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Rapid communication

Pulmonary delivery of a GLP-1 receptor agonist, BMS-686117

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ABSTRACT

Alternate delivery route of therapeutic peptides is an attractive non-invasive option to patients who must chronically self-administer their medication through injections. In recent years, much attention has centered on pulmonary peptide delivery of peptide drugs such as insulin and GLP-1 mimetic peptides in the treatment of type II diabetes. In this study, we assessed the feasibility of delivering BMS-686117, an 11-mer GLP-1 receptor peptide agonist, to the lung in rats via intratracheal administration. The pharmacokinetic profiles of three spray-dried, prototype inhaled powder formulations, 80/20 BMS-686117/trehalose (I), 100% BMS-686117 (II), and 20/80 BMS-686117/mannitol (III), as well as a lyophilized BMS-686117 powder, were compared with intravenously and subcutaneously administered peptide. The spray-dried formulations were mostly spherical particles with narrow particle size distribution between 2 to 10 μm, which are better suited for inhalation delivery than the lyophilized, irregular shape powder with a wide particle size distribution between 2 to 100 μm. Prototype III exhibited the best physical characteristics and *in vivo* performance, with bioavailability of 45% relative to subcutaneous administration. The T_{max} for lung delivered peptide formulations were almost twice as fast as subcutaneous injection, suggesting potential for rapid absorption and onset of action. This study demonstrated that pulmonary delivery is a promising, non-invasive route for the administration of BMS-686117.

Glucagon-like peptide-1 (GLP-1) is a 30-mer peptide hormone secreted by intestinal L-cells that bind to GLP-1 receptor on the β-cell and stimulates insulin secretion in response to ingested carbohydrates and lipids (Elliott et al., 1993; Drucker, 2001). GLP-1 has a number of advantages that make it a highly promising therapeutic agent for type II diabetes treatment: GLP-1 stimulates insulin secretion in a glucose-dependent manner, and exerts multiple antidiabetic actions such as inhibition of glucose-dependent glucagon secretion, delayed gastric emptying, weight loss, and increased β-cell mass (Nauck et al., 1993; Elliott et al., 1993; Buteau et al., 1999; Drucker, 2001; Kjems et al., 2003). Native GLP-1 peptide has limited application as a therapeutic agent due to its rapid elimination in vivo (Drucker, 2001; Kjems et al., 2003). A number of GLP-1 analogs with longer half-lives have been synthesized (Drucker, 2001; Ribel et al., 2002; DeFronzo et al., 2005). GLP-1 mimetic peptide exenatide is approved as an adjunct treatment that requires multiple daily subcutaneous injections for meal-time glycemic control (Drucker and Nauck, 2006). For patients looking to avoid daily self-injections, either sustained release products that lower the injection frequency to once-weekly or once-monthly (Okada and Toguchi, 1995; Robinson and Talmadge, 2002; Shi and Li, 2005), or products that non-invasively deliver drugs systemically via an alternate routes of administration are potential strategies that can be exploited. Small peptides and proteins are generally not orally bioavailable due to their large molecular weight, polarity, and metabolic instability in the gastrointestinal tract, although pulmonary and nasal delivery have shown promise in clinical development. The pulmonary route is of particular interest given the large surface area for absorption, modest enzymatic activity relative to the GI-tract, and permeability to small peptides. The precedence, knowledge and experience gained with inhaled insulin (powder and liquid products), exenatide and other small peptides in various stages of development further stimulated investigation of this drug delivery route (Davies and Muir, 1966; Quattrin, 2006; Hollander, 2007; Patton and Byron, 2007; Gedulin et al., 2008).

BMS-686117 is an 11-mer GLP-1 receptor agonist with molecular weight of 1528.7 g/mol. The aqueous solubility of the peptide is about 1 μg/mL or less between pH 4 and pH 6.5, and the peptide is not orally bioavailable. The current investigation assessed the feasibility of pulmonary delivery of BMS-686117 in rats.

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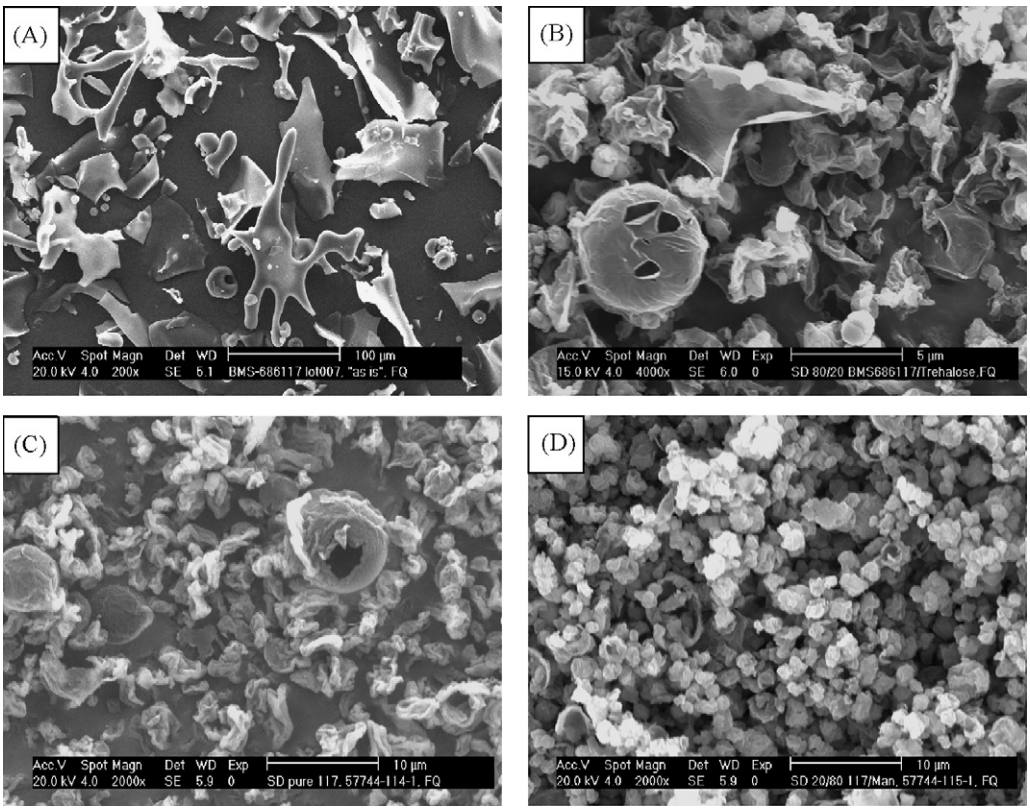

Fig. 1. SEM morphology of (A) lyophilized BMS-686117 powder as received, (B) spray-dried 80/20 BMS-686117/trehalose (prototype I), (C) spray-dried 100% BMS-686117 (prototype II), and (D) spray-dried 20/80 BMS-686117/mannitol (prototype III).

Lyophilized BMS-686117 powder as received was compared with three prototype dry powder formulations prepared by spray drying (Buchi B-191 mini spray dryer, Brinkmann Instruments, Westbury, NY) the ethanol or ethanol/water solutions containing: (I) 80/20 (w/w) BMS-686117/trehalose; (II) 100% BMS-686117; and (III) 20/80 (w/w) BMS-686117/mannitol. BMS-686117 was dissolved in ethanol first and then mixed with aqueous solution of excipients. The final concentration of the total solids in the ethanol/H_2O (40/60, v/v) mixture was 0.2% w/v. Spray drying conditions were as follows: inlet temperature 100 °C, solution pump rate 7 mL/min, atomizing nitrogen flow rate 500 Nl/h, aspirator 100%. A scanning electron microscope (Philips XL 30ESEM, FEI Philips, Hillsboro, OR) was used to study the morphology of the spray-dried powder.

This animal study adhered to the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985) and the Bristol-Myers Squibb animal care use committee guidelines. Male Sprague–Dawley rats (Harlan, Indianapolis, IN) were anesthetized with pentobarbital (50 mg/kg, IP). A ventral midline incision was made in the neck to expose the carotid artery and a polyethylene (PE-50) cannula filled with heparinized saline was inserted into the artery and secured with surgical silk to facilitate blood sampling through the duration of the experiment. A 10% blend of lyophilized BMS-686117 or spray-dried powder (protoypes I, II and III) with inhalation grade lactose (Respitose SV003) was delivered intratracheally (IT) with a model DP-4 dry powder insufflator (Penn Century, Philadelphia, PA). A dose of 1 mg/kg was administered via a small incision in the trachea. Respiration was maintained with a PE-205 tube inserted into the trachea immediately post-dose. Blood samples were collected in heparinized vacutainers, centrifuged at 5000 rpm at 4 °C for 10 min, and the supernatant isolated for BMS-686117 estimation. Plasma concentration of the peptide was determined by LC/MS/MS on a Shimadzu HPLC (Columbia, MD) interfaced with a Sciex API 4000 mass spectrometer (Applied Biosystems, Foster City, CA). Pharmacokinetic parameters were determined using Kinetica™ software (InnaPhase, Philadelphia, PA).

BMS-686117 as received was a lyophilized, fluffy powder that exhibited morphology of irregular flakes under SEM (Fig. 1A). The size of the flakes ranged widely from 2 to 100 μm and exhibited poor flow characteristics. Spray drying or co-spray drying BMS-686117 with trehalose or mannitol, produced free flowing fine powders with particle size ranging from 2 to 10 μm (Fig. 1, B–D). Carbohydrates such as trehalose, lactose, glucose or mannitol, have been commonly used in the development of inhaled dry pow-

Table 1
Pharmacokinetic parameters of IV, SC and IT delivery in rats.

Treatment	Dose (mg/kg)	C_{max} (nM)	T_{max} (h)	AUC_{last} (nM h)	%F
IV	0.1	961.3 ± 144	–	195 ± 47	100
SC	0.08	11 ± 2	1.2 ± 0.5	55 ± 5	34.4 ± 3.1
Lyophilized powder	1	55.9 ± 51	0.3	49.4 ± 55	2.5 ± 2.8
Prototype I (n = 4)	1	91.4 ± 14.1	0.44 ± 0.13	103 ± 15	5.6 ± 0.5
Prototype II (n = 4)	1	152.8 ± 43.3	0.30 ± 0.17	151 ± 51	7.7 ± 2.6
Prototype III (n = 4)	1	191.5 ± 48.1	0.67 ± 0.29	299 ± 43	15.3 ± 2.2

Values are mean ± S.D., n = 3–4 rats. Additional IV PK parameters: $T_{1/2}$ = 0.84 ± 0.34 h; Cl = 6.3 ± 1.5 mL/(min kg^{-1}); V_{ss} = 0.19 ± 0.006 L/kg.

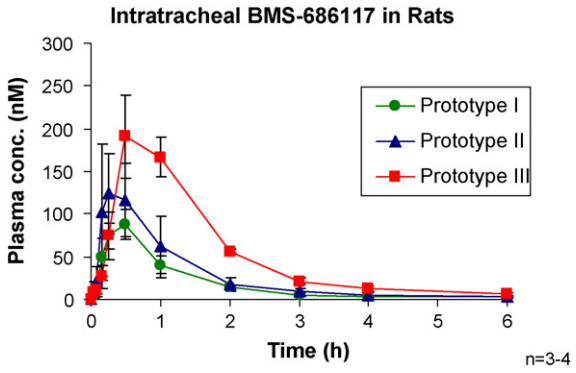

Fig. 2. Pharmacokinetics results of spray-dried BMS-686117 prototypes dosed in rats intratracheally at 1 mg/kg. Values are means ± standard deviation, $n = 3–4$ rats.

der formulations (Cryan, 2005). However, lactose and glucose are reducing sugars and cannot be co-processed with BMS-686117, due to the poor chemical compatibility between them. On the other hand, trehalose and mannitol are chemically compatible with BMS-686117. Mannitol was shown to offer better dispersability than the more hygroscopic sugars (Steckel and Bolzen, 2004).

By visual comparison of the three spray-dried prototypes, prototype III showed the most regular, spherical appearance with narrowest particle size distribution around 2–3 μm. While particle size of both prototypes I and II was smaller than 5 μm, they appeared to be more collapsed, hollow and somewhat irregular particles with broader size distribution. Prototypes I, II and III were spray-dried from same ethanol/H_2O solutions with identical solid concentrations and processing parameters. Presumably, the difference between their morphologies could be attributed to the different solubility of these solids in the solvent, different solution viscosity, and different drug-excipient miscibility (in cases of prototypes I and III). Systematic studies are needed to identify the key factors controlling the particle morphologies.

Particle deposition studies in the lung have shown that delivery of therapeutic agents to the "deep lung" requires aerodynamic diameter between 0.5 and 5.8 μm and optimally between 1 and 3 μm (Davies and Muir, 1966; Muir and Davies, 1967; Chege et al., 1997; Clark et al., 1998). Assuming the aerodynamic diameters were close to the geometric diameters, all three free flowing prototypes appeared suitable for pulmonary delivery with prototype III exhibiting the best characteristics for an inhalable powder.

Fig. 2 summarizes the pharmacokinetic time course profiles of the intratracheally administered BMS-686117 in rats. The pharmacokinetic parameters of these formulations were compared with SC and IV delivered BMS-686117 in Table 1. Compared with the lyophilized powder, all three spray-dried BMS-686117 prototypes showed significantly enhanced exposure. The absolute bioavailability of prototype III was 15.3%, which was 3-times and twice as high as prototypes I and II, respectively. Relative to subcutaneous administration, prototype III was 45% bioavailable with a coefficient of variation around 14%, suggesting a robust potential for pulmonary delivery with variability that appears appropriate for a potent peptide. For reference, inhaled insulin has been reported to show 8–25% bioavailability relative to SC depending on the formulation, device and method of delivery (Patton et al., 1999), and exenatide reported to be 13.6% bioavailable relative to IV administration in normal rats (Gedulin et al., 2008).

All pulmonary delivered formulations were more rapidly absorbed as noted by the shorter T_{max} values ranging from 0.3 to 0.67, relative to subcutaneous T_{max} of 1.2 h. BMS-686117 was detected in plasma for at least 6 h for all three powder prototypes with plasma half-life in the same range as injected peptide (0.84–1.4 h). Taken together, these results suggest that inhalable dry powders of BMS-686117 can be successfully made and intrapulmonary delivery of these formulations may achieve meal-time control of glycemia, as previously demonstrated with other GLP-1 mimetic peptides (Gedulin et al., 2008).

In conclusion, pulmonary delivery is a viable non-invasive alternative to systemically delivery BMS-686117. Inhalable, spray-dried powder formulation 20/80 BMS-686117/mannitol exhibited the best physical characteristics (particle size, morphology and flow) and in vivo performance of all the formulations tested. A bioavailability of 45% relative to subcutaneous injection, modest variability *in vivo*, and potential for rapid onset of action all support the pulmonary route as an alternate to the injection route for BMS-686117 in the treatment of type II diabetes.

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