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Effect of Volatile Halocarbons on Lymphocytes and Cells of the Urinary Tract

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Volatile halocarbons in body fluids are found in areas with chlorinated surface water rich in organic material (Eklund et al. 1978).

Improved chemical methodology has made it possible to quantify volatile halocarbons in drinking water, blood and urine (Dowty et al. 1975, Pfaffenberger et al. 1980, Bauer 1981, Reunanen & Kroneld 1982, Kroneld & Reunanen 1986).

Knowledge of the toxicity of, and long-term exposure of humans to, volatile halocarbons found in drinking water and body fluids is limited (Davidsson et al. 1982). Toxicity in mice and rats has been investigated in several studies (Bowman et al. 1978, Chu et al. 1982).

Several reports show carcinogenic activity of these substances (Tardiff et al. 1978, Davidsson et al. 1982). A link between the total trihalomethane concentration in drinking water and increased cancer mortality, especially cancer of the urinary organs (Cantor et al. 1980, Williamson 1981) has been suggested.

This paper deals with the in vitro effects of different volatile halocarbons shown to exist in drinking water and body fluids on lymphocyte stimulation and surface properties of cells of the urinary tract.

MATERIALS AND METHODS

Human peripheral blood lymphocytes were separated according to Gesner et al. (1967). Volatile halocarbons reflecting the concentrations found in drinking water and body fluids were added to the started lymphocyte cultures. Each lymphocyte culture was then stimulated with 10 µg/ml phytohaemaglutinine (PHA).

The control lymphocyte cultures received only buffer and PHA. After three days 10 µl labelled thymidine was added to all the lymphocyte cultures and the uptake of ^{3}H-thymidine was measured according to Strannegård et al. (1976). The inhibition of stimulation is shown in absolute numbers as well as a percentage of the control. The concentrations of the volatile halocarbons in the cultures were followed by gas chromatography and electrone capture detection analysis according to Reunanen and Kroneld (1982).

The uroepithelial cells used were harvested from the sediment of urine from healthy donor (Svanborg-Eden et al. 1977). After addition of volatile halocarbons as above the human uroepithelial cells were incubated overnight. The viability of the cells was monitored at times zero, thirty minutes and twenty-four hours by taking an aliquot of the cell mixture and adding a drop of trypan blue. The number of cells excluding dye per hundred cells counted was taken as the viability index. The uroepithelial cells were subsequently tested for receptivity to bacteria, using Eschericcia coli 36692 (Svanborg-Eden et al. 1977). Adhesion is given as a mean number of bacteria attacted to forty uroepithelial cells.

Student's t-test was used to analyse the significance of the differences.

RESULTS AND DISCUSSION

The volatile halocarbons added to the cultures were shown to reduce the PHA stimulation in the experiments (table 1). The inhibiting effects were most pronounced at the highest concentrations of volatile halocarbons tested.

A decreased viability of the uroepithelial cells was also noted after incubation with volatile halocarbons (table 2). Both the PHA lymphocyte stimulation and the viability of uroepithelial cells were altered at the lowest concentrations of volatile halocarbons tested. These concentration levels could also be found in body fluids of persons in the Turku area in Finland (Reunanen & Kroneld 1982, Kroneld & Reunanen 1986). The receptivity of human uroepithelial cells treated with volatile halocarbons to bacteria was, on the other hand, not shown to be altered by the test concentrations used (table 2).

Volatile halocarbons accumulate in the body and are

Table 1. Effects of uptake of ^{3}H-thymidine by human peripheral blood lymphocytes exposed to volatile halocarbons (ug/l)

	Thymidine uptake							
n=18	concentration of volatile halocarbons		concentration of volatile halocarbons		concentration of volatile halocarbons		concentration of volatile halocarbons	
	µg/l	dpm	µg/l	dpm	µg/l	dpm	µg/l	dpm
$CHCl_3$	0.2	135.1±12.2	1.5	76.4±20.1**	15.0	49.9±15.4**	150.0	12.5±10.1**
CCl_4	0.2	128.4±15.2	1.6	78.2±22.3**	16.0	57.5± 6.7**	160.0	15.2± 7.3**
$ClCH=CCl_2$	0.2	141.3±20.1	1.5	67.4±14.9**	15.0	44.8±22.7**	150.0	8.6± 4.4**
$CHBrCl_2$	0.2	129.6±28.1	2.0	86.7±24.1**	20.0	41.1±12.5**	200.0	16.4± 7.1**
$CBrCl_2$	0.2	134.3±13.4	2.0	65.3±15.8**	20.0	34.3±20.1**	200.0	12.1± 7.1**
$CHBr_2Cl$	0.2	132.5±13.4	2.4	57.1±24.1**	24.0	24.2± 9.1**	240.0	14.1± 9.7**
$Cl_2C=CCl_2$	0.2	142.2±10.6	1.6	72.4±27.1**	16.0	38.7±12.1**	160.0	17.1±14.1**
$CHBr_3$	0.3	140.7±26.1	2.9	76.1±30.1**	29.0	41.7±19.3**	290.0	19.7± 6.1**
TTHM	0.5	120.4±10.1*	5.0	61.3±22.1**	50.0	23.1±11.2**	150.0	5.1± 3.1**

significance *) $p < 0.05$, **) $p < 0.001$

Table 2. Viability of human uroepithelial cells and their receptivity to volatile halocarbons (ug/l)

	Viability (No of cells per 100 cells excluding trypan blue)					
	concentration of volatile halocarbons µg/l	% viable cells	concentration of volatile halocarbons µg/l	% viable cells	concentration of volatile halocarbons µg/l	% T cells
$CHCl_3$	0.2	80	1.5	50	15.0	30
CCl_4	0.2	60	1.6	40	16.0	20
$ClCH = CCl_2$	0.2	40	1.5	60	15.0	30
$CHBrCl_2$	0.2	60	2.0	40	20.0	20
$CBrCl_3$	0.2	80	2.0	10	20.0	10
$CHBr_2Cl$	0.2	50	2.4	40	24.0	20
$Cl_2 = CCl_2$	0.2	60	1.6	40	16.0	20
$CHBr_3$	0.3	50	2.9	10	29.0	10
TTHM	0.5	40	5.0	-	50.0	-
	Receptivity (No of bacteria attached to 40 uroepithelial cells)					
	concentration of volatile halocarbons µg/l	number of bacteria	concentration of volatile halocarbons µg/l	number of bacteria	concentration of volatile halocarbons µg/l	number of bacteria
$CHCl_3$	0.2	114	1.5	96	15.0	71
CCl	0.2	112	1.6	82	16.0	88
$ClCH = CCl_2$	0.2	71	1.5	104	15.0	69
$CHBrCl_2$	0.2	71	2.0	64	20.0	82
$CBrCl_3$	0.2	71	2.0	71	20.0	82
$Cl_2C = CCl_2$	0.2	82	1.6	98	29.0	69
$CHBr_3$	0.3	96	2.9	65	29.0	71
TTHM	0.5	64	5.0	56	50.0	71

either eliminated or accumulated in tissues relatively rapidly (Pfaffenberger et al. 1980, Vogt et al. 1980). They are lipophilic and quickly penetrate membranes. Elimination of such halocarbons from the blood and urine is not complete in cases of prolonged exposure. Consequently, the effects on lymphocyte stimulation due to these compounds in vitro may also be found in vivo. The ensuing effects may be alterations in cell surface characteristics or impared function. Decreasing lymphocyte function may lead to increased infection susceptibility, autoimmunity, cancer etc. Indirect evidence suggests a link between especially carcinogenity and exposure to volatile halocarbons via drinking water.

The mechanism of the action of volatile halocarbons on target cells is not well understood. At this stage, therefore, borderline concentrations should be based on sufficiently such small levels that the volatile halocarbons are not even detectable in body fluids. Future experiments might be based on lymphocytes from persons with known amounts of volatile halocarbons in their body fluids to reveal the relevance of these findings.

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