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The formation of acetate from ethanol with and without prior chlorpropamide intake in diabetic and non-diabetic subjects

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Summary

It has been suggested that raised post-ethanol plasma acetaldehyde levels, from inhibition of aldehyde dehydrogenase, underlie the liability to chlorpropamide alcohol flushing (CPAF). We tested the hypothesis that acetate formation from acetaldehyde, the reaction catalysed by that enzyme, was also likely to be affected by chlorpropamide (CP) medication. In six healthy non-diabetic 'non-flushers', fasting acetate (Ac ± s.d. mmol/l) was 0.22 ± 0.12, and increased by 0.47 ± 0.14 to peak levels by 30 min after intake of 40 ml dry sherry, which increased plasma ethanol (mmol/l) levels to 10.2 ± 6.0. After 5 days of CP (250 mg daily), fasting Ac (0.17 ± 0.05) and increase to peak of Ac and ethanol after 40 ml sherry (0.56 ± 0.12 and 8.9 ± 7.2 respectively), were not changed (*P* n.s.). There was no correlation between Ac and ethanol at any time point. When the studies were repeated in five non-insulin-dependent diabetic 'flushers', both on regular CP medication and after 3 days without CP, there was again no significant difference in fasting and post-ethanol Ac levels between the two studies (fasting 0.18 ± 0.04 *v.* 0.17 ± 0.02, and increase to peak 0.62 ± 0.13 *v.* 0.72 ± 0.18, *P* n.s.). These results indicate that the conversion of ethanol to acetate is unaffected by CP medication, and furthermore that post-ethanol acetate levels do not predict liability to CPAF.

Résumé

Il a été suggéré que des niveaux plus élevés d'acétaldéhyde du plasma après éthanol, à partir de l'inhibition de déshydrogénases d'aldéhyde, étaient sous-jacents à la tendance à l'enchymose d'alcool chlorpropamide (CPAF). Nous avons expérimenté l'hypothèse selon laquelle la formation d'acétate à partir d'acétaldéhyde, réaction catalysée par cet enzyme, avait aussi des chances d'être affectée par le traitement chlorpropamide (CP). Chez 6 non-diabétiques en bonne santé et 'sans enchymose', l'acétate à jeun (Ac ± s.d. mmol/l) était de 0.22 ± 0.12 et s'est élevée à 0.47 ± 0.14 au niveau maximum 30 minutes après la prise de 40 ml de sherry sec, ce qui a entraîné l'augmentation du niveau d'éthanol (mmol/l) à 10.2 ± 6.0. Après 5 jours de CP (250 mg par jour), l'acétate à jeun (0.17 ± 0.05) ainsi que l'augmentation au maximum d'acétate et d'éthanol, respectivement 0.56 ± 0.12 et 8.9 ± 7.2, après absorption de 40 ml de sherry, n'étaient pas changées (*P* n.s.). Il n'y avait pas de corrélation à aucun moment entre l'acétate et l'éthanol. Quand l'étude a été répétée chez 5 diabétiques 'avec enchymose' et non-insuline-dépendants, à la fois sous traitement par CP et après 3 jours sans CP, il n'y avait toujours pas de différence significative dans les niveaux d'Ac à jeun et aprés éthanol entre les deux études (à jeun: 0.18 ± 0.04 *v.* 0.17 ± 0.02 — augmentation au maximum: 0.62 ± 0.13 *v.* 0.72 ± 0.18, *P* n.s.). Ces résultats indiquent que la transformation d'éthanol en acétate n'est pas affectée par le traitement par CP et, de plus, que les niveaux d'acétate après éthanol ne déterminent pas une tendance à l'enchymose d'alcool chlorpropamide (CPAF).

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Introduction

It is well known that free acetate is formed by mammalian liver when ethanol is ingested [1–

4]. The process involves an initial oxidation of ethanol to acetaldehyde by the enzyme alcohol dehydrogenase, and subsequent further oxidation to acetate by aldehyde dehydrogenase. Both enzymes are present in most mammalian tissues, but with the greatest activity in the liver [2].

This study attempts to investigate the possible effects of the sulphonylurea drug, chlorpropamide (CP), known to inhibit hepatic aldehyde dehydrogenase (ALDH) activity [5], on acetate levels as measured in human peripheral blood after consumption of moderate doses of ethanol, particularly because of observations previously published in regard to chlorpropamide alcohol flushing. This latter group of subjects was studied because of earlier suggestions that the chlorpropamide alcohol flush (CPAF) had a distinct biochemical basis in increased acetaldehyde levels after taking ethanol [6].

Patients and methods

Six healthy Caucasian non-diabetic subjects (five male), aged 34.7 ± 14.4 years, volunteered for the study. Their body-mass index (BMI; weight/height^2) was 24.1 ± 2.9 kg/m^2. None had ever experienced any unpleasant facial sensation (flush) on taking ethanol.

Five well-controlled non-insulin-dependent diabetic patients (three male), controlled on dietary measures and CP (aged 53.5 ± 7.7 years, BMI 27.5 ± 3.3 kg/m^2, glycosylated haemoglobin (HBAlc) 8.4 ± 1.3%) and with a definite history of alcohol-induced flushing (hereafter referred to as flushers) were also recruited into the study. The diabetic subjects were older and heavier (both $P < 0.01$) than the non-diabetic subjects, but those variables (age and weight) have not been shown to influence the liability to CPAF.

Diabetic 'non-flusher' controls were not recruited into the study because it was thought that their post-ethanol acetate production rates would be similar to those of the non-diabetic controls. In fact, it had been demonstrated earlier that acetaldehyde levels after ethanol ingestion were similar in diabetic and non-diabetic 'non-flushers'.

All the subjects (diabetic and non-diabetic) drank 40 ml dry sherry (containing 17.5% ethanol) after an overnight fast (from 22.00 h the previous night, with water *ad libitum*). Blood specimens were collected fasting and at 10-min intervals for 30 min. These studies were repeated 1 week later after each non-diabetic subject took 250 mg CP orally daily for 5 days, and the diabetic subjects had been without their daily CP for 3 days. This time interval was chosen since the half-life of CP is about 30 h, and levels in the blood should have decreased significantly by 72 h after the last dose.

All the diabetic subjects flushed on taking ethanol when on CP, but not on taking ethanol alone. The non-diabetic subjects did not flush on either occasion as judged both subjectively and objectively (by cheek colour). As all the subjects were white Caucasians, the subjective changes of flushing were easy to observe. None of the subjects took any alcoholic drink during the study period.

Plasma ethanol levels in the non-diabetic subjects were measured by gas chromatography [7]. Similar estimations were not done on the diabetic 'flushers'. Also, plasma CP levels were not measured in any of the subjects but compliance of the subjects to the drugs was good, as assessed by telephone calls and direct questioning. Similarly, plasma acetaldehyde levels were not estimated because of lack of facilities. It was however, believed that the inability to estimate these two parameters (CP and acetaldehyde) should not influence the significance of any observations made on changing plasma acetate levels in the diabetic and non-diabetic subjects.

Other assays done were: glucose (Beckman Analyser, Beckman Instruments, High Wycombe, U.K.), insulin by double antibody radioimmunoassay [8], acetate by an enzymatic spectrophotometric method [9], and glycosylated haemoglobin HBAlc by isoelectric focusing [10].

The results are expressed as means ± s.d. The within-subject comparisons were made by paired Student's *t*-tests. The difference in response between the non-diabetic and diabetic subjects was examined by a three factor repeated measures design analysis of variance with one between (diabetes) and two within (treatment and time) variables.

Results

Before taking CP, the fasting acetate level in the non-diabetics was 0.22 ± 0.12 mmol/l. The

mean increases in acetate and ethanol levels from baseline were 0.38 ± 0.12 and 7.7 ± 3.4 respectively (both $P < 0.001$) over 30 min, and mean rises to peak were acetate 0.47 ± 0.14 and ethanol 10.2 ± 6.0 (both $P < 0.001$) after ingesting 7 g ethanol in dry sherry. After 5 days of CP, fasting acetate in the same subjects was essentially unchanged at 0.17 ± 0.05. The mean rises in acetate and ethanol levels from fasting over 30 min were respectively 0.46 ± 0.14 and 6.6 ± 6.0, and increases to peak 0.56 ± 0.12 and 8.9 ± 7.2, respectively, after taking ethanol. These were not significantly different from the changes seen before CP (Fig. 1a). Glucose and insulin levels were essentially unchanged during both studies. There was no significant correlation between acetate and ethanol at any of the time points or during the whole study.

When these studies were repeated in the diabetic 'flushers', similar observations were made, with no difference in acetate levels between the two studies (with and without prior CP intake) at any of the time points (fasting 0.18 ± 0.04 *v.* 0.17 ± 0.01 mmol/l, mean increase from fasting over 30 min 0.48 ± 0.11 *v.* 0.41 ± 0.09, and to peak 0.62 ± 0.13 *v.* 0.72 ± 0.18, all P n.s.) (Fig. 1b). As in the non-diabetic non-flushers, plasma glucose and insulin levels did not change during either study in the diabetic flushers.

Although these values for mean increase and increase to peak in plasma acetate levels tended to be higher in the diabetic flushers, they were not significantly different from those for the non-diabetic non-flushers.

Discussion

The results confirm previous observations that acetate is produced from ethanol in humans [1,3,4]. The rise in acetate levels was not influenced by prior CP medication in either the non-diabetic subjects or the diabetic flushers, an observation of some interest.

CPAF remains controversial [11–13]. While its aetiology is still speculative, the liability to flushing has been related to plasma CP concentration [14], increased post-ethanol acetaldehyde [15], reduced hepatic ALDH activity [5],

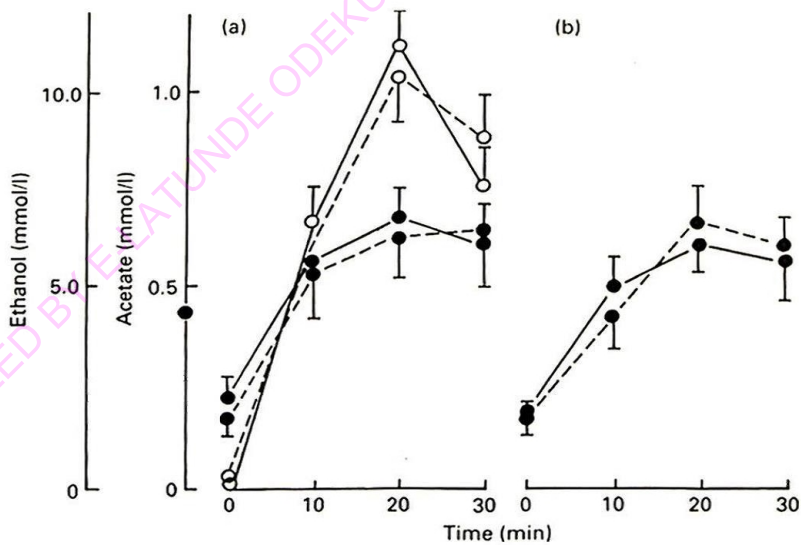

Fig. 1. Plasma ethanol and acetate levels after ethanol intake with and without prior chlorpropamide — CP (mean ± s.e.m.). (a) Non-diabetic subjects ($n = 6$); (b) diabetic flushers ($n = 5$). (○——○) Ethanol without CP; (○- - -○) ethanol with CP; (●——●) acetate without CP; (●- - -●) acetate with CP. Mean values for 10, 20 and 30 min significantly different from 0 min ($P < 0.001$).

genetic susceptibility [16], and, in non-insulin-dependent diabetes, some protection against microangiopathy [17]. Central to all these possibilities is ALDH, the inhibition of which would result in elevated acetaldehyde levels, and whose isoenzymes [18] could explain the genetic influences. Thus, a raised post-ethanol level of acetaldehyde was proposed as a marker for liability to flushing, since it differentiated 'flushers' from 'non-flushers' [6]. However, reduced ALDH activity should also result in decreased formation of acetate when ethanol is taken with CP.

The results obtained in this study are not in keeping with this hypothesis, although obviously concerned with plasma concentration and not total acetate production. Acetate levels were not influenced by CP intake in either the 'non-flushers' or the 'flushers'. If acetaldehyde levels were raised in the flushers, as they might well have been, any inhibition of ALDH activity did not affect acetate formation. This could be due to several factors. Raised acetaldehyde levels may have saturated a concentration-dependent inhibition of ALDH. The possibility of other pathways of acetaldehyde metabolism also exists. Aldehyde oxidases, capable of converting acetaldehyde to acetate, are present in mammalian liver and capable of aldehyde conversion to acetate [2], and it is conceivable that these enzymes are activated in conditions where ALDH activity is reduced. In any case, ALDH is not at the rate-limiting step of ethanol oxidation as alcohol dehydrogenase activity more consistently correlates with ethanol oxidation rates [19]. Otherwise, as proposed by Hillson *et al.* [20], ALDH may not be consistently inhibited by CP.

Conclusion

This study suggests that plasma acetate has no value as a marker for chlorpropamide alcohol flushing.

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