results of bone marrow aspiration were compatible with primary thrombocytopenia. The ulcer healed successfully after treatment with oral fluocoxacin and local application of aureomycin cream. He was started on a course of busulphan for his blood disorder.

Seven weeks later, there was a pronounced recurrence of the ulceration, now covering the entire outer aspect of the left lower leg. On examination the ulcer had a ragged undermined margin with a bluish tinge, and the edge resembled a collapsed bulla. The appearance was suggestive of pyoderma gangrenosum and biopsy confirmed this diagnosis. Rheumatoid factor was absent, serum protein concentrations and immunoglobulin electrophoresis were normal, and sigmoidoscopy (up to 18 cm) showed no abnormality. He was given prednisolone 40 mg daily, which produced a rapid improvement in the lesion in two weeks and complete healing in five. He now remains in good health without any medication.

Discussion

Pyoderma gangrenosum is most commonly associated with inflammatory bowel disease and rheumatoid arthritis, though it has been reported in association with various forms of leukaemia, myelofibrosis, and polycythaemia rubra vera.

References


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Chlorpropamide-alcohol flushing, aldehyde dehydrogenase activity, and diabetic complications

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Abstract

Many diabetics who take chlorpropamide (a sulphonylurea compound) experience facial flushing after drinking even small amounts of alcohol. These flushers have a noticeably lower prevalence of late complications of diabetes (microangiopathy, macroangiopathy, and neuropathy) than non-flushers. This flush reaction is accompanied by increased blood acetaldehyde concentrations, suggesting an inhibition of aldehyde dehydrogenase activity. In the present study the activity of this enzyme in erythrocytes was assessed in the absence of chlorpropamide. Erythrocyte homogenates obtained from flushers and non-flushers were incubated with acetaldehyde and the rate of metabolism studied. Flushers eliminated acetaldehyde more slowly at a low range of concentrations (0-30 μmol/l), suggesting a difference in aldehyde dehydrogenase activity. Further studies are needed to clarify the role of this enzyme in the pathogenesis of diabetic complications.

Introduction

For 25 years facial flushing after intake of small amounts of alcohol has been a well-known side effect of chlorpropamide medication, a sulphonylurea derivative used in the treatment of type II diabetes (non-insulin-dependent or maturity-onset diabetes). The implications of the flush were not known until 1978, however, when Leslie and Pyke reported that chlorpropamide-alcohol flushing was inherited as an autosomally dominant trait in type II diabetes. This was followed by reports of a lower prevalence of diabetic retinopathy, large-vessel disease, peripheral neuropathy, and diabetic nephropathy in the flushers than in the non-flushers. The prevalence of chlorpropamide-alcohol flushing in type II diabetes has varied considerably at around 30%.

Since the flush reaction seemed to be related to the pathogenesis of angiopathy, studies of its biochemical basis were warranted. We found that flushers had higher blood concentrations of acetaldehyde, the first metabolite of ethanol, than non-flushers during a chlorpropamide-alcohol challenge test. We also found higher concentrations of chlorpropamide in flushers than in non-flushers, which accords with the fact that chlorpropamide-induced inhibition of aldehyde dehydrogenase leads to a rise in blood acetaldehyde concentrations. Barnett et al did not detect this difference in chlorpropamide concentrations between flushers and non-flushers, although they confirmed our finding that acetaldehyde concentrations were increased during the flush. This discrepancy can be explained by the different size of the populations studied (105 subjects in our study and 21 in theirs). Furthermore, we found a considerable overlap in serum chlorpropamide concentrations, whereas the difference in blood acetaldehyde concentrations was highly significant with a minimal overlap. Obviously other factors might be of importance. This prompted us to study the role of aldehyde dehydrogenase in diabetes.

Acetaldehyde is converted to acetate by aldehyde dehydrogenase, which is distributed in various organs, the highest activity being found in the liver. Two major isoenzymes have been isolated from mammalian livers; one is mitochondrial with a low Km for aldehydes, the other is cytosolic with a high Km.
for aldehydes. Inoue et al. isolated aldehyde dehydrogenase from human erythrocytes and found that it shared several characteristics with the cytosolic enzyme. It had a high $K_m$ for aldehydes, was strongly inhibited by disulfiram, and had a biphasic nature of kinetics which suggested both high and low affinity sites for acetaldehyde.

**Patients and methods**

Ten type II diabetes with chlorpropamide-alcohol flushing (eight women and two men) and 10 type II diabetics without (five women and five men) were studied. The mean age for men and women was 69 and 62 years respectively, the mean duration after the diagnosis of diabetes six and nine years respectively, and the mean body weight 117% and 123% of ideal weight respectively. None of the differences was statistically significant. Five flushers and four non-flushers were treated with diet only whereas five flushers and six non-flushers were treated with glibenclamide or glipizide. All diabetic treatment was stopped for at least 24 hours before any part of the study and the patients were told to refrain from alcohol before the test.

**CHALLENGE TEST**

The patients were studied with a standardised chlorpropamide-alcohol flushing challenge test. Twelve hours after taking 250 mg chlorpropamide the patients were given 8 g of alcohol in fruit juice. The flush reaction was assessed by measuring skin temperature and determining blood acetaldehyde concentrations. One month later when no chlorpropamide metabolites remained from the challenge test blood samples were collected to study aldehyde dehydrogenase activity.

**ALDEHYDE DEHYDROGENASE ACTIVITY**

To assess the activity of erythrocyte aldehyde dehydrogenase in flushers and non-flushers we used the method of Inoue et al. slightly modified as follows. Blood samples were collected from the patients in the morning in heparinised vacuum tubes, kept at 4°C, and within one hour centrifuged at 1000 $\times$ $g$ at 4°C for 10 minutes. Plasma and erythrocytes were mixed in fixed proportions (5 : 1) and homogenised by freezing and thawing. Three millilitres of the homogenate was incubated at 37°C with 100 $\mu$mol acetaldehyde/l and 1 mmol nicotinamide-adenine dinucleotide (NAD)/l in 5-ml airtight glass vials. Samples were taken at fixed intervals and the reaction stopped by cooling the vials in iced water for 10 minutes followed by deproteinization with 0.5 ml 35% perchloric acid. After centrifugation at 4°C the clear supernatant was assayed for acetaldehyde using head-space gas chromatography.

Student's $t$ test was used to analyse data from the challenge test and Wilcoxon's test for the data on aldehyde dehydrogenase activity.

**Results**

During the chlorpropamide-alcohol flushing challenge test the flushers had positive reactions and the non-flushers did not. The mean increase in facial skin temperature was 2.2 ± 0.3°C and 0.5 ± 0.1°C for the flushers and non-flushers respectively ($p<0.001$). The mean increase in blood acetaldehyde concentration 25 minutes after the intake of 8 g of ethanol was 6.3 ± 1.6 $\mu$mol/l and 1.6 ± 0.2 $\mu$mol/l respectively ($p<0.01$) for the flushers and non-flushers without any overlap between the two groups (fig 1). There was no difference in acetaldehyde concentrations between those who were receiving anti-diabetic drugs and those who were treated with diet only. The decline of acetaldehyde concentrations in erythrocyte homogenates was exponential with two phases (fig 2). During the first phase no difference between the two groups was seen, whereas the flushers had a slower metabolism of acetaldehyde during the second phase (fig 3).

**Discussion**

The enzymatic nature of acetaldehyde metabolism in erythrocytes has been established by Inoue et al. The reaction is NAD-dependent and can be blocked by disulfiram, an inhibitor
of aldehyde dehydrogenase. Our finding of a biphasic acetaldehyde metabolism accords with earlier studies on purified sheep liver and human liver cytosolic enzyme. Biphasic kinetics may have several causes: (a) enzymes may exhibit co-operative behaviour, (b) different isoforms may be present, and (c) one enzyme may consist of non-equivalent subunits which have a different affinity for the substrate. The difference in metabolic rates between flushers and non-flushers was unlikely to be due to a difference in enzyme concentration, since the elimination in phase 1 was almost identical for the two groups. Our data support the hypothesis of a difference in aldehyde dehydrogenase activity between flushers and non-flushers, which is probably due to two types of high affinity binding sites for acetaldehyde. Hypothetically this difference in enzyme activity between those at risk of developing late complications and those who are not could be used as an additional refinement to identify patients at risk early by using a simple blood test. Determining the biochemical basis of protection against late complications through the change in enzyme activity will be an even more important step.

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References

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High serum vitamin B12 binding capacity as a marker of the fibrolamellar variant of hepatocellular carcinoma

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Abstract
Ten (9.3%) of 107 patients with hepatocellular carcinoma had considerably increased serum unsaturated vitamin B12 binding capacity. All 10 were young (mean 21 years), had no serum alpha-fetoprotein, and no underlying cirrhosis; all had a longer survival compared with patients without increased serum unsaturated vitamin B12 binding capacity in the study. Seven of the 10 patients had fibrolamellar hepatocellular carcinoma, a recently recognised histological variant, which was found in only one young patient without increased serum unsaturated vitamin B12 binding capacity and no alpha-fetoprotein among the remaining 97. This high degree of correlation between increased serum unsaturated vitamin B12 binding capacity and fibrolamellar hepatocellular carcinoma has not been reported before. Increased serum unsaturated vitamin B12 binding capacity may be of considerable help in diagnosis, prognosis, and monitoring treatment of this well-defined group of patients with hepatocellular carcinoma but no alpha-fetoprotein.

Introduction
A fibrosing variant of hepatocellular carcinoma found in young people was first noted by Edmondson in 1958, but the clinical features of this variant, including its development in the non-cirrhotic liver and its relatively good prognosis, were not reported until 1976. Because of the distinct eosinophilic cytoplasm of the tumour cells and the parallel arrangement of the collagen in the conspicuous fibrous septa the tumour was called eosinophilic hepatoma with lamellar fibrosis, which was later shortened to fibrolamellar carcinoma. There have also been reports of...