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Comparing the effects of treatment with ammonium molybdate versus ammonium molybdate and phenoxy-2-methyl-2-propionic acid on liver functions in natural copper poisoning of sheep

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ABSTRACT

Aim of the presented study is to compare the effects of treatment with ammonium molybdate versus ammonium molybdate and phenoxy-2-methyl-2-propionic acid on liver functions in natural copper poisoning of sheep and overall treatment responses in sheep naturally poisoned with copper (Cu). Study was conducted on 80 yearlings aging between 6–9 months. AM + PMPA group (n = 50) received ammonium molybdate and PMPA and AM group (n = 30) received only ammonium molybdate. First blood samples were collected before the treatments. PMPA was administered once daily intramuscularly at dose of 10 mg/kg for the first three days of the study to AM + PMPA group. AM + PMPA and AM groups both received ammonium molybdate two times with one week interval at dose of 1.34 mg/kg (1 cc per 10 kg BW, of %1.34 ammonium molybdate in saline solution) subcutaneously. Second blood samples were collected from all 80 animals on day 21 of the study. Cu levels were measured in a subgroup of randomly selected 9 (5 from AM + PMPA and 4 from AM group) animals on days 0 and 21 of the study. Mean Cu levels were 158.25 ± 14 μg/dl and 156.75 ± 9 μg/dlon day 0 and 129 ± 9 μg/dl and 154.5 ± 22 μg/dl in AM + PMPA and AM group respectively. AST levels decreased from 502 ± 67.2 IU/L to 168 ± 10.1 IU/L in AM + PMPA group ($P < 0.001$) and from 423 ± 71.1 IU/L to 202 ± 17.1 IU/L in AM group ($P = 0.005$) on day 21 of the study. GGT levels were 250 ± 24.2 IU/L and 248 ± 28.1 IU/L on day 0 and decreased to 160 ± 16.4I U/L and 166 ± 22.2 IU/L on day 21 in AM + PMPA and AM group with significance of $P = 0.001$ and $P = 0.037$ respectively. Two animals from AM group and one from AM + PMPA group died during the study period. Based on the more pronounced decrease in AST and GGT levels in AM + PMPA group we conclude that PMPA has beneficial effects on liver functions in chronic copper poisoning of sheep probably as a result of decreased lipid peroxidation in hepatocytes and/or increased Cu elimination by cholerectic effects of PMPA.

1. Introduction

Copper (Cu) plays a role in variety of vital enzymes such as cytochrome coxidase, tyrosinase, *p*-hydroxyphenyl pyruvatehydrolase, dopamine beta-hydroxylase, lysyl oxidase and copper-zinc superoxidase dismutase (Gaetke and Chow, 2003). Cu deficiency is clinically characterized by anemia, decreased milk yield, infertility, decreased wool quality in sheep and enzootic ataxia in lambs (Darrell et al., 2012). On the other hand Cu intake that exceeds the mobilization capacity of sheep results with Cu poisoning. Cu ingested with feed and water is absorbed from intestines by binding to amino acids and is carried to liver by bloodstream bond to albumin and transcupreine (Turnlund, 1998). Cu entering liver is used in enzymatic reactions, eliminated via bile or is stored in lysosomes binded to metallothionine. In case of excess Cu intake, Cu could not be carried to lysosomes, thus Cu levels in cytoplasm increases resulting with lipid oxidation and oxidative damage in hepatocytes and then erythrocytes (Britton, 1996; Fernandes et al., 1988; Gaetke and Chow, 2003). Clinical findings associated with mentioned cellular damage are hepatitis and anemia, icterus and haemoglobinuria associated with acute intravascular haemolysis (Radostits et al., 2007).

Metabolic elimination of Cu is slower and Cu absorbed tend to accumulate in the liver of sheep when compared to other species and thus sheep are the most susceptible species for Cu poisoning (Sommerville and Mason, 1978). Cu poisoning in sheep occurs

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mainly as primary Cu toxication caused by excess ingestion of Cu. Primary Cu poisoning could be caused by ingestion of feed stuff contaminated with Cu used in agriculture (Oruc et al., 2009), industrial wastage, feeding broiler or pig manure (Kerr and McGavin, 1991). Meanwhile, vitamin and mineral supplements formulated for other species containing high Cu levels could also result with Cu poisoning when fed to sheep (Navvarre et al., 2012; Radostits et al., 2007). Molybdenum, iron and sulfur decrease intestinal absorption of Cu, thus low levels of mentioned elements results with higher intestinal absorption of Cu (Hidiroglou et al., 1984; Moshtaghinia et al., 1989). Feeding sheep's in pasture with plants containing pyrrolyzidine alkaloids causes hepatitis and Cu accumulation in liver (Cheeke, 1988).

Molybdenum compounds are widely and efficiently used in treatment of Cu poisoning of sheep (García-Fernández et al., 1999; Hidiroglou et al., 1984; Humphries et al., 1988). D-penicillamine, sodium sulfate, sodium thiosulfate are other options that could be preferred for treatment of Cu toxication (Navvarre et al., 2012; Radostits et al., 2007). In case of haemolytic crysis prognosis of Cu posioning is guarded thus treating subclinical cases in prehaemolytic period is crucial for control of the poisoning. Ammonium molybdate and sodium thiosulfate are recommended for treatment of subclinical cases (García-Fernández et al., 1999; Hidiroglou et al., 1984; Humphries et al., 1988; Navvarre et al., 2012). Aspartate aminotransferase (AST) and Gamma-glutamyl transpeptidase (GGT) levels are indicators of hepatic damage and could be used for detection of subclinical animals in Cu poisoning; prophylactic treatment of animals with elevated blood AST and GGT levels in herds with Cu poisoning is strictly recommended (Ortolani et al., 2003).

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors involved in energy metabolism (Jia et al., 2015). Especially PPARα is responsible for catabolism of fatty acids in liver, heart, kidney and skeletal muscles (Harano et al., 2006). PPARα agonists decrease lipid oxidation and increase hepatocyte proliferation and regeneration in mouse liver (Bayly et al., 1994; Harano et al., 2006). Along with that PPARα agonists also enhance liver functions by increasing chloresis (Huber et al., 1997).

Aim of the presented study was to compare the effects of PPARα agonist phenoxy -2-methyl-2-propionic acid (PMPA) plus ammonium molybdate versus only ammonium molybdate treatment on liver functions in naturally occurring Cu poisoning.

2. Material and methods

The study was conducted in a flock with 350 sheep in which 6 yearlings between 6 and 9 months died due to a disease characterized with icterus, haemoglobinuria and anemia in last two weeks. Owner of the flock presented two sheep, one dead and one living to our clinic before our visit to the flock. Clinical examination of the living animal which was sick for three days revealed anemia, haemoglobinuria and icterus. Animal died at time of examination. Necropsy of the both animals revealed icterus, haemoglobinuria, pale and friable liver and gunmetal colored kidneys. Owner reported that only animals aged between 6–9 months fed in the same group suffered from the disease. Animals in that group were fed a vitamin mineral supplement formulated for cattle containing 800 mg Cu per kilogram. Flock had been previously vaccinated against leptospirosis and clostridial infections. Blood smear of animal was evaluated for babesiosis and anaplasmosis. According to the history and clinical findings combined with high serum copper concentration, disease was diagnosed as chronic copper poisoning.

Eighty yearlings aged between 6–9 months from the effected group, not showing any clinical signs were randomly selected for the study. Premix use was immediately ceased. Blood samples were collected from all 80 animals, on day 0 of the study before beginning of the treatments. Fifty yearlings were randomly assigned to AM + PMPA group which recieved ammonium molybdate and PMPA, other 30 were assigned to AM group which recieved only ammonium molybdate. PMPA (Live-R®, Vetas, Istanbul-Turkey) was administered once daily intramuscularly at dose of 10 mg/kg for the first three days of the study to AM + PMPA group. AM + PMPA and AM groups both received ammonium molybdate two times with one week interval at dose of 1.34 mg/kg (1 cc per 10 kg BW, of %1.34 ammonium molybdate in saline solution) subcutaneously. Second blood samples were collected from all 80 animals on day 21 of the study.

Serum AST and GGT levels of samples collected before and on day 21 of treatment were measured by using an automated analyzer (Reflotron™, Roche® Diagnostics, Mannheim, Germany). Serum Cu levels of totally 9 animals; 5 from AM + PMPA and 4 from AM group on day 0 and 21 of the study were determined by using Pye Unicam 2900® Atomic Absorption Spectrophotometer equipped with a PU9090® data graphics system.

Statistical analysis of the results was performed using Sigma Plot 12® software (Systat Software Inc., USA). Normality test was performed using Shapiro-Wilk test and the data were found to be normally distributed. Serum AST and GGT levels between the days of the study were compared using repeated measures analysis of variance (RM ANOVA). Students *t*-test was performed for comparison AST and GGT levels between groups in the same day of the study. For all analyses, $P \leq 0.05$ was considered significant.

3. Results

Anemia, yellowish brown mucous membranes and conjunctivae and haemoglobinuria were the main findings in one animal examined before the beginning of the study and three animals that died during the study period after initiation of the treatment. Body temperatures of mentioned cases were normal. Serum AST was measured >2000 IU/L, GGT 561 IU/L and serum Cu 324 μg/dl in the animal that died before beginning of the study. Three animals died during the study period, one from AM + PMPA group and two from AM group. GGT levels of animals that died were 428 IU/L, 76 IU/L, 106 IU/L and AST levels 357 IU/L, 231 IU/L, 200 IU/L respectively. No deaths related with copper toxicity were reported after the 21. day of the study. Mean Cu concentrations of 9 animals were 240 ± 82.7 μg/dl at the beginning of the study and although not statistically significant ($P = 0.092$) mean Cu concentrations decreased to 140.6 ± 10.7 μg/dl on day 21 of the study. Serum Cu concentration of one animal from AM + PMPA group was 900 μg/dl on day 0 of the study. Mean serum Cu levels of animals was 157.5 ± 7.8 μg/dl when the animal with extremely high Cu level was excluded (Table 1). Mean Cu concentrations did not differ in AM group on day 21, however although not statistically significant Cu levels decreased from 158 ± 14 μg/dl on day 0–129 ± 9 μg/dl on day 21 of the study. In fact serum Cu levels increased in two animals in AM group, on the other hand serum Cu decreased in all animals in AM + PMPA group (Table 1).

As shown in Table 2, statistical significance of decrease in serum AST and GGT levels was higher in AM + PMPA ($P < 0.001$ and $P = 0.001$ for AST and GGT respectively) group when compared to AM group ($P = 0.005$ and $P = 0.037$ for AST and GGT respectively).

4. Discussion

Chronic poisoning occurs in sheep with daily intake of 3.5 mg/kg BW of Cu. However with deficiency of molybdenum and/or sulphate doses as low as 15 ppm in the diet could result with chronic