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Research Article

Regression of Symptoms of Trypanosomosis in Mice Dosed Cotrimoxazole-Antivirt® $\{Al_4(SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3(SiO_4)_3\}$ Formulation

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Abstract

Protozoa require Folic acid for replication and Cotrimoxazole inhibits synthesis of the B-vitamin. So, the drug could be effective for treatment of trypanosomosis (tropical protozoan disease of man and animals). To improve efficacy of such treatment on symptoms of the disease, a formulation of the medicine and The Medicinal synthetic Aluminum-magnesium silicate (Antivirt®) was used to treat mice. Cotrimoxazole improved ($P \leq 0.05$) PCV, RBC and HB in uninfected mice (from 47.60 ± 0.51, 7.93 ± 0.09 and 15.87 ± 0.17 to 59.60 ± 0.51, 9.93 ± 0.09 and 19.93 ± 0.12) and in trypanosome-infected mice (from 34.75 ± 0.48, 5.79 ± 0.08 and 11.67 ± 0.14 to 37.67 ± 0.88, 6.28 ± 0.15 and 12.56 ± 0.29) but it worsened fever in trypanosome-infected mice (from rectal temperature: 36.30 ± 0.35 to 46.27 ± 0.79). Stabilizing the drug with Antivirt® reduced ($P \leq 0.05$) that adverse effect (fever) from temperature of 46.27 ± 0.79 to 39.50 ± 0.29. Weight of the mice remained approximately ($P \geq 0.05$) same in all the groups. Formulation of Cotrimoxazole (anti-protozoan) and Antivirt® could be a new medicine for trypanosomosis (sleeping sickness).

Keywords: *Trypanosomes; Cotrimoxazole; Pyrogens; Trypanosomosis-symptoms (Fever, Anemia)*

Introduction

Trypanosomosis is a protozoan disease of man and animals, caused by infections of pathogenic parasites of the genus *Trypanosoma* [1,2]. The disease is found mainly in sub-Saharan Africa, between latitude 14^0N and 29^0S [3]. It affects wide range of animals [4]. The parasites are transmitted by inoculation of its infective metacyclic stages into blood of animals and man by *Glosina spp* during meals. Infection is also possible through biting flies like *Tabanids* and *Stomoxys* or by vampire bats. Dourine in Equines is sexually transmitted. Tsetse transmitted African trypanosomosis is responsible for 55,000 human and 3 million livestock deaths, annually [5].

Trypanosoma brucei and *T. congolense* are the most pathogenic species affecting domestic animals in Nigeria [6]. Socio-economic effects of trypanosomosis are on the increase not withstanding huge amount of money spent researching on the disease. The disease is a major cause of mortality in animals in Eastern Nigeria [7] and contributes greatly to under-development of region of Sub-Saharan Africa [8,9]. Over 60 million people and 48 million livestock are reported to be at risk [10].

Control for trypanosomosis is by controlling the vectors or by controlling the parasite or a combination of both. In poor-rural communities, affected by the disease, control is mainly by use of trypanocidal drugs. Trypanocides currently employed in treatment of trypanosomosis are: Homidium salts (Ethidium-Novidium®); Quinapyraminesulfate (Antrycide®); Diminazene aceturate (Berenil®); Isometamidium (Samorin-Trypamidium®) and Suramin sodi-

um. These drugs have been in use for more than half a century. It is estimated that 35 million doses of the drugs are used in Africa each year, with about 50 - 70 million animals, at risk.

When microorganisms/cells are exposed to drugs for such a long time, they develop resistance against the drugs. Mechanisms for drug-resistance include: loss of surface specific receptors or transporters for the drugs, specific metabolism of the drugs, alteration (mutation) of specific sites for the drugs on the organisms. These changes result in resistance to individual drugs or to a number of related drugs. More often, cells express mechanisms of resistance that confer simultaneous resistance to many different structurally and functionally unrelated drugs.

Cotrimoxazole is a fixed-dose combination of two antimicrobial drugs (sulfamethoxazole and trimethoprim) and its activities cover antibacterial, antifungal and anti-protozoa. It consists of one part, trimethoprim and five parts, sulfamethoxazole. In the late 1960s, this drug-combination was introduced into clinical practice for treatment of many infectious diseases, such as urinary tract infections, respiratory infections, sexually transmitted diseases, Gram-negative sepsis, enteric infections and typhoid fever [11].

The drug was introduced based on advantages of the combination over each one, used individually. Trimethoprim and sulfamethoxazole inhibit synthesis of tetrahydrofolic acid, the physiologically active form of Folic acid which is a necessary cofactor in the synthesis of thymidine, purines and bacterial DNA [12,13]. Sulfamethoxazole inhibits synthesis of the intermediary dihydrofolic acid from its precursors [14]. Trimethoprim inhibits dihydrofolate reductase and consequently, production of tetrahydrofolic acid from dihydrofolic acid. The sequential blockade of the bacterial Folic acid synthesis pathway produces *in vitro* synergy [15] and it was postulated that such synergy would also occur, *in vivo* [16,17].

The drug-combination has greater effects than using the drugs separately, because they inhibit successive stages in Folic acid synthesis. They are formulated in a one-to-five ratio so that when they enter the body their concentration in blood and tissues is exact ratio required for a peak synergistic effect between the two [18].

Molecules of Aluminum-magnesium silicate (AMS: A pharmaceutical absorbent, viscosity-enhancing agent, anti-caking agent, stabilizing agent and tablet binder/capsule des-integrant) are 0.96 nanometer thick and some hundred Nanometers across [19]. As a Nanomedicine, AMS may help in delivering drug-molecules to target cells. Drug molecules in "corridors" of AMS "house of cards" are also bound by charged faces and edges of its platelets. So, they are protected from degradation by physical factors and from metabolic processes but are released gradually into blood of treated patients. So, high bioavailability of such drugs would be prolonged [20]. This prolonging of high bioavailability is a very good advantage for chemotherapeutics since prolonging high bioavailability improves efficacy of antimicrobials [21,22].

Silicates are immune stimulants [25] and by stabilizing drugs AMS also increases their potency. So, using it in drug formulations reduces doses of the drugs needed to achieve desired effects [23,24].

AMS is not found in Nigeria but the country has large deposits of Aluminum silicate ($Al_4(SiO_4)_3$) and Magnesium silicate (Mg_2SiO_4). These solid minerals were used for a reaction [25] to get the Medicinal synthetic AMS {MSAMS; Antivirt®: $Al_4(SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3(SiO_4)_3$}. The MSAMS has been shown to increase efficacy of Cotrimoxazole against *Salmonella pullorum* [26] hence the need to test its anti-trypanosome efficacy, too.

Materials and Methods

Forty mice were assigned to eighty (8) groups of five (5) each, as follows:

- Group 1: Uninfected/untreated
- Group 2: Uninfected/treated with 100%-dose of Cotrimoxazole
- Group 3: Uninfected/treated with 100%-dose of Cotrimoxazole in Antivirt® drug formulation
- Group 4: Infected/Untreated
- Group 5: Infected/Treated with 100%-dose of Cotrimoxazole
- Group 6: Infected/Treated with 75% -dose of Cotrimoxazole
- Group 7: Infected/Treated with 100% -dose of Cotrimoxazole in Antivirt® drug formulation
- Group 8: Infected/Treated with 75%-dose of Cotrimoxazole in Antivirt® drug formulation.

Treatment was started 7 days post infection and lasted for 5 days while blood samples were collected 2 days, post treatment, for

determination of packed Cell Volume (PCV). Hemoglobin Concentration (Hb) and total Red Blood Cell-counts (RBC). Body weights of the mice were determined using a weighing balance and the values recorded in grams (g). While their rectal temperatures were determined using clinical thermometer and the values recorded in degree Celsius (°C).

Data analysis

The PCV, Hb, RBC, weight and temperature were presented as Means ± SEM and tested for statistical differences by one way analysis of variance (ANOVA). Significance was accepted at $P \leq 0.05$.

Results

Means of rectal temperature: 36.30 ± 0.35, 46.27 ± 0.79, 35.77 ± 0.34, 39.50 ± 0.29 and 36.03 ± 0.48 of trypanosome-infected mice were significantly ($P < 0.05$) higher than 29.67 ± 4.40, 30.14 ± 1.77 and 20.95 ± 0.80 of the uninfected groups (fever). There was no significant ($P > 0.05$) difference in the mean rectal temperature (30.14 ± 1.77) of the group of uninfected-treated with 100%-dose of Cotrimoxazole and 29.67 ± 4.40 of the uninfected-untreated group but mean rectal temperature, 46.27 ± 0.79, of the group of trypanosome-infected mice treated with the same 100% -dose of Cotrimoxazole was significantly ($P < 0.05$) higher than 29.67 ± 4.40 and 30.14 ± 1.77 of the two uninfected groups and rectal temperatures of all the other infected groups. However, the 39.50 ± 0.29 of the group of trypanosome infected mice treated with 100%-dose of Cotrimoxazole in Antivirt® showed significant ($P \leq 0.05$) drop of the fever. There was no significant ($P > 0.05$) difference in rectal temperatures: 39.50 ± 0.29, 35.77 ± 0.34, 36.03 ± 0.48 of the groups of infected/100%-dose of Cotrimoxazole in Antivirt®, infected/75%-dose of Cotrimoxazole, infected/75% -dose of Cotrimoxazole in Antivirt® respectively and 29.67 ± 4.40, 30.14 ± 1.77 and 20.95 ± 0.80 of the infected-untreated group and the two uninfected groups, respectively (Figure 1).

Mean weight (20.95 ± 0.80) of uninfected/treated with 100%-dose of Cotrimoxazole in Antivirt® was significantly ($P \leq 0.05$) less than 29.67 ± 3.46 and 30.14 ± 4.77 of the uninfected/untreated group and the uninfected/treated with 100%-dose of Cotrimoxazole whereas there was no difference between it and 28.96 ± 4.23, 27.68 ± 0.79, 22.04 ± 0.85, 23.10 ± 0.60 and 23.39 ± 1.44 of the infected groups (Figure 2).

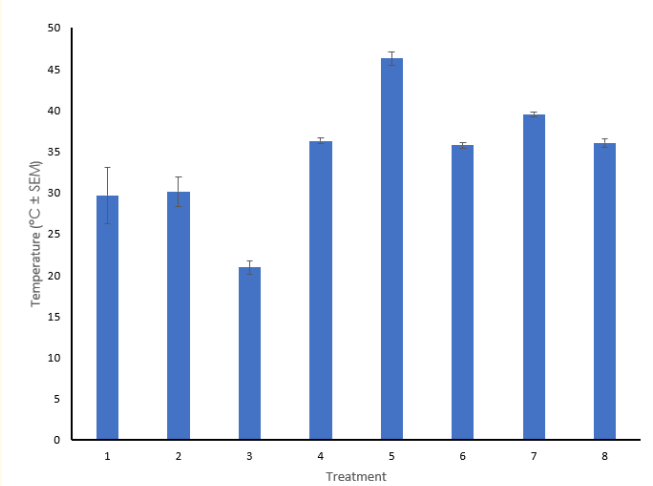

Figure 1: *Mean rectal temperatures of trypanosome-infected mice treated with different doses of Cotrimoxazole and Cotrimoxazole-Antivirt® drug formulation.*

Legend: 1: Uninfected/Untreated, 2: Uninfected/treated with 100%-dose of Cotrimoxazole, 3: Uninfected/treated at 100% Cotrimoxazole-dose with Cotrimoxazole- Antivirt drug formulation, 4: Infected/Untreated, 5: Infected/Treated with 100%-dose of Cotrimoxazole, 6: Infected/Treated with 75%-dose of Cotrimoxazole, 7: Infected/ Treated at 100% Cotrimoxazole-dose with Cotrimoxazole-Antivirt® drug formulation, 8: Infected/Treated at 75% Cotrimoxazole-dose with Cotrimoxazole- Antivirt® drug formulation.

Mean PCV of trypanosome-infected mice treated with 75%-dose Cotrimoxazole- Antivirt® drug formulation was significantly ($P < 0.05$) lower than that of the group infected and treated with 100%-dose Cotrimoxazole in Antivirt® but not significantly ($P > 0.05$) different from that of the group infected and treated with 75%-dose of Cotrimoxazole alone. There was no significant ($P > 0.05$) difference in mean PCV of trypanosome infected mice treated with 100%-dose Cotrimoxazole in Antivirt® and that infected and treated with 100%-dose Cotrimoxazole alone when compared with the group infected but not treated. Mean PCV was significantly ($P < 0.05$) higher in group of infected mice treated with 100%-dose Cotrimoxazole alone when compared with the group infected and

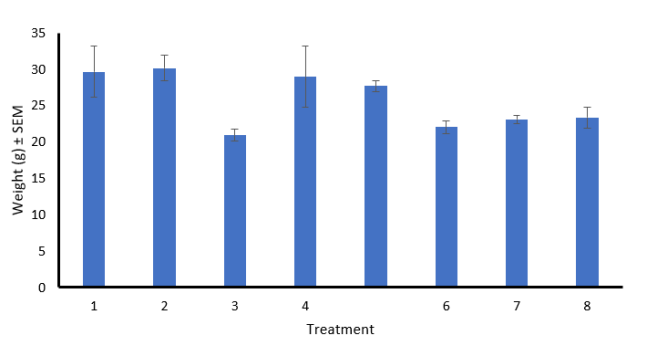

Figure 2: *Mean body weights of trypanosome-infected mice treated with different doses of Cotrimoxazole and Cotrimoxazole -Antivirt® drug formulation.*
Legend: 1: Uninfected/Untreated, 2: Uninfected/treated with 100%-dose of Cotrimoxazole, 3: Uninfected/treated at 100% Cotrimoxazole-dose with Cotrimoxazole- Antivirt drug formulation, 4: Infected/Untreated, 5: Infected/Treated with 100%-dose of Cotrimoxazole, 6: Infected/ Treated with 75%-dose of Cotrimoxazole, 7: Infected/Treated at 100% Cotrimoxazole-dose with Cotrimoxazole -Antivirt® drug formulation, 8: Infected/Treated at 75% Cotrimoxazole-dose with Cotrimoxazole- Antivirt® drug formulation.

treated with 75%-dose Cotrimoxazole alone as well as that of the group infected but not treated (Table 1).

Antivirt® significantly ($P \leq 00.05$) improved RBC counts of both uninfected mice (from 7.93 ± 0.09 to 9.93 ± 0.09) and of trypanosome infected mice (from 5.79 ± 0.08 to 6.28 ± 0.15) and there was no difference between the counts (6.28 ± 0.15 and 5.97 ± 0.37: 4.79 ± 0.12 and 4.79 ± 0.12) in the infected mice treated with Cotrimoxazole alone and those treated with the Cotrimoxazole-Antivirt® drug-formulation (Table 1).

There was a significant ($P < 0.05$) increase in mean HBC of the group infected and treated with 100%- dose Cotrimoxazole in Antivirt® when compared with that of the group infected and treated with 75%-dose Cotrimoxazole in Antivirt® as well as the group infected and treated with 75%-dose Cotrimoxazole alone, but was comparable ($P \geq 0.05$) to that of the group infected and treated with 100%-dose Cotrimoxazole alone and the infected/untreated (Table 1).

Group	Treatment	PCV (%)	HB(g/dL)	RBC ($X10^6$/µL)
1	Uninfected/ Untreated	47.60 ± 0.51^c	15.87 ± 0.17^c	7.93 ± 0.09^c
2	Uninfected/ treated with 100%-dose of Cotrimoxazole	59.60 ± 0.51^a	19.93 ± 0.12^a	9.93 ± 0.09a
3	Uninfected/ treated with 100 %-dose of Cotrimoxazole in Antivirt®	53.60 ± 1.17^b	17.87 ± 0.39^b	8.93 ± 0.19^b
4	Infected/ Untreated	34.75 ± 0.48^e	11.67 ± 0.14^e	5.79 ± 0.08^e
5	Infected/ Treated with 100%-dose of Cotrimoxazole	37.67 ± 0.88^d	12.56 ± 0.29^d	6.28 ± 0.15^d
6	Infected/ Treated with 75% -dose of Cotrimoxazole	30.33 ± 0.88^f	10.11 ± 0.29^f	6.28 ± 0.15^d
7	Infected/ Treated with 100%-dose of Cotrimoxazole in Antivirt®	35.69 ± 0.33^{de}	12.30 ± 0.15^{de}	5.97 ± 0.37^{de}
8	Infected/ Treated with 75%-dose of Cotrimoxazole in Antivirt®	28.75 ± 0.75^f	9.58 ± 0.25^f	4.79 ± 0.12^f

Table 1: Mean packed cell volume, hemoglobin concentration and red blood cell counts of trypanosome-infected mice treated with cotrimoxazole and cotrimoxazole in antivirt® drug formulation.

Discussion

Main symptoms of trypanosomosis are anemia and fever. So, in addition to medicines terminating the infection, they should be effective in managing these symptoms. In this study, hemoglobin

concentration, total red blood cell counts, packed cell volume (anemia) and rectal temperature (fever) were used to assess efficacy of Cotrimoxazole and Cotrimoxazole-Antivirt® drug formulation for treatment of trypanosmosis in experimentally infected mice. Weight was used as a measure of general health status of the mice.

The results showed that Cotrimoxazole significantly improved HB, RBC and PCV both in uninfected mice and in trypanosome-infected mice. Weight of Cotrimoxazole-treated uninfected mice significantly increased when compared with the uninfected/untreated group while weights remained fairly same between the infected/treated and infected/untreated groups thus suggesting that Cotrimoxazole does not have serious side effects.

However, whereas Cotrimoxazole reduced anaemia in trypanosome infected mice, it worsened ($P \leq 0.05$) fever (increase in rectal temperature from 36.30 ± 0.35 to 46.27 ± 0.79). Since there was no significant difference between mean rectal temperature of uninfected mice treated with Cotrimoxazole and those not treated (30.14 ± 1.77 and 29.67 ± 3.40), the increase in body temperature of infected mice treated with Cotrimoxazole may not be a side effect of the drug. It is possible that there is a pyrogen produced by reaction of trypanosome-toxins and Cotrimoxazole which worsens fever in trypanosome-infected animals when treated with the drug. Also, when parasitaemia in trypanosome infected mice treated with Cotrimoxazole was assessed, recommended dose of the drug achieved only 54% reduction of the infection load [27]. The worsening of fever observed in present study and that failure o to achieve cure (≥ 95% parasitaemia-reduction) may be reason the drug has not been recommended for treatment of trypanosmosis in spite of the fact that it is known to inhibit synthesis of Folic acid which is essential for replication of trypanosomes.

Temperature of the infected mice treated with cotrimoxazole (46.27 ± 0.79) significantly reduced ($P \leq 0.05$) to 39.50 ± 0.29 when the treatment was at same dose of the drug stabilized with the Antivirt®. This suggests that Antivirt® is able to mop whatever pyrogen that causes the increase in fever.

Aluminum-magnesium silicate is both an adsorbent and a stabilizing agent. As an adsorbent it mops toxins while as a stabilizing agent it improves efficacy of other medicines. The Antivirt® may have mopped the pyrogen to reduce the fever and enhanced efficacy of cotrimoxazole to achieve clearance of the infection.

Bibliography

1. Barret MP., *et al*. "Human African Trypanosomiasis: Pharmacological re-engagement with a neglected disease". *British Journal of Pharmacology* 152 (2007): 1155-1171.

2. Rashid A., *et al*. "Trypanosomiasis in dogs: A case report". *Iranian Journal of Arthropod-Borne Diseases* 2.2 (2008): 48-51.

3. Samdi SM., *et al*. "Impact of trypanosomosis on food security. Security in Nigeria: A review". *International Journal of Animal and Veterinary Advances* 2.2 (2010): 47-50.

4. Soulsby EJI Helminthes. "Arthropods and Protozoa of domesticated animals, 7th edition". Balliere Tindall London (1982): 203-206.

5. Abenga JN., *et al*. "Prevalence of trypanosomosis in trade cattle at slaughter in Kaduna". *Nigeria Journal of Parasitology* 23 (2002): 107-110.

6. Anene BM., *et al*. "Preliminary efficacy trial of Cymelersan in dogs and mice artificially infected with Trypanosoma brucei isolated from dogs in Nigeria". *Revue D'elevage Et De Medicine Veterinaire Des Pays Tropicaux* 52 (1999): 123-128.

7. Anene BM., *et al*. "A Diminazene-Resistant strain of Trypanosoma brucei isolated from a dog in cross resistant to Pentamidium in experimentally infected albino rats". *Parasitology* 132 (2006): 127-133.

8. Onyiah JA. "African animal trypanosomosis: An overview of the current status in Nigeria". *Tropical Veterinarian* 155 (1997): 111-116.

9. Oyewusi JA., *et al*. "The course of trypanosomiasis in laboratory rabbits following experimental infection, treatment and re-infection: haematological study". *European Journal of Scientific Research* 42 (2010): 411-419.

10. WHO. Control and surveillance of African trypanosomiasis. Report of WHO Expert Committee, World Health Organization Technical Report series (1998): 881.

11. Kristjanson PM., *et al*. "Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research". *Agricultural Systems* 59 (1999): 79-98.

12. Delespaux V and De Koning HP. "Drugs and drug resistance in African trypanosomiasis". *Drug Resist* 10 (2007): 30-50.

13. Goldberg E and Bishara J. "Contemporary unconventional clinical use of cotrimoxazole". *Clinical Microbiology and Infection* 18.1 (2012): 8-17.

14. Gleckman R., *et al*. "Trimethoprim: mechanisms of action, antimicrobial activity, bacterial resistance, pharmacokinetics, adverse reactions, and therapeutic indications". *Pharmacotherapy* 1 (1981): 14.

15. Hong YL., *et al*. "Inhibition of recombinant Pneumocystis carinii dihydropteroate synthetase by sulfa drugs". *Antimicrobial Agents and Chemotherapy* 39 (1995): 1756.

16. Bushby SRM. "Trimethoprim-sulfamethoxazole: in vitro microbiological aspects". *Journal of Infectious Diseases* 128.3 (1973): S442-S462.

17. Bushby SR and Hitchings GH. "Trimethoprim, a sulphonamide potentiator". *British Journal of Pharmacology* 33.1 (1968): 72-90.

18. Böhni E. "Chemotherapeutic activity of the combination of trimethoprim and sulfamethoxazole in infections of mice". *Postgraduate Medical Journal* 45 (1969): 18-21.

19. Vanderbilt Report. "Technical Information: VEEGUM - The versatile ingredient for Pharmaceutical Formulations". R.T. Vanderbilt Company Bulletin 91R. R.T. Vanderbilt Company Inc., Norwalk (2012).

20. Brent W., *et al*. "What Do We Really Know about Antibiotics Pharmacodynamics?" *Pharmcotherapy* 21 (2001): 28-31.

21. Ezeibe MCO., *et al*. "Effect of Aluminum Magnesium Silicate on anticoccidial activity of sulphadimidine". *Tropical Veterinarian* 29 (2011): 41-44.

22. Suni L., *et al*. "Immunostimulation by Silica Particles and the Development of Autoimmune Dysregulation". In Tech Open (2014).

23. Ezeibe MCO., *et al*. "Adjuvant effect of a synthetic Aluminium-Magnesium Silicate on chloroquine phosphate, against plasmodium berghei". *Nature Proceedings* (2012).

24. Ezeibe MCO., *et al*. "Aluminium-Magnesium Silicate enhances antibacterial activity of Ampicillin trihydrate, against Salmonella. Gallinarum". *Nature Proceedings* (2012).

25. Ezeibe. "Medicinal synthetic Aluminum-magnesium silicate (Nanoparticles)-Antiviral agent and adjuvant to chemotherapeutics". Federal Republic of Nigeria Patents and Designs.

26. Ezeibe MCO., *et al*. "Restoring efficacy to cotrimoxazole against resistant Salmonella pullorum with Medicinal synthetic Aluminum-magnesium silicate: Al4 (SiO4)3 + 3Mg-2SiO4 →2Al2 Mg3 (SiO4)3]". *Health* 11.9 (2019): 1162-1168.

27. Akpan CA. "Prevalence of Trypanosomes in Cattle, Sheep and Goats in Rivers state and Abia state Nigeria and Exploitation of anti-Foliate effect of Cotrimoxazole for treatment of Trypanosomosis". PhD Dissertation, Michael Okpara University of Agriculture, Umudike, Nigeria (2020).