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THE EFFECTS OF LOW CHLORINE DOSES IN DRINKING WATER ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN PERIPHERAL LYMPHOCYTES IN EWE LAMBS

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We observed the effects of low chlorine doses (i.e. 0.3 mg/l drinking water), on the frequency of chromosome aberrations in peripheral lymphocyte cells in ewe lambs for 4 weeks. The frequency of aberrant cells in the experimental and control groups was 3.0 ± 2.1 % AB.C and 3.2 ± 1.3 % of AB.C respectively. No significant effect was recorded ($p>0.05$) in either group. Our results may help in finding solutions to health troubles in both animals and humans as well as in preventing contamination of the food chain.

Key words: drinking water, chlorination, ewe lambs, chromosome aberrations, genotoxicity

INTRODUCTION

A variety of synthetic compounds containing chlorine are used in industries and agriculture, as well as human and veterinary medicine in the form of preparations, such as water disinfectants etc. These compounds represent potential environmental pollutants. The action of such compounds in the environment may be a serious cause of health disturbances in living creatures (Koisuvalo et al., 1995; Hildesheim et al., 1995; Gerhard et al., 1999).

Drinking water is essential for life and therefore it must be safe. Chlorination is the standard method for disinfecting drinking water supplies and is responsible for the improvement of public health through reduction of waterborne microbial pathogens. On the other hand, chlorination byproducts result from disinfection of drinking water with chlorine. The U.S. National Cancer Institute has published results showing that chloroform (one of the trihalomethanes), occurring as a byproduct of drinking water disinfection was carcinogenic in rodents and could also pose a chemically-induced cancer risk for humans etc. (Boorman et al., 1999).

According to van der Schalie et al. (1999) the use of non-human organisms, as early warming systems for human health risk is not new. Sentinel animal models could involve mammalian or non-mammalian species, domestic animals, or wildlife. Sentinel animal populations would be exposed to a single chemical or a complex mixture, or to different media (e.g. air, water, soil, sediment)

in various locations. Although it is unlikely that sentinel-animal data will be used as the sole determinative in evaluating human health, such data may be useful as additional evidence in risk assessment. The action of low doses of genotoxic compounds for a long time is especially likely to be the cause of somatic mutations with potential carcinogenesis. In such a way, a number of genetic diseases may occur (Natarajan et al., 1996). It is for these reasons too, that health protection in vertebrates is of increasing interest in different ecosystems, using proper biomarkers and bioindicators (Lessire et al., 1997). Chromosome aberrations from peripheral lymphocytes are accepted as indicators of the biological effects of genotoxic compounds (Tucker and Preston, 1996).

Ruminants may be exposed to various environmental pollutants via pasture, air, water, etc. Therefore this work assesses the effects of exposure to low chlorine doses given as the disinfectants chlorine gas and Savo-Super (sodium hypochlorite) in drinking water on the frequency of chromosomal aberrations in ewe lamb peripheral lymphocytes.

MATERIALS AND METHODS

A total of 12 two-month-old Merino lambs weighing 11.0 - 14.5 kg was included in the study. The lambs were obtained from a private breeder in a village (N.B.). Prior to the study, the lambs were nursed by their mothers in such a way that they had limited access to drinking water and received no additional feed. Two weeks before the study, the lambs were adapted to the experimental housing conditions as follows: all the animals had access to spring non-chlorinated water *ad libitum* obtained from the East Slovakian Waterworks and Sewerage (Košice, Slovak Republic). The lambs also had free access to a feed mixture (COJ 2) and meadow hay at 250-300 g and 350 g per head per day respectively. The control group consisted of 5 ewe lambs. The control animals were supplied with spring water without any additions throughout the experiment.

In the experimental group, 6 ewe lambs were offered chlorinated water containing 0.3 mg active chlorine/l. The water supplied was firstly treated with chlorine gas (up to 0.2 mg/l) and then adjusted to 0.3 mg/l of active chlorine with the preparation of Savo-Super (Biochemie a.s., Bohumin, Czech Republic) in 950 ml bottles, sodium hypochlorite min. 47.2 g/l) up to the final concentration. The animals were offered feed in the same way as described for the pre-treatment period. The study lasted four weeks.

Heparinised blood (100 IU/ml), obtained by jugular vein puncture, was used for chromosome analysis. Whole blood samples (0.4 ml) were cultured at 37.5 °C for 48 h. in 7.0 ml of chromosome medium-S-chromo cell supplemented with FCS, PHA-L, L-glutamine (PAN Systems GMBH Biotechnologische Produkte, Germany), antibiotics (penicillin G 100 IU/ml and streptomycin, 100 µg/ml and 7.5% $NaHCO_3$ (Serva p.l.c., Czech Republic). Two hours prior to the end of cultivation, colchicine (10 µg/ml) was added. The squashes were stained with a 10 % solution of Giemsa-Romanowski stain in 2.5×10^{-2} mol x l^{-1} phosphate buffer (pH 7.0). One hundred well spread metaphase cells per animal were examined for the presence of different types of chromosome aberrations. Coded slides were scored blindly using a microscope (Nikon) at x 1000 enlargement. The aberrations were scored in metaphase according to the classification criteria suggested by

Savage (1975) and Carrano and Natarajan (1988). Gap aberrations were excluded from the total number of chromosomal aberrations and considered separately.

Data were analysed using the statistical software of Sigma Stat (Jandel Scientific®). The significance of the difference in results between the experimental and control groups was calculated using the t-test.

RESULTS AND DISCUSSION

The effects of four weeks of chlorine administration in prophylactic doses in drinking water on the frequency of chromosomal aberrations are presented in Table 1. *In vivo* chromosomal analysis of peripheral ewe lymphocytes indicated that no significant differences occurred when comparing the frequency of aberrant cells of the experimental and control groups ($p>0.05$).

The frequency of aberrant cells in the experimental group was 3.0 ± 2.1 % AB.C and in the control group it was 3.2 ± 1.3 % AB.C. It is quite difficult to analyse the risk of low doses of different xenobiotics from the responses of some biochemical and molecular markers. Most biomarkers are not proportionally related to the bodyžs response to toxins. From this it follows that there is no assumption of a simple interaction between molecular and biochemical parameters and the toxic response of the body immediately following the action of low doses of xenobiotics (Anderson and Barton, 1998). Au (1991) points out that chromosome analyses in this field prove to be one of the most sensitive and relevant assays to identify genotoxic compounds. According to Đelic et al (1995) drinking water from different surfaces has different chemical properties depending mainly on pollutants found in the particular environment. Moreover, SCE frequency in trials was influenced by the dose of organic condensates used in water. According to the above authors, different chemical compositions of three fractions (neutral, acid, alkaline) correspond with different signs in cytotoxicity and genotoxicity. Neither cytotoxic nor cytostatic effects at the lowest doses of condensates (20 μl/ml) in drinking water on SCR frequency were observed in human lymphocytes. On the other hand, at higher doses of the agent (50 μl/ml and 100 μl/ml), there was lowering of mitotic activity.

In the experimental ewe lambs, we recorded a higher number of chromatid breaks (17) and gaps (6) compared to the control group. In one control lamb, there was a higher occurrence of chromatid breaks (4). According to Anwar (1991), there is considerable variation in individual susceptibility and there is a long and complicated interaction between an organism and subsequent cytogenetic damage.

This is why it would be better to use the same animals prior to the intake and immediately after the end of the experiment. We are of a similar opinion. This follows from our studies. Gaps are considered to be an indicator of the bodys susceptibility to exposure to different genotoxic compounds. Gaps help mainly to assess the actions of low doses as a "guardian" parameter in such studies (Brgger, 1982). This was confirmed in our trials.

The genotoxic effects of various byproducts in drinking water can be detected using *in vitro* and *in vivo* assays (Fuscoe et al., 1996; Miettinen et al., 1998). For instance, Miettinen et al. (1998) studied mutagenicity and the amount of chloroform in drinking water after chlorination against the strain of *Salmonella typhimurium* T A 100. Reduction of chloroform amounts as well as mutagenicity

Table 1 The frequency of chromosome aberrations in ewe lamb peripheral lymphocytes after a four-week intake of low chlorine doses in drinking water

Group of animals	Statistical values	Number of metaphase cells observed	Types of aberrations						Number of AB.C	AB.C	B/C	G/C	Other aberrations
			B_1	B_2	E_1	E_2	G_1	G_2					
Control		500	14	5	-	-	3	-	16		0.038	0.006	
n=5	mean									3.200			3.400
Ewe lamb	Std Dev									1.304			1.140
Merino	SEM									0.583			0.510
													fragments, centric fusion, associations
Experimental		594	17	1	-	-	6	1	18		0.0303	0.0118	
n=6	mean									3.000			2.333
Ewe lamb	SD									2.191			1.366
Merino	SEM									0.894			0.558
	P (Normality test)									0.479			0.278
	P (Equal variance test)									0.572			0.561
	P									>0.05			>0.05
													associations, fragments, centric fusion

Legend: AB.C - aberrant cells; B_1,B_2, E_1, E_2 - chromatid and isochromatid breaks and exchanges; G_1, G_2 - gaps; B/C - number of breaks per cell; G/C - number of gaps per cell; P - statistical significance

of chlorinated drinking water were found to be partially influenced by the total decrease in the organic carbon content. Sabouni and Ziaee (1995) studied the genotoxic effects of drinking water on SCE frequency and on the frequency of chromosome aberrations in CHO fibroblasts and also in V79 fibroblast cells. Doses up to 50 µl of the extract did not enhance his^+ spontaneously, reversible colonies in *Salmonella typhimurium* of the tested strains (T A 98, T A 100, and T A 102) but influenced the culture of S 9 by a fraction. At the same time, the above authors showed that the SCE test is considered to be the most reliable assay for detection of genotoxic compounds in drinking water.

It should be mentioned too that in the control groups of animals, the frequency of aberrant cells was 3.2 ± 1.3 % AB.C. According to the findings of Nemec (1987) in sheep breeds in Slovakia, the level of spontaneous chromosome aberrations is 1.7 %, irrespective of the breed. When comparing three age categories (1-2, 3-4, and 5-9 year-old animals) the spontaneous frequency of the aberrant cells was 1.01 do 1.37 % both in males and females (Holečkova et al., 1993). Forni (1992) recommends comparison of the results of such studies with one´s own control group with the same methodological requirements.

The action of low doses of different xenobiotics on the health of living creatures may be exhibited by different biological effects such as endocrine system disorders, cancerogenic processes etc. Therefore it is useful to make such studies (Ashford, Miller, 1998).

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UTICAJ NISKIH DOZA HLORA U VODI ZA PIĆE NA UČESTALOST HROMOZOMSKIH ABERACIJA U PERIFERNIM LIMFOCITIMA JAGNJADI

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U ovom radu su proučavani efekti niskih doza hlora u vodi za piće (0.3 mg/L) na učestalost hromozomskih aberacija u perifernim limfocitima jagnjadi tokom 4 nedelje. Frekvenca pojavljivanja aberantnih ćelija u eksperimentalnim i kontrolnim grupama je iznosila 3.0 ± 2.1% AB,C odnosno 3.2 ± 1.3% AB.C i nisu uočene razlike između grupa. Ovi rezultati mogu da doprinesu iznalaženju rešenja u zdravstvenoj problematici ljudi i životinja vezanoj za zagađenja u lancu ishrane.