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The influence of liver disease on the methylation of arsenite in humans

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Abstract. The capacity for inorganic arsenic (AS_i) methylation in 13 healthy volunteers and in 30 patients with different types of liver disease has been assessed by measuring the amount of unmetabolized As_i, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) excreted in urine within 24 h after the IV injection of 7.14 μg/kg AS_i. Liver disease does not affect the percent of the injected dose excreted within 24 h but has striking and opposite effects on the proportions of MMA and DMA. MMA excretion is highly correlated with the ^{14}C-aminopyrine breath test ($r = 0.73$; $P < 0.05$). The reduction in the proportion of MMA excreted in urine and the increase in that of DMA are similar with regard to sensitivity and specificity for detecting liver impairment. Unlike the ^{14}C-aminopyrine breath test, the inorganic arsenic methylation test offers the advantage of being unaffected by treatment with microsomal enzyme inducers.

Key words: Inorganic arsenic – Methylated arsenic metabolites – Liver disease

Introduction

The study of the metabolism of inorganic arsenic in man has recently been made possible by the development of analytical methods for the specific measurement of arsenical derivatives in urine (Lauwerys et al. 1979). It has been demonstrated that exposure to inorganic arsenic leads to the urinary excretion of monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), also called cacodylic acid, and unchanged inorganic arsenic (As_i).

After the oral administration of a moderate dose of As_i (500 μg As_i as $NaAsO_2$), about 75% of the arsenic excreted in urine within 4 days following ingestion is methylated arsenic (33% as MMA and 67% as DMA) (Buchet et al. 1980, 1981a; Crecelius 1977). While excretion occurs in the form of As_i during the first hours following exposure, the methylation process is rapidly triggered and this leads after about 8 h to a preponderant excretion of the methylated species. The production of MMA and DMA can be saturated by high doses of As_i, as observed in cases of acute intoxication (Mahieu et al. 1981). The metabolites MMA and DMA are much less toxic than As_i, and therefore the methylation process represents a true detoxication mechanism.

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The main site of arsenic methylation is probably the liver, but the detailed biochemical mechanism has not yet been elucidated. It is not yet known whether the methylation reactions proceed sequentially from As_i to MMA and then to DMA, or whether MMA and DMA are formed independently. The methylation reaction, however, requires the production of high energy cofactors and the integrity of mitochondrion function.

We have investigated the capacity for inorganic arsenic methylation in patients with different types of liver disease. Our results indicate that urinary excretion of MMA and of DMA following a single IV administration of a very low dose of inorganic arsenic is a good index of hepatocellular function.

Materials and methods

Control subjects. The control group included six women (mean age 41.3 years; range 24–51 years) and seven men (mean age 31.4 years; range 23–44 years) without any previous history of hepatic or renal dysfunction. They were not taking any medication, nor did they exhibit excessive alcohol intake. All were negative for HBs antigen and HBc antibodies and had normal serum levels of aspartate aminotransferase-GOT (< 30 IU/l), alanine amino-transferase-GPT (< 40 IU/l) and γ-glutamyltranspeptidase- GT (< 45 IU/l). A ^{14}C-aminopyrine breath test performed in four subjects was normal. Two female and three male volunteers were used as subjects for experiments designed to test the influence of microsomal enzyme induction on inorganic arsenic methylation. These studies were performed before and after the administration of 50 mg sodium phenobarbital three times (PO) per day for 10 days, a dose known to induce a significant increase in microsomal function (Remmer 1970). At the time of the second test, on day 11, serum concentration of phenobarbital averaged 14.7 μg/ml (SD 1.8 μg/ml) in the five subjects.

Patients. The inorganic arsenic methylation test was performed in 30 patients with miscellaneous liver diseases, ranging in age from 18 to 72 years ($\bar{X} = 48.5$; SD = 14.8). On the basis of clinical, sero-biochemical, and histological work-up, those cases were classified into alcoholic cirrhosis (six males and three females), postnecrotic cirrhosis (three males and six females), hemochromatosis (three males) biliary cirrhosis (two females) and steatosis (one male and one female). In addition, the potential influence of drugs known to affect the mixed-function oxydase was investigated in two patients: one

female who had been treated for tetanus with high doses of phenobarbital for more than 1 month (serum phenobarbital level between 20 and 50 μg/ml) and another female who had been treated for several years with phenylhydantoin for epilepsy (serum phenylhydantoin level of 3.7 μg/ml). All the patients had normal renal function.

Sero-biochemical and liver function tests. Serological and liver enzyme determinations were performed following routine laboratory methods, and included serum GOT ($n < 30$ IU/l), GPT ($n < 40$ IU/l), GT ($n < 45$ IU/l), alkaline phosphatase (Alk. Phos.; $n < 60$ IU/l), leucine aminopeptidase (LAP; $n < 60$ IU/l), total and direct bilirubin ($n < 1$ μg/ml) and protein electrophoresis. Prothrombin time (PTT) was determined by the Quick method (Quick 1935). Sulfobromophtalein (BSP) retention was determined 45 min after the injection of 5 mg/kg body weight of the dye ($n < 5\%$). The ^{14}C-aminopyrine breath test was performed following a previously described technique (Pauwels et al. 1982) and the data were expressed as percent $^{14}CO_2$ cumulative excretion during the 1st hour (C.E.; $n > 2.8\%$).

Inorganic arsenic methylation test. To prevent possible misinterpretations related to variable rates of gastrontestinal absorption of AS_i in controls and patients with liver disease, the IV route of administration was used throughout the study. A sterile aqueous solution containing 0.68 mg sodium arsenite ($NaAsO_2$) and 0.5 mg ascorbic acid per milliliter was prepared and injected IV at a dose of 0.2 ml/10 kg. This provides an equivalent dose of 500 μg As_i for a 70-kg person (7.14 μg As_i/kg body weight) which is about 5,000 times less than the minimum integrated dose reported to increase the risk of skin lesion (Tseng et al. 1968). Ascorbic acid was added in order to maintain inorganic arsenic in its trivalent state during storage. A sample of urine was obtained just before injection to determine the baseline level of arsenic metabolites (usually less than 3 μg As_i, 1 μg MMA and 12 μg DMA/g urinary creatinine). Urine was collected for 24 h; after volume measurement, an aliquot was kept at 4° C and As_i, MMA, and DMA concentrations were determined following a previously described method (Buchet et al. 1980). Care was taken to prevent any additional exposure to As_i, MMA or DMA during the test, e.g., consumption of mineral water containing As_i (Vichy water) or use of over-the-counter tonic preparations which may contain significant amount of arsenic derivatives. Intraindividual variability in inorganic arsenic methylation capacity was assessed in five control subjects who were given the test three times at 3 monthly intervals. In order to assess the potential for saturation of the methylation reaction, the proportion of arsenic excretion as MMA and DMA was compared following IV administration of 7.14 or 14.28 μg/kg As_i to one healthy subject.

Statistical methods. Statistical evaluation of data was performed using Student's t test and Pearson correlation coefficients.

The design of the study was approved by the ethical committee of the University on April 16, 1980.

Results

1. Control subjects

The results of the inorganic arsenic methylation test performed in six female and seven male volunteers are summarized in Table 1. No significant difference was found between males and females with regard to the percentage of the injected dose excreted within 24 h and the proportion of the different metabolites. In a similar test during which the same dose of arsenic was administered orally to three of the same male volunteers (Buchet et al. 1981a), the percentage of the administered dose excreted in urine within 24 h was lower (IV: 34% vs PO: 20%), but the proportion of the methylated derivatives was smaller after IV injection (IV: 37% vs PO: 60%). This is consistent with the hypothesis that the liver is the main site of methylation. With both routes of administration, the amount of DMA excreted is about twice that of MMA. The test was repeated three times at 1-month intervals in five control subjects. The mean coefficient of variation was 15% for MMA and 16% for DMA. The interindividual variability in MMA and DMA excretion follows a normal distribution. The range of normal values was established by considering the mean value ± 2 SSD of the control population. The ranges for percent total arsenic excreted within 24 h as MMA and DMA were 7.6–18.0 and 12.5–36.1 respectively.

When injecting a higher dose, the results were rather similar (lowest dose: MMA 10.1%; DMA 35.7%; highest dose: MMA 11.1%, DMA 30.3%). This suggests that the dose selected for this study (7.14 μg/kg) does not saturate the methylation reaction in control subjects (see also Buchet et al. 1981b).

In the five subjects in whom the As_i methylation test was performed before and after phenobarbital administration, the mean excretion of As_i was reduced by 6% whereas that of MMA and DMA was increased by 1% and 5% respectively. Although all these differences were statistically significant (paired t test, $P < 0.005$), they were very small and probably have no practical relevance.

2. Patients

The results of arsenic methylation tests performed in patients with liver disease are presented in Table 2. When compared to controls (Table 1), the percent of the injected dose excreted within 24 h was not significantly different (t-test, $P > 0.4$) but the proportion of As_i excreted as DMA was significantly increased (t-test, $P < 0.001$) whereas that excreted as MMA was significantly decreased (t-test, $P < 0.001$). The excretion of MMA (monomethylation test) was highly correlated with the results of the ^{14}C-aminopyrine breath test ($r = 0.73$; $P < 0.05$, Fig. 1). The results of both parameters were also found to be significantly correlated with the serum γ-globulin level ($r = -0.43$ for MMA excretion and -0.46 for aminopyrine demethylation), the serum urea level ($r = +0.73$ for MMA excretion and $+0.51$ for aminopyrine demethylation) and the prothrombin time ($r = +0.37$ for MMA excretion and $+0.72$ for aminopyrine demethylation). On the contrary to MMA excretion, that of DMA is not correlated with the results of the ^{14}C-aminopyrine breath test. Although MMA excretion was decreased and DMA excretion increased in patients with liver disease, there was no statistically significant negative correlation between these parameters ($n = 30$; $r = -0.177$; $P > 0.3$). The excretion of DMA (but not of MMA) was, however, inversely correlated with that of As_i ($n = 30$; $r = -0.94$; $P < 0.001$). Statistically significant positive correlations were also found between the DMA excretion and serum AP ($r = 0.54$), GT ($r = 0.44$) and direct bilirubin ($r = 0.38$). The respective sensitivity (proportion of true abnormal values) and specificity (proportion of true normal values) of the As_i mono- and

Table 1. Methylation of inorganic arsenic injected IV (0.5 mg As/70 kg) in female and male control subjects[a]

	Total As excreted within 24 h		Urinary metabolites excreted within 24 h (% of total)		
	µg	% of the injected dose	As_i	MMA	DMA
Women (n = 6)	178.8 ± 19.9 (107 – 232)	29.9 ± 2.8 (22.8 – 42.6)	62.2 ± 3.7 (44.5 – 69.1)	12.6 ± 1.2 (9.5 – 16.3)	25.2 ± 3.4 (16.3 – 40.4)
Men (n = 7)	163.9 ± 20.9 (107 – 264)	29.8 ± 1.5 (23.3 – 34.9)	63.5 ± 1.8 (57.7 – 70.3)	12.9 ± 0.9 (10.1 – 16.2)	23.5 ± 1.1 (19.2 – 26.5)
Total population	170.5 ± 14.1 (107 – 264)	29.8 ± 1.4 (22.8 – 42.6)	62.9 ± 1.9 (44.5 – 70.3)	12.8 ± 0.7 (9.5 – 16.3)	24.3 ± 1.6 (16.3 – 40.4)

[a] Mean ± SEM, with ranges in parentheses

Table 2. Methylation of inorganic arsenic injected IV (0.5 mg As/70 kg) in patients with liver disease[a]

Patients	n	Total As excreted within 24 h		Urinary metabolites excreted within 24 h (% of total)		
		µg	% of the injected dose	As_i	MMA	DMA
Alcoholic cirrhosis	9	129.2 ± 15.2 (69.3 – 203.1)	27.5 ± 2.7 (19.2 – 48.2)	53.9 ± 3.3 (39.2 – 70.9)	5.1 ± 0.7 (2.5 – 10.0)	40.6 ± 3.3 (23.0 – 56.2)
Chronic hepatitis	5	156.7 ± 9.2 (143.8 – 192.3)	39.4 ± 3.0 (29.2 – 46.8)	57.3 ± 2.8 (48.8 – 66.3)	9.2 ± 1.4 (3.7 – 12.1)	33.5 ± 3.4 (21.6 – 41.0)
Hemochromatosis	3	142.8 ± 19.6 (114.3 – 180.3)	32.3 ± 3.0 (28.3 – 38.3)	45.1 ± 9.3 (29.7 – 61.8)	4.1 ± 2.0 (0 – 6.3)	50.9 ± 7.6 (38.2 – 64.4)
Postnecrotic cirrhosis	9	135.8 ± 16.9 (60.2 – 210.5)	28.8 ± 3.0 (13.5 – 40.5)	57.2 ± 3.1 (39.5 – 65.3)	6.5 ± 1.6 (0.3 – 14.6)	36.3 ± 2.2 (28.6 – 51.6)
Steatosis	2	116.2 (153.2;79.1)	30.6 (254;35.9)	47.1 (55.2;39.0)	1.6 (1.1;3.4)	50.7 (43.7;57.6)
Biliary cirrhosis	2	180.8 (187.8;173.8)	44.8 (38.8;50.7)	38.8 (36.1;41.4)	7.3 (4.1;10.4)	54 (48.2;59.8)
Total	30	139.7 ± 7.7 (60.2 – 210.5)	31.7 ± 1.7 (13.5 – 50.7)	53.1 ± 1.9 (29.7 – 70.9)	6.1 ± 0.7 (0.0 – 14.6)	40.7 ± 1.9 (21.6 – 64.4)

[a] Mean ± SEM, with ranges in parentheses

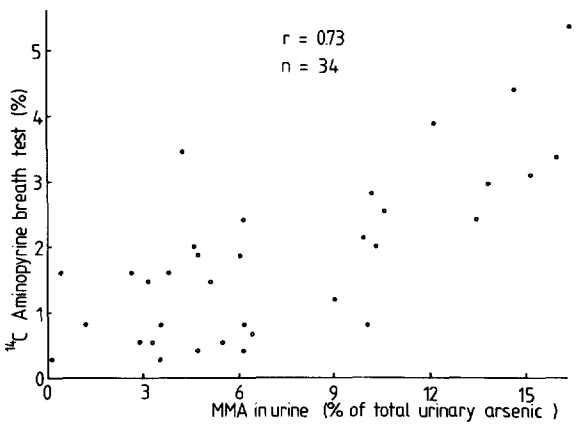

Fig. 1. Relationship between the ^{14}C-aminopyrine demethylation breath test and the arsenic monomethylation test in four control subjects and 30 patients with liver disease

dimethylation and ^{14}C-aminopyrine demethylation tests in detecting abnormal values in the various biological parameters has been determined. For this computation, the results of the methylation tests and ^{14}C-aminopyrine breath test were considered abnormal when MMA excretion was below 7.6%, that of DMA above 36.1% and the cumulative excretion of $^{14}CO_2$ in breath did not exceed 2.8%. Depending on the biological parameter considered, the sensitivity of the tests ranged from 0.8 to 1 (mean value: 0.91) for the ^{14}C-aminopyrine demethylation test, from 0.7 to 1 (mean value 0.87) for the As_i monomethylation test and from 0.5 to 0.87 (mean value 0.74) for the As_i dimethylation test respectively (Table 3). Their specificity varies from 0 to 0.37 (mean value 0.19) for the ^{14}C-aminopyrine demethylation test, from 0 to 0.63 (mean value 0.39) for the As_i monomethylation test and from 0.29 to 1 (mean value 0.52) for the As_i dimethlyation test, respectively.

In the female patient who received high doses of phenobarbital for more than 1 month before the test, the

Table 3. Prevalence (%) of decreased ^{14}C-aminopyrine demethylation and As_i monomethylation and of increased As_i dimethylation in patients with abnormal sero-biochemical variables

Variable	N[a]	^{14}C-breath[b] test < 2.8%	MMA[c] < 7.6%	DMA[d] > 36.1%
GPT > 40 IU/L	18	83.3	77.8	72.2
GOT > 40 IU/L	16	87.5	81.2	87.5
LAP > 60 IU/L	5	80	100	80
AIK.Phosph. > 60 IU/L	10	90	100	80
GT > 45 IU/L	18	88.9	83.3	83.3
Total bilirubin 1 > mg/100 ml	16	93.7	87.7	68.7
Direct bilirubin > 1 mg/100 ml	10	90	100	70
Serum globulin > 1.5 g/100 ml	21	95.2	86.7	72.5
Serul albumin < 3.5 g/100 ml	10	100	80	70
PTT < 50%	8	100	87.5	50
BSP retention > 5%	10	90	70	80

[a] Number of patients
[b] ^{14}C-Aminopyrine demethylation
[c] Methylation of inorganic arsenic to monomethylarsonic acid
[d] Methylation of inorganic arsenic to dimethylarsinic acid

percentages of metabolites excreted within 24 h amounted to 3 and 27 in the case of MMA and DMA respectively, reflecting impaired liver function indicated by increased serum GOT, GPT, GT, Alk. Ph., and total bilirubin values. In the female epileptic patient treated for some years with phenylhydantoin 13.2% and 28.6% of the total amount of arsenic excreted within 24 h was in the form of MMA and DMA, respectively.

Discussion

After the IV administration of 0.5 mg As_i/70 kg to healthy men and women, approximately 30% of the injected dose is eliminated within 24 h by the urinary route as As_i, MMA, and DMA. The proportion of the three metabolites is similar in both sexes (on the average 62.5% as As_i, 12.5% as MMA and 25% as DMA respectively). The methylation capacity of arsenic by the organism is not appreciably influenced by pretreatment with two microsomal enzyme inducers (phenobarbital, diphenylhydantoin). Liver disease has striking but opposite effects on the excretion of the two methylated arsenic metabolites, increasing the production of DMA and decreasing that of MMA. At first sight, it would seem logical to hypothesize that both effects are directly related, an inhibition of the monomethylation reaction leading to an increased availability of the substrate for the dimethylation reaction. However, the absence of a statistically significant negative correlation between the proportion of both metabolites in patients with liver disease does not support this hypothesis. Furthermore, the fact that only MMA production is correlated with ^{14}C-aminopyrine demethylation suggests that two independent metabolic pathlays are responsible for the production of MMA and DMA, although both may be modified in patient with liver diseases. The reduction in the proportion of MMA excreted in urine and the increase in that of DMA have approximately the same discriminatory power for detecting liver impairment. The arsenic methylation test has the same sensitivity as the ^{14}C-aminopyrine breath test, but offers the advantage of being more specific and unaffected by treatment with microsomal enzyme inducers. This may be due to the dependence of arsenic methylation on mitochondrial integrity, whereas aminopyrine demethylation requires microsomal activity.

The present study suggests that measurement of MMA and DMA excretion in urine following As_i administration may constitute a useful test for evaluating liver function. Further study is required to evaluate its potential place in the battery of liver function tests.

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