Asthmagenic properties of a newly developed detergent ingredient: sodium iso-nonanoyl oxybenzene sulphonate

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Abstract

The suspicion that a newly developed detergent ingredient, sodium iso-nonanoyl oxybenzene sulphonate (SINOS), was inducing asthma among a workforce led to a series of inhalation challenge tests to determine the specificity and dose response characteristics of its asthma provoking properties. Three previously exposed workers, three non-exposed non-asthmatic controls, and three non-exposed asthmatic controls were challenged with SINOS 0.01–100 µg and another chemically similar surface active detergent ingredient, linear alkyl benzene sulphonate (LAS) 0.01–100 µg. Asthmatic symptoms, late falls in FEV₁, and increases in non-specific bronchial responsiveness were seen after the inhalation of SINOS in all three workers, confirming SINOS as a cause of occupational asthma. No changes were seen after the inhalation of SINOS in either group of control subjects nor after LAS in any subject. These findings suggest that SINOS causes asthma through a specific hypersensitivity mechanism unrelated to its surface active properties.

During the industrial development of a new detergent ingredient (sodium iso-nonanoyl oxybenzene sulphonate, SINOS, (fig 1)), which has bleach activating and surface active properties, rashes, rhinitis, conjunctivitis, and wheezing developed in several workers over 12–18 months. In one worker with respiratory symptoms late asthmatic reactions and increases in non-specific bronchial responsiveness (NSBR) were noted both on returning to the workplace after a two week absence and after a series of inhalation challenge tests in the laboratory.¹

Occupational asthma attributable to SINOS was consequently diagnosed.

The challenge tests entailed the daily inhalation of nebulised SINOS with increasing doses. Whereas clear cut late asthmatic reactions (LARs) were seen after two separate challenges with the highest SINOS dose (32 µg), there were also late falls in ventilatory function of smaller magnitude after lower doses. An apparently linear dose response relation relating the magnitude of the late declines in ventilatory function to the logarithm of the challenge dose was observed.¹ Such a dose response relation for LARs after inhalation challenge with common allergens or industrial agents had not previously been published and it raised the question whether SINOS might be acting through an unusual mechanism.

The present study has investigated the specificity of SINOS in causing occupational asthma using a further series of inhalation challenge studies involving (1) the index case and two other workers with symptoms suggesting occupational asthma, (2) three unexposed non-asthmatic control subjects, and (3) three unexposed control subjects with symptomatic asthma. To determine whether any asthmagenic properties could be related to the surface active properties of SINOS—that is, its ability to reduce surface tension—each subject also underwent a series of inhalation challenges with the structurally similar detergent ingredient linear alkyl benzene sulphonate

SINOS

LAS

Figure 1 SINOS: sodium iso-nonanoyl oxybenzene sulphonate and LAS: linear alkyl benzene sulphonate.

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(LAS, fig 1).² LAS has been widely used throughout the detergent industry for many years without any suspicion of it causing occupational asthma.³ It is chemically similar to SINOS and shares its surface active but not its bleach activating properties.

Methods

SUBJECTS

Three development technicians who had been occupationally exposed to SINOS within a research and development plant and had symptoms suggesting occupational asthma were studied along with three non-asthmatic control subjects selected from the investigating team and three asthmatic subjects from our clinic population. None of the six control subjects had had previous exposure to SINOS. The asthmatic control subjects were chosen to have similar levels of NSBR to those measured in the workers at the time of their symptoms. The non-asthmatic controls all had low levels of NSBR which could not be quantified using our standard methacholine challenge technique⁴—that is, they did not show a 20% fall in FEV₁ after the maximum cumulative dose of 6.4 mg. The mean age of the workers was 36, of the non-asthmatic controls 38, and of the asthmatic controls 26. Two workers and two control subjects were atopic (at least one skin prick test to common allergens giving a weal diameter greater than that of a 0.1% solution of histamine). All nine subjects had pre-study FEV₁ at least 70% of the predicted value and all subjects gave fully informed consent. The study was approved by the local ethics committee.

CHALLENGE PROTOCOL

Each subject underwent one series of daily inhalation challenge tests with SINOS and one series with LAS, the sequence being chosen randomly. For each agent the starting dose was 0.01 µg. Doses were increased daily by 3.2-fold ($\sqrt{10}$) increments until a reaction causing characteristic symptoms and a 20% fall in FEV₁ occurred or the maximum dose of 100 µg was administered. At least three saline control challenges were interspersed within each challenge series, all challenges being administered double blind with respect to the subject and the immediately supervising physician. The dose range for SINOS (0.01–100 µg) was based on environmental measurements in the manufacturing plant. In the weeks immediately before the initial investigation, maximum exposures of the order 0.02–1.00 µg/hour (0.1–10 µg/day) had been tolerated without undue discomfort. A starting dose of 0.01 µg was consequently considered to be both safe and suitable. Earlier exposures were thought to have been higher and a failure to respond to 100 µg was considered improbable if SINOS hypersensitivity truly existed.

The dose range for LAS was chosen to match that for SINOS. Although occupational exposure to both agents had been as a dry powder of respirable size, each was prepared as a solution for inhalation tests to allow greater precision of dose.

PREPARATION OF SOLUTIONS AND CHALLENGES

Stock solutions of SINOS and LAS 2 mg/ml in 0.4% phenol saline were prepared weekly and appropriate serial dilutions made using the phenol saline solution. Diluted samples were then labelled and stored at 4°C. The challenge sequence (LAS series first or SINOS series first) having been chosen in advance, a decision was made each day by one of us whether to use the next dose of SINOS/LAS or a saline control, and the appropriate solution was placed in an unlabelled container.

Nebulised challenges were performed using an inhalation dosimeter; a total of 50 µl of challenge solution being administered from five inhalations. The dosimeter was calibrated each morning and delivered 10 µl $\pm$ 5% to the mouth with each inhalation. The phenol effectively disguised any smell/taste of the detergent and the body of the nebuliser was covered to mask any frothing provoked by the higher doses of detergent. Thus neither the subject nor the immediately supervising physician was aware of the identity of the challenge agent, its possible dose, or whether a control challenge was being administered.

MONITORING TESTS

NSBR was measured as PD₂₀FEV₁ to methacholine using a dosimeter technique during the week before and at intervals after each challenge series (see table 1).⁴ Measurements were made on different days at 1000.

On each challenge day, ventilatory function was monitored as FEV₁ (mean of three technically satisfactory measurements). It was measured at 10 minute intervals from 0920 to 1000 using a dry wedge bellows spirometer (Vitalograph, Buckingham). Inhalation challenge was at 1000, after which FEV₁ was measured at 10 minute intervals until 1120 to detect any early asthmatic reaction. It was then measured hourly to 2200, at 0300 if there had been any symptoms during the day, and from 0800 to 1000 the next day to complete a 24 hour period of surveillance. All respiratory symptoms over the 24 hour monitoring period were recorded.

SUPERVISION

Before the challenge protocol, subjects measured FEV₁ as above on three days. For each hour, the lowest FEV₁ recorded over these three days was selected and from this 90% and 80% "reference" values were calculated. After each inhalation challenge, subjects were observed in the laboratory until 1200 when the possibility of an immediate

Table 1 Changes in bronchial responsiveness in the workers and asthmatic controls

Challenge test	PD ₂₀ FEV ₁ (µg methacholine)		
Sequence	Asthmatic control 1	Worker 1	Worker 3
Before challenge:			
1	130	800	230
2	260	1300	250
After LAS:			
3	420	950	6400
4	580		> 6400
After SINOS:			
5	700	185 (day 1)	110 (day 1)
6		200 (day 3)	440 (day 2)
7		270 (day 6)	320 (day 3)
8		400 (day 10)	460 (day 8)
9		500 (day 13)	1250 (day 11)
10		2000 (day 29)	3800 (day 45)
Sequence	Asthmatic control 2	Asthmatic control 3	Worker 2*
Before challenge:			
1	400	320	0%
2	600	152	5%
3		400	
After SINOS:			
4	530	145	13% (day 1)
5	540		15% (day 12)
6			12% (day 14)
After LAS:			
7	380	340	6%

*Bronchial responsiveness for worker 2 expressed as % fall of FEV₁ after 6.4 mg methacholine.

A decreasing PD₂₀ indicates an increasing level of bronchial responsiveness.

Three subjects were challenged with LAS first and three with SINOS first. Each challenge series followed immediately from the previous: thus for workers 1 and 3, the post-LAS measurements of bronchial responsiveness are the pre-SINOS measurements.

No non-asthmatic control subject showed a measurable PD₂₀ at any stage.

Day 0 is the day of the last challenge in the series.

asthmatic reaction had passed. They then returned home with a spirometer to record FEV₁ values without immediate supervision. They were instructed to contact the supervising doctor if FEV₁ measurements fell below the 90% reference value. If the FEV₁ fell below the 80% reference value the immediately supervising physician was required to meet the subject, confirm the accuracy of the measurements, and arrange further supervision or treatment as necessary.

EXPRESSION OF RESULTS

Late declines in ventilatory function were expressed cumulatively by the 2-12 hour "area decrement" from the FEV₁/time plot. This comprised the area in litre × hours between a line extrapolated from the mean baseline for the day and the hourly measurements of FEV₁ between two and 12 hours after challenge. It takes into account all measurements during the observation period and is subject to less variation than a single measurement of ventilatory function—for example, the lowest FEV₁ after challenge or the maximum fall in FEV₁ from baseline.

SUBTILISIN CHALLENGE

For worker 1, a series of similar inhalation challenge tests was performed using the detergent enzyme, subtilisin, to which he had also been exposed occupationally. Challenges over the dose range 0.001-0.1 µg were performed with saline controls interspersed in a double blind manner as described above.

Results

Challenge with SINOS produced unequivocal LARs with symptoms of wheeze/ breathlessness and late declines in FEV₁ at the highest challenge doses in all three workers (table 2, fig 2). With workers 1 and 3 there were significant increases in NSBR that persisted for up to six weeks (table 1); a doubling or halving of the PD₂₀ suggesting a significant change.⁴ With worker 2, a PD₂₀FEV₁ was never obtained. A PD₁₀FEV₁ was briefly obtainable immediately after the SINOS challenge series suggesting that there had been an increase in NSBR if not into the conventionally measured range. Dose response analyses suggested log linear relation between the challenge dose of SINOS and the magnitude of the LAR for two workers, though for the third (worker 2), such a relation was not obvious (fig 3).

No symptoms, falls in FEV₁, or increases in NSBR were seen after challenge with SINOS in the three non-asthmatic control subjects or in the three asthmatic controls. FEV₁ area decrements after the maximum SINOS doses in all six control subjects did not exceed those seen on control days (table 2). Table 1 shows PD₂₀FEV₁ values for the three asthmatic control subjects. Subject 1 showed a steady im-

Table 2 Changes in ventilatory function following challenge with SINOS and LAS

Test subject	2-12 Hour FEV ₁ area decrement (l × h)		
	Control days (median & range)	SINOS (100 µg)	LAS (100 µg)
Workers:			
1	0.69 (-0.96; 1.22)	7.47*	0.50
2	-0.17 (-1.50; 0.53)	2.64	0.98
3	-0.11 (-2.58; 1.23)	7.62	0.10
Non-asthmatic controls:			
1	0.31 (-1.11; 1.39)	0.26	-0.28
2	0.55 (-0.16; 1.40)	0.71	-0.06
3	0.22 (-0.02; 1.41)	-1.63	-0.68
Asthmatic controls:			
1	-0.02 (-1.66; 1.24)	0.04	-0.59
2	-0.95 (-2.17; 2.19)	-0.47	-2.03
3	0.47 (-0.46; 2.30)	0.42	1.24

*Maximum dose of SINOS = 32 µg.

1 All 2-12 hour FEV₁ area decrements after challenge with SINOS 100 µg or LAS 100 µg in the control subjects were within the range observed on control days.

2 Similar results were noted with the 2-24 hour FEV₁ area decrements.

3 A negative area decrement implies a net improvement in FEV₁, compared with baseline values.

Figure 2 Late asthmatic reactions after challenge with SINOS in three workers. — challenges with highest challenge doses of SINOS for each worker, — a control challenge with saline. All challenges were administered at 1000.

provement in NSBR (increase in $PD_{20}FEV_1$) throughout the challenge series, possibly due to his removal from domestic environmental agents during the study period; and the other asthmatic controls showed no significant changes. None of the non-asthmatic controls showed a $PD_{20}FEV_1$ at any stage of the study.

No symptoms, sustained falls in FEV_1 , or increases in NSBR were seen after LAS challenge in any

subject (table 2, fig 3). Worker 3 showed a considerable increase in $PD_{20}FEV_1$ after challenge with LAS. This may have been due to his removal from the workplace and consequent reduced exposure to SINOS during the LAS challenge series. Worker 2 showed an increase in 2–12 hour area decrement after LAS 100 µg which just exceeded those on the control days (table 1). This value, however, was not beyond the 95% confidence limit established for these areas.

Figure 3 Dose response relations for late asthmatic responses to SINOS in three workers and subtilisin in one worker. C = Control; FEV_1 monitoring without any challenge or after a saline control challenge; L = challenges with LAS 0.01–100 µg. Linear regression lines are plotted for workers 1 and 3. Regression equations are area decrement = $2.85 + 0.6 \times \text{dose increment}$ for worker 1 and area decrement = $-0.6 + 0.7 \times \text{dose increment}$ for worker 3. Highest control measurement associated with subtilisin challenges occurred during challenge sequence after subtilisin 0.32 µg and was probably influenced by reaction on preceding day.

decrements on control days, did not have a pattern typical of an LAR, and was not accompanied by symptoms or changes in NSBR.

Figure 3 shows the results of the subtilisin challenges in worker 1. The threshold for LARs appeared to occur at about 0.01 μg . There was only a small increase in the magnitude of the reaction at 0.032 μg , followed by a more rapid increase in the response with symptoms and a maximum fall in FEV_1 of 24% after the 0.1 μg challenge. This latter response was reproduced on retesting.

Discussion

Unequivocal symptoms, LARs, and increases in NSBR occurred after the SINOS challenge tests in the three workers confirming the role of SINOS as a potential inducer of occupational asthma. These results coupled with the lack of symptoms for several months after exposure first occurred, are typical features of occupational asthma. The apparent log linear dose response relation for the LARs was, however, unexpected when the index case was first studied in 1985.¹ Such a relation had not then been reported for occupational or natural inducers of asthma and the question that arose was whether this relation indicated an unusual pathogenetic mechanism or whether with the refinements of our investigatory protocol we had simply clarified the normal pattern of reactivity at dose levels preceding the threshold of the clinically unequivocal reaction.

The original finding in worker 1 was reproduced in this study but the slope of the dose response relation had diminished slightly from 0.8 to 0.6 $1 \times \text{h}/\text{dose}$ increment over the intervening year spent without further exposure to SINOS. The data still suggested, however, that whereas moderate and clinically obvious LARs occurred after 32 μg SINOS, LARs of smaller magnitude were occurring from the 0.032 μg dose onwards. The size of the LAR, measured as the 2–12 hour area decrement, increased only fourfold whereas the dose of SINOS increased 3200-fold from 0.01 to 32 μg —seemingly in a log linear fashion.

The results obtained using the same protocol in workers 2 and 3 showed a similar picture. With worker 3, the slope of the regression equation was somewhat greater than that of worker 1, and the points on the dose response plot were less closely deployed about the regression line. The findings were, nevertheless, similar to those of worker 1. With worker 2, the principal symptoms had been of rhinitis and conjunctivitis and the clinical evidence for asthma before this study was less convincing than for workers 1 and 3. The dose response relation was less clearly log linear and its slope was flatter. There was a curious step in the dose response graph from 0.32 to 1 μg but we could not find any artefact to

account for it. There was a suspicion of a LAR after 0.32 μg SINOS but it was not until the 100 μg dose that there was a clinically unequivocal and symptomatic LAR. This analytical approach provides the framework for a statistical input to the recognition of LARs. A full description of the use of statistics in analysing asthmatic reactions is beyond the scope of this paper but is being published in detail elsewhere.⁵

It was of interest to examine the response to a more commonly recognised inducer of occupational asthma—subtilisin.⁶ Worker 1 had previously worked with this well recognised asthma inducer and had developed positive immediate skin prick test reactivity to it. The more rapid increase in response seen with increases in subtilisin dose is perhaps the pattern which is more widely expected. None the less increasing LARs over a tenfold dose range were shown and we cannot be confident that this pattern differs from that seen with SINOS, or that the SINOS pattern is unique.

In fact a similar dose response relation has also been shown for tetrachlorophthalic anhydride.⁷ It consequently appears that dose response relations for LARs may be elicited for a wider variety of occupational agents and possibly common allergens⁸ if study protocols are sufficiently robust. It should also be possible to elicit threshold values at which reactions first occur, though such “postsensitisation” values are not probably of much use in establishing conventional threshold limit values (or other statutory exposure limits) designed to prevent sensitisation occurring initially.

The closeness of fit of the dose response plots to the regression line is interesting. With all three workers there were examples of a dose increment producing a lesser area decrement. Part of this may be attributed to biological and measurement variability but part is likely to result from the daily challenge protocol. In general, challenges were not carried out at weekends and this would have allowed changes in NSBR induced by each SINOS challenge to subside partially or even fully before the next SINOS dose was administered. Similarly, a SINOS challenge after a saline control challenge was likely to produce a lesser reaction than one immediately after another SINOS challenge.

In two workers considerable changes in $\text{PD}_{20}\text{FEV}_1$ to methacholine were noted in association with the SINOS challenges. These persisted for up to six weeks. In the third worker a $\text{PD}_{20}\text{FEV}_1$ was never obtained. The development of a $\text{PD}_{10}\text{FEV}_1$ after the SINOS series does suggest, however, that an increase in NSBR occurred. The provocation of late asthmatic reactions in the absence of pre-existing NSBR measurable by $\text{PD}_{20}\text{FEV}_1$ has been reported but is an exception rather than the rule.⁹ It is consistent with the belief that the strength of asthmatic reactions to specific environmental agents

(whether occupational or natural) is dependent on the dose, the degree of specific hypersensitivity, and the level of NSBR. In the presence of low levels of NSBR (below those measurable as $PD_{20}FEV_1$ to methacholine) late asthmatic reactions may still be observed if the challenge dose and the degree of specific hypersensitivity are sufficiently high.

The lack of any reactions after LAS challenges in the workers suggests that the ability of SINOS to induce asthma is not directly related to its surface active properties. It has been suggested that such agents are capable of disrupting gastrointestinal epithelial surfaces¹⁰ and whereas we are unaware of any similar studies in the lungs, airway epithelial disruption is increasingly being recognised in association with asthma.¹¹ Our results, however, are consistent with the widespread industrial experience with LAS during which few respiratory problems¹² (and no occupational asthma) have been reported.

No symptoms, LARs, or changes in NSBR occurred in the control subjects after SINOS. This suggests that whatever the mechanism of action of SINOS, it is a specific one and accords with the latent period between the onset of occupational exposure and the development of symptoms. SINOS asthma therefore appears similar to other varieties of occupational asthma where immunological hypersensitivity is presumed. Immune reactivity to this chemical has not been shown (personal communication with manufacturer) and the mechanism of SINOS asthma remains undefined. Detergents formulated for domestic use are less likely to be respirable and this together with the specificity of SINOS asthma suggests that it is unlikely to pose any

hazard to the consumer. Furthermore, improved industrial hygiene standards during manufacture should allow SINOS to be used safely in the occupational setting.

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