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Improved Efficacy of Cotrimoxazole with Medicinal Synthetic Aluminum-Magnesium Silicate for Effective Treatment of Trypanosomosis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The mechanism of Cotrimoxazole (Sulfamethoxazole and Trimethoprim combination) as an anti-bacterial drug is inhibition of Folic Acid but its anti-trypanocidal efficacy has not been investigated though Trypanosomes also require Folic Acid for replication. Development of resistance by Trypanosomes against drugs, frequent relapse infections and toxicity of most trypanocides demand that the search for new Trypanocidal drugs be continuous. Aluminum

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magnesium silicate (AMS) and its formulation named Medicinal Synthetic AMS (MSAMS) have been reported to stabilize other medicines. Stabilizing medicines improves the length of time they remain at high bioavailability and when drugs remain at high concentration in plasma for a long time, their efficacies improve. Improving the anti-folic acid efficacy of Cotrimoxazole may deny Trypanosomes Folic Acid to the extent that their replication could be inhibited.

Study Design: Fifteen West African Dwarf (WAD) sheep allotted to 3 groups of 5 animals each were used. One group was infected and treated, the second group was infected and untreated while the third group was uninfected and untreated.

Place and Duration of Study: the study was conducted in the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike/ for a period of 7 months.

Methodology: Five (5) Trypanosoma b. brucei infected sheep (WAD) were treated with Cotrimoxazole MSAMS formulation at dose of 120mg/kg orally for 5 days while another group was left untreated.

Results: Mean parasitaemia of 80.49±7.56 million parasite per ml of blood was recorded just before commencement of the treatment but nine days after treatment with the Cotrimoxazole-MSAMS formulation the parasitaemia of the treated group reduced (P≤ 0.05) from 2.25±1.50 in the control to 0.00± 0.00). Cotrimoxazole-MSAMS terminated parasitaemia in Trypanosoma b. brucei infected WAD sheep 9 days post-treatment. Relapse infection was not observed up to 100 days post-treatment.

Conclusion: MSAMS enhanced the anti-folic acid efficacy of Cotrimoxazole so that it cleared Trypanosome parasitaemia and there was no relapse.

Keywords: Efficacy; cotrimoxazole; medicinal synthetic aluminum magnesium silicate; trypanosomosis; West African Dwarf Sheep.

1. INTRODUCTION

Co-trimoxazole is a well-established compound that is extensively used for various indications in countries with limited resources, offering an additional option in the battle against many pathogens, owing to its low cost, acceptable toxicity profile, availability by both oral and intravenous routes, and bactericidal activity [1]. Cotrimoxazole inhibits synthesis of folic acid which is important in replication of bacteria, protozoa, and fungi; it has been used to prevent several opportunistic infections among HIV/AIDS patients [2, 3]. Co-trimoxazole is a combination of two antimicrobial drugs (sulfamethoxazole and trimethoprim) in a fixed dose that covers a variety of bacterial, fungal, and protozoan infections [4]. Besides its use in the treatment of bacterial diseases, co-trimoxazole has been used successfully in the treatment of some fungal diseases as well as protozoal diseases including malaria (*Plasmodium falciparum and P. vivax*) [5, 6], *Isospora belli* especially in immunosuppressed HIV patients [7], *Acanthamoeba* [8], *Cyclospora* [7] and Toxoplasmosis [9, 10]. Its use in treatment of trypanosomosis was tried recently and only resulted in a 54 % reduction of parasitaemia in mice [11].

Co-trimoxazole is a product of the potentiation of sulfamethoxazole with trimethoprim. The potentiation results in lowering the quantity of sulfonamides required to inhibit specific susceptible bacteria and reduce toxicity and microbial resistance [12,13].

Sulfamethoxazole is a sulfonamide drug and works by inhibiting synthesis of the intermediary dihydrofolic acid from its precursors [14]. Trimethoprim is a diaminopyrimidine compound and works by competitively inhibiting dihydrofolate reductase and, consequently, the production of tetrahydrofolic acid from dihydrofolic acid [15].

Oral administration of Cotrimoxazole have certain advantages over intravenous (IV) or intramuscular (IM) injection. Intramuscular injection of trimethoprim often causes considerable tissue irritation, depending on the formulation used [16], besides economic reasons. On the other hand, the bioavailability of trimethoprim after oral administration is extremely low in ruminants, mainly as a result of chemical reduction by the ruminal microflora and fauna [14].

Use of Cotrimoxazole alone to treat *Trypanosoma brucei* infected mice significantly (P≤0.05) reduced trypanosome parasitaemia but could not terminate infection; but, the same dose of the drug in formulation with Medicinal

Synthetic Aluminum magnesium silicate (MSAMS) cleared trypanosome parasitaemia 2 days post-treatment in the mice [11]. Aluminum magnesium silicate (AMS) and its formulation MSAMS have been reported to stabilize other medicines [17, 18]. Stabilizing medicines improves the length of time they remain at high bioavailability; when drugs remain at high concentration in plasma for a long time, their efficacies improve [17, 19].

Medicinal synthetic aluminum magnesium silicate (MSAMS) is a formulation of aluminum magnesium silicate (a nanoparticle) incorporated with dextrose monohydrate (simple sugar) to convey the electrically charged *nanoparticles* across mucous membranes into blood-circulation by active transport, since aluminum magnesium silicate is not absorbable [20-22]. Aluminum Magnesium silicate is a naturally occurring mineral obtained from silicate ores of the montmorillonite group [23] and consists of Nanoparticles which have negative electrical charges on their surfaces and positive charges on their edges [22]. Aluminum magnesium silicate has been synthesized from aluminum silicate ($Al_4(SiO_4)_3$) and magnesium silicate (Mg_2SiO_4), which are two other medicinal minerals found in many countries including Nigeria, since Nigeria does not have deposit of natural Aluminum magnesium silicate (AMS) [24]. Aluminum silicate and magnesium silicate are also medicines that are already being used for treatments [25]. Aluminum silicate $Al_4(SiO_4)_3$ and magnesium silicate Mg_2SiO_4 are reacted to form the synthetic and purer form of aluminum magnesium silicate, $Al_2Mg_3(SiO_4)_3$: $\{Al_4(SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3(SiO_4)_3\}$ [26].

Dextrose monohydrate was formulated with the Medicinal Synthetic AMS (MSAMS), to carry its molecules, by active transport [20], across mucous membranes of the gastro-intestinal tract, into blood which carries them to all organs/tissues and to all body systems.

As a nanomedicine, Aluminum-magnesium silicate also enhances the delivery of drugs to their desired targets [27]. Aluminum magnesium silicate is a stabilizing and potentiating agent [17, 18, 22]. By stabilizing drugs, Aluminum Magnesium Silicate increases the potency of drugs [17]. Stabilizing drugs protects them against rapid degradation by metabolic processes thus prolonging time of their high bioavailability. AMS prolongs the bioavailability of drugs that are in its formulations and prolonging bioavailability improves the efficacy of antimicrobials [17]. Since it is found to potentiate drugs, use of Aluminum Magnesium Silicate may reduce drug dosages and thus save cost, especially in veterinary practice [17, 28]. Potentiated drugs may also be useful in handling drug resistant micro-organisms.

Trypanosomosis is a debilitating and often fatal disease of man and animals, resulting from infection with pathogenic protozoan parasites of the genus *Trypanosoma* [29]. The disease occurs majorly in sub Saharan Africa, between latitude 14^0N and 29^0S [30]. Human infections with trypanosomes are called Human African Trypanosomosis (HAT) while animal infections are known as African Animal Trypanosomosis (AAT). These infections occur more in the rural areas [31]. In Africa, Trypanosomosis is of great significance to both human health and animal production [32]. Several trypanosome species cause diseases in animals including *Trypanosoma brucei brucei, T. congolense* and *T. vivax* [33, 34]. Livestocks are susceptible to infection with one or more of these Trypanosoma species [33]. The disease, in the absence of treatment, causes high morbidity, mortality and infertility and can occur as acute and/or chronic forms [35].

Transmission of trypanosomes is by inoculation of infective metacyclic stages of the parasite into blood vessels of susceptible hosts during blood meals by tsetse (*Glossina spp)*; mechanical tansmission can occur when biting flies begin a blood meal on an infected host and end it on another one so long as the interval between the two meals is short enough to ensure survival of parasites in the insect mouthparts [36]. Infection with *T. vivax* is also possible, mechanically, through biting flies like *Tabanids* (horse flies) and *Stomoxys* (stable flies) as well as by vampire bats. Non-tsetse transmitted *T. vivax* infection in cattle is common in some parts of Africa including Chad, Sudan and some regions of Ethiopia [37]. *Trypanosoma congolense* has been reported to be also transmitted mechanically under experimental conditions [38] implying that its mechanical mode of transmission might have contributed to its spread in Africa [39]. Goats have been described as reservoir hosts of *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense,* the causative agents of human trypanosomosis and are transmissible to humans from goats [40].

Anaemia is a major consequence of African Animal Trypanosomosis [41]; and together with other systemic lesions, can contribute to death through eventual congestive heart failure [42]. Other clinical signs of AAT include intermittent fever, lymphadenopathy, splenomegaly, ataxia, lethargy, progressive weight loss, oedema, immunosuppression, abortion, and a decrease in milk production. Haematological changes reported in trypanosomoses, include anaemia, leukopaenia, and increased immunoglobin levels [43, 44].

Parasitaemia in susceptible animals may be influenced by the quantity or number of parasites inoculated, the level of stress the host is subjected to, inter-current/concurrent infections, the host's immunity and the pathogenicity of the strain of trypanosome species [41].

The undulating parasitaemia characteristic of trypanosomoses is a protective mechanism exhibited by trypanosome parasites; the parasites are shielded from the host immune system by changing their surface coat, once the previous one is attacked by the host's defense mechanism [45].

Persistence of parasitaemia is possibly due to the protozoan evasion mechanism by antigenic variation of variant surface glycoproteins (VSG) [46] as well as refuge points (made inaccessible by natural barriers) with subsequent lapses in infection such as cerebrospinal fluid and aqueous humor [47]. These variants surface glycoproteins are very immunogenic and hide from the immune system`s low variables or invariant proteins [48]. Recurrence of parasitaemia occurs owing to this variation.

Control of trypanosome parasite currently relies on a small group of trypanocidal compounds which include diminazene aceturate, isomethamidium chloride, homidium salts and suramin [49].

Drug resistance, relapse of infections and toxicity of trypanocidal drugs are major challenges encountered in the treatment of trypanosomosis [50, 51]. Challenges in or lack of effective control of African animal trypanosomosis [52] may have contributed to the lack of success in achieving the World Health Organization's target of eradicating Human African trypanosomosis by the year 2020 [53-55]; more so, since ruminants, especially goats, that survive trypanosome (*Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*) infection often become reservoirs of the parasite for humans and other animals [40]

There is increasing evidence that efficacies of trypanocidal drugs are becoming reduced by the widespread development of trypanosome-drug-resistance [56,57]. Rapid development of resistance to available drugs by trypanosomes presents a serious threat to effective control of trypanosomosis [58, 59]. The inability of most of these trypanocidal drugs to cross the blood-brain barrier, certainly, is a factor in the control of the tissue-invasive trypanosome parasites (*T. bucei* group of trypanosomes). There is little progress in the introduction of new drugs against trypanosomes [60]. The above mentioned reasons underscore the need for more research and introduction of new and effective trypanocidal drugs.

The aim of this study, therefore, was to test the efficacy of Cotrimoxazole when in formulation with medicinal synthetic aluminum magnesium silicate (MSAMS) to see if the already known anti-folic acid effect of Cotrimoxazole would increase to trypanocidal effects in trypanosome infected sheep.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Fifteen (15) adult West African Dwarf (WAD) sheep of both sexes were used. They were purchased from a local market (Ariam market) in Ikwuano LGA of Abia State, Orba market in Nsukka, Enugu State and Udua Ekponwa market in Akwa Ibom State. They were kept in the animal house belonging to Veterinary Medicine Department Michael Okpara University of Agriculture, Umudike. The sheep were acclimatized for two months. During this period, they were prophylactically treated for ecto- and endo- parasites with Albendazole (Tuyil Pharm Ind. Ltd., Nig), Ivermectin, Tick and Flea Powder (Propets Product & Serv., Nig) and long acting Oxytetracycline (TETROXY LA®, Bimeda, Holland). The animals were screened and confirmed negative for trypanosome. The sheep were fed with guinea grass (*Panicum maximum*) and elephant grass *(Pennisetum purpureum*); kitchen wastes including plantain peel, banana peels, yam peels, dried cassava (*Manihot esculentum*) in addition to commercial dry preparations containing mixture of cereal bran, husks of legumes and flour powder. Feed and water were provided *ad libitum* except during 5

days of treatment in which animals were fasted for about 12 hours before treatment and fed 2 hours after treatment [61,62]. This was done in other to relatively decongest the gastrointestinal tract so as to eliminate or reduce trapping of the drug after oral administration by the ruminal contents before absorption.

2.2 Trypanosome Parasites

Trypanosoma b. brucei used to infect sheep in this experiment was local isolate obtained from a clinically infected dog presented to the Veterinary Teaching Hospital, University of Nigeria, Nsukka. The isolate was identified in the Department of Veterinary Parasitology and entomology, University of Nigeria, Nsukka. The parasites were maintained in albino mice until used in the experiment. Each infected sheep was challenged with approximately 1 x 10^6 of the parasites, intravenously.

2.3 Drugs

Medicinal Synthetic Aluminum Magnesium Silicate (MSAMS) at a dose of 50 mg/kg was formulated with Cotrimoxazole for treatment of some group per os for five (5) days at a dose of 120mg/kg.

2.4 Experimental Design

The sheep were assigned to three treatment groups of five (5) sheep per group as follows:

Group 1: Uninfected-untreated control.

Group 2: Infected-untreated control.

Group 3: Infected and treated with 100 % Cotrimoxazole-dose with Cotrimoxazole-MSAMS formulation (120 mg/kg).

2.5 Infection of Experimental Animals

Infected blood from donor mice was collected from the retrobulbar plexus via the medial canthus of donor-mice eyes into a blood sample bottle containing ethylene-diamine tetra acetate (EDTA). The infected blood was then diluted in phosphate-buffered saline (PBS). Each sheep was infected with approximately 1 × 10^6 trypanosomes suspended in 2 ml of PBS intravenously via the jugular vein.

2.6 Diagnosis of Infection

Blood samples obtained from sheep infected with trypanosomes were examined daily until parasitaemia was established in all infected groups and thereafter weekly till the end of the experiment. The wet blood film method, as described by OIE, [63] was used for initial detection of parasitaemia. Micro-haematocrit buffy-coat method [64] was used to confirm infection when parasites are absent in wet blood film. The degree of parasitaemia was estimated by the rapid matching method, as described by Herbert and Lumsden [65].

2.7 Statistical Analysis

Data obtained were computed into means and analyzed using Analysis of Variance (ANOVA). The means were separated at *post hoc* using Duncan's Multiple Range test [66] at 95 % confidence interval.

3. RESULTS AND DISCUSSION

The inoculum containing approximately 1.0 × 10^6 parasites, given intramuscularly to each infected animal, was able to infect experimental animals, with parasites being detected by wet mount and micro-haematocrit buffy-coat methods. Parasitaemia was observed in infected animals 4 weeks post infection and progressed until treatment was commenced by day 36 post inoculation in the treated group. Mean parasitaemia of 80.49±7.56 million parasite per ml of blood was recorded just before commencement of treatment in the treated group (Table 1).

At 2 days post treatment, mean parasitaemia, 1.00 ± 0.00^b of the treated group (100 % Cotrimoxazole dose in MSAMS) was significantly ($P\leq0.05$) lower than mean parasitaemia (81.60 ± 27.71^a) of the untreated group. Parasitaemia was reduced by 98.76 % in the treated sheep but it increased by 128.48% in the untreated infected (control) group, on day 2 post-treatment, as shown in Table 2. At 9 days post treatment, zero mean parasitaemia (0.00 ± 0.00^b) was recorded in the treated group against 2.25 ± 1.50^a ($P\leq 0.05$) of the untreated group. There was 100 % reduction (zero parasitaemia), in the treated sheep 9 days post treatment (Table 2). No relapse of infection was observed up to 100 days post treatment.

An average prepatent period of 28 days was observed in infected sheep in this study. This is in contrast with the report of Akpan *et al*. [67] who recorded a prepatent period of 5 days in *T.b. brucei-infected* WAD sheep, 3.5 days in *T. congolense-infected* sheep [68]; 7 days in *T. brucei-infected* Yankasa rams [69] and 6 days in

T. vivax infected Zebu cattle [70]; but somehow similar to 20 days reported by Wada *et al*. [69] in *T. evansi* infected Yankasa ram. The pre-patent period as well as the level of parasitaemia obtained in experiments can be affected by the concentration of inoculum or the number of trypanosome parasites inoculated. Prepatent periods of 4 days to 8 weeks could be observed in trypanosomosis of ruminants [42]. The length of the prepatent period can be affected by the strain and virulence of the trypanosome isolate, the infective dose of the parasite, nutritional and immune status as well as the degree of susceptibility to isolate, of the host [70, 71, 69].

The long prepatent period observed in infected sheep in this study could be due to a low virulence and pathogenicity of the trypanosome isolates used [69]. Increasing the concentration of the inoculum above the 1 X 10^6 used in this study could have shortened the prepatent period. It is possible that the locally sourced WAD sheep used mounted strong resistance against the trypanosome parasite. This apparent trypanosome resistance by the experimental animals may related to the zero trypanosome prevalence rate recorded in an earlier prevalence study conducted in the area [72].

Table 1. Parasitaemia (X10^6/ml) in *Trypanosoma brucei* - infected Sheep treated with Cotrimoxazole-medicinal synthetic aluminum-magnesium silicate formulation

Days Post Treatment	Cotrimoxazole MSAMS	Infected/Untreated (Control)
Pre-treatment Parasitaemia	**80.49±7.56^a**	**35.72±2.36^b**
2	1.00±0.00^b	81.60±27.71^a
9	0.00±0.00^b	2.25±0.15^a
16	0.00±0.00^b	0.50±0.00^a
23	0.00±0.00^b	0.43±0.03^a
30	0.00±0.00^b	0.37±0.05^a
37	0.00±0.00^b	0.43±0.03^a
44	0.00±0.00^b	0.37±0.05^a
51	0.00±0.00^b	0.43±0.03^a
58	0.00±0.00^b	0.43±0.03^a
65	0.00±0.00^b	0.34±0.02^a
72	0.00±0.00^b	0.43±0.03^a
79	0.00±0.00^b	0.43±0.03^a
86	0.00±0.00^b	0.43±0.03^a
93	0.00±0.00^b	0.34±0.02^a
100	0.00±0.00^b	0.34±0.02^a

Data presented as means ± SEM. Different Superscripts $^{(a, b, c)}$ in a row indicate significant difference between the means of the groups at ($P \leq 0.05$)

Table 2. Parasitaemia reduction rates (%) in *Trypanosoma brucei* - infected Sheep treated with Cotrimoxazole-medicinal synthetic aluminum-magnesium silicate formulation

Days post treatment	Cotrimoxazole MSAMS	Infected-Untreated
2	98.76	-128.48
9	100	93.69
16	100	98.60
23	100	98.80
30	100	98.96
37	100	98.80
44	100	98.96
51	100	98.80
58	100	98.80
65	100	99.05
72	100	98.80
79	100	98.80
86	100	98.80
93	100	99.05
100	**100**	**99.05**

The chemotherapy (Cotrimoxazole-MSAMS) was successful as it achieved a significant (P≤ 0.05) reduction of parasitaemia, 2 days post-treatment, from 80.49±7.56 to 1.00±0.00 (98.76 % infection-reduction) but completely (P≤0.05) cleared the parasitaemia (100 % infection reduction) 9 days PT in trypanosome-infected sheep.

Cotrimoxazole-MSAMS formulation was able to clear parasitaemia in the infected experimental sheep; this indicates that it is efficacious against trypanosomes. This result is in line with the report of Ezeibe *et al.* in mice [11] in which Cotrimoxazole-MSAMS formulation cleared parasitaemia in *Trypanosoma b. brucei* infected mice 2 days post-treatment.

Use of Cotrimoxazole alone to treat trypanosomosis in sheep has not been reported; however Ezeibe *et* al. [11] reported a significant reduction (54 %) in trypanosome parasitaemia in *Trypanosome b. brucei* infected mice treated with Cotrimoxazole alone. This suggests that use of Cotrimoxazole alone may also lead to cure of trypanosome infections, in sheep, if the dose is increased or properly adjusted. However, use of Cotrimoxazole-MSAMS formulation is still better because it achieves that complete cure at lower dose which means lower cost of treatment and lower drug residues in the food animals. In addition, Cotrimoxazole-MSAMS formulation could have the added capability of crossing blood-brain barriers, consequently eliminating relapse infections, in addition to clearing resistant infections [27] since MSAMS is a nanoparticle [22]. Medicinal Synthetic Aluminum Magnesium Silicate (MSAMS) may have enhanced ability of Cotrimoxazole to inhibit synthesis of Folic acid and so terminated the trypanosome infections in the WAD breed of sheep used in this study.

Lack of relapse in parasitaemia up to 100 days post-treatment supports the formulation (Cotrimoxazole-MSAMS) as a new therapy for treatment and control of African Animal Trypanosomosis.

4. CONCLUSION

Cotrimoxazole-MSAMS effectively treated *Trypanosoma brucei brucei* infected WAD sheep 9 days post-treatment, and relapse infection was not observed up to 100 days post-treatment..

ETHICAL APPROVAL

All applicable international, national, and institutional guidelines for the care and use of animals were followed in this study.

Approval for conduct of the work was obtained from the College of Veterinary Medicine Research Ethics Committee of Michael Okpara University of Agriculture, Umudike. Ref: MOUAU/CVM/REC/202109.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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