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Twenty-eight-day Double-blind Safety Study of an HFA-134a Inhalation Aerosol System in Healthy Subjects

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Abstract

A 28-day double-blind parallel group study has been conducted to compare the safety and tolerability of HFA-134a, a chlorofluorocarbon-free propellant in a pressurized metered-dose inhaler (MDI A), with a chlorofluorocarbon propellant (MDI C).

Sixteen subjects were randomly assigned to receive one of the two MDIs, either four inhalations four times per day for 14 days or eight inhalations four times a day for 14 days, and were then crossed over to the alternative exposure regime with the same propellant for the next 14-day period. No clinically significant changes occurred in blood pressure, heart rate, electrocardiograms, pulmonary function (FEV_1, FVC, $FEF_{25-75\%}$), haematology or serum chemistry. One subject in the MDI A group had elevated eosinophil counts throughout the study; there were no other remarkable clinical laboratory data. Fifty six adverse events were related to the study propellants; 34 of these occurred in the MDI C group and 22 in the MDI A group. For each adverse event no statistically significant differences were detected between propellant systems or between exposure levels. The most frequent adverse event was headache, which was reported by four subjects with each propellant system. Blood samples for HFA-134a in the MDI A group were collected on day 28 to measure systemic absorption. Blood levels of HFA-134a were detected in all subjects given this propellant within 1 min post-exposure, and these levels decreased to one-tenth of the original value by 18 min after the start of exposure.

The safety and tolerability of an HFA-134a chlorofluorocarbon-free system was demonstrated over 28 days of exposure in healthy subjects. These negative results are clinically important because they indicate it will be safe to proceed with the study of this chlorofluorocarbon-free system in asthmatic patients.

It is now widely accepted that the global depletion of stratospheric ozone can be attributed to a large extent to the generation of atmospheric chlorine by the commercial use of chlorofluorocarbons (CFCs) (Manzer 1990). As a result of heightened environmental concerns, a global effort is being made to eliminate the manufacture of CFCs (D'Souza 1995). The ban on CFC production will have a major effect on the medical community, which uses CFCs as propellants to deliver drugs to the lungs of such patients as asthmatics (Newman 1990).

A consortium of pharmaceutical companies is jointly developing alternative propellants to CFCs. The development effort began in the late 1980s with the identification of the suitability of 1,1,1,2-tetraflouroethane (HFA-134a). HFA-134a contains no chlorine and is considered to have essentially no ozone-depleting potential (Ravishandara et al 1994). This hydrofluoroalkane has recently completed a rigorous programme of safety studies in mice, rats, rabbits, and dogs (Alexander 1995; Finch et al 1995). Because of the chemical properties of HFA-134a, however, more difficulty was encountered in preparing stable aerosol formulations with this propellant than with current CFC propellants (Tansey 1994). Despite this an HFA-134a metered-dose inhaler (MDI) of salbutamol sulphate (Airomir) using a proprietary CFC-free system has recently been introduced in several European countries, and additional drug formulations are currently under development.

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During product development, clinical testing of this CFC-free system without active drug for at least one month was required to provide data demonstrating the system's safety and tolerability. It was hoped that the results of a comparative study of the safety profiles of the CFC-free system and a typical CFC 11/12 propellant blend would be completely negative. Such a result would be of clinical importance as it relates to the safety of the alternative propellant system to be used in future CFC-free medicinal aerosols. To enable objective assessment of the results, the study was performed double-blind.

Methods and Materials

Inhalers

3M Health Care Ltd (Loughborough, UK) prepared all MDIs. The CFC-free MDI (MDI A) contained HFA-134a as propellant, ethanol as co-solvent, and oleic acid as surfactant. The reference MDI (MDI C) contained the CFC propellants 11 and 12 in the ratio of 1:2.6 and the surfactant oleic acid. Each MDI was primed just before first use.

Study population

Sixteen subjects entered and completed the study. Subjects were healthy, non-smoking males between 18 and 55 years of age

(mean ± s.d.; 27·6 ± 5·3 MDI A group; 23·8 ± 4·7 MDI C group), and within ± 15% of ideal body weight (73·0 ± 7·0 kg MDI A group; 66·4 ± 7·3 kg MDI C group). Subjects had to have at least 90% of predicted normal FEV_1 (Quanjer et al 1993) and demonstrate correct use of a placebo MDI. Subjects were prohibited from taking any drug within two weeks of the study and from ingesting ethyl alcohol and methylxanthine-containing foods and beverages for 24 h before and during the study. Paracetamol was an exception which was allowed throughout the study.

All subjects gave written informed consent before inclusion, in accordance with the Declaration of Helsinki; in addition, each subject's personal physician was informed and asked to record his or her lack of objection. The study protocol was approved by the Independent Ethics Review Committee of Inveresk Research International (Tranent, UK).

Study design

The study was a double-blind, parallel group design, with 28 days of continuous exposure. Subjects were randomly assigned into one of two propellant system groups, MDI A or MDI C, eight subjects per group. Within each group, subjects were further randomized to receive either four inhalations four times per day (16 inhalations per day) for 14 days or eight inhalations four times per day (32 inhalations per day) for 14 days; and then were crossed over to the alternative exposure level with the same propellant system for the next 14-day period. Each subject was domiciled for the entire study. All inhalations were administered by the subjects under the supervision of a nurse. Subjects were instructed to hold their breath for 10 s following each inhalation and to wait 30 s before taking the next inhalation. The times when the subjects were exposed were 0800, 1200, 1600 and 2000 h.

Safety assessments

Blood pressure and heart rate were determined 15 min after each exposure on days 1, 7, 8, 14, 15, 21, 22 and 28. Twelve-lead electrocardiograms (ECGs) were recorded just before the study, before the 2000 h exposure on days 14 and 28, and post-study. Haematology and serum chemistry were measured before the 0800 h exposure on days 1, 14, 28 and post-study.

Pulmonary function tests were performed immediately before and 20 min after the 0800 h exposure on each of the 28 study days using an Alpha Vitalograph spirometer. Pulmonary function was measured in triplicate, and the largest volumes of FEV_1, and FVC were recorded. The $FEF_{25-75\%}$, value was taken from the test which gave the largest sum of FEV_1, and FVC. All manoeuvres had to be technically acceptable in accordance with the current statement of spirometry standardization from the American Thoracic Society (1987).

Blood level monitoring of HFA-134a and ethanol

Blood samples (3 mL) were drawn into sealed head-space vials for the determination of HFA-134a levels immediately before exposure, at 1 min post-exposure, and at 18 min and 2 h following the first inhalation of the 1200 and 2000 h exposures on ay 28. A post-study sample was collected at 0800 h on day 29. HFA-134a levels in whole blood were determined by head-space-capillary gas chromatography with flame ionization detection (Cooper et al 1995).

Blood samples (5 mL) were drawn for the determination of ethanol levels pre-exposure on day 1, 3 min after the last inhalation and 20 min after the first inhalation of the 2000 h exposures on days 14 and 28, and post-study at 0800 h on day 29. The whole-blood samples were immediately analysed by gas chromatography with flame ionization detection (Cooper 1971). The limit of detection was 5 $\mu g\ mL^{-1}$.

Statistical analysis

The primary responses were the mean changes from baseline in pulmonary function tests, vital signs, ECGs, haematology, and serum chemistry. These mean values were compared using analysis of variance with sequences, subjects within sequences, periods, and propellant system as factors in the model. The subjects-within-sequences mean square was used as the error term in the test of sequence effect. Tests with a P value of less than 0·05 were considered significant.

The incidence of adverse events was also examined. The incidence of adverse events was compared between propellant system groups using Fisher's exact test and between exposure levels (within a propellant system) using McNemar's test for paired binomial data.

Results

Cardiovascular responses

No clinically significant abnormalities or trends in heart rate or blood pressure measurements were noted for either propellant system at either exposure level. Four subjects had elevated heart rate (> 90 beats min^{-1}) with each propellant system. The only other abnormality occurring in more than one subject was elevated systolic blood pressure (> 90 mmHg), which occurred in two subjects given MDI C. For the abnormalities reported by multiple subjects in a propellant system group, equal distribution of reports were seen between the two exposure levels.

No clinically significant changes in ECGs occurred during this study in relation to propellant system or exposure level in either atrial or ventricular rate, or in P-R, QRS, or QT intervals. Three subjects were noted as having incidental occurrences of sinus bradycardia in the MDI C group, and none in the MDI A group. There was one report of ventricular extrasystole in the MDI A group.

Pulmonary function tests

Actual pre-exposure pulmonary function test values were statistically higher in the MDI C than in the MDI A group, beginning before the 0800 h exposure on day 1 (before randomization) and continuing throughout the study. The daily pre-0800 h exposure pulmonary function test values did not, however, change significantly during each 14-day exposure period with either propellant system. Fig. 1 shows the daily pre-0800 h exposure results for all 28 days of the FEV_1, tests. Pre-0800 h exposure results for the FVC and $FEF_{25-75\%}$ tests were similar.

No clinically or statistically significant changes in pulmonary function test values from the pre-0800 h exposure baseline values were seen for any parameter (between propellant system

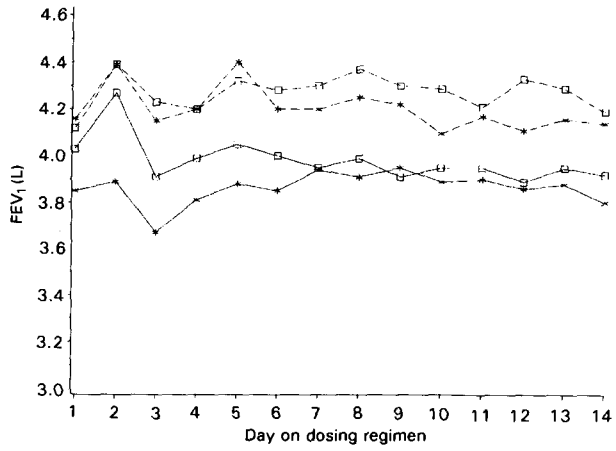

FIG. 1. Mean baseline FEV_1 values (pre-0800 h dose) on each dosing day in subjects given either four inhalations four times a day (*) or eight inhalations four times a day (□) for 14 days of either MDI A (—) or MDI C (---). Each subject received 28 days of dosing with one MDI.

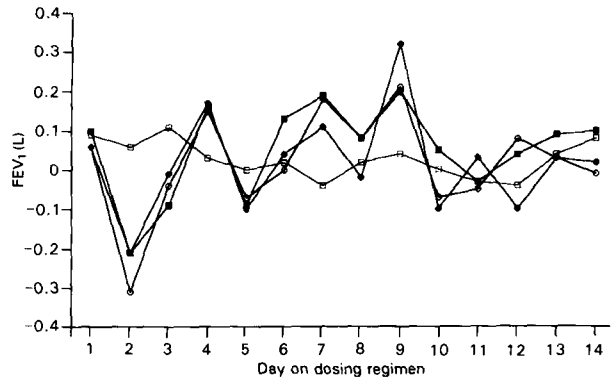

FIG. 2. Mean change from baseline FEV_1 values on each dosing day in subjects given four inhalations four times a day (□, ■) or eight inhalations four times a day (○, ●) for 14 days of either MDI A (□, ○) or MDI C (■, ●).

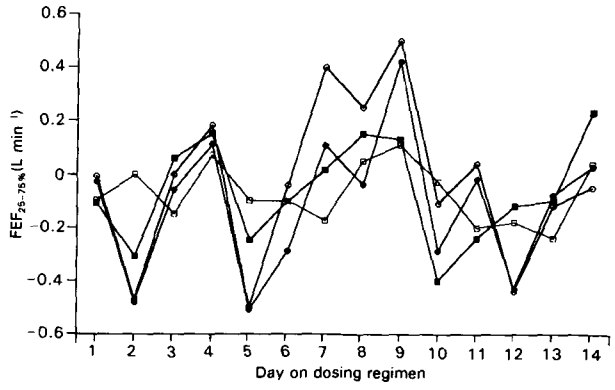

FIG. 3. Mean change from baseline FEF_{25-75} values on each dosing day in subjects given either four inhalations four times a day (□, ■) or eight inhalations four times a day (○, ●) for 14 days of either MDI A (□, ○) or MDI C (■, ●).

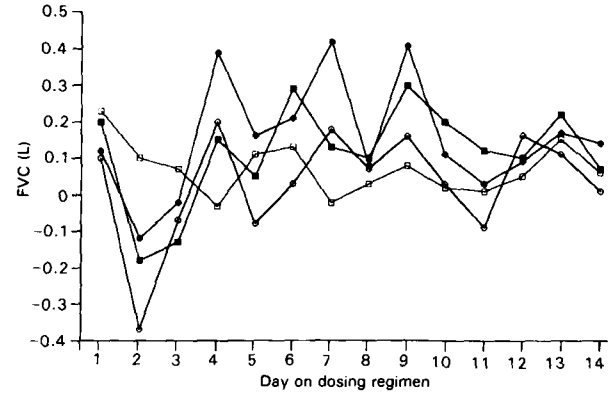

FIG 4. Mean change from baseline FVC values on each dosing day in subjects given either four inhalations four times a day (□, ■) or eight inhalations four times a day (○, ●) for 14 days of either MDI A (□, ○) or MDI C (■, ●).

or exposure level) when these pre-exposure values were compared with those obtained 20 min post-exposure. Fig. 2 shows the change from baseline results for the FEV_1 tests. Change from baseline results for the other pulmonary function tests are given in Figs 3 and 4.

Clinical laboratory data

As expected because of the large number of tests performed, several minor abnormalities, most within 15% of the normal range, were observed in the haematology and serum chemistry parameters. All were judged to be clinically insignificant and not propellant-related, with one exception. One subject in the MDI A group had his eosinophil count increase throughout the study, from $0{\cdot}70 \times 10^9$ counts L^{-1} on day 1, to $1{\cdot}38 \times 10^9$ counts L^{-1} on day 14, to $3{\cdot}46 \times 10^9$ counts L^{-1} on day 28. His eosinophil count was $3{\cdot}02 \times 10^9$ counts L^{-1} on day 29 (post-study); when re-tested six months later it was in the normal range.

Adverse events

Table 1 lists the 56 events that were classified as possibly or probably related to the study propellant systems; 34 occurred in the MDI C group and 22 in the MDI A group. Nearly half of the adverse events (26) occurred following eight inhalations with MDI C. For each type of adverse event, no statistically significant differences were detected between the propellant systems or between the exposure levels (within a propellant system). The most frequent adverse event was headache, reported by four subjects with each propellant system. All adverse events were of mild or moderate intensity with one exception. One subject while receiving eight inhalations of MDI A reported two instances of severe headache. One instance was resolved by treatment with paracetamol, the other required no medication. There were no serious adverse events.

HFA-134a and ethanol blood levels

Table 2 lists the blood concentrations of HFA-134a on the last day of exposure with each exposure level in the MDI A group.

Table 1. Treatment-related adverse events.

Body system	Adverse event	Number of adverse events: MDI A By subject	Total reported	MDI C By subject	Total reported
Cardiovascular	Chest tightness	1	1	0	0
Respiratory	Cough	1	2	2	11
	URTI[a]	1	1	1	1
	Sore throat	1	1	1	2
	Dry throat	0	0	2	2
	Sneezing	0	0	1	1
Gastrointestinal	Nausea	0	0	2	6
	Oral ulceration	2	3	1	1
	Indigestion	0	0	1	1
	Bitter taste	1	1	0	0
Central nervous system	Headache	4	10	4	8
	Dizziness	1	1	1	1
	Light-headedness	1	1	0	0
	Malaise	1	1	0	0

[a] Upper respiratory tract infection.

As no differences in blood concentration were seen between the 1200 h and 2000 h exposures at any time, the data from the two exposures were combined. The HFA-134a blood concentrations achieved within 1 min of completion of the four-inhalation exposure ranged from 331 to 1222 ng mL^{-1}. Blood concentrations approximately twice as high, 546 to 2357 ng mL^{-1}, were seen at similar times after eight-inhalation exposure. A larger difference between the exposure levels was seen with the blood samples that were collected 18 min after the start of each exposure. No HFA-134a was detectable in the 2 h samples.

No post-study samples or blood samples from the MDI C group contained quantifiable levels of HFA-134a. Analysis of blood CFC levels was not performed.

Ethanol was not detected in any blood sample from either propellant system group. Blood sampling times included 3 min post-exposure, when the HFA-134a levels were expected to be high (Ventresca 1995), and 20 min post-exposure, when ethanol levels following low oral doses of ethanol were expected to be high (Wilkinson et al 1977).

Discussion

This 28-day study was designed for objective examination of systemic changes after inhalation of a novel CFC-free pressurized MDI propellant system. The amount of propellant administered represented the upper limit of that which might be expected during chronic MDI use by severe asthmatics. For example, the commercially available CFC-free system formulation of salbutamol sulphate (Airomir) uses a 25 μL valve; that used in this study used a 63 μL valve. The high daily exposure in this study was equivalent to 80 inhalations per day from Airomir.

As hoped from animal toxicology studies, results from this clinical study were negative for the CFC-free system for all measured safety parameters. There were no serious or unexpected adverse events recorded throughout the study and there were no observations of bronchospasm or hyperactivity with either propellant. Although there was a suggestion from one subject that MDI A could affect the eosinophil count, subsequent results from studies as long as 12 weeks with MDI A have not found any significant change in eosinophil count (Bleecker et al 1995). This study is of clinical importance in providing part of the documentation needed to enable the mandatory change to ozone-sparing propellants.

Because this was the first long-term study in a CFC-free aerosol development programme, normal subjects, rather than asthmatics, were enrolled. The selection of healthy subjects afforded certain advantages over asthmatics – a stable pulmonary function baseline and no confounding concomitant medication. This enabled sensitive measurement of the systemic

Table 2. Blood levels of HFA-134a (ng mL^{-1}) following four or eight puffs four times per day.

Time	HFA-134a blood-level: 4 inhalations Mean ± s.d.	Median	8 inhalations Mean ± s.d.	Median
1 min post-exposure	717 ± 359	521	1381 ± 694	1107
18 min after start of exposure	29 ± 9[a]	27	107 ± 46[b]	107

[a] Approximately 15·5 min post-exposure. [b] Approximately 13·5 min post-exposure.

effects of the HFA-134a CFC-free system. Future studies are necessary involving patients with hyper-reactive airways to enable examination of the local effects of the CFC-free system on the lungs.

Stable but statistically higher pulmonary function test values in the MDI C group than in the MDI A group were observed before and throughout the study. Because the higher values were seen pre-exposure, it is likely that the differences between the groups occurred by chance. Since all pulmonary function test statistical comparisons involved change from baseline, the absolute value of each pulmonary function test parameter did not affect the analyses.

Blood samples on day 28 were collected during the study for the determination of HFA-134a levels. The purpose of these samples was to establish the range of blood levels of propellant HFA-134a that would be expected in man following multiple exposure. Within 1 min of exposure on day 28, HFA-134a levels were detectable in all subjects and were reasonably proportional to the exposure. The actual blood levels observed were in the same range as has been reported for CFC propellants 11 and 12 (Paterson et al 1971) and for HFA-134a after a single exposure (Donnell et al 1995).

Eighteen minutes after the start of exposure, HFA-134a blood levels had decreased to one-tenth or less of the original concentration, in most instances for both exposure levels. This result is also in agreement with observations for CFC propellants 11 and 12 (Paterson et al 1971) and for HFA-134a after a single exposure (Ventresca 1995) and suggests rapid removal of HFA-134a from the blood and no apparent accumulation upon chronic exposure.

Ethanol was a component of the MDI A formulation and each 63-μL actuation of MDI A contained 9 μL ethanol. Assuming complete pulmonary absorption and a volume of distribution of 0·54 L kg^{-1} (Benet & Williams 1990), maximum blood ethanol concentrations of about 0·5 $\mu g\ mL^{-1}$ were projected in the highest exposure group with MDI A. In actuality, studies have shown that pulmonary deposition from the CFC-free system is about 55% of that delivered from the canister, which would reduce the estimated blood concentration approximately in half (Leach 1996). It was not surprising that incomplete pulmonary absorption combined with extensive metabolism (Holford 1987) resulted in no detectable ethanol levels after 14 days of exposure with MDI A. From a safety viewpoint, this level of ethanol is in the same range as that seen endogenously on the breath of volunteers who abstained from ethanol and is not associated with any toxicity (Jansson & Larsson 1969; Jones 1985).

Overall, the safety and tolerability of the HFA-134a CFC-free system was demonstrated over 28 days of exposure in healthy subjects. These results provide support for the continued development of this system.

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