Assessment of Antioxidative Ability in Brain: Technetium-99m-*meso*-HMPAO as an Imaging Agent for Glutathione Localization

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To visualize the regional localization of glutathione (GSH) in the brain, the relationship between the concentrations of tissue GSH and uptake of [^{99m}Tc]*meso*-hexamethyl propyleneamine oxime ([^{99m}Tc]*meso*-HMPAO) or [^{99m}Tc]*d,l*-hexamethyl propyleneamine oxime ([^{99m}Tc]*d,l*-HMPAO) was studied in mice. **Methods:** The uptake of [^{99m}Tc]*meso*-HMPAO in the mouse brain was decreased to 35% of control paralleling the decrease in GSH content by pre-loading of diethyl maleate (DEM), an agent to reduce GSH. In contrast, pre-treatment with DEM scarcely affected the ^{99m}Tc-*d,l*-HMPAO uptake in the brain. **Results:** The DEM treatment decreased the GSH content in liver, kidney, spleen, fat and lung but did not affect the uptake of [^{99m}Tc]*meso*-HMPAO in those tissues except lung. The images of rat brain acquired with a gamma camera showed a significant reduction of [^{99m}Tc]*meso*-HMPAO uptake by DEM treatment. **Conclusion:** Technetium-99m-*meso*-HMPAO may be a potential tool to assess GSH content and to estimate antioxidative ability in the brain.

Key Words: glutathione; localization; technetium-99m-*meso*-hexamethyl propyleneamine oxime; brain

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Oxidative stress is postulated to play an important role in the tissue injury caused by ischemia and reperfusion, inflammation, aging and various diseases (*1–3*). Because the brain is one of the most metabolically active organs consuming oxygen, oxidative stress is believed to be involved in the pathogenesis of various disorders, including Alzheimer's and Parkinson's disease (*1*). Oxidative stress is a reaction process of biomolecules with O_2 or reactive oxygen species (ROS). Since O_2 and ROS are chemically highly active, they rapidly react with neighboring molecules, particularly with antioxidants. Therefore, it would be valuable to estimate the oxidative stress in the brain in vivo.

Two approaches have been considered to the estimation of oxidative stress in vivo. One is to detect free radicals such as ROS with electron spin resonance computed tomography (*4*). This technique, however, is still under development and has not been materialized yet. The other is to estimate the amount of antioxidants in the tissue, which is an indicator of antioxidative reserve and of the history of exposure to ROS and free radicals. Nonenzymatic antioxidants include glutathione (GSH), cysteine, ascorbate, tocopherols, carotenoids, quinones and metal-binding protein, of which GSH plays the major role in the brain (*5*). In vivo measurement of GSH or any other antioxidants, however, has not been successful yet.

Technetium-99m-*d,l*-hexamethyl propyleneamine oxime ([^{99m}Tc]*d,l*-HMPAO) has been widely used as a cerebral blood flow imaging agent (*6*). As a lipophilic compound, [^{99m}Tc]*d,l*-HMPAO diffuses across the blood-brain barrier and is rapidly converted to a hydrophilic form retainable within the brain tissue. The conversion rate of [^{99m}Tc]*d,l*-HMPAO to the hydrophilic form in brain (k_3) is faster than the diffusion from blood to brain (k_1) or backdiffusion from brain to blood (k_2). Since the delivery and diffusion is the rate limiting step, the uptake of [^{99m}Tc]*d,l*-HMPAO depends mainly on the cerebral blood flow (*7,8*). GSH is supposed to be responsible for the metabolism and retention of [^{99m}Tc]*d,l*-HMPAO in the brain (*9–11*). The bases of these experiments are, however, only done in vitro and the relation of GSH to the retention of ^{99m}Tc has not been completely clarified.

Two stereoisomers of [^{99m}Tc]HMPAO are known: *d,l*-isomer (*12,13*), which is commercially available as a blood flow agent, and *meso*-isomer, which is not suitable as a flow tracer because of the slower conversion to the retained form. If the rate limiting step for the retention of [^{99m}Tc]*meso*-HMPAO is at the reaction with GSH, it could be utilized as an imaging agent of GSH concentration.

In this study, we examined the possibility of [^{99m}Tc]*meso*-HMPAO as an imaging agent for GSH localization in the brain by a comparative study of *meso*- and *d,l* form in the animals treated with diethyl maleate (DEM), a GSH-depleting agent (*14*).

MATERIAL AND METHODS

The precursor of *meso*-HMPAO and *d,l*-HMPAO was labeled with [^{99m}Tc]pertechnetate according to the method of Sharp et al. (*6*). The radiochemical purity was checked by three different chromatographic systems (*6*) and was >90% for *meso*- and *d,l*-HMPAO.

GSH-depleted mice (a group of five ddY mice each weighing about 30 g) were produced by intraperitoneal injection of 55, 137.5, 275, 550 mg/kg body weight of DEM dissolved in corn oil. Control animals were injected with the vehicle of corn oil alone. One hour after the DEM treatment, 1.85 MBq [^{99m}Tc]*meso*- or *d,l*-HMPAO were injected intravenously into the mouse. Thirty minutes after the tracer injection, the animals were killed by decapitation, and the radioactivities in the brain, liver, kidney, spleen, lung and fat were measured by an auto-well scintillation counter.

The GSH and thiols contents, which had been produced as described above, were measured in another set of DEM-treated and control animals. The tissues were homogenized with 10 volumes of 1 *M* PCA or 50 m*M* Hepes buffer (pH 7.4). The GSH content in PCA soluble fraction was measured using a high-performance liquid chromatographic-electrochemical detection method (HPLC-ECD) with a glassy carbon electrode at 1.1V (cation-exchange column; Shodex SP-825; 8 mm I. D. × 75 mm; elution with 10 m*M* citric acid and 10 m*M* disodium hydrogen phosphate solution adjusted to pH 2.1 with metaphosphoric acid; at the flow rate of 1

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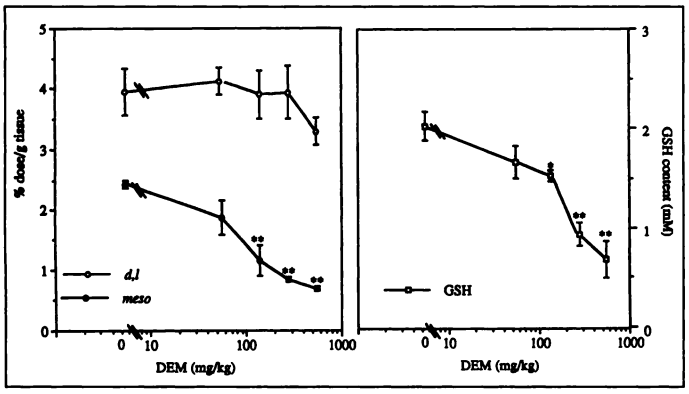

FIGURE 1. Effect of various doses of DEM on uptake of [^{99m}Tc]*meso*- and *d,l*-HMPAO and GSH content in the brain. DEM was dissolved in corn oil and administered intraperitoneally into ddY mice 1 hr before intravenous injection of 1.85 MBq [^{99m}Tc]*meso*- and *d,l*-HMPAO. Asterisks indicate significance in the decrease from the control values (*$p < 0.05$; **$p < 0.01$).

mliter/min) (*15*). The content of thiols in the whole homogenate (Hepes buffer, pH7.4) (total thiols) and those in PCA soluble fraction (nonprotein thiols) were determined by the DTNB-method (*16*). The thiols content in PCA precipitated fraction (protein thiols) was calculated by subtracting nonprotein thiols from total thiols.

As an in vivo imaging study, DEM-treated and control Wistar rats (body weight 200 g) were imaged with a gamma camera equipped with a pin-hole collimator. DEM (550 mg/kg body weight) was injected intraperitoneally into a rat, and an equal volume of corn oil was injected into a control rat. One hour after the DEM administration, 111 MBq of [^{99m}Tc]*meso*-HMPAO was injected intravenously into the rat. The rats were anesthetized with pentobarbital. Static images were obtained 30 min after the [^{99m}Tc]*meso*-HMPAO injection.

RESULTS

Figure 1 shows the relationship between the content of GSH and the uptake of [^{99m}Tc]*meso*- or *d,l*-HMPAO in the mouse brain. Increasing the load of DEM led to a dose-dependent decrease of GSH and [^{99m}Tc]*meso*-HMPAO uptake in the brain. At the highest dose of DEM (550 mg/kg), brain uptake of [^{99m}Tc]*meso*-HMPAO was decreased to about 35% of the control mice. On the other hand, the reduction in [^{99m}Tc]*d,l*-HMPAO uptake was much smaller, being about 85% of the control at even the highest dose of DEM.

Table 1 presents the biodistribution of [^{99m}Tc]*meso*- and *d,l*-HMPAO in normal and DEM-(550 mg/kg body weight) treated mice. No significant reduction was observed in the uptake of either [^{99m}Tc]*meso*- or *d,l*-HMPAO in liver, spleen fat or blood. In the lung, however, the uptake of [^{99m}Tc]*meso*- and *d,l*-HMPAO was decreased to about 68% and 79% of the control mice, respectively, by DEM treatment. The uptake of [^{99m}Tc]*meso*-HMPAO in the kidney was also decreased to 75% of the control mice by DEM treatment.

The content of nonprotein thiols and protein thiols in normal and DEM-(550 mg/kg body weight) treated mice brain are presented in Table 2. In the normal mouse brain, 23.4% and 76.6% of tissue thiols existed as nonprotein thiols and protein thiols, respectively, and 94% of the nonprotein thiols existed in the form of GSH. The DEM treatment led to a decrease in the nonprotein thiols, almost all (>95%) of which was attributable to GSH depletion. On the other hand, concentrations of protein

TABLE 1
Effect of Diethyl Maleate (DEM) on the Biodistribution of Technetium-99m-*meso*- and *d,l*-HMPAO in Mice

		% dose/g tissue	
	Tissue	Control	DEM (550 mg/kg)
[^{99m}Tc]*meso*-HMPAO	Liver	16.86 ± 1.09	22.96 ± 3.33 (136)
	Kidney	6.614 ± 1.038	4.931 ± 0.373 (74.6)*
	Spleen	1.996 ± 0.068	2.270 ± 0.138 (113)
	Lung	5.025 ± 0.392	3.430 ± 0.386 (68.3)*
	Fat	0.396 ± 0.102	0.489 ± 0.069 (123)
	Blood	0.665 ± 0.061	0.801 ± 0.051 (120)
[^{99m}Tc]*d,l*-HMPAO	Liver	7.207 ± 0.583	10.637 ± 2.390 (148)
	Kidney	10.426 ± 1.037	9.865 ± 1.012 (94.6)
	Spleen	3.337 ± 0.445	4.585 ± 0.862 (137)
	Lung	12.530 ± 0.705	9.918 ± 0.660 (79.2)*
	Fat	0.439 ± 0.191	0.604 ± 0.096 (138)
	Blood	1.386 ± 0.066	1.410 ± 0.046 (102)

Significance in the decrease from the control values ($p < 0.05$).
Numbers in parentheses indicate percentage of the control level.

TABLE 2
Effect of Diethyl Maleate (DEM) Administration on the Content of GSH, Nonprotein Thiols and Protein Thiols in the Mouse of Brain

		DTNB-reactable thiols	
	GSH (m*M*)	Nonprotein thiols (m*M*)	Protein thiols (m*M*)
Control	2.030 ± 0.142	2.156 ± 0.098	7.049 ± 0.552
DEM (550 mg/kg)	0.758 ± 0.158* (37.3%)	0.874 ± 0.071* (40.5%)	7.057 ± 0.226 (100.1%)

Significance in the decrease from the control values ($p < 0.01$).
Numbers in parentheses indicate percentage of the control level.

TABLE 3
Effect of Diethyl Maleate (DEM) Administration on GSH and Nonprotein Thiols Content in Mouse Liver, Kidney, Spleen, Lung and Fat

Tissue		GSH (m*M*)	DTNB-reactable thiols nonprotein thiols (m*M*)
Liver	Control	7.333 ± 0.227	7.360 ± 0.604
	DEM (550 mg/kg)	2.918 ± 0.329† (39.8%)	3.110 ± 0.201† (42.3%)
Kidney	Control	4.693 ± 0.236	4.711 ± 0.353
	DEM (550 mg/kg)	2.730 ± 0.285† (58.2%)	2.813 ± 0.273† (59.7%)
Spleen	Control	3.380 ± 0.209	3.384 ± 0.160
	DEM (550 mg/kg)	2.111 ± 0.325* (62.5%)	2.208 ± 0.149† (65.2%)
Lung	Control	1.880 ± 0.067	1.880 ± 0.087
	DEM (550 mg/kg)	0.820 ± 0.355* (43.6%)	1.107 ± 0.101† (58.9%)
Fat	Control	0.312 ± 0.052	0.473 ± 0.062
	DEM (550 mg/kg)	0.198 ± 0.047 (63.5%)	0.249 ± 0.017† (52.6%)

*Significance in the decrease from the control values *$p < 0.05$; †$p < 0.01$.
Numbers in parentheses indicate percentage of control level.

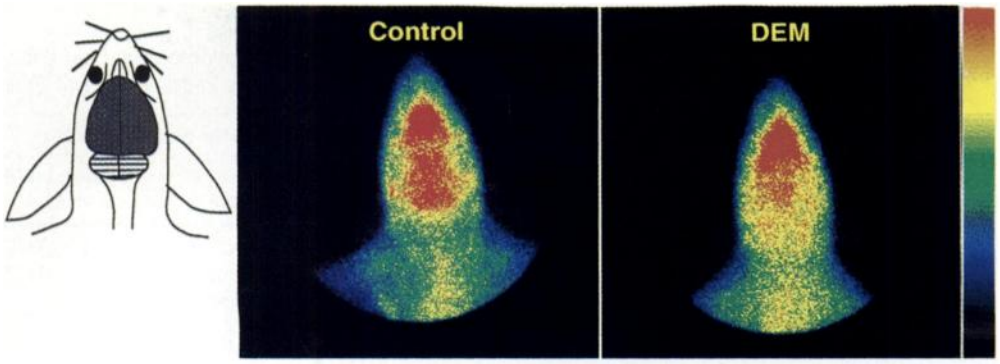

FIGURE 2. Control and DEM-treated rat brain by a gamma camera equipped with pin-hole collimator. DEM, solubilized in corn oil (550 mg/kg body weight), was intraperitoneally injected. Control rat was also administered an equal volume of corn oil.

thiols in the mice brain did not change by DEM treatment. Similarly, concentration of nonprotein thiols in liver, kidney, spleen, lung and fat was reduced by DEM treatment, most of which was attributable to GSH (Table 3).

Figure 2 shows scintigraphic images of the control and DEM-treated rats. Brain uptake of [^{99m}Tc]*meso*-HMPAO was significantly reduced by DEM treatment to 47% of that for the control rat. The GSH content in DEM-treated rat brain was decreased in agreement with [^{99m}Tc]*meso*-HMPAO uptake (control; 1.97 ± 0.02 versus DEM; 1.14 ± 0.11 m*M*).

DISCUSSION

Neirinckx et al. (*10*) investigated the kinetics of [^{99m}Tc]-*meso*- and *d,l*-HMPAO and estimated the rate constants for the washout of diffusible form (k_2) and for conversion to the retainable form (k_3) in various organs. Their findings indicate that for the *meso*-isomer in brain and lung and for the *d,l*-isomer in lung, where k_3 is much smaller than k_2, tissue uptake is determined by k_3, which depends on intracellular conversion of *meso*-isomer to nondiffusible forms. On the other hand, for the *d,l*-isomer in brain, heart and liver, where k_3 is large enough, the tissue uptake is related to blood flow.

The uptake of [^{99m}Tc]*meso*-HMPAO in the mouse brain was decreased to 35% of the control paralleling the decrease in the GSH content by pre-loading of DEM (Fig. 1). In contrast, pretreatment with DEM scarcely affected the [^{99m}Tc]*d,l*-HMPAO uptake in the brain. Because the GSH content was reduced to 37% of the control by DEM treatment in this experiment (Fig. 1), it is considered sufficient to decrease the uptake of *meso*-isomer but not that of the *d,l*-isomer. The DEM treatment decreased the GSH content in liver, kidney and spleen, but did not affect the uptake of [^{99m}Tc]*meso*- or *d,l*-HMPAO in these organs (Tables 1 and 3). We speculated that GSH concentration in liver, kidney and spleen under DEM treatment is still high enough to retain the radioactivity of [^{99m}Tc]HMPAO, and that the level of GSH concentrations and reduction of GSH by DEM treatment in fat are too low to retain and too little to reduce [^{99m}Tc]HMPAO. On the other hand, the lung uptake of *meso*- as well as *d,l*-isomer responded to DEM-induced GSH depletion, indicating that the retention in the lung is related to GSH concentration. All those findings are compatible with the measurement by Neirinckx et al. (*10*). We presume that the respondent concentration of GSH to the level of [^{99m}Tc]*meso*-HMPAO uptake in tissue is in the range of 0.7 to 2.1 m*M*.

It is known that the concentrations of human brain GSH is variable in the range of about 2.2 for normal to 0.8 m*M* for certain diseases by the analysis of autopsied or biopsied brain (*17,18*). Thus we consider that [^{99m}Tc]*meso*-HMPAO could be utilized as an imaging agent of GSH concentration for human brain.

Evidence is limited to the specificity of GSH to the trapping mechanism of [^{99m}Tc]HMPAO in the brain (*9–11*). We have confirmed that [^{99m}Tc]*meso*- and *d,l*-HMPAO is degradable by GSH (Glu-Cys-Gly), cysteine or Gly-Cys-Glu (synthesized GSH-analog) but not by GSSG (oxidized GSH) or ascorbate (data not shown). Moreover, a large portion of thiols in various tissues are attributable to protein thiols (*19*). Therefore, we determined the thiols in the nonprotein and protein-fraction isolated from the DEM-treated mouse brain. The nonprotein fraction was responsible for [^{99m}Tc]*meso*-HMPAO retention and GSH accounted for almost all nonprotein thiols (Table 1). These findings indicate that GSH is the major determinant of [^{99m}Tc]*meso*-HMPAO uptake in vivo.

The reaction mechanism of [^{99m}Tc]HMPAO with GSH has not been completely elucidated. Two hypotheses are considered in the mechanism of interaction of [^{99m}Tc]HMPAO with GSH. One hypothesis is that [^{99m}Tc]HMPAO, lipophilic ^{99m}Tc-chelate is decomposed by coordination of GSH and then it becomes a nondiffusible form within the brain. An alternative is that the ^{99m}Tc atom coordinated to HMPAO molecule is transferred to the thiol group on GSH.

The nonprotein thiols, GSH and cysteine, react with DTNB more rapidly than protein thiols (*19*). On oxidation of thiols by chemicals, GSH and cysteine are more rapidly oxidized than the protein thiols (*14*). We found that cysteine, as a simple molecule, reacts more rapidly with [^{99m}Tc]HMPAO than GSH, as a tripeptide, in vitro. These findings indicate that the conformation around the thiol group on proteins and peptides is related to reactivity with [^{99m}Tc]HMPAO, and protein thiols have less reactivity against [^{99m}Tc]HMPAO than nonprotein thiols.

A close relationship to oxidative stress is suggested in ischemia and Parkinson's disease. The GSH level is reduced to 35%–80% of the control animal brain (*20–22*) in the cortex of reperfused animal brain and 10%–70% of the age-matched control brain (*23,24*) in the substantia nigra of Parkinson's disease. Its relationship with Alzheimer's disease and brain aging is also speculated. Technetium-99m-*meso*-HMPAO is expected to be a potential tool for those clinical and basic studies.

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REFERENCES

1. McIntosh TK. Neurochemical sequela of traumatic brain injury: therapeutic implications. *Cerebrovasc Brain Metab Rev* 1980;6:109–162.
2. Gotz ME, Kunig G, Piederer P, Youdim MBH. Oxidative stress: free radical production in neural degeneration. *Pharmac Ther* 1994;63:37–122.
3. Fahn S, Cohen GC. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neurol* 1992;32:804–812.

4. Ishida S, Matsumoto S, Yokoyama H, et al. An ESR-CT imaging of the head of a living rat receiving an administration of a nitroxide radical. *Magn Reson Imaging* 1992;10: 109–114.
5. Slivka A, Spina MB, Cohen G. Reduced and oxidized glutathione in human and monkey brain. *Neurosci Lett* 1987;74:112–118.
6. Sharp PF, Smith FW, Gemmell HG, et al. Technetium-99m-HMPAO stereoisomers as potential agents for imaging regional cerebral blood flow: human volunteer studies. *J Nucl Med* 1987;27:171–177.
7. Matsuda H, Oba H, Seki H, et al. Determination of flow and rate constants in a kinetic model of [^{99m}Tc]-hexamethyl-propyleneamine oxime in the human brain. *J Cereb Blood Flow Metabol* 1988;8:S61–S68.
8. Lassen NA, Andersen AR, Friberg L, Paulson OB. The retention of [^{99m}Tc]-*d,l*-HMPAO in the human brain after intracarotid bolus injection: a kinetic analysis. *J Cereb Blood Flow Metabol* 1988;8:S13–S22.
9. Ballinger JR, Reid RH, Gulenchyn KY. Technetium-99m-HMPAO stereoisomers: differences in interaction with glutathione. *J Nucl Med* 1988;29:1998–2000.
10. Neirinckx RD, Burke JF, Harrison RC, Forster AM, Andersen AR, Lassen NA. The retention mechanism of technetium-99m-HMPAO: intracellular reaction with glutathione. *J Cereb Blood Flow Metabol* 1988;8:S4–S12.
11. Andersen AR, Friberg H, Lassen NA, Kristensen K, Neirinckx RD. Assessment of the arterial input curve for [^{99m}Tc]-*d,l*-HMPAO by rapid octanol extraction. *ibid.* 1988; 8:S23–S30.
12. Neirinckx RD, Canning LR, Piper IM, et al. Technetium-99m d,l-HMPAO: a new radiopharmaceutical cerebral blood perfusion. *J Nucl Med* 1987;28:191–202.
13. Nowotnik DP, Canning LR, Cumming SA, et al. Development of a ^{99m}Tc-labeled radiopharmaceutical for cerebral blood flow imaging. *Nucl Med Commun* 1985;6:499–506.
14. Plummer JL, Smith BR, Sies H, Bend JR. Chemical depletion of glutathione in vivo. *Methods Enzymol* 1981;77:50–59.
15. Demaster EG, Shirota FN, Redfern B, Goon DJW, Nagasawa HT. Analysis of hepatic reduced glutathione, cysteine and homocysteine by cationе-exchange high-performance liquid chromatography with electrochemical detection. *J Chromatogr* 1984; 308:83–91.
16. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70–77.
17. Marstein S, Jellum E, Nesbakken R, Perry TL. Biochemical investigations of biopsied brain tissue and autopsied organs from a patient with pyroglutamic acidemia (5-oxoprolinemia). *Clin Chim Acta* 1981;111:219–228.
18. Perry T, Yong VW. Idiopathic Parkinson's disease, progressive supranuclear palsy and glutathione metabolism in the substantia nigra of patients. *Neurosci Lett* 1986;67:269–274.
19. Boyne AF. A methodology for analysis of tissue sulfhydryl components. *Anal Biochem* 1972;46:639–653.
20. Cooper AJL, Pulsinelli WA, Duffy TE. Glutathione and ascorbate during ischemia and postischemic reperfusion in rat brain. *J Neurochem* 1980;35:1242–1245.
21. Rehncrona S, Folbergrova J, Smith DS, Siesjo BK. Influence of complete and pronounced incomplete cerebral ischemia and subsequent recirculation on cortical concentrations of oxidized and reduced glutathione in the rat. *J Neurochem* 1980;34: 477–486.
22. Slivka A, Cohen G. Brain ischemia markedly elevates levels of the neurotoxic amino acid, cysteine. *Brain Res* 1993;608:33–37.
23. Perry TL, Godin DV, Hansen S. Parkinson's disease: a disorder due to nigral glutathione deficiency? *Neurosci Lett* 1982;33:305–310.
24. Riederer P, Sofic E, Rausch W-D, et al. Transition metals, ferritin, glutathione and ascorbic acid in parkinsonian brains. *J Neurochem* 1989;52:515–520.

Coronary Flow and Flow Reserve by PET Simplified for Clinical Applications Using Rubidium-82 or Nitrogen-13-Ammonia

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To validate routine, noninvasive determination of absolute myocardial perfusion and coronary flow reserve (CFR), cardiac PET was performed in animals using a simplified imaging protocol, high-dose dipyridamole and a simplified quantitative algorithm specific for ^{82}Rb and ^{13}N-ammonia. **Methods:** One hundred thirty-five PET scans were obtained in eight dogs after intravenous ^{13}N-ammonia or ^{82}Rb using serial dynamic PET or a simple two-image dataset. A simple flow model using the two-image dataset was developed for each radionuclide to account for varying arterial input function, flow-dependent myocardial extraction and increased permeability surface area (PS) product due to capillary recruitment at high flows not incorporated into previous models. Myocardial perfusion by the simple model was compared to standard, complete, two-compartment kinetic models validated by comparison to electromagnetic flow meter. **Results:** For ^{13}N-ammonia, myocardial perfusion by the simple PET model correlated with that by complete compartmental analysis of multiple serial PET images with r = 0.94, slope = 0.96; CFR by compartmental analysis correlated with CFR by electromagnetic flow meter with r = 0.94, slope = 0.97. For ^{82}Rb, myocardial perfusion determined by the simple model correlated with that determined by complete compartmental analysis of multiple serial PET images with r = 0.98, slope = 1.06; CFR determined by compartmental analysis correlated with CFR by electromagnetic flow meter with r = 0.88, slope = 1.13. **Conclusion:** A simplified PET protocol using ^{13}N-ammonia or ^{82}Rb and simple flow models provide noninvasive measurement of CFR up to six times baseline flow throughout the heart and diagnostic image quality for routine clinical application.

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Current pharmacologic or stress perfusion imaging reflects relative distribution of maximum coronary flow or relative coronary flow reserve (CFR) (*1,2*) which may be normal in the presence of diffuse or balanced coronary artery disease. However, absolute CFR, defined as maximum flow normalized to resting flow (*1,2*), is reduced by diffuse disease and therefore reflects its presence and severity. Based on a new approach for quantitative arteriographic analysis of the entire epicardial coronary arterial tree (*3,4*), we have demonstrated that patients with localized coronary artery stenoses have coronary arterial lumens that are diffusely 30% to 50% smaller than normal subjects (*3*).

Although multistenotic or diffuse coronary artery disease is common, there has been no clinical method of quantifying such disease or its cumulative fluid dynamic, functional severity. Intracoronary echocardiography may identify atherosclerosis in the wall of the coronary artery and reduced lumen size (*5*), but it is invasive and does not provide information on functional impairment of flow capacity. We have demonstrated the central role of PET in the comprehensive noninvasive management of coronary artery disease (*6–9*), particularly for changes in severity during reversal treatment (*7–9*). However, absolute coronary flow and flow reserve have not been routinely mea-