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The effect of inhaling a dry powder of sodium chloride on the airways of asthmatic subjects

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ABSTRACT: Wet aerosols of 4.5% sodium chloride (NaCl) are often used to assess the bronchial responsiveness associated with asthma. We questioned whether dry NaCl could be used as an alternative.

Dry powder NaCl was inhaled from capsules containing either 5, 10, 20 or 40 mg to a cumulative dose of 635 mg. The powder was delivered *via* an Inhalator™ or Halermatic™. The airway sensitivity to the dry and wet NaCl was compared in 24 patients with asthma aged 19–39 yrs.

All subjects responded to both preparations and the geometric mean (95% confidence intervals) for the provocative dose of NaCl causing forced expiratory volume in one second (FEV_1) to fall 20% from baseline ($PD_{20,NaCl}$) for dry NaCl was 103 mg (68–157) *versus* 172 mg (102–292), $p<0.03$ for the wet NaCl. The response to dry NaCl was reproducible and on repeat challenge the PD_{20} was 108 mg (75–153). The mean maximum fall in FEV_1 was approximately 25% on each of the two test days. Spontaneous recovery occurred within 60 min after challenge with dry NaCl and within 5 min after bronchodilator. There were no serious side-effects requiring medical attention, however some patients coughed on inhalation of the 40 mg dose and three gagged. Arterial oxygen saturation remained within normal limits.

We conclude that a suitably prepared dry powder of sodium chloride could potentially replace wet sodium chloride to assess bronchial responsiveness in patients with asthma, but further studies are required to establish the long-term stability of the dry powder preparation.

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Bronchial provocation testing, is well established as a technique for identifying and assessing the severity of airway hyperresponsiveness in persons suspected of having asthma [1]. In 1981, SCHOEFFEL *et al.* [2] reported that patients with asthma were sensitive to the inhalation of wet aerosols of hypotonic and hypertonic saline. This observation led to the development of a standardized bronchial provocation challenge with hypertonic saline both in adults and children [1, 3–8]. Studies comparing responses to hypertonic saline and other provocative stimuli commonly used for bronchial provocation testing have shown good concordance between responses to hypertonic saline, exercise and hyperventilation [9–11]. Patients responsive to hypertonic saline have been shown to have bronchial hyperresponsiveness to inhaled aerosols of methacholine and histamine with a 20% fall in forced expiratory volume in one second (FEV_1) at concentrations <8 $mg \cdot mL^{-1}$ or a dose <4 μmol [5].

There are, however, disadvantages when using wet aerosols of hypertonic saline. An ultrasonic nebulizer is needed and this requires maintenance and cleaning. Furthermore, a weighing machine is needed to measure the output for each challenge test as output differs over time, between nebulizers, and between patients. Another disadvantage, as with other wet aerosols, is that the person administering the test is also exposed to the aerosol. For these reasons we investigated the possibility that a dry powder preparation of sodium chloride (NaCl) could be substituted for the wet aerosol preparation of 4.5% NaCl.

The aim of this study was to compare the airway sensitivity to a suitably prepared dry powder inhalation of NaCl with that of an inhaled wet aerosol preparation of 4.5% NaCl in patients known to be responsive to challenge with hypertonic saline. The reproducibility of the airway responses to the dry powder and the time-course of spontaneous recovery of the airways after challenge were also investigated.

Subjects

Twenty four asthmatic subjects (seven males and 17 females) aged 19–39 yrs, were recruited from the local community (table 1). All subjects had a baseline FEV_1

Table 1. – Anthropometric details

Subj. No.	Age	Sex	Height	Predicted FEV_1*	Daily medication	Dose ICS	Atopic	4.5% saline	Dry NaCl challenge	
								Control PD_{20}	1 PD_{20}	2 PD_{20}
	yrs		cm	L		$\mu g \cdot day^{-1}$		mg	mg	mg
1	27	F	160	3.05	Sal *p.r.n.*		Yes	296.1	73.7	172.1
2	29	F	159	2.96	Sal *p.r.n.*	BDP 400	Yes	32.85	22.1	111.8
3	25	M	188	4.87	Fen 800	BDP 1000	Yes	7.2	50.1	18.2
4	19	F	161	3.13	Sal *p.r.n.*	Bud 1600	Yes	163.35	67.2	151
5	39	M	172	3.78	Sal *p.r.n.*	BDP 100	Yes	955.8	107.7	103.8
6	22	F	167	3.37	Sal *p.r.n.*	Bud 2400	Yes	99.9	168.2	138.22
7	29	F	169	3.35	Sal *p.r.n.*	Bud 800	Yes	45.9	105.6	55.08
8	22	F	176	3.73	Sal *p.r.n.*	Bud 800	Yes	378	151.5	56.39
9	29	M	189	4.8	Fen 0.4 mg *p.r.n.*		-	651.15	146	127
10	20	F	157	2.98	Sal 600 µg	Bud 800	Yes	149.4	161	96
11	23	F	166	3.33	Sal 100 µg *p.r.n.*			295.2	155	162
12	21	F	167	3.37	Sal 200 µg 3–4 per week		Yes	249.75	121	118
13	21	M	191	5	Sal 600 µg, Terf 240 mg			508.5	423	588
14	28	M	186	4.7	Sal 200 µg 3–4 per week			232.65	126	105
15	25	F	167	3.37	Sal 200 µg *p.r.n.*			153	20.45	75.69
16	25	F	166	3.33	Sal 200 µg *p.r.n.*			79.65	29	40
17	25	F	163	3.21	Terbutaline	Bud 1200		913.5	493.5	502.9
18	23	M	183	4.65	Sal *p.r.n.*			725.4	36.1	79.5
19	25	F	164	3.25	Sal 400 µg and *p.r.n.*	BDP 500		515.25	340.7	268.6
20	20	F	158	3.02	Terbutaline *p.r.n.*	Bud 800		205.65	257.99	128.8
21	26	F	167	3.35	Sal 800 µg	BDP 1000		58.5	87.93	132.03
22	19	F	151	2.74	Sal *p.r.n.*	Bud 2000		16.65	19.4	62.9
23	19	F	165	3.29	Sal *p.r.n.*			254.25	630	283.1
24	24	M	181	4.57	Sal 600 µg, Theo 600 mg	BDP 400	Yes	137.7	52.6	19.16
Mean	24.4	F=17	169.7				GM	172.3	102.8	108.0
SEM	0.9	M=7	2.3				95% CI	(101.8–291.7)	(67.6–156.5)	(75.2–152.8)
Median	24.5		167.0							
SD	4.5		11.2							
Range	19–39		151–191							
n	24		24							

Subj.: subject; FEV_1: forced expiratory volume in one second; PD_{20}: provocative dose causing a 20% fall in FEV_1; ICS: inhaled corticosteroid; Fen: fenoterol; Sal: salbutamol; BDP: beclomethasone dipropionate; Bud: budesonide; Theo: theophylline; Terf: terfenadine; 95% CI: 95% confidence intervals; GM: geometric mean. *: values are those of QUANJER *et al*. [15].

>60% and a 20% fall in lung function (FEV_1) during challenge with 4.5% saline. They were all nonsmokers and none had experienced a chest infection in the previous 6 weeks. Subjects were asked to refrain from taking short acting bronchodilators for 6 h and long acting bronchodilators for 12 h prior to the study days. No corticosteroids were taken by the subjects on the day of the study and no antihistamines were taken for at least 3 days before the study day. All medications (including the daily dose of inhaled steroids) are recorded in table 1. The healthy subjects had no personal or family history of asthma, were nonsmokers and did not have a positive skin-test to common allergens (dust, grasses, animal dander, moulds). The study was approved by the Central Sydney Area Health Service Ethics Committee (X93-0061) and all subjects signed a consent form prior to commencement of the study. The study was carried out under the Clinical Trial Notification Scheme of the Therapeutics Goods Administration of Australia (CTN 94-633).

Methods

NaCl powder and capsule preparation

The NaCl powder (Mallinckrodt AR; Paris, KY, USA) was prepared for inhalation at Genentech Inc (So San Francisco, CA) by the method of micronization using a Trost air impact pulverizer (Trost Equipment Corporation, Newtown, PA, USA). The mill uses compressed nitrogen to break up the NaCl crystals by collision. Prior to

milling, all parts of the mill were washed with Mili-Q water (Millipore Corporation, Bedford, MA, USA), rinsed with ethanol, and dried under a stream of compressed nitrogen. NaCl was then fed to the micronizer and milled. The powder was collected and milled once more. The powder was then dried in a vacuum oven at 140°C and a 5.05 kPa (38 mmHg) vacuum for 1 h, followed by transfer to glass vials and shipment to Sydney. The particle size was measured using a multi-stage liquid impinger (Astra Draco, Lund, Sweden) and by measuring sodium and chloride content by flame photometry on each stage of the impactor. The Halermatic™ device (Fisons Pharmaceuticals, Loughborough, UK) used was loaded with 120 mg of NaCl (3×40 mg capsules) and, by using a pump, a flow rate of 60 $L \cdot min^{-1}$ was generated through the device. The particle size analysis was repeated for the Halermatic™ device and performed for the first time with the Inhalator™ (Boehringer Ingelheim Pty Ltd., Ingelheim, Germany) after transport of the NaCl to the Sydney laboratory. The same type of multistage liquid impinger was used as in California, but the sodium and chloride content was measured at each stage using a vapour pressure osmometer (5500 Vapour Pressure Osmometer; Wescor Inc., UT, USA). The bioburden analysis was carried out by Northview Pacific Laboratories Inc (Berkeley, CA, USA). The results for both yeast and mould showed a value of less than 10 colony-forming units $(CFU) \cdot g^{-1}$ and no coliforms or other pathogens were detected. The gelatine capsules (No. 2; Gallipot, St Paul, Minnesota, USA) were hand-filled with 5, 10, 20 and 40 (±0.2) mg on an analytical balance (BA11OS; Sartorius, Gottingen, Germany) as required under controlled conditions (relative humidity 40%, temperature 20±1°C) in Sydney. The capsules were held in plastic containers that were stored in a larger glass container with silica gel and kept in a cool environment.

Delivery device

Two devices were used to deliver the NaCl powder. Subjects No. 1–8 received the NaCl powder *via* an Inhalator™ (Boehringer Ingelheim Pty Ltd) and subjects No. 9–24 received the NaCl powder *via* a Halermatic™ (Fison's Pharmaceuticals Pty Ltd). Both the Halermatic™ and the Inhalator™ are single dose devices permitting different doses to be loaded during the challenge. These devices were chosen as they were readily available and many of their delivery characteristics are known [12–14].

Flow measurement

For the Halermatic™ the subjects were required to inhale the NaCl powder at a flow rate between 50–120 $L \cdot min^{-1}$. As the design of the Halermatic™ precludes the in-line measurement of flow at each inhalation, a pressure transducer (DTX Disposable Pressure Transducer; Viggo-Spectromed Oxnard, CA, USA) was used to approximate flow changes. To calibrate the pressure transducer flows of 50–120 $L \cdot min^{-1}$ were generated through a rotameter (Series 2000, GEC-Elliott, Croydon, UK) in line with the Halermatic™ device which was used to deliver the powder. Pressure changes were measured through a side port of the Halermatic™ at each flow rate (50–120 $L \cdot min^{-1}$ in 10 $L \cdot min^{-1}$ intervals) and the data were graphically correlated to provide an estimate of flow measurements for each known pressure change. Pressure tracings were recorded during the challenge, on a chart recorder (Miniwriter Type WTR771A, Watanabe Instruments Corp., Tokyo, Japan) to provide instantaneous readings. The data was analysed more accurately after testing.

For the Inhalator™ the subjects were required to inhale the NaCl powder at a flow rate >28 $L \cdot min^{-1}$. The inspired flow rate was checked by having the subject inhale maximally from the Inhalator™ while it was attached to an anemometer (Minato AS 800, Minato Medical Science Co Ltd, Osaka, Japan) prior to challenge on all of the test days. The best of three attempts was recorded. To calibrate the anemometer flows of 25–95 $L \cdot min^{-1}$ were generated through a rotameter (Series 2000, GEC-Elliott, Croydon, UK).

Lung function measurements

Spirometry was performed on an Autospiro AS-300 spirometer (Minato Medical Science Co Ltd., Osaka, Japan) and the FEV_1 measurement was used as an index of change in airway calibre. The predicted FEV_1 values used were taken from QUANJER *et al*. [15]. The spirometer was calibrated each morning using a 2 L syringe.

Oxygen saturation

Oxygen saturation (S_{a,O_2}) was measured by oximetry (Ohmeda Biox 3700e, BOC Health Care, Louisville, CO, USA) as an index of safety. S_{a,O_2} was measured during the dry NaCl capsule challenges in 22 subjects and for 11 subjects during the wet challenges.

Challenge duration and number of capsules

The median (and range) time taken to perform the challenge and the number of capsules used was calculated for the two devices.

Study design

Subjects were asked to attend the laboratory on four or five occasions with at least 48 h between visits. The first visit was a control day with a 4.5% NaCl was challenge performed to determine eligibility for the study. Thereafter the subject performed either two dry powder NaCl capsule challenges (subjects 1–8) or a further wet aerosol followed by a further dry powder challenge (subjects 9–24).

Wet aerosols of 4.5% sodium chloride challenge

The sensitivity of the subjects to a wet aerosol of 4.5% NaCl was measured on the control day visit. The aerosols was generated by a $Mist O_2 gen$ EN 143a Ultrasonic Nebulizer (Timeter, PA, USA). Subjects inhaled the aerosols at resting ventilation rates through a two-way valve (No. 2700; Hans Rudolph, Kansas City, MO, USA) connected to the nebulizer by Bennetts smooth

bore tubing (Cat. No. TV 2723) 67.5 cm in length with an internal diameter of 2.2 cm. This unit was weighed with the tubing, but not the valve (Sartorius, 1216 MP, Gottingen, Germany), before the bronchial challenge and after the final dose of challenge aerosol had been delivered. Thus the output of the nebulizer over time was known for each subject on each occasion. The dose of wet aerosol delivered is expressed in milligrams of NaCl. This value was obtained by multiplying the dose of aerosol delivered in grams by 45 (*i.e.* 45 mg NaCl·g aerosol delivered^{-1}).

The protocol used to perform the challenge with 4.5% NaCl is described in detail by SMITH and ANDERSON [4], although the protocol was modified so that the maximum dose of aerosol delivered on the control day was greater than the usual dose of 15 g. A further modification was made by measuring FEV1 in duplicate at only 60 s after each challenge period. This procedure was followed as the maximum response usually occurs within 1 min after each challenge interval.

Subjects inhaled the challenge aerosol for 0.5 min and waited 60 s before the FEV1 measurement was performed. If there was a 20% fall in FEV1 from the baseline value, the challenge was stopped and the subject included in the study. If a 20% fall was not recorded, the challenge continued for further exposures of l, 2, 4, 8, 8 and 8 min or part thereof, or ceased when a fall in FEV1 ≥20% was recorded. The subjects were eligible for the study if they had a 20% fall in FEV1 after <22 g of wet aerosol containing 990 mg of NaCl had been delivered.

Following this challenge, subjects were given 0.5 mg terbutaline sulphate *via* a pressurized metered dose inhaler actuated into a Nebuhaler™ (Astra Pharmaceuticals, Lund, Sweden).

Dry powder NaCl challenge

Subjects performed two challenges with dry powder NaCl. Lung function, as measured by FEV1, was recorded on arrival at the laboratory, and 10 min later to establish its stability. The dose protocol consisted of inhaling 0 (empty capsule acting as a placebo) then 5 and/or 10, then 20, 40, 80, 160, 160 and 160 mg NaCl. The doses of 40, 80 and 160 was given in multiples of either 20 or 40 mg capsules. Two FEV1 manoeuvres were performed 60 s after the completion of each dose and the highest FEV1 measurement was used in calculations. The FEV1 value taken after the inhalation of the 0 mg capsule was used to calculate the percentage fall in FEV1 in response to the dry NaCl. If the subject had a fall >10% in response to a single dose, then, for safety reasons the causative dose was repeated. The challenge was stopped when a 20% fall in FEV1 was measured or a total cumulative dose of 635 mg had been given.

Time course of recovery of lung function following challenge

Spontaneous recovery of FEV1 to baseline values (before any capsules were given) following the completion of the first capsule challenge was assessed in all subjects by performing spirometry 5 min after completion of the test, and then at 10 min intervals for at least 30 min or until the FEV1 had returned to within 5% of the baseline FEV1 value. Subjects 1–8, following the completion of the second capsule challenge, received 0.5 mg terbutaline sulphate, actuated into and inhaled from a Nebuhaler™ and then performed spirometry 5 min later and at 10 min intervals for 30 min, or until the subject had returned to within 5% of the baseline FEV1 value.

Statistical analysis

Baseline FEV1, expressed as a percentage of predicted normal, and post-placebo capsule percentage predicted FEV1 values were expressed as mean±SD and compared using an analysis of variance (ANOVA) and Student's paired t-test.

Airway sensitivity (PD20,NaCl). Airway sensitivity was measured as the provoking dose of NaCl that caused a 20% fall in FEV1 (PD20,NaCl). These values were calculated by linear interpolation using the cumulative dose of NaCl causing a 20% fall in FEV1 from the pre-challenge value.

The geometric mean (GM)±95% confidence interval (95% CI) and range of values were calculated for the PD20,NaCl (mg) values and the log PD20,NaCl values and compared using Students paired t-test for both inhaler devices and for the wet aerosol challenges. The Pearson correlation coefficient (r_p) and significance values were calculated for the relationship between the 4.5% saline and the first and second NaCl capsule challenge for each of the two devices. The repeatability of the two NaCl capsule challenges was calculated using the log PD20,NaCl. The equation previously described [16] was used to express repeatability as fold change. The data were also expressed in the manner described by BLAND and ALTMAN [17].

The ratio of the wet PD20,NaCl challenge:dry PD20,NaCl challenge was calculated to make a relative comparison between the two devices.

The peak inspiratory flow rates (L·min^{-1}) and duration of the challenge (min) were calculated for the Inhalator™ (n=8) and the Halermatic™ (n=16) separately and expressed as median and range of values.

Student's paired t-test, was used to compare the spontaneous recovery values (n=15) at 30 and 60 min and the recovery values following bronchodilator at 5 and 60 min. For statistical and graphical reasons all values above the baseline FEV1 were considered to be 0.

Results

Pre-challenge lung function

There was no significant difference for the baseline mean±SD FEV1 values expressed as the percentage of predicted FEV1 between any of the test days either within or between the two groups (table 2).

Airway sensitivity to NaCl

Individual dose-response curves for the dry powder NaCl are illustrated for each device in figure 1 and the individual values for the wet and dry PD20,NaCl challenge are given in table 1. The GM (95% CI) for the

Table 2. – Values obtained using the two different devices for inhalation of the dry powder of NaCl

	Inhalator™	Halermatic™
Subject Nos. total	1–8 (8)	9–24 (16)
Peak inspiratory flow $L \cdot min^{-1}$ median (range)	58 (46–65)	65 (38–103)
Duration of challenge for $PD_{20,NaCl}$ median time min (range)	10 (6–15)	13.5 (3–38)
Number of capsules median (range)	6 (3–11)	5 (2–17)
First dry powder NaCl baseline FEV_1 % pred±SD	84±10%	88±20%
Second dry powder NaCl baseline FEV_1 % pred±SD	83±13%	88.3±18%
First dry powder NaCl geometric mean PD_{20} (95% CI)	80 (46–138)	117 (64–212)
Second dry powder NaCl geometric mean PD_{20} (95% CI)	84 (45–156)	123 (77–196)
First wet aerosol 4.5% NaCl baseline FEV_1% pred±SD	83±16%	87±19%
Second wet aerosol 4.5% NaCl baseline FEV_1 % pred±SD	-	90±17%
First wet aerosol 4.5% NaCl geometric mean PD_{20} (95% CI)	274 (137–550)	213 (117–385)
Second wet aerosol 4.5% NaCl geometric mean PD_{20} (95% CI)	-	253 (139–459)
Pearson's correlation (r_p) wet *versus* dry	0.53	0.61
Repeatability fold change 95% CI		
dry powder NaCl	0.3–1.8	0.6–1.5
wet aerosol NaCl	-	0.7–1.7

PD_{20}: provocative dose causing a 20% fall in forced expiratory volume in one second (FEV_1); % pred: percentage of predicted value; 95% CI: 95% confidence interval.

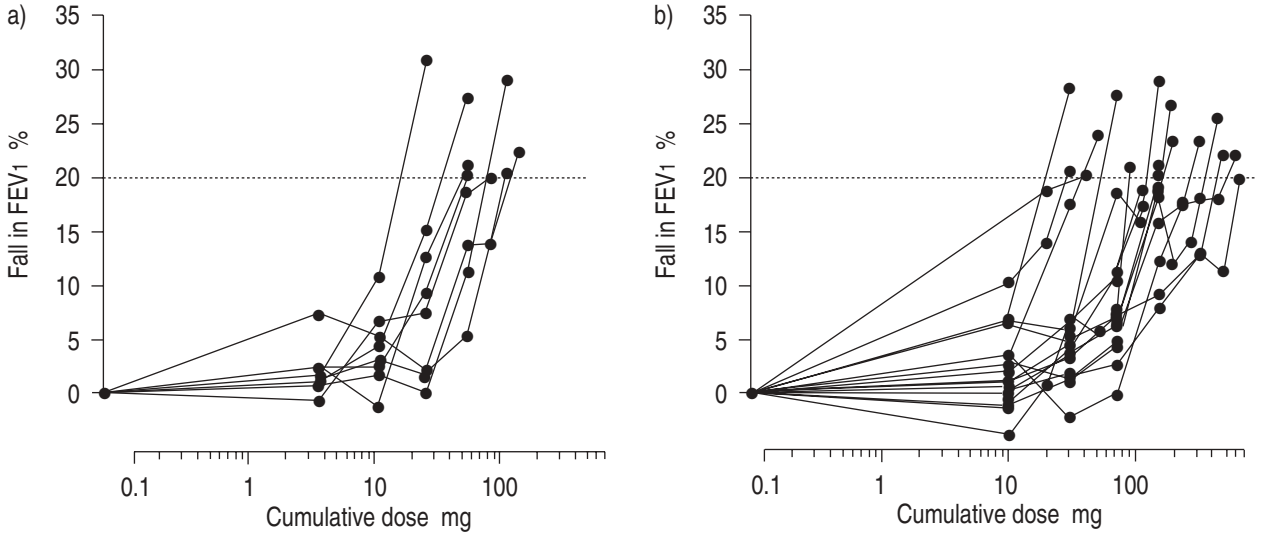

Fig. 1. – Individual dose-response curves for a) the eight subjects who inhaled from the Inhalator™ and b) the 16 asthmatics who inhaled from the Halermatic™. The provoking dose of dry powder NaCl causing a 20% reduction in FEV_1 (PD_{20}) in the 24 asthmatic subjects represented a wide range in severity of airway responsiveness. FEV_1: forced expiratory volume in one second.

$PD_{20,NaCl}$ for both the 4.5% saline challenge and the dry powder challenges for the two devices is given in table 2. The relationship between the values for $PD_{20,NaCl}$ on the initial wet and dry challenges for the whole group was r_p=0.55, p<0.01 (fig. 2) and is given separately for the two inhalers in table 2. The relationship between the values for $PD_{20,NaCl}$ on the initial dry challenge and the second wet challenge for the 15 subjects who performed two wet challenges was r_p=0.61, p<0.05. The ratio of wet $PD_{20,NaCl}$ challenge:dry $PD_{20,NaCl}$ challenge was 2.55 (0.14–8.87) for the Inhalator™ and 3.12 (0.4– 20.1) for the Halermatic™. These values were not significantly different.

There was no significant difference in values for $PD_{20,NaCl}$ measured between repeated challenges either for the wet or the dry aerosol (table 2). The repeatability expressed as fold-change is given in table 2, and expressed as a Bland and Altman plot as illustrated in figure 3.

Oxygen saturation during challenge

The initial challenge value and the lowest saturation value measured were used to calculate the fall in saturation during challenge. The S_{a,O_2} was measured in 11 of the 24 subjects during the wet NaCl aerosol challenge. The lowest S_{a,O_2} during the wet challenge was 95% and none of the subjects fell more than 2%. During the first dry capsule two subjects had no S_{a,O_2} measurements. Of the remaining 22 subjects, two fell by 3% during challenge and one fell by 6%. The lowest S_{a,O_2} measured during dry NaCl capsule challenge was 92% (recorded for the subject who fell 6%). The remaining 19 subjects fell less than 3% during challenge. During the second dry capsule challenge three of the 24 subjects fell 3% and the remaining 21 subjects fell less than 3% during challenge. The lowest S_{a,O_2} recorded during the second challenge was 94% which may be considered "normal" or just below.

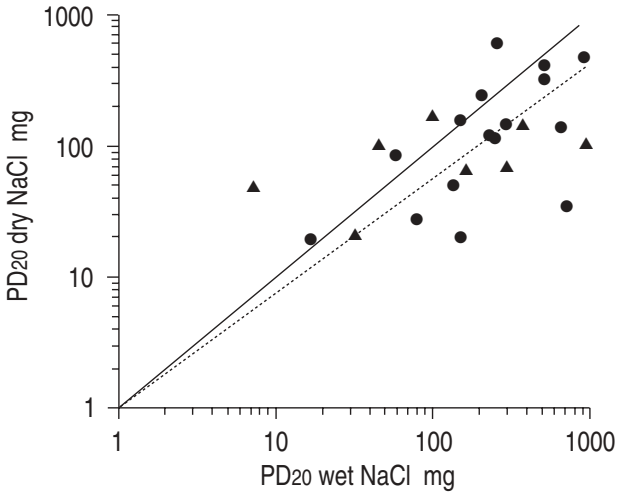

Fig. 2. – Individual values obtained for the provoking dose delivered in milligrams of the wet aerosol of 4.5% NaCl to induce a 20% fall in forced expiratory volume in one second (wet challenge PD_{20}) in relation to the PD_{20} obtained for the dry powder preparation. ▲: subjects who inhaled from the Inhalator™; ●: subjects who inhaled from the Halermatic™. ———: line of identity; ·····: line of correlation (r_p=0.55, p<0.01, n=24). The PD_{20} for the dry powder was significantly less compared with the wet aerosol although eight of the 24 subjects required a greater dose when the dry powder was used.

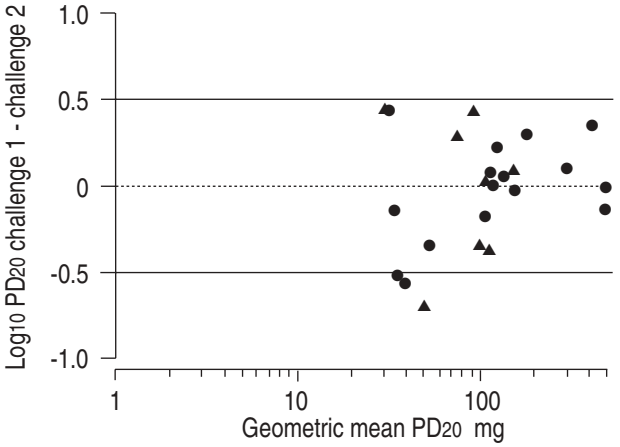

Fig. 3. – A Bland and Altman plot relating the geometric mean for the provoking dose of NaCl causing a 20% fall in forced expiratory volume in one second (FEV_1) (PD_{20}) for the first and second challenge with dry powder NaCl plotted against the difference between the $\log_{10}$ PD_{20} values for the 24 subjects who performed repeated challenge. ▲: subjects who inhaled from the Inhalator™; ●: subjects who inhaled from the Halermatic™; ·····: the point of no difference between the first and second challenge. The repeatability was independent of the dose. The difference in $\log_{10}$ PD_{20} values for all but two subjects was ±0.5.

Peak inspiratory flow rate (PIFR)

The median values for the PIFR measured for both dry powder devices are given in table 2. PIFR exceeded 38 $L \cdot min^{-1}$ in all subjects.

Aerosol characteristics of powder preparations and devices

For the initial powder preparation the analysis of the particle size, using the Halermatic™, revealed that <7% of the NaCl was in the respirable range of ≤7 μm by the time it had reached Sydney, although at the time of preparation in California 19% was in the respirable range. This initial batch was unsuccessful in provoking an airway response when delivered by a Halermatic™. The second batch of dry powder had 37% of particles below 7 μm at the time of preparation and 30% at the time of use in Sydney when measured with the Halermatic™ and this was used successfully in subjects 9–24. The third batch received a year later was also a failure going from 38% at source to 13% at the time of use in Sydney. The fourth batch had 15.8% of the mass below 7 μm and was used successfully when inhaled from the Inhalator™.

Challenge duration and number of capsules

The median duration of the challenge and number of capsules used is given in table 2.

Recovery following dry powder NaCl capsule challenge

Spontaneous recovery was analysed in 23 subjects following the first NaCl challenge. Subject No. 16 was not included in the analysis as she did not spontaneously recover and required the administration of bronchodilator at 30 min. For n=23 at 30 min post-challenge the mean ±SD percentage reduction from baseline FEV_1 was still -15±8.8% but at 60 min it was only -7±5.4% below baseline. That is, the FEV_1 had returned spontaneously to 93% of the baseline value 60 min after challenge.

Recovery after the first capsule challenge following bronchodilator was compared to spontaneous recovery in the eight subjects who used the Inhalator™ (fig. 4). Five minutes after the bronchodilator had been given the mean±SD percentage change from baseline FEV_1 following bronchodilator was -5±5.8% compared to -22±6.5% without bronchodilator (n=8, p<0.003). At 60 min the

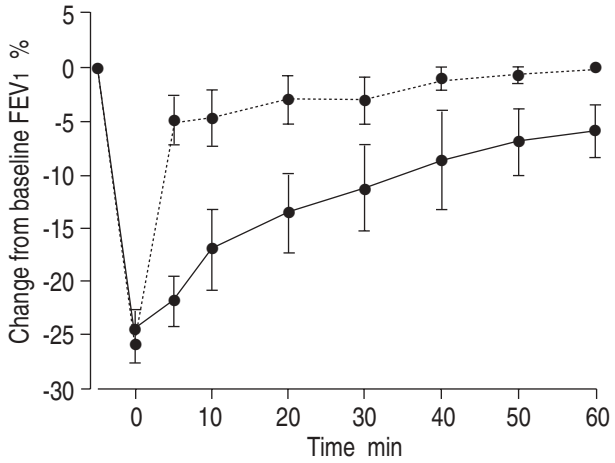

Fig. 4. – The mean±SEM for the forced expiratory volume in one second (FEV_1) expressed as a percentage reduction from the baseline prechallenge value in the eight subjects who spontaneously recovered after the first challenge with dry powder sodium chloride (———) and were given 0.5 mg of terbutaline aerosol immediately after the second challenge (·····). The value at time 0 was the maximum reduction in FEV_1 recorded and the time after bronchodilator or spontaneous recovery is shown. For seven of the eight subjects the FEV_1 had returned to within 6% of baseline within 5 min after taking bronchodilator. There was a significant difference in the values for FEV_1 5 min after bronchodilator had been administered (p<0.003). For the remaining subject recovery took 50 min.

subjects who had received bronchodilator had all returned to baseline whereas those who recovered spontaneously had an FEV_1 compared with baseline of -6±6.8% (n=8, p=0.05).

Healthy control subjects

Five healthy control subjects (aged 19–22 yrs) performed an inhalational challenge using the dry powder NaCl administered from the Halermatic™ device. Four received a dose of 620 mg and one a dose of 540 mg. None of these healthy volunteers, who acted as control subjects, recorded a $PD_{20,NaCl}$ and the maximum fall in FEV_1 was 6.5% with the range being 0–6.5%.

Discussion

In this study we have shown that a dry powder preparation of NaCl, delivered from a capsule *via* either a Halermatic™ or an Inhalator™ device, can provoke airway narrowing in the same asthmatic subjects who are sensitive to the wet aerosol preparation of 4.5% NaCl. Furthermore, the airway response to the dry powder had good repeatability and spontaneous recovery from the challenge occurred over 60 min. With the aid of a bronchodilator FEV_1 recovered to 95% of the baseline value in less than 10 min.

The wet aerosol was always performed first on the control day because the entry criteria required that. However, the difference in $PD_{20,NaCl}$ values for the first dry challenge and either the first or the second wet challenge was similar, suggesting that there was no order effect.

In order to evaluate the dry powder we used subjects who had a wide variation in their wet $PD_{20,NaCl}$ and two different devices. Thus, the subject who was most sensitive to the 4.5% NaCl wet aerosol required less than 30 s exposure with a $PD_{20,NaCl}$ of 7 mg while the least sensitive subject required 22 min exposure and had a $PD_{20,NaCl}$ of 955 mg. A $PD_{20,NaCl}$ of less than 90 mg is regarded as consistent with severe bronchial responsiveness, 90–270 mg as moderate and greater than 270 mg as mild responsiveness to wet NaCl challenge [5]. All our asthmatic subjects displayed the expected airway response and none of the healthy subjects responded. Healthy nonasthmatic subjects do not demonstrate bronchial responsiveness to 4.5% saline and the mean±SD percentage reduction from baseline FEV_1 for a group of 75 people has been previously reported as 4.6±3.1% [5]. The limited number of healthy control subjects investigated in this study related to the limited availability of dry powder.

The two inhalers used were selected because they were both commercially available. However, their characteristics were different in the way in which the capsules were pierced and the powder dispersed and they had differing inspiratory resistances (low for the Halermatic™ and high for the Inhalator™). The airway responses to dry NaCl were not compared in the same subjects using both devices, because only a small amount of dry NaCl was available and 12 months separated the two studies. However there did not appear to be any qualitative differences between the devices, as evident from the individual dose-response curves. While there were some differences between the $PD_{20,NaCl}$ for the two devices this was more likely to have been related to the severity of their asthma, as their responses to the wet aerosol were similarly smaller. Furthermore, the ratio of the wet $PD_{20,NaCl}$:dry $PD_{20,NaCl}$ was similar for both devices. The values observed for repeatability on the Halermatic™ were somewhat better than the Inhalator™ but this may have been due to the small numbers of subjects studied on the Inhalator™. Both devices were adequate for the delivery of the salt although the Inhalator was easier to use because it pierced the capsules more easily. We also found that the Inhalator™ caused less cough compared with the Halermatic™. This may be because of the higher inspiratory resistance of the Inhalator™, resulting in less deposition of the powder on the back of the throat.

We do not know where in the respiratory tract the NaCl was deposited or what percentage of the inhaled dose was deposited in the lower respiratory tract. The relatively small changes in S_{a,O_2}, in most subjects, suggest that the site of deposition of the NaCl was more likely to be the larger airways. Further studies with labelled NaCl are required to determine the ratio of peripheral to central deposition of the powder in the airways [18].

We have measured the size of the wet aerosol particles of the NaCl after passing through the tubing and valve to 3.6 μm with a geometric SD less than 1.1 [19]. We have also measured the amount of wet aerosol reaching the mouth as 47% of that reaching the inspiratory port of the two-way valve [5]. On the basis of these measurements we have estimated the volume of wet aerosol reaching the lower respiratory tract to be approximately 10% of the volume generated by the nebulizer [5]. This is a value similar to that which has been measured for jet nebulizers [20]. The percentage of particles of dry powder of NaCl less than 7 μm measured at the site of testing was 15.8% for the Inhalator™ and 30% for the Halermatic™. It is possible that a higher proportion of the dry powder aerosol entered the lower respiratory tract with the Inhalator™ compared with the Halermatic™ [21] but we have no *in vivo* data on deposition.

For 50% of the subjects, the dose required to record a $PD_{20,NaCl}$ was less than 100 mg when the dry powder was used. For one subject with very mild asthma, receiving 1,200 $\mu g \cdot day^{-1}$ of the aerosol corticosteroid budesonide, a cumulative dose of 493 mg was required, but this was reproducible with 503 mg being required on the second challenge. This was substantially less than the dose required by wet aerosol in the same subject. However there were three subjects who required substantially more dry powder than wet aerosol. Some subjects showed much greater variation in their $PD_{20,NaCl}$ on the two test days. However, the repeatability for the dry powder compared well with the repeatability for wet aerosol challenge performed in the same subjects. Furthermore, the repeatability compares well with other challenge tests such as histamine or methacholine [22, 23].

There were no adverse experiences requiring medical intervention with the dry powder. The S_{a,O_2} as measured

by ear oximetry remained above 94% in all but one subject. Three subjects did gag with the 40 mg dose but even with this there was no significant fall in their S_{a,O_2}. We were not required to give bronchodilator immediately at the end of challenge in any subject. One subject was given a bronchodilator 30 min after challenge on the day spontaneous recovery was being documented. The dry NaCl powder was well tolerated at the lower doses but with the 40 mg capsule some subjects found difficulty with inhaling quickly and coughed. The coughing could have reduced deposition and be the reason that some subjects required a much higher dose of dry NaCl compared with the wet NaCl aerosol. Furthermore, it is possible that the dry powder provided a potent pharyngeal stimulus contributing to the airway narrowing by causing reflex bronchoconstriction.

From our studies using the liquid impinger we had 40–70% recovered on the "throat" and Stage 1 (particles above 13 μm) This is probably the reason that many patients coughed while inspiring the 40 mg capsules. Ideally a greater percentage of the dose would have a particle size in the respirable range. Indeed the most important issue relating to this study was the reliability and stability of the dry powder preparations. We received four batches of NaCl from California and only two of these were used successfully in Sydney. Further studies are required to establish the long-term stability of the powder preparation before studies are performed on sensitivity and specificity of the challenge in large numbers of subjects.

The precise mechanism whereby hyperosmolarity leads to airway narrowing is not known. At present it is thought that mast cell mediators [9, 24–26] and neuropeptides from sensory nerves are released in response to this stimulus. The evidence to support this contention comes from *in vitro* and *in vivo* studies. An increase in osmolarity is a potent stimulus for human lung mast cell release of histamine [27] and in humans the airway responses to hyperosmolarity are markedly inhibited by specific antihistamines [28, 29]. The only evidence in support of neuropeptide comes from work in animals. It shows indirectly that C-fibres are stimulated by hyperosmolarity and there is an increase in microvascular permeability that can be accounted for by the release of neuropeptides [30, 31].

We believe that hypertonic saline challenge is an attractive alternative to the pharmacological challenges with histamine and methacholine that are currently used most widely, both in routine hospital laboratories [1, 32] and in the field of epidemiology [22, 23, 33, 34]. We consider that the hypertonic saline challenge is not only useful for identifying persons with asthma, but will be particularly important in following response to treatment with aerosol corticosteroids [35, 36]. We predict that the advantage in using a hypertonic challenge in epidemiology is in its high specificity yet comparable sensitivity to other challenge tests to identify current asthma [6, 8, 37]. We believe that a hypertonic challenge test would adapt well to use outside the hospital laboratory if an appropriate dry powder preparation of an osmotic agent could be found.

There are many potential advantages in using dry powders for delivering substances used for bronchial provocation testing. One advantage is the reduced exposure of the test aerosol to the investigator. Another is the safe disposable nature of the device and the substance. There would be considerable time saved in using dry powders compared with wet aerosol preparations in that the equipment used for nebulization and to determine output is expensive and requires cleaning and regular maintenance.

This is the first report of the airway narrowing effects of dry particles of sodium chloride in known asthmatic subjects. The challenge with sodium chloride would appear as safe as any other challenge with which we have had experience, *i.e.* methacholine, histamine, exercise and hyperventilation. Further studies are required to establish safety and efficacy and acceptability in larger groups of subjects and to compare responses with other stimuli commonly used for bronchial provocation testing. From a technical standpoint, long-term stability of the dry powder of sodium chloride and achieving a greater proportion of substance in the respirable range (to reduce coughing) are important issues that need to be addressed before commercial development is considered.

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