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EFFECTS OF PRETREATMENT WITH VITAMIN E AND SELENIUM ON CCL_4 INDUCED MICRONUCLEI IN SHEEP IN VIVO

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Carbon tetrachloride (CCl_4) is a well-known carcinogen. Free radicals resulting from of CC_{l4} metabolism react with a variety of cel1 constituents and can lead as well as to the breakage of chromosomes. Vitamin E and selenium have fundamental and complementary roles in the prevention of oxidative damage. In sheep pretreated with vitamin E and selenium and subsequently exposed to CCl_4 increases in the frequencies of BNMN (binucleated micronucleated cells) were induced but without statistical significance. When the sheep were exposed to CCl_4 in the absence of vitamin E and selenium, the increases in frequencies of BNMN were statistically significant ($P<0.05$, $P<0.01$, respectively). In this study, we have observed that vitamin E and selenium decreased CCl_4-induced number micronuclei in sheep in vivo. The results presented from part of an investigation on the effect of carbon tetrachloride intoxication and the role of vitamin E and selenium in influencing this effect in sheep

Key words: vitamin E, selenium, carbon tetrachloride, micronuclei, sheep

INTRODUCTION

Aerobic metabolism entails the generation of reactive oxygen species. These include the superoxide anion radical, hydrogen peroxide, the hydroxyl radical, and singlet molecular oxygen. Under physiological conditions, about 3% to 10% of molecular oxygen seems to be converted to reactive oxygen species (Chance at al.,1979). Moreover, they originate in diverse biological processes such as inflammation, carcinogenesis, aging and radiation damage. Most but not all reactive oxygen species rapidly react with a variety of molecules and thereby interfere with cellular functions. Reactions producing free radicals have been shown to lead to the breakage of chromosomes (Emerit,1982). It has been suggested that hydroperoxyicosatetraenoic acids represent major components of the clastogenic factor (Ochi and Cerutti, 1987), indicating that hydroperoxide metabolism is potentially important in chromosome breakage.

Carbon tetrachloride (CCl_4) is one of the most commonly used toxins in the experimental study of liver disease (Edwards et al.,1993). The effects of CCl_4 are attributed to its metabolism by cytochrome P450, especially by P450 2E1. According to Castillo et al. (1992) the toxic trichloromethyl radicals can react with polyunsaturated fatty acids or other cellular targets, and can act as free radical initiators. This can result directly or indirectly in damage to lipids, nucleic acids, and other molecules (Johnston and Kroening,1998).

One of the most attractive approaches to disease prevention involves the use of antioxidants to protect tissues against toxic and carcinogenic injuries and degenerative diseases. The most widely studied protective agents are vitamin E, vitamin C, vitamin A, carotenoids and others. Because these compounds directly scavenge reactive oxidants, they are hypothesized to constitute a vital endogenous defense against oxidative cell and tissue injury caused by toxic and carcinogenic chemicals (Ames,1983, Diplock et al.,1991).

In the present study we attempted to investigate the protective effects of pretreatment with vitamin E and selenium to reduce CCl_4-induced micronuclei in sheep *in vivo*. CCl_4 is also a well-known carcinogen (Johnston and Kroening, 1998) and most chemical carcinogens are known to be mutagens (Ames and McCann,1976). Our principal presumption was that vitamin E and selenium are generally associated with decreased cytogenetic damage.

In recent years the use of the micronucleus technique has come into the limelight as a practical endpoint for measuring the physical effects of ionizing radiation or chemical agents both *in vivo* and *in vitro* (Almássy et al.,1987). The *in vivo* micronucleus test allows an effective assessment of both chromosomal damage and chromosome loss induced by chemicals. Additionally, it provides the advantage of taking metabolism into account as the genotoxicity of a substance actually results from the dynamic balance between its enzymatic activation and its enzymatic detoxification.

MATERIAL AND METHODS

Ten healthy non-pregnant Merino sheep, 3-4 years old and weighing 57 to 63 kg, were used in this experiment. Concentrates for sheep (BAK) were given and water and meadow hay were provided ad libitum. The sheep were randomly divided into two groups. The first group was injected with vitamin E - 400-mg s.c. as tocopheryl acetate and selenium -12 mg as sodium selenite (Vitamin E and selenium, Bremer Pharma, GmbH, Bremerhaven, Germany). Nothing was given to the second group. After 24 hours both groups were dosed orally with 0.05 ml/kg b. w. carbon tetrachloride (Mikrochem s r.o. Bratislava, Slovakia) via a stomach tube into the rumen.

Peripheral blood was taken from the jugular vein before any treatment (control 1 and control 2) and then on the sixteenth day following the administration of vitamin E and selenium (experiment 1 and experiment 2). Lymphocyte cultures were prepared by adding 0.5 ml of heparinized whole blood from healthy donors to 5 ml of chromosome medium RPMI 1640 supplemented with L-glutamine, 15

μM/l HEPES (Sigma), 15% fetal calf serum (BOFES, Workplace for Special culture Sera, Brno, Czech Republic), antibiotics (penicillin 250 U/ml and streptomycin 250 μg/ml) and phytohaemagglutinin (PHA,180 μg/ml, Welcome, Darford, England). Lymphocyte cultures were incubated at 37° C for 72 h. Cytochalasin B (Cyt. B, Sigma, St. Louis, MO, USA) was added at 44 h after initiation of the culture, at a concentration of 6 μg/ml.

Slides for the MN test were stained with 5% Giemsa (Merck, Darmstadt, Germany) in Sörensen phosphate puffer (pH 6.8) for 15 min.

For the identification of MN the published criteria were applied (Countryman and Heddle, 1976). The induction of MN was evaluated by scoring a total of 1000 binucleated (BN) cells per donor. Micronuclei (MN) in binucleated cells containing one MN, two MN and three micronuclei were scored in a total number of 1000 CB. The MN yields can be attributed to cytogenetic damage.

For statistical evaluation of the results we used the X^2 test for BNMN. Student's test was performed to compare the differences between the pretreated donors with vitamin E and selenium and those treated with CCl_4 only.

RESULTS AND DISCUSSION

One hour after CCl_4 exposure some animals became anorexic with muscular fasciculation, convulsions and with increased respiratory rate. During the three hour trial all sheep recovered clinically.

The data obtained after pretreatment with vitamin E and selenium and subsequent administration of carbon tetrachloride are shown in Table 1. The frequencies of BNMN were increased but mainly without attaining statistical significance ($P>0.05$) as only donor number four showed statistical significance ($P<0.05$).

Table 1. Induction of micronuclei in sheep exposed to CCl_4 after pretreatment with vitamin E and Se (experiment 1)

Donor	Before experiment I (control I)					After preteartment with vit.E, Se and subsequent exposition to CCl_4				
	BN	NNMN	Distrbution of MN in BN cells			BN	BNMN	Distrbution of MN in BN cells		
			1	2	3			1	2	3
1	1000	13	12	1		1000	24	21	3	
2	1000	14	13	1		1000	27	27		
3	1000	14	14			1000	26	26		
4	1000	14	12	2		1000	31*	30	1	
5	1000	18	18			1000	21	17	4	

BN-binucleated cells, BNMN-binucleated micronucleated cells
Statistical significance: $P>0.05$ (x^2 test)
*$P<0.05$

In control group 1 the spontaneous frequency of MN was 15.4 ± 1.49 per 1000 binucleated cells and 27.6 ± 2.8 after administration of vitamin E, selenium and CCl_4.

The results for the sheep treated only with CCl_4 are summarized in Table 2. Statistically significant increases in BNMN frequencies were seen in four treated sheep ($P<0.05$ or $P<0.01$, respectively).

Table 2. Induction of micronuclei in sheep exposed to CCl_4 in vivo (experiment 2)

Donor	Before experiment I (control I)					After preteartment with vut.E, Se and subsequent exposition to CCl_4				
	BN	NNMN	Distrbution of MN in BN cells			BN	BNMN	Distrbution of MN in BN cells		
			1	2	3			1	2	3
6	1000	13	12	1		1000	30*	30		
7	1000	15	15			1000	30*	29	1	
8	1000	14	13	1		1000	32*	29		3
9	1000	13	13			1000	28*	27	1	
10	1000	14	14			1000	24	22	2	

BN-binucleated cells, BNMN-binucleated micronucleated cells
Statistical significance *$P<0.05$, **$P<0.01$ (x^2 test)

In control group 2 the spontaneous frequency of MN was 14.2 ± 0.75 per 1000 binucleated cells and 30.8 ± 3.97 after administration of CCl_4.

Finally, the comparisons between differences in the MN frequencies in both experiments are presented in Figure 1. The sheep in the group pretreated with vitamin E and selenium and subsequently exposed to CCl_4 showed an induced MN rate 1.8-fold over the control group. When sheep were exposed to CCl_4 in the absence of vitamin E and selenium, the frequencies of MN were 2.1-fold more than in the control group. Thus, we observed that pretreatment of sheep with vitamin E and selenium decreased CCl_4-induced micronuclei, but without statistical significance ($P>0.05$, Student t' test).

Vitamins and trace elements give some antioxidant activities of central importance in protection against free radical attack. Both vitamin E and selenium have fundamental and complementary roles in the prevention of oxidative damage. They protect the cell from the detrimental effects of peroxidation but each has its own mechanism. Thus, vitamin E is present in the membrane components of the cell and prevents free radical formation, while selenium functions throughout the cytoplasm to destroy peroxides (Milad and Kováč,1998). In addition to the above, selenium has been shown to be an integral component of the enzyme glutathione GSH-Px glutathione hydrogen oxidoreductase EC 1.11.1.9 (Rotruck et al.,1973). Each mole of this enzyme contains 4 g-atoms of selenium (Flohe et al.,1973).

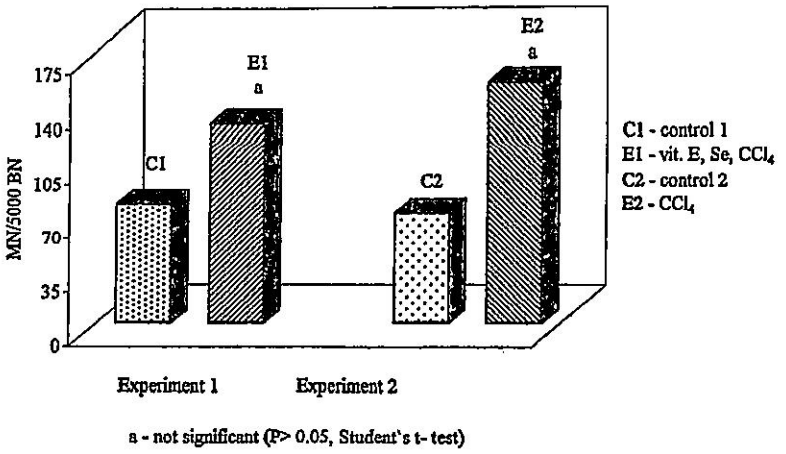

Figure 1. Effect of vitamin E and selenium on CCl_4-induced micronuclei in shee

Vitamin E has been shown to be effective in preventing chromosomal breakage induced by carcinogens (Carney et al.,1991) and to act also as a nitrite scavenger and inhibit actions of nitroso compounds (Shamberger et al., l973). Similarly, vitamin E has also been shown to reduce the carcinogenicity of methylbenzylnitrosamine (Odeleye et al.,1992), dimethylbenzanthracene (Shamberger, 1970) and methylcholanthrene (Haber et al.,1962).

In vitro studies have shown that tocopherol inhibits growth and/or induces morphological differentiation in several types of cells, including murine neuroblastoma (Helson et al.,1983) and rat glioma (Prasad et al.,1980).

The mechanism(s) whereby vitamin E inhibits tumor cell proliferation remain unclear but a well-characterized function for vitamin E is its free-radical scavenging and antioxidant effect (Odeleye et al.,1991).

Grosse et al. (1997) reported that pretreatment of mice by vitamin E decreased DNA adducts by 80 % in kidney. The DNA adducts were detected in mice after a single administration of ochratoxin A, produced by Penicillium viridicatum and Aspergillus ochraceus.

Selenium is an essential trace element for animals that protects cells from oxidation and from the adverse effects of some heavy metals. Recently, there has been growing interest in selenium as a naturally occurring substance with the capacity to exhibit potential antimutagenic and/or anticarcinogenic properties (Shamberger, 1985). Thus, Jacobs et al. (1978) in Salmonella and Ray et al. (1978) and Shamberger et al.(1973) in human lymphocyte cultures have shown that sodium selenite acts as an antimutagen if is applied together with some known mutagen.

In this study, we have observed that pretreatment of sheep with vitamin E and selenium decreased CCl_4-induced micronuclei. These results confirmed those obtained by Köteles (1993) demonstrating that micronuclei induced by X-irradiation were reduced by two compounds of selenium (sodium selenite and selenium dioxide). The results presented are a part of an investigation on the effect of carbon tetrachloride intoxication and role of vitamin E and selenium in influencing this effect in sheep.

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UTICAJ TRETMANA VITAMINOM E I SELENOM NA UGLJEN TETRAHLORIDOM INDUKOVANA MIKROJEDRA KOD OVACA *IN VIVO*

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SADRŽAJ

Ugljen-tetrahlorid (CCl_4) je dobro poznat karcinogen a slobodni radikali, koji nastaju njegovom metaboličkom razgradnjom reaguju sa različitim ćelijskim komponentima i mogu dovesti do prekida hromozoma. Kao što je poznato, Vitamin E i selen imaju bitnu ulogu u prevenciji oštecenja oksidacionim sredstvima. Kod ovaca prethodno tretiranih vitaminom E i selenom, kojima je zatim sondom aplikovan ugljen tetrahlorid, bila je povećana frekvencija pojave dvo-

jedarnih limfocita sa mikrojedrima, ali ova razlika nije bila statistički značajna u odnosu na vrednosti dobijene pre početka ogleda. Kad su ovce bile tretirane ugljn-tetrahloridom bez premdikacije vitaminom E i selenom, frekvencija pojave limfocita sa mikrojedrima je bila statistički značajno veća ($P<0.05$, $P<0.01$) u odnosu na vrednosti pre početka ogleda.U ovom radu je dokazano da vitamin E i selen smanjuju broj mikrojedara indukovanih ugljen tetrahloridom u limfocitima ovaca u *in vivo* uslovima.