

Ethanol as a cause of hypersensitivity reactions to alcoholic beverages

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Summary

Background Adverse reactions after ingestion of alcoholic beverages are common. Metabolic differences in individuals and also the histamine content in alcoholic beverages have been implicated. By contrast pure ethanol has rarely been reported as a cause of hypersensitivity reactions and its mechanism has not been clarified yet.

Objective To determine whether ethanol itself accounts for alcohol hypersensitivity in patients with anaphylactic reactions after alcohol intake. In search of possible pathomechanisms all patients were analysed by skin prick testing and sulfidoleukotriene production of peripheral leucocytes using ethanol and its metabolites.

Methods Double-blind, placebo-controlled food challenges with a cumulated amount of 30 mL ethanol were performed in 12 adult patients with a positive history of adverse reactions after consumption of different alcoholic beverages. Skin prick tests and measurement of sulfidoleukotriene production were performed using different concentrations of ethanol and acetaldehyde from 50 to 1000 mM.

Results Oral challenges with pure ethanol were positive in six out of eleven patients. All challenge-positive patients, but also four out of five challenge-negative patients, showed an increased sulfidoleukotriene production *in-vitro* compared with healthy controls. Skin prick tests using alcoholic beverages, ethanol, acetaldehyde and acetic acid were negative in all patients (12/12).

Conclusion Our study shows that ethanol itself is a common causative factor in hypersensitivity reactions to alcoholic beverages. These reactions occur dose-dependent and a non-IgE-mediated pathomechanism is likely, because skin prick tests were negative in all cases. Increased sulfidoleukotriene production was determined in some patients, but is no reliable predictor. Therefore oral provocation tests remain indispensable in making the diagnosis of ethanol hypersensitivity.

Keywords double-blind placebo-controlled challenge, ethanol, hypersensitivity, sulfidoleukotriene production

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Introduction

Adverse reactions induced by the ingestion of alcohol are common. Some reactions are related to the genetic background of a population resulting in an impaired alcohol metabolism [1, 2]. In persons from an oriental origin, for example, intolerance to alcohol is common. The primary symptom in these patients is flushing, which led to the term 'oriental flushing syndrome'. However, Caucasians also sometimes experience unpleasant reactions after alcohol consumption [3]. If these reactions only occur after intake of certain alcoholic beverages, it is most likely that ingredients such as food additives [4–6] or food allergens [7–9] but not ethanol or its metabolites themselves are responsible for the symptoms. Alcoholic beverages

with a high histamine content or alcohol ingested together with histamine-rich food may also result in adverse reactions in persons with an impaired diamine oxidase function [10]. By contrast, if hypersensitivity reactions are experienced after the ingestion of small amounts of any type of alcohol, an allergic pathomechanism has been suggested by some authors [3, 11]. These previous studies have focused primarily on the ethanol metabolites, namely acetic acid [11–14] and acetaldehyde [3, 15], which are believed to be recognized by the immune system via products of protein condensation [3], although some authors were reluctant to specify this as type I allergy [12].

To investigate whether ethanol by itself causes allergic reactions we conducted a prospective study in patients with reported adverse reactions after intake of small amounts of alcoholic beverages. In order to elucidate the nature and eliciting substance of these adverse reactions, ethanol and its metabolites were tested *in vivo* by skin prick test (SPT) and *in vitro* by sulfidoleukotriene (sLT) production in the presence ethanol and acetic acid.

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Materials and methods

Patients

12 patients (five males, seven females) were recruited by their history for the study. The age of the patients ranged between 30 and 62 years (mean age: 46 years). All patients experienced adverse reactions within 1 to 2 h after the intake of different alcoholic beverages. As most of them avoided drinking alcohol thereafter, detailed history regarding the time of onset of symptoms after ingestion of alcohol could not be documented. Reported symptoms were angioedema, urticaria and conjunctivitis, but not flushing (Table 1). All of our patients tolerated vinegar according to their history. Only one out of twelve patients had a known type I allergy (grass pollen), the mean total IgE level in the study group was 46 kU/L (range: <0.1–116 kU/L).

Skin prick tests

SPTs were performed in all patients with ethanol at two different concentrations (10% and 96%) and with its metabolites at three different concentrations each (acetaldehyde at 0.1%, 1.0% and 10%, and acetic acid at 0.6%, 1.2% and 9.6%). In addition, the alcoholic beverages that were reported to elicit hypersensitivity reactions were tested by SPT. Histamine (10 mg/mL) was used as a positive control and NaCl (0.9%) as a negative control.

Double-blind placebo-controlled oral challenge using ethanol and food additives

Oral challenges with ethanol were performed in a titrated and double-blind, placebo-controlled fashion. The alcohol was blinded in 200 mL black currant juice and orange flavour (SHS, Heilbronn, Germany), which was also used as placebo. The starting dose was 5 mL of ethanol (96%), and every 30 min 5 mL of ethanol were added until an objective positive reaction

occurred or until the cumulated dose of 30 mL was reached. At both days of double-blind, placebo-controlled food challenge (DBPCFC), the patients were observed for at least 30 min after the last challenge dose or after symptoms had been resolved. Active and placebo drinks were impossible to differentiate as evaluated in 10 volunteers prior to the study and were given in a randomized order on two different days. All patients were advised to avoid coming by car or bike and were asked to use public transportation.

In patients with suspected hypersensitivity against food additives (FAs), DBPCFCs with a mixture of FAs were performed additionally, as described previously [15]. The mixture contained FAs such as colourants (azo-dyes and natural colours), preservatives (benzoate, sulfite, nitrite) and antioxidants (butylhydroxytoluol-anisol), which might be present in alcoholic beverages and may cause adverse reactions in some patients. DBPCFCs with a single FA were intended to follow in case of a positive reaction after the challenge of the mixture. Placebo capsules contained silicium oxide and mannitol.

Determination of sulfidoleukotriene production *in vitro*

For detection of sLT production *in vitro*, the cellular antigen stimulation test (CAST-ELISA) was used according to the manufacturer's instructions (DPC, Biermann GmbH, Bad Nauheim, Germany). Briefly, 10 mL EDTA blood of each patient was drawn after informed consent. The cells were then mixed with dextrane solution and after 90 min of sedimentation the upper phase was transferred into another tube and centrifuged (150g, 20 min at 25 °C). Thereafter, the platelet rich supernatant was removed and the cell pellet was resuspended into stimulation buffer containing IL-3 (20 ng/mL). The cell suspension was transferred to separate tubes and ethanol was added at different concentrations (2.5, 25, 50, 500, 1000 mM) with and without presence of C5a, as it has been shown previously that in the presence of C5a sLT production may be

Table 1. Demographical data, history of the patients and outcome of oral food challenge with ethanol

Patient	Sex	Age	Reported symptoms	Ingested amounts	Frequency of alcohol consumption	Outcome of oral ethanol challenge
1	F	41	Dizziness, angioedema	100 mL of sparkling wine or red wine	None since 1999, mastocytosis was diagnosed in 9/2000	Angioedema
2	F	34	Angioedema, urticaria	2 to 3 sips of any alcohol	Almost none	Negative
3	F	50	Urticaria	1 glass of wine, sparkling wine or beer	Rare	Negative
4	F	30	Rhinitis	Small amounts of any alcohol	Rare	Rhinitis
5	M	47	Redness around the eye	Small amounts of any alcohol	Rare	Conjunctivitis
6	F	60	Redness around the eye	1 glass of red wine	Very rare since 1995	Negative
7	F	54	Angioedema, redness around the eye, rhinitis	1 glass of beer or wine	Rare since 1989	Negative
8	M	34	Angioedema	1 glass of beer or wine	Rare	Conjunctivitis
9	F	41	Angioedema, wheeze	100 mL of red wine or sparkling wine	None	No challenge performed
10	M	47	Urticaria, anaphylaxis, pruritus	1 glass of beer or wine	None Alcohol-free beer tolerated	Urticaria
11	M	62	Urticaria	1 glass of wine	Rare	Negative
12	M	33	Conjunctivitis, pruritus	1 glass of beer or wine	Rare	Conjunctivitis, pruritus

enhanced in intolerance reactions [16]. Cell toxicity was ruled out by staining the cells with trypan blue. Also it has been shown previously that incubation of basophils and mast cells with ethanol, even up to a concentration of 1000 mM, did not markedly affect cell viability [17]. Spontaneous sLT production was determined using unstimulated cells in the presence of IL-3 (negative control or basal level release). The stimulation control represents anti-IgE-R (2.75 µg/mL) stimulated cells. The anti-IgE-R antibody binds to domain 1 of the alpha subunit and is a stimulating antibody.

The cells were incubated for 40 min at 37 °C and were finally centrifuged (600 g, 5 min at 4 °C). The supernatants were subsequently measured for sLT production by an ELISA using antileukotriene mAb pre-coated plates. After adding the enzyme conjugate solution and antibody, samples and standards were incubated for 24 h at 4 °C. After a washing step, substrate solution was added and incubated for another 30 min at 25 °C. Finally, stop solution was added and the absorbance was measured at 405 nm in an ELISA-reader. SLT production is expressed in pg/mL after subtracting spontaneous sLT production. Reactions were considered positive if the sLT concentrations were greater than the mean production of 12 healthy controls $\pm$ two standard deviations (cut-off range).

Statistical analysis

The Mann–Whitney *U*-test was used for statistical comparison between the groups. Sensitivity, specificity and predictive values were calculated by using a 2 $\times$ 2 contingency table.

Results

Skin prick tests with ethanol and its derivatives

The skin prick tests that were performed with the different concentrations of ethanol, acetic acid and acetaldehyde were negative in 12/12 patients. Also the suspected alcoholic beverages including red wine and beer, which were skin prick tested according to patient's history, did not show a positive result in any patient.

Oral challenge using ethanol and food additives

DBPCFC with ethanol was performed in 11/12 patients; one patient refused oral challenge.

In six of these eleven patients the DBPCFC with ethanol was positive, although the symptoms were less severe than reported (Table 1). Mostly eye symptoms were observed (conjunctivitis $n = 3$, angioedema $n = 1$), one patient developed an urticaria, one rhinitis and one conjunctivitis with generalized pruritus. No other systemic reactions such as dyspnoea or hypotension were seen after titrated oral challenge tests. Interestingly, all these DBPCFC-positive patients did not develop symptoms before reaching a cumulated dose of 30 mL ethanol. Their symptoms appeared within 10 to 20 min following the final challenge dose in all cases.

Furthermore, DBPCFCs with the FA mixture were performed in six out of eleven patients in order to exclude intolerance to FAs. They were negative in all but one patient, which was the patient who refused oral challenge with ethanol. This patient reported to react with angioedema, sore throat, hoarseness and dyspnoea when challenged with all food additives at the same time. As this patient refused to perform further challenges with single food additives, we were unable to identify the eliciting food additive.

Sulfidoleukotriene production in vitro

To study whether ethanol hypersensitivity may be a result of direct effects of ethanol on effector cells resulting in the production of pro-inflammatory molecules like leukotrienes, which are capable of contributing to the observed reactions in our patients, the sLT production from enriched peripheral basophils in the presence of ethanol was determined in all 12 patients. Twelve age- and sex-matched, non-atopic individuals without a history of alcohol hypersensitivity were studied as controls.

As shown in Fig. 1, in all 12 studied patients the median sLT release after stimulation with 1000 mM ethanol alone (328 pg/mL) was significantly higher compared with healthy controls ($n = 12$, 0 pg/mL) ($P < 0.01$). sLT release was also increased in

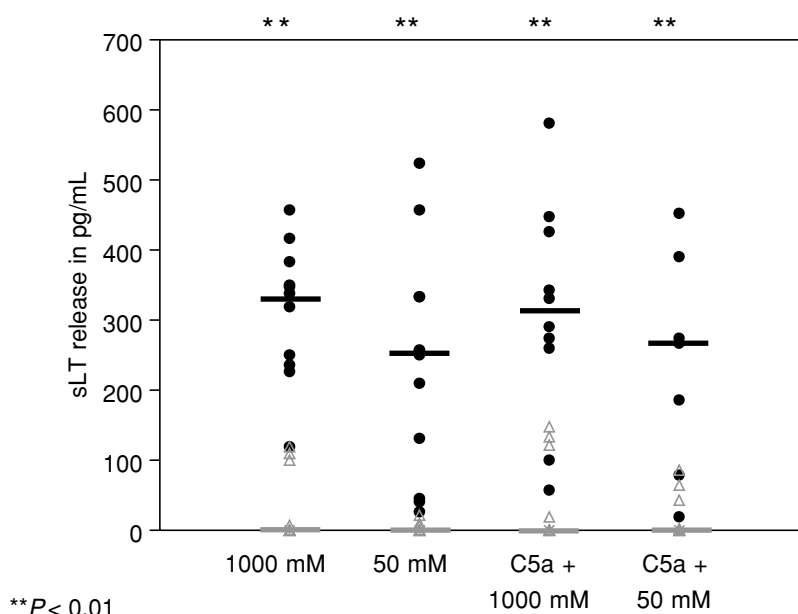


Fig. 1. Sulfidoleukotriene (sLT) release in 12 patients (●) after stimulation with different concentrations of ethyl alcohol (and C5a) compared with controls (△). Median values are depicted as horizontal bars (–) compared with median sLT production of 12 healthy controls.

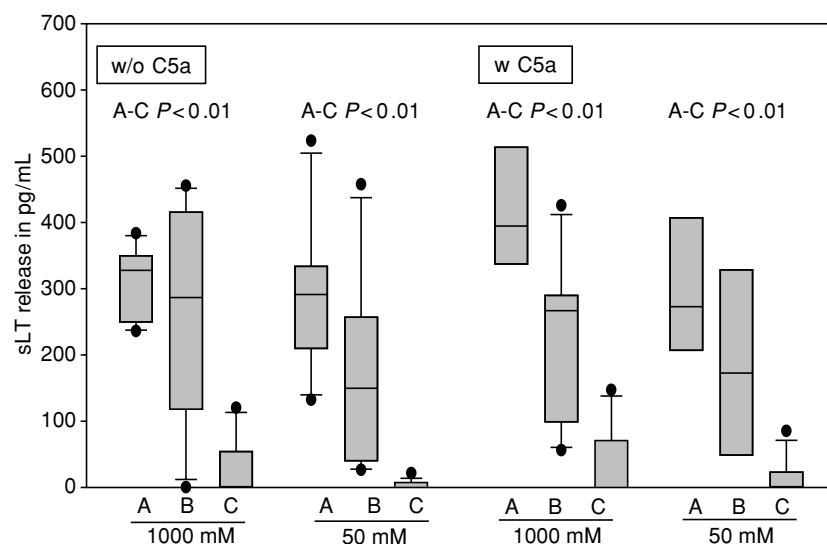


Fig. 2. Sulfidoleukotriene release (sLT) in DBPCFC (double-blind, placebo-controlled food challenge)-positive patients (a, $n = 6/12$) compared with DBPCFC-negative patients (b, $n = 5/12$) and with healthy controls (c, $n = 12$) after stimulation with ethyl alcohol at different concentrations (and C5a), depicted in boxplot analysis. One patient refused to perform DBPCFC.

the presence of 50 mM ethanol ($n = 12$, 252 pg/mL) in the patient group compared with the control group ($n = 12$, median 0 pg/mL at 1000 and 50 mM ethanol, respectively).

In the presence of C5a, which has been shown to enhance sLT production, e.g. in ASA-sensitive individuals, the median sLT production in the presence of 1000 mM ethanol was 311 pg/mL and in the presence of 50 mM ethanol 266 pg/mL, which was also significantly higher ($P < 0.01$) compared with the control group (medians of the controls were 0 pg/mL for 1000 mM and 50 mM ethanol + C5a. We did not observe a significant difference of sLT production in the presence of C5a of stimulated cells.

After dividing the patients into two groups according to the results of the oral challenge tests and comparing them with controls [Fig. 2], all DBPCFC-positive patients showed an increased sLT release in the presence of different concentrations of ethanol, again regardless of the presence of C5a. This was significant compared with the control group at the different concentrations of ethanol (1000 and 50 mM) with or without C5a. However, sLT release was also found to be increased in four out of five DBPCFC-negative patients (Fig. 2). The degree of sLT production did not predict the outcome of the oral challenge. In the patient who refused oral challenge with ethanol, no increased sLT release was observed.

Further evaluation of the data regarding sensitivity and specificity showed that increased sLT production was found in all DBPCFC-positive individuals, indicating a sensitivity of 100%. In all healthy controls studied no increased sLT production was observed. However, the specificity is low, considering that four out of five DBPCFC-negative patients showed an increased sLT production *in vitro* in the presence of ethanol.

Discussion

The results of our study show that even though ethanol itself is rarely suspected as an eliciting agent in allergic and non-allergic hypersensitivity reactions, more than half of our patients with a history of adverse reactions against alcoholic beverages react positively to DBPCFC with ethanol.

The results of DBPCFC with ethanol were supported by increased sLT release *in vitro* in DBPCFC-positive patients after stimulation with different concentrations of ethanol, which was not seen in any healthy controls. Due to the significant difference in sLT release between DBPCFC-positive patients and individuals without hypersensitivity reactions after alcohol consumption, we propose that our DBPCFC-positive patients did react rather to ethanol itself and not, as previously described, to its metabolites, namely acetic acid and acetaldehyde. Previous investigations focused primarily on ethanol metabolites and not on ethanol itself, which might explain the considerable difference in challenge dose [11–14]. Koivisto et al. [17] observed in an *in vitro* study in rats that ethanol is needed at molar levels to alter histamine release from mast cells, whereas stimulation with 50 μ M of acetaldehyde already results in a significant increase of histamine release in mast cells.

The observation that not only DBPCFC-positive but also most DBPCFC-negative patients showed increased sLT production after ethanol stimulation, might suggest that ethanol induces sLT production without clinical significance. However, this is contradicted by the normal sLT production in all healthy controls. Therefore, we hypothesize, that in DBPCFC-negative patients the individual threshold was not reached and therefore no symptoms were seen, even though the cumulated challenge dose in our study was much higher than in earlier studies on adverse reactions to alcohol [11–14]. The finding of an increased sLT production by ethanol in some DBPCFC-negative patients may be also explained by the possible necessity of a cofactor. The observation that some hypersensitivity reactions are induced only in the presence of cofactors is well known for exercise [18] or drugs [19], but a complex phenomenon involving several mechanisms has also been suggested to play a role in wine-induced asthma [5]. Although other ingredients of alcoholic beverages such as FAs [4, 6] or cereals (in beer) [7–9] have been shown to elicit adverse reactions, in our patients, FAs were only relevant in the one patient who refused ethanol challenge. Type I allergy against ingredients of alcoholic beverages as described by several authors [7–9] is unlikely to play a role as a cofactor in our patients, because none of the DBPCFC-negative patients had known sensitizations to food allergens.

Histamine, which has often been claimed to cause adverse reactions below its toxicity level, is unlikely to be an eliciting agent in alcoholic beverages as a recent study by Kanny et al. [20] showed no correlation between wine intolerance and histamine content of wine.

It has been suggested by Wantke et al. [10] that adverse reactions to alcoholic beverages might be a result of an impaired diamine oxidase function, which is competitively inhibited by ethanol. This mechanism is unlikely to be relevant in our patients as we performed our DBPCFCs with pure alcohol and not with red wine, which may contain high amounts of histamine. Furthermore, all our patients were challenged after breakfast, which usually consists of bread, butter and jam or honey and therefore does not contain high amounts of histamine.

In the literature, most hypersensitivity reactions following ethanol challenge have been suggested to be type I-like allergic reactions [3, 11, 17]. Israel et al. [3] demonstrated that acetaldehyde-protein-adducts are recognized by the immune system as foreign and are found in high amounts in Caucasians with adverse reactions after alcohol consumption. Other authors identified acetic acid [11–14] to be responsible for symptoms after ethanol ingestion. In all these studies positive skin prick tests to acetic acid in the patients were detected and positive history of vinegar intolerance was reported in all but one study [13]. Reactions were severe in almost all patients and occurred immediately after 1 to 5 mL of pure ethanol. In spite of positive skin prick tests and the immediate strong clinical reactions after challenge with small amounts of ethanol, some authors [12, 13] were reluctant to use the term type I allergy. In contrast to these studies, we did not detect any positive skin prick test reaction in our patients and no immediate reactions to small amounts of ethanol. None of our patients reacted before the ingestion of the cumulated amount of 30 mL of pure ethanol, which is a considerably higher dose than in the studies incriminating acetic acid as causative agent. Furthermore, all our patients tolerated by history the ingestion of vinegar. Therefore in our patients, we suggest that a pseudoallergic rather than a type I allergic pathomechanism is responsible for their adverse reactions to ethanol. However, different forms of hypersensitivity reactions towards alcohol may exist and will need to be considered in the diagnostic work-up.

In conclusion, we demonstrated by DBPCFC that ethanol itself is a causative factor in patients with hypersensitivity reactions after intake of alcoholic beverages. Due to the negative skin prick tests and the high amounts of alcohol given in order to elicit a positive reaction, a type I allergic pathomechanism is unlikely. In spite of the increased sLT production that was observed in these patients, sLT production did not prove to be a predictor of DBPCFC outcome. Oral challenge remains indispensable for the diagnosis of hypersensitivity reactions to alcoholic beverages.

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