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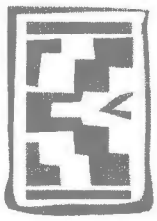

Attempted induction of chronic copper poisoning in boma confined impala

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ABSTRACT

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Induction of chronic copper poisoning in ten boma-confined impala was attempted in a randomized, single dose, parallel designed, titration study using five increasing oral doses, ranging between 125 mg/kg to 1000 mg/kg, of copper oxide needles. Two untreated impala were kept as controls. Impala ($n = 1$) from each treatment group were culled 52 d and 105 d after treatment and examined for tissue copper accumulation and signs of chronic copper poisoning. Despite the high doses of copper administered to the impala and liver copper concentrations above 150 ppm WM achieved in two animals, no clinical signs related to chronic copper poisoning were observed. Faecal copper concentrations indicated that the major portion of copper oxide particles was excreted in the faeces.

Keywords: Chronic poisoning, copper, experimental, impala

INTRODUCTION

Experimental chronic copper poisoning in domestic livestock is well documented, especially in sheep (Buckley & Tait 1981; Kumaratilake & Howell 1989), but also in cattle (Claypool, Adams, Pendell, Hartmann & Bone 1975; Suttle 1987b) and goats (Zervas, Nikolaou & Mantzios 1990). These studies were used to clarify and understand the pathogenesis of chronic copper poisoning. Various copper salts and different routes of administration have been used to induce chronic copper poisoning (Ishmael, Gopinath & Howell 1972; Allen & Mallinson 1984).

Copper oxide needles are high quality copper oxide wire particles (about 4 mm long and 0,6 mm in diameter) which are widely used in sheep and cattle to increase liver copper stores. It has been shown to alleviate hypocupraemia very effectively in cattle (Suttle 1987b) and sheep (Judson, Trengrove, Langman & Vandergraaff 1984; Suttle 1987a; Rogers & Poole 1988). The copper oxide needles lodge in the abomasal wall and in the acidic environment results in a sustained release of copper (Suttle 1981). Chronic copper poisoning has been induced with copper oxide needles in sheep administered oral doses of 200–400 mg/kg (Suttle 1987a). In another study, mortalities were reported in ewes when 0,8 g/kg copper oxide needles were administered (Judson, Brown, Gray, Dewey & Babidge 1984). In both trials, although faecal copper excretion was high, the remaining copper oxide particles in the abomasum were still able to increase liver copper concentration and induce chronic copper poisoning.

Chronic copper poisoning following administration of copper oxide needles has not been reported in cattle, even though high doses (36 g/100 kg) have been administered (Rogers & Poole 1988). A possible explanation given was that the copper oxide needles were initially retained within the rumen and were thus not exposed to the acidic environment of the abomasum. It has also been postulated that the retention rate of copper in the liver is higher in sheep than in cattle (Suttle 1987b). Furthermore, it appears that

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cattle have a better ability to excrete excess copper via the bile and therefore should be able to tolerate more copper originating from a sustained release source, such as copper oxide needles (Suttle 1987b).

The occurrence of chronic copper poisoning has been confirmed in impala in the Phalaborwa area of the Kruger National Park (KNP) (Gummow, Botha, Basson & Bastianello 1991; Grobler 1996). However, it is unknown what the critical copper concentration in the liver is for chronic copper poisoning to occur in impala. This information is important for the successful surveillance of airborne copper pollution within the KNP using impala as sentinels for monitoring of critical rates of exposure. Since impala have successfully been maintained in captivity (Knox, Hattingh & Raath 1990) a study was undertaken to induce chronic copper poisoning under conditions of boma-confinement. Copper oxide needles administered orally to impala were used to supply a continuous source of copper in an attempt to reduce unnecessary handling of the animals and thereby avoiding death due to capture or stress.

MATERIALS AND METHODS

Experimental animals

Twelve apparently healthy, free roaming impala rams, approximately 15 months of age, were chemically immobilized in the Skukuza area of the KNP. Immobiliation was achieved with a combination of 1,5 mg etorphine hydrochloride (HCl) ((M99, R&C Pharmaceuticals) and 10 mg xylazine (Rompun, Bayer SA) using a Telinject capture system (Knox *et al.* 1990). The immobilized animals were transported to the boma and prior to reversal of anaesthesia, individually weighed, identified, baseline blood samples and faecal samples collected, allocated to treatment groups and the various treatments administered. Anaesthesia was reversed by administering 3 mg diprenorphine HCl (M5050, R&C Pharmaceuticals) intravenously and yohimbine HCl (Yohimbine, Kyron) at 0,125 mg/kg, intramuscularly.

Allocation and treatment

The impala were randomly allocated to six parallel treatment groups of two animals each. In five of the groups the impala were dosed orally with copper oxide needles at doses of either 125, 250, 500, 750 or 1 000 mg/kg, while the animals in the remaining group were left untreated. The copper oxide needles were administered in gelatin capsules by means of a stomach tube to the impala at the time of capture, while the animals were under anaesthesia. The gelatin capsules were placed in the tip of the tube at the end inserted into the rumen. Animals within each group were identified with different coloured plastic collars and eartags. An anticoccidial, diclazuril (Vecoxan, Janssen), was dosed orally to each animal at a dose of 5 mg/kg. Venous blood samples to establish copper concentration, serum gamma-glutamyltransferase (GGT) activity and haematology baseline values, were collected from each animal into two 10-mℓ heparinized vacuum tubes (Vacutainer), two 10-mℓ plain glass tubes and one 5-mℓ EDTA vacuum tube, respectively. Plasma and serum samples collected after centrifugation of the blood samples were stored in labelled plastic vials at –8 °C until analyzed. A faecal sample for determination of baseline copper concentrations was also collected from the rectum of each impala.

Housing and feeding

All animals were housed in a single boma measuring 12 x 25 m. The animals were fed dried lucerne hay, and fresh branches of *Ziziphus mucronata, Grewia hexamita* and *Grewia monticola*, collected daily and supplied *ad libitum*. Water was always *ad libitum* in a cement water crib. Feed and water samples were collected every 2 weeks for analysis of copper, molybdenum and zinc concentration. The boma was cleaned twice weekly.

Faecal copper excretion

The animals were observed each morning and afternoon and fresh faecal samples were collected as soon as an animal was seen to defaecate. An aliquot of the faecal sample (20 g) was taken for copper analysis, while the remainder was scrutinized for the presence of copper oxide particles. Since it was practically not possible to collect faecal samples daily from each animal, at least one sample was collected for each impala per week. Faecal samples for copper analysis were dried in a force draft dry-oven (Memmert, Model 2260) at 60 °C for 24 h, labelled and stored in a cool, dry place before being submitted for analysis.

Necropsy

One animal from each group was culled on day 52 (day 0 is the day of treatment) using 1,1 mℓ (500 mg) saturated suxamethonium chloride (Scoline, Labethica). The second animal of each group was culled on day 105. Immediately after recumbency each animal was removed from the boma and the blood vessels of the neck severed with a sharp knife. Blood samples were collected at this time. During necropsy the entire gastro-intestinal tract was examined for copper oxide particle remnants according to a method described by Bang, Familton & Sykes (1990).

Liver, kidney and lung samples were collected for copper analysis. Samples of these organs, as well as samples from the spleen, adrenal glands, triceps muscle, lymph nodes of the small intestine, spinal cord, brain and testis were collected for histopatho-

logical examination. All tissue samples were preserved in a 10-% buffered formalin solution.

Analyses

Copper concentrations (organs and blood), were determined by atomic absorption spectrophotometry (Boyazoglu, Barrett, Young & Ebedes 1972). Wet mass (WM) copper concentration were determined for all organ analyses.

Haematological examination included analysis of haemoglobin concentration, haematocrit and red blood cell count. Serum samples were analyzed for GGT activity, using a Boehringer Mannheim kit, (France SA, CBR kits) according to the method described by the manufacturers (Persijn & Van der Slik 1976).

Faecal, feed and water samples were analyzed for copper content by atomic absorption spectrophotometry according to the method of the Perkin-Elmer Corporation (Norwalk, Connecticut, USA).

Statistical analysis

Statgraphics 4.0 programme (STSC, 1989, Statistical Graphics Corporation, Maryland, USA) was used for the statistical calculations. One-way analysis of variance was used to compare differences between the various treatment groups and untreated control.

RESULTS

All the impala adapted very well to the boma conditions and showed body mass increases of between 1 and 4 kg at the time of culling (Table 1). No mortalities or clinical signs associated with chronic copper poisoning were encountered during the experiment.

Tissue copper concentrations

Tissue copper concentrations of liver, kidney and lung samples collected on days 52 and 105 of the trial are presented in Table 1.

TABLE 1 Organ copper concentrations (ppm WM) from impala dosed with copper oxide needles at different rates and an untreated control group

Group	Day when culled*	Animal live mass		Cu 0 dose (g)	Copper concentrations (ppm WM)		
		Initial	Slaughter		Liver	Kidney	Lungs
Untreated Control	52 105	32,5 32,0	34,0 33,0	0,00 0,00	40,0 36,0	4,0 4,0	3,0 2,0
Mean		32,3	33,5	0,00	38,0[a]	4,0	2,5
125 mg/kg	52 105	38,5 32,0	41,0 33,0	4,75 4,00	68,0 75,0	3,0 4,0	2,0 2,0
Mean		35,2	37,0	4,38	71,5[a,b]	3,5	2,0
250 mg/kg	52 105	30,0 33,0	32,0 33,0	7,37 8,25	157,0 89,0	4,0 3,0	2,0 2,0
Mean		31,5	32,5	7,81	123,0[b]	3,5	2,0
500 mg/kg	52 105	35,5 32,0	37,0 33,0	17,75 16,00	118,0 70,0	4,0 4,0	3,0 3,0
Mean		33,7	35,0	16,88	94,0[b]	4,0	3,0
750 mg/kg	52 105	30,0 33,0	32,0 34,0	22,50 24,75	121,0 95,0	6,0 4,0	2,0 2,0
Mean		31,5	33,0	23,63	108,0[b]	5,0	2,0
1 000 mg/kg	52 105	32,0 27,0	32,0 29,0	32,00 27,00	169,0 148,0	6,0 9,0	3,0 3,0
Mean		29,5	30,5	30,50	158,5[b]	7,5	3,0

[a, b] Values with different superscripts are significantly different ($P \leq 0,05$)
* Day when culled refers to days after treatment

Liver copper concentrations were markedly higher in all impala receiving copper oxide needles compared to the control group and were similar in all impala receiving copper oxide needles at doses of 250 mg/kg and higher. A significantly higher ($P \leq 0,05$) mean liver copper concentration of 120,8 ± 26,8 ppm WM was observed in these treated impala ($n = 8$) in comparison to the 68,0–75,0 ppm WM and 36,0–40,0 ppm WM recorded in the animals ($n = 2$) treated at 125 mg/kg and untreated animals ($n = 2$), respectively. Lower liver concentrations were generally noted in impala culled on day 105 compared to day 52. There were no significant differences ($P > 0,05$) in kidney copper concentration between the treatment groups, with the highest concentrations found in the highest dose group. The kidney copper concentrations ranged between 3 and 9 ppm WM.

Lung copper concentrations remained very low in all experimental animals, with values ranging between 2 and 3 ppm WM.

Pathology

No macropathological lesions indicative of chronic copper poisoning were observed. Remnants of copper oxide needles, all fragmented, were found in the rumen and reticulum of three impala culled on day 52, all from the three high-dose groups (500, 750, 1 000 mg/kg). In one of the impala, dosed at 1 000 mg/kg, 1,76 g of copper oxide particles were recovered, representing 6,5 % of the original dose. In the 500 and 750 mg/kg groups, 1,2 g (7,5 %) and 1,35 g (6,0 %) of copper oxide particles were recovered, respectively. Minute fragments were observed in the abomasum and small intestine of one animal. No visible copper oxide particles could be found in any of the impala culled on day 105.

Apart from a few parasitic lesions, which included *Pneumostrongylus calcaratus,* in the lungs of some individual animals and a mild cholangitis caused by a *Stilezia* spp. in other animals, no other microscopic lesions could be detected in any of the organs sampled.

Plasma copper

No increase in plasma copper concentrations occurred in impala administered copper oxide needles relative to untreated impala (Table 2).

Plasma GGT activity

Plasma GGT activities remained within the normal range for the duration of the experiment (Table 2). GGT activities of treated impala ($n = 10$) ranged from 26–45 U/ℓ at the start of the trial, with a mean of 36,16 ± 5,44 U/ℓ. At the time of culling the activities measured between 29 and 52 U/ℓ, with a mean of 36,25 ± 6,23 U/ℓ. There were no significant differences ($P > 0,05$) between the results.

Haematological parameters

All haematological parameters remained within normal limits for the duration of the experiment.

Faecal copper excretion

Baseline faecal copper concentrations, collected from impala on day 0, ranged between 8 and 14 ppm,

TABLE 2 Clinical pathological results in impala dosed with copper oxide needles at different rates and an untreated control group

Group	Plasma copper concentrations (mmol/ℓ)		GGT activity (U/ℓ)	
	Initial	Slaughter	Initial	Slaughter
Untreated Control	13,6 16,2	14,2 18,6	30 26	37 33
125 mg/kg	12,0 14,4	12,8 15,2	35 37	31 43
250 mg/kg	11,2 15,8	16,8 14,2	41 45	36 38
500 mg/kg	15,4 14,6	16,4 15,8	32 39	33 31
750 mg/kg	13,8 15,2	13,2 18,8	32 38	37 35
1 000 mg/kg	14,4 11,8	14,0 12,6	42 37	52 29

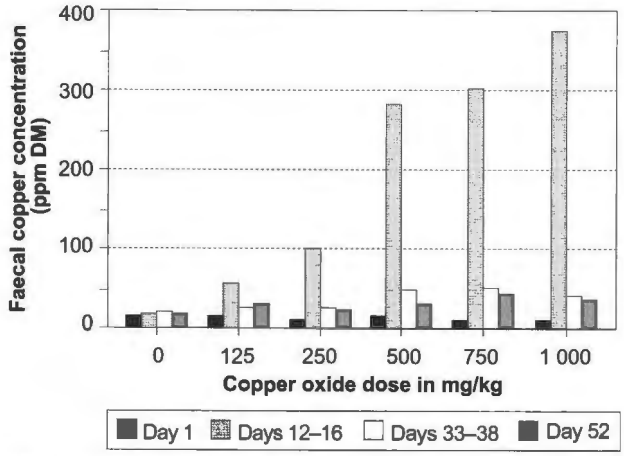

FIG. 1 Faecal copper concentration (ppm) DM in impala dosed with copper oxide needles at doses of 125 mg/kg to 1 000 mg/kg

with a mean of 11,47 ± 2,18. Faecal copper concentrations increased markedly from days 12–16 in all treated experimental groups, ranging from 68 ppm DM (125 mg/kg) to 400 ppm (1 000 mg/kg), with a mean of 204,9 ± 133,56 ppm DM. This was significantly higher ($P \leq 0,05$) than the faecal copper concentration of the two impala from the control group (14 and 20 ppm DM). Copper excretion decreased sharply from days 33–37, but was nevertheless still significantly higher than the control animals. At the end of the trial, faecal copper excretion in experimental impala was only slightly higher than control animals (Fig. 1). Fragments of copper oxide particles were found in faeces of six animals between days 9 and 17. Although in very small quantities, it appears that not all copper particles were retained in the alimentary tract, and some were lost via the faeces.

Copper in feed and water

Molybdenum, copper and zinc concentrations of the feed and water were within normal ranges for domestic stock (Suttle 1987a & b).

DISCUSSION

Copper oxide particles at all dose levels, were very effective in increasing liver copper stores in impala. Liver copper concentrations above 150 ppm WM are regarded as diagnostic for copper poisoning in cattle (Gummow *et al.* 1991) and sheep (Bath 1979). The same value was used as a diagnostic concentration to confirm and class the severity of microscopical pathology in suspected chronic copper poisoning cases in impala. This value is well above the normal mean liver copper concentrations of impala, i.e 26,9 ± 11,9 ppm WM reported by Boyazoglu *et al.* (1972).

Although liver copper concentrations exceeding 150 ppm WM occurred in some of the impala ($n = 2$), no clinical signs or indications of chronic copper poisoning were observed. Kidney copper concentrations also remained within normal limits. No cases of chronic copper poisoning were induced. Even at the highest of 1 g/kg (total dose of 32 g), no clinical signs or pathological lesions of chronic copper poisoning could be found.

According to Suttle (1987a), three factors influence hepatic copper storage after copper oxide administration. They are the duration of time that particles are retained in the gut, the fractional absorption of copper from the retained pool, and the excretion rate of copper from the liver. Suttle (1987a) also reported marked differences in faecal excretion patterns between sheep and cattle, as well as a lower efficiency of cattle in retaining copper from copper oxide particles in their livers. The authors further concluded that hepatotoxicity seems unlikely to occur when cattle are given a slow release source of copper, such as copper oxide needles, because of the greater ability of cattle to excrete any excess copper absorbed via the bile. This situation may be similar for impala.

A fairly large proportion of copper particles were excreted in the faeces of the experimental impala, especially in the three higher (500, 750, 1 000) dose groups. This was also reported in certain breeds of sheep, receiving a total dose of 20 g (0,4 g/kg) of copper oxide particles, however, clinical signs of chronic copper poisoning did occur in these cases (Suttle 1987a).

The faecal excretion rate of copper was rapid during the first 3 weeks of the trial, which is reflected by the high faecal copper concentrations. These high values represent mainly a loss of copper oxide particles, which were found in fragments in the faeces of six impala between days 9 through 17, as well as biliary excretion of absorbed copper. Faecal copper excretion decreased from day 37 onwards, but was still significantly higher ($P \leq 0,05$) than that of control impala. The remaining pool of particles in the gut was still sufficient to increase and maintain high liver copper concentrations. It appears that there is an optimum single dose of copper oxide needles needed to increase liver copper stores in impala (between 125 and 250 mg/kg) and that larger doses (500, 750, 1 000), will result in no additional increase in the liver copper stores (Fig. 1).

The bioavailability of copper is dependent on the prolonged retention of cupric oxide particles within the acid environment of the abomasum in sheep (Suttle 1987a). In the case of impala in this trial, it would appear that the main retention site in the gastrointestinal tract was the rumen and reticulum, which is similar to cattle (Suttle 1987b). In the three higher dosage groups copper oxide particles were

recovered from the rumen and reticulum of all animals, whereas only fragments were found in the abomasum of one impala.

The lung copper concentrations measured in the impala of all treated groups were below 3 ppm WM, and therefore similar to concentrations measured in impala culled within the KNP but outside the Phalaborwa copper mining air-pollution risk zones (Grobler 1996). There was no correlation between the liver and lung copper concentrations of impala receiving copper oxide needles at different dosage levels.

An alternative method will have to be considered to induce chronic copper poisoning in impala, such as consecutive six-weekly dosages of copper oxide needles, or daily feeding of copper-containing pelleted rations in conjunction with stressing the impala.

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