

Increases in Urinary $9\alpha,11\beta$ -Prostaglandin F_2 Indicate Mast Cell Activation in Wine-Induced Asthma

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Key Words

Asthma • Mast cells • Prostaglandin D_2 • Sulphite, wine

Abstract

Background: Wine-induced asthmatic symptoms may be caused by sulphite additives. Prostaglandin (PG) D_2 and cysteinyl leukotrienes (cysLT) are important mediators of asthmatic responses. To determine whether the sulphite additives in wine alter the production of PG D_2 and cysLT, asthmatic patients with compelling histories of wine sensitivity were challenged with high- and low-sulphite wines; the urinary metabolites of PG D_2 and cysLT were measured before and after challenge. **Methods:** Eight self-reporting wine-sensitive asthmatic patients completed double-blind challenges with high- and low-sulphite wines on separate days. Urine samples were collected before and after consumption of 150 ml of wine. Urinary concentrations of $9\alpha,11\beta$ -PGF $_2$ and leukotriene (LT) E_4 were measured by enzyme immunoassay. **Results:** Urinary $9\alpha,11\beta$ -PGF $_2$ concentrations increased in all subjects following challenge with high-sulphite wine, and the median concentration increased 1.6-fold ($p < 0.01$). Urinary $9\alpha,11\beta$ -PGF $_2$ also increased 1.5-fold after low-sulphite wine challenge, although this did not reach statistical significance ($p = 0.08$). The median difference in $9\alpha,11\beta$ -PGF $_2$ concentration after high-sulphite wine challenge was not

significantly different compared with that after low-sulphite wine challenge. Median urinary LTE $_4$ concentrations did not change significantly after either wine challenge. **Conclusions:** Increased urinary $9\alpha,11\beta$ -PGF $_2$ concentrations following wine challenge suggest mast cell activation as a possible mechanism for wine-induced asthma, although this did not appear to be related to the sulphite additives in wine. Urinary $9\alpha,11\beta$ -PGF $_2$ may warrant further assessment as a potential biomarker of reactivity to wine in asthmatic subjects.

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Introduction

Epidemiological studies suggest that wine consumption may be associated with worsening of asthma symptoms in as many as 30% of asthma patients [1–3]. In addition, sensitivity to alcoholic drinks is also relatively common in the general population, with 7.6% of Northern European adults reporting upper-airway symptoms and 3.2% reporting lower-airway symptoms following consumption of alcoholic drinks [4]. However, wine challenges of self-reporting wine-sensitive asthmatics in a clinical setting have usually resulted in few positive responses, as assessed by changes in forced expiratory vol-

ume in 1 s (FEV₁) [5–7]. A possible explanation is that changes in lung function may not provide a reliable indication of reactivity to wine in many asthmatics. Some individuals may react to wine only when their asthma has been aggravated by exposure to other irritants or triggers in the environment, or conversely, wine drinking may increase the reactivity of sensitive individuals to other irritants or triggers [8]. In either case, wine challenge under controlled conditions may not elicit a measurable change in lung function.

Sulphite additives have been implicated as a cause of wine-induced asthma [3, 8], and studies were therefore designed to assess alternative methods for investigating the role of sulphite additives in wine-induced asthmatic responses. The utility of measuring changes in bronchial reactivity following challenge of wine-sensitive asthmatic patients with high- or low-sulphite wines has been reported previously [9].

Prostaglandin (PG)D₂ and cysteinyl leukotrienes (cysLT) are involved in asthmatic responses to a number of triggers, including allergens, aspirin and exercise [10–13], and urinary 9 α ,11 β -PGF₂ and leukotriene (LT)E₄ have been shown to be reliable markers for the generation in the lungs of PGD₂ and cysLT, respectively [10, 14]. Some studies suggest that PGD₂ and cysLT may also mediate the bronchoconstrictive effects of sulphur dioxide and sodium metabisulphite [15–17]. Therefore, the primary aim of this study was to determine whether the sulphite additives in wine alter the production of PGD₂ and cysLT in asthmatic patients with compelling histories of wine sensitivity. Consequently, a double-blind placebo-controlled study was performed to measure the concentrations of the respective urinary metabolites, 9 α ,11 β -PGF₂ and LTE₄, following challenge of wine-sensitive asthmatic patients with high- and low-sulphite wines.

Methods

Subjects

Thirteen subjects with stable asthma and compelling histories of asthmatic responses following wine consumption were screened for participation in the study, which was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee. All subjects reported multiple episodes of worsening asthma symptoms within 1 h of consuming either white or red wine, or both. The inclusion and exclusion criteria have been detailed previously [9]. During screening, all potential study participants were asked whether spirits or other alcoholic beverages also worsened their asthma. Of the subjects who completed the study, 5 answered no to this question, 2 reported previous reactions to beer and 1 reported a reaction to rum. Subjects with general in-

Table 1. Characteristics of the wine-sensitive asthmatic subjects who completed the study

Sub- ject	Gen- der	Age years	Atopic	Asthma medica- tions	Wine sensitivity	Baseline FEV ₁	
						litres	% of predicted
1	F	42	yes	BA, BC	white	2.60	109.5
2	F	38	yes	BA, BU	white	2.03	82.5
3	F	53	yes	BA, BU, SM celebrex	white + red	2.36	103.5
4	M	32	yes	BA, FL	red	3.55	92.2
5	M	51	yes	BA, BU	white	2.78	86.0
6	F	26	yes	BA, FL	red + white	3.73	117
7	F	56	yes	BA	white	1.97	97.5
8	F	31	no	BA, BC	red	2.70	100.4

Baseline FEV₁ is the mean value for the 2 study days.

BA = β_2 -adrenoceptor agonists; BC = beclomethasone; BU = budesonide; FL = fluticasone; SM = salmeterol.

tolerance to alcohol, which is relatively common among subjects of Asian ethnicity, were excluded, and all participating subjects were of Caucasian ethnicity. None of the subjects who completed the study reported sensitivity to aspirin.

Skin prick tests were performed for grass mix, house dust mite (*Dermatophagoides pteronyssinus*), cat dander, dog dander and mould mix. Most subjects showed positive skin prick responses (≥ 3 mm wheal) to multiple allergens, with 5 subjects being sensitive to grass mix, 5 to house dust mite, 4 to cat dander, 1 to dog dander and 1 to mould. All subjects had their baseline spirometry assessed during initial screening and were required to have an FEV₁ >1.0 litre and $>70\%$ of their predicted value to be eligible to participate in the study. Of the 13 subjects screened for the study, 5 were excluded because they did not satisfy lung function or bronchial reactivity criteria, either before or after they had commenced the study. Consequently, 8 subjects completed the study and were challenged with both high- and low-sulphite wines. The demographic details, asthma characteristics, medication use and wine sensitivity histories of these subjects are presented in table 1. None of the subjects was receiving antileukotriene therapy.

Study Design

This was a randomized double-blind, placebo-controlled crossover study comparing the effects of challenge with high- and low-sulphite wines, in which the low-sulphite wine was a placebo for the challenge with sulphite-containing wine. Subjects were asked to abstain from alcoholic drinks for 24 h before each study day and were instructed to consume a light meal 1 h prior to baseline testing. Inhaled corticosteroids and short-acting β_2 -agonists were withheld for 8 h, long-acting β_2 -agonists and antihistamines were withheld for 48 h, and other oral medications were withheld for 3 days prior to wine challenge. On each study day, baseline spirometry was performed, bronchial reactivity to histamine was assessed using the standard method described by Cockcroft et al. [18], and a pre-challenge urine sample was collected. After a 30-min recovery period, and if FEV₁ was within 10% of the baseline

value, the subject consumed 150 ml of wine over a 5-min period. For each subject the choice of white or red wine was based on the subject's history of wine sensitivity, with the type of wine causing most problems being used for both challenges. Spirometry was performed immediately after, and at 15 and 30 min after wine consumption. If the FEV₁ at 30 min was within 10% of the baseline FEV₁, post-challenge bronchial reactivity was assessed. A second urine sample was collected approximately 1 h after wine consumption. On a second study day, at least 48 h after the first in order to avoid any possibility of a carry-over effect, an identical challenge was performed using the low- or high-sulphite wine, according to the randomization schedule. For 1 subject, a urine sample could not be obtained after the low-sulphite wine challenge. In subject 5, clinically significant changes in bronchial reactivity were observed after consumption of both high- and low-sulphite wines, and consequently on a 3rd study day, the subject consumed a control white wine drink in a single-blind challenge.

Challenge Drink Preparation

Low-sulphite white and red wines containing <20 ppm of sulphite were obtained from BRL Hardy Wine Co., Reynella, SA, Australia. High-sulphite wine was prepared by adding sodium metabisulphite to the low-sulphite wines, to obtain a final sulphite concentration of 250–300 ppm [7]. A control white wine drink based on the model wine of Halpern et al. [5], as modified by Vally et al. [7], was also prepared for subject 5 as described previously [9].

Measurement of Urinary 9 α ,11 β -PGF₂ and LTE₄

Urine samples collected before and after wine consumption were stored at –80°C until analyzed. Urinary 9 α ,11 β -PGF₂ concentrations were assayed in unextracted diluted (1:10) urine samples using a specific enzyme immunoassay (EIA) kit according to the manufacturer's instructions (Cayman Chemical Co., Ann Arbor, Mich., USA) [14]. Urinary LTE₄ concentrations were measured using a previously validated method based on immunoaffinity purification followed by EIA (Cayman Chemical Co.) [14]. To correct for variations in diuresis, creatinine concentrations were measured and urinary 9 α ,11 β -PGF₂ and LTE₄ concentrations were expressed as picograms per milligram of creatinine.

Data Analysis and Statistics

Median values and interquartile ranges were calculated for urinary 9 α ,11 β -PGF₂ and LTE₄ concentrations, and differences were assessed for statistical significance using the Wilcoxon signed-rank test, with a *p* value < 0.05 being regarded as significant.

Results

As reported previously [9], FEV₁ did not change by >15% following challenge with either high- or low-sulphite wine in any of the subjects. Although clinically significant changes in bronchial reactivity were observed in some subjects, mean bronchial reactivity to histamine

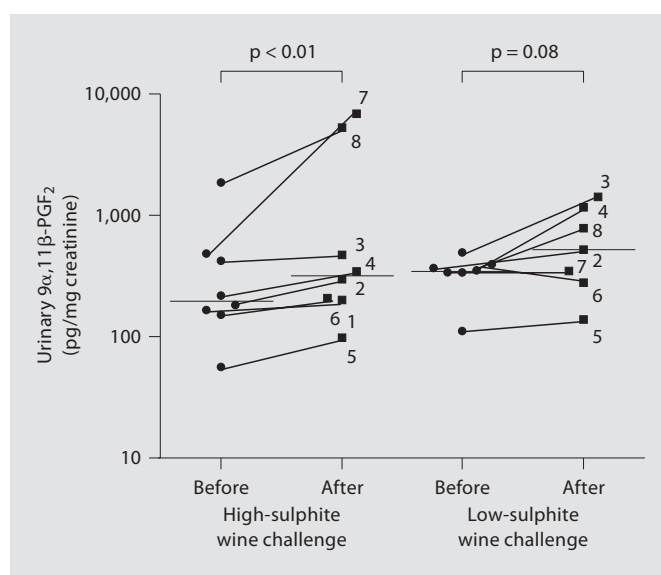


Fig. 1. Urinary 9 α ,11 β -PGF₂ concentrations in 8 wine-sensitive asthmatic subjects (identified numerically) before and after challenge with high- or low-sulphite wine. Horizontal bars indicate median values. For subject 1, a urine sample could not be obtained after the low-sulphite wine challenge.

also did not change significantly after either high- or low-sulphite wine challenge [9].

Urinary 9 α ,11 β -PGF₂ concentrations increased in all subjects following challenge with high-sulphite wine, with the median concentration increasing 1.6-fold from 198.2 to 317.6 pg/mg creatinine (*p* < 0.01, fig. 1). After high-sulphite wine challenge there were >2-fold increases in 9 α ,11 β -PGF₂ concentration in subjects 7 and 8. The median urinary 9 α ,11 β -PGF₂ concentration also increased 1.5-fold following low-sulphite wine challenge, from 345.3 to 517.5 pg/mg creatinine, although this change did not quite reach statistical significance (*p* = 0.08). After low-sulphite wine challenge there were >2-fold increases in 9 α ,11 β -PGF₂ concentration in subjects 3, 4 and 8. Because the overall increases in 9 α ,11 β -PGF₂ after high- and low-sulphite wine challenges were similar, but statistically significant only for the high-sulphite wine challenge, the individual changes in 9 α ,11 β -PGF₂ concentrations after the two wine challenges were compared statistically. This analysis showed that the median change in 9 α ,11 β -PGF₂ after high-sulphite wine challenge (+84.7 pg/mg creatinine) was not significantly different compared with that after low-sulphite wine challenge (+155.4 pg/mg creatinine).

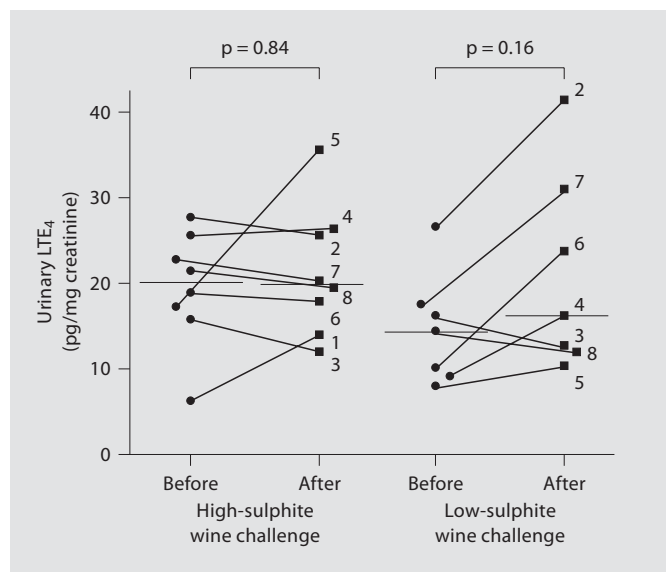


Fig. 2. Urinary LTE_4 concentrations in 8 wine-sensitive asthmatic subjects (identified numerically) before and after challenge with high- or low-sulphite wine. Horizontal bars indicate median values. For subject 1, a urine sample could not be obtained after the low-sulphite wine challenge.

The median urinary LTE_4 concentration did not change appreciably following challenge with high-sulphite wine (20.2–19.9 pg/mg creatinine) or low-sulphite wine (14.4–16.2 pg/mg creatinine). However, there were >2-fold increases in urinary LTE_4 concentration in subjects 1 and 5 after high-sulphite wine challenge, and in subject 6 after low-sulphite wine challenge (fig. 2).

In subject 5, challenge with the control white wine drink resulted in little change in urinary $9\alpha,11\beta\text{-PGF}_2$ (822–723 pg/mg creatinine) or LTE_4 concentrations (9.9–13.8 pg/mg creatinine).

Discussion

The aim of this study was to determine whether there would be a change in urinary $9\alpha,11\beta\text{-PGF}_2$ or LTE_4 levels following challenge with high- and low-sulphite wines in subjects reporting wine-induced asthmatic reactions. Whilst the subjects recruited for this study all provided compelling histories of sensitivity to white or red wine, or both, our previous studies have demonstrated that challenge of such patients in a clinical setting resulted in relatively few positive responses, as assessed by a clini-

cally significant decline in FEV_1 (>15% from pre-challenge values) [1, 7, 19].

In this study, the urinary concentrations of $9\alpha,11\beta\text{-PGF}_2$ and LTE_4 were generally similar to the levels previously measured in large populations of asthmatic and healthy subjects [14]. There was a significant overall increase in urinary excretion of the PGD_2 metabolite, $9\alpha,11\beta\text{-PGF}_2$, following challenge of wine-sensitive asthmatic subjects with high-sulphite wine. However, there was also an increase in the median urinary $9\alpha,11\beta\text{-PGF}_2$ concentration following low-sulphite wine challenge, although this did not quite reach statistical significance. The overall increases in urinary $9\alpha,11\beta\text{-PGF}_2$ after both wine challenges were similar in magnitude (~1.5-fold) to those previously observed after allergen challenge of asthmatic subjects [10], although, as in that study, there was wide variation between individual subjects. Importantly, the time course of urinary $9\alpha,11\beta\text{-PGF}_2$ formation after challenge appears to vary between individuals [10], although for logistical reasons and because most wine-sensitive asthmatic subjects report rapid onset of reactions to wine [1], the post-challenge urine sample was collected about 1 h after wine consumption. Nevertheless, the peak in urinary $9\alpha,11\beta\text{-PGF}_2$ levels may occur at or later than 2 h after challenge in some individuals [10], and it is possible that even more conclusive results would have been obtained in this study if samples had been collected at a later time point.

The median urinary $9\alpha,11\beta\text{-PGF}_2$ concentration before the low-sulphite wine challenge was higher than that before high-sulphite wine challenge. Although we cannot be certain of the exact explanation for this difference, there is wide inter- and intra-individual variation in basal urinary $9\alpha,11\beta\text{-PGF}_2$ concentrations [14]. Factors that may contribute to this variability are differences or changes in the numbers or degree of activation of PGD_2 -producing mast cells, or physiological variation in the metabolism of PGD_2 to urinary $9\alpha,11\beta\text{-PGF}_2$. Importantly, however, despite the differences in median baseline concentrations, the magnitude of the overall increase in $9\alpha,11\beta\text{-PGF}_2$ levels from baseline was similar following both high- and low-sulphite wine challenges. It therefore seems unlikely that these increases in urinary $9\alpha,11\beta\text{-PGF}_2$ represent a response to the sulphite additive. The results from this study are therefore consistent with previous observations from clinical challenge studies, that wine-induced asthmatic responses are not solely due to sensitivity to the sulphite additives, and suggest that the role of sulphites in wine sensitivity may have been overestimated [7, 19]. Because there was an increase in uri-

nary $9\alpha,11\beta$ -PGF₂ in most subjects following both wine challenges, we cannot exclude the possibility that such an increase may occur, irrespective of wine sensitivity, and this needs further investigation in subjects who are not sensitive to wine. However, urinary $9\alpha,11\beta$ -PGF₂ did not increase following challenge of one subject with a control wine drink, suggesting that consumption of alcohol per se does not result in increased levels of this urinary metabolite and that another component of wine may be responsible for this effect.

Both PGD₂, which is produced exclusively by mast cells [11], and its metabolite, $9\alpha,11\beta$ -PGF₂, are potent contractile agonists for human airways [20], and measurement of $9\alpha,11\beta$ -PGF₂ is currently the most sensitive method for detecting mast cell activation in vivo [21]. Therefore, the findings from the present study suggest that activation of mast cells in the lungs may be playing a role in wine-induced asthmatic responses. Mast cells may release PGD₂ when IgE bound to the high-affinity Fc receptor is cross-linked by allergen [22]. The plausibility of allergen-IgE-mediated mast cell activation in some instances of wine-induced asthma is supported by recent reports that specific allergens in grapes and wine may cause allergic and anaphylactic reactions [23–26]. There is, however, increasing evidence for IgE-independent activation of mast cells by molecules such as stem cell factor, IL-4, neuropeptides and Toll-like receptor ligands [27].

Based on our observations of the clinical characteristics of wine-sensitive asthmatic subjects, it appears more likely that IgE-independent mast cell degranulation is important. Such a mechanism would be analogous to the acetaldehyde-induced release of histamine from mast cells, which has been postulated as a cause of alcohol intolerance among Asian subjects [28, 29].

This study has demonstrated increases in urinary $9\alpha,11\beta$ -PGF₂ following both high- and low-sulphite wine challenges, suggesting that these increases were unlikely to be due specifically to the sulphite additives. Nevertheless, the increases in urinary $9\alpha,11\beta$ -PGF₂ observed in this study suggest a role for mast cells in wine-induced asthma, and this PGD₂ metabolite may also warrant further assessment as a potential biomarker for wine sensitivity in asthmatic subjects.

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