STUDIES ON EFFICACY OF COPPER GLYCINATE IN CLINICAL BABESIOSIS IN CROSSBRED CATTLE

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INTRODUCTION

Bovine babesiosis, a tick borne infectious haemoprotozoan disease of cattle, has been reported to be prevalent in Europe, Africa, South America and Asia including India[1,2]. The disease is of immense importance in tropical and subtropical countries including India, causing heavy mortality and morbidity in exotic and crossbred animals. A large number of babesicidal compounds have been used effectively for treatment of bovine babesiosis[1]. Copper glycinate has been extensively used for the effective treatment of post-parturient haemoglobinuria (PPH) as well as for the treatment of certain copper responsive primary or secondary copper deficiency conditions[3,4]. However, results of preliminary clinical observations revealed its efficacy against bovine babesiosis[5].

The haemoglobinuria in bovine babesiosis has been ascribed to increased intravascular destruction of red cells by the parasites with the release of free haemoglobin in urine[6]. It was reported that the degree of anaemia in clinical babesiosis was out of proportion to the degree of parasitaemia which could indicate that intravascular haemolysis was not only of parasitized red cells but also of some unparasitized red cells[5]. The present investigation was carried out to confirm the preliminary observations of the efficacy of copper glycinate against clinical bovine babesiosis and to elucidate the role of lipid peroxidation in the genesis of haemolysis in bovine babesiosis by measuring the levels of malondialdehyde in the red blood cells, plasma and urine of affected and healthy cattle. Malondialdehyde, being one of the product, is a sensitive indicator of the lipid peroxidation.

MATERIALS AND METHODS

Animals

Forty-six clinical cases of bovine babesiosis were included in the present investigation. All the affected cattle were over 3 years of age and the disease was having no relationship with advanced pregnancy or recent parturition. The diagnosis of babesiosis was based on characteristic clinical findings to be subsequently confirmed on blood smear examination.

Sampling Procedure

Blood samples were collected at the peak of febrile reaction in heparinized glass vials by jugular venepuncture along with the urine samples from all clinical cases of bovine babesiosis. Subsequently, blood and urine samples were also collected 48 hrs after clinical recovery. Blood and urine samples from eight healthy crossbred cattle confirmed to be negative for blood protozoan infection were collected for establishing base values.

Analysis Procedure

Fresh blood smears fixed in methanol were stained with Giemsa's stain for detection of Babesia spp. Haematological parameters, viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total leucocytic count (TLC) were determined immediately with the help of Erma blood cell counter (Japan, Model PC-604). The blood samples were also stained with new methylene blue stain for detection of Heinz bodies[7]. Washed red blood cells, plasma and urine samples, collected from 16 of the affected cattle at the peak of febrile reaction and subsequently 48 hrs after clinical recovery were used for estimation of malondialdehyde[8]. The results of the haemato-biochemical and urinary analysis of the babesiosis affected crossbred cattle before and after treatment were compared with the control values established from eight healthy crossbred cattle. The data were statistically analysed.

Treatment Procedure

Single dose of copper glycinate @ 1.5 mg/kg body weight, to a maximum of 500 mg dissolved in 540 ml of normal saline was administered by slow intravenous drip at the peak of febrile reaction in cattle tentatively diagnosed to be affected with clinical babesiosis.

RESULTS AND DISCUSSION

All the affected crossbred cattle exhibited characteristic clinical symptoms of babesiosis characterised by acute onset of high fever ranging between 105° to 107°F, acute dullness, depression, inappetance associated with passing of brownish red to chocolate coloured urine. All the present cases were recorded between April to September, 1991 and the animals were moderately to heavily infested with ticks mostly of Boophilus spp. Freshly prepared blood smears stained with Giemsa's stain revealed moderate to severe degree of parasitaemia with detection of mostly intraerythrocytic Babesia bigemina. The characteristic clinical findings observed in the present investigation were similar to those described earlier[1,2]. The disease was subsequently confirmed by blood smear examination.

Haematological analysis revealed severe degree of anaemia reflected by significant ($P<0.05$) decline in mean Hb (5.23±0.58 g/dl), PCV (16.88±0.90%) and TEC (2.42±0.31x10^6/mm^3) values as compared to respective mean values of 11.64±0.24 g/dl, 34.22±0.69% and 7.46±0.25x10^6/mm^3 recorded in healthy control group. Comparable haematological alterations in bovine babesiosis has been recorded earlier[2,5]. The average MCV and MCHC values in affected cases were 61.77±3.62 fl and 36.10±1.72% as compared to respective values of 50.02±0.96 fl and 31.13±0.91% recorded in healthy control group, which revealed regenerative macrocytic normochromic anaemia with extensive poikilocytosis similar to that described by Jain and Randhawa et al.[5,9]. The average TLC in affected animals was within normal range. The degree of anaemia recorded in the present investigation was out of proportion to the degree of parasitaemia which supported the earlier observations that intravascular haemolysis was not only of parasitized red cells but also of some unparasitized red cells.

The malondialdehyde levels (MDA) in red blood cells,plasma and urine of affected and healthy crossbred cattle are presented in Table 1.

The malondialdehyde levels in red blood cells, plasma and urine of diseased cattle were significantly increased ($P < 0.05$) than those recorded in healthy controls. Mean MDA content in red blood cells of affected cattle was about two times higher than that of healthy controls,whereas, plasma and urinary malondialdehyde contents in diseased cattle was approximately six and nine times higher than that of healthy controls (Table 1).

Although MDA levels in red blood cells, plasma and urine of healthy and postparturient haemoglobinuric buffaloes have been reported earlier[10], but the present investigation is the first report of measurement of MDA levels in red blood cells, plasma and urine of healthy and babesiosis affected cattle.

Significant increase in MDA levels recorded in the present investigation confirmed that the mechanism of intravascular haemolysis of red blood cells involved a high degree of lipid peroxidation. Significant increase in plasma malondialdehyde concentration was also recorded in experimentally induced Babesia equi infection in splenectomized donkey at and beyond 5-15 per cent parasitaemia[11].

Table 1. Malondialdehyde levels in red blood cells, plasma and urine (n moles/ml) in healthy crossbred cows and babesiosis affected cows before and after therapy with copper glycinate (Mean±S.E.)

Parameters	Healthy crossbred cows	Bovine babesiosis	
		Before therapy	After clinical recovery
Red Blood Cells	196.72±9.40	450.64±56.70*	237.60±12.50
Plasma	2.32±0.29	14.82±2.33*	4.06±1.26
Urine	2.39±0.18	22.64±3.02*	5.50±0.84*

*$P < 0.05$

Lipid peroxidation is considered to be involved in the genesis of haemolytic anaemia in disorders of glutathione metabolism, thallasaemia, primaquine toxicity and vitamin E and glucose-6-phosphate dehydrogenase enzyme deficiency in human beings[12]. Significant haemolysis has been ascribed to the severity of MDA production. In the present investigation also, significant rise in MDA levels in red blood cells, plasma and urine samples indicated the involvement of lipid peroxidation in the genesis of haemolytic crisis besides the direct effect of the intraerythrocytic parasite on the red cell membrane.

Heinz bodies were detected in all babesiosis affected animals; their number being variable between 1 to 7 per cent of erythrocytes. D.Chiu et al. reported that superoxide radicals which are normally produced in small amounts by auto-oxidation of Hb and in large amounts under oxidant stress, if not destroyed could result in inactivation of vital enzymes, denaturation of Hb, production of Heinz bodies and peroxidation of red cell membrane lipids with subsequent haemolysis. Detoxification of these oxidants is carried by several enzymatic viz. superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, glutathione peroxidase and glutathione reductase as well as non-enzymatic antioxidant mechanisms, viz. reduced glutathione, vitamin E and C[12]. Copper is associated with

activity of superoxide dismutase, a copper metalloenzyme, protecting red cells from deleterious effects of superoxide anions. The increased activity of this enzyme following treatment could prevent subsequent haemolysis.

It was postulated that either increased intraerythrocytic concentration of copper ions attained following single intravenous administration of the drug or due to its effect on the Babesia organisms during the extraerythrocytic phase could have accounted for babesicidal effect of copper glycinate as observed in the present investigation. Ceruloplasmin, a copper containing serum protein, is a major antioxidant component of serum. Cerulplasmin has been shown to inhibit Cu^{++} induced lysis of rat erythrocytes[13]. In vitro studies revealed that ceruloplasmin binds to erythrocytic membrane, presumably at a ceruloplasmin receptor[14]. It was proposed that increased ceruloplasmin concentration following copper glycinate administration could prevent copper induced lysis in association with increased activity of superoxide dismutase enzyme. It was also postulated that the intraerythrocytic concentration of copper ions attained following intravenous administration of copper glycinate at the dose rate followed in the present investigation might not induce lysis of erythrocytes. This hypothesis was supported by the clinical observations that haemoglobinuria associated with babesiosis disappeared within 12-24 hrs following infusion of copper glycinate. Thus, the proposed mechanism accounting for babesicidal effect of the drug along with its protective effect on erythrocytic membrane needs confirmation.

The clinical recovery in all the treated cases was recorded within 12-24 hrs after single infusion; the majority of the cases (over 75%) responding by 12 hr. Fresh blood smears examined after 48 hrs of clinical recovery revealed absence of Babesia bigemina. Haematological parameters following clinical recovery reflected significant (P 0.05) improvement in mean Hb (7.74±0.99 g/dl), TFC ($4.12\pm0.41\times10^6/mm^3$) and PCV (24.55±1.28%) values, but were still below the levels recorded in healthy controls. Malondialdehyde levels in red blood cells, plasma and urine following clinical recovery revealed a parallel decline but the values were still above the levels recorded in healthy crossbred cattle.

Thus, it is concluded from the present investigation that a single intravenous administration of copper glycinate @ 1.5 mg/kg body weight to a maximum dosage of 500 mg resulted in complete sterilization of Babesia bigemina organisms from the blood and was highly effective for the treatment of clinical babesiosis in bovines.

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SUMMARY

The efficacy of copper glycinate in the treatment of clinical babesiosis was tested in 46 crossbred cattle. After laboratory confirmation of babesiosis, on the detection of Babesia bigemina in blood smears, all the animals were injected intravenously with a single dose of copper glycinate administered @ 1.5 mg/kg body weight dissolved in 540 ml of normal saline. The affected animals exhibited characteristic clinical sings of babesiosis. However, following single treatment with copper glycinate, all the animals showed complete clinical recovery reflected by normal temperature, improvement in appetite and general condition of the animals with absence of haemoglobin in urine within 12-24 hrs of infusion. Blood smears examined after 48 hrs of the clinical recovery revealed absence of parasites in the erythrocytes. Haematological studies revealed severe degree of anaemia which improved following recovery. Malondialdehyde levels were significantly increased in red blood cells, plasma and urine of babesiosis affected cows as compared to healthy control which returned towards base values following clinical recovery. It was postulated that copper glycinate exerted babesicidal effect and might also be providing protection to red blood cells against oxidative stress through antioxidant mechanisms.

RESUME

La efficacite' de glycinate de cuiver dans la treatment de babesiasis clinique etait teste' dans 46 animaux croise'. Apre's la confirmation de, babesiosis à la labotoire et en de'couvrir le Babesia bigemina dans le sang, tous les animaux etait injecte' intravenousment @ 1.5 mg/kg poids de animaux, dissondre' dans 540 ml saline normale. Tous les animaux effecte' montre' recourvrement complet appre's la treatment de glycinate de cuiver, c'est a dire, il ya la progre's d'appetit et en condition

generale des animaux sans trouven le haemoglobin dans la urine pen dans 12-24 heurs de infusion. Le examen de enduire de sang aprés 48 heurs de recouvrement dans la labotoire revéle, la absence de parasite en erytheocytee. Le etude Haematalogical, revéle la anémie severe, qui a montre la progréss aprés recouvrement.

Le niveau de malondialdehyde etait significatif haut dans sang et plasma et en urine de animaux effecte par babesiosis en comparaison des animaux normales. Il etait postulante que glycinate due cuiver déployé de effect babesicidal et aussi-grade le cels rouge contre accent oxidative par mechanism antioxidant.