ASTHMA

Genetic polymorphism of *GSTM1* and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City

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Background: We recently reported that antioxidant supplementation with vitamins C and E mitigated ozone related decline in forced expiratory flow ($FEF_{25–75}$) in 158 asthmatic children in an area with high ozone exposure in Mexico City.
Methods: A study was undertaken to determine whether deletion of glutathione S-transferase M1 (*GSTM1* null genotype), a gene involved in response to oxidative stress, influences ozone related decline in $FEF_{25–75}$ and the benefit of antioxidant supplementation.
Results: *GSTM1* null children receiving placebo had significant ozone related decrements in $FEF_{25–75}$ (percentage change per 50 ppb of ozone 2.9 (95% CI −5.2 to −0.6), p = 0.01); *GSTM1* positive children did not. Conversely, the effect of antioxidants was stronger in children with the *GSTM1* null genotype.
Conclusions: Asthmatic children with a genetic deficiency of *GSTM1* may be more susceptible to the deleterious effects of ozone on the small airways and might derive greater benefit from antioxidant supplementation.

Ozone is a powerful oxidant associated with impairment of pulmonary function and increased airway inflammation.[1] We recently reported that asthmatic children in Mexico City, an area with high ozone exposure, have significant ozone related decrements in the index of lung function, forced expiratory flow ($FEF_{25–75}$), that were mitigated by supplementation with the antioxidant vitamins C and E in a double blind randomised trial.[2]

Genetic factors probably contribute to the substantial variability between individuals in the effects of ozone on lung function and airway inflammation observed in humans.[1] Glutathione transferases (GSTs) play a major role in protecting cells against damage from reactive oxygen species—such as those produced by ozone—by conjugating them with glutathione so that they can be rapidly eliminated.[3] The glutathione S-transferase, *GSTM1*, may be especially important in response to oxidative stress,[3] including ozone exposure.[4] A common homozygous deletion polymorphism of the *GSTM1* gene (*GSTM1* null genotype) abolishes enzyme activity and may increase susceptibility to oxidative stress.[3 5]

We hypothesised that asthmatic children genetically lacking in *GSTM1* activity might have greater susceptibility to reductions in $FEF_{25–75}$ associated with ozone exposure and greater benefits from antioxidant supplementation. We tested this hypothesis by genotyping DNA samples from asthmatic children in our earlier randomised double blind trial of antioxidant supplementation in Mexico City.[2]

METHODS

Study design

Details of the randomised double blind antioxidant supplementation study have recently been published.[2] In brief, 158 asthmatic children were recruited through the allergy clinic of a large public hospital in Mexico City and followed over a 12 week period with two spirometric tests per week. At baseline, parents completed an interviewer administered questionnaire on diet and risk factors for asthma. Randomisation to receive the supplement (vitamin C 250 mg/day and vitamin E 50 mg/day) or placebo was conducted in a double blind manner. Children provided blood samples for measurements of vitamins C and E at baseline and at several points during the follow up period to verify compliance.[2] Pulmonary function was measured at each visit with a spirometer (Medifacts Pneumotachograph, San Clemente, CA, USA) according to American Thoracic Society specifications. The best of three technically acceptable manoeuvres was used. Institutional review boards of the collaborating institutions approved the study and parents gave written informed consent for their child's participation.

Measurements of ambient ozone and climate variables were obtained from the Mexican government's air monitoring stations. All children resided within 5 km of a monitoring station whose values were assigned to that child. We also classified exposure to particles less than 10 μm and nitrogen dioxide but we found no evidence for an effect of antioxidant intervention in response to these agents[2] and thus they are not considered in this analysis.

DNA was extracted from whole blood or buffy coat using a standard Gentra Puregene protocol (Gentra Systems, Minneapolis, MN, USA). *GSTM1* genotype was determined as previously described.[6] The method distinguishes subjects with homozygous deletion of *GSTM1* (*GSTM1* null genotype) from those with either one or two copies of the gene (*GSTM1* positive genotype).

Statistical analysis

The effect of ozone exposure on the day before spirometric tests was examined using the maximum 1 hour concentration.[2] Generalised estimating equation models (GEEs) were used to assess the relation between ozone exposure and changes in lung function tests ($FEF_{25–75}$) by *GSTM1* genotype (null versus positive) within the two treatment groups (placebo and antioxidant supplemented). Confounding was evaluated as previously described.[2]

Table 1 Difference in $FEF_{25–75}$ related to ozone concentration on the day before spirometric testing according to *GSTM1* genotype in asthmatic children in Mexico City given antioxidant supplementation or placebo

		All asthmatics			Moderate and severe asthmatics		
Group	Subgroup	n*	Coefficient (95% CI)†, ml/s 50 ppb O_3	Percentage (95% CI) change‡ $FEF_{25–75}$/50 ppb O_3	n	Coefficient (95% CI), ml/s 50 ppb O_3	Percentage (95% CI) change $FEF_{25–75}$/50 ppb O_3
Placebo	*GSTM1* null	29	−50.5 (−90.1 to−10.9)§	−2.9 (−5.2 to −0.6)§	18	−80.8 (−132.7 to −28.9)§	−4.7 (−7.7 to−1.7)§
	GSTM1 positive	49	−10.5 (−38.7 to 17.7)	−0.6 (−2.1 to 0.9)	17	−34.4 (−75.6 to 6.8)	−1.9 (−4.4 to 0.5)
	Genotype effect		40.0 (−7.5 to 87.5)¶	2.3 (−0.2 to 4.9)¶		46.4 (−20.7 to 113.4)	2.8 (−1.2 to 6.7)
Supplement	*GSTM1* null	33	−3.0 (−40.7 to 34.7)	−0.2 (−2.3 to1.9)	21	−7.3 (−54.0 to 39.5)	−0.3 (−3.1 to 2.4)
	GSTM1 positive	47	5.1 (−29.8 to 40.1)	0.3 (−1.6 to 2.2)	26	2.0 (−43.8 to 47.8)	0.1 (−2.4 to 2.6)
	Genotype effect		8.1 (−44.1 to 60.4)	0.5 (−2.4 to 3.3)		9.3 (56.5 to 75.2)	0.4 (−3.2 to 4.1)

*The number of subjects in each group is given. The number of observations is: *GSTM1* null (placebo 654, supplement 732), *GSTM1* positive (placebo 1065, supplement 1039).
†Results and 95% confidence intervals from generalised estimating equation models adjusted for age, height, use of bronchodilator, respiratory symptoms on the day of the spirometric test, minimum temperature, and time since the beginning of the study. Ozone level is 1 hour maximum. During the observation period the mean (SD)1 hour maximum ozone was 102 (47) ppb (range 12–309). The Mexican standard is 110 ppb.
‡Percentage change from baseline $FEF_{25–75}$ for the individual stratum.
§$p \leqslant 0.01$. All p values were obtained by *t* test comparing coefficients between the two groups.
¶$0.05 < p < 0.10$

We first compared the effect of ozone on lung function by *GSTM1* genotype within the two supplementation groups, and then compared the effect of supplementation on the relation between ozone and lung function by genotype. Regression coefficients were compared using a *t* test to estimate the modifying effect of *GSTM1* polymorphisms/or supplementation groups on ozone mediated responses. We also tested the significance of interaction terms between genotypes, supplementation groups, and ozone. These analyses were conducted in all children and in the subsample of children with moderate and severe asthma. All analyses used Stata Release 7.0 (Stata Corporation, College Park, TX, USA).

RESULTS

The *GSTM1* null genotype was present in 39% of children. Although more children with moderate and severe asthma had a *GSTM1* null genotype ($p = 0.03$), baseline pulmonary function did not differ and other characteristics were balanced between the two *GSTM1* genotype groups (male 63.5% versus 66.1%; mean age 9.2 years in both groups; mother smoking 17.0% versus 18.3%). The number of spirometric tests per child was similar between the supplement and placebo groups (mean 22, range 12–28). All but three participants had at least one positive skin test for common antigens. The 1 hour maximum ozone level during the study period ranged from 12 to 309 ppb with a mean (SD) of 102 (47) ppb.

In the placebo group ozone levels were significantly and inversely associated with $FEF_{25–75}$ in children with the *GSTM1* null genotype; no significant decrement was observed in *GSTM1* positive children (table 1). Expressed as a percentage of baseline $FEF_{25–75}$, for an increase of 50 ppb in 1 h maximum ozone concentration, this decrement in children with the *GSTM1* null genotype taking placebo corresponded to −2.9% (95% CI −5.2 to -0.6, $p = 0.01$). This effect was stronger in children with moderate to severe asthma (−4.7%, 95% CI -7.7 to −1.7; $p = 0.002$). The difference in the relation between ozone and $FEF_{25–75}$ by genotype among all asthmatic children receiving placebo was of borderline statistical significance ($p = 0.10$). Children receiving antioxidant supplementation had no statistically significant ozone related decrement in $FEF_{25–75}$, regardless of genotype. When we considered the effect of antioxidant supplementation by genotype, the beneficial effect was seen primarily in the *GSTM1* null individuals (difference of 2.7% between the placebo and supplement groups, $p = 0.09$) rather

Table 2 Difference in $FEF_{25–75}$ related to ozone concentration on the day before spirometric testing according to antioxidant supplementation groups within *GSTM1* genotypes among asthmatic children in Mexico City

		All asthmatics			Moderate and severe asthmatics		
Group	Subgroup	n*	Coefficient (95% CI)†, ml/s 50 ppb O_3	Percentage (95% CI) change‡ $FEF_{25–75}$/50 ppb O_3	n	Coefficient (95% CI), ml/s 50 ppb O_3	Percentage (95% CI) change $FEF_{25–75}$/50 ppb O_3
GSTM1 null	Placebo	29	−50.5 (−90.1 to−10.9)§	−2.9 (−5.2 to −0.6)§	18	−80.8 (−132.7 to −28.9)§	−4.7 (−7.7 to −1.7)§
	Supplement	33	−3.0 (−40.7 to 34.7)	−0.2 (−2.3 to1.9)	21	−7.3 (−54.0 to 39.5)	−0.3 (−3.1 to 2.4)
	Supplement effect		47.5 (−7.2 to 102.2)**	2.7 (−0.2 to 5.8)**		73.5 (3.8 to 143.2)¶	4.4 (0.3 to 8.4)¶
GSTM1 positive	Placebo	49	−10.5 (−38.7 to 17.7)	−0.6 (−2.1 to 0.9)	17	−34.4 (−75.6 to 6.8)	−1.9 (−4.4 to 0.5)
	Supplement	47	5.1 (−29.8 to 40.1)	0.3 (−1.6 to 2.2)	26	2.0 (−43.8 to 47.8)	0.1 (−2.4 to 2.6)
	Supplement effect		15.6 (−29.2 to 60.4)	0.9 (−1.6 to 3.3)		36.4 (−29.0 to 101.9)	2.0 (−1.7 to 5.7)

*The number of subjects in each group is given. The number of observations is: *GSTM1* null (placebo 654, supplement 732), *GSTM1* positive (placebo 1065, supplement 1039).
†Results and 95% confidence intervals from generalised estimating equation models adjusted for age, height, use of bronchodilator, respiratory symptoms on the day of the spirometric test, minimum temperature, and time since the beginning of the study. Ozone level is 1 hour maximum. During the observation period the mean (SD) 1 hour maximum ozone level was 102 (47) ppb (range 12–309). The Mexican standard is 110 ppb.
‡Percentage change from baseline $FEF_{25–75}$ for the individual stratum.
§$p \leqslant 0.01$. All p values were obtained by *t* test comparing coefficients between the two groups.
¶$0.01 < p < 0.05$.
**$0.05 < p < 0.10$.

than in the *GSTM1* positive children (difference of 0.9% between placebo and supplement groups, p = 0.49). Among *GSTM1* null children with moderate and severe asthma, the effect of supplementation was enhanced (4.4%, p = 0.04; table 2). In a general model, interactions between genotypes and ozone and supplement group and ozone were marginally significant (p = 0.14 and p = 0.093, respectively).

DISCUSSION

The findings are consistent with the role of *GSTM1* in oxidative stress. *GSTM1*, along with other GSTs, appears to be critical in protecting against oxidative stress by catalysing the conjugation of glutathione to reactive oxygen species.[3] *GSTM1* null genotype appears to contribute to increased levels of biomarkers of oxidative stress after ozone exposure.[4] In our population, as in other populations of Mexican descent,[5 7] nearly 40% of the children had a *GSTM1* null genotype. However, given our sample size, we could not stratify by other genotypes that might interact with *GSTM1* in relation to the response to ozone. A larger intervention study would be necessary for this type of analysis.

Our data provide preliminary evidence that asthmatic children who may be genetically impaired in their ability to handle oxidative stress, by virtue of deletion of the *GSTM1* gene, are more susceptible to the impact of ozone exposure on small airways function. Furthermore, supplementation with the antioxidants vitamins C and E above the minimum daily requirement might compensate for this genetic susceptibility.

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LUNG ALERT

Environmental nitrogen dioxide causes a pro-allergic response in the bronchial epithelium of normal subjects in vivo

▲ Pathmanathan S, Krishna MT, Blomberg A, *et al*. Repeated daily exposure to 2 ppm nitrogen dioxide upregulates the expression of IL-5, IL-10, IL-13 and ICAM-1 in the bronchial epithelium of healthy human airways. *Occup Environ Med* 2003;**60**:892–6

High levels of nitrogen dioxide (NO_2) are associated with asthma related morbidity and mortality although the precise mechanisms surrounding this remain unclear. This study evaluated the effects of in vivo NO_2 exposure on expression of a variety of biomarkers.

Twelve non-asthmatic subjects were randomly exposed, with an intervening 3 week period, to 2 ppm NO_2 or filtered air on four successive days. Bronchial biopsies were taken after exposure and subsequently immunostained and their expression quantified using computerised image analysis. Expression of IL-5, IL-10, IL-13, and ICAM-1 were significantly greater ($p \leqslant 0.05$) in subjects exposed to NO_2 than in those exposed to filtered air.

The study showed that NO_2 exposure results in an upregulation of pro-allergic Th2 cytokines. This suggests a mechanism by which environmental agents may trigger changes in bronchial epithelium which are associated with the asthmatic inflammatory process. In turn, this raises the possibility of future targets for disease prevention and treatment. Larger studies in asthmatic subjects incorporating the effects of more long term NO_2 exposure on cytokines in conjunction with parameters such as lung function, airway hyperresponsiveness, and inflammatory surrogates are required.

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THORAX

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