Quantification of osmotic water transport in vivo using fluorescent albumin

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Peritoneal dialysis (PD) is a life-sustaining therapy that applies the principle of osmosis across the peritoneal membrane to remove water and solutes accumulated in patients with end-stage renal disease. The efficiency of osmosis during PD is crucial for technique and patient survival (3, 7, 20). The high expression of AQP-1 (AQP1) in the endothelial cells lining peritoneal capillaries led to the early suggestion of a potential role for water channels in water transport generated during PD (6, 30). The development of a mouse model of PD (18) and its application to Aqp1 mice indeed demonstrated that AQP1 accounts for ~50% of osmotic water transport when hypertonic glucose is used as osmotic agent (19). Mouse models of PD have also contributed to the development and characterization of the first identified AQP1 agonist (31), opening perspectives for the treatment of conditions associated with defective water handling.

During the last decades, radioiodinated (125I) serum albumin (RISA) has been the most widely used tracer for intraperitoneal volume (IPV) measurement in rodent models of PD (22, 33). However, the use of RISA is limited by the emission of ionizing radiation, imposing stringent safety regulations for storage, use, and waste handling. Fluorescent probes have been developed to overcome the limitations of radioactive agents and are increasingly used to assess various processes in vivo, including plasma volume changes in rodents (8, 11). In the present study, we developed and validated a highly sensitive method using fluorescently labeled BSA tracers and fluorescence spectroscopy to monitor IPV and osmotic water transport across the peritoneal membrane. The relevance of this new technique to measure osmotic water transport and ultrafiltration (UF) was substantiated by using various osmotic solutions and mice lacking AQP1 and by comparing the accuracy of fluorescent tracers to direct volumetry and RISA.

METHODS

Animals. Adult male SV129 mice (Charles River, Brussels, Belgium), aged 8–14 wk, weighing 20–30 g, were used to assess the kinetics of fluorescently labeled BSA and 125I-human serum albumin and to evaluate the influence of the fluorescent tracer on transport and inflammation parameters in a mouse model of PD (18). Gender-matched Aqp1 mouse littermates generated as previously described (15), aged 8–12 wk, were used to assess the influence of the water channel AQP1. All animals had access to a standard diet and tap water ad libitum. The experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and the Animal Ethics Committee of the Université Catholique de Louvain Medical School.

Peritoneal transport studies and tissue sampling. Osmotic water transport across the peritoneum was investigated using a peritoneal equilibrium test, as previously described (18). Briefly, following anesthesia with ketamine (100 mg/kg sc, Merial) and xylazine (10 mg/kg sc, Merial), adult male SV129 mice were intraperitoneally injected with 1% dextran (500 KDa) and with 50% DMSO in saline to achieve intraperitoneal volumes of 10%–15% body weight. After 24 h, the animals were euthanized and organs were sampled for histological and biochemical analyses.
mg/kg sc, Bayer), mice were laid in a supine position and the fur over the abdominal wall was shaved after desinfec- tion with 70% alcohol. A silicon catheter (Venflon 22 G, 0.9-mm diameter, Terumo) was inserted percutaneously into the right lower quadrant of the peritoneal cavity. This catheter was used for the infusion and sampling of PD solution. Mice were intraperitoneally injected with 2.5 ml of glucose-based dialysate containing 100 µg of albumin labeled with either a fluorescent dye (Alexa Fluor 488-, Alexa Fluor 555-, or Alexa Fluor 647-BSA; Molecular Probes, Invitrogen), FITC-albumin (Sigma-Aldrich), or RISA (Serlab-125, IBA, Cis-BioInternational). The instilled volume of 2.5 ml is between that obtained when scaling to body weight (~0.7 ml) and to body surface area (~10 ml), when considering a standard infusion of 2,000 ml in a 70-kg adult. Previous observations have shown that using a 2.5-ml instilled volume in mouse models of PD induced very similar glucose disappearance rates and net ultrafiltration across species, including humans (18). The content of free $^{131}$I in the spent dialysate was ~0.6% as checked by trichloroacetic acid (5% vol/vol) precipitation. A neutral pH dialysis solution (Physioneal 3.86%, pH ~7.4, Baxter Healthcare, Nivelles, Belgium) was used for tracer kinetics studies, while further experiments using Alexa Fluor 555-BSA were conducted with conventional dialysate [Dianeal 1.36 (344 mOsm/l) or 3.86% (483 mOsm/l), pH ~5.2, from Baxter Healthcare] or glucose 7% (657 mOsm/l). No intermediate sampling was performed during the assessment of the kinetics of the tracer mass to prevent the disappearance of the osmotic agent and tracer by multiple sampling (27). Microsampling (10 µl) was used at intermediate time points in other experiments to assess fluorescence. Before each sampling, the abdomen was gently agitated to facilitate fluid mixing. Instilled and recovered dialysate volumes were weighed on a precision balance (AG35, Mettler Toledo; margin of error during experiments is 0.1 mg). Dialysate recovery was performed at the end of the dwell, using syringes and preweighed safe-lock tubes; the remaining dialysate in the peritoneal cavity was collected using gauze tissue and weighed using the same technique. The fluid taken from the gauze tissue accounted for ~5% of total volume of the dialysate in both groups. Provided a 3.86% glucose dialysate density of 1.020 ± 0.001 g/cm$^3$ in experimental conditions, the volume margin of error can be calculated as 0.1 µl. Values rounded off to 1 µl were used for both instilled and recovered volumes. These parameters were used to measure net UF by volumetry, as previously described (18). Mean arterial blood pressure was recorded using a pressure transducer (Harvard Apparatus, Holliston, MA) connected to the right common carotid artery in anesthetized mice at baseline and during 2-h dwells with 3.86% glucose. The significant water removal and the resulting drop in blood pressure observed during PD had no effect on peritoneal water or solute transport in this mouse model (Fig. 1).
a subset of experiments, IPP was modulated by external abdominal compression to assess the effect of IPP on transperitoneal tracer kinetics. IPP was raised using a neonatal sphygmomanometer (Phillips, Eindhoven, the Netherlands) that was applied around the abdomen of mice and inflated to produce and external abdominal compression, a method that has previously been applied to rats (32) and adapted to mice. IPP was continuously monitored, as previously described, raised to 10 mmHg, and maintained constant for the dwell duration.

Fluorescence and radioactivity assessment. Relative fluorescence intensity in the dialysate, plasma, and peritoneal membrane extracts was assessed on a LS55 fluorescence spectrometer (PerkinElmer) using appropriate standards to prevent quenching and nonspecific interference after tracer extraction, as described (14). Radioactivity in the dialysate, blood, and visceral peritoneum was assessed using a γ-counter (Cobra II, Canberra-Packard, Downers Grove, IL) with statistics ensuring <1% Poisson error (i.e., at least 10,000 counts/positive sample).

Quantification of Alexa Fluor 555-BSA by HPLC. Proteins were separated by reverse-phase HPLC on a GRACE Vydac C4 column (214TP54, 4.6 mm × 25 cm) with an acetonitrile gradient in solvent A [2% (vol/vol) acetonitrile in 0.1% (vol/vol) trifluoroacetic acid]. Elution was performed with the following gradient program: 0–90% solvent B [98% (vol/vol) acetonitrile in 0.085 trifluoroacetic acid] over 50 min at a flow rate of 500 μl/min generated by a model 1260 Agilent solvent delivery system. Absorbance was monitored simultaneously at three wavelengths, namely, 214, 280, and 555 nm. Quantification of protein was performed by integration of the area under the curve for each absorbance peak.

Free fractions of the fluorescent dye in the recovered dialysate. Ultramicrofiltration was performed on the recovered dialysate of mice to confirm the absence of free fraction of the fluorescent dye. Amicon columns with a nominal cutoff of 30,000 Da (Millipore, Bedford, MA) were loaded with 3.86% Dianeal containing Alexa Fluor 555-BSA sampled at the end of a 2-h dwell in SV129 mice, and centrifuged at ×4,000 g for 10 min. Fluorescence was assessed on both the supernatant and the ultrafiltrate.

Nitrite/nitrate and albumin concentrations in the dialysate. The production of nitrate and nitrate in the dialysate was measured using a colorimetric assay (Cayman Chemical, Ann Arbor, MI). After 3-h incubation with nitrate reductase (670 mU/ml) and NADPH (160 μmol/l) at room temperature, total nitrite concentration in the samples was measured by the Griess reaction at 540 nm (Benchmark Plus Microplate Reader, Bio-Rad, Hercules, CA). Albumin concentration was measured by the Bradford assay (Bio-Rad Protein Assay, Bio-Rad).

Statistical analysis. Data are presented as means ± SE. Comparisons between the means from different groups were performed using paired t-tests or one-way ANOVA, followed by Bonferroni’s multiple comparison tests, as appropriate. The significance level is indicated in each figure.

RESULTS

Intraperitoneal kinetics of tracer albumin labeled with 125I or fluorescent dyes. We first investigated the recovery of RISA and BSA labeled with fluorescent dyes (FITC, Alexa Fluor 488, Alexa Fluor 555, and Alexa Fluor 647) (Fig. 2A) at the end of 2-h dwells with 3.86% glucose-based dialysate (pH 7.4) in a mouse model of PD (Table 1). The recovery of the mass of RISA, as well as all types of fluorescent BSA, was almost complete (~99%), indicating negligible disappearance of the tracer from the peritoneal cavity in this model. This conclusion was supported by the minimal (~1%) appearance of the instilled mass of RISA in plasma, with only traces (<0.1%) of RISA retrieved in the visceral peritoneum of exposed mice (data not shown).

According to the mass-balance principle, since the mass transfer of tracer albumin outside the peritoneal cavity is insignificant, IPV at time t can be calculated using the following equation:

\[ V_t = \frac{C_0 \times V_0}{C_t} \]

where \( V_0 \) is the instilled volume of dialysate, and \( C_0 \) and \( C_t \) are the concentrations of labeled albumin in the dialysate at time 0 and t, respectively. The IPV value derived from the tracer concentration at the end of the dwell was concordant with direct volumetric measurement of IPV, considered as the gold standard, for all tracers (Table 1).

Since the conventional dialysate solutions are acidic, we next evaluated the pH sensitivity of fluorescent albumin tracers in vitro. As expected, FITC-labeled BSA showed the most significant pH sensitivity whereas the fluorescence intensity of Alexa Fluor-labeled BSA is less (Alexa Fluor 488 and Alexa Fluor 647) or even not (Alexa Fluor 555) affected by acidic pH changes (ranging from 7.0 to 5.2) (Fig. 2B). Accordingly, the linear relationship between fluorescence intensity and dialysate concentration of Alexa Fluor 555-BSA remained unaffected by changes in dialysate pH (Fig. 2C).

Alexa Fluor 555-BSA to assess osmotic water transport during peritoneal dialysis. The pH insensitivity of Alexa Fluor 555-BSA, associated with its long wavelength, preventing potential interference from autofluorescence, justified the further characterization of Alexa Fluor 555-BSA as a tracer to assess IPV in a mouse model of PD using conventional dialysis solutions. The standard dialysis procedure (2-h dwell with 3.86% glucose-based dialysate) by itself did not affect the fluorescence intensity of the dye, and no free fractions were detected at the end of the dwells (data not shown). The addition of Alexa Fluor 555-BSA to the dialysate had no effect on transport (net ultrafiltration: 48.4 ± 1.9 μl/g vs. 47.8 ± 1.9 μl/g; glucose disappearance rate (D120/D0); 0.44 ± 0.01 vs. 0.43 ± 0.02 in mice exposed or not to Alexa Fluor 555-BSA, respectively; n = 6 in each group) and inflammation [nitrite/nitrate (NOx): 19.0 ± 2.2 vs. 22.8 ± 1.3 μM; albumin leakage (D120 albumin): 0.45 ± 0.03 vs. 0.45 ± 0.03 μg/μl in mice exposed or not to Alexa Fluor 555-BSA, respectively; n = 6 in each group] parameters during the exchange, further supporting its safe use in mouse models of PD.

We next confirmed that the instilled intraperitoneal mass of Alexa Fluor 555-BSA is completely recovered at the end of the procedure, independently of dwell duration and dialysate osmolality (Fig. 3A). We used fluorescence spectroscopy (Fig. 3B) and HPLC (Fig. 3C), two independent and highly sensitive techniques (lower limit of detection of <1% of the total injected mass), to confirm the virtual absence of Alexa Fluor 555-BSA in extracts from visceral peritoneum and plasma of exposed mice.

The validity of Alexa Fluor 555-BSA as an IPV tracer during PD was assessed by the direct comparison of the estimated values with volumetric IPV taken as the gold standard. The absolute values and kinetics of IPV estimated using Alexa Fluor 555-BSA were concordant to volumetric IPV (Fig. 3D), irrespective of dialysate osmolality (Fig. 3E). A close
correlation between the estimated IPV and the volumetric IPV (Pearson coefficient, \( r = 0.91, \ P < 0.001, \ n = 23 \)) was observed after dwells of different durations and using various concentrations of glucose (Fig. 3f).

Since transperitoneal macromolecule absorption depends on intraperitoneal pressure (IPP) (32), we hypothesized that the very low IPP (≤ 2 mmHg up to 10 ml of instilled dialysate) (Fig. 4A) accounts for the lack of tracer disappearance from the peritoneal cavity in our mouse model of PD. We therefore evaluated the effect of modulating IPP (by external abdominal compression) on tracer kinetics. Increasing IPP from 0 to 10 mmHg resulted in a significant reduction of Alexa Fluor 555-BSA mass recovery in the dialysate at the end of 2-h dwells (from 1.00 ± 0.02 to 0.85 ± 0.02, respectively; \( P = 0.001 \)) (Fig. 4B), and in a significant increase in the amount of Alexa Fluor 555-BSA detected in the plasma using fluorescence spectroscopy (Fig. 4C). Similarly, mice exposed to elevated IPP had a higher Alexa Fluor 555-BSA content in their visceral peritoneum at the end of the dwell (data not shown). These observations support the hypothesis that low

Table 1. Fluorescent emission properties and intraperitoneal kinetics of albumin tracers

<table>
<thead>
<tr>
<th>Albumin Label</th>
<th>Excitation, nm</th>
<th>Emission, nm</th>
<th>Extinction coefficient (^1)</th>
<th>Recovery, %</th>
<th>Volumetric IPV(_{120}), (\mu)l</th>
<th>Estimated IPV(_{120}), (\mu)l</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{125})I</td>
<td>491</td>
<td>515</td>
<td>75,000</td>
<td>98.7 ± 1.0</td>
<td>3,690 ± 51</td>
<td>3,741 ± 39</td>
</tr>
<tr>
<td>FITC</td>
<td>491</td>
<td>515</td>
<td>73,000</td>
<td>99.1 ± 1.1</td>
<td>3,511 ± 29</td>
<td>3,501 ± 22</td>
</tr>
<tr>
<td>Alexa Fluor 488</td>
<td>491</td>
<td>515</td>
<td>73,000</td>
<td>99.7 ± 0.7</td>
<td>3,610 ± 48</td>
<td>3,721 ± 75</td>
</tr>
<tr>
<td>Alexa Fluor 555</td>
<td>555</td>
<td>565</td>
<td>155,000</td>
<td>99.1 ± 1.5</td>
<td>3,504 ± 55</td>
<td>3,535 ± 46</td>
</tr>
<tr>
<td>Alexa Fluor 647</td>
<td>650</td>
<td>668</td>
<td>270,000</td>
<td>99.8 ± 1.0</td>
<td>3,667 ± 123</td>
<td>3,663 ± 67</td>
</tr>
</tbody>
</table>

\(^1\) Extinction coefficient of the reactive dye at emission maximum in cm/M.

Values are means ± SE. IPV, intraperitoneal volume. All exchanges were performed with 3.86% glucose-based dialysate during 120 min in SV129 mice. For intraperitoneal kinetics studies, \( n = 6 \) mice/group for radioiodinated \(^{125}\)I human serum albumin and Alexa Fluor 555-labeled BSA and \( n = 3 \) for FITC-, Alexa Fluor 488-, and Alexa Fluor 647-labeled BSA. 1. Extinction coefficient of the reactive dye at emission maximum in cm/M. 2. IPV\(_{120}\), IPV at 120 min of the dwell measured volumetrically or estimated from tracer dilution.

Fig. 2. Spectral characteristics and pH sensitivity of fluorescent dyes. A: fluorescence excitation spectrum of BSA labeled with FITC, Alexa Fluor (AF) 488, Alexa Fluor 555, or Alexa Fluor 647. B: effect of varying pH (from 7.4 to 5.2) of the dialysis solution in vitro on the relative fluorescence intensity of BSA labeled with FITC, Alexa Fluor 488, Alexa Fluor 555, or Alexa Fluor 647 (\( n = 4 \) measures/group). C: linear relationship between fluorescence intensity measured with a LS55 spectrometer (PerkinElmer) in arbitrary units (A.U.) and Alexa Fluor 555-BSA concentration in the dialysis solution. The relationship is unaffected by changes in pH in the studied range (pH 5.2–7.4).
IPP accounts for the negligible disappearance of macromolecular tracers in our mouse model of PD.

**Alexa Fluor 555-BSA to assess IPV kinetics: applications to mouse models of PD.** Having established the validity and accuracy of Alexa Fluor 555-BSA to assess IPV kinetics, we next assessed the usefulness of the fluorescent indicator dilution technique to detect changes in osmotic water transport over time. Modulation of water transport across the mouse peritoneum was achieved in two independent models, first by varying the transperitoneal osmotic gradient (Fig. 5), and second by genetic deletion of the water channel AQP1 (Fig. 6).

Increasing glucose concentration in the dialysate from 1.36 to 3.86 and further to 7% was reflected by a faster movement of water across the peritoneum and increased final IPV (Fig. 5A, Table 2). Both the osmotic water transport over 120 min, estimated by the area under the IPV curves (Fig. 5B), and the AQP1-mediated water transport, corresponding to the UF rate during the first 30 min of the dwell (Fig. 5C), showed a linear relationship with dialysate osmolality (Pearson coefficient, $r = 0.98$, $P < 0.0001$) (Fig. 6, A and B, Table 2). In agreement with the role of AQP1 in mediating water transport during PD, the use of Alexa Fluor 555-BSA as a tracer yielded the same reduction in net UF, area under the IPV curve, and initial UF rate than RISA in the $Aqp1^{-/-}$ mice compared with their wild-type littermates (Fig. 6C).
DISCUSSION

Fluorescent dyes are increasingly used in biomedical research to avoid limitations and safety risks of radiolabeled tracers (8, 11, 14). In this study, we used well-established models of PD to demonstrate that Alexa Fluor 555-BSA is an easy-to-use, neutral, and accurate IPV tracer that allows the study of osmotic water transport in vivo.

Alexa Fluor dyes are a new family of bright, photostable, and water-soluble fluorophores obtained by sulfonation of rhodamine or coumarin molecules (2, 21). Sulfonation makes Alexa Fluor molecules negatively charged and hydrophilic, contributing to the stability of these dyes, and to their strong pH insensitivity, allowing their efficient use in a broad range of pH (21). Among Alexa Fluor dyes, Alexa Fluor 555 was shown
to be the more pH insensitive in the studied range of pH (5.2–7.4), in agreement with previous observations in which Aqp1

Parameters of osmotic water transport during PD in mice using Alexa Fluor 555-BSA as an indicator dilution

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changes in intraperitoneal pH occur, as a result of transperitoneal buffers and gas transport (12). Covalent conjugation of Alexa Fluor dyes to specific proteins such as BSA (2) prevents the appearance of free fractions, limiting the risk of free dye absorption. The addition of tracer concentrations of fluorescent albumin to the PD solution had no effect on parameters of transport and inflammation during PD.

The validity and accuracy of Alexa Fluor 555-BSA to assess osmotic water changes in vivo are demonstrated by the close correlation between IPV based on the dilution of the fluorescent tracer Alexa Fluor 555-BSA and IPV measured by volumetry, considered as the gold standard technique (27). The use of Alexa Fluor 555-BSA as an indicator of osmotic water transport is further validated by the similar IPV values over time obtained with Alexa Fluor 555-BSA and RISA in Aqp1 mice, which represent a critical tool to assess osmotic water transport in rodent models of PD. The serial assessment of IPV over time in the same animal yielded IPV curves exactly similar to those previously obtained using the RISA technique (19, 31).

Similar values of osmotic water transport and initial UF rates, two parameters that are directly linked to the nature of the dialysate and the availability of AQP1 water channels, respectively, were also obtained using either Alexa Fluor 555-BSA or RISA (19, 31).

Application of a macromolecular IPV tracer to assess osmotic water changes in mouse models of PD warrants a careful evaluation of the kinetics of the intraperitoneal mass of the tracer during the dwell. In the present model, the intraperitoneal mass of fluorescent albumins used as IPV tracers is constant throughout all experiments, independently of the fluorescent dye, dwell time, and dialysate osmolality. Minimal disappearance of Alexa Fluor 555-BSA from the peritoneal cavity is corroborated by the lack of significant tracer detection in plasma or visceral peritoneum of exposed mice based on fluorescence spectroscopy and HPLC, which are both highly sensitive (detection limit ~1 µg/ml) whereas HPLC allows to rule out any artifact due to fluorescence. The lack of disappearance of RISA from the peritoneal cavity in this mouse model confirms these results. The data presented here suggest that the very low IPP in basal conditions accounts for the lack of disappearance of fluorescent albumin tracers from the peritoneal cavity. Conversely, increasing IPP by external abdom-

Table 2. Parameters of osmotic water transport during PD in mice using Alexa Fluor 555-BSA as an indicator dilution technique

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Dialysate Glucose, %</th>
<th>Net UF, µl/g body wt</th>
<th>Volumetric IPV120, µl</th>
<th>Estimated IPV120, µl</th>
<th>AUC Net UF, ml/min</th>
<th>UF Rate 0–30 min, µl/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqp1+/+</td>
<td>6</td>
<td>3.86</td>
<td>38.7 ± 3.2</td>
<td>3,473 ± 53</td>
<td>3,505 ± 36</td>
<td>99 ± 5</td>
<td>23.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.36</td>
<td>11.1 ± 0.9***</td>
<td>2,898 ± 79***</td>
<td>2,767 ± 24***</td>
<td>39 ± 3***</td>
<td>3.5 ± 0.6***</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7</td>
<td>60.8 ± 1.1***</td>
<td>4,402 ± 129***</td>
<td>4,274 ± 38***</td>
<td>163 ± 3***</td>
<td>31.9 ± 0.5***</td>
</tr>
<tr>
<td>Aqp1−/−</td>
<td>6</td>
<td>3.86</td>
<td>18.2 ± 2.8</td>
<td>2,881 ± 42***</td>
<td>2,918 ± 41***</td>
<td>46 ± 6***</td>
<td>8.5 ± 1.0***</td>
</tr>
</tbody>
</table>

Values are means ± SE. UF, ultrafiltration; AUC, area under the curve. ***P < 0.001 vs. Aqp1+/+ mice using 3.86% glucose.
Innovative Methodology

FLUORESCENT ALBUMIN TO ASSESS WATER TRANSPORT IN VIVO

inal compression results in a significant reduction in tracer mass recovery in the dialysate, and a parallel increase in tracer appearance in the plasma and in the peritoneal membrane. In other rodent models of PD, disappearance of the macromolecular IPV tracer (RISA, dextrans, or autologous hemoglobin) from the peritoneal cavity (up to 10–30%) can occur as a result of an uptake into lymphatic vessels (mainly subdiaphragmatic) and interstitial tissues lining the peritoneal cavity (10, 34). In those models, transperitoneal transport of macromolecules from dialysate to plasma indeed depends on the nature of the tracer (5, 17) and, more importantly, on IPP, which directly determines tracer disappearance (9, 23, 32, 35). When present, tracer disappearance requires correcting equations to avoid overestimation of IPV, and to accurately assess IPV over time (5, 16, 22). We speculate that differences in abdominal wall compliance among species, in the type of anesthesia and related effects on diaphragmatic contraction, and in the use or not of mechanical ventilation, influence IPP and account, at least partly, for the variability in macromolecular tracer disappearance in rodent models of PD (13, 24–26). Careful evaluation of tracer mass kinetics is therefore a prerequisite for the application of indicator dilution techniques assessing IPV in animal models of PD.

Altogether, these data support the appropriateness of fluorescently labeled macromolecules, and more specifically Alexa Fluor 555-BSA, as alternative IPV tracers to RISA to accurately assess IPV in mouse models of PD, decipher the molecular mechanisms of water transport, and evaluate interventions modifying water transport in vivo. Further investigations are required to investigate the potential use of fluorescently labeled macromolecules to assess IPV over time in PD patients.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


