptake by the lungs after the insult, but also show by tissue autoradiography that [3H]deoxyglucose uptake was specifically limited to neutrophils.

Chen and Schuster (10) hypothesized an increased rate of [18F]FDG uptake by the lungs in the setting of acute lung injury and, indeed, found that the rate of [18F]FDG uptake by the lungs in animals exposed to endotoxin was eight times higher than that seen in normal controls and four times higher than the uptake in animals exposed only to oleic acid, a common method of experimentally producing acute lung injury (71). The rate of [18F]FDG uptake correlated with in vitro measurements of deoxyglucose uptake in neutrophils accessible by bronchoalveolar lavage.

Despite such data, increased lung uptake of [18F]FDG is not necessarily limited to neutrophils. In a recent study in mice, the increased uptake of [18F]FDG by the lungs in response to endotoxin was diminished only by ~50% in response to neutrophil depletion (93). And significant increases in the lung uptake of [18F]FDG can occur during inflammatory lung diseases not characterized by neutrophilic infiltration, such as sarcoidosis (3, 35). Nevertheless, the available evidence at this point strongly suggests that, in the appropriate experimental or clinical context, an increased uptake of [18F]FDG is a highly reliable biomarker of the neutrophilic inflammatory burden within the lungs.

Jones et al. (26–28) were the first to suggest that FDG-PET imaging could be used to study pulmonary inflammation in patients. More recently, FDG-PET imaging was used in a study of 20 adult patients with stable CF (8). The imaging results were grouped according to the patients’ rate of decline in pulmonary function during the previous 4 yr (66). The rate of [18F]FDG uptake was higher in CF patients than in normal controls, and the highest rates of [18F]FDG uptake occurred in those patients with the most rapid decline in lung function. Additionally, the imaging signal correlated strongly with neutrophil numbers in bronchoalveolar lavage fluid. These results not only imply that the rate of [18F]FDG uptake may be useful as a biomarker of active lung inflammation, but that it may also predict the onset of more rapid deterioration in pulmonary function in CF patients.

A previous study by Taylor et al. (82) suggested the possibility that FDG-PET imaging could be used to quantify focal inflammatory responses in the airways of the lungs induced by bronchoscopic deposition of grass pollen or other allergens. Therefore, Chen et al. (9) used FDG-PET imaging in a dose-escalation study in a recently reported model of focal, limited lung inflammation, induced by the direct bronchial instillation of small amounts of endotoxin into the airway of a single lung segment in normal humans (47). An example of the images obtained is shown in Fig. 5. The rate of [18F]FDG uptake increased postendotoxin in the right lung of each research volunteer in the high-dose group.

Altogether, these various studies suggest FDG-PET imaging may be useful as a biomarker of the inflammatory response, for instance in testing new anti-inflammatory agents or in quantifying clinical responses to treatment.

While the use of [18F]FDG-PET is the most frequent example to date of molecular imaging of pulmonary inflammation, other approaches are also being developed (25). These include markers of macrophage infiltration, adhesion molecule expression, and apoptosis, among others.

GENE EXPRESSION

“Structural imaging” includes those methods, like X-ray CT and magnetic resonance imaging, that depict anatomic structures. Spatial rather than temporal resolution is the critical variable determining the value of such techniques. In contrast, “functional imaging,” as with many of the PET imaging methods described thus far, is commonly used to characterize a transport process (like pulmonary blood flow, ventilation, vascular permeability, or rate of glucose uptake). For these methods, spatial resolution may be important but sometimes...
Fig. 5. Transmission (top row) and [18F]fluorodeoxyglucose (FDG)-PET (bottom row) images from one healthy volunteer, before and 24 h after direct bronchial instillation of 4 ng/kg of endotoxin into the right middle lobe. An area of increased density (presumably due to inflammation) and [18F]FDG uptake are clearly visible (arrows). Also shown is a subtraction [18F]FDG image, in which activity of the before and after images are normalized for injected dose, and the “before” image is subtracted from the “after” image. [Reproduced with permission from Chen et al. (9)].

Fig. 6. PET images (top), light microscopic (middle), and corresponding fluorescence micrographs (bottom) obtained in one rat infected with AdCMVnull (panel 1) and 3 rats, respectively, infected with 1 × 10^{10}, 5 × 10^{10}, and 1 × 10^{11} VP of AdCMV-mNLS-xr39tk-egfp (panels 2, 3, and 4 from left to right). The PET images are transverse slices obtained at the midchest level. Light and fluorescent micrographs (×10 magnification) represent identical (ID) lung areas in the left lung from adjacent sections. Exposure time for each fluorescent micrograph was the same (960 ms). Fluorescence was mainly seen in distal parenchymal (alveolar) cells, rather than in airway epithelium or microvessel endothelium. VP, viral particles. [Reproduced with permission from Richard et al. (64)].
less so than with anatomic imaging, as regions of an organ with similar function (which is often the case in the lungs) can be adjacent to one another (thus may not need to be distinguished from one another spatially). However, as the derivation of the functional parameter frequently requires a mathematical analysis of the time-dependent behavior of some contrast agent or tracer, the temporal resolution of the imaging method is usually of special importance.

The value of molecular imaging is directly dependent on its “specificity,” i.e., the ability to assume that the accumulation of a tracer or contrast agent into a tissue is entirely dependent on the expression or activity of a specific molecule, be it an enzyme, receptor, membrane transporter, etc. While this specificity may be conferred by the contrast agent or tracer itself (for instance, a highly specific radiolabeled ligand for a receptor), nonspecific uptake of such probes is almost invariably a factor that reduces the ratio of imaging signal-to-background noise.

In contrast, very high degrees of specificity can be achieved by using modern molecular biology methods to introduce reporter genes into target tissues, the products of which trap an imaging signal that can be detected and recorded by PET (or other) instrumentation. Using such an approach, molecular imaging methods are freed from the time-consuming process of developing, implementing, and validating the pharmacokinetics and pharmacodynamics of the imaging probe (the contrast agent or tracer) and instead become simply dependent on the ability to link the reporter gene to the target gene, a process that can be achieved with great efficiency using modern molecular tools. Broad overviews that cover general concepts, strategies, and imaging platforms for molecular imaging can be found elsewhere (2, 11, 12, 37, 52, 56, 76, 79).

Thus far, studies involving PET reporter gene (PRG) imaging in the lungs have almost exclusively used mutant variants of the Herpes simplex virus (HSV) type 1 thymidine kinase (mHSV1-1k) as the “PRG” and 9-[(18)F]-fluoro-3-hydroxymethylbutylguanine ([18]F-FHBG) as the “PET reporter probe” (PRP) (11, 58, 59, 63, 64). All of these studies have used intratracheal delivery of adenovirus carrying the mHSV1-1k gene, followed by intravenous administration of [18]F-FHBG before imaging. The [18]F-FHBG is trapped in cells, if it is phosphorylated by an appropriate kinase. Mammalian kinases have a restricted substrate specificity relative to the HSV viral kinase. Thus mammalian thymidine kinase will not phosphorylate, or trap, [18]F-FHBG intracellularly. In contrast, cells expressing the mHSV1-1k gene produce a thymidine kinase capable of phosphorylating (and therefore trapping) [18]F-FHBG. As the [18]F-FHBG accumulates in these tissues, an imaging signal is generated from the radiation emitted by fluorine-18 decay.

The feasibility of using this PRG-PRP combination as a reporter system to monitor transgene function in the lungs was demonstrated by Richard et al. (64) using previously transfected normal rodent lungs with various doses of adenovirus carrying the mHSV1-1k gene administered intratracheally (Fig. 6). Using this approach, they then showed that gene delivery in a surfactant vehicle is at least 40% greater than when saline is used as the vehicle (59), providing proof of principle that imaging could be used to monitor gene delivery in clinical trials of gene therapy.

In conclusion, PET has emerged as an important technology in the noninvasive study of regional VA, Q, shunt, gas content, and alveolar-capillary permeability and is already showing promise as a tool for imaging inflammation and gene expression. Linking structure to function in complex biological systems has become increasingly important for biomedical research. Making this link requires understanding of how the components interact to produce a group behavior that cannot be predicted from that of isolated components. PET has the potential of integrating gene activation, cell signaling, and cell trafficking to whole lung organ function, thus advancing our understanding of pulmonary physiology.

REFERENCES


