Similitude of transperitoneal permeability in different rodent species

Michael F. Flessner, Kimberly Credit, Xiarong Li, and Jarred Tanksley
Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi
Submitted 15 May 2006; accepted in final form 14 July 2006

Flessner MF, Credit K, Li X, Tanksley J. Similitude of transperitoneal permeability in different rodent species. Am J Physiol Renal Physiol 292: F495–F499, 2007. First published July 18, 2006; doi:10.1152/ajprenal.00169.2006.—Transgenic mice facilitate mechanistic studies of altered peritoneal transport, but the majority of transport studies have been carried out in rats. We hypothesized that mouse transport parameters, normalized to the peritoneal contact area, would be similar to those of the rat. To address this, we affixed small (∼10-mm diameter) plastic chambers to the serosa of the abdominal wall of anesthetized CD1 and C57BL mice. The chamber constrained transfer across the area of the chamber base and facilitated mixing, volumetric, and concentration measurements vs. time for mannitol, serum albumin, and osmotic and hydrostatic pressure-driven convection. The mass transfer coefficient of mannitol (MTCM) and of serum albumin (MTCSA), hydrostatic pressure-driven flux (Jp), and osmotic filtration (Josm) were calculated from the time-dependent volume and concentration data. The units of all parameters (µl·min⁻¹·cm⁻²) were compared with previously derived parameters from SD rats with a one-way ANOVA. Results indicated small but significant differences in MTCSA (x10²); CD1, 9.72 ± 1.97, n = 6; C57BL, 7.13 ± 1.52, n = 10; rat, 12.5 ± 1.6, n = 17 (P = 0.03). ANOVAs of all other parameters were not significant and confirmed our hypothesis: MTCM (CD1, 3.20 ± 0.38, n = 7; C57BL, 2.34 ± 0.41, n = 6; rat, 2.72 ± 0.23 n = 19), Jp (CD1, 0.77 ± 0.15, n = 10; C57BL, 0.33 ± 0.13, n = 15; rat, 0.51 ± 0.16, n = 9), or Josm (CD1, 0.92 ± 0.35, n = 6; C57BL, 0.49 ± 0.35, n = 6; rat, 1.72 ± 0.35, n = 6). We conclude that elimination of the variable peritoneal transfer area normalizes calculated transport characteristics and facilitates comparison between species.

The rat has been one of the chief species utilized in animal models of peritoneal dialysis, but the recent introduction of transgenic mice has propelled the mouse into this arena of applied physiology. While initial experiments in normal mice must be utilized as a baseline for measurements in transgenic or pathogenic states in mice, with the 30 years of data from multiple investigators in rat models it should be possible to scale to the mouse (4). The typical experiments in rats and mice mimic dialysis in humans, with an injection of dialysis solution into the peritoneal cavity via a catheter and the collection of samples of blood and peritoneal fluid and measurement of peritoneal volume vs. time. The following equation is fitted to this data to derive parameters, such as the mass transfer area coefficient (MTAC), to characterize the rate of solute transfer across the peritoneum

\[
\frac{d(V_{pc}C_{pe})}{dt} = \text{MTAC} \cdot (\text{Cplasma} - C_{pc})
\]

where \(V_{pc}\) is the fluid volume in the peritoneal cavity, \(C_{pc}\) is the peritoneal concentration of the solute, \(t\) is time, \(C_{plasma}\) is the plasma concentration of the solute, \(MTC\) is the mass transfer coefficient or “intrinsic permeability” of the surface \(i\) of the peritoneum during a dialysis, and \(A_i\) is the area of contact of surface \(i\). From Eq. 1, it is easily seen that

\[
\text{MTAC} = \sum_i MTC_i A_i (C_{plasma} - C_{pc})
\]

Animal and human studies have demonstrated that only 30–40% of the anatomic peritoneum is in contact with a relatively large peritoneal volume (2–3 liters in humans or 35–50 ml in rats); furthermore, with movement or change in body position, the duration of contact with any one surface is variable. While the MTCs of different surfaces are relatively the same magnitude (8), the true contact area in a “whole-cavity” experiment is typically unknown.

The peritoneal volume used in an animal or human study determines the surface area of contact and the MTAC (2, 11). Different laboratories utilize different volumes in rats and different durations of experiments to describe the transport phenomena (1, 13, 15, 17). In the case of mice, there is similar variation in a wide variation of techniques utilized in these animals (12, 16). Ni and colleagues (16) utilize a very small volume of 2 ml of hypotonic solution (7.5% glucose in an isotonic salt solution). Flessner (11), on the other hand, has used as much as 10 ml in similar-size animals. This wide variation in volumes can lead to significant differences in the contact surface area and estimated MTAC, and it makes the studies from different laboratories difficult to compare. Acute or chronic studies in which the peritoneum is altered may result in increased or decreased surface contact area and produce even more variation in the calculation of transport parameters.

We hypothesize that elimination of the peritoneal contact area as a variable will result in transport rates or parameters, which are of the same order of magnitude. To address this hypothesis, we used transport chambers (9) to fix the contact area in two species of mice to investigate the differences between different species and to compare these to our previous rat studies. Our findings support the hypothesis.

METHODS

Materials. The tracer molecule used for small-solute experiments was [14C]mannitol purchased from Moravek Biochemicals (Brea, CA), and the tracer was stated to be at least 97% pure by the manufacture and was used as received. FITC-BSA was purchased from Sigma (St. Louis, MO) and was used as delivered. Checks of label purity (9) have demonstrated that there is essentially no separation of the label from the protein.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
The chamber solution for studying mannitol transport in the osmotically induced, hypertonic flux from the tissue consisted of 4% mannitol in a Krebs-Ringer-bicarbonate (KRB) solution (see Ref. 7 for details of solution). $^{[14C]}$mannitol in a concentration of 1 μCi/ml was mixed in this solution. The solution for macromolecular transport was made up of 20 mg of FITC-BSA in 0.5 ml of KRB. For studying hydrostatic pressure-driven convection into the tissue, 5% unlabeled BSA+0.05% Evans blue dye were mixed in KRB with 1 μCi of $^{125}$I-labeled IgG per ml (see Ref. 13 for details of this protein and the daily process of removal of free $^{125}$I label).

**Animals:** surgical preparation. CD1 and C57BL mice were purchased from Charles River Laboratories and had weights of 23–30 and 22–28 g, respectively, at the time of experiment. Sprague-Dawley (SD) rats were purchased from Charles River Laboratories and had weights of 180–220 g. A minimum of five animals was utilized in every experimental protocol. Each animal was weighed and anesthetized with 2% isoflurane introduced via a Euthanex Small Animal Anesthesia System (model 2500, Palmer, PA). Rectal temperature was maintained at 35–38°C with overhead heating lamps and the Euthanex water-jacketed warming system. A catheter was placed in the carotid artery for sampling and blood pressure monitoring; mean arterial blood pressure was always 70 mmHg and in a similar range to that of SD rats. An internal jugular catheter was placed for infusion of fluids and tracer molecules. Diffusion chambers were constructed of polystyrene centrifuge tubes and were scaled down from our previous rat chambers because of the smaller size of the mouse (8); the internal diameter of the chamber was typically 9–10 mm. To access the peritoneum, a bloodless, vertical incision was made along the linea alba, which separates the two sides of the abdominal wall and the respective circulations of each side. The animal was then placed on its side, and one side of the abdominal wall was carefully stretched to its original size; abdominal viscera were retracted with warm, moist gauze pads, and the abdominal wall was exposed for chamber placement. The chamber was affixed to the abdominal wall with cyanoacrylate glue. After adhesion, 1 ml of KRB was placed into the chamber for 30 min to allow tissue to recover from the procedure. Warned KRB was dripped onto gauze pads surrounding the chamber and the remaining abdominal tissues to keep them at physiological temperatures. Details of chamber placement and an extensive investigation of effects of the chamber on the tissue can be found elsewhere (5, 8).

All animal procedures were reviewed and approved by the University of Mississippi Institutional Animal Care and Use Committee.

**Experimental protocols.** Figure 1A displays the method for hydrostatic pressure-driven convection, and Fig. 1B displays the method for large- and small-solute transfer and osmotic ultrafiltration. Methods were analogous to our previous techniques in rats (8–10).

The initial experiment was designed to study $^{[14C]}$mannitol transport from the chamber into the tissue and to induce hypertonic fluid flux from the tissue. A preweighed volume of the hypertonic solution containing the labeled mannitol was injected into the chamber. Previous data have demonstrated that fluid flux from the tissue has negligible influence on the diffusion of mannitol into the tissue (10). The height of this volume was <1 cm to minimize any hydrostatic pressure. Chamber solution samples of 20 μl were obtained every 30 min, and the chamber volume was measured by complete withdrawal of the solution every 30 min and then reinjection for a 120-min duration of the experiment. Osmolality of the solution was checked at 60 and 120 min. The osmolality (mosmol/kgH2O) decreased 11–20% over the 2 h and averaged 533 ± 1 for CD-1 and 506 ± 3 for C57BL at the beginning of the experiment and 437 ± 3 (CD-1) and 451 ± 11 (C57BL) at the end of the experiment. At the end of the experiment, the entire volume was removed and weighed. Because there is residual volume left on the walls of the chamber, the residual volume needed to be determined. This was calculated by determining the residual $^{14}$C counts/min (cpm) in the chamber at the end of the experiment and dividing by the final chamber concentration. One milliliter of isotonic Krebs-Ringer without tracer was injected into the chamber to mix with the residual fluid; then it was withdrawn and the total residual cpm were determined by scintillation counting. Dividing the total residual cpm in the chamber by the final concentration (cpm/μl) in the chamber approximates the residual volume in microliters. The typical residual volume was 5–20 μl. At the end of the experiment, the chamber was removed and the chamber area was determined. $^{[14C]}$mannitol was determined by scintillation counting (LS Tri-Carb, Beckman Instrument, Fullerton, CA). To validate the comparison between the rat data, which was obtained with larger chambers (diameter = 1.2–1.3 cm), the measurements of mannitol transport and osmotic filtration with the smaller “mouse chamber” were repeated in SD rats ($n = 9$).

To study the transport of serum albumin from the blood into the chamber, the FITC-BSA solution was injected in a volume of ∼0.3 ml of isotonic KRB intravenously over 2–3 min. The chamber was filled with a preweighed volume of ∼0.6 ml of isotonic KRB. The height was <1 cm to prevent any hydrostatic pressure-driven convection into the tissue (13). The volume of the chamber was checked hourly over 3 h. Thirty-microliter samples of the blood were taken hourly. At 3 h, the volume of the chamber fluid was measured, while the fluorescence was determined for each sample of plasma and the chamber solution.

---

**Fig. 1.** A: tall chamber used in measurement of hydrostatic pressure-driven convection from the chamber, which is filled to 6 cm to exert a constant pressure of ∼4 mmHg. The clearance of labeled protein into the tissue divided by the area of the base of the chamber provides an estimate of the flux per unit area into the tissue. B: smaller chamber used to study small-solute transfer, protein transfer, and osmotic filtration into the chamber. The height of the fluid in the chamber is restricted to <1 cm to preclude the force of convection. UF, ultrafiltration. See text for further details.
(Turner Designs TD700, Sunnyvale, CA). At the end of the experiment, the chamber was removed and the chamber area was determined.

To determine the hydrostatic pressure-driven convection into the tissue, a tall chamber of 7-cm height was utilized and filled with the solution of KRB with 5% BSA plus Evans Blue dye and 125I-IgG. The tracer is a volume marker, and its disappearance correlates with the fluid transfer to the tissue (13). The Evans Blue dye, bound to the albumin, provides a visual check on where the convection occurs within the tissue. Fifty-microliter samples of the solution volume at time 0 and then hourly for 180 min were collected to determine the concentration of the tracer, and the solution was removed hourly and weighed to follow the volume change, as a check for leaks at the chamber base. At 180 min, the fluid was removed and the chamber was removed and its area was measured. After death of the animal, the tissue underlying the chamber, including the muscle and skin in the surrounding area, was removed and counted with a gamma counter (Beckman Instrument, Fullerton, CA) to determine total deposition of IgG into the tissue. The clearance of labeled IgG to the tissue was used to calculate the hydrostatic pressure-driven flux into the tissue (see Eq. 5 below).

Calculations. Statistical calculations were carried out using NCSS 97 (Number Crunching Statistical System, Kaysville, UT). One-way ANOVA was utilized to compare different data sets. Probability of a type I error was set at \( P \leq 0.05 \) for significance. The rat data were obtained in the same manner as these experiments or taken from previous publications for comparison (5, 8).

The following equation (similar to Eq. 1) is fitted to the mass transfer data for mannitol or FITC-BSA using the program Scientist (Micromath, Salt Lake City, UT).

\[
\frac{d(V_{chamber} \cdot C_{chamber})}{dt} = -MTC \cdot A_{chamber}(C_{chamber} - C_{plasma}) \quad (3)
\]

where \( V_{chamber} \) is the volume in the chamber, \( C_{chamber} \) is the tracer concentration in the chamber, \( t \) is time, MTC is the mass transfer coefficient for the abdominal wall, \( A_{chamber} \) is the area of the base of the chamber, and \( C_{plasma} \) is the plasma tracer concentration.

The osmotically induced fluid from the tissue to the chamber (Flux_{osm}) was calculated as follows

\[
\text{Flux}_{osm} = \frac{dV_{chamber}}{dt} = \frac{\Delta V_{chamber}}{\Delta t \cdot A_{chamber}} \quad (4)
\]

where \( \Delta V_{chamber} \) is the change in chamber volume, and \( \Delta t \) is time.

Fluid flux from the chamber into the tissue due to hydrostatic pressure (FluxP)-driven convection is calculated as a clearance term for the IgG from the chamber into the underlying tissue as follows

\[
\text{Flux}_P = -\frac{dV_{chamber}}{dt} = -\frac{\text{Total Tissue CPM}_{IgG}}{\Delta t \cdot C_{IgG} \cdot A_{chamber}} \quad (5)
\]

where \( C_{IgG} \) is the mean concentration of IgG in the chamber (cpm/ml), and total tissue CPM_{IgG} is the total 125I counts from the tissue (including subcutaneous and skin) under and surrounding the chamber.

RESULTS

Figure 2 displays the results for mass transfer of mannitol and the osmotic fluid flux. The one-way ANOVA displayed no significant trends or differences between the CD1 mice, C57BL mice, and the SD rat data (nos. of animals per experiment are noted under each bar). The use of the smaller mouse chamber in the SD rats did not change the results for mannitol mass transfer or osmosis.

Figure 3 displays for the different species the values of the pressure-driven fluid flux and mass transfer coefficient for serum albumin. There were no significant differences among the different species for fluid flux, but there was a statistically significant difference in the mass transfer coefficient for serum albumin. Note, however, that these are relatively small differences, and the results are of similar magnitude.

DISCUSSION

Studies in both rodents and humans have demonstrated that the surface contact area during peritoneal dialysis has a significant effect on the rate of transport. Comparison of whole-
cavity rat studies from different laboratories demonstrates the effects of different peritoneal dwell volumes on the rate of mass transfer. Flessner (6) calculated a MTAC_{urea} of 26.5 ml/h from experiments using 50-ml volumes in 200-g rats. In experiments in rats with an average weight of 350–400 g, a 30-ml volume was used in transport experiments, yielding a calculated MTAC_{urea} of ~8 ml/h (18). Flessner and colleagues (11) showed in measurements in separate sets of animals close correspondence between the measured surface contact area and the MTAC. Keshaviah and colleagues (14) found a linear correspondence between the measured surface contact area and the MTACs for urea, creatinine, and glucose over a range of fill volumes from 0.5 to ~ 3 liters in 10 patients and concluded that this was due to an increasing peritoneal contact area. Chagnac and colleagues (2) studied the solute exchange in 10 patients dialyzed with either 2- or 3-liter dwell. They measured the peritoneal contact area for a 2-liter solution as 0.57 ± 0.03 m^2, which corresponded to a MTAC for creatinine of 10.6 ± 0.7 ml/min; the corresponding values for the 3-liter dwell were 0.67 ± 0.04 m^2 with a MTAC of 13.6 ± 1.2 ml/min. A comparison of the ratio of the MTACs (0.77) with that of the areas (0.85) provides further clinical support for Eq. 1.

Transport data obtained from whole-cavity experiments in mice depend on the osmolality and volume of the solution and vary with the technique employed. Ni and colleagues (16) injected 2 ml of 7% glucose solution into 30-g mice and obtained ~34 μl/min of volume flow into the cavity; the MTAC for urea was calculated to be 28 μl/min. Using the scaling factor of (peritoneal volume)^2/3 (4), a 10-ml volume would increase the surface area by a factor of ~2.9 and would theoretically (see Eq. 2) raise the rate of mass and fluid transfer by the same amount. The discordance between methods used by different laboratories makes comparisons within a species difficult and between the rat and the mouse almost impossible. The range of MTAC_{area} for rats is 8–25 ml/h (6, 18), while the value for mice with a 2-ml volume is ~1.7 ml/h (16). We hypothesized that the differences between experiments within rodent species and between rodent species (rat vs. mouse) were due to the different surface contact areas. Elimination of this variable might simplify experimental comparison and extend data sets from different rodents.

To address the question of similarity of transport between rodent species, we have utilized a scaled-down version of the transport chamber technique that we developed in rats (8) and have applied this to mice. To validate the comparison between results for the rat with a larger chamber and determinations in mice with the smaller chamber, experiments were repeated in rats with the chambers used in mice. For MTC_{mannitol} and Flux_{osm}, no differences were noted. This demonstrates that data obtained from the smaller chambers are a valid comparison with that obtained in the larger chambers.

To address our hypothesis that elimination of the peritoneal contact area as a variable will result in transport rates or parameters, which are of the same order of magnitude, we carried out one-way ANOVAs to compare data from both groups of mice and SD rats. Only one of the parameters measured (MTC_{BSA}) was significantly different. Comparisons with a one-way ANOVA between the rat measurements and either the CD1 mice or the C57BL did not reveal any other significant differences in the MTC_{M}, Flux_{osm}, and Flux_{p}. Because of the differences in Flux_{osm} between C57BL mice and the SD rats, we combined all of the rat data and compared it to the mice data with a one-way ANOVA; with the increase in the number of data points (n = 15 vs. n = 6 or 9), the difference between C57BL and rats became statistically significant. However, there was not a significant difference for Flux_{osm} between the CD1 and SD rats. These variations in statistical analyses are most likely due to the nature of invasive animal procedures, which are subject to variations in animal batches, preoperative state of hydration, and surgical technique of different technicians.

The physiological principle of the importance of the surface contact area is not so much dependent on statistics but on the relative magnitude of the measured variables. All the measurements were well within an order of magnitude for each category. These results further demonstrate that once the surface contact area is removed as a variable, all of these species appear to be quite similar in their transport characteristics for the four different types of transport that occur during peritoneal dialysis. While there is variation in these measurements from animal to animal, our hypothesis of these measurements being of the same order of magnitude has been upheld. With these techniques, we can compare and translate results from mice to rats and rats to mice.

ACKNOWLEDGMENT

The authors greatly appreciate the technical assistance of Ravi Deverkada.

GRANT

This work was supported by US Public Health Service Grant ROI-DK-048479.

REFERENCES


