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Volatile Halocarbons in Haemodialysis Therapy

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Volatile halocarbons in chlorinated drinking water and in human blood have attracted increasing attention during the past years (Rook 1974; Dowty et al. 1975; Symons et al. 1977; Kroneld et al. 1980; Kroneld et al. 1983).

Several investigations have suggested that the main source of the volatile halocarbons found in human body fluids is chlorinated drinking water (US EPA 1978; Cotruvo 1980; Pfaffenberger et al. 1982).

The lipophilic nature of these substances give them a strong tendency to accumulate in the body tissues (Vogt et al. 1980). Several of these substances are also carcinogenic, mutagenic and teratogenic (Kuzma et al. 1977; Tardiff 1977; Cantor et al. 1978; Davidson et al. 1982). Our toxicological data on these substances and the hazard of exposure of humans for long periods to concentrations found in the body fluids is, on the other hand, limited.

Haemodialysis therapy is commonly used in the therapy of chronic uremia (Kolff 1947; Bailey 1972). The blood of the patients is dialysed in an artificial kidney against a dialysis fluid consisting of a haemodialysis concentrate and tap water. 100 l of such fluid flows through the apparatus each time and the therapy is repeated 2-3 times a week for each patient and may continue many years. Methods to eliminate harmful substances have on the other hand been developed (Cowty et al. 1978), especially considering water prehandling.

This paper deals with the exposure and the absorption of volatile halocarbons in patients receiving haemodialysis therapy.

MATERIALS AND METHODS

The volatile halocarbons were analysed through eluation

with n-pentane (Kroneld et al. 1983). A 1 ml portion of plasma or blood cells was for the actual analyses measured into a glass test tube, and 100 µl of n-pentane containing 100 ng/ml of 1-iodobutane as an internal standard was added. The extraction was performed by turning the tube until the pentane phase had been passed about 100 times throughout the tube. After a short period of centrifugation at 1000 rpm, one 3 µl aliquot of the pentane phase was injected into the gas chromatograph (GC) equipped with a split injector and an ^{63}Ni electron capture detector (ECD). The actual determinations in water were carried out by extracting 100 ml (22 °C) of dialysis fluid with 5 ml of n-pentane containing the internal standard (400 ng/ml) for 5 min, and then injecting a 2 µl aliquot of the pentane phase into the gas chromatograph. A Schimadzu C-R1B calculating integrator calculated the concentrations of identified compounds according to the sorted calibration date (figure 1). Mass spectrometry was used to confirm the analyses as the method of preference.

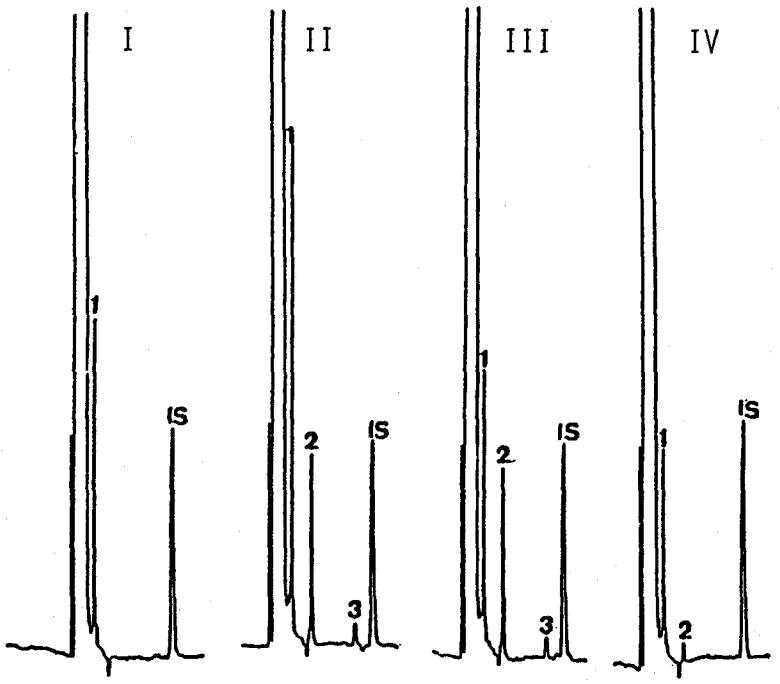

Figure 1. Series of chromatograms obtained from blood samples of patients before dialysis (I), after two hours of dialysis (II), at the end of dialysis (III), and one hour after dialysis (IV). 1 = $CHCl_3$, 2 = $CHBrCl_2$, 3 = $CHBr_2Cl$, IS = internal standard. Column: SE-52, 20-m x 0.3-mm i.d; temperature: 50 °C; carrier gas: 1.7 ml hydrogen/min.

The haemodialysis patients consisted of groups of 5 to 6 patients each. 3 to 4 ml blood in heparinized test tubes were collected before, during and after such therapy. The specimens were gently shaken and then centrifuged. After haemolysis through freezing of the separated cells, both fractions were analysed according to the method described. The samples of dialysis fluid were taken at the inlet to the dialysis coil and at the drain. The samples were collected before, during and at the end of treatment. The tap water used in the dialysis fluid was subjected to softening, charcoal filtering, reverse osmosis and paper filtering before use. Travenol CF 15.11 and Secon MTL 104 artificial kidneys were used for haemodialysis therapy.

The Student's t-test was used to analyse the significance of the differences between independent and dependent samples.

RESULTS AND DISCUSSION

The concentrations of volatile halocarbons in dialysis fluid and in the blood of the patients receiving haemodialysis therapy are shown in table 1.

Only chloroform was found before dialysis was started, but one hour after dialysis started dichlorobromomethane and dibromochloromethane could also be found. At the same time the mean value for the chloroform concentrations had further increased. All the mean values for these three compounds reached a maximum concentration both in plasma and blood cells two hours after dialysis started. Three and four hours after dialysis started,

Table 1. Concentrations of volatile halocarbons in dialysis fluid and plasma and cells of patients before dialysis started, 1 to 4 hours during and after dialysis. P values (dependent) by comparison with initial values.

	hours	0	1	2	3	4	5
$CHCl_3$	dialysis fluid µg/l (n=18)			37.3±1.2			
	patients nmol/l (n=12) plasma	155.4±16.1	206.1±16.4^{x}	249.1±20.3^{xxx}	189.4±18.6	126.3±23.8	116.7±18.1
	cells	313.2±23.6	340.1±19.8	367.3±26.8	289.2±28.6	230.9±15.6	177.7±23.6
		p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	N.S.
$CHBrCl_2$	dialysis fluid µg/l (n=18)			7.5±0.5			
	patients nmol/l (n=12) plasma	-	4.7±2.8	7.8±2.5^{xxx}	4.2±1.7	1.4±1.2	-
	cells	-	6.9±2.9	14.2±3.0^{xxx}	6.74±1.8^{xxx}	4.0±2.2^{x}	-
			N.S.	N.S.	N.S.	N.S.	
$CHBr_2Cl$	dialysis fluid µg/l (n=18)			1.4±0.3			
	patients nmol/l (n=12) plasma	-	0.6±0.5	1.6±0.6^{x}	0.7±0.5	0.4±0.3	-
	cells	-	0.7±0.4	1.6±0.4^{x}	1.1±0.7	0.8±0.3^{x}	-
			N.S.	N.S.	N.S.	N.S.	

x p < 0.05, xxx p < 0.01, xxx p < 0.001
- = not detected

Table 2. Concentrations of volatile halocarbons in inflow and outflow water during dialysis.

	minutes	0	15	30	60	120	140	160	180	210	240	300
water	µg/l											
$CHCl_3$	in-flow (n=18)	36.3±0.4			36.3±0.4	36.3±0.4			36.3±0.4		36.3±0.4	36.3±0.4
	out-flow (n=16)	36.3±0.4	23.3±0.6 p<0.001	17.9±0.5 p<0.001	13.2±0.3 p<0.001	10.2±0.5 p<0.001	9.3±0.3 p<0.001	9.2±0.2 p<0.001	9.3±0.3 p<0.001	9.5±0.3 p<0.001	10.3±0.4 p<0.001	
$CHBrCl_2$	in-flow (n=18)	8.7±0.3			8.7±0.3	8.7±0.3			8.7±0.3		8.7±0.3	8.7±0.3
	out-flow (n=16)	8.7±0.3	7.4±0.3 p<0.001	6.4±0.2 p<0.001	4.5±0.4 p<0.001	3.4±0.3 p<0.001	3.4±0.3 p<0.001	2.5±0.3 p<0.001	2.6±0.3 p<0.001	3.2±0.2 p<0.001	3.3±0.3 p<0.001	
$CHBr_2Cl$	in-flow (n=18)	2.8±0.3			2.8±0.3	2.8±0.3			2.8±0.3		2.8±0.3	2.8±0.3
	out-flow (n=16)	2.8±0.3	2.3±0.2 N.S.	1.7±0.3 p<0.05	1.0±0.3 p<0.01	1.3±0.3 p<0.01	0.6±0.2 p<0.001	0.5±0.2 p<0.001	0.7±0.3 p<0.001	0.6±0.1 p<0.001	0.8±0.2 p<0.001	

P values by comparing with the initial values. N.S. = not significant

the mean values had decreased. One hour after cessation of dialysis the mean concentrations in both plasma and cells had decreased further. Bromine compounds were not detected in the blood samples.

The mean value for the chloroform concentrations in cells was higher two hours after the start of dialysis than the concentration measured in dialysis fluid. This difference was, however, not significant. The concentrations of volatile halocarbons were,in general during dialysis, lower in the blood samples than in the dialysis fluid.

The differences (dependent samples) between the chloroform concentrations in plasma and cells before and during dialysis were statistically highly significant ($p < 0.001$) at 0,1, 2 and 4 hours and significant ($p < 0.01$) at 3 hours, with higher concentrations in the cell samples. After dialysis no significance was found for chloroform or the bromine compounds in this respect.

The concentrations of chloroform were higher and statistically significant in plasma samples at one hour ($p < 0.05$) and two hours ($p < 0.01$) of dialysis, but not in the cell samples.

The dichlorobromomethane concentrations in both plasma and blood samples were significantly higher ($p < 0.001$) at two hours of dialysis compared with the initial value and also for cells at 3 hours ($p < 0.001$) and 4 hours ($p < 0.05$). Otherwise, no significant differences could be found for either dichlorobromomethane or dibromochloromethane during the study, when comparing the concentrations of plasma and blood samples.

Exposure to volatile halocarbons was also studied by comparing the concentrations in dialysis fluid and in waste water (table 2).

The concentrations of volatile halocarbons were measured and found to be equal before dialysis started. The decrease found in the waste water or outflow after 15 minutes of dialysis was, compared with the concentrations in the dialysis fluid or inflow for all three substances, significantly lower during the whole dialysis process, except for dibromochloromethane at 15 minutes. The significance for dibromochloromethane was $p < 0.05$ at 30 minutes, $p < 0.01$ at 1 and 2 hours. The decrease in all three substances in the outflow fluid was otherwise highly significant compared with the initial values.

The difference between inflow and outflow represented the intake or exposure to the patients during dialysis. Hence about 50-60 % of the amounts in the inflow was absorbed during dialysis. This could lead to a yearly

Table 3. Concentrations of volatile halocarbons in dialysis fluid and plasma and cells of patients after two hours of dialysis during the study.

months	I	III	V	VII	IX	XI	I
dialysis fluid n = 18							
µg/l							
$CHCl_3$	6.8±0.4	9.7±0.7 p<0.01	18.2±1.0 p<0.001	33.4±0.8 p<0.001	36.2±0.6 p<0.001	37.3±1.2 p<0.001	5.4±1.6 N.S.
$CHBrCl_2$	1.6±0.2	3.2±0.3 p<0.001	5.4±0.4 p<0.001	6.8±0.7 p<0.001	7.1±0.4 p<0.001	7.5±0.5 p<0.001	0.8±0.5 N.S.
$CHBr_2Cl$	0.5±0.2	0.6±0.3 N.S.	0.6±0.2 N.S.	1.0±0.4 N.S.	1.3±0.2 N.S.	1.4±0.3 p<0.05	-
patients n = 10							
nmol/l							
plasma							
$CHCl_3$	33.1±12.8	45.4±15.3 N.S.	70.6±16.2 N.S.	223.2±18.8 p<0.001	246.5±22.2 p<0.001	249.1±20.3 p<0.001	26.1±10.6 N.S.
$CHBrCl_2$	-	-	+	6.3±3.1 p<0.05	7.6±2.7 p<0.05	7.8±2.5 p<0.05	-
$CHBr_2Cl$	-	-	-	1.3±0.2 p<0.001	1.5±0.4 p<0.01	1.6±0.6 p<0.01	-
cells							
$CHCl_3$	72.6±14.3	85.4±24.1 N.S.	123.2±30.2 N.S.	361.1±32.8 p<0.001	366.9±25.7 p<0.01	367.3±26.8 p<0.01	44.2±12.3 N.S.
$CHBrCl_2$	-	-	1.2±0.2 p<0.001	11.2±1.7 p<0.001	13.8±3.1 p<0.001	14.2±3.0 p<0.001	-
$CHBr_2Cl$	-	-	+	1.4±0.3 p<0.001	1.6±0.3 p<0.001	1.6±0.4 p<0.05	-

+ = traces, - = not detected
P values by comparing with the initial values. N.S. = not significant

absorption of about 2-3 g/year for chloroform, 1-1.5 g/year for dichlorobromomethane and 250-300 g/year for dibromochloromethane, which is far more than 85 mg/year for TTHM exposure calculated as an average level in U.S.A. (Cotruvo 1980).

The concentrations of volatile halocarbons in dialysis fluid and the patients during the study period are shown in table 3.

The concentrations of chloroform and dichlorobromomethane analysed in dialysis fluid were significantly higher ($p < 0.001$) compared with the initial or lowest values analysed in January. The concentrations for dibromochloromethane were in this respect significant ($p < 0.05$) only in November. The chloroform concentrations were significantly higher ($p < 0.001$) in both plasma and cell samples in July, September and November compared with the initial values in January. The dichlorobromomethane concentrations were significantly higher in this respect in plasma samples in July, September and November ($p < 0.05$) and in cell samples in May, July, September ($p < 0.001$) and in November ($p < 0.01$). The dibromochloromethane concentrations were significantly higher in plasma samples in July ($p < 0.001$) and September and November ($p < 0.01$) and in cell samples in July and September ($p < 0.001$) and in November ($p < 0.05$).

There is obviously a positive link between the increase in volatile halocarbons in dialysis fluid and in blood samples during different seasons.

RESULTS AND DISCUSSION

Volatile halocarbons accumulate in the body and are either eliminated or accumulated in tissues relatively rapidly (Vogt et al. 1980; Pfaffenberger et al. 1980). They are lipophilic and quickly penetrate membranes. Elimination of such halocarbons from the blood may not be complete in cases of prolonged exposure.

The chloroform concentrations were highest of all volatile halocarbons detected in plasma and blood cells of dialysis patients. The differences between the chloroform concentrations in plasma and cells during dialysis was significant, with higher concentrations in the cell fractions. After dialysis was ended no significance in this respect could be found for chloroform, while bromine substances could not be detected at all.

Exposure to volatile halocarbons was also confirmed by comparing the concentrations in dialysis fluid and in waste water.

Evidently volatile halocarbons are absorbed in the body. After two hours of dialysis all three compounds studied or detected reached the maximum concentrations of means, even though these values were not statistically significant. The chloroform concentration in blood and tissues normally increases with increasing values of chloroform in water (Pfaffenberger et al. 1980; Vogt et al. 1980; Combs et al. 1982). The maximum absorption of chloroform from the gastrointestinal tract into the blood is registered approximately one to two hours after a single dose (Brown et al. 1974; Vogt et al. 1980). The amounts of volatile halocarbons are lowered at the end of dialysis. The maximum of means at two hours followed by a fall in the actual concentrations could then possibly be explained by enzymatic activation and/or an increased absorption in tissues in the patients. In addition to accumulation, elimination by excretion through the lungs and metabolic conversion into carbon dioxide, urea and other products may also explain the observations. The absorption was over-all much higher during dialysis than the average given in U.S.A. (Cotruvo 1980). There was obviously a positive link between the increase in tap water and in the blood sample in different seasons.

The negative effects of chloroform used as an anaesthetic agent have been well documented. Negative effects of bromine-containing halocarbons, including mutagenic activity in bacterial tests, have also been documented. Even though we do not yet know all the possible consequences of exposure to humans of volatile halocarbons through drinking water, the conclusions of recent studies (Chu et al. 1980) show that the trihalomethanes can produce biochemical, haematological and histological changes even in small concentrations. These were reversible in rats after exposure was terminated. Absorption during dialysis over long periods may also be partly connected with the problems of mental retardation appearing in patients, although no such connection has been reported for volatile halocarbons.

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