Supplemental Material for

Phloretin Improves Ultrafiltration and Reduces Glucose Absorption during Peritoneal Dialysis in Rats

Karin Bergling[1], Giedre Martus[1], Carl M. Öberg[1]

[1] Lund University, Skåne University Hospital, Clinical Sciences Lund, Department of Nephrology, Lund, Sweden

Supplemental Material File Listing

Intraperitoneal volume and GFR estimation methodology

Table S1 - Complementary results

Figure S1 – Mizagliflozin effect on volume and small solute transport

Corresponding Author

Dr. Karin Bergling, Njurmottagningen SUS Lund, Barngatan 2a, SE-221 85 LUND, Sweden +4673-52 53 313, karin.bergling@med.lu.se

Intraperitoneal volume estimation using radioactive iodine 125-labeled human serum albumin (RISA)

The intraperitoneal volume during the dialysis dwell was determined using dilution of ^{125}I human serum albumin (RISA) by a modified method described by Zakaria and Rippe.[1] The initial mass (M_I) of RISA in the peritoneal cavity was assessed as instilled mass (M_O) – bound mass (M_B) as follows

$$M_I = 0.956 M_O \quad (1)$$

The mono-exponential elimination of RISA mass from the dialysate was determined from

$$f(k_E, t) = e^{-k_E t} \quad (2)$$

Thus, if the mass M_t (Bq) is known at some timepoint T, then the mass at *t* minutes after this timepoint is $M_t \cdot e^{-kE \cdot (t-T)}$. Accordingly, the intraperitoneal volume at the first sample can be determined from where t_1 is the time between the first sample and the start of dialysis and $C_{RISA,1}$ is the activity of RISA (Bq/mL) in this sample. To calculate subsequent volumes, corrections to the intraperitoneal mass of RISA need to be made to account for lost RISA mass in samples. The intraperitoneal volume at the n:th sample was calculated as follows

$$V_n = \frac{(V_{n-1} - V_{sample,n-1}) C_{RISA,n-1} f(k_E, t_n - t_{n-1})}{C_{RISA,n}} \quad (3)$$

Here $V_{sample,n}$ is the sampling volume of the n:th dialysate sample and t_n is the time at which this sample was collected. The elimination coefficient (k_E) was determined using a root finding algorithm to determine the root of the function $F(k) = V_N(k) -$

$(V_{out} + V_{sample,N})$ where N is the total number of samples. Lastly, to approximate the volume curve had there been no sampling, calculated volumes were corrected for the cumulative sampling volume as follows:

$$V_n = V_n + \sum_{k=1}^{n-1} V_{sample,k} \quad (4)$$

This of course neglects the extra amount of UF that would have resulted from the sampled fluid, but since sampling volumes were small, between 45 to 140 µL, we regard this as a negligible error (underestimating total UF by ~50-100 uL) given other sources of variation in the data. The net ultrafiltration volume was calculated from:

$$UF = V_{out} - V_{in} + \sum_{k=1}^{N} V_{sample,k} \quad (5)$$

Again, N is the total number of samples. To determine the clearance of albumin from the dialysate to plasma, the first-order dissipation of the total intra-vascular mass (M_{RISA}) of ^{125}I-albumin was described by the boundary value problem

$$\frac{dM_{RISA}}{dt} = -TER \cdot \frac{M_{RISA}}{PV} + LM_I f(t)/IPV \quad (6a)$$

$$C(0) = 0 \quad (6b)$$

$$C(t) = PV \cdot C_{P,RISA}(60) \quad (6c)$$

Here TER (min^{-1}) represents the transcapillary escape rate of intravascular ^{125}I concentration (estimated to 10% min^{-1} corresponding to a normal transcapillary escape rate in rats) and L is mass clearance out from the peritoneal cavity; t the total treatment time from the start of filling.[2] The boundary value problem (6a-c) was solved using a shooting method, solving the initial value problem (6a-b) using a 4:th order Runge-Kutta algorithm and then finding the root of $F(k_L)=m(T,k_L)/PV-C_{P,RISA}$.

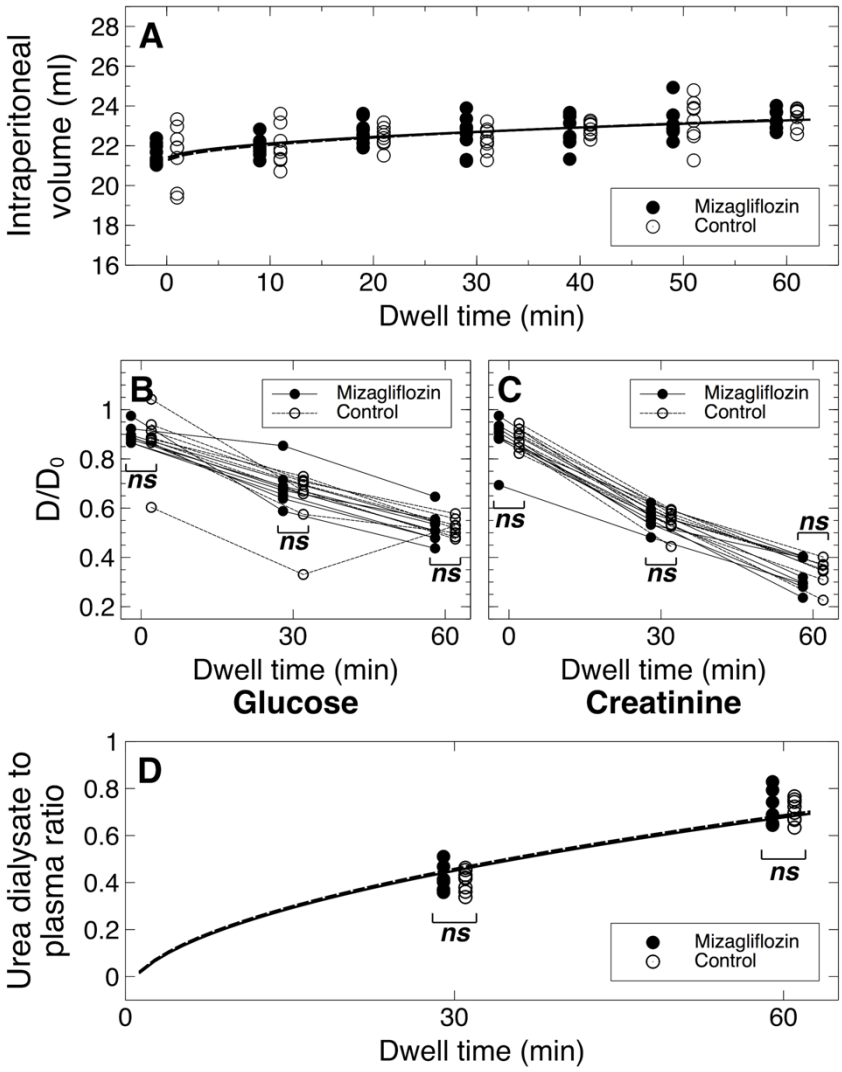
GFR was estimated from the plasma to urine clearance of Cr-EDTA.

Table S1 - Effects of mizagliflozin and phloretin on plasma concentrations, D/D0 and D/P.

	Mizagliflozin Median (IQR)	**Control** Median (IQR)	*P*	95% CI for drug effect	**Phloretin** Median (IQR)	**Control** Median (IQR)	*P*	95% CI for drug effect
Plasma concentrations								
Glucose, mmol/ L								
Pre-dialysis	14 (13 to 15)	14 (13 to 15)	ns	-2 to 2	12 (11 to 13)	13 (12 to 14)	ns	-3 to 1
Post-dialysis	14 (13 to 15)	13 (13 to 14)	ns	-1 to 2	14 (12 to 14)	13 (13 to 14)		-2 to 1
Creatinine, µmol/L								
Pre-dialysis	21 (20 to 22)	19 (18 to 21)	ns	-1 to 3	22 (18 to 24)	18 (18 to 20)	ns	-2 to 5
Post-dialysis	38 (37 to 44)	33 (32 to 33)	*	1 to 13	32 (30 to 34)	33 (28 to 38)	ns	-8 to 6
Urea, mmol/L								
Pre-dialysis	6 (5 to 6)	6 (6 to 6)	ns	-1 to 1	6 (6 to 7)	6 (6 to 7)	ns	-1 to 1
Post-dialysis	7 (6 to 8)	7 (7 to 7)	ns	-1 to 2	7 (6 to 8)	7 (6 to 7)	ns	-1 to 1
Sodium, mmol/L								
Pre-dialysis	136 (134 to 137)	135 (134 to 135)	ns	-1 to 2	136 (135 to 136)	136 (136 to 136)	ns	-1 to 3
Post-dialysis	136 (135 to 138)	136 (135 to 137)	ns	-1 to 2	137 (136 to 138)	137 (136 to 137)	ns	-1 to 2
Chloride, mmol/L								
Pre-dialysis	97 (97 to 99)	98 (98 to 99)	ns	-2 to 1	98 (97 to 100)	100 (98 to 100)	ns	-3 to 1
Post-dialysis	100 (99 to 101)	100 (100 to 101)	ns	-2 to 2	101 (100 to 102)	102 (100 to 103)	ns	-2 to 3

	Mizagliflozin Median (IQR)	**Control** Median (IQR)	*P*	**Phloretin** Median (IQR)	**Control** Median (IQR)	*P*
Solute D/D0						
Glucose						
t = 1 min	0.89 (0.88 to 0.9)	0.88 (0.87 to 0.92)	ns	0.89 (0.86 to 0.9)	0.88 (0.86 to 0.9)	ns
t = 30 min	0.67 (0.65 to 0.7)	0.68 (0.64 to 0.71)	ns	0.66 (0.63 to 0.71)	0.64 (0.64 to 0.66)	ns
t = 60 min	0.52 (0.48 to 0.55)	0.52 (0.5 to 0.54)	ns	0.55 (0.52 to 0.58)	0.49 (0.47 to 0.5)	**
Creatinine						
t = 1 min	0.9 (0.88 to 0.93)	0.88 (0.85 to 0.91)	ns	0.93 (0.92 to 0.93)	0.91 (0.88 to 0.93)	ns
t = 30 min	0.56 (0.53 to 0.58)	0.56 (0.53 to 0.58)	ns	0.57 (0.53 to 0.58)	0.54 (0.51 to 0.58)	ns
t = 60 min	0.31 (0.29 to 0.4)	0.35 (0.33 to 0.37)	ns	0.27 (0.22 to 0.38)	0.32 (0.32 to 0.38)	ns
Urea D/P						
t = 30 min	0.39 (0.36 to 0.43)	0.42 (0.37 to 0.44)	ns	0.36 (0.33 to 0.38)	0.43 (0.38 to 0.46)	ns
t = 60 min	0.69 (0.65 to 0.75)	0.71 (0.67 to 0.75)	ns	0.62 (0.57 to 0.63)	0.73 (0.69 to 0.76)	**

Figure S1



Mizagliflozin effect on volume and small solute transport. A. Intraperitoneal volume as a function of dwell time estimated in mizagliflozin treated animals compared to sham. Solid line represents non-linear regression in drug exposed animals while dashed represent control group. **B-C.** D/D0 ratios of glucose and creatinine at 1, 30 and 60 min. **D.** Urea dialysate to plasma (D/P) ratio at 30 and 60 min in mizagliflozin exposed animals and controls. Solid line represents non-linear regression in drug exposed animals while dashed represent control group.

REFERENCES

1. Zakaria ER, Rippe B. Intraperitoneal fluid volume changes during peritoneal dialysis in the rat: Indicator dilution vs. Volumetric measurements. Blood Purif 13: 255-70, 1995
2. Martus G, Bergling K, Oberg CM. Dual sglt1/sglt2 inhibitor phlorizin reduces glucose transport in experimental peritoneal dialysis. Perit Dial Int 8968608221080170, 2022