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Research Article

Development of Acute Tolerance to Bumetanide: Bolus Injection Studies

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Bumetanide was administered intravenously to four mongrel dogs, in a random crossover fashion, at doses of 0.05 mg/kg (I), 0.15 mg/kg (II), and 0.5 mg/kg (III) where urinary losses were replaced with lactated Ringer's solution at 1.5 ml/min (hydropenic conditions) or at a dose of 0.5 mg/kg (IV) where urinary losses were replaced with lactated Ringer's solution isovolumetrically (euvolemic conditions). Serial plasma and urine samples were assayed for bumetanide by high-performance liquid chromatography (HPLC) and for sodium by flame photometry. There were no significant differences in the pharmacokinetic parameters of bumetanide among Treatments I–IV. The dynamic parameters E_{max} (maximum effect attributable to the drug) and s (slope factor) were not different between treatments. However, a consistent, demonstrable increase in ER_{50} (urinary excretion rate of drug producing 50% of E_{max}) was observed among Treatments I (2.34 μg/min), II (3.92 μg/min), and III (6.54 μg/min); also, a significant decrease in ER_{50} was observed between Treatment III (6.54 μg/min) and Treatment IV (2.66 μg/min). These results show that hydration status has a marked effect on natriuretic and diuretic response and that tolerance can rapidly develop within a single intravenous dose of bumetanide.

KEY WORDS: acute tolerance; bumetanide; kinetics; dynamics.

INTRODUCTION

The determinants of diuretic response have been rigorously investigated for furosemide in the past and more recently for bumetanide. Animal and human studies have demonstrated that these two loop diuretics exert their effect from the luminal rather than from the peritubular side of the nephron (1–5). As a result, the overall response can be modified by changes in either the total amount of drug delivered to its site of action or the time course of drug delivery. Obviously, an important factor in the dose–response relationship of these drugs involves the regulation of normal salt and water homeostasis (6,7).

In studying the pharmacokinetics and clinical response of bumetanide in healthy subjects and chronic renal failure patients, Lau *et al.* (8) observed that the drug excretion–response curve was significantly shifted to the right for the control group. This was surprising since, at face value, it implies that patients were more responsive to bumetanide than healthy subjects. However, in light of the results concerning the development of acute diuretic tolerance to furosemide (9–11), it seems more probable that a similar phenomenon was occurring here. Since this previous study was not specifically designed to test for tolerance, interpretation of the dose–response data for bumetanide remains speculative.

Therefore, the following objectives were proposed:

(1) to study the kinetics and dynamics of bumetanide as a function of hydration status and
(2) to elucidate the effect of acute tolerance development on the dose–response relationships of this drug.

MATERIALS AND METHODS

Materials

An aqueous solution of bumetanide (lot 8193311811, Hoffmann-La Roche, Inc., Nutley, N.J.) in normal saline was prepared immediately prior to use with the aid of 0.4 *N* NaOH. All other chemicals and solvents were reagent grade or better, as previously reported (12).

Experimental Methods

Four male, mongrel, conditioned, unanesthetized dogs weighing from 21.4 to 27.3 kg received intravenous injections of bumetanide at doses of 0.05 mg/kg (I), 0.15 mg/kg (II), and 0.5 mg/kg (III) where urinary losses were replaced with lactated Ringer's solution at 1.5 ml/min (hydropenic conditions) or at a dose of 0.5 mg/kg (IV) where urinary losses were replaced with lactated Ringer's solution isovolumetrically (euvolemic conditions). Each dog fasted the night before and throughout the entire study period. Bumetanide was intravenously infused (Harvard Compact Infusion Pump, Harvard Apparatus Co., Inc., South Natick, Mass.) over an approximate 2-min period, with the beginning of the infusion being considered time zero. Replacement fluids were given intravenously (IVAC 560, IVAC Corp., San

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Diego, Calif.) The four treatments for each dog were performed in a random crossover fashion and an interval of at least 1 week elapsed between experiments. Bumetanide was administered to each dog at approximately the same time of day for all treatments. Identical lots of drug were used throughout.

Heparinized catheters (Abbocath-T, 18 G × 2 in., Abbott Hospitals Inc., North Chicago, Ill.) were placed in each foreleg of the dogs, one for the administration of bumetanide and replacement fluids and one for obtaining blood samples. Blood samples (5 ml) were collected just prior to bumetanide dosing (blank) and at 3, 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min. Blood samples were centrifuged immediately and the plasma was harvested and frozen. Voided urine was collected predose (blank) via an indwelling bladder catheter (Swan-Ganz flow-directed monitoring catheter, Model 93-111-7F, American Edwards Laboratories, Santa Ana, Calif.) and at 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min after bumetanide dosing. The bladder was flushed with 10 ml of air at the end of each urine collection period to ensure a complete catch. Plasma and urine samples were stored at −20°C until subsequent analysis.

Analytical Methods

Plasma and urine samples containing bumetanide were analyzed using a sensitive and specific high-performance liquid chromatographic (HPLC) assay developed by Smith (12). Plasma and urine samples were assayed for sodium with a flame photometer (Model 455, Corning Medical and Scientific, Medfield, Mass.) and creatinine was determined colorimetrically using a commercial kit (Sigma Chemical Co., St. Louis, Mo.). Plasma aldosterone levels were measured with a commercially available solid-phase radioimmunoassay (Diagnostic Products Co., Los Angeles, Calif.).

Calculations

Plasma concentration–time curves of bumetanide were fit to the general polyexponential equation for post-constant-rate infusion data (13).

$$C_p = \sum_{i=1}^{n} Y_i \cdot e^{-\lambda_i \cdot t} \quad (1)$$

In Eq. (1), C_p represents the bumetanide plasma concentration at time t, n is the number of exponential terms needed for a given data set, Y_i is the coefficient of the ith exponential term for post-constant-rate infusion data, and λ_i is the exponent of the ith exponential term. Initial estimates for the coefficients and exponential terms in Eq. (1) were obtained using the computer program RSTRIP (5); their final estimates were obtained using the nonlinear least-squares regression program NONLIN (14). The number of exponents needed for each data set was determined by the application of Akaike's information criterion (15). The final choice for a weighting scheme (unity, $1/C_p$, $1/C_p^2$) was based upon R^2, COR, and visual examination of the residuals.

Since

$$Y_i = \sum_{i=1}^{n} (1 - e^{\lambda_i \cdot T}) C_i / (-\lambda_i \cdot T) \quad (2)$$

where T is the constant-rate infusion time and C_i is the coefficient of the ith exponential term for bolus intravenous data, once the values of the coefficients and exponential terms in Eq. (1) are determined by computer fitting, the values of C_i in Eq. (2) can be calculated (13).

The following pharmacokinetic parameters were calculated using standard Eqs. (3)–(10) (13,16).

$$V_i = D / \sum_{i=1}^{n} C_i \quad (3)$$

$$Vd_{ss} = D \sum_{i=1}^{n} C_i/\lambda_i^2 / \left[\left(\sum_{i=1}^{n} C_i/\lambda_i\right)^2\right] \quad (4)$$

$$Vd_{area} = D / \left(\lambda z \sum_{i=1}^{n} C_i/\lambda_i\right) \quad (5)$$

$$CL_p = D / \sum_{i=1}^{n} C_i/\lambda_i \quad (6)$$

$$CL_r = A_e(0 - t)/AUC(0 - t) \quad (7)$$

$$CL_{nr} = CL_p - CL_r \quad (8)$$

$$T_{1/2} = \ln(2)/\lambda z \quad (9)$$

$$F_e = CL_r/CL_p \quad (10)$$

In Eqs. (3)–(10), V_1 is the volume of the central compartment; Vd_{ss} is the volume of distribution steady state; Vd_{area} is that volume which, when multiplied by C_p in the log-linear phase, is equal to the amount of drug in the body; D is the intravenous dose (equal to the product of the zero-order infusion rate and the time of infusion); CL_p is the total plasma clearance; CL_r is the renal clearance; CL_{nr} is the nonrenal clearance; $A_e(0\text{-}t)$ is the amount of unchanged drug recovered in the urine after 4 hr; AUC(0-t) is the area under the plasma concentration–time curve from time zero to 4 hr [calculated as $\Sigma(Ci - Yi \cdot e^{-\lambda i \cdot t})/\lambda i$, where $i = 1$ to n and t = 4 hr]; $T_{1/2}$ is the biologic half-life; λ_z is the smallest of the λ_i values; and F_e is the fraction of the dose excreted unchanged in the urine.

Creatinine clearance (CL_{cr}) was calculated by dividing the urinary excretion rate of creatinine by its plasma concentration at the midpoint of the urine collection interval.

The relationship between the sodium excretion rate (E; mEq/min) and the urinary bumetanide excretion rate (ER; μg/min) was evaluated using the sigmoid E_{max} model (17):

$$E = E_{max} \cdot ER^s/(ER_{50}{}^s + ER^s) + E_0 \quad (11)$$

where E_{max} is the maximum effect attributable to the drug, ER_{50} is the urinary excretion rate of drug producing 50% of the E_{max}, E_0 is the baseline effect, and s is the parameter influencing the slope of the dose–effect curve. The unknown parameters (E_{max}, ER_{50}, and s) were determined after each intravenous dose of bumetanide for all four dogs using NONLIN (14) and a weighting factor of unity. E_0 was taken as the baseline sodium excretion rate observed at the end of Treatment I (last four or five data points in the experiment). Due to the counterclockwise hysteresis or time lag for equilibration between the urine and the effect compartments (11,18), the first collection period (0–15 min) was omitted from the fit.

The pharmacodynamic response to bumetanide was reported as the 4-hr cumulative excretion of sodium and urine.

Table I. Pharmacokinetic Parameters for Bolus Injections of Bumetanide

Treatment	ml/kg			ml/min/kg			T_{V2} (min^{-1})	F_e (%)	CL_{cr} (ml/min/kg)
	V_1	Vd_{ss}	Vd_{area}	CL_p	CL_r	CL_{nr}			
I	118	303	668	12.7	5.49	7.18	44.7	46.1	1.45
	(72.2)	(111)	(481)	(4.25)	(1.19)	(3.80)	(39.3)	(14.9)	(0.38)
II	109	311	941	10.8	4.95	5.89	62.2	46.7	1.61
	(26.9)	(72.1)	(602)	(1.70)	(0.68)	(2.04)	(40.0)	(9.9)	(0.40)
III	94.3	363	967	8.42	4.12	4.31	98.7	51.5	1.78
	(44.8)	(89.6)	(303)	(3.41)	(1.56)	(2.58)	(77.8)	(16.3)	(0.32)
IV	102	388	1151	9.80	4.89	4.91	88.1	50.7	4.22
	(57.4)	(169)	(500)	(2.52)	(1.12)	(1.73)	(54.9)	(7.3)	(2.56)

The efficiency of the natriuretic response (Eff) was calculated as follows:

$$\text{Eff} = (\Delta E - \Delta E_0)/\Delta A_e \quad (12)$$

where ΔE and ΔA_e are the amount of sodium and drug excreted in the urine, respectively. ΔE_0 was estimated by multiplying the baseline effect, E_0, by 240 min.

Statistical differences for the effect of the dose on the kinetic and dynamic parameters were determined by a single-factor analysis of variance and a Newman–Keuls multiple-range test. A P value of ≤ 0.05 was considered to be significant. Unless otherwise stated, data throughout the study are expressed as means (±SD).

RESULTS

Plasma concentrations of bumetanide were fit to a biexponential equation for seven data sets and to a triexponential equation for the remaining nine sets. The goodness of fit as determined by R^2 and COR was 0.989 or higher.

The mean values for the pharmacokinetics of bumetanide in all four treatments are displayed in Table I. There were no significant differences in any of the kinetic parameters, although the creatinine clearance (a measure of GFR) is significantly increased in Treatment IV vs Treatment I ($P < 0.05$). This may reflect the intrinsic hemodynamic action of bumetanide in the well-hydrated dog.

The cumulative effects of bumetanide-induced natriuresis and diuresis are presented in Table II. The 4-hr cumulative sodium excretion and urine volume were significantly greater with increasing doses (Treatment III vs Treatment I, $P < 0.005$) for natriuresis and $P < 0.01$ for diuresis) and showed over a twofold increase between the hydropenic conditions of Treatment III and the euvolemic conditions of Treatment IV ($P < 0.001$). This difference between Treatment III and Treatment IV may be indicative of the development of acute diuretic tolerance in Treatment III, as both treatments use an identical bumetanide dose of 0.5 mg/kg. Sodium imbalance also showed an increase as doses of bumetanide were increased in hydropenia; it was a positive value when urinary losses were replaced isovolumetrically in Treatment IV (all four treatments were significantly different from each other). This positive value resulted because, on average, the sodium content per volume of lactated Ringer's is slightly higher than that of urine. In addition, efficiency tended to decrease with increasing dose (Treatments I, II, and III) and increase when comparing Treatment III (hydropenia) and Treatment IV (euvolemia). Although these differences were not statistically significant, the trends observed in efficiency were consistent in all four dogs and may reflect a Type II error due to the limited number of animals studied.

In Table III the results of the pharmacodynamic modeling are shown. Goodness of fit as determined by R^2 and COR was 0.931 or greater. There was no significant difference between treatments in the maximal effect, E_{max}, or in the slope factor, s. However, there was a significant difference in the value for ER_{50} between Treatment III and two of the three treatment groups ($P < 0.025$ for Treatment I vs Treatment III; $P < 0.05$ for Treatment III vs. Treatment IV). Consequently, a parallel shift to the right is observed for the dose–response curves of bumetanide as the diuretic's dose is increased under the hydropenic conditions of Treatments I, II, and III (Fig. 1). A similar shift to the right is observed for the same dose of bumetanide when administered under the euvolemic and hydropenic conditions of Treatments IV and III, respectively (Fig. 2).

The mean values for urine flow rate were found to range from 11.3 to 0.2 ml/min for Treatment I, from 13.8 to 0.5 ml/min for Treatment II, from 11.2 to 2.2 ml/min for Treatment III, and from 17.5 to 7.6 ml/min for Treatment IV.

DISCUSSION

Several studies have demonstrated that the critical determinant with respect to furosemide's natriuretic and diuretic effect is the drug's luminal concentration or amount rather than its plasma concentration. In particular, Kao-

Table II. Cumulative Pharmacodynamic Effects for Bolus Injections of Bumetanide

Treatment	Sodium excretion (mEq/4 hr)	Urine volume (ml/4 hr)	Efficiency (mEq/μg)	Sodium balance (mEq)
I	61.7	522	0.127	−14.8
	(9.89)	(82.5)	(0.046)	(9.7)
II	99.3	824	0.073	−52.9
	(19.3)	(170)	(0.027)	(19.2)
III	147	1194	0.027	−99.6
	(20.8)	(125)	(0.010)	(20.2)
IV	327	2730	0.056	23.9
	(44.5)	(445)	(0.009)	(20.4)

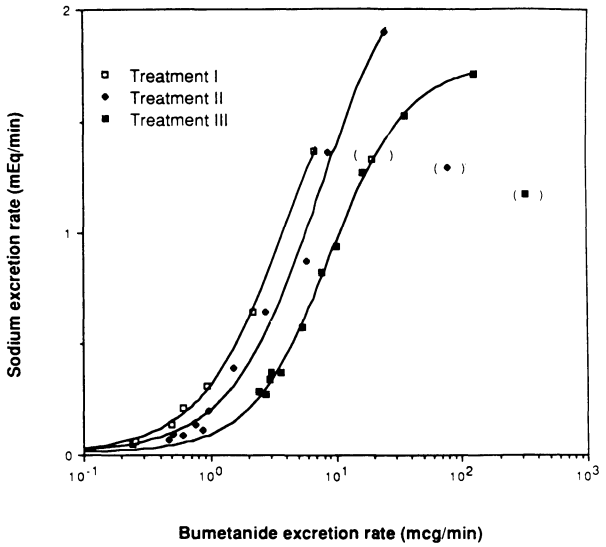

Fig. 1. Dose–response curves for dog 1: (□) 0.05 mg/kg, (◆) 0.15 mg/kg, and (■) 0.5 mg/kg under hydropenic conditions. Data in parentheses were not fit to the sigmoid E_{max} model.

jarern *et al.* (2) elucidated the importance of the "slope factor" (i.e., power term of the sigmoid E_{max} model, s) in determining the contribution of the time course of drug delivery to the overall response of furosemide. This discovery could explain two phenomena: (i) that pretreatment of subjects with probenecid caused a greater overall natriuresis without affecting the total amount of drug excreted into the urine and (ii) that oral doses of furosemide caused the same cumulative natriuretic effect as identical doses administered intravenously, despite delivering only half as much drug to the active site as the intravenous dose. These investigators calculated the furosemide excretion rate with maximum efficiency to be 21.5 μg/min. Since the excretion rate of furosemide was closer to this value for a longer period of time following the oral dose, as opposed to the intravenous dose, a greater effect was obtained for each amount of drug excreted after the oral route (i.e., higher efficiency). However, the concept of a maximally efficient dose or excretion rate, as proposed by Kaojarern *et al.* (2), is valid only if the excretion–response curves after different administrations of diuretic are superimposable. This is not always possible, as discussed below.

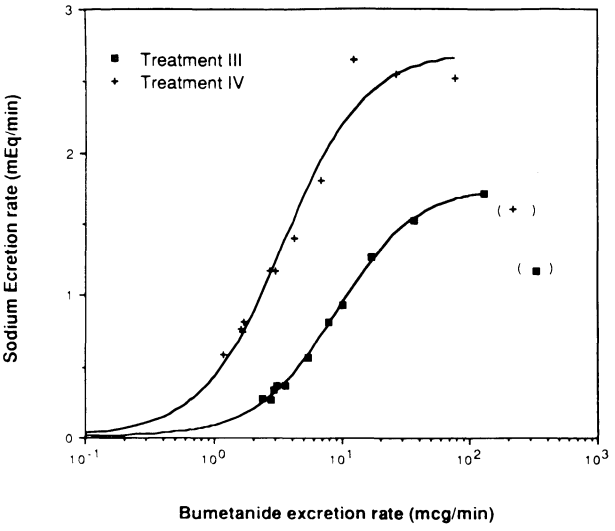

Fig. 2. Dose–response curves for dog 1: (+) 0.5 mg/kg under euvolemic conditions and (■) 0.5 mg/kg under hydropenic conditions. Data in parentheses were not fit to the sigmoid E_{max} model.

In 1985, Hammarlund and Paalzow (9) reported that an acute tolerance developed to the diuretic effect of furosemide in rats. In the first set of experiments, five different groups of rats were administered intravenous doses of furosemide, ranging from 2.5 to 100 mg/kg. In fitting the data to the sigmoid E_{max} model, the ER_{50} of furosemide increased by a factor of 20 as the dose increased. This was reflected by a parallel shift to the right in the furosemide excretion–response curves. In the second set of experiments, these same investigators infused furosemide at a constant rate to achieve steady-state plasma concentrations of 14 μg/ml. They observed that while the furosemide excretion rate was relatively constant or somewhat increasing, the effect gradually decreased over the same 6-hr period. As a result, the efficiency (sodium excretion/drug excretion) was reduced to 20% of its initial value.

Subsequently, Hammarlund *et al.* (10) reported that the acute tolerance observed in rats was also being observed in healthy volunteers. In the human studies, 40 mg of furosemide was administered to eight subjects as intravenous and oral doses (tablet and solution), with and without food. In these experiments, a clockwise hysteresis was noted when the oral doses were taken postprandially. In addition, the drug excretion–response curves showed parallel shifts to the right, depending on the mode of administration of furosemide. As a result, the authors attributed these within-dose and between-dose discrepancies to the acute volume depletion that occurs postdose, resulting in tolerance. It appears that the brisker the initial diuresis, the bigger the acute volume depletion and the more the compensatory mechanisms are triggered in an attempt to preserve the extracellular fluid volume.

Although we do not disagree with these authors' conclusions (9,10), the results may be compromised because the oral replacement of fluid and electrolyte losses were not controlled in one study (9), while fixed volumes of water were orally substituted for urinary losses in the second study (10). As suggested by Li *et al.* (11), the development of diuretic tolerance may depend upon the time and mode of fluid replacement as well as its rate, extent, and composition.

In the present study, bumetanide was administered to mongrel dogs as a function of dose and hydration status under conditions of well-controlled fluid replacement. Although the pharmacokinetics of bumetanide did not differ among treatment groups, dramatic differences were observed for the drug's dynamic parameters. In particular, a consistent, demonstrable increase in ER_{50} occurred over the 10-fold dose range, resulting in a parallel shift to the right in the dose–response curves of bumetanide in Treatments I, II, and III (Fig. 1). This finding is in agreement with previous studies involving furosemide tolerance in the rat (9) and human (10). The shift of the dose–response curve back to the left between Treatment III (hydropenic conditions) and Treatment IV (euvolemic conditions) shows that water and

sodium balance play an important role in the development of acute diuretic tolerance and that this tolerance effect is reversible (Fig. 2). Furthermore, the data clearly demonstrate that the calculation of *one* maximally efficient excretion rate for a diuretic may be an idealized concept, unless strict water and sodium balance is maintained. This condition is highly unlikely in the clinics.

Creatinine clearances were measured in an attempt to see what role, if any, filtration played in acute tolerance development after single intravenous doses of bumetanide. As shown in Tables I and III, creatinine clearances were not significantly different among Treatments I, II, and III even though the mean values for ER_{50} increased almost threefold (from 2.34 to 6.54 mEq/min) as larger doses of bumetanide were administered. In addition, the ER_{50} values for Treatments I, II, and IV were not statistically different despite the higher creatinine clearance observed in Treatment IV. These findings argue against the possibility that changes in glomerular filtration of solute are responsible for the rapid development of tolerance to bumetanide in these bolus injection studies.

Plasma aldosterone levels were also measured in an attempt to explain this tolerance development (Table IV). As observed, when the first 2 hr of Treatments I–IV were compared (a time when tolerance to bumetanide occurred), no obvious relationship could be found between the shift to the right in ER_{50} values and the aldosterone levels (Tables III and IV). However, it should be noted that several samples

Table III. Pharmacodynamic Fits for Bolus Injections of Bumetanide[a]

Dog No.	Treatment	E_{max} (mEq/min)	ER_{50} (μg/min)	s	R^{2b}	COR[c]
1	I	2.07	4.04	1.27	0.999	0.999
		(0.24)	(0.86)	(0.10)		
	II	2.34	7.47	1.20	0.994	0.994
		(0.24)	(1.62)	(0.13)		
	III	1.75	8.89	1.39	0.999	0.999
		(0.03)	(0.37)	(0.06)		
	IV	2.69	3.39	1.36	0.994	0.985
		(0.14)	(0.43)	(0.21)		
2	I	1.66	1.57	1.91	0.999	1.000
		(0.04)	(0.07)	(0.11)		
	II	2.19	3.68	1.40	0.993	0.995
		(0.20)	(0.72)	(0.18)		
	III	1.78	6.83	1.39	0.997	0.995
		(0.07)	(0.55)	(0.10)		
	IV	1.87	3.66	1.76	0.997	0.980
		(0.07)	(0.24)	(0.28)		
3	I	1.27	1.68	1.55	1.000	0.999
		(0.04)	(0.10)	(0.09)		
	II	1.22	2.16	2.00	0.993	0.993
		(0.06)	(0.20)	(0.22)		
	III	1.35	5.24	1.22	0.999	0.998
		(0.04)	(0.36)	(0.06)		
	IV	2.27	1.87	1.65	0.992	0.931
		(0.14)	(0.25)	(0.05)		
4	I	1.17	2.07	1.91	0.998	0.998
		(0.06)	(0.18)	(0.18)		
	II	1.35	2.35	2.14	0.999	0.999
		(0.03)	(0.12)	(0.14)		
	III	1.05	5.19	1.88	0.996	0.988
		(0.04)	(0.32)	(0.25)		
	IV	1.52	1.70	1.71	0.998	0.933
		(0.06)	(0.28)	(0.51)		
Mean	I	1.54	2.34	1.66	—	—
(SD)		(0.41)	(1.15)	(0.31)		
Mean	II	1.78	3.92	1.69	—	—
(SD)		(0.57)	(2.46)	(0.46)		
Mean	III	1.48	6.54	1.47	—	—
(SD)		(0.35)	(1.74)	(0.28)		
Mean	IV	2.09	2.66	1.62	—	—
(SD)		(0.51)	(1.01)	(0.18)		

[a] Data reported as parameter estimates (± SD).

[b] $R^2 = [\Sigma(\text{Obs})^2 - \Sigma(\text{Dev})^2]/\Sigma(\text{Obs})^2$, where $\Sigma(\text{Dev})^2$ is the residual sum of squares.

[c] The correlation between the calculated and the observed sodium excretion rates.

Table IV. Range of Plasma Aldosterone Levels for the First Two Hours After Bolus Injections of Bumetanide

Dog No.	Treatment	Aldosterone (pg/ml)[a]
1	I	<25–63
	II	<25–90
	III	<25–66
	IV	<25–48
2	I	<25
	II	<25–34
	III	<25
	IV	<25
3	I	44–247
	II	<25–129
	III	96–201
	IV	64–104
4	I	<25
	II	<25–117
	III	<25–69
	IV	<25–74
Range	I	<25–247
Range	II	<25–129
Range	III	<25–201
Range	IV	<25–104

[a] Assay sensitivity of 25 pg/ml.

were below the assay sensitivity for plasma aldosterone, thereby making comparisons between treatments difficult.

Although this study has demonstrated that an acute diuretic tolerance does develop to single bolus injections of bumetanide, care must be used in interpreting how this development affects the dose–response curve. For example, the E_{max} is biased toward not changing between treatments since the data points that have the most influence on this parameter are those at the beginning of each experiment. This represents the time when sodium and water losses are lowest and least variable between treatments. Under conditions where hydropenia is produced prior to bumetanide dosing, one may expect this parameter also to change as a function of hydration status.

In summary, acute diuretic tolerance can rapidly develop within a single intravenous dose of bumetanide. Water and sodium depletion play an important role in this development. However, the exact mechanisms that affect the dose–response relationship of bumetanide are unclear. Nevertheless, our data demonstrate that the acute tolerance to bumetanide dosing is not a consequence of changes in the drug's pharmacokinetic properties.

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