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Research paper

Diuretic bioactivity optimization of furosemide in rats

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ABSTRACT

Furosemide is a loop diuretic widely used by patients with congestive heart failure (CHF) to rid excess body water, reducing blood pressure, and mobilizing edemas. However, due to the narrow window of furosemide absorption, occurring only in the proximal gastrointestinal tract, only immediate release oral formulations are clinically available. Comparisons of bolus and continuous administration of furosemide in intravenous settings demonstrate that continuous administration at lower concentrations produced greater diuretic efficiency and reduced subsequent hospitalization rates in patients experiencing severe CHF. We report a systematic investigation of the diuretic bioactivity profiles of phase inversion micronized furosemide and furosemide co-precipitated with Eudragit L100, as well as their blends with stock furosemide, targeted at reducing the rapid spike in diuresis associated with immediate release formulations while maintaining cumulative urine output. Of the formulations tested, an equal parts blend of micronized furosemide and stock furosemide demonstrated optimal diuretic bioactivity profiles in a rat model.

1. Background and introduction

In its current pharmaceutical formulation, Lasix or furosemide, the most commonly used diuretic for treating congestive heart failure (CHF), is only absorbed in the proximal small intestines [8]. Because it is a weak acid (pKa 3.9), furosemide is protonated only in the acidic lumen of the stomach and proximal small intestines [23]. In the more distal gastrointestinal (GI) tract, furosemide becomes deprotonated and carries a negative charge that significantly reduces its ability to cross biological membranes [8]. Compounding the site specificity of absorption, furosemide has very low water solubility leading to its classification as a class IV narrow absorption window therapeutic [4,16,26].

In keeping with its absorptive properties, furosemide bioactivity is characterized by a sharp onset of diuresis, sometimes referred to as the "Niagara effect" in Depomed press releases, which occurs when furosemide blocks the Na–K–2Cl co-transporter (NKCC) in the thick ascending limb of the kidneys causing diuresis [11]. Patients with CHD experience rapid diuresis daily from Lasix followed by an increase in water intake over the course of the day that leads to peaks and troughs in blood pressure and often leads to patient tolerance of Lasix that requires increased dosing over time [12]. The bioactivity profile of current formulations of furosemide is inconvenient for patients, produces inefficient diuresis, and causes increased renal stress with dose escalation.

Due to similarities in gastrointestinal anatomy and the therapeutic action, rats have been shown to be a good model for humans with respect to furosemide. The primary difference highlighted by previous research comparing intravenously administered furosemide to rats and humans is the markedly reduced potency in rats. Andreasen et al. report that the dose at which rats achieve 60% of their maximal diuretic response is 230 times lower than in humans.

Given the magnitude of the problem, more than 5.8 million Americans are living with CHF, and numerous investigators have created controlled release formulations of furosemide with varying degrees of success [3]. Further evidencing the need for a controlled release formulation, continuous release intravenous administration of furosemide has demonstrated benefit when compared with bolus intravenous control patients in the form of reduced hospitalization to human patients [10,20]. The primary obstacle to creating a controlled release furosemide formulation is the limited residence in the proximal GI. In 1997, Santus et al. studied GR delivery of furosemide to validate a generalized mucoadhesive gastroretentive drug delivery system that relies on the mucoadhesion of a blend of carbomer and hydroxypropyl methyl cellulose first in a rabbit model and then in six healthy human volunteers [21]. Santus et al. observed an insignificant increase in gastric residence time with bioadhesive formulations [21]. In more recent studies, Sakkinen et al. in 2003 and again in 2005 studied the ability of bioadhesive microcrystalline chitosan to increase gastric residence time and reached similar conclusions to Santus et al. [18,19,21].

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In 2000, Ozdemir et al. prepared a floating dosage form of furosemide to enhance bioavailability in six healthy human volunteers [17]. Residence time of the floating pill was determined by radiography [17]. Each subject imbibed 100 ml of water hourly, and although 100 ml of water hourly may not be excessive during diuretic testing, it may dramatically affect the residence time of the floating pill within the stomach [17]. Davis asserts that in the fasted state, very little fluid remains within the stomach, which has led to the irreproducibility and failure of previous floating-pill studies [8]. While Ozdemir et al. present convincing data estimating gastric retention of up to 6 h, Davis indicates that the results are of questionable clinical relevance because of the prescribed drinking schedule [17]. Depomed formulated furosemide into a swelling gel that once ingested became too large to pass through the pyloric sphincter, leading to gastric retention [8]. Phase II clinical results reported in press releases showed inconsistent reduction in urinary urgency in patients with CHF. In addition to gastroretentive strategies, Terao et al. tested the ability of methacrylate-derivative, pH-altering polymers to widen the absorption window of furosemide by lowering the pH in the distal GI [24]. And Shin et al. increased the water solubility of furosemide by cogrinding and co-precipitation with the hydrophilic polymer, crospovidone [22].

While gastric retention of furosemide has proven challenging, spherical crystallization and co-precipitation with pH-altering polymers show great potential to alter the diuretic profile of furosemide without requiring prolonged gastric residence. We focused our efforts on utilizing phase inversion-based precipitation techniques in combination with GI pH-altering polymers and blends with stock furosemide to achieve to reduce the Niagara effect without reducing the total urinary output over a 10 h period in a rat model. Ideally, a linear urine mass output as a function of time profile could reduce the Niagara effect, increase diuretic efficiency, and reduce renal stress without requiring gastric retention.

2. Materials and methods

2.1. Furosemide micronization

Furosemide (Sigma Aldrich, St. Louis, MO) was dissolved in ethyl acetate (Fluka, St. Louis, MO) at a concentration of 4 mg/ml, near its maximum solubility. The furosemide solution was poured into an excess of miscible non-solvent, petroleum ether (Sigma Aldrich, St. Louis, MO), at a volume ratio of 1:20 ethyl acetate to petroleum ether causing phase inversion of the furosemide [6]. Once phase inverted, the petroleum ether suspension is filtered using a Millipore stainless steel filter column (Billerica, MA) fitted with a 0.2 μm mixed cellulose ester filter membrane (Millipore, Billerica, MA). The furosemide retentate is then transferred on the membrane into 50 ml conical tubes topped with Kimwipes (Kimberly-Clark, Mississaua, Ontario) held in place by rubber bands and wrapped in aluminum foil to reduce light exposure. The phase inverted furosemide is then placed inside a lyophilization jar (VirTis, Gardiner, NY) and lyophilized for 24 h until the powder is fully dried. Dried powder is then separated from the filter membrane and stored in amber glass containers to minimize light-induced degradation [25].

2.2. Co-precipitation with Eudragit

Furosemide was dissolved in ethyl acetate as in the precipitation procedure and then mixed with 1 w/v% Eudragit L100 (Rohm GmbH, Darmstadt, Germany) pH-sensitive, acrylic acid derived polymer in ethanol (Sigma Aldrich, St. Louis, MO) to create a 1:1 dissolved mass ratio of furosemide to Eudragit. The furosemide and Eudragit solution was poured into an excess of petroleum ether (Sigma Aldrich, St. Louis, MO), a non-solvent for both furosemide and Eudragit, leading to co-precipitation of the polymer and drug. After co-precipitation, the polymer and drug are filtered and lyophilized as with phase inversion micronized furosemide. Because Eudragit L100 has a pKa of 6.0, at small intestinal pH > 6.0, protonated carboxylic acid residues liberate hydrogen ions locally reducing pH temporarily as the polymer dissolves [7,9,13,15].

2.3. Scanning electron micrograph (SEM) analysis

Conductive, double-sided carbon tape was overlaid on top of aluminum SEM stubs. Dry powder samples of stock furosemide, phase inversion micronized furosemide, and co-precipitated furosemide and Eudragit L100 were transferred onto the carbon tape. The SEM stubs were then sputter coated with 50–100 Å of gold–palladium (Emitech K550, Kent, UK). Each stub was imaged by SEM (Hitachi S-2700, Tokoyo, Japan) with an accelerating voltage of 8 kV. The electron beam was aligned, and digital images were obtained at 1000× and 5000× (Quartz Imaging Corporation, Vancouver, BC).

2.4. Differential scanning calorimetry (DSC) analysis

Three to five milligram of each furosemide powder dosage form was weighed in aluminum sample pans (Perkin–Elmer, Waltham, MA). Each sample was covered with an aluminum lid (Perkin–Elmer, Waltham, MA) and crimped to seal the sample within the pan (Perkin–Elmer, Waltham, MA). Sealed pans were then placed into a DSC7 (Perkin–Elmer, Waltham, MA), controlled by Pyris software (Perkin–Elmer, Waltham, MA). Samples were cooled to −25 °C, then heated to 250 °C, and compared with an empty reference pan to quantify heat flow as a function of sample temperature during thermal transitions.

2.5. Fourier transform infrared spectroscopy (FTIR) analysis

Infrared transmittance of the powdered furosemide samples was measured using total internal reflectance FTIR (Spectrum One, Perkin–Elmer, Waltham, MA). Absorption peaks were labeled using Spectrum software (Perkin–Elmer, Waltham, MA). IR spectra were analyzed to evaluate the presence of functional groups.

2.6. Dose preparation

At the start of each experiment, the rats were weighed. Each formulation was prepared and then loaded into size 9 gelatin capsules using the gelatin capsule filler (Torpac, Fairfield, NJ). Each gelatin capsule was weighed on a microbalance (AD-4 Autobalance, Perkin Elmer, Waltham, MA) prior to and after drug loading to create oral doses of 2.5, 5, or 10 mg of drug per kilogram of body mass within 0.5 mg of dose mass.

2.7. Oral administration

Each rat was induced in an induction chamber with 3.5% isoflurane (Novation, Irving, TX) for 5–10 min. Once anesthetized, the rat was removed from the induction chamber and dosed with a size 9 gelatin capsule containing one of the furosemide formulations using the gelatin capsule dosing syringe (Torpac, Fairfield, NJ). As a negative control, the same rats were administered empty gelatin capsules without furosemide to quantify the basal urine mass output for comparison. Upon recovery from anesthesia, each rat was then transferred to a metabolic cage for urine collection over a 10 h period.

2.8. Diuretic bioactivity

Twelve albino, male, Sprague–Dawley rats (450–750 g) were employed in the bioactivity analysis study. Between dosing with furosemide, rats were housed in standard bedded cages in accordance with NIH and IACUC guidelines. Immediately after oral gavage with a furosemide formulation, rats were housed individually in a metabolic cage rack (Unifab Cages, Kalamazoo, MI). Subjects had access to food and water *ad libitum* throughout the study. Metabolic cages were equipped with wire grating floors that allowed for free passage and collection of excreted material while containing the rat. Feces were caught beneath the large opening wire grating floor by smaller opening wire mesh screen and urine continued through the screen into a funnel for collection in pre-weighed glass scintillation vials (Cole-Parmer, Vernon Hills, IL). At 2 h intervals after dosing, the glass scintillation vials were weighed (Mettler Toledo, Columbus, OH) to quantify urine output as a function of time and then replaced. Increased urine output above baseline values is used as a non-invasive measure of diuretic activity of the various furosemide formulations examined. After each 10 h study, rats were housed in bedded cages for a recovery period of at least 48 h between metabolic cage studies.

3. Results and discussion

3.1. Furosemide dose analysis

Scanning electron microscopy (SEM) shows the angular, crystalline nature of stock, pharmaceutically available furosemide having individual crystals with length of ~5 μm (Fig. 1A, i). Image analysis shows that phase inversion of furosemide alone decreases the majority of crystal lengths, or micronized the furosemide, from ~5 μm in the stock formulation to <1 μm (Fig. 1A, ii). Co-precipitation of furosemide with L100 in equal masses results in more needle-like crystal formation than stock furosemide (Fig. 1A, iii). SEM reported by Aceves and Hernandez of furosemide dissolved in methanol and phase inverted by evaporation also resulted in reduced crystal size [1].

Differential scanning calorimetry (DSC) shows that the thermal decomposition temperature of stock furosemide, 217 °C, is unchanged by phase inversion micronization (Fig. 1B) [5]. Eudragit L100, as a thermoplastic polymer, exhibits a glass transition temperature at 65 °C and a melting temperature at 220 °C. Due to the similarity of the thermal decomposition temperature of furosemide and the melting temperature of L100, mixed formulations show the beginning of an endothermic melting transition interrupted by the exothermic decomposition. Both the co-precipitated and physically mixed solid dispersion of furosemide and Eudragit L100 demonstrate very similar thermal behavior, indicating that neither co-precipitation nor physical mixing yields covalent bonding.

FTIR analysis of phase inversion micronized furosemide shows no substantial difference from stock furosemide (Fig. 1C). L100 has a broad peak at 1705 cm^{-1}, corresponding to carbonyl stretching that is not present in either the co-precipitated or physically mixed formulations. Disappearance of the carbonyl peak indicates possible interaction and stabilization by the amine group of the furosemide. Aceves and Hernandez report that both solid dispersion and precipitation of furosemide with Eudragit R/L-100 showed a loss of the amine peak at 3400 cm^{-1} and translation of the 1900 cm^{-1} carbonyl peak indicating a secondary interaction between the quaternary ammonium groups of R/L-100 and the carbonyl groups of furosemide [1].

Each of the furosemide formulations analyzed was then administered at varying doses alone and mixed with stock furosemide and were compared with basal urine output and stock furosemide to quantify changes in the bioactivity profiles resulting from the physiochemical alterations induced by phase inversion.

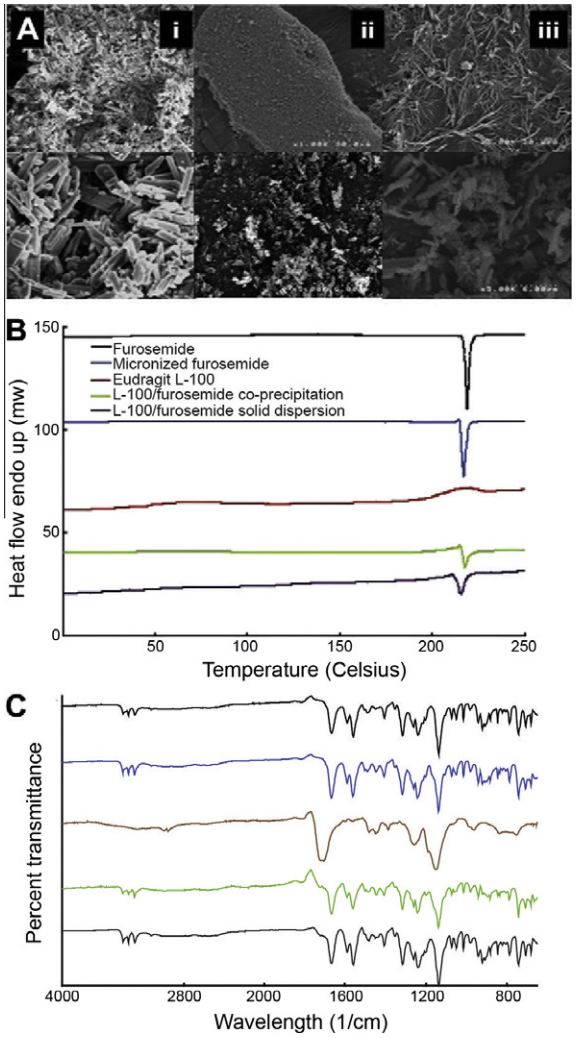

Fig. 1. Characterization of oral furosemide doses. (A) Scanning electron micrographs of stock furosemide (i), micronized furosemide (ii), and furosemide co-precipitated with Eudragit L-100 (iii) at 1000× (top) and 5000× (bottom) magnification. Rod-like crystals shown in (i) are characteristic of stock furosemide [1]. The crystal size appears smaller in the micronized doses (ii) and more needle like when co-precipitated with Eudragit L-100 (iii). (B) Plot of heat flow as a function of temperature acquired by differential scanning calorimetry of oral furosemide doses. Stock and micronized furosemide thermally decompose ~220 °C. Eudragit L-100 undergoes glass-to-rubber transition ~65 °C and melts ~220 °C. When Eudragit and furosemide are co-precipitated or physically mixed into a solid dispersion, the glass transition and melting temperature of L-100 are still apparent, and the polymer melt is overtaken by the thermal decomposition of furosemide both occurring at ~220 °C. (C) Stacked line plot of percent transmittance as a function of wavelength acquired by Fourier transform infrared spectroscopy of the same oral doses analyzed in (B). Stock and micronized formulations show nearly the same absorption pattern. Eudragit L-100 shows similar absorption to furosemide with the most notable exceptions of the additional peak at 1705 cm^{-1} and lack of peaks ~3400 cm^{-1}. Both the co-precipitated and physically mixed formulations show similar absorption patterns, indicating that neither formulation yields additional chemical bonds, although physical bonding between furosemide and L-100 remains plausible.

3.2. Dose escalation

In order to quantify how phase inversion micronized and L100 mixtures with furosemide altered urine output as compared with stock furosemide, rats were orally dosed gelatin capsules contain-

ing furosemide doses and housed in metabolic cages enabling urine isolation and collection every 2 h for a total of 10 h. Each dose was administered to three rats, and the average urine mass produced in the 2 h time periods is reported as compared with the average output of the same rats that received a sham dosage without any furosemide, referred to in the figures as the basal output. To determine the minimum necessary dose required to sufficiently emulate the Niagara effect, a dose escalation study was performed. The bioactivity profile of stock furosemide was compared with that of micronized furosemide and an equal parts mixture of stock and micronized furosemide. Additionally, the bioactivity response of stock furosemide was compared with that of furosemide co-precipitated with Eudragit L100, an equal parts mixture of stock furosemide and co-precipitated furosemide with L100, and a physically mixed solid dispersion of equal parts stock furosemide and L100. All furosemide formulations produced greater cumulative urine output 10 h after dosing than basal output at 2.5 mg/kg (Table 1) and 5 mg/kg (Table 1). Additionally, 5 mg/kg doses produced greater urine output than 2.5 mg/kg doses. However, the trends were statistically insignificantly different from basal output ($p > 0.05$), indicating that higher furosemide dosing was required to mimic the bioactivity profile observed in humans. The stock furosemide formulations are meant to represent commercially available furosemide formulations. Unlike commercial formulations, stock formulations administered in this study do not contain excipients and are administered in gel cap rather than tablet form.

When the dose was increased to 10 mg/kg ($N = 3$), the mean urine mass output 2 h after dosing was statistically significantly 12.2× greater than that of basal urine output and 8.6× greater than the output produced after administration of an equivalent dose of micronized furosemide ($p < 0.01$). The sharp increase in urine production observed 2 h following administration of 10 mg/kg furosemide mimics the Niagara effect reported in clinical use. Yet by hour 10, the cumulative urine output caused by the administration of stock and micronized doses were statistically insignificantly different with the stock furosemide producing 1.02× the total urine output of the micronized dose (Fig. 2A). Therefore, the micronized dose demonstrated similar diuretic activity without the Niagara effect in hour 2. However, the increase in cumulative urine output in hour 4 of the micronized dose indicates that micronization alone may merely delay the Niagara effect. Given the delay in the Niagara effect produced by micronization, combinations of stock furosemide and micronized were mixed at lower doses to produce a combined effect of maintaining diuresis while reducing the Niagara effect. Toward that end, the equal parts mixture of stock and micronized furosemide demonstrates 47% less average urine output at hour 2 than stock.

While phase inversion micronization reduces crystal size as compared with stock, it also causes re-crystallization of dissolved furosemide in the hydrophobic non-solvent, petroleum ether. Crystallization of furosemide in hydrophobic media may yield a more hydrophobic molecular organization to minimize interfacial energy with the non-solvent. Therefore, although phase inversion reduces particle size, it may also increase hydrophobicity leading to delayed water dissolution that corresponds to the delayed onset of pharmacological action.

With the administration of co-precipitated and solid dispersion furosemide and L100 doses at 10 mg/kg, the co-precipitated dose produces 1.28× the diuresis at 2 h than the stock furosemide (Fig. 2B). The addition of L100 may temporarily, locally reduce pH increasing the amount of time that the furosemide spends in the protonated state, which is more apt to cross biological membranes than the anionic, deprotonated form. L100 may also act as a bioadhesive promoting prolonged intimate contact of furosemide with the GI mucosa as the crystals hydrate and dissolve. Administering 10 mg/kg of L100 alone did not significantly increase urine output above basal levels, indicating that the polymer alone has little or no effect on diuresis. The physical mixture of equal parts stock furosemide and L100 produced 0.15× the mean urine output of stock furosemide at 2 h and 0.96× the mean cumulative diuresis after 10 h of stock furosemide.

Table 1
Summary of cumulative urine output at 2 h and 10 h after oral dosing with 2.5 and 5 mg/kg control and experimental doses of furosemide formulations. The initial time point is indicative of the rapid diuretic response associated with the control formulation, and the cumulative 10-h time point is a basis of comparison for determining whether the experimental formulations achieved similar levels of diuresis to the control formulation, which mimics clinically available oral formulations.

Dose (mg/kg)	Description	Urine output 2 h after dose (g)	SEM	Cumulative urine output 10 h after dose (g)	SEM
0	Basal	0.47	0.22	4.33	0.74
2.5	Stock furosemide	0.87	0.41	6.77	2.77
2.5	Micronized furosemide	1.27	0.55	9.03	2.03
2.5	50% Stock:50% micronized furosemide	0.47	0.37	5.03	0.52
2.5	L-100 co-precipitated furosemide	0.33	0.20	7.87	2.65
2.5	50% L-100:50% stock furosemide	0.13	0.13	4.50	0.31
2.5	50% Stock 50% L-100 co-precipitated furosemide	0.90	0.71	5.10	1.76
5	Stock furosemide	0.23	0.12	7.50	2.02
5	Micronized furosemide	0.90	0.47	6.87	1.37
5	50% Stock 50% micronized furosemide	2.50	1.57	11.83	2.64
5	L-100 Co-precipitated furosemide	0.87	0.62	11.03	4.01
5	50% L-100: 50% stock furosemide	2.97	1.49	9.40	3.18
5	50% Stock 50% L-100 co-precipitated furosemide	0.73	0.23	7.53	0.50

3.3. Bioactivity optimization

The two lead candidate formulations, 10 mg/kg of an equal parts mixture of stock and micronized furosemide and a solid dispersion of equal parts stock furosemide and Eudragit L100, were administered to six additional rats for a total of $N = 9$ to directly compare the doses with a larger cohort (Fig. 3). Both doses continued to demonstrate reduced diuresis compared with stock furosemide at hour 2 and similar diuresis to stock furosemide at hour 10. The mixture of stock and micronized furosemide produced less of a Niagara effect than the stock and L100 mixture with 0.56× the mean urine output at hour 2. Additionally, at hour 10, the mixture of stock and micronized furosemide produced 0.95× the cumulative diuresis of the same dose of stock alone. By the hypothesized parameters for the optimal bioactivity profile, the equal parts mixture of stock and micronized furosemide performed the best of all formulations tested. It is important to note that because the urine was collected every 2 h ($\Delta t = 2$ h) and the formula for calculating the area under the urine output bioactivity curve (AUC) using the trapezoid method is $AUC_{t2} = \frac{Output_{t1} + Output_{t2}}{2} * \Delta t$, the AUC has the same magnitude as the cumulative urine mass output. Therefore, the plot of cumulative urine output over time has the same shape

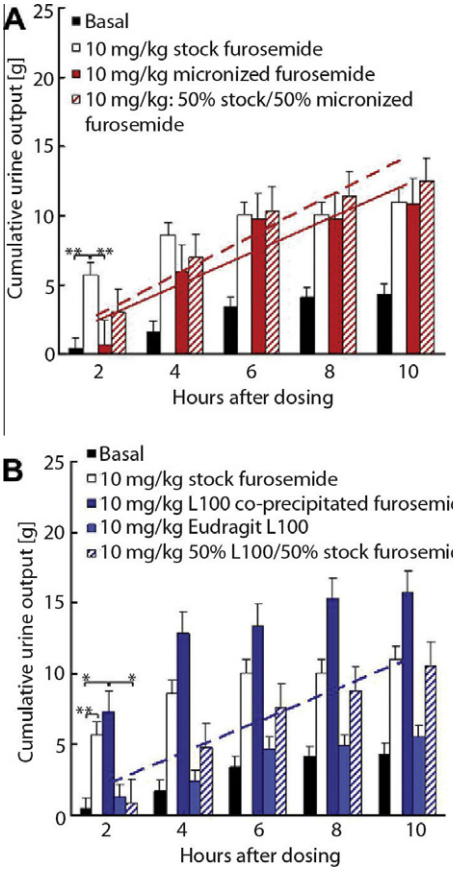

Fig. 2. Comparison of bioactivity in response to 10 mg/kg oral doses of micronized and L-100 mixed formulations. (A) Stock furosemide induces a statistically significantly higher urine output 2 h after oral dosing than in response to micronized and basal output with 10 mg/kg doses ($p < 0.01$, $N = 3$). Increased urine output within the first 2 h mimics the Niagara effect experienced clinically, indicating that 10 mg/kg is the appropriate dose for testing the effectiveness of formulations to reduce the Niagara effect without reducing cumulative urine output 10 h after administration. Both micronized and an equal parts mixture of stock and micronized cause less urine output than stock furosemide at 2 h, and both produce similar cumulative diuresis by hour 10. (B) Although co-precipitated furosemide and L100 produce the greatest cumulative diuresis, it also produces the greatest diuresis at hour 2, statistically significantly greater than basal and that produced by a physically mixed solid dispersion of L100 and stock furosemide ($p < 0.05$, $N = 3$). Therefore, the co-precipitated dose produces a greater Niagara effect than stock furosemide. Error bars depict s.e.m, $^{*}p < 0.05$, $^{**}p < 0.01$.

as the cumulative AUC over time plot with the only difference being that urine mass output is measured in grams while AUC is expressed in gram $*$ hours.

3.4. Clinical potential

Physiochemical analysis by FTIR and DSC indicates that furosemide does not undergo any chemical change in response to phase inversion micronizatin, co-precipitation with L100, or physical mixing with L100. Therefore, the safety master file from the widely clinically used furosemide should apply to the described doses. The lack of a spike in urine output within 2 h of oral administration observed in the equal parts mixture of stock and micronized, as well as the equal parts mixture of L100 and stock, has excellent clinical potential. Unlike a previous study by Terao et al. that reports the bioavailability of 15 mg/kg aqueous furosemide solution co-administered with 400 mg/kg aqueous

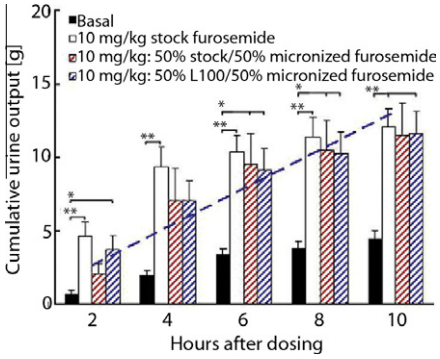

Fig. 3. Bioactivity response to the leading micronized and L100-incorporated oral furosemide dose candidates tested in an increased size cohort ($N = 9$) to determine the optimal oral furosemide formulation that reduces the Niagara effect, while maintaining cumulative diuresis at 10 h. Both the equal parts mixture of micronized and stock furosemide, and L100 and stock furosemide produce less urine output than stock furosemide alone at hour 2 and similar cumulative urine output at hour 10. Error bars depict s.e.m, $^{*}p < 0.05$, $^{**}p < 0.01$.

Eudragit L100–55 solution, the orally administered doses contain at most 10 mg/kg Eudragit polymer [24]. While Eudragit polymers are well tolerated in clinical practice, minimizing Eudragit incorporation is important in a clinical, daily dosing regimen. Additionally, the Terao et al. study utilizes an isolated loop such that the pH of the entire loop is significantly altered by the incorporation of Eudragit L100-55 [24]. In this study, furosemide is delivered orally in a gelatin capsule with little L100 polymer and therefore is unlikely to significantly alter the pH of a large segment of the intestines. Instead, we hypothesize that physical bonding occurs between the carbonyl groups of the L100 and the amine groups of furosemide promoting physical proximity within the intestines. The L100 may locally reduce pH and supply hydrogen ions to protonate furosemide at higher pH than the drug alone [15]. Additionally, the temporary bioadhesiveness of Eudragit L100 prior to its dissolution as an acrylic acid derived polymer may serve to promote intimate contact of the furosemide with the absorptive epithelium [14,21].

Al Gohary and El Gamal administered furosemide and Eudragit R/L-100 to humans and it reduced the Niagara effect at a dose of constant mass dose of 40 mg of furosemide in healthy human volunteers [2]. However, the cumulative urine output 10 h after oral administration was only ~53% of the stock furosemide dose. Therefore, higher furosemide doses would be required to achieve the same diuretic efficiency. If the bioactivity profile translates from the small animal trials conducted in this study to the clinic, the described doses have the potential to reduce the Niagara effect and maintain diuretic efficiency obviating the need for higher doses to achieve similar diuresis.

Regarding translational potential, rats have been shown to be a good model for humans with respect to diuretic activity following oral and intravenous administration of furosemide. Andreasen et al. provide a careful analysis of the pharmacokinetics and dynamics of intravenously administered furosemide. Furosemide was found to be 230 times less potent in rats than in humans with respect to the dose required to produce 60% of the maximal diuretic output. Based on that correlation with the assumptions provided therein, the 10 mg/kg dose administered to rats roughly correlates to a 3 mg dose administered to a 70 kg human. A 3 mg dose of furosemide is on the order of a daily dose administered to a patient beginning diuretic treatment for a non-severe case of congestive heart failure.

4. Conclusions

Mixtures of phase inversion micronized and stock furosemide demonstrated the ability to reduce the Niagara effect while maintaining diuretic efficiency in rats. Moreover, an equal parts mixture of stock and micronized furosemide not only reduced the Niagara effect, but also produced similar cumulative urine output 10 h after oral administration as compared with stock furosemide alone. If the optimal bioactivity profile observed in rats, produced by mixtures of stock furosemide with micronized furosemide, translates to larger animals and humans, it may improve diuretic efficiency, reduce the risk of ototoxicity, and ameliorate concerns of acute tolerance currently associated with clinical use of furosemide.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2011.04.014.

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