SHORT COMMUNICATION

Effect of acute and chronic alcohol ingestion on the rate of folate catabolism and hepatic enzyme induction in mice

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Summary

1. Folate deficiency is commonly found in alcoholic subjects although the causative mechanism is uncertain. It has been suggested that microsomal enzyme induction resulting from chronic alcohol ingestion might accelerate the rate of folate catabolism thus causing deficiency.

2. By using an experimental animal model to determine the rate of catabolism of \([^3H]\)pteroylglutamate (folic acid) by the quantitative estimation of the two urinary catabolites \(p[^3H]\)aminobenzoylglutamate and \([^3H]\)acetamidobenzoylglutamate, we have measured both the rate of folate catabolism and the extent of microsomal-enzyme induction in mice after acute and chronic alcohol ingestion.

3. Despite significant evidence of enzyme induction in the chronic alcohol group, there was no difference in the rate of folate catabolism after acute or chronic alcohol ingestion when compared with that of the controls.

Key words: alcohol, folate catabolism, hepatic enzyme induction.

Introduction

The development of folate-dependent megaloblastic anaemia in alcoholic patients was first reported by Jandl (1956). Since then numerous studies have confirmed this finding (Herbert, Zalusky & Davidson, 1963; Klipstein & Lindenbaum, 1965; Eichner, 1973). The exact mechanism for alcohol-induced folate depletion, however, is not yet certain. It is likely that dietary deficiency is important (Klipstein & Lindenbaum, 1965; Hines, 1969; Eichner & Hillman, 1971), but it is also clear that alcohol has a direct toxic effect on serum folate levels (Eichner & Hillman, 1973; McGuffin, Goff & Hillman, 1975), on bone marrow function (Sullivan & Herbert, 1964) and on the enterohepatic circulation of folates (Hillman, McGuffin & Campbell, 1977). An alternative possibility is that, as chronic alcohol ingestion is known to induce hepatic microsomal enzymes (Conney, 1967; Matsuzaki, Teschke, Ohnishi & Lieber, 1977), alcohol might increase the folate catabolic rate either by inducing enzymes dependent on folate for their synthesis (Labadarios, Dickerson, Parke, Lucas & Obuwa, 1978) or by increasing the requirement for folate cofactors (Maxwell, Hunter, Stewart, Ardeman & Williams, 1972). Although there are many studies documenting the effect of alcohol on microsomal induction there are no studies of its association with folate catabolism.

We have developed an experimental animal model to determine the rate of folate catabolism in mice by the quantitative estimation of the two urinary catabolites \(p\)-aminobenzoylglutamate (\(p\)ABGlut) and acetamidobenzoylglutamate (\(A\)pABGlu) (Kelly, Reed, Weir & Scott, 1979). Subsequently we demonstrated that the anti-convulsant drug, phenytoin, significantly increased the rate of catabolism whereas administration of phenobarbitone did not (Kelly et al., 1979), implying that microsomal-enzyme induction was not the mechanism for the accelerated folate catabolism. The aim of this study was to investigate the effect of acute and chronic alcohol ingestion on the rate of folate catabolism and microsomal induction.
Methods

Radiochemicals

\[^{3}H\]Pteroylglutamate (3',5',7,9(n)-[^{3}H]PteGlu), sp. radioactivity 93 mCi/mg; \[^{3}H\]hexadecane, sp. radioactivity 2-17 mCi/mg; \[^{53}Cr\]EDTA, sp. radioactivity 700 μCi/mg. These were supplied by The Radiochemical Centre, Amersham, Bucks., U.K.

Chemicals

Carbon monoxide (CO) (Irish Industrial Gases, Dublin, Ireland); NADPH and cytochrome c (Sigma Chemical Company, Poole, Dorset, U.K.).

Animals and procedure

Lace mice weighing 20 g were used. In each experiment the mice were matched for sex and then randomly assigned to groups, with two cages of five mice in each group. All mice received a single intraperitoneal injection of 4 μCi of \[^{3}H\]PteGlu and 5 nCi of \[^{53}Cr\]EDTA and the pooled urine from mice of each cage was collected daily for 9 days. Test groups received an 8% ethanol solution in water; control groups received tap water only. All mice were fed a standard diet containing 0.5 mg of folic acid/kg body wt. (Coates, O'Donoghue, Payne & Ward, 1969). Weight, dietary intake and fluid balance were measured daily for each cage of five mice. Dietary and fluid intake were assessed by subtracting the residue from the daily aliquot.

Acute alcohol ingestion. Test mice received alcohol daily for 10 days after being given the radioactive injection (group A).

Chronic alcohol ingestion. Mice were pretreated with alcohol for 7 (group B) and for 13 weeks (group C).

Estimation of p-[\(^{3}H\)ABGlu and p-[\(^{3}H\)AABGlu

Estimation of these catabolites was performed by alkaline hydrolysis of all p-[\(^{3}H\)]AB-containing catabolites in the urine to p-[\(^{3}H\)]aminobenzoic acid, which was subsequently extracted by a chemical procedure (Kelly et al., 1979). Radioactivity was estimated as previously described (Kelly et al., 1979). Estimation of hepatic microsomal enzyme induction

The livers from mice of each cage were pooled for analysis. The method of Omura & Sato (1964) was used to prepare the microsomes and assay cytochrome P-450. The protein concentration was determined by the biuret method (Layne, 1957); cytochrome c reductase was assayed by the method of Phillips & Langdon (1962).

Statistics

The difference between the mean daily excretion and cumulative excretion of p-[\(^{3}H\)]AB catabolites in the control and test groups was determined (Snedecor & Cochran, 1967). Student's t-test was used to estimate the significance of the microsomal-enzyme induction (Snedecor & Cochran, 1967).

Results

Animals

Once the mice had become adjusted to the metabolic cages there was no obvious difference in dietary and fluid intake, urinary output or weight loss in any of the groups. Each test mouse ingested approximately 0.25 g of alcohol/day.

Excretion of[^{53}Cr]EDTA

In a typical experiment 72% of the dose was excreted over 24 h in the control group; 78% in group A; 76% in group B; 70% in group C. This implies comparable injection and collection techniques (Chantler, Garnett, Parsons & Veall, 1969; Kelly et al., 1979).

Estimation of total radioactivity

Total excreted radioactivity was highest in the first 3 days in all groups, stabilizing after this time to an almost constant level (Kelly et al., 1979). There was no significant difference between the daily radioactive excretion after either acute or chronic alcohol ingestion. The mean daily excretion after day 3 was 9.0 × 10^4 c.p.m./day (SEM 0.4, n = 40).

Estimation of p-[\(^{3}H\)]AB catabolites

Estimation of p-[\(^{3}H\)]AB catabolites was highest in the first 3 days, stabilizing after this time to a constant level. The mean daily excretion of these catabolites after day 3 was as follows.

Acute alcohol ingestion: controls, 2.7 × 10^4 c.p.m./day (SEM 0.3, n = 8); group A, 2.6 × 10^4 c.p.m./day (SEM 0.2, n = 8). Chronic alcohol ingestion: controls, 3.6 × 10^4 c.p.m./day (SEM
Effect of alcohol on folate catabolism

| Effect of alcohol ingestion on (i) cumulative excretion of \( p^{3}H \)AB catabolites and (ii) hepatic microsomal enzyme induction in mice

Mean ± SEM (n = 8) values are shown. Significance of differences: * \( P < 0.05 \); ** \( P < 0.001 \). † Not significant.

<table>
<thead>
<tr>
<th></th>
<th>( p^{3}H )AB catabolites (0–3 days)</th>
<th>( 10^{9} ) × c.p.m. (4–9 days)</th>
<th>Cytochrome P-450 (nmol/mg of protein)</th>
<th>Cytochrome c reductase (nmol min(^{-1}) mg(^{-1}) of protein)</th>
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<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>7.7 ± 0.7</td>
<td>1.6 ± 0.2</td>
<td>0.3 ± 9</td>
<td>220 ± 15</td>
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<tr>
<td>Acute alcohol ingestion</td>
<td>7.7 ± 0.3†</td>
<td>1.6 ± 0.2†</td>
<td>0.3 ± 6†</td>
<td>210 ± 8†</td>
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<tr>
<td>Group B</td>
<td></td>
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<tr>
<td>Control</td>
<td>4.6 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>0.21 ± 0.01</td>
<td>76 ± 4</td>
</tr>
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<td>7 week ingestion</td>
<td>4.6 ± 0.2†</td>
<td>2.0 ± 0.3†</td>
<td>0.32 ± 0.04*</td>
<td>90 ± 4†</td>
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<tr>
<td>Group C</td>
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<tr>
<td>Control</td>
<td>4.6 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>0.15 ± 0.02</td>
<td>63 ± 5</td>
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<td>13 week ingestion</td>
<td>5.5 ± 0.2†</td>
<td>1.6 ± 0.2†</td>
<td>0.25 ± 0.07**</td>
<td>85 ± 5†</td>
</tr>
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0.2, n = 8); group B, 3.4 × 10\(^4\) c.p.m./day (SEM 0.3, n = 8); group C, 2.6 × 10\(^4\) c.p.m./day (SEM 0.2, n = 8).

Comparison of cumulative excretion of radioactivity after acute and chronic alcohol ingestion

There was no significant difference in the cumulative excretion of total excreted radioactivity or of \( p^{3}H \)AB radioactivity after acute or chronic alcohol ingestion (Table 1).

Estimation of microsomal enzyme induction

There was an increase in microsomal protein concentration in all the test groups but this was not statistically significant (mg): controls, 10.9; group A, 14.9; group B, 12.9; group C, 13.2 (\( P = 1.0 \)). There was no evidence of enzyme induction in group A, whereas there was significant induction of enzymes in groups B and C (Table 1). It was considered more appropriate to express these results per mg of microsomal protein rather than per g of liver because the alteration in liver weight might be due to fatty change as well as increased microsomal protein. The mean percentage induction above the control values for cytochrome P-450 was 55% (B) and 17% (C) and for cytochrome c reductase was 58 (B) and 36% (C).

Discussion

The aim of this study was to investigate the hypothesis that the folate deficiency associated with alcoholism might be due to increased folate catabolism secondary to hepatic induction of folate-related enzymes. Accordingly, both the rate of folate catabolism and the extent of microsomal-enzyme induction in mice after acute and chronic alcohol ingestion was measured.

There was no evidence of enzyme induction in the acute alcohol group whereas satisfactory levels were obtained in the chronic group (Table 1), comparable with other studies (Joly, Ishin, Teschke, Hasnumen & Lieber, 1972; Matsuzaki et al., 1977). Despite significant evidence of enzyme induction in the chronic alcohol group (Table 1), there was no alteration in the rate of folate catabolism when compared with that of either the controls or the acute alcohol group. This suggests that neither alcohol ingestion nor the subsequent microsomal-enzyme induction had any effect on the rate of folate catabolism as previously suggested (Maxwell et al., 1972).

We have previously shown that phenytoin significantly increased the rate of folate catabolism at three separate doses whereas administration of phenobarbitone did not (Kelly et al., 1979), implying that microsomal-enzyme induction was not the mechanism involved (Richens & Waters, 1971; Maxwell et al., 1972). The present study further supports this implication.

References


