Effect of high and sustained zinc supplements on trace element metabolism in sheep

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ABSTRACT

Three groups of 2th Romney wethers were given 0, 12g ZnO fortnight^{-1} and 12g ZnO week^{-1} respectively for 18 months. Plasma trace element levels were monitored regularly over this period. At the end of the trial the animals were slaughtered and various organs, muscle, skin and wool were removed and analysed for a wide range of elements and for metallothionein.

Zinc treatment resulted in significantly elevated Zn in plasma, red blood cells, liver, kidney, intestine and pancreas. Copper concentrations in liver (272 ± 44 v 471 ± 45 μg g^{-1} dry weight) and kidney (17 ± 0.8 v 24 ± 1.6μg g^{-1} dry weight) were significantly ($P<0.001$) increased in the weekly Zn treatment group compared with the control group. Plasma Cu levels were not affected. Metallothionein levels in livers from the weekly treatment group were increased 10-20 fold over those from the control group (3.6 ± 0.9 and 0.26 ± 0.1 mg g^{-1} dry weight respectively). In the weekly treatment group 76% of the total liver Cu and Zn was bound to MT compared with 10% in control group livers.

Keywords Zinc, copper, trace element metabolism, sheep, metallothionein, liver, tissues.

INTRODUCTION

High doses of Zn (> 10 mg/kg/day), as ZnO, have been recommended as a method to prevent or reduce the severity of facial eczema in sheep (Smith *et al.*, 1977). However, excess Zn purportedly induces Cu deficiency and influences the metabolism of other trace elements, for example, Fe (O'Dell, 1989). Aspects of the Zn-Cu antagonism, both in absorption and storage of Cu, are mediated via Zn-induced metallothionein (Mills, 1974). In ruminants, the effects on Cu status depend on the nature, level and chemical form of Zn administration, and its duration. Increased Zn intakes have been observed to decrease Cu plasma levels in cattle (Ivan and Grieve, 1976; Towers *et al.*, 1981), and decrease liver Cu (Ott *et al.*, 1966; Bremner *et al.*, 1976). Other studies have shown no effect on liver Cu levels but markedly increased Cu concentrations in the kidney (Allen and Masters, 1985; Allan *et al.*, 1986). In the latter studies Zn toxicity effects were also observed. Increasing Zn intakes 2-fold (40-70 mg/day), that is within a range normally encountered by the grazing sheep, had no effect on Cu levels in plasma or soft tissues (Grace and Lee, 1990).

As part of a larger study on Zn metabolism and wool growth in sheep, tissues from a group of grazing animals which had been regularly dosed with ZnO for a sustained period (12-18 months), were analysed for a wide range of elements. This paper reports on the effect of Zn on Cu levels in plasma, liver and kidney, induction of metallothionein, and on its interaction with other elements in various tissues.

EXPERIMENTAL

Three groups of 14 month old Romney wethers (initial average liveweight, 55 kg) each of 15 sheep, were given 0, 12g ZnO fortnight^{-1} and 12g ZnO week^{-1} respectively for 18 months. The Zn was administered in gelatin capsules using a balling gun. All the animals were grazed together on a white clover/ryegrass pasture and weighed at fortnightly intervals. Blood samples were taken over short periods during the first and last weeks of the study. Wool from a midside patch was collected monthly.

At the end of the experiment the animals were killed with an overdose of sodium pentobarbital. The liver, pancreas, heart, kidneys, small intestine, hind limb muscle and associated skin patch were quickly removed, dissected, and sub-samples washed 3 x in physiological saline, frozen immediately in liquid

nitrogen and stored at -85°C prior to analysis.

Elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) in the tissues were analysed in duplicate by plasma emission spectrometry as previously described (Lee, 1983; Grace and Lee, 1990). Total metallothionein (MT) in liver sample extracts was isolated and purified on a Sephadex G-75 column using a Tris-HCl, pH 8.6 buffer (Mulder *et al.*, 1990). Total MT was estimated on the basis of its Cu and Zn content.

RESULTS

Growth Rates

There were no significant differences in growth rates among the control and Zn treated animals. A marked seasonal growth pattern was observed. Little change in liveweight occurred between February and September (mean, 55 kg), with an increase over the period October to December of 280 gd^{-1}, after which there was again little change in liveweight (mean, 76 kg).

No significant differences in (liver and kidney) weights were found among the treatment groups.

Elemental Plasma Concentrations

Changes in mean plasma Zn concentrations over a 6 day period immediately after dosing are shown in Figure 1, for periods at the beginning and near the end of the experiment for sheep from the weekly treatment group. These are compared with plasma Zn concentrations in control sheep which showed no change over the same period. Plasma Zn increased rapidly over the first 8 hours subsequent to the dose being administered, then declined approximately linearly over the next 3 days. However, while at the start of the experiment plasma levels in the weekly group reached a maximum 5-fold higher than basal levels (2.65 ± 0.15 µg ml^{-1}) before falling back to a level similar to the control group, 18 months later this increase was only 2-fold, with levels falling back to a significantly higher level than the control group. This indicates an adaptive effect over the trial period. Also notable were the large variations in plasma Zn levels of individual sheep compared with the control group. This variation was markedly reduced over the treatment period.

No significant changes to plasma Cu levels (nor any other of the elements measured) occurred subsequent to the Zn dosing.

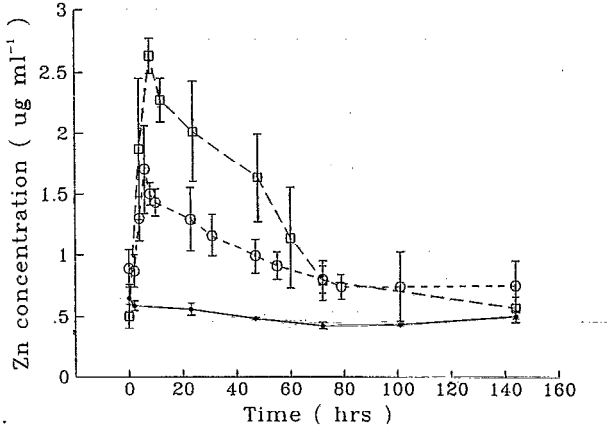

FIG 1 Mean zinc concentration in plasma from Romney wethers over 6 days after dosing with 12g ZnO in gelatin capsules compared with an untreated control group. Treatment: (□) weekly Zn group at start of experiment, February 1989 (n=3); (○) weekly Zn group 18 months later, July, 1990 (n=3), (●) control group, (n=4).

TABLE 1 Mean (± s.e.) Zn and Cu concentrations in tissues (µg g^{-1} dry wt) and in plasma (µg ml^{-1}) from sheep not dosed with Zn (C), and dosed with 12g ZnO $fortnight^{-1}$ (F) and with 12g ZnO $week^{-1}$ (W).

Tissue		Zn		Cu	
Liver	C	114 ± 7		272 ± 44	
	F	190 ± 13	***	279 ± 28	ns
	W	234 ± 17	***	471 ± 45	**
Kidney	C	96 ± 3		17 ± 0.8	
	F	267 ± 5	***	18 ± 1.4	ns
	W	390 ± 58	***	24 ± 1.6	***
Plasma	C	0.56 ± 0.1		1.14 ± 0.1	
	F	0.91 ± 0.08	*	1.00 ± 0.1	ns
	W	1.06 ± 0.18	*	1.04 ± 0.1	ns

ns = not significant; * = P<0.05; ** = P<0.01; *** = P<0.001.

Elemental Tissue Concentrations

Table 1 gives mean Zn and Cu concentrations in liver, kidney and plasma from each treatment group. Zinc

concentrations were significantly increased in all three tissues (2 to 3 fold) from both Zn treated groups compared with the control group. Copper concentrations were significantly ($P<0.001$) increased in the liver and kidney, but only in those tissues from the weekly treatment group.

TABLE 2 Mean (± s.e) Zn concentrations in various tissues ($\mu g\ g^{-1}$ dry wt) from sheep not dosed with Zn (C), and dosed with 12g ZnO fortnight^{-1} (F), and with 12g ZnO week^{-1} (W).

		Zn	
Wool	C	114 ± 6	
	F	109 ± 4	ns
	W	142 ± 12	ns
Skin	C	35 ± 3	
	F	33 ± 2.5	ns
	W	26 ± 1.3	*
Red Blood Cells	C	3.4 ± 0.2	
	F	4.3 ± 0.2	**
	W	5.2 ± 0.2	**
Muscle	C	69 ± 4	
	F	81 ± 4	*
	W	83 ± 4	*
Small Intestine	C	72 ± 3	
	F	97 ± 6	**
	W	141 ± 15	***
Pancreas	C	63 ± 9	
	F	330 ± 25	***
	W	466 ± 101	***
Heart	C	52 ± 3	
	F	62 ± 1.5	**
	W	62 ± 3	**

ns = not significant; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

Zinc concentrations of a number of other tissues from the treatment groups were also increased. These are shown in Table 2. In small intestine and pancreatic tissue especially, Zn concentrations were markedly increased in both the fortnightly and weekly treatment groups, while in heart, muscle and red blood cell smaller, but significant, increases were measured. Although Zn levels in wool from the weekly group were higher than that of the control group (142 ± 12 and 114 ± 6 $\mu g\ g^{-1}$ respectively), this change was not significant. A small but significant ($P<0.05$) decrease in Zn concentration of skin from animals in the weekly group was measured.

Although the main effect of Zn dosing on tissue element concentrations was on Cu and Zn itself, a number of interactions of Zn on other elements were also observed. These are summarized in terms of their level of significance in Table 3. Notable are the large number of highly significant positive correlations of Zn on other elements (especially Fe, Mg, Mn, P and S) in muscle, skin and heart tissue. Apart from the expected, large and positive interaction of Zn on Cu in liver and kidney, of interest also are the highly significant ($P<0.001$) Zn/S positive correlations in kidney, skin and wool.

TABLE 3 Correlation matrix of significant interactions between Zn concentrations and other elements in various tissues of sheep across all treatments (n=30).

Zn	Ca	Cu	Fe	K	Mg	Mn	Na	P	S
Liver		**		(-)**					
Kidney		***				(-)**			***
Muscle		*	***		***	**	*	*	*
Skin			**	***	***	***	*	***	***
Pancreas	*								*
Heart			***		***			***	***
Wool									***
Plasma									
RBC	*	*					***		
Small Intestine									

Much of the increased Cu content in liver and kidney tissue in response to the Zn dosing may be accounted for by increased synthesis of Cu-Zn containing metallothionein (MT) in these tissues. Preliminary data for levels of total MT in liver tissue of sheep from the control and weekly treatment groups is given in Table 4 and shows more than a 10-fold increase for animals in the weekly group. Also given is the amount of Zn and Cu calculated to be associated with liver-MT. Although individual animals in the weekly treatment group showed a large variation in the relative proportions of Zn and Cu

they incorporated in liver MT (data not shown), the total amounts of these metals associated with MT approximately accounts for their overall increase in liver tissue (Table 4).

DISCUSSION

In many previous studies investigating metabolic effects of Zn dosing to sheep, weight losses coupled with other toxicity symptoms have often been encountered when zinc doses have exceeded 20mg Zn kg^{-1} day^{-1}, or where dosing has been via oral drenching with $ZnSO_4$ (Ott *et al.*, 1966; Smith, 1977; Allen *et al.*, 1986). Treatments which result in liver Zn concentrations exceeding approximately 1500 μg g^{-1} dry weight are associated with kidney and pancreatic lesions, reduced organ weights and depressed Cu levels (Allen and Masters, 1985). Furthermore, excessive drenching stimulates closure of the reticular groove and may cause abomasitis (Allen *et al.*, 1986) with subsequent effects on metal ion transport processes.

TABLE 4 Mean (± s.e.) total metallothionein (MT) concentration in liver of sheep not dosed with Zn (C) and dosed with 12g ZnO $week^{-1}$ (W).

	C (n=2)	W (n=4)
Total MT (mg g^{-1} dry wt)	0.26 ± 0.1	3.6 ± 0.9
Total MT Cu, Zn (μg g^{-1})	40 ± 11	538 ± 135
(Cu, Zn) - MT as percentage of total Cu and Zn in liver	10	76

In this study, using ZnO in gelatin capsules, liver Zn concentrations did not exceed 300 μg g^{-1} dry weight at the highest dose rate. No impairment to animal performance was noted. Plasma Cu levels were unaffected by the Zn treatments, but both kidney and liver Cu levels were increased. Although changes to Cu absorption could not be measured directly, normal Cu plasma and small intestine tissue levels suggest that increased Zn intake did not suppress Cu transport. Rather, Cu accumulation in kidney and liver tissue may have occurred through either increased Cu absorption and/or decreased endogenous loss in association with enhanced metallothionein synthesis.

It is noteworthy that increased total Zn in digesta changes the speciation of metals in the available pool, and in particular, increases soluble Cu by inhibiting insoluble CuS formation (J. Lee, unpublished data).

It is now well established that Zn induces metallothionein (MT) protein synthesis in a range of species, including sheep (Bremner and Beattie, 1990). In this work, the increased MT measured in the liver of sheep from the weekly Zn treatment groups accounts for all the accumulated Zn and Cu in this tissue. Further work on the quantitative analysis for MT and its isoforms and for MT-mRNA on a range of tissues from the treatment groups is currently in progress. This will lead to a better understanding of how sheep respond to increased and sustained Zn intakes.

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