Short communication

Metal (molybdenum, copper) accumulation and retention in brain, pituitary and other organs of ammonium tetrathiomolybdate-treated sheep

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Ammonium tetrathiomolybdate (TTM) is the treatment of choice for chronic Cu poisoning in sheep and is recommended in Wilson's disease. However, the long-term effects have not been fully evaluated and some evidence questions the long-term safety of the drug. The aim of the present study was to investigate the systemic distribution and retention of Cu and Mo in TTM-treated sheep of different breeds and Cu status. Low-Cu Cambridge sheep were divided into a TTM trial group (3·4 mg/kg, subcutaneously, on three alternate days per month, for 5 months) and a control group, and were killed at the end of the course or 7 months later. High-Cu sheep consisting of a Cu-supplemented (150 mg/kg) Cambridge group and a North Ronaldsay group were administered TTM as before and compared with untreated controls. Brain, liver, kidney, heart, skeletal muscle, pituitary, adrenals, testes and ovaries were retained for metal analysis. Mo accumulated in all organs including brain and pituitary ($P < 0·02$) in all TTM trial groups and was retained after cessation of treatment, except in liver, kidney and skeletal muscle. Cu was increased ($P < 0·02$) and retained in the cerebellum and medulla oblongata in the TTM-treated high-Cu Cambridge groups. Brain Cu *v.* Mo concentrations showed a strongly positive correlation (r 0·7) in the high-Cu Ronaldsay group 7 months after TTM treatment. It is concluded that TTM is not all excreted but (Mo) is widely distributed and retained in many organs including brain and pituitary. In addition TTM may redistribute some displaced excess liver Cu (Cu-TTM) to the brain. The consequences of these disturbances await clarification.

Tetrathiomolybdate: Molybdenum: Copper: Toxicity

Ammonium tetrathiomolybdate (TTM) is used for the treatment of Cu poisoning in sheep, promoting rapid clearance of liver Cu by the biliary–faecal route (Mason *et al.* 1988). TTM has also been given to patients with penicillamine-intolerant Wilson's disease (Brewer *et al.* 1991). Recently, doubts were raised as to the long-term safety of the drug during the investigation of a field case of unthrifty, TTM-treated, Cu-poisoned Bleu de Maine sheep with suspected endocrinopathy. On subsequent necropsy 3 years later these sheep were shown to contain considerable excesses of Cu and Mo in their brains (Haywood *et al.* 1993; Dincer, 1994) and it was speculated that TTM had effected a redistribution of Cu (Cu-TTM) to the brain. It was decided to investigate the systemic distribution and retention of Cu and Mo in TTM-treated sheep of different breeds and Cu status.

Materials and methods

Experimental protocol

Three groups of twelve adult sheep (of either sex) were used in the trials: (1) low liver-Cu (< 400 μg/g) Cambridge sheep fed on a pelleted diet (11 mg Cu/kg) in addition to hay and water were equally divided into a trial group which received TTM

Abbreviations: TTM, ammonium tetrathiomolybdate.

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subcutaneously (3·4 mg/kg) on three alternate days (Humphries *et al.* 1988) per month for five successive months and a control group given subcutaneous saline at the appropriate time intervals. At the end of the fifth course of TTM treatment, three sheep from each group were killed and necropsied immediately. The remaining six animals (TTM trial and controls) were killed 7 months later. (2) High liver-Cu (> 500 μg/g) Cambridge sheep were given a controlled quantity of a diet containing 150 mg Cu/kg (BOCM Pauls Ltd, Bury St Edmunds, Suffolk, UK) for 2·5 months before the onset of the trial, plus hay and water. They were subsequently taken off the supplemented diet and divided into three groups of four animals, one group of which was killed immediately because of incipient haemolytic crisis and used as the control. The remaining sheep were given TTM (3·4 mg/kg on three alternate days per month for five successive months). Four were killed immediately after the course of treatment and the rest 4 months after TTM was discontinued. (3) High liver-Cu (> 500 μg/g) North Ronaldsay sheep maintained on hay and water alone were subdivided into a trial group, which received the five course TTM treatment, and a control group. Trial and control sheep (*n* 6) were killed immediately after TTM treatment and the remainder 7 months later.

Samples from liver, kidney, heart, skeletal muscle, pituitary, adrenals, ovaries, testes and brain regions (cerebrum, cerebellum, hypothalamus, medulla oblongata) were collected for metal analysis.

Metal analysis

Wet-ashed samples were prepared from dried tissues with concentrated HNO_3 and $HClO_4$ (2 : 1, v/v). Cu concentrations were measured using an IL 157 atomic absorption spectrophotometer (Instrumentation Laboratories Ltd, Wellington, Somerset, UK) and Mo in a graphite furnace atomic absorption spectrophotometer (Varian Instruments, Walton-on-Thames, Surrey, UK).

Results

Molybdenum distribution

Immediately after TTM treatment Mo concentrations increased ($P < 0·02$) in all tissue samples from the high-Cu Cambridge sheep and also in their low-Cu counterparts (results not shown). After discontinuation of TTM treatment (Table 1) Mo elevations in both high and low-Cu Cambridge sheep were maintained in the pituitary, brain, adrenals, heart and gonads but Mo had declined relatively in both liver and kidney and had fallen to control values in skeletal muscle. Pituitary Mo elevations were particularly marked at both time points ($P < 0·02$) in all trial groups.

Copper distribution

Mobilization of Cu in low-Cu Cambridge sheep was confined to minor renal elevations ($P < 0·02$) both immediately after TTM treatment and later. By contrast, in high-Cu Cambridge sheep immediately after TTM treatment, increases ($P < 0·02$) in kidney Cu were accompanied by a decline in liver Cu ($P < 0·02$) and elevated Cu in cerebellum and medulla oblongata ($P < 0·02$) (results not shown). After discontinuation of TTM treatment elevations in regional brain Cu were maintained (Table 1).

A comparable pattern of Mo and Cu redistribution was identified in the high-Cu North Ronaldsay sheep; moreover regional brain Cu concentrations plotted against equivalent

Table 1. Copper and molybdenum concentrations (μg/g dry weight) in organs of copper-supplemented (high-copper) and unsupplemented (low-copper) Cambridge sheep after cessation of treatment with ammonium tetrathiomolybdate (TTM)

(Values are means and standard deviations for three (low-copper) or four (high-copper) sheep; values in parentheses are for control non-TTM-treated sheep)

	Low-Cu group				High-Cu group			
	Cu		Mo		Cu		Mo	
Organ	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cerebrum	13·7	5·6	0·20**	0·02	15·0	1·8	0·09**	0·02
	(11·1)	(1·4)	(0·10)	(0·01)	(13·6)	(4·2)	(0·01)	(0·00)
Hypothalamus	17·0	1·8	0·12**	0·03	14·6	5·4	0·13**	0·04
	(17·1)	(3·6)	(0·04)	(0·02)	(12·9)	(4·5)	0·02	(0·01)
Cerebellum	18·3	6·1	0·11**	0·01	26·6**	5·9	0·12**	0·01
	(19·7)	(0·3)	(0·06)	(0·01)	(16·8)	(1·3)	(0·03)	(0·01)
Medulla oblongata	9·6	3·1	0·17**	0·04	14·1**	1·9	0·17**	0·07
	(12·5)	(2·9)	(0·08)	(0·01)	(9·4)	(0·2)	(0·05)	(0·02)
Pituitary	42·7	1·9	3·26**	0·89	109·7	24·2	3·26**	0·83
	(34·1)	(2·1)	(0·23)	(0·09)	(72·6)	(22·4)	(0·19)	(0·11)
Liver	127	35	1·56*	0·49	338**	146	6·52**	2·30
	(137)	(106)	(3·46)	(0·97)	(932)	(327)	(1·69)	(0·27)
Kidney	12·6*	0·7	1·56	0·68	33·6	11·9	8·95*	5·29
	(9·5)	(0·4)	(1·31)	(0·35)	(18·0)	(5·0)	(1·19)	(0·22)
Heart	20·5	1·4	0·15**	0·04	17·9	1·3	0·19**	0·01
	(18·4)	(2·3)	(0·05)	(0·00)	(18·3)	(0·4)	(0·05)	(0·01)
Skeletal muscle	4·3	0·2	0·02	0·01	4·3	0·7	0·02	0·01
	(5·0)	(0·9)	(0·02)	(0·00)	(3·5)	(0·6)	(0·03)	(0·01)
Adrenal	30·2	2·8	0·38**	0·07	79·5	19·4	1·16**	0·26
	(30·4)	(4·7)	(0·20)	(0·02)	(65·3)	(24·2)	(0·38)	(0·12)

Mean values were significantly different from those for the corresponding controls (given in parentheses): *$P < 0·05$, **$P < 0·02$.

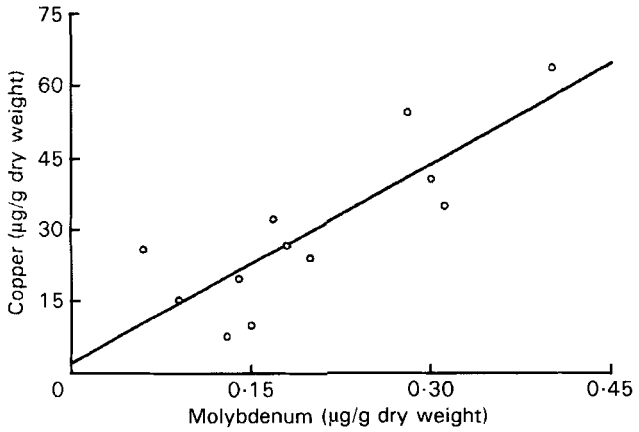

Fig. 1. Copper *v.* molybdenum correlation in different regions of the brains of North Ronaldsay sheep, 7 months after administration of ammonium tetrathiomolybdate (*r* 0·7).

Mo concentrations showed a strong positive correlation (*r* 0·7) 7 months after discontinuation of treatment (Fig. 1).

Discussion and conclusions

Systemic Mo retention after TTM treatment indicates that not all TTM is excreted as had been previously maintained (Mason *et al.* 1988). In particular the magnitude of pituitary Mo enhancement has parallels with earlier observations of reproductive disorders with pituitary luteinizing hormone dysfunction consequent on Mo supplementation to cattle (Phillippo *et al.* 1986).

Mobilization of Cu from the liver by TTM appears to depend on the initial liver-Cu status of the sheep and TTM had no effect on basal tissue Cu levels, confirming the findings of Phillippo *et al.* (1986). However, in sheep with liver Cu excess the rise in brain Cu after TTM administration, together with the strongly positive brain Cu *v.* Mo correlation suggests that some displaced liver Cu is redistributed to the brain in complexed form (Cu-TTM). This is in contrast with untreated Cu-poisoned sheep in which no rise in brain Cu occurs and supposes that Cu-TTM bypasses the blood–brain barrier as has been shown to occur with other similar non-polar lipophilic chelates such as diethyldithiocarbamate (Allain & Krari, 1991).

In conclusion, the present study has shown that Mo (TTM) is selectively distributed and retained by many organs, including the brain and pituitary which may be target organs. Second, the findings confirm that TTM does not deplete Cu from essential sites in sheep but support the contention that TTM may redistribute some excess liver Cu (Cu-TTM) to the brain, in which site it is retained. The consequences of these metal displacements are under investigation.

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