CONCISE

PUBLICATIONS

The Pathogenesis of Coronary Artery Disease

A POSSIBLE ROLE FOR METHIONINE METABOLISM

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ABSTRACT Homocystinuria, an abnormality of methionine metabolism, is associated with severe vascular disease in infancy and childhood. Homocysteine is formed during the metabolism of methionine and accumulations of this and of cysteine-homocysteine mixed disulfide in the plasma indicate a partial block in the methionine degradation pathway. Methionine metabolism was investigated in 25 patients aged under 50 with angiographically proved coronary artery disease and in 22 control patients, of whom 17 had normal coronary arteries at angiography and 5 were healthy volunteers.

After an overnight fast, venous blood was drawn before and 4 h after oral L-methionine, 100 mg/kg. Plasma methionine levels at 4 h were not different in the two groups, but there were significant differences in the levels of cysteine-homocysteine mixed disulfide. This was detected in 5 of 22 in the noncoronary group and in higher concentration in 17 of the 25 coronary patients ($P < 0.01$). Age, weight, height, body-mass index, glucose tolerance, fasting serum urate, and triglycerides were not different, but serum cholesterol was higher in the coronary patients ($P < 0.01$).

These results suggest a reduced ability to metabolise homocysteine in some patients with premature coronary artery disease when this pathway is stressed.

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INTRODUCTION

The development of coronary heart disease is associated with a number of established risk factors (1, 2). Yet it is not uncommon for individual patients to have a paucity, or complete absence, of known risk factors, a finding more readily apparent to the practicing physician than to the epidemiologist.

We undertook the present study to explore the possibility that premature coronary artery disease (in patients under the age of 50) is associated with abnormal methionine metabolism. Abnormalities of methionine metabolism are responsible for homocystinuria, in which arteriosclerotic disease in infancy and childhood, with a high incidence of thromboembolism, is a prominent feature (3, 4). The principal biochemical features in the plasma in homocystinuria due to cystathionine synthetase deficiency are an elevated level of methionine, and the detection of homocysteine and cysteine-homocysteine mixed disulfide. Homocystinuria, a recessively inherited inborn error of methionine metabolism, is rare, but the incidence of heterozygotes for the condition may approach 1% of the population (5, 6). Heterozygotes exhibit abnormalities of methionine metabolism if tested appropriately (7) and may be prone to develop premature vascular disease (8). Several other abnormalities of methionine metabolism are known (9, 10) and some, e.g. cystathioninemia, may be more common than homocystinuria.

In the present study we found evidence of impaired methionine metabolism in patients with coronary artery disease. This paper reports our data.

TABLE I

Mean Values ±SE, for Age, Weight, Height, Body-Mass Index, Cholesterol, Triglycerides, Urate, and Blood Glucose; and for Methionine, before and after Load

	Age	Weight	Height	Body-mass index	Cholesterol	Triglycerides	Urate	Glucose 1 h after 50 g load	Methionine	
									Fasting	4 h after 100 mg/kg
	yr	*kg*	*cm*	*kg/m²*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*μmol/liter*	
Control patients, $n = 22$	39±2	69.8±1.5	173.4±1.4	23.2±0.4	194± 9	116± 9	6.9±0.3	132±9	17±3	409±41
Patients with coronary artery disease, $n = 25$	39±1.5	72.0±2.1	172.9±1.3	24.0±0.6	237±11*	150±14	7.0±0.3	136±7	19±3	493±45

* $P < 0.01$.

METHODS

Subjects aged 50 and under with and without coronary artery disease were studied. There were 25 male patients in the coronary artery disease group referred for coronary angiography because of angina pectoris. All were normotensive, none was in heart failure, and none had had recent myocardial infarction (within the previous 4 mo). All patients had at least 70% obstruction of the lumen in one or more major coronary vessels. There were 7 patients with 3-vessel, 5 with 2-vessel, and 13 with 1-vessel coronary artery disease. In 12, coronary artery surgery was undertaken subsequent to this investigation.

There were 22 patients with normal or presumed normal coronary arteries; 17 (15 men and 2 women) were referred with a diagnosis of possible coronary artery disease because of chest pain. Coronary angiography established that the coronary arteries, left ventriculograms, and intravascular pressures and flows were entirely normal. There were in addition five normal male volunteers, all staff doctors. Coronary angiography was not performed in these, but all were asymptomatic and physical examinations, chest X-rays, and resting electrocardiograms were normal.

Glucose tolerance and fasting serum cholesterol, triglycerides, and urate were measured in all patients and normal subjects by standard techniques. Height, weight, and body-mass index (weight/height²) were recorded as well as medical histories, including drug therapy and dietary history. All drugs were stopped at least 72 h before the study. The patients and controls were eating a standard ward diet and in none had there been radical dietary changes, such as weight-reducing programs over the previous 4 wk. The response to a methionine load was assessed in each as follows:

A control venous blood sample (10 ml) was obtained at approximately 8 a.m. after a 12-h fast. L-methionine, 100 mg/kg, was then given orally in orange juice. 4 h later a second blood sample was taken. The patient took nothing by mouth between the giving of methionine and the 4-h sample. The heparinized blood samples were centrifuged within 5 min, deproteinized with 1% picric acid, and stored at −10°C until analysis on a Beckman Unichrome amino acid analyzer (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), as described by Spackman, et al. (11). To assess reproducibility, in five patients load tests were repeated at intervals of from 3 mo to 2 yr.

Student's t test and the χ^2 test were used in the statistical calculations.

RESULTS

The values (means±SE) obtained in the coronary and noncoronary groups for cholesterol, triglycerides, urate, glucose tolerance as assessed by the 1-h after 50 g glucose blood level, and total methionine (methionine plus methionine sulphoxide) before and after loading are shown in Table I; age and body-mass index are also included. Whereas age, height, weight, body-mass index, the 1-h blood sugar, and triglycerides were not different in the two groups, cholesterol was significantly higher in the coronary group ($P < 0.01$). Total methionine was greatly increased at 4 h in both coronary and noncoronary patients. The mean 4-h value for the coronary group was higher than that for the controls but the difference was not significant.

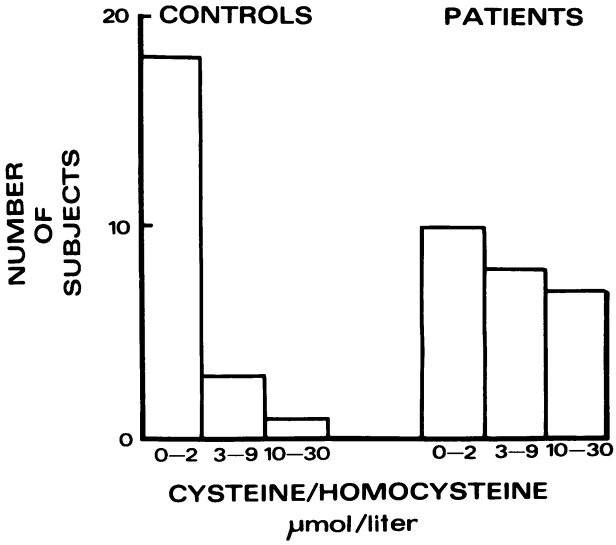

FIGURE 1 The distribution within the noncoronary ($n = 22$) and coronary ($n = 25$) patients of levels for cysteine-homocysteine mixed disulfide 4 h after methionine, 100 mg/kg. The distributions are different at the $P < 0.02$ level by a 3×2, χ^2 test; with a 2×2, χ^2 test the level is $P < 0.01$; see text.

There was a significant difference in cysteine-homocysteine mixed disulfide between the two groups. In the 4-h blood samples, mixed disulfide was detected in only 5 of 22 in the noncoronary group, whereas it was present in 17 of the 25 coronary patients.

To evaluate the significance of these findings, the values in the coronary and noncoronary patients were assessed by the distribution of 4-h mixed disulfide concentrations within each group. As shown in Fig. 1, 7 patients had high values, between 10 and 30 μmol/liter, whereas only 1 of the 22 controls was in this range. Intermediate values of between 3 and 9 μmol/liter were found in 8 patients and 3 controls; and mixed disulfide was absent or very low, less than 3 μmol/liter, in 10 of 25 patients and 18 of 22 controls. The distributions are significantly different at the $P < 0.02$ level when assessed by a 3×2, χ^2 test. If a 2×2 analysis is performed of the distribution in each group of values above and below a level of 2 μmol/liter the difference is significant at the $P < 0.01$ level. Neither the presence in the plasma of mixed disulfide nor the level were correlated with the prior administration of beta-adrenergic blocking drugs (propranolol).

Mixed disulfide was not detected in any fasting, premethionine sample.

Methionine load tests were repeated in the five patients with the highest mixed disulfide levels. There was no significant difference between first and second determinations; and in individual patients values were within ±15% of the mean of the two determinations.

DISCUSSION

The main biochemical features of the metabolism of methionine are well known (12). The step from homocysteine to cystathionine requires the enzyme cystathionine synthetase. This enzyme has greatly reduced activity in patients with homocystinuria, leading to an accumulation in the plasma of methionine, homocysteine (excreted in the urine as the oxidised form, homocystine), and the cysteine-homocysteine mixed disulfide.

The accumulation in the plasma of cystine-homocysteine mixed disulfide that we found in some coronary patients could be due to a partial block in one of several stages in the methionine degradation pathway. The abnormally high mixed disulfide levels suggest a reduced ability to metabolize homocysteine, and this may be relevant to the development of premature vascular disease. It was shown by McCully and Ragsdale that homocysteine thiolactone and homocysteic acid produced arteriosclerotic plaques when administered to rabbits (13). Further, in a recent study, Harker and his associates demonstrated a threefold increase in platelet consumption in four homocystinuric patients (14). However, measurements of platelet function were normal. But Harker and his associates also showed that experimental homocysteinemia in baboons caused patchy desquamation of vascular endothelium; circulating endothelial cells could be identified, platelet consumption was increased, and arterial thrombosis occurred (14). They concluded that homocysteine-induced endothelial injury was responsible for arterial thrombus formation in homocystinuric patients. Evidence is gradually accumulating in support of the proposition that intimal injury is the initial event in the development of atherosclerosis (15–18).

Sardharwalla and his colleagues (7) have also reported results of methionine loads. They tested 12 obligate heterozygotes for homocystinuria due to cystathionine synthatase deficiency and 12 normal subjects. The heterozygotes had much higher levels of mixed disulfide at 4 h than the normals and there was no overlap. They found that mixed disulfide levels were the most discriminatory plasma measurement. Their results in the normals were somewhat different from ours, in that they found some mixed disulfide, albeit frequently at very low levels, in all subjects. This could be because their method for analysis may have been more sensitive than the one we used, in that they employed an iodoplatinate indicator in parallel with Ninhydrin in the amino acid analysis. Their mean level for mixed disulfide at 4 h in normal subjects was 6 μmol/liter ±2 (SD), with a range of 3-9 μmol/liter. In obligate heterozygotes the mean was 18±8 μmol/liter with a range of 11-37 μmol/liter, and these latter values are similar to results obtained in 7 of our 25 coronary patients. We confirmed our five highest values by repeating methionine load tests, obtaining good agreement (see Results). Their plasma methionine levels showed considerable overlap between normals and heterozygotes, as was also the case wtih our values for the controls and patients with coronary artery disease. Both fasting and 4-h plasma methionine levels in our study were lower than those found by Sardharwalla and his associates, and we have no explanation for this. The fasting levels they recorded were higher than those reported by several authors (19, 20) and higher than the normal range for our laboratory.

Although published figures for the incidence of homocystinuria have suggested that it is extremely rare, McKusick calculated a theoretical incidence of at least 1 in 50,000, which corresponds to a frequency of heterozygotes of just less than 1 in 100 (5). Recent experience in New South Wales bears this out (6). The incidence of heterozygotes for other forms of homocystinuria and for other abnormalities in the methionine pathway, such as cystathioninemia, is not known.

The present studies do no more than establish that methionine metabolism in patients with premature coronary artery disease, selected solely by age at referral and by suitability for coronary artery surgery, differs from

that found in a matched control group. The method of selection excluded patients with hypertension, diabetes, and hereditary hyperlipoproteinemia. The timing of the post-methionine sample (4 h) was chosen arbitrarily, and other samples were not taken in this study because of lack of available amino acid analyzer time. Clearly, more detailed studies are required to explore this observation.

Methionine is an essential amino acid, obtained only from dietary sources and found mainly in animal protein, the intake of which is high in affluent societies. Abnormalities of lipid and carbohydrate metabolism constitute well-established risk factors for coronary and other vascular disease. We suggest that altered protein metabolism in societies in which methionine intake is high may well constitute an important additional risk factor in genetically susceptable subjects by contributing towards the production of endothelial damage in key areas of the vasculature. Those particularly affected by hemodynamic disturbance seem most vulnerable (21). It is noteworthy that platelet survival time is frequently reduced in patients with coronary artery disease but that this cannot be related to the known risk factors (22), findings consistent with this hypothesis. Several co-factors are involved in methionine metabolism (12), which, if subsequent studies support this hypothesis, could extend therapeutic possibilities for the prevention of premature coronary artery disease.

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