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Administration of an insulin powder to the lungs of cynomolgus monkeys using a Penn Century insufflator

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Abstract

A powder formulation of live-attenuated measles vaccine is being developed for administration to the lungs. The safety and efficacy of the powder will be assessed by insufflation into cynomolgus monkeys. A Penn Century insufflator has been evaluated for powder dosing to the monkeys using an insulin formulation having similar physicochemical characteristics to the vaccine powder. Insulin pharmacokinetics were compared following dosing by powder insufflation, solution instillation into the trachea and subcutaneous injection. The insulin dosed to the lungs and trachea was more rapidly absorbed than that administered subcutaneously. Insulin bioavailability was greater from the inhaled powder than from the instilled solution. The findings confirm that the Penn Century device is suitable for vaccine powder dosing to the deep lung.

Keywords: Pulmonary administration; Insulin; Monkey; Measles vaccine; Powder insufflation

1. Introduction

The standard route of administration for measles vaccine is subcutaneous injection. For mass vaccination campaigns, particularly in areas where hepatitis B and HIV are highly prevalent, needle-free administration of the vaccine would be desirable to avoid transmission of blood-borne pathogens. In addition, there are indications that mucosal measles vaccination can result in increased vaccine efficacy (Cutts et al., 1997).

Inhalation of nebulised measles vaccine provides an effective means of mass vaccination (Dilraj et al., 2000; Bennett et al., 2002). Such administration requires reconstitution of freeze-dried vaccine and cold chain maintenance to preserve biological stability. Vaccines can, however, be formulated with carbohydrates, such as trehalose, to enhance powder stability at normal ambient temperatures (Aguado et al., 1998).

Dry powder inhalers are well-established for dosing anti-asthma drugs (Steckel and Müller, 1997). There is considerable interest in extending powder inhaler use to the administration of peptides and proteins, as an alternative to injections (Patton, 1996); the large surface area of the alveolar epithelium facilitating absorption of systemically acting drugs. A powder formulation of insulin, spray dried with trehalose, has been shown to be effective in reducing blood glucose following inhalation to the deep lung (Hardy et al., 2002). The inclusion of trehalose in the formulation increased insulin thermal stability.

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There is interest in extending the application of pulmonary delivery to administration of live-attenuated respiratory virus vaccines in dry powder form. Measles vaccine spray dried with trehalose is being developed as a powder formulation for inhalation. The efficacy of inhaled measles vaccine powder will be compared with nebulised solution and intra-muscular controls in cynomolgus monkeys. The present study has been undertaken to assess the suitability of a Penn Century dry powder insufflator (Penn Century, Philadelphia, USA) for delivery of the vaccine powder to the deep lung. Insulin has been selected as a tracer for use in the evaluation, since it is well-absorbed from the lung, can be readily assayed in the blood and is well tolerated following inhalation so long as hypoglycaemia is avoided. Additionally, it can be prepared as a powder having similar physicochemical properties to the proposed vaccine powder.

2. Materials and methods

A placebo vaccine powder was prepared by spray drying an aqueous solution containing trehalose. An insulin powder was also prepared by spray drying and comprised 20% (w/w) human insulin in trehalose. The particles of both powders were examined by scanning electron microscopy and found to be spherical. The particle size distributions were determined using a Coulter LS particle size analyzer. The placebo vaccine powder had a median particle diameter of 5 μm with more than 70% of the particles in the range 2–8 μm. The median diameter of the insulin particles was 2 μm with over 70% in the range 1–5 μm. The placebo vaccine and insulin powders were blended in a ratio of 1–5.2, to achieve a target dose of 5 units insulin in approximately 10 mg powder.

Insulin formulations were administered to seven male cynomolgus monkeys (*Macaca fascicularis*), weighing between 2.8 and 4.3 kg. Three monkeys received insufflated powder doses, three monkeys subcutaneous insulin injections and one monkey solution instilled into the trachea. Immediately before dosing the monkeys were anaesthetised by intra-muscular injection of a solution of ketamine, xylazine and atropine (2.5% (w/v), 0.25% (w/v) and 0.025% (w/v), respectively) at a dose of 0.4 ml/kg body weight. Throughout the study the animals were breathing spontaneously. The animals were dosed supine and remained in this posture for about 70 min, until the effect of the anaesthetic began to diminish.

Each powder dose was administered as two shots from a Penn Century powder insufflator having a 12-cm long straight delivery tube. For each shot 5.5 mg powder was loaded into the device. The device was inserted into the trachea via an oropharyngeal tube and positioned with the tip of the delivery tube approximately 1 cm proximal to the carina. The depth of insertion required had been previously established by inserting a delivery tube to the carina, withdrawing it 1 cm and marking the position on the tube. The powder was delivered with 3 ml air over approximately 0.5 s at the beginning of inspiration, the device withdrawn, reloaded and the second shot administered. Following dosing of the second shot, an additional 3 ml air was administered to aid delivery of any residual powder from the device. Washings from the Penn Century device were assayed for insulin in order to calculate the dose delivered to the monkey.

A solution for instillation into the trachea was prepared by dissolving 10 mg powder mixture in 0.5 ml water to provide a dose of 5 units insulin. The solution was injected into the neck and dripped into the trachea. Subcutaneous dosing into the thigh was undertaken using a commercial preparation of insulin for injection (NovoRapid, Novo Nordisk), each monkey receiving 0.1 unit insulin/kg body weight.

Venous blood samples for insulin and glucose assay were taken immediately before insulin dosing and at intervals up to 2 h after dosing. Samples for insulin assay were collected into EDTA tubes, centrifuged and the plasma assayed using a microparticle enzymatic immunoassay (AxSYM, Abbott Diagnostics, Hoofddorp, The Netherlands). Whole blood glucose measurements were obtained using a portable glucose monitor (Precision Xtra, MediSense, kindly provided by Abbott Diagnostics) to provide instantaneous values. Glucose solution was administered intravenously if the blood glucose concentrations decreased to less than 1.4 mmol/l.

3. Results

Insulin recoveries from the Penn Century devices following dosing indicated that two animals

Table 1
Insulin administrations to monkeys—blood glucose and plasma insulin concentrations

Time (min)	Insufflation						Time (min)	Instillation		Time (min)	Subcutaneous					
	Dose, 0.9 unit/kg		Dose, 0.9 unit/kg		Dose, 0.7 unit/kg			Dose, 1.8 unit/kg			Dose, 0.1 unit/kg		Dose, 0.1 unit/kg		Dose, 0.1 unit/kg	
	Glucose (mmol/l)	Insulin (μIU/ml)	Glucose (mmol/l)	Insulin (μIU/ml)	Glucose (mmol/l)	Insulin (μIU/ml)		Glucose (mmol/l)	Insulin (μIU/ml)		Glucose (mmol/l)	Insulin (μIU/ml)	Glucose (mmol/l)	Insulin (μIU/ml)	Glucose (mmol/l)	Insulin (μIU/ml)
Pre-dose	5.7	1.2	4.8	0.8	6.3	1.0	Pre-dose	5.7	1.1	Pre-dose	4.9	1.5	6.0	1.2	6.9	0.5
15	3.2	433	3.9	79	5.2	34	15	4.8	51	25	4.8	6.5	6.7	5.9	6.3	4.6
25	1.7	236	2.3	53	3.1	40	20	3.0	47	40	3.4	6.6	5.8	7.7	6.0	5.8
35	<1.1[a]	120	1.6	27	1.4	32	35	1.8	49	55	2.9	7.4	5.3	9.8	5.4	6.3
45	5.0	62	1.3[a]	15	<1.1[a]	27	45	1.6	49	70	3.0	7.0	4.7	9.2	4.4	5.7
60	1.9	25	5.6	11	4.0	18	60	<1.1[a]	38	95	3.1	9.1	3.9	14	4.1	4.2
										120	4.7	12	4.6	19	5.8	6.7

[a] Glucose i.v. injection after sample taken.

had received 0.9 unit/kg body weight and the third 0.7 unit/kg. The dose instilled into the trachea equated to 1.8 units/kg. The higher instilled dose was intended to compensate for the lower bioavailability anticipated for this route of administration (Colthorpe et al., 1992).

The plasma insulin and blood glucose concentrations are listed in Table 1. The mean maximum insulin concentrations (C_{max}) and estimated mean areas under the curves over the initial 90 min ($AUC_{0–90\,min}$), normalised to doses of 1 unit/kg body weight, are presented in Table 2. Intravenous glucose was administered to counteract hypoglycaemia in all three monkeys dosed by powder insufflation and the animal dosed by instillation of solution into the trachea. On each occasion the need for glucose intervention occurred after the peak insulin plasma concentration had been reached. The insulin concentrations measured following the administration of glucose have not been included in the pharmacokinetic calculations. None of the animals dosed with insulin subcutaneously became hypoglycaemic. All the animals made complete recoveries with no apparent adverse effects.

As shown in Table 2, the extent of insulin absorption was greater for the insufflated powder than for the solution instilled into the trachea. Additionally, the absorption profile was much flatter for the instilled dose. The variability in insulin absorption from the powder doses probably relates to difficulties in coordinating actuation of the device with the onset of inspiration due to the relatively high breathing rate of approximately 30 breaths/min. Additionally, with a low tidal volume of about 20 ml, the 3 ml bolus of air required to deliver the dose may have resulted in powder failing to reach the alveoli. On several occasions powder was observed on expiration. Although insulin from the insufflated powder was more rapidly absorbed from the lungs than from the subcutaneous injection site, the extents of absorption over the initial 90 min were similar. These findings indicate extensive penetration of the powder from the Penn Century device to the target site, the deep lung.

4. Discussion

Aerosol inhalation dosing provides an attractive alternative to injection for the administration

Table 2
Insulin absorption over initial 90 min

Dosing	T_{max} (min)	C_{max} (μIU/ml)	$AUC_{0–90\,min}$ (μIU min/ml)
Powder insufflation	15, 15, 25	481, 88, 57	11251, 2214, 2474
Solution instillation	15	27	1773
Subcutaneous injection	>90, >90, >90	74, 98, 63 (120, 190, 67)[a]	4530, 6210, 3856 (7125, 10600, 5234)[a]

Normalised to doses of 1 unit insulin/kg body weight.
[a] 120 min values.

of vaccines, especially those against respiratory pathogens. It has been successfully applied to the dosing of measles vaccine by nebulisation (Dilraj et al., 2000; Sepúlveda-Amor et al., 2002). Dry powder formulations can provide increased vaccine stability (Aguado et al., 1998) and may, therefore, offer advantages over nebulised solutions for dosing by inhalation (LiCalsi et al., 1999).

The present study has been undertaken to determine the suitability of a Penn Century insufflator for delivering dry powder measles vaccine to the deep lung of cynomolgus monkeys. Insulin was chosen as a tracer, since it is well-absorbed from the lungs, can be readily assayed in blood and can be prepared as a powder formulation (Hardy et al., 2002) having physicochemical properties similar to those proposed for the measles vaccine powder. It has been shown that inhaled insulin is more rapidly absorbed than doses administered by subcutaneous injection; the time to the maximum plasma concentration (T_{max}) following pulmonary administration to man occurring at about 30 min compared with 100 min following subcutaneous dosing (Hardy et al., 2002; Heinemann et al., 1997). The bioavailability of inhaled insulin in man is dependent on the efficiency of the inhaler and on the extent of absorption from the lungs. Typically 20–40% of insulin deposited in the lungs is absorbed (Patton et al., 1999). The findings in the monkeys are in agreement with those reported for man.

The T_{max} values for insulin dosed to rabbits by nebulisation and instillation into the trachea were very similar at 11 and 12 min, respectively (Colthorpe et al., 1992). The instilled solution was shown by gamma scintigraphy to distribute mainly in the central airways, whereas the nebulised solution deposited more peripherally in the lungs. In the monkeys, the insulin T_{max} values were similar for powder insufflated into the lungs and solution instilled into the trachea. The volume of solution instilled into the rabbits was 1 ml; twice that dosed to the monkeys. The smaller instilled volume, along with the larger size of the monkeys, can be expected to have resulted in a more localised distribution to the larger airways. The differences in distributions of the insufflated and instilled doses have been confirmed by the greater insulin absorption following powder administration. The absorption profiles for the insulin powder doses are in agreement with those reported for other inhalation studies (Patton et al., 1999). Over the initial 90 min, the extent of the insulin absorption from the powder doses to the monkeys was similar to that following subcutaneous dosing.

The findings confirm that powder dosed from the Penn Century device was distributed to the deep lung. This validates the use of the technique for dosing vaccine powder, having similar physiochemical properties and in similar quantities, from the Penn Century device to the lungs in monkeys.

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