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A study of the pharmacodynamic differences between immediate and extended release bumetanide formulations

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Abstract

Optimized bumetanide extended (ER) and immediate release (IR) formulations were developed using fluid bed layering and coating techniques. We postulated that the ER bumetanide formulation would have more effective and sustained diuretic and saluretic effects than IR. The diuretic/saluretic effects of both formulations were measured in rabbits ($n = 8$) for two days after dosing with 1 mg/kg bumetanide. During the first day, both formulations produced 2–3 times more urine volume and sodium excretion than baseline. In the first 24 h, despite less bumetanide excretion from the ER formulation (101 ± 13.9 μg/kg compared to 146 ± 14.6 μg/kg for the IR formulation; $P < 0.04$); the ER formulation produced diuresis and natriuresis that was equivalent to that of the IR formulation. In contrast, urine production in the IR formulation group fell below that of placebo controls on day 2. During the second day, the ER formulation was noted to produce persistent bumetanide excretion; the diuretic and natriuretic effects were not statistically significant from baseline control. We speculate that the decrease in response to bumetanide observed especially for the IR formulation during the second day may be due to the activation of compensatory counter-regulatory homeostatic mechanism(s). We conclude that the ER formulation had similar diuretic/saluretic effects but better drug excretion to urine production efficiencies than the IR formulation in the healthy rabbit model. The ER formulation, while providing comparable diuretic/saluretic effect to the IR formulation, offers the advantage of avoiding the initial, rapid and robust diuretic effect experienced with the IR formulations. Taken together, the data provide sufficient basis to warrant further investigation and refinement of our ER bumetanide formulation in humans.

Keywords: Loop diuretic; Bumetanide; Extended release; Rabbits; Fluid bed; Coating

1. Introduction

The term "loop diuretic" is used to describe a class of drugs that block the reabsorption of sodium, potassium and chloride from the ascending limb of Henle in the kidney nephron. In doing so, the loop diuretics (also known as (a.k.a.) high ceiling diuretics) effect a greater diuresis and saluresis than any other class of diuretic/saluretic drug. Loop diuretics are mainly used to relieve edema associated with congestive heart failure, hepatic cirrhosis and renal impairment diseases (Ives, 1998). Furosemide (a.k.a. frusemide) was the first loop diuretic introduced to the world market in the 1960s. Other loop diuretics include bumetanide,

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torsemide, ethacrynic acid, azosemide, muzolimine, piretanide, and tripamide.

Shaping the diuretic response to loop diuretics for effective use in edemateous patients is important for the successful treatment in these patients who are more resistant to loop diuretics than normal subjects (Brater et al., 1980; Fuller et al., 1981; Beermann, 1984; Voelker et al., 1987; Schwartz et al., 1993; Vargo et al., 1995). Available evidence suggests that slower delivery of loop diuretics to the urinary tubules would improve the efficiency of the drugs (effect per unit stimulus; i.e. urine volume effect in relation to the molecules of loop diuretic excreted in the urine) (Alvan et al., 1990; Rudy et al., 1991; Ryoo et al., 1993). Moreover, the slow delivery of loop diuretic is expected to offer the advantage of avoiding the initial rapid and robust diuresis manifest with both the immediate release oral formulations and intravenous injection. The extended release (ER) formulation could also offer the potential advantage of not evoking compensatory homeostatic countermeasures typically activated by immediate release (IR) or parenteral formulations owing to the slow and sustained loss of water and electrolytes with the ER formulation (Castaneda-Hernandez et al., 1994).

In attempt to deliver loop diuretics in extended and slower fashion to improve their diuretic and saluretic responses, furosemide has been formulated as extended release peroral formulations. However, when tested in humans, ER furosemide formulations have lower diuretic and saluretic responses than IR formulations ($P < 0.05$) (Alvan et al., 1992). The decrease in response is attributed to decrease in the bioavailability of the drug from the extended release formulation, as furosemide is only absorbed from the stomach and the upper part of the small intestine (Alvan et al., 1992; Menon et al., 1994).

Bumetanide, on the other hand, has been shown to be absorbed from different segments of the gastrointestinal tract (Lee et al., 1994). In addition, the bioavailability of bumetanide in humans is not decreased, as it is with furosemide, when co-administered with food (McCrindle et al., 1996). Taken together, these results suggest that bumetanide may not follow the same pattern of absorption as furosemide. Therefore, because of its pharmacokinetic and biopharmaceutical characteristics, bumetanide would appear more suited for ER formulations. Accordingly, we selected bumetanide as the model loop diuretic for this study in which we tested the hypothesis that its peroral ER formulation would have more effective and sustained diuretic and saluretic effects than those produced by an IR formulation.

Multiparticulate immediate and extended release bumetanide formulations with predetermined and optimized in vitro release profiles were developed (Hamed and Sakr, 2001, 2003). The study was designed to compare and contrast the diuretic and saluretic responses to both ER and IR bumetanide formulations in a laboratory animal model.

2. Materials and methods

2.1. Materials

The following materials were used as received: bumetanide (generously donated by American Pharmaceutical International, Cincinnati, OH), bumetanide USP reference standard (USPC, Inc., Rockville, MD), nupariels sugar pellets (CHR Hansen, Vineland, NJ), acetonitrile optima, methanol HPLC grade, glacial acetic acid HPLC grade, water HPLC grade, sodium chloride (Fisher Scientific, Fair Lawn, NJ), IL Test^{TM} flame photometry standard 100 mmol Na/l 100 mmol K/l (Instrumentation Laboratory Company, Lexington, MA), poly-ethylacrylate-methylmethacrylate-trimethylammonioethylmethacrylate chloride (Eudragit RS 30D, generously donated by Rohm GmbH, Darmstadt, Germany), polyvinylpyrrolidone (Plasdone K29/32, International Specialty Products (ISP), Wayne, NJ), talc powder (J.T. Baker, Phillipsburg, NJ), and triethyl citrate (Morflex Inc., Greensboro, NC).

2.2. Methods

2.2.1. Manufacture of bumetanide IR and ER formulations

The details of the manufacture of IR and ER bumetanide formulations are described elsewhere (Hamed and Sakr, 2001, 2003). In summary, bumetanide was layered on nupariel sugar pellets using fluid bed equipment with the Wurster insert (GPCG-1, Glatt Air Technique, Ramsey, NJ). The

loaded pellets were subsequently coated with 6% Eudragit RS plasticized with 20% triethylcitrate using the same equipment. Sodium chloride (5% of the polymer dry weight) was incorporated in the coating dispersion to serve as channeling agent. When the coated particles come in contact with aqueous medium, sodium chloride leaches out creating channels within the film coat. Therefore, it is expected that the level of sodium chloride in the film coat can affect the release rate of bumetanide and can be used to optimize the in vitro release to a predetermined release profile.

2.2.2. Testing the release of bumetanide from coated pellets

The bumetanide pellets were tested for their release profiles in USP purified water using Vankel 7000 dissolution apparatus (Vankel Technology Group, Cary, NC) utilizing the USP XXV basket dissolution method (Apparatus 1) at a rotation speed of 50. Samples removed after 0.5, 1, 2, 4, 8, and 12 h were analyzed for their bumetanide contents using fluorescence spectrophotometer (Hitachi f-2500 fluorescence spectrophotometer, Hitachi Instruments Inc., Naperville, IL) at an excitation wavelength of 326 nm and emission wavelength of 406 nm.

2.2.3. Testing IR and ER bumetanide formulations in laboratory animals

2.2.3.1. Animal study design. Eight male white New-Zealand rabbits (Myrtle's Rabbitry Inc., Thompson Station, TN) weighing 2.1 ± 0.36 kg were used for the study. Each rabbit received both the IR and ER bumetanide formulations according to a randomized two-treatment cross over design with one-week washout period. All studies were conducted in accordance with applicable federal, state and local regulations and research subject guideline consistent with an institutionally approved protocol (#10-10-08-03). Calculations were made so that each rabbit received 1 mg bumetanide per kg body weight. Each rabbit was used as its own control; urine samples were collected for at least 48 h before the administration of bumetanide formulations.

2.2.3.2. Drug administration. Bumetanide multiparticulate formulations were administered orally using 12-french gauge gastric gavage together with 15 ml water. Elizabethan collars were used to prevent drug re-administration by fecal ingestion.

2.2.3.3. Urine sample collection. After dosing with bumetanide formulations, the rabbits were introduced into a metabolism cage specifically designed to separate urine from feces. Urine samples were collected for 48 h after dosing.

2.2.3.4. Analysis of urine samples.

Volume and osmotic pressure. Urine sample volume was measured to the nearest 0.1 ml using 10-ml volumetric cylinder. The osmotic pressure (mOsm/kg) of the urine samples collected was determined in 20 μl aliquots using the freezing point depression method with an Advanced™ 3300 Micro Osmometer (Advanced Instrument Inc., Norwood, MA).

Bumetanide concentration in urine. The bumetanide content in urine was determined using the HPLC method described by Ryoo et al. (1993). The HPLC system consisted of a 126 solvent module and a 507-2 autosampler coupled with a 157 Fluorescence Detector (Beckman Coulter Instruments Inc., Fullerton, CA). Urine samples were centrifuged at $4000 \times g$ for 5 min (Marathon 21K/BR Centrifuge, Fisher Scientific, Fair Lawn, NJ), 0.2 ml of the supernatant was mixed with 0.5 ml acetonitrile by shaking followed by centrifugation at $10{,}000 \times g$ for 10 min. The supernatant was carefully transferred into vials from which 20 μl was injected first through C_{18} guard column (MetaGuard 4.6 mm Inertsil ODS-3 5 μm (particle size), Ansys Technologies Inc., Torrance, CA) then through C_{18} HPLC reverse phase column (Inertsil SDS 5, 3.9 mm × 30 mm, 5 μm particle size, Ansys Technologies Inc., Torrance, CA). The mobile phase consisted of methanol: water: acetic acid in a volume ratio of 70:30:1 and was run at a flow rate of 1 ml/min. Bumetanide was detected using Beckman 157 fluorescence detector using excitation filters of 305–395 nm and emission filters of 350–650 nm wavelengths. The bumetanide peak had a retention time of around 9 min. Chromatograms were recorded using Varian 4270 integrator (Varian Inc., Palo Alto, CA).

Sodium and potassium concentration in urine. Urine samples were analyzed for their sodium and potassium content using flame photometer (Instrumentation Laboratory 943 flame photometer, Instrumentation Laboratory Company, Lexington, MA). The flame was sparked using propane fuel and the equipment was calibrated using standard sodium and potassium solution (100 mmol/l of each). Urine samples were injected into the flame and their sodium and potassium content was measured directly. The equipment was re-calibrated after each 14 samples.

Calculation of diuretic/saluretic efficiency. Efficiency, as a concept, reflects the ratio of useful work done to the total energy expended or heat taken in. In pharmacology the definition of drug efficiency is modified to designate pharmacological effect per unit stimulus (Alvan et al., 1999). For loop diuretics, efficiency can be calculated by dividing the diuretic/saluretic effect by the amount of drug excreted within a specific period of time (Alvan et al., 1990, 1992; Rudy et al., 1991; Yoon et al., 1995). In this study, the diuretic/saluretic efficiency of bumetanide was calculated by dividing the urine volume or urine sodium content by the amount of bumetanide excreted. The diuretic/saluretic efficiency was expressed as ml-urine/μg-bumetanide or mmol-sodium/μg-bumetanide.

2.2.3.5. Statistical analysis. The following statistical analyses were applied to the data:

1. Analysis of variance (ANOVA) using SAS software (SAS Institute Inc., Cary, NC) to test for carryover and period effects as well as to compare the effects of both formulations to control.
2. Paired *t*-test using SAS software to compare the difference in responses to the IR and ER formulations among the rabbits. Paired *t*-test is preferred in cross-over design as it can limit the effect of inter-subject variability by using the variance of the difference in the calculations rather than the variance among each group. The differences were considered significant at *P*-values less than the universally accepted value of 0.05, marginally significant at *P*-values between 0.05 and 0.1, and insignificant at *P*-values above 0.1.

3. Results and discussion

3.1. Optimization of bumetanide in vitro release

The in vitro release profiles of bumetanide from uncoated and coated pellets with and without the incorporation of sodium chloride in the coat are shown in Fig. 1. Coating with 6% Eudragit RS plasticized with 20% triethyl citrate significantly extended the release of bumetanide in water. The release profile obtained was slower than the target profile as seen in Fig. 1. The target release profile was selected as part of in progress in vitro/in vivo correlation study. Three different release profiles were targeted for the in vitro/in vivo correlation study. The release profile selected for this study was the fastest among the three target profiles. Selection was based on our belief that the gastrointestinal residence time in rabbits would be faster than that in humans (Kararli, 1995). Therefore, the faster target profile was selected to assure reliable comparison to the immediate release formulation in terms of the amount of bumetanide released in vivo. Coated pellets with faster in vitro release profile were expected to release most of their drug contents before they reach the lower part of large intestine where drug absorption is known to be limited (Lee et al., 1994).

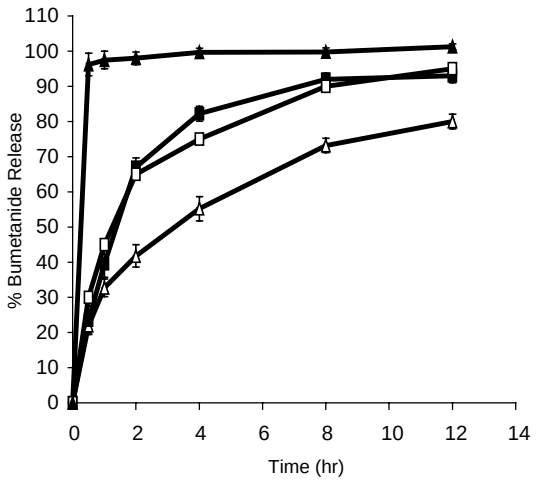

Fig. 1. Effect of incorporating sodium chloride as channeling agent in the coat on the in vitro release for bumetanide from the coated pellets. (▲) Uncoated pellets; (■) coated pellets with sodium chloride; (□) target release; (△) coated pellets without sodium chloride.

The use of water soluble salts as channeling agents in coating systems to accelerate and optimize drug release has been reported in the literature (Ghebre-Sellasie et al., 1987; Bodmeier and Paeratakul, 1991; Tirkkonen and Paronen, 1992). The addition of 5% sodium chloride as the channeling agent accelerated bumetanide release from the coated pellets. As seen in Fig. 1, the predetermined target release profile was intermediate between those of the coated pellets with and without sodium chloride. A 1:1 mixture of coated pellets with and without sodium chloride was prepared and tested for its release in USP purified water. The pellet mixture had a release profile that coincided with the target release profile. The mixture of pellets was used in laboratory rabbits to test our hypothesis that an ER bumetanide formulation would produce a more efficient and sustained diuresis and saluresis than an IR formulation containing an equivalent amount of active drug.

3.2. Comparison of the rabbit responses to IR and ER bumetanide formulations

3.2.1. Urine output

Both IR and ER bumetanide formulations significantly increased the urine output during the first 24 h after dosing compared to control (Table 1). A comparable diuretic effect was produced by both the IR and ER formulations during the first 24 h after dosing (P-value = 0.3570). Statistical analysis using ANOVA revealed no significant carryover effect (P-value = 0.7240) or period effect (P-value = 0.3666). An insignificant carryover effect reflects independence of the response to the second treatment in relation to the response to the first treatment, while an insignificant period effect removes any concern that treatment sequence influenced the outcome.

When the urine flow rate was calculated separately for the first and second 12-h period, the ER formulation maintained constant flow rate during both periods (1st 12 h = 2.0 ± 0.5 ml/h/kg; 2nd 12 h = 2.5 ± 0.5 ml/h/kg; P-value = 0.6606). As expected, the IR formulation produced a rapid and robust diuresis in the first 12 h (3.9 ± 0.2 ml/h/kg) compared with what occurred during the second 12-h period (1.6 ± 0.3 ml/h/kg, P-value = 0.0115).

The diuretic effect during the second 24 h after dosing with the IR formulation was significantly less than control. In contrast, the ER was associated with a comparable diuretic effect to control during the second 24-h period. During the second 24-h period after dosing, animals treated with the ER formulation produced more urine compared to those treated with the IR formulation. When comparing the overall 48-h diuretic effect, both the ER and the IR bumetanide formulations produced equivalent diuretic effects.

3.2.2. Urinary excretion of bumetanide

The amount of bumetanide excreted in the urine after the peroral administration was formulation-dependent (Table 2). During the first 24-h period, more bumetanide was excreted in the urine following

Table 1
Effect of formulation on the urine output (ml/kg) in rabbits (n = 8)

	Control	Immediate release	Extended release
0–24 h			
Average ± standard error	23.0 ± 2.8	66.3 ± 3.8	57.2 ± 4.9
Percentage of the total response		89.8	69.0
P-value (comparison to control)		0.0001	0.0009
P-value (comparison to ER)		0.3570	
24–48 h			
Average ± standard error	21.9 ± 3.3	7.5 ± 1.6	25.7 ± 5.4
Percentage of the total response		10.1	31.0
P-value (comparison to control)		0.0006	0.7891
P-value (comparison to ER)		0.0642	
Total response (0–48 h)			
Average ± standard error		73.8 ± 4.3	82.9 ± 5.2
P-value (comparison to ER)		0.4383	

Table 2
Effect of formulation on the urinary excretion of bumetanide (μg/kg) in rabbits ($n = 8$)

	Immediate release	Extended release	*P*-value
0–24 h			
Average ± standard error	145.8 ± 7.5	101.4 ± 13.9	0.0387
Percentage of the total dose	14.6	10.1	
24–48 h			
Average ± standard error	23.0 ± 4.5	79.1 ± 14.9	0.0215
Percentage of the total dose	2.3	7.9	
0–48 h			
Average ± standard error	168.8 ± 8.7	180.5 ± 7.5	0.2045
Percentage of the total dose	16.9	18.1	

administration of the IR formulation than following the ER formulation. During the second 24-h period, more bumetanide was excreted in the urine of animals treated with the ER formulation than in the urine of animals treated with the IR formulation. Taken together, the 48-h period data shows equivalent amounts of bumetanide excretion for the IR and ER formulations.

Similar to bumetanide excretion, the cumulative urine output was temporally different for each formulation (Fig. 2). The cumulative amount of urine excreted after the IR formulation was produced more rapidly and robustly in the first 16 h than that which followed administration of the ER formulation. In the subsequent period of 16–48 h, urine volume excretion continued in the ER-treated animals, while that in the IR-treated animals abated. The net result of these different urine excretion patterns was that equivalent urine volumes were collected from IR and ER formulation-treated animals over a 48-h collection period. The same pattern was noticed with the cumulative amounts of bumetanide excreted from both formulations. The amount of bumetanide excreted into the urine from the IR formulation rose faster than that of the ER formulation within the first 24 h after dosing. Bumetanide excretion from the ER formulation continued to rise at almost the same rate throughout the following 24 h while that excreted from the IR formulation rose at slower rate. At the end of the 48-h urine collection period, equivalent amounts of bumetanide were excreted from both formulations.

Bumetanide was excreted into the urinary tubules from the ER formulation at a relatively high level during the second 24-h period however, no associated diuretic response higher than control was observed. For the IR formulation, somewhat surprising, less urine output than the daily animal control was obtained during the second 24-h period after dosing. This response is a presumed result of the activation of compensatory homeostatic countermeasures evoked by the more rapid and robust diuresis and saluresis produced during the first 24-h observation period following the administration of the IR formulation. Such compensatory regulatory countermeasures are postulated to include activation of the renin–angiotensin–aldosterone, sympatho-adrenal, natriuretic peptides and possibly other systems that would, individually or in concert with increase sodium and water reabsorption from the distal and collecting tubules, decrease the sustained diuretic/saluretic response to bumetanide (Hammarlund and Paalzow, 1985; Hammarlund et al., 1985; Li et al., 1986; Cook and Smith, 1987; Wakelkamp et al., 1996; Vadlamani and Abraham, 2000). The activation of compensatory mechanisms following single dose administration of loop diuretics has been reported in the literature and described as acute tolerance (Hammarlund and Paalzow, 1985; Hammarlund et al., 1985; Li et al., 1986). When there is inadequate timely replacement of extracellular water volume consequent to urinary loss, homeostatic mechanisms are rapidly brought into play. However, the relative contribution of these homeostatic regulatory countermeasure systems to the development of acute tolerance to loop diuretics remains speculative but highly probable (Wakelkamp et al., 1996).

3.2.3. Sodium and potassium excretion

The subacute (first 24-h) response in urinary excretion of sodium was enhanced, while that of potassium

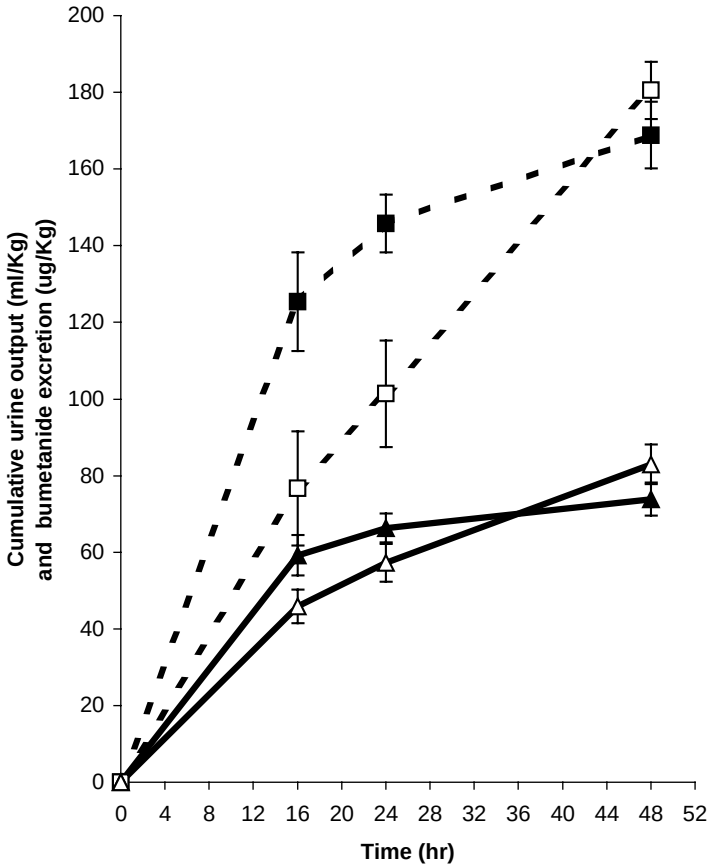

Fig. 2. Effect of formulation on the cumulative urine output (ml/kg) and bumetanide excretion (μg/kg) in rabbits ($n = 8$). (▲) Urine output after the immediate release formulation; (△) urine output after the extended release formulation; (■) bumetanide excreted from the immediate release formulation; (□) bumetanide excreted form the extended release formulation.

was not changed following both IR and ER formulations (Table 3). The amount of sodium excreted within the first 24 h after dosing with either the IR or ER formulation was significantly more than control. The amount of potassium excreted after dosing with either formulation did not significantly differ from control.

The results are in agreement with other reports in the literature where intravenous bumetanide induced significant increase in the amount of sodium excreted while exerted no effect on the amount of potassium excreted in rabbits (Ryoo et al., 1993; Yoon et al., 1995). The insensitivity of potassium excretion to bumetanide was attributed to the constant rate of potassium excretion in the distal tubules (Ryoo et al., 1993). In contrast to rabbits, bumetanide has been shown to increase both sodium and potassium excretion in humans. However, the increase in sodium excretion is higher than that of potassium (Marcantonio et al., 1982). Sodium rather than potassium excretion has been reported to parallel water diuresis in humans particularly during the short period of time after dosing (Marcantonio et al., 1982).

The ER bumetanide formulation had better saluretic efficiency during the first 24 h since comparable amounts of sodium and potassium were excreted despite less bumetanide was available in the urinary tubules. Our results are in agreement with those comparing IR to ER release furosemide formulations. Similar saluretic effects were obtained from both

Table 3
Effect of formulation on the urinary excretion of sodium and potassium (mmol/kg) in rabbits ($n = 8$)

	Control	Immediate release	Extended release
0–24 h (sodium)			
Average ± standard error	2.1 ± 0.2	6.4 ± 0.2	5.4 ± 0.4
P-value (comparison to control)		0.0001	0.0009
P-value (comparison to ER)		0.1064	
24–48 h (sodium)			
Average ± standard error	2.3 ± 0.2	0.3 ± 0.05	0.8 ± 0.2
P-value (comparison to control)		0.0006	0.0070
P-value (comparison to ER)		0.1776	
0–24 h (potassium)			
Average ± standard error	4.9 ± 0.8	3.6 ± 0.2	3.7 ± 0.5
P-value (comparison to control)		0.1991	0.3213
P-value (comparison to ER)		0.8316	
24–48 h (potassium)			
Average ± standard error	5.0 ± 0.4	3.1 ± 0.7	5.8 ± 1.4
P-value (comparison to control)		0.1628	0.6918
P-value (comparison to ER)		0.1065	

formulations despite furosemide was less available to the urinary tubules from ER formulation relative to the IR formulation (Beerman, 1982).

On the second 24-h period (sustained response period) after dosing, IR formulation had lower sodium but comparable potassium excretion to control. Similarly for the ER formulation, less sodium was excreted during the second 24 h compared to control. However, comparable amounts of potassium were excreted compared to control.

During the second 24 h after dosing, more bumetanide was available to the urinary tubules from ER formulation however, it induced comparable sodium and potassium excretion to that obtained from the IR formulation and the natriuretic effect was less than the control value. Again, the results can be explained by considering the activation of compensatory regulatory countermeasures during the second 24-h period with a subsequent increase in sodium and water reabsorption.

Both formulations did not differ statistically in the amount of sodium and potassium excreted during the 48-h period after oral administration (for sodium excretion, *P*-value = 0.1064 for the first 24 h and 0.1776 for the second 24 h, for potassium excretion, *P*-value = 0.8316 for the first 24 h and 0.1065 for the second 24 h).

3.2.4. Diuretic/saluretic efficiency

The diuretic/saluretic efficiency is an estimation of how much effect (i.e. urine output and sodium content) is obtained per unit stimulus (i.e. amount of bumetanide excreted in the urine). The efficiency concept is used to evaluate the performance of different loop diuretics and their various formulations (Alvan et al., 1990, 1992; Rudy et al., 1991; Yoon et al., 1995). It is worth mentioning that the rabbits used in the study were conscious, ambulatory and not catheterized for urine collection. Therefore, rabbits voided voluntarily and irregularly during the 48-h urine collection period. Consequently, there was some variability in the timing of urine collection among the rabbits particularly within the first 12 h after dosing. This variability affects the rigorous calculation and assessment of the diuretic/saluretic efficiency. However, the data collected and presented are highly suggestive that bumetanide ER formulation had better diuretic/saluretic efficiency than IR formulations during the first 24 h after dosing (Fig. 3) (diuretic efficiency = 0.5 ± 0.03 ml-urine/μg-bumetanide/24-h for the IR formulation compared to 1.0 ± 0.3 ml-urine/μg-bumetanide/24-h for the ER formulation; saluretic efficiency = 0.04 ± 0.01 mmol-sodium/μg-bumetanide/24-h for the IR formulation compared to 0.1 ± 0.04 mmol-sodium/μg-bumetanide/24-h for the

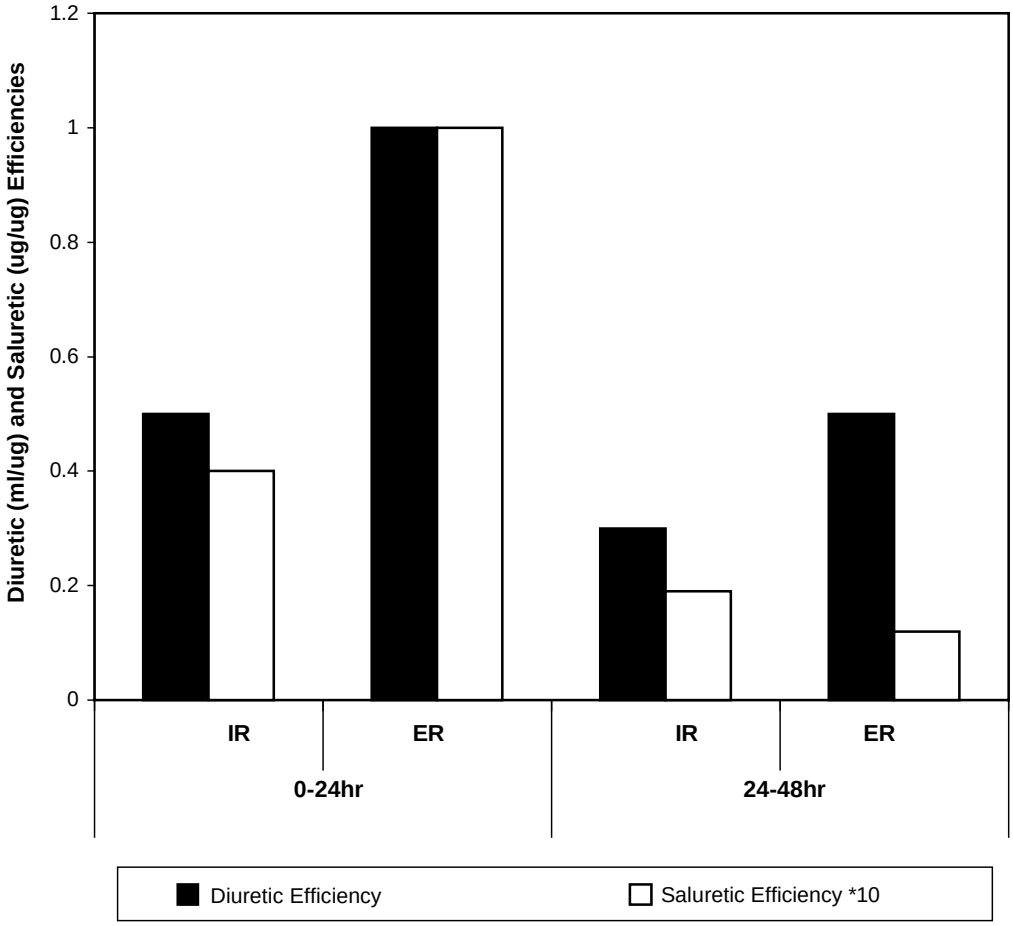

Fig. 3. Effect of formulation on the diuretic/saluretic efficiency of bumetanide during the first and second 24 h after dosing.

ER formulation). The observed trends of differences in efficiencies between the two formulations were not confirmed by statistical analysis (P-value = 0.2855 and 0.2743 for the diuretic and saluretic efficiencies, respectively).

The results suggest that slow delivery of bumetanide from the gastrointestinal tract to the bloodstream and consequently to the urinary tubules improves the diuretic/saluretic effect obtained per molecule of bumetanide excreted in the urine. The findings are in agreement with other reports (Rudy et al., 1991; Ferguson et al., 1997) where the slow infusion of bumetanide to patient with chronic renal failure and congestive heart failure had better natriuretic efficiency than i.v. bolus. Similar results have also been reported for other loop diuretics (Alvan et al., 1992; Van Mail et al., 1992; Piantaud et al., 1995). The results can be explained by considering the concept of diminishing return reported with loop diuretic (Alvan et al., 1999). The concept hypothesizes the decrease in response to loop diuretics compared with the input stimulus (i.e. molecules of loop diuretic excreted in the urine) with the gradual increase in the concentration of the drug at the receptor sites (presumably at the loop of Henle) due to gradual saturation of the receptors (Alvan et al., 1999). The slower increase in bumetanide concentration at the receptor sites from the ER formulation compared to the IR formulation may have led to less receptor saturation and better utilization of the bumetanide molecules excreted

Table 4
Effect of formulation on the urine osmolarity in rabbits ($n = 8$)

	Control	Immediate release	Extended release
0–24 h			
Average ± standard error	1131.1 ± 22.9	482.9 ± 31.8	445.0 ± 8.5
P-value (comparison to control)		0.0001	0.0001
P-value (comparison to ER)		0.4555	
24–48 h			
Average ± standard error	1131.1 ± 22.9	1605.5 ± 136.6	1065.3 ± 89.0
P-value (comparison to control)		0.0608	0.6229
P-value (comparison to ER)		0.0460	

reflected in an improvement of the diuretic/saluretic efficiency.

The diuretic/saluretic efficiency of both formulations decreased during the second 24-h period of urine collection as compared to the first 24-h period. For the IR formulation, diuretic efficiency = 0.5 ± 0.03 ml-urine/μg-bumetanide/24-h for the first 24 h compared to 0.3 ± 0.03 ml-urine/μg-bumetanide/24-h for the second 24 h; saluretic efficiency = 0.04 ± 0.01 mmol-sodium/μg-bumetanide/24-h for the first 24 h compared to 0.02 ± 0.01 mmol-sodium/μg-bumetanide/24-h for the second 24 h. For the ER formulation, diuretic efficiency = 1.0 ± 0.3 ml-urine/μg-bumetanide/24-h for the first 24 h compared to 0.5 ± 0.1 ml-urine/μg-bumetanide/24-h for the second 24 h; saluretic efficiency = 0.1 ± 0.04 mmol-sodium/μg-bumetanide/24-h for the first 24 h compared to 0.01 ± 0.001 mmol-sodium/μg-bumetanide/24-h for the second 24 h. Again the reported differences are trends and were not confirmed by statistical analysis. The decrease in diuretic/saluretic efficiency during the second 24 h after dosing can be explained by our earlier discussion that the activation of compensatory mechanisms in response to water and electrolyte loss during the first 24 h, particularly relevant to the IR formulation, may have led to the sharp decrease in both the diuretic/saluretic response of bumetanide during the second 24-h period of urine collection.

3.2.5. Urine osmolarity

The urine osmolarity was determined using Advanced™ Micro Osmometer and reported as mOsm/kg water (Table 4). The urine osmolarity decreased significantly during the first 24 h after dosing with both formulations. The two formulations were equivalent in reducing the urine osmolarity (*P*-value = 0.4555). The results suggest that bumetanide induces more diuresis than saluresis during the first 24 h.

In the second 24 h after dosing with the IR bumetanide formulation, hyper-osmotic urine was collected (*P*-value = 0.0608 reflecting marginal significance). The ER formulation, on the other hand, maintained iso-osmotic urine excretion compared to control. The results are in agreement with our previous discussion that compensatory mechanisms triggered by water and electrolyte loss during the first 24 h lead to more water reabsorption from the urinary tubules (and perhaps electrolyte retention) during the second 24 h. Consequently, the urine collected during the second 24 h after dosing with the IR formulation was hyper-osmotic compared to control urine. For the ER formulation, bumetanide continued to be excreted during the second 24 h at a level that maintained water excretion at normal level (suggesting a lesser activation of compensatory mechanisms) and therefore, the urine collected during the second 24 h was iso-osmotic with control urine collected prior to dosing with bumetanide formulation.

4. Summary and conclusions

The ER formulation was successful in presenting the drug to the urinary tubules of rabbits in an extended fashion. During the first 24 h after dosing, comparable diuretic and saluretic effects were produced by both formulations despite less bumetanide was excreted from the ER reflecting improvement in the diuretic/saluretic efficiency with the ER formulation. The diuretic/saluretic effects of both formulations

decreased significantly in the second day after dosing. The decrease in response to bumetanide during the second 24 h is postulated to be due, in part, to compensatory mechanisms activation including but not limited to renin–angiotensin–aldosterone, sympathoadrenal and natriuretic peptides in response to water and electrolytes loss during the first 24 h. The exact mechanisms involved and the extent of their contribution are still to be thoroughly investigated.

The results presented here suggest the potential applicability of the ER formulations in improving the diuretic/saluretic efficiency of loop diuretics in general and bumetanide in particular in treating edema associated with congestive heart failure, liver cirrhosis and renal failure. The ER formulation can offer the advantage of providing less intense but sustained diuretic response which can be of great benefit to the patients life style and timing of dosing.

Another aspect of great interest will be the interaction of the developed ER formulation with other medications used in combination with loop diuretic in treatment of edema such as ACE inhibitors and beta-blockers. These drugs inhibit the proposed compensatory mechanisms that reduced the diuretic/saluretic effect of the ER formulation in the second 24 h and therefore, expected to have augmenting interaction with the ER formulation.

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References

Alvan, G., Helleday, L., Lindholm, A., Sanz, E., Villen, T., 1990. Diuretic effect and diuretic efficiency after intravenous dosage of frusemide. Br. J. Clin. Pharmacol. 29, 215–219.

Alvan, G., Paintaud, G., Eckernas, S.A., Grahnen, A., 1992. Discrepancy between bioavailability as estimated from urinary recovery of frusemide and total diuretic effect. Br. J. Clin. Pharmacol. 34, 47–52.

Alvan, G., Paintaus, G., WakelKamp, M., 1999. The efficiency concept in pharmacodynamics. Clin. Pharmacokinet. 36, 375–389.

Beerman, B., 1982. Kinetics and dynamics of furosemide and slow-releasing furosemide. Clin. Pharmacol. Ther. 32, 585–589.

Beermann, A., 1984. Aspects of pharmacokinetics of some diuretics. Acta Pharmacol. Toxicol. 54, 17–29.

Bodmeier, R., Paeratakul, O., 1991. Process and formulation variables affecting the drug release from chlorpheniramine maleate-loaded beads coated with commercial and self-prepared adueous ethyl cellulose pseudolatexes. Int. J. Pharm. 70, 59–68.

Brater, D.C., Chennavasin, P., Seiwell, R., 1980. Furosemide in patients with heart failure: shift in dose–response curves. Clin. Pharmacol. Ther. 28, 182–186.

Castaneda-Hernandez, G., Caille, G., du Souich, P., 1994. Influence of drug formulation on drug concentration–effect relationships. Clin. Pharmacokinet. 26, 135–143.

Cook, J.A., Smith, D.E., 1987. Development of acute tolerance to bumetanide: bolus injection studies. Pharm. Res. 4, 379–384.

Ferguson, J.A., Sudbald, K.J., Becker, P.K., 1997. Role of duration of diuretic effect in preventing sodium retention. Clin. Pharmacol. Ther. 62, 203–208.

Fuller, R., Hoppel, C., Ingalls, S.T., 1981. Furosemide kinetics in patients with hepatic cirrhosis with ascites. Clin. Pharmacol. Ther. 30, 461–467.

Ghebre-Sellasie, I., Gordon, R.H., Nesbitt, R.U., Fawzi, M.B., 1987. Evaluation of acrylic based modified release film coatings. Int. J. Pharm. 37, 211–218.

Hamed, E., Sakr, A., 2001. Application of multiple response optimization technique to extended release formulations design. J. Contr. Rel. 73, 329–338.

Hamed, E., Sakr, A., 2003. Effect of curing conditions and plasticizer level on the release of highly lipophilic drug from coated multiparticulate drug delivery system. Pharm. Dev. Tech. 8, 397–407.

Hammarlund, M., Odlind, B., Paalzow, L.K., 1985. Acute tolerance development to the diuretic effect of furosemide in human: pharmacokinetic–pharmacodynamic modeling. J. Pharmacol. Exp. Ther. 233, 453–477.

Hammarlund, M., Paalzow, L.K., 1985. Acute tolerance development to the diuretic effect of furosemide in the rat. Biopharm. Drug Dispos. 6, 9–21.

Ives, H.E., 1998. Diuretic agents. In: Katzung, B.G. (Ed.), Basic & Clinical Pharmacology, 7th ed. Lange Medical Books/McGraw-Hill, New York, pp. 242–261.

Kararli, T.T., 1995. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dispos. 16, 351–380.

Lee, S.H., Lee, M.G., Kim, N.D., 1994. Pharmacokinetics and pharmacodynamics after intravenous and oral administration to rats: absorption from various GI segments. J. Pharm. Biopharm. 22, 1–17.

Li, T., Lee, M.G., Chiou, W.L., 1986. Effect of rate and composition of fluid replacement on the pharmacokinetics and pharmacodynamics of intravenous furosemide. J. Pharmacokinet. Biopharm. 14, 495–509.

McCrindle, J.L., Likam, T.C., Barron, W.A., Prescott, L.F., 1996. Effect of food on the absorption of frusemide and bumetanide in man. Br. J. Clin. Pharmacol. 42, 743–746.

Marcantonio, L.A., Auld, W.H., Skellern, G.G., Howes, C.A., Murdoch, W.R., Purohit, R., 1982. The pharmacokinetics and pharmacodynamics of bumetanide in normal subjects. J. Pharmacokinet. Biopharm. 10, 393–409.

Menon, A., Ritschel, W.A., Sakr, A., 1994. Development and evaluation of a monolithic floating dosage form for furosemide. J. Pharm. Sci. 83, 239–245.

Piantaud, G., Alvan, G., Eckernas, S.-A., WakelKamp, M., Grahnen, A., 1995. The influence of food intake on the effect of two controlled release formulations of furosemide. Biopharm. Drug Dispos. 16, 221–232.

Rudy, D.W., James, R.V., Greene, P.K., Esparza, A., Brater, D.C., 1991. Loop diuretics for chronic renal insufficiency: a continuous infusion is more efficacious than bolus therapy. Ann. Int. Med. 115, 360–366.

Ryoo, S.H., Lee, M.G., Lee, M.H., 1993. Effect of intravenous infusion time on the pharmacokinetics and pharmacodynamics of the same total dose of bumetanide. Biopharm. Drug Dispos. 14, 245–255.

Schwartz, S., Brater, D.C., Pound, D., Greene, P.K., Kramer, W.G., Rudy, D., 1993. Bioavailability, pharmacokinetics and pharmacodynamics of torsemide in patients with cirrhosis. Clin. Pharmacol. Ther. 54, 90–97.

Tirkkonen, S., Paronen, P., 1992. Enhancement of drug release from ethylcellulose microcapsules using solid sodium chloride in the wall. Int. J. Pharm. 88, 39–51.

Vadlamani, L., Abraham, W.T., 2000. Insights into pathogenesis and treatment of cytokines in cardiomyopathy. Currr. Cardiol. Rep. 2, 120–128.

Van Mail, J.J., Smith, P., Russel, F.G., Gerlag, P.G., Tan, Y., Gribnau, F.W.J., 1992. Diuretic efficiency of furosemide during continuous administration versus bolus injection in healthy volunteers. Clin. Pharmacol. Ther. 51, 440–444.

Vargo, D.L., Kramer, W.G., Black, P.K., Smith, W.B., Serpas, T., Brater, D.C., 1995. Bioavailability, pharmacokinetics and pharmacodynamics of torsemide and furosemide in patients with congestive heart failure. Clin. Pharmacol. Ther. 57, 601–609.

Voelker, I.R., Cartwright-Brown, D., Andreasen, S., 1987. Comparison of loop diuretics in patients with chronic renal insufficiency. Kidney Int. 32, 572–578.

Wakelkamp, M., Alvan, G., Gabrielsson, J., Paintaud, G., 1996. Pharmacodynamic modeling of furosemide tolerance after multiple intravenous administration. Clin. Pharmacol. Ther. 60, 75–88.

Yoon, W.Y., Lee, S.H., Lee, M.G., 1995. Effect of the rate and composition of fluid replacement on the pharmacokinetics and pharmacodynamics of intravenous bumetanide. J. Pharm. Sci. 84, 236–242.