Relationship Between In Vitro Transendothelial Permeability and In Vivo Single-Pass Brain Extraction

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In vitro transendothelial permeability was compared to in vivo rat single-pass cerebral extractions to evaluate which method would best estimate the blood-brain barrier (BBB) permeability of several SPECT imaging agents. **Method:** Six ^{99m}Tc complexes and seven non-Tc complexes were tested in vitro using monolayers of primary bovine brain microvessel endothelial cells and in vivo using the rat single-pass cerebral extraction model. In vitro transendothelial permeability indices (PI) were determined by measuring the average percent of radioactivity traversing the monolayers as a function of time. In vivo single-pass cerebral extractions were determined using an indicator fractionation method. **Results:** A positive correlation between extraction and PI was found for the non-Tc complexes (r^2 = 0.96). The CBF imaging agents ^{99m}Tc-ECD and ^{99m}Tc-PnAO have high values for E and PI, demonstrating that these agents penetrate the BBB and have a high membrane permeability, while the heart imaging agent ^{99m}Tc-sestamibi had low values for both E and PI. The low PI and E values for ^{99m}Tc-sestamibi are consistent with a low brain uptake for this agent, except in cases of disruption of the BBB. In contrast to ^{99m}Tc-ECD, ^{99m}Tc-PnAO and ^{99m}Tc-sestamibi, which had concordant values for E and PI, two highly lipophilic boronic acid adducts of technetium dioxime (BATOs), ^{99m}Tc-teboroxime and ^{99m}TcCl(DMG)$_3$2MP, had low negative values for PI, but high values for E. In addition, after 3 hr of incubation, the monolayer-to-medium concentration ratio of the BATOs was 642:1 and 744:1, respectively. This compares with values of 89:1 (^{99m}Tc-PnAO), 25:1 (^{99m}Tc-ECD) and 34:1 (^{99m}Tc-sestamibi). **Conclusion:** These data suggest that the high in vivo single-pass extraction of the BATOs may be explained by a hydrophobic interaction with the luminal surface of the capillary endothelial cell plasma membrane. We conclude that a high single-pass extraction cannot necessarily be used to infer high BBB or membrane permeability.

Key Words: blood-brain barrier; transendothelial; permeability index; single-pass extraction

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It is generally believed that an agent for imaging blood flow should have a high single-pass extraction and be rapidly cleared from the blood (*1*). The single-pass cerebral extraction of several radiopharmaceuticals for external imaging of blood flow has been evaluated in various animal models (*2–7*). As predicted by the Renkin-Crone model, agents with higher single-pass cerebral or myocardial extraction exhibit greater fidelity to true blood flow measured by co-injected microspheres (*8–10*). Contemplation of putative radiopharmaceutical agents designed to target intracellular metabolic processes raises a question, however, concerning whether a high single-pass extraction necessarily implies high-membrane or transcellular permeability. For example, very lipophilic agents might become trapped in the hydrophobic membrane interior after penetrating the hydrophilic region occupied by phospholipid polar head groups at the membrane's outer surface (*11,12*). Thus, the possibility arises that a high lipophilicity may retard repartitioning of an agent into the aqueous cytosolic compartment. In this case, a low membrane permeability would be associated with a high apparent single-pass extraction.

In the Renkin-Crone equation, E is an exponential function of CBF and capillary permeability-surface area product (PS) according to: $E = 1 - e^{-PS/CBF}$ (*13,14*). In this equation, E is a function of two variables, one of which, PS, is unknown and cannot be measured directly using in vivo single-pass methodology. Rather, the value of PS is inferred by substitution of measured values of E and CBF into the equation shown above (*9,10*). The purpose of the present experiments was to develop an in vitro model to measure PS for these agents directly, thus permitting a comparison of in vivo single-pass extraction and in vitro transendothelial permeability determinations. Bovine brain microvessel endothelial cells, which form tight intercellular junctions and posses few pinocytotic vesicles (*15–17*), have been used as an in vitro model of the BBB when grown on porous supports (*15,18–20*). Adhesion of lipophilic compounds to cell supports typically used in this type of experiment limits the utility of this approach for determining the transendothelial permeability of lipophilic radiopharmaceuticals. Herein, we describe a modification of existing methods, accomplished by substituting a new, inert,

weakly anionic, highly porous, aluminum oxide tissue culture cell support (*21*) for supports used previously such as nylon mesh, polycarbonate filter or other types of plastic cell supports (*16*). Due to its weakly anionic hydrophilic surface, preliminary studies established that the aluminum oxide insert was characterized by the lowest binding of the neutral highly lipophilic agent ^{99m}Tc-teboroxime. At morphological confluence, an asymptotic level of high electrical resistance was used to indicate tight junction formation (*22–24*), which allowed selection of wells with similar resistances. This provided the advantage of reproducible permeabilities of the monolayers to our controls, ^{3}H-water and ^{14}C-sucrose. These controls, were used to calibrate high and low permeabilities, respectively, within our model. Using these modifications, we analyzed seven nonmetal agents and six ^{99m}Tc-complexes.

MATERIALS AND METHODS

Cell Support Membrane Selection

Preliminary experiments were performed to determine which of the commercially available cell support membranes would have the lowest level of nonspecific binding for lipophilic Tc-complexes. Nonspecific binding studies of the lipophilic BATO ^{99m}Tc-teboroxime (*25*) to various cell support membranes was performed using a Millipore 1225 sampling Manifold. Polymeric (MF-mixed esters of cellulose membrane, Millipore), polycarbonate (Isopore membrane, Millipore) and aluminum oxide (Anocell anapore membrane, Whatman, Inc., Maidstone, UK) were used. Four samples of each membrane type were placed onto the sampling manifold and washed with 5 ml of 0.9% isotonic saline. A volume of 5 ml containing 5 μCi of ^{99m}Tc-teboroxime in 0.9% isotonic saline was passed through each of the membranes. Each membrane was then rinsed twice with 5 ml of 0.9% isotonic saline, removed from the manifold and counted in an LKB gamma counter. The amount of bound ^{99m}Tc-teboroxime was expressed as a percentage of the total amount passed through the membrane.

Cell Culture

Bovine brain microvessel endothelial cells were isolated from the cerebral gray matter of fresh bovine brains and characterized using a modification of the method described by Audus and Borchardt (*20*). After the meninges and large vessels were removed from the surface of the brains, the gray matter was removed and finely minced until the texture of a slurry was achieved. This tissue slurry was then incubated in a shaking water bath for 3 hr at 37.5°C with 0.17 g/ml of Dispase (Boehringer-Mannheim, Indianapolis, IN). The cell suspension was then mixed with a 13% dextran solution and centrifuged at 5800× g for 10 min. The resulting tissue pellet was resuspended in 20 ml of MEM (Gibco-BRL) containing 1.5 mg/ml of collagenase/dispase (Boehringer-Mannheim) and incubated in a shaking water bath for 1 hr at 37.5°C. This modification of increasing the collagenase/dispase concentration from 1 mg/ml to 1.5 mg/ml significantly reduced the incubation time from 4 hr to 1 hr, and reduced the number of days required for the monolayers to reach confluence from 14 to 20 days postseeding to 9–10 days. The resulting microvessel cell suspension was diluted up to 50 ml with fresh MEM and centrifuged at 1000× g for 10 min. The resulting capillary pellet was resuspended in 8 ml of fresh MEM from which, 2-ml aliquots were layered over 35 ml of a pre-established 50% percoll gradient in each of four 50-ml centrifuge tubes. These four tubes were then centrifuged at 1000× g for 10 min. The resulting microvessel layer was separated and washed twice in MEM by centrifugation at 1000× g for 5 min. A portion of the isolated microvessels was used for seeding and the remaining microvessels were cryopreserved, using a cryo 1°C freezing container (Nalgene Corp., Rochester, NY) at −80°C, using Cellvation (Celox Corp., Hopkins, MN) as the cryopreservation medium, for future use. Cellvation increased the viability of the frozen cells from an average of 45%–70%, throughout the 6-wk period we tested.

Fresh or thawed microvessels were seeded at approximately 1×10^6 cells/cm^2 onto Anocell inserts (8 mm diameter, 0.2 μm pore size; Whatman Inc.), which were precoated with 6 mg/cm^2 of rat tail collagen (Sigma, St. Louis, MO) and 5 mg/cm^2 of bovine fibronectin (Sigma). These inserts were then placed into a Falcon primara 24-well tissue culture plate (BD Scientific, Lincoln Park, NJ) and grown in primary culture medium at 37.5°C with 95% humidity and 5% CO_2. The primary culture medium contained 10% plasma-derived equine serum, 1:1 DMEM/F12 medium, 10 m*M* HEPES, 13 m*M* Sodium Bicarbonate, 200 μg/ml Heparin, 100 μg/ml of Penicillin G—Streptomycin, 2.5 μg/ml of Amphotericin B and 50 μg/ml of Polymyxin B. (All medium components, if not otherwise specified, are from Gibco BRL). Three days after seeding, endothelial cells migrated out from the microvessels onto the Anocell insert. At this time, the primary culture medium was replaced with growth medium (primary culture medium without Amphotericin B and Polymyxin B). Expression of factor VIII antigen and gamma glutamyl transpeptidase activity on endothelial cells was determined as previously described (*26,27*). Electrical resistances were determined when the monolayers became morphologically confluent at 4 days after plating and every day thereafter with a Millipore-ERS electrical resistance probe (Millipore Inc., Bedford, MA). To minimize contamination once the time-course of transmonolayer electrical resistance was established, measurements were limited to the period of peak asymptotic resistance (9–12 days after plating). Only monolayers that reached a peak resistance of >500 Ohm/cm^2 were used for permeability studies.

Permeability and Single-Pass Extraction Studies

On the day of the experiment, the medium inside the inserts and the corresponding bottom wells was aspirated and replaced with 400 μl of growth medium. When testing ^{99m}Tc-complexes, 5 μCi of ^{3}H-water, 2 μCi of ^{14}C-sucrose and 20 μCi of the ^{99m}Tc-complex were added to a total of 0.4 ml of medium inside the inserts. Alternatively, when testing ^{14}C-labeled agents, 5 μCi of ^{3}H-water, 20 μCi of a ^{99m}Tc-complex, which had previously been shown not to cross or associate with the monolayers, and 2 μCi of the ^{14}C-agent were added to a total of 0.4 ml of the medium inside the inserts. For each experiment, we used one 24-well tissue culture plate containing an experimental group consisting of four inserts with monolayers, as well as a control group consisting of four inserts without monolayers. After the addition of radioactive compounds, the 24-well plate was agitated using an orbital plate shaker (Thomas Scientific, Swedesboro, NJ) between sampling points in a 37.5°C incubator with 95% humidity and 5% CO_2. Ten-microliter samples were removed from the inner and outer compartments of the insert using a multiple microliter pipette 1, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 120 and 180 min after the addition of radioactivity to the inserts.

Technetium-99m counts in the samples were measured in an LKB gamma counter. Four days later, after decay of ^{99m}Tc, ^{14}C

and ^3H, counts were determined using Ecoscint (National Diagnostics, Inc., Atlanta, GA) in a liquid scintillation counter, with dual label counting capabilities (Beckman LS 7500). After rinsing all inserts twice with 1 ml of phosphate-buffered saline, we separated the aluminum oxide membranes from the insert housings. The percent of experimental agent associated with the monolayer at 3 hr was determined by subtracting the average value associated with the control inserts from the amount of activity associated with the experimental inserts. The average percent of radioactivity that had traversed the monolayers by 3-hr approximately: TcO_4^- (33%), ^{99m}Tc-PnAO (50%), ^{99m}Tc-ECD (43%), ^{99m}Tc-sestamibi (25%), ^{99m}Tc-Cl(DMG)$_3$2MP (18%) and ^{99m}Tc-teboroxime (20%). From these values, it is apparent that only PnAO was near equilibrium. The monolayer:media ratio was calculated according to Equation 1:

$$\text{Monolayer:Media} = \left(\frac{\%\ \text{Bound}}{100}\right)\left(\frac{\text{Total media vol.}}{\text{Monolayer vol.}}\right), \quad \text{Eq. 1}$$

where the monolayer volume of 0.085 μl was obtained by multiplying the approximate endothelial monolayer thickness of 1.7 μm (*28*) by the insert growth area of 50 mm^2. The total media volume was 800 μl.

The average percent of radioactivity traversing the monolayers was plotted as a function of time. The slope for the first five sampling points, representing 10 min of the clearance curves of ^{14}C-butanol and ^3H_2O, was determined by linear regression analysis. Since an r^2 value of 0.99 was obtained for both of these highly permeating agents, it was clear that back-diffusion would not be significant for any of the agents in the first 10 min and that linear regression of the clearance curves over this interval could provide an estimate of unidirectional flux across the monolayers. The slope of the clearance curve for inserts without monolayers was designated PS_i, where PS is the permeability-surface area product. The slope of the clearance curve for inserts containing monolayers was designated PS_m. The PS value for just the monolayer (PS_e) was computed for water, sucrose and the experimental agent using Equation 2 (*18*).

$$\frac{1}{PS_e} = \frac{1}{PS_m} - \frac{1}{PS_i}. \quad \text{Eq. 2}$$

After correction for the permeability to sucrose, the permeability to the experimental agent was expressed as a fraction of the permeability to water, thus generating a permeability index (PI) as shown in Equation 3:

$$PI = 100 \times \left(\frac{PS_{e\{agent\}} - PS_{e\{sucrose\}}}{PS_{e\{water\}} - PS_{e\{sucrose\}}}\right). \quad \text{Eq. 3}$$

In vivo rat single-pass cerebral extractions (E) for all agents tested were determined by the indicator fractionation method as previously described (*9,29*). In brief, all ^{99m}Tc-agents were mixed with ^{85}Sr-labeled microspheres and co-injected into the left ventricle of an anesthetized rat. Six seconds after injection, the rat was decapitated and the brain removed within 3–4 min and weighed. Technetium-99m and ^{85}Sr activities in the whole brain and an arterial blood reference organ were measured with an LKB gamma counter, which corrected for crossover counts for each radionuclide. In vivo E for all ^{14}C-agents and ^{3}H-water were similarly determined with the exception that we substituted ^{99m}Tc-albumin aggregated (Macrotec®, Squibb Diagnostics, New Brunswick, NJ) for ^{85}Sr-labeled microspheres. In addition, we tested all rats at normal p_aCO_2 (~35 mmHg), thus assuring that whole-brain CBF would approximate 0.5 ml/g/min.

TABLE 1
Comparison of Extraction Values for Carbon-14-Labeled Agents

Agent	E_{eq}	E_{av}	CBF_{av}
Caffine	72	72	0.50
Butanol	101	101	0.53
Diphenylhydantoin	53	53	0.36
Iodoantipyrine	96	96	0.49
Water	90	88	0.46
Thiourea	20	19	0.39

*Comparisons were calculated by substituting a CBF value of 0.5 ml/g/min into the linear regression equation (E_{eq}) or by averaging extraction values (E_{av}) over the normal range of CBF for each group. Also shown is the average cerebral blood flow (CBF_{av}) for each group.

A positive correlation (r^2 = 0.94) exists between CBF measured with Macrotec® and CBF measured with ^{85}Sr-labeled microspheres. Each vial of Macrotec® contained 100 μCi in a volume of 1.0 ml (0.9% isotonic saline). Ninety-five percent of the mean particle size fell between 10–90 μ with none above 150 μ and the number of particles per 1.0-ml vial was approximately 4.5×10^6 depending on the batch. Technetium-99m activity was measured in a LKB gamma counter. Four days later, after ^{99m}Tc decay, ^{14}C counts were determined by first solubilizing the tissue in Solvable® (New England Nuclear, N. Billerica, MA) and then counted in a liquid scintillation counter (Beckman LS 7500). Extractions were calculated in two ways. The first method was accomplished by substituting a CBF value of 0.5 ml/g/min into the linear regression equation describing the regression of E on CBF. However, because a very narrow range of flows was studied in each group, some of the regression equations had slopes that were close to 0 and correspondingly low values for r. For this reason, we also calculated the extraction as an average value over the range of CBFs observed for each group.

Radiolabeled Compounds and Log p Determination

Carbon-14-sucrose (200 mCi/mmole), [^{3}H]water (1.1 mCi/g), [^{14}C]butanol (1 mCi/mmole), [^{14}C]caffeine (50 mCi/mmole), [^{14}C]iodoantipyrine (50 mCi/mmole), [^{14}C]diphenylhydantoin (50 mCi/mmole) and [^{14}C]thiourea (50 mCi/mmole) were purchased from New England Nuclear. Technetium-99m-L,L,-ethyl cysteinate dimer (^{99m}Tc-ECD), ^{99m}Tc-sestamibi, ^{99m}Tc-teboroxime, ^{99m}Tc-(chloro [bis-{2,3-butanedionedionedioxime (1-)-} {2,3-butanedionedioximato (2-)- N, N′, N″, N‴, N⁗, N′⁗} (2-methylpropylborato (2-)]) (^{99m}Tc-Cl(DMG)$_3$2MP) and ^{99m}Tc-propylene amine oxime (^{99m}Tc-PnAO) were synthesized, as previously described (*9,10,30–32*). Technetium-99m-O_4^- was obtained as eluent from a ^{99m}Tc-generator. The lipohilicity of $^{99m}TcO_4^-$ and ^{99m}Tc-sestamibi were determined using a shake-flask method, since these values were not readily available in the literature. Lipophilicities for the other ^{99m}Tc agents were obtained from the literature.

RESULTS

Values for E, calculated by the two methods described above, are given in Table 1, which shows that the values

TABLE 2
Relationships Among Extraction, Permeability, Lipohilicity and Membrane Uptake of Various Technetium-Labeled Radiopharmaceuticals*

Agent	E	PI	Log p	Monolayer-to-medium	% Bound
TcO_4^-*	9.0	9.1	−0.74	3:1	0.03
ECD	68.0	48.2	1.11 (ref 32)	25:1	0.26
PnAO	85.0	64.0	1.76 (ref 31)	89:1	0.97
Sestamibi*	5.9	−2.9	2.70	34:1	0.36
DMG-2MP	70.0	−9.0	3.51 (ref 32)	744:1	7.91
Teboroxime	48.0	−4.0	4.56 (ref 32)	642:1	6.81

*Lipohilicity of $^{99m}TcO_4^-$ and ^{99m}Tc-sestamibi were determined using a shake flask procedure. Lipophilicity of the other agents were obtained from the indicated references.

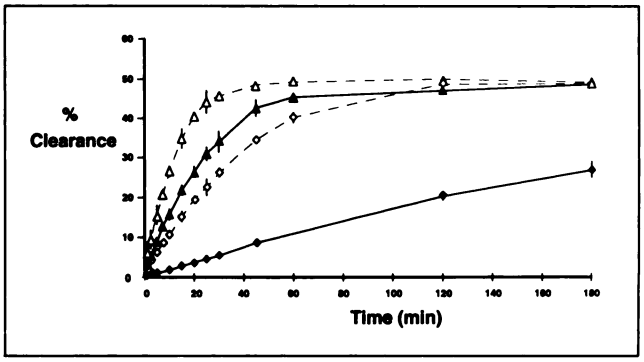

FIGURE 1. Clearance of ^{14}C-sucrose and ^{3}H-water: Reproducibility of the Anocell system. (▲) ^{3}H-water, (◆) ^{14}C-sucrose. Closed symbols represent the clearance of water and sucrose in the presence of an endothelial cell layer. Open symbols with dashed lines represent the clearance of water and sucrose in the absence of an endothelial cell monolayer.

determined by the different methods were identical in four cases, and very similar in the remaining two cases.

The log p of the ^{99m}Tc-complexes is shown in Table 2, which indicates that ^{99m}Tc-teboroxime, one of the most lipophilic BATOs, has a log p of 3.51 (*32*). Accordingly, this agent has a high affinity for organic materials, such as the polymeric and polycarbonate membranes, and provides 20% and 15% binding of ^{99m}Tc-teboroxime, respectfully. In contrast, the inert surface of the Anocell aluminum oxide membrane provides <1% binding of ^{99m}Tc-teboroxime. In addition, evaluation of the amount of material associated with the control membranes indicates <0.1% binding of even the lipophilic cation ^{99m}Tc-sestamibi when serum is present. In addition, owing to the presence of nontortuous pores in the Anocell membrane and a greater density of pores, it exhibits a 38% faster diffusion rate than the Polycarbonate membrane (*21*). These results demonstrate that the Anocell membrane does not disproportionately restrict the diffusion of highly lipophilic agents and can, therefore, be used to measure the membrane permeability of these agents.

Electrical resistance reached an asymptotic level of approximately 300 Ohms per monolayer (600 Ohms/cm^2) 9 days after plating, which was sustained for about 4 days before resistances declined. Approximately 90% of the inserts seeded according to our procedures reached electrical resistance of >500 Ohms/cm^2 or more within 9–12 days. At peak electrical resistance, the endothelial cell monolayers tested positive for Factor VIII antigen and gamma-glutamyl transpeptidase activity. Figure 1 shows the clearance time-course of [^{14}C]sucrose and [^{3}H]water passage through inserts with and without monolayers at peak electrical resistance (n = 20). The difference between sucrose and water clearance in the absence of monolayers reflects the difference in the diffusion coefficient of these molecules. As expected, the difference between water and sucrose clearance increased when monolayers were present. The use of monolayers at peak electrical resistance was clearly associated with reproducible clearance rates for [^{14}C]sucrose and [^{3}H]water, which were comparable to previously reported values (*20*). This allowed us to use [^{14}C]sucrose and [^{3}H]water, subsequently, as standards delineating high (100%) and low (0%) permeabilities for ^{99m}Tc-agents tested in the same inserts. Similarly, a ^{99m}Tc-agent with a PI of zero could be substituted for the [^{14}C]sucrose, when testing the PI of ^{14}C agents. The relationship between PI and E is shown in Figure 2 for seven nonmetal compounds including [^{14}C]sucrose and [^{3}H]water. E-values were obtained by the linear regression method described above. As found in previous investigations (*18,19*), linear regression of E on PI revealed a strong linear relationship between these variables (r^2 = 0.96).

The in vivo brain extraction (E), PI, log p, monolayer-to-medium concentration ratio and percent bound to the endothelial monolayers for all technetium-labeled agents tested are listed in Table 2. In contrast to TcO_4^-, ^{99m}Tc-PnAO, ^{99m}Tc-ECD and ^{99m}Tc-sestamibi, which had concordant values for E and PI, ^{99m}Tc-Cl(DMG)$_3$2MP and ^{99m}Tc-teboroxime had high and moderate E values, respectively, but negative values for PI. In addition, the monolayer-to-medium concentration ratio of these BATOs was 744:1 and 642:1, corresponding to 7.91% and 6.81% of radioactivity associated with the monolayers, respectively.

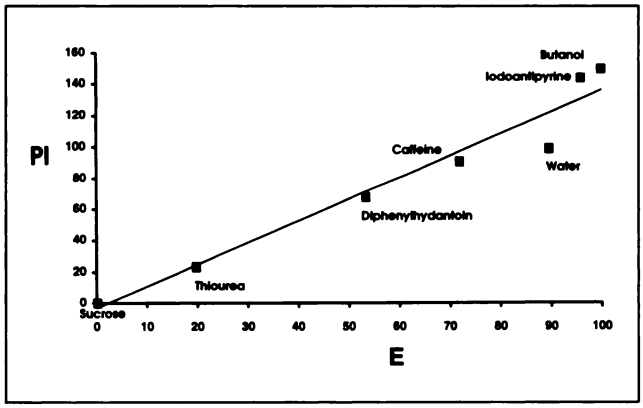

FIGURE 2. In vivo single-pass cerebral extractions (E) versus permeability indexes (PI) for seven nonmetallic agents. (r^2 = 0.96)

This compares with values of 89:1 (^{99m}Tc-PnAO), 25:1 (^{99m}Tc-ECD) and 34:1 (^{99m}Tc-sestamibi). Three of these compounds have a negative PI. For ^{99m}Tc-Cl(DMG)$_3$2MP and ^{99m}Tc-teboroxime, this is caused by their high association with the monolayer which leads to a slight underestimation of PS_m in Equation 2, thereby resulting in a negative PI when correcting for the permeability of the monolayers to sucrose. The high level of BATO association with the monolayers suggests that the high in vivo single-pass extraction of these agents may be explained by a hydrophobic interaction with the luminal surface of capillary endothelial cells and that a high single-pass extraction cannot be used to infer high BBB or trans-membrane permeability.

DISCUSSION

The idea that lipophilicity and high single-pass cerebral extraction imply high transendothelial permeability was encouraged by early work on drug delivery through the BBB that demonstrated the importance of lipophilicity as a necessary requirement of penetration (*33,34*). This perspective was further supported by the initial experience with the lipophilic radiopharmaceutical ^{99m}Tc-PnAO, which revealed a high single-pass cerebral extraction and a high rate of brain washout or back-diffusion through the BBB owing to the lack of a trapping mechanism (*31*). On the other hand, the discrepancy shown here between E and PI for the two BATOs, ^{99m}Tc-teboroxime and ^{99m}Tc-Cl(DMG)$_3$2MP, shows that high lipophilicity and significant single-pass cerebral extraction do not necessarily imply high BBB or trans-membrane permeability.

Given the low PI and high monolayer association of ^{99m}Tc-teboroxime and ^{99m}TcCl(DMG)$_3$2MP, one must consider the possibility of membrane trapping as an explanation for the discrepancy between E and PI. This is especially true for highly lipophilic agents that may not readily partition into the aqueous nonmembrane compartment after solvation in the hydrophobic membrane interior. In addition, a significant possibility of hydrophobic BATO interaction with membrane surface molecules should also be considered. Washout from membrane interior and surface compartments may correspond to the slow and fast components, respectively, of BATO washout kinetics previously described (*25,30*). Variation in the relative proportions of membrane phospholipids and hydrophobic surface molecules in different endothelia may help explain variation in organ uptake and washout pharmacokinetics of the BATOs (*25,30*).

The low brain residence time for ^{99m}Tc-PnAO necessitated the development of radiopharmaceutical agents, such as ^{99m}Tc-HMPAO (*35,36*) and ^{99m}Tc-ECD (*37–39*), that were characterized by longer residence times owing to the operation of various intracellular trapping mechanisms (*37,40*). The ability of ^{99m}Tc-PnAO and ^{99m}Tc-ECD to reach the intracellular compartment is suggested by the data in Table 2, which shows moderately high values for both the PI and E of these agents. The case of ^{99m}Tc-sestamibi is more complicated, however. Uptake of members of this class of lipophilic cation initially appeared to be dominated by a nonsaturable physical diffusion determined by lipophilicity (*41,42*). In addition, uptake by noncontractile cells (fibroblasts) appeared to be 7–10 times lower than that by contractile cultured myocytes (*42,43*). The uptake difference between contractile and noncontractile cells could be explained by the previously documented mitochondrial uptake of ^{99m}Tc-sestamibi (*44*), since mitochondria are more numerous in contractile cells. Nevertheless, the noncontractile nature of brain capillary endothelial cells cannot explain their apparent impermeability to ^{99m}Tc-sestamibi, since even noncontractile cells exhibit an uptake approximating 10% of that found in contractile cells. The extremely low PI for ^{99m}Tc-sestamibi (PI = −2.9), would seem to suggest that this agent reaches the myocardial parenchyma primarily by inter-endothelial passage.

Technetium-99m-sestamibi may, nevertheless, reach the endothelial intracellular compartment. For example, as shown in Table 2, we found that a significant amount of ^{99m}Tc-sestamibi was associated with the monolayers relative to ^{99m}Tc-ECD and ^{99m}Tc-PnAO, both of which have substantial values for PI. If this comparison indicates that ^{99m}Tc-sestamibi does cross the endothelial cell plasma membrane, the question arises why it does not then penetrate through the endothelium completely? Recent findings demonstrate that ^{99m}Tc-sestamibi is a substrate for the P-glycoprotein multidrug-resistance transporter (MDR) (*45*), which is heavily expressed in brain capillary endothelial cells (*46*). This raises the possibility that the MDR may pump ^{99m}Tc-sestamibi out of the brain capillary endothelial cells before it is able to cross through the monolayer completely. The MDR is not likely to affect ^{99m}Tc-sestamibi uptake through myocardial capillary endothelium, since the heart is not among those organs and tissues that express high levels of P-glycoprotein (*47*). Accordingly, it is likely that ^{99m}Tc-sestamibi reaches the myocardial parenchyma by a combination of inter-endothelial and transendothelial passage.

CONCLUSION

We have found that the inert Anocell cell culture support membrane provides an ideal substrate for sustaining the growth and differentiation of bovine brain capillary endothelial cells. In addition, the low binding of even extremely lipophilic complexes of ^{99m}Tc such as ^{99m}Tc-teboroxime, allowed us to determine the transendothelial permeability of several radiopharmaceutical agents that are currently in use clinically. These experiments served to illustrate the shortcoming of relying upon in vivo single-pass organ extraction data as an index of transendothelial permeability by demonstrating that the association of an agent with the endothelium can lead to an apparent high extraction when in fact true permeability is near zero. The findings reported herein demonstrate that a complete understanding of the transendothelial permeability of radiopharmaceutical agents is enhanced

by in vitro measurements to supplement and explain in vivo results.

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