The effect of long-term high heavy metal intake on lipid peroxidation of gastrointestinal tissue in sheep

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ABSTRACT: The present study was designed to determine the effects of dietary heavy metal intake on malondialdehyde levels (MDA, product of cellular lipid peroxidation) in the tissues of gastrointestinal tract, kidneys and liver of sheep. The experiment was carried out on twelve female sheep of mixed breed, weighing from 30 to 35 kg, one year old. The first group of sheep was bred in the conditions of laboratory farm. They had free access to hay and 300 g of barley per sheep was added daily. The second group grazed on a natural pasture in the environs of a factory processing copper ore. The second group of sheep had significantly higher concentrations of copper and cadmium in the tissues of rumen wall, duodenum, colon wall, liver and kidneys. The concentration of lead was significantly higher in the rumen wall, colon, liver and kidneys, but no differences were found in duodenum. The concentration of mercury was significantly higher only in the kidney tissue, but no differences were found in rumen wall, duodenum, colon wall and liver. The concentration of malondialdehyde was found significantly higher in reticulum epithelium, omasum, duodenum and colon. The significantly higher concentration of MDA was measured in colon wall and kidney medulla. The highest concentration of MDA was found in liver. Our results confirm that lipid peroxidation is one of the molecular mechanisms of cell injury in chronic heavy metal poisoning. The epithelium of the gastrointestinal tract of sheep is more sensitive to the oxidative damage induced by dietary heavy metal intake.

Keywords: malondialdehyde; thiobarbituric acid; reticulorumen; intestine

Lipid peroxidation is a well-established mechanism of cellular injury in animals and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxidation degrades polysaturated fatty acids of cell membranes with consequent disruption of membranes. Strubelt et al. (1996) studied the toxic effect of cadmium, mercury and copper using the isolated perfused rat liver. All metals caused similar changes in various parameters used to describe general toxicity but only copper significantly increased the tissue malondialdehyde content. Lipid peroxidation is induced by Cu-overload in liver of sheep (Sansinanea et al., 1997). Cadmium induced histopathological changes and lipid peroxidation in the liver and kidneys of rodents (El-Maraghy et al., 2001; Wlostowski et al., 2003). Lipid peroxidation measured in the human hepatic line increased as the $HgCl_2$ concentration and time of exposure increased (Bucio et al., 1999). A simple intraperitoneal injection of $Hg^{(2+)}$ in mouse increased hepatic and renal TBARS (thiobarbituric acid reactive substances) levels in 12 and 48 h after treatment (Farina et al., 2003). Occupational exposure to elemental mercury leads to increased lipid peroxidation in erythrocytes in humans (Bulat et al., 1998). Lipid peroxidation is one of the molecular mechanisms of cell injury in acute CH_3HgCl poisoning (Lin et al., 1996). Parenteral administration of mercuric chloride to rats enhanced lipid peroxidation in liver, kidneys, lung, testis and serum, but not in heart, spleen or muscle in rats (Huang et al., 1996) and in mice (Mahboob et al., 2001).

Supported by the Science and Technology Assistance Agency of Slovak Republic (Grant APVT No. 51012602).

The purpose of this study was: (1) to determine the distribution of mercury, copper, cadmium and lead in the tissues of the gastrointestinal tract of sheep after long-term intake of a contaminated diet and (2) to determine the effect of food intake contaminated by heavy metals on lipid peroxidation in the tissues of the gastrointestinal tract in sheep.

MATERIAL AND METHODS

Animals and diet. The experiment was carried out on twelve female sheep of mixed breed, weighing from 30 to 35 kg, one year old. The animals were divided into two groups. Six sheep of the first group were from a laboratory farm and were fed hay *ad libitum* and 300 g of barley per sheep/day and they had free access to water. The second group grazed on a natural pasture in the environs of a factory processing copper ore.

Experimental procedure. Sheep were stunned with a captive bold gun, exsanguinated, and the visceral organs were removed. The reticulorumen complex was emptied and rinsed with cold tap water to remove digesta and then rinsed with cold saline. Rumen, reticulum, omasum and abomasum tissues were identified and the epithelium was separated from underlying musculature. Small and large intestines were separated from the mesentery and rinsed with cold saline. Two sections from duodenum, ileum, caecum and colon were removed. The cut section was laid on a glass plate and the tissue was scraped with a glass slide to remove the epithelium. The kidneys were divided into cortex and medulla. Total and epithelial pieces (approximately 5 g) from all removed segments of the digestive tract were frozen in liquid nitrogen and stored (–70°C) until analysed.

Sample analysis. The lipid peroxide concentration was determined by the method measuring the amount of thiobarbituric acid (TBA) reactivity by the amount of malondialdehyde (MDA) formed during acid hydrolysis of the lipid peroxide compound. Thiobarbituric acid-reactive substances (TBARS) in tissues of digestive tract, kidneys and liver were measured using a modification of the method of Uchiyama and Mihara (1978). Small pieces of the whole wall of rumen, reticulum, omasum, abomasum, duodenum, ileum, caecum, colon and the epithelium of the same parts and kidney cortex, kidney medulla and liver were thawed and homogenized with cold 1.15 mol KCl to make a 10 % (w : v) homogenate. In a fresh tube, 0.5 ml of this homogenate were combined with 3 ml of 1 mol phosphoric acid and 1 ml of 0.6 % thiobarbituric acid solution. This mixture was heated in a boiling water bath for 45 min and then cooled in cold water for 10 minutes. After cooling 4 ml of n-butanol was added. The mixture was vortexed and centrifuged at 2000 rpm for 10 min to separate the phases. The absorbance of butanol phase was measured at 520 and 535 nm on a spectrophotometer SPECOL 11 and the difference was used to quantify MDA. 1.1.3.3. tetraethoxypropane was treated in the same manner as the samples and used as external standard curve and the level of lipid peroxides was expressed as micromoles MDA per gram of protein.

Protein concentration in homogenate was measured by the method of Bradford (1976), using BSA as a standard.

The concentration of mercury was determined by atomic absorption spectrophotometry (AMA-254), copper was determined by flame atomic absorption spectrophotometry (AAS UNICAM 939), cadmium and lead were determined by graphite-cell atomic absorption spectrophotometry (AAS UNICAM 939 QZ) and the values are expressed in μg/kg of tissue.

Statistical analysis. Statistical analysis of the differences was carried out using unpaired Student's *t*-test. The results are expressed as mean values ± S.E.M.

RESULTS

The concentrations of mercury, copper, cadmium and lead in the tissue of rumen wall, duodenum, colon wall, liver and kidneys are summarised in Table 1.

The second group of sheep, which consumed natural grass, had significantly higher concentrations of copper and cadmium in the tissue of rumen wall, duodenum, colon wall, liver and kidneys. The concentration of lead was significantly higher in the rumen wall, colon, liver and kidneys, but no differences were found in the duodenum. The concentration of mercury was significantly higher only in the kidney tissue, but no differences were found in rumen wall, duodenum, colon wall and liver.

The largest differences between the first and second group were found in the concentration of cadmium which was 12 times higher in rumen wall, 10 times higher in duodenum, 13 times higher in colon

Table 1. Concentrations of mercury, copper, cadmium and lead in the tissues of sheep from a laboratory farm (first group) and from an exposed area (second group). Each value represents the mean ± S.E.M; $n = 6$

Tissue	Elements (μg/kg)	First group	Second group	$P <$
Rumen	Hg	1.10 ± 0.057	1.03 ± 0.033	NS
	Cu	955.0 ± 26.5	2 711.0 ± 57.74	0.001
	Cd	6.66 ± 1.76	83.0 ± 1.15	0.001
	Pb	49.33 ± 5.54	119.7 ± 4.91	0.001
Duodenum	Hg	1.03 ± 0.033	1.06 ± 0.066	NS
	Cu	788.7 ± 98.46	2 620.0 ± 3.18	0.001
	Cd	12.33 ± 4.66	121.0 ± 0.57	0.001
	Pb	48.33 ± 5.17	42.67 ± 0.88	NS
Colon	Hg	1.10 ± 0.057	1.03 ± 0.033	NS
	Cu	493.0 ± 30.66	2 240.0 ± 11.84	0.001
	Cd	7.0 ± 1.52	93.0 ± 1.52	0.001
	Pb	43.33 ± 7.42	92.33 ± 0.88	0.01
Liver	Hg	2.33 ± 1.33	3.66 ± 0.33	NS
	Cu	76 980.0 ± 5 909.0	108 000 ± 5 581.0	0.05
	Cd	84.33 ± 19.77	434.7 ± 109.9	0.05
	Pb	83.33 ± 8.19	360.3 ± 51.96	0.01
Kidneys	Hg	2.33 ± 0.33	20.33 ± 1.76	0.001
	Cu	3 281.0 ± 164.3	6 555.0 ± 282.3	0.001
	Cd	282.7 ± 75.03	596.0 ± 66.96	0.05
	Pb	115.7 ± 33.18	564.3 ± 12.99	0.001

NS = not significant

in the second group than in the first group. Smaller differences were found in liver (5 times) and kidneys (twice). A larger difference was also observed in the concentration of mercury in kidneys (9 times). Differences in the other elements were only from 1.5 to 4.5 times higher in the second group.

Table 2 shows MDA levels in tissue homogenates from sheep from the laboratory farm (first group) and from the exposed area (second group). Significantly higher values were found in the epithelia of reticulum, omasum, duodenum and colon, in colon wall, kidney medulla and liver in the second group of sheep. The highest concentration of MDA was found in liver.

DISCUSSION

The present study provides evidence of oxidative damage to the gastrointestinal tract tissue in sheep after one-year intake of pasturage contaminated by heavy metal emissions. Numerous evidences support the hypothesis that acute or chronic heavy metal toxicity is mediated primarily via the generation of injurious free radical species in various tissues. In the kidneys of rats this process causes increased lipid peroxidation (Xiao et al., 2002). Tandon et al. (2003) reported a higher level of MDA in blood, liver and brain in cadmium intoxicated rats. The changes in oxidant defence systems associated with cadmium exposure could increase the steady-state level of oxidants in the testis. Moreover, ROS are produced in cells continuously as a by-product of mitochondrial and microsomal electron transport and other metabolic processes (Hales, 2002). Metals are known to influence the oxidative status of sea organisms. Cadmium increased cellular activities such as MDA production in the clam *Ruditapes decussatus* (Geret et al., 2002). A significant increase in the level of substances reac-

Table 2. Malondialdehyde levels in tissue homogenates of sheep from a laboratory farm (first group) and from an exposed area (second group). Each value represents the mean ± S.E.M; $n = 6$

Tissue	First group	Second group	$P <$
	(μmol MDA/g protein)		
Rumen epithelium	8.03 ± 2.04	9.56 ± 4.79	NS
Rumen wall	3.23 ± 0.27	3.33 ± 1.59	NS
Reticulum epithelium	3.53 ± 2.03	9.16 ± 0.83	0.05
Reticulum wall	7.23 ± 0.83	10.0 ± 1.44	NS
Omasum epithelium	8.76 ± 1.45	12.9 ± 0.43	0.05
Omasum wall	6.71 ± 1.24	11.7 ± 2.27	NS
Abomasum epithelium	7.38 ± 0.94	9.20 ± 0.78	NS
Abomasum wall	6.58 ± 0.96	7.6 ± 1.92	NS
Duodenum epithelium	14.0 ± 4.05	27.5 ± 1.95	0.05
Duodenum wall	3.17 ± 0.51	4.43 ± 0.86	NS
Ileum epithelium	7.33 ± 2.6	7.03 ± 0.29	NS
Ileum wall	6.15 ± 1.14	9.05 ± 1.58	NS
Caecum epithelium	11.2 ± 3.71	11.8 ± 1.9	NS
Caecum wall	5.3 ± 2.8	5.2 ± 1.6	NS
Colon epithelium	2.44 ± 0.24	6.71 ± 1.71	0.05
Colon wall	1.50 ± 0.61	7.11 ± 0.94	0.01
Kidney cortex	15.4 ± 1.8	14.1 ± 1.21	NS
Kidney medulla	9.12 ± 1.44	15.5 ± 1.36	0.01
Liver	14.9 ± 1.64	48.3 ± 4.42	0.001

NS = not significant

tive to thiobarbituric acid was measured in liver of *Gymnogeophagus gymnogenys* living in the area with a high concentration of total mercury and chromium in the sediment and interstitial water in the Feitoria rivers in Brazil (Tagliari et al., 2004).

Long-term occupational exposure to mercury is one of the risk factors for increased lipid peroxidation in miners (Kobal et al., 2004). The prevention of lipid peroxidation is essential for all aerobic organisms, and so the organism is well equipped with antioxidants that directly or indirectly protect cells against the adverse effects of xenobiotics, carcinogens and toxic radicals. Production of reactive oxygen species such as superoxide ion, hydroxyl radicals and hydrogen peroxide is one of the toxic effects of metal ions. These reactive oxygen species promote lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA and membrane damage, altered gene expression and apoptosis (Stohs et al., 2000). Lipid peroxidation may be a direct consequence of membrane damage (Casalino et al., 1997) or of weakened antioxidant defence (Jamall and Sprowls, 1987).

In conclusion, our results confirm that lipid peroxidation is one of the molecular mechanisms of cell injury in chronic heavy metal poisoning. Our results do not indicate which element measured by us had a higher toxic effect on epithelial cells. We presented total toxic effects of contaminated pasture on the health of animals living in the industrial area.

Acknowledgements

The authors gratefully acknowledge Zuzka Makarova's skilled technical assistance.

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Received: 05–01–28
Accepted: 05–09–09

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