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Effects of Furosemide on Body Water Compartments in Infants with Bronchopulmonary Dysplasia[1]

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ABSTRACT. We studied the effects of a single dose of intravenous furosemide on the body water compartments of nine infants with bronchopulmonary dysplasia. We measured total body water, extracellular water, and plasma volume using deuterium oxide, sodium bromide, and Evans blue dye, respectively. From the results of these measurements, we calculated cell water, interstitial water, red cell volume, and total blood volume. We performed these measurements on the first day of the study and again 28 h later, 4 h after an intravenous dose of furosemide (1 mg/kg). All infants had a brisk diuresis in the first hour after the dose, but urine output was no greater during the 24-h period after the dose than during the preceding 24-h period. Total body water, extracellular water, and interstitial water were significantly decreased 4 h after furosemide. There was no change in plasma volume, red cell volume, or total blood volume (*Pediatr Res* 26: 121–124, 1989)

Abbreviations

BPD, bronchopulmonary dysplasia
TBW, total body water
IW, interstitial water
ECW, extracellular water
PV, plasma volume
CBS, corrected bromide space
Br, bromide
IW, interstitial water
BV, blood volume
D_2O, deuterium oxide
ICW, intracellular water
Hct, hematocrit
RCV, red cell volume
OD, optical density

Infants with BPD have interstitial pulmonary edema (1). Furosemide has been shown to improve lung function and speed recovery of these infants (2–4), but the mechanism of these effects is not known. Although the water mobilized and excreted after furosemide administration is assumed to be extracellular in origin, little is known about the effects of furosemide on specific body water compartments in health and disease.

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Studies in adults with pulmonary edema (5–7) have shown variable effects of furosemide on plasma volume. Davidor *et al.* (5) measured plasma vol in patients with refractory edema 2 h after an intravenous dose of furosemide. These patients had a mean decrease of 25% in plasma volume. Figueros and Weil (6) found that the plasma volume of adults with acute cardiogenic pulmonary edema was lower than normal before therapy but rose after treatment with furosemide, oxygen, and morphine. Schuster *et al.* (7) measured blood volume in 21 patients with pulmonary edema who had either normal or decreased renal function. The patients who had normal renal function, as indicated by a good diuresis, had no change in plasma volume 4 to 6 h after a single intravenous dose of furosemide. Patients who did not have a good diuresis had a rise in plasma volume.

There have been no similar studies in infants. Infants with BPD are often fluid restricted with the hope that this will reduce pulmonary edema. It would be of practical value to know whether furosemide causes a decrease in plasma volume in these infants, because hypovolumeemia might compromise organ perfusion and activate the compensatory mechanisms that stimulate water and sodium retention. Our study was undertaken to assess the effects of a single intravenous dose of furosemide on the plasma volume and other body water compartments of infants with BPD.

MATERIALS AND METHODS

Nine infants with BPD were studied (Table 1). Their birth wt ranged from 0.57 to 2.24 kg (median 0.74 kg), with gestational ages from 24 to 36 wk (median 27 wk). At the time of study their body wt ranged from 0.92 to 3.98 kg (median 2.12 kg); their ages were from 31 to 130 d (median 65 d). BPD was diagnosed if an infant still required supplemental oxygen or mechanically assisted ventilation beyond 21 d of age and had typical changes of BPD on chest x-ray. Infants who were receiving chronic diuretic therapy or who had received any furosemide within the previous 48 h were excluded, as were infants with abnormal renal function or major congenital anomalies. The study was approved by the Human Subjects Review Committee of the University of Iowa, and informed consent was obtained from the parents of each subject.

The experimental period lasted 52 h. The rate of fluid intake was not standardized among infants, but in each case the intravenous infusion rate or enteral feeding volume was not changed during the 52-h experimental period. At the beginning of the first day, TBW, ECW, and PV were measured as the dilution volume of D_2O, sodium bromide, and Evans blue dye, respectively. Then 24 h later a single 1-mg/kg dose of furosemide was given intravenously. Four h after this dose, the body water compartments were measured again; 4 h was chosen because most of the diuresis resulting from furosemide was expected within that period (8, 9). The D_2O and Evans blue dye were

administered 4 h after the furosemide. The sodium Br was given 1 h after the furosemide, allowing measurement of Br space 3 h later, at the same time as the determinations of deuterium space and PV. Before each administration of D_2O, sodium Br, and Evans blue dye, baseline urine, serum, and plasma samples were collected. The infant was weighed before the administration of marker substances on the first day.

Deuterium oxide (99.8%, MSD Isotopes, Montreal, PQ) was administered intragastrically in a dose of 1 g/kg except for infant 3, who received this dose intravenously. If a gastric tube was used, the deuterium was flushed through the tube using 3 mL of formula or milk; the regular feeding was then given 20 min later through the same tube. If the intravenous route was used, the stopcock and tubing were flushed with sterile normal saline. The syringe containing the D_2O was weighed to the nearest 0.0001 g before and after the dose was given. Urine samples were collected by positioning the infant on a mattress over a recessed collection vessel. We used a modified colostomy bag to collect urine from the larger infants who were more active. The volume and time of each urine sample were recorded. The last urine collected before the administration of D_2O and aliquots from each spontaneous voiding between 8 and 24 h after administration were saved for analysis of deuterium oxide enrichment. D_2O enrichment in the urine samples was measured using nuclear magnetic resonance spectroscopy (10). After plotting the logarithm of D_2O enrichment in urine *versus* time, the TBW at the time of administration of the D_2O was calculated by extrapolating the urine enrichment back to 0 time using linear regression analysis. The formula for calculation of TBW volume (mL) is shown below.

$$TBW = \frac{D_2O \text{ dose} \times 18.02 \times 0.998 \times 100}{APE\ D_2O \times 20.02 \times 1.015 \times 0.993}$$

where D_2O dose is the wt (g) of the D_2O administered, 18.02 is the mol wt of water, 0.998 is the purity of the D_2O administered, 100 is a factor to convert atom percent to atomic ratio, APE D_2O is the rise in atom percent excess from predosing to the 0-h extrapolated value, 20.02 is the mol wt of D_2O, 1.015 is the correction for deuterium distributed into nonaqueous tissues (11–14), and 0.993 is the specific gravity of water at body temperature.

At the time of D_2O administration on each day of the experiment, sodium Br was also administered either by gastric feeding tube or, in infant 3, intravenously (after the baseline serum sample was drawn). Oral and intravenous administration of sodium Br have been shown to yield similar estimates of ECW in adults (15). The dose used was 4.5 mL/kg of a 0.333 M solution of sodium Br (1.5 mmol/kg). The exact dose given was determined by weighing the syringe to the nearest 0.0001 g before and after administration. Three h later, a single serum sample was taken for bromide analysis. From the time of administration of the dose to the second serum sample, all voided urine was pooled for determination of Br excretion. The excreted Br was then subtracted from the dose injected. Br was analyzed using a spectrophotometric method based on the reaction of Br with fluorescein (16). The ECW was assumed to be equal to the CBS. The CBS (mL) was calculated using the formula below (17).

$$CBS = \frac{\text{Br dose}}{[Br]_s} \times 0.90 \times 0.95 \times 0.94 \times 1000$$

where Br dose is the amount (mmol) of Br given minus the amount excreted in the urine from 0 to 3 h, $[Br]_s$ is the serum Br concentration (mM), 0.90 is the fraction of the dose assumed to remain extracellular, 0.95 is the correction for the Donnan equilibrium, 0.94 is the assumed water content in serum, and 1000 is the factor to convert liters to mL.

ICW (mL) was calculated as the difference between TBW and ECW.

$$ICW = TBW - ECW$$

Plasma volume was determined as the volume of distribution of Evans blue dye (T-1824) administered intravenously. The dye concentration was measured spectrophotometrically using the method of Nielsen and Nielsen (18) as adapted by Parving *et al.* (19) and Linderkamp *et al.* (20). The original method (18) requires that the linear relationship between the negative logarithm of the absorbance of undyed plasma at the wave lengths 620 and 740 nm be established for the spectrophotometer being used. Inasmuch as Evans blue dye has its highest absorbance at 620 nm and no absorbance at 740 nm, the blank OD for each plasma sample can be calculated from the absorbance at 740 nm. We measured these absorbance values for 14 plasma samples from normal infants. The actual absorbance values rather than negative logarithms were plotted against each other (19, 20). The resulting relation is described by the following equation.

$$OD_{620} = 1.40\ OD_{740} + 0.010$$

The OD of Evans blue dye at 620 nm for each plasma sample was then calculated using the following formula.

$$OD_{620}\ (\text{corrected}) = OD_{620}\ (\text{measured}) - [1.40\ OD_{740} + 0.010]$$

where OD_{620} (corrected) is the sample OD at 620 nm (for Evans blue dye alone), OD_{620} (measured) is the sample OD at 620 nm (for dye and plasma) as experimentally determined, and OD_{740} is the sample OD at 740 nm.

A blank plasma sample was collected before administration of Evans blue dye on days 1 and 2. Evans blue dye (4.5 mg/mL, Harvey Laboratories, Philadelphia, PA) is packaged in 5-mL

Table 1. *24-h water balance before and after furosemide administration*

Subject	Gestational age (wk)	Postnatal age (d)	Intake		Output	
			Before furosemide	After furosemide	Before furosemide	After furosemide
			($mL \cdot kg^{-1} \cdot h^{-1}$)		($mL \cdot kg^{-1} \cdot h^{-1}$)	
1	24	46	6.9	6.9	3.6	5.1
2	31	31	5.3	4.6	4.0	2.7
3	26	100	5.2	5.2	1.7	3.2
4	30	65	5.7	5.8	3.6	3.4
5	27	67	6.5	6.4	3.9	4.1
6	26	130	5.7	6.3	2.3	3.1
7	25	60	6.8	6.3	3.8	4.2
8	30	52	7.8	8.0	4.6	4.9
9	36	82	5.7	6.0	1.5	1.5
Mean	28	70	6.2	6.2	3.2	3.6
(SD)	(4)	(30)	(0.9)	(1.0)	(1.1)	(1.1)

vials for injection. We diluted one vial with normal saline 1:5 (vol/vol resulting concentration 0.75 mg/mL) and gave 0.5 mL/kg intravenously at 0 time on the first day. The precise dose given was determined by weighing the syringe to the nearest 0.0001 g before and after administration. Ten min later a second sample was taken. Four hours after the dose of furosemide on the second day, Evans blue dye was given again in a similar dose, with blood samples taken before and 10 min after administration. Each injectate was diluted 1:100 vol/vol with distilled water; the OD at 620 nm and the specific gravity of each resulting solution were measured.

PV (mL) was calculated using the following formula.

$$PV = \frac{\text{injectate OD}_{620} \times 100 \times \text{injectate dose}}{\text{sample OD}_{620}\text{ (corrected)} \times \text{injectate SG}}$$

where injectate OD_{620} is the optical density at 620 nm of the diluted injectate, 100 is the dilution factor for the injectate, injectate dose is the wt (g) of injectate administered, sample OD_{620} (corrected) is the difference between the OD at 620 nm (corrected as described above) of the 10-min sample and the predosing sample, and injectate SG is the specific gravity (g/mL) of the injectate.

IW (mL) was calculated as the difference between ECW and PV.

$$IW = ECW - PV$$

At the time of each measurement of PV, a venous Hct was obtained. The total BV (mL) was then calculated.

$$BM = \frac{PV \times 100}{100 - (Hct \times 0.98 \times 0.90)}$$

where Hct is the venous Hct (%), 0.98 is the correction for trapped plasma (20), and 0.91 is the correction for whole body Hct (21).

Then RCV (mL) was calculated as follows.

$$RCV = BV - PV$$

The body water compartments before and after furosemide administration were compared using the *t* test for paired observations.

RESULTS

All infants had a significant diuresis during the first 4 h after administration of furosemide with the greatest diuresis occurring in the first hour. The mean diuresis during the first 4 h was 7.8 $mL \cdot kg^{-1} \cdot h^{-1}$. The overall urine output for the 24 h after furosemide administration, however, was not different from the previous 24-h period (Table 1).

Tables 2 and 3 illustrate the values for body water compartments before and 4 h after 1 mg/kg of intravenous furosemide. The results in Tables 2 and 3 are expressed in mL and mL/kg initial body wt, respectively. There was a significant decrease in TBW, ECW, and IW. Although intracellular water tended to increase, this change was not significant. PV, BV, and RCV were unchanged.

Serum Br concentrations 3 h after administration varied from 1.35 to 4.81 mM on d 1, with a mean of 2.52 mM (SD 1.12). Serum concentrations 3 h after dosing on d 2 ranged from 3.74 to 7.13 mM with a mean of 5.31 mM (SD 1.05). No adverse effects of D_2O, Br, or Evans blue dye were recognized in any of the infants.

DISCUSSION

The measured diuresis during the 4 h between furosemide administration and body water measurement was, on average, 7.8 $mL \cdot kg^{-1} \cdot h^{-1}$, or 31 mL/kg. This value is considerably less than the mean decrease in TBW of 87 mL/kg, suggesting error in the determination of urine output or change in TBW or both. Unfortunately, body wt was not measured at the correct times

Table 2. *Changes in body water compartments after intravenous furosemide, with compartments expressed in absolute volume (mL)**

Subject		Wt (kg)	TBW	ICW	ECW	IW	PV	RCV	BV
1	Before furosemide	0.92	701	325	376	313	63	21	84
	After furosemide	631	339	292	234	58	21	79	
2		1.47	1300				110	45	155
			1081				105	45	150
3		2.06	1707	592	1115	977	138	60	198
			1466	542	924	783	141	55	196
4		2.34	1732	612	1120	957	163	58	221
			1845	1338	507	339	168	63	231
5		2.28	1817				166	55	221
			1374				162	47	209
6		3.98	3290	1386	1904	1675	229	69	298
			2662	1397	1265	1086	179	52	231
7		1.50	1176	430	746	635	111	54	165
			1138	670	468	346	122	60	182
8		2.12	1664	991	673	505	168	60	228
			1445	779	666	500	166	61	227
9		3.94	2759	1076	1683	1452	231	128	359
			2739	1263	1476	1260	216	101	317

* Bromide space measurements were not done for subjects 2 and 5.

Table 3. *Changes in body water compartments after intravenous furosemide, with compartments expressed in mL/kg initial body wt*

Subject		TBW	ICW	ECW	IW	PV	RCV	BV
1	Before furosemide	762	353	409	340	68	23	91
	After furosemide	686	368	317	254	63	23	86
2		884				75	31	105
		735				71	31	102
3		829	287	541	474	67	29	96
		712	263	449	380	68	27	95
4		740	262	479	409	70	25	94
		788	572	217	145	72	27	99
5		797				73	24	97
		603				71	21	92
6		827	348	478	421	58	17	75
		669	351	310	273	45	13	58
7		784	287	497	423	74	36	110
		759	447	312	231	81	40	121
8		785	467	317	238	79	28	108
		682	367	314	236	78	29	107
9		700	273	427	369	59	32	91
		695	321	375	320	55	26	80
Mean before furosemide		790	325	450	382	69	27	96
(SD)		(54)	(72)	(73)	(76)	(7)	(6)	(11)
Mean after furosemide		703*	384	329*	263*	67	26	93
		(54)	(100)	(71)	(74)	(11)	(7)	(18)

* Significantly lower ($p < 0.05$) after furosemide.

during the study to help resolve this discrepancy. We speculate that our 4-h urine collections underestimated the cumulative diuresis up to the time of deuterium equilibration because of incomplete urine collection and because of urine that had already been cleared by the kidney but not yet voided before the D_2O was administered. It is also possible that the magnitude of the change in TBW was overestimated; however, this seems less likely because the decrease in ECW (corrected Br space) was even greater than the decrease in TBW.

Furosemide has been previously shown to cause an increase in lung compliance and a decrease in airway resistance in infants with BPD (2). These effects were detectable 1 h after an intravenous dose of furosemide but did not persist. Patel *et al.* (22) also found clinical respiratory improvement that occurred only transiently 2 h after intravenous furosemide. The improvement in lung function produced by furosemide has been postulated to result from increased venous capacitance (23) or mobilization of excess IW from the lungs (24). Our finding of reduced whole body IW supports the latter hypothesis. Although we did not measure water compartments beyond 4 h after dosing, the 24-h water balance results suggest that the interstitial dehydration induced by furosemide probably lasted only a few hours; the same would presumably be true of the effects on lung function. Although it is possible that changes in intravascular volume occurred closer to the period of maximum diuresis at 1 h (8, 9), we found no change in PV or calculated BV 4 h after the dose of furosemide.

The exact mechanism of fluid mobilization with furosemide is not known. It presumably results from the direct renal effects of furosemide, but it may also involve drug effects on the secretion of prolactin (25), vasopressin (26), or atrial natriuretic peptide. The mobilized water arises from the interstitial compartment and is not limited to pulmonary IW. Although the increase in cell water was not statistically significant, our results suggest that there may also have been some movement of interstitial water into cells after furosemide administration.

Furosemide acutely decreased TBW and ECW in infants with BPD. This water was derived primarily from the interstitial space, presumably including lung IW. We postulate that the mobilization of IW observed in this study accounts, at least partly, for the acute effects of furosemide on lung function observed by other investigators (2–4).

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