Volume 20 Issue 52
Originally issued as G3

Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 20 Issue 3 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Antivirt ® $\{Al_4\ (Sio_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2\ Mg_3\ (Sio_4)_3\}$ Enhances Efficacy of Co-trimoxazole to Terminate Experimental Trypanosome-Infections in Mice

By M. C. O. Ezeibe, M. I. Ezeja, C. A, Akpan, F. I.O. Onyeachonam, M. E. Sanda, I. J. Ogbonna, E. Kalu, N.U. Njoku & M.I. Udobi

Michael Okpara University of Agriculture

Abstract- Both bacteria and protozoa require Folic acid for replication and Cotrimoxazole inhibits synthesis of the vitamin. For its mechanism of inhibiting Folic acid, the medicine has been in use as antibacterial drug for many decades but it is not being used to treat trypanosomosis (protozoan disease). To enhance anti-Folic acid activity of the medicine in order to improve its anti-trypanosome efficacy and make it function as new medicine for sleeping sickness (tropical disease of man and animals) it was stabilized with Antivirt® (Medicinal synthetic Aluminum-magnesium silicate). At 100 % of its antibacterial dose, Cotrimoxazole significantly reduced ($P \leq 0.05$) trypanosome parasitemia in mice, from 12.76±1.20 to 5. 87± 0.43. When it was stabilized with the Antivirt®, 75 % of the antibacterial dose had slight reduction ($P \geq 0.05$) in the trypanosome parasitemia (11.30±1.01) while the 100 %-dose achieved zero (0.00±00) trypanosome parasitemia and improved total WBC counts (immunity) from 1.50±0.16 to 2.86±0.38.

Keywords: cotrimoxazole; folic acid-inhibition; anti-trypanosomes efficacy; antivirt®; sleeping sickness, new medicine.

GJMR-G Classification: NLMC Code: QW 70



Strictly as per the compliance and regulations of:

© 2020. M. C. O. Ezeibe, M. I. Ezeja, C. A, Akpan, F. I.O. Onyeachonam, M. E. Sanda, I. J. Ogbonna, E. Kalu, N.U. Njoku & M.I. Udobi. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Antivirt ® {Al_4 (Sio_4)$_3$ + 3Mg_2SiO_4 → 2Al_2 Mg_3 (Sio_4)$_3$} Enhances Efficacy of Co-trimoxazole to Terminate Experimental Trypanosome-Infections in Mice

M. C. O. Ezeibe [α], M. I. Ezeja [σ], C. A, Akpan [ρ], F. I.O. Onyeachonam [ω], M. E. Sanda [¥], I. J. Ogbonna [§], E. Kalu [χ], N.U. Njoku [ν] & M.I. Udobi [Θ]

Abstract- Both bacteria and protozoa require Folic acid for replication and Cotrimoxazole inhibits synthesis of the vitamin. For its mechanism of inhibiting Folic acid, the medicine has been in use as antibacterial drug for many decades but it is not being used to treat trypanosomosis (protozoan disease). To enhance anti-Folic acid activity of the medicine in order to improve its anti-trypanosome efficacy and make it function as new medicine for sleeping sickness (tropical disease of man and animals) it was stabilized with Antivirt® (Medicinal synthetic Aluminum-magnesium silicate). At 100 % of its antibacterial dose, Cotrimoxazole significantly reduced (P≤ 0.05) trypanosome parasitemia in mice, from 12.76±1.20 to 5. 87± 0.43. When it was stabilized with the Antivirt®, 75 % of the antibacterial dose had slight reduction (P≥ 0.05) in the trypanosome parasitemia (11.30±1.01) while the 100 %-dose achieved zero (0.00±00) trypanosome parasitemia and improved total WBC counts (immunity) from 1.50±0.16 to 2.86±0.38.

Keywords: cotrimoxazole; folic acid-inhibition; anti-trypanosomes efficacy; antivirt®; sleeping sickness, new medicine.

I. Introduction

Trypanosomosis is debilitating and often fatal in both man and animals [1] [2]. The disease is found mainly in sub-Saharan Africa [3]. It has wide range of animal-hosts [4] [5] and is transmitted by *Glossina spp*, flies (*Tabanids* and *Stomoxys*) and Vampire bats. Dourine (caused by *T. equiperdum*) is sexually transmitted in Equines.

African trypanosomosis has been reported to be responsible for 55,000 human and 3 million livestock deaths, annually [6]. In Nigeria, *Trypanosoma brucei* and *T. congolense* are the most pathogenic species for domestic animals [7].

The disease is a major cause of mortality in animals in Nigeria and contributes greatly to under-development of Sub-Saharan Africa and other socio-economic consequences despite huge amounts being spent on research to control it [8]. World health organization [9] reported that over 60 million people and 48 million livestock in Africa, are at risk of \trypanosomosis. Estimated losses due to trypanosomosis in Africa run into billions of Dollars.

Of the different species of Trypanosomes which affect man and animals, *Trypanosoma. congolense* is strictly a parasite of the microcirculation, producing primary lesions in blood vessels and lymph nodes [10] [11] [12]. *Trypanosoma brucei* is found in connective tissues, producing inflammation, cellular degeneration and necrosis which lead to tissue and organ damage [4] [12].

Control measures for trypanosomosis are either by controlling the vectors or by use of chemotherapy or a combination of both. In poor, rural communities, affected by the disease, control is mainly by use of trypanocidal drugs [13]. Drugs currently employed in treatment of trypanosomosis are: Homidium salts (Ethidium-Novidium®:); Quinapyramine sulfate (Antrycide®:); Diminazene aceturate (Berenil®:); Isometamidium (Samorin-Trypamidium®:) and Suramin sodium. These drugs have been in use for more than half a century now [14]. It is estimated that 35 million doses of the drugs are used in Africa each year, with about 50–70 million animals at risk [14].

Microorganisms exposed to drugs over such a long time usually develop resistance to the drugs. Mechanisms for drug-resistance include, loss of surface specific receptors or transporters for the drugs, increased metabolism of the drugs and alteration (by mutation) of specific targets for the drugs on the organisms. These result in resistance to a small number of related drugs, too. More often, cells express mechanisms of resistance that confer simultaneous resistance to many different structurally and functionally unrelated drugs [15]. For the problem of drug-resistance by pathogens, there is need for constant search for new drugs for treatment of important diseases such as trypanosomosis. Cotrimoxazole is a combination of five parts of sulfamethoxazole and one part of trimethoprim based on dose of each of the two drugs. It is being used for treatment of bacterial, fungal and protozoan

Author α: College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria. e-mail: maduikeezeibe@yahoo.com

© 2020 Global Journals

infections [16]. The drug-formulation was introduced into clinical use in the late 1960s and it is for treatment of urinary tract infections, respiratory infections, sexually transmitted diseases, enteric infections and typhoid fever. Advantages of Cotrimoxazole include combination of the two components. The drug inhibits synthesis of tetrahydrofolic acid which is needed as a cofactor in synthesis of thymidine and purines which are components of bacterial DNA [17] [18] [19]. Sulfamethoxazole inhibits synthesis of the intermediary dihydrofolic acid from its precursors [20] while Trimethoprim competitively inhibits dihydrofolate reductase and consequently, production of tetrahydrofolic acid from dihydrofolic acid [21]. Potentiating sulfamethoxazole with trimethopriim reduces toxicity and microbial resistance [22]. The synergy between trimethoprim and sulfamethoxazole was first described in the late 1960s [23]. Trimethoprim and sulfamethoxazole have a greater effect when given together than when given separately, because they inhibit successive stages in the folate synthesis pathway. They are formulated in a ratio of one-to-five so that when they enter the body their concentrations in the blood and tissues are exact ratio required for a peak synergistic effect between the two [24]. Trimethoprim causes a backlog of dihydrofolate and this backlog can work against inhibitory effect the drug has on tetrahydrofolate biosynthesis. This is where the sulfamethoxazole comes in. Its role is in depleting the excess dihydrofolate by preventing it from being synthesized in the first place.

Trimethoprim-sulfamethoxazole (Cotrimoxazole) has proved effective in the treatment of infections of coccidian protozoa parasites, Isospora and Cyclospora [25] [26]. So, by potentiating its anti-folic acid effects its protozoan efficacy may be enhanced to achieve cure for trypanosmosis.

Molecules of Aluminum magnesium silicate (AMS: clay) are 0.96 nanometer thick and some hundred Nanometers across [27] [28] [29]. As a Nanomedicine, AMS helps 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 both physical and physiological factors but are released gradually into blood of treated patients. Also, silicates are immune stimulants [30] and AMS is a stabilizing/potentiating agent [31] [32]. By stabilizing drugs, AMS increases potency of the drugs [33]. When drugs are potentiated their doses required for desired effects reduce and using lower doses for treatments reduces side effects of drugs so that immune responses of patients improve. With enhanced efficacy of drugs and improved immune responses of patients both sensitive infections and drug-resistant infections could be effectively treated. So, using The Medicinal synthetic Aluminum-magnesium silicate (Antivirt®) to stabilize Cotrimoxazole may enhance its efficacy against trypanosomes enough so that the medicine being commonly used for treatment of bacterial diseases and amebiasis (protozoan disease) can also function as a medicine for trypanosomosis (sleeping sickness).

II. Materials and Methods

Twenty-five mice were assigned to five (5) groups of five (5) each, as follows:

Group 1: Infected/Untreated

Group 2: Infected/Treated with 100%-dose of Cotrimoxazole

Group 3: Infected/Treated with 75% -dose of Cotrimoxazole

Group 4: Infected/Treated with 100% -dose of Cotrimoxazole in Antivirt®

Group 5: Infected/Treated with 75%-dose of Cotrimoxazole in Antivirt®

Blood samples were collected from each of the mice and examined daily until parasitemia was established in all infected groups. Treatment was started 7 days post-infection and lasted for 5 days while assessment of parasitemia was on day-2 post-treatment.

a) Data analysis

The parasitemia and total WBC were presented as means ± SEM and analyzed for statistical differences by one way analysis of variance and the significant differences were accepted at the level of $P \leq 0.05$.

III. Results

Parasitemia was observed in all the groups from four days post infection and it increased steadily until treatment was commenced by day-7 post infection. Zero mean parasitaemia (0.00±00) of the group of trypanosome-infected mice treated at 100 % dose of Cotrimoxazole with Cotrimoxazole- Antivirt® drug formulation was significantly ($P \leq 0.05$) less than 5.87±0.43 of the group treated with 100 % dose of Cotrimoxazole with Cotrimoxazole alone. Both the 0.00±00 parasitaemia of the 100 % -dose of Cotromoxazole in Antivirt® group and 5.87±0.43 of the 100 % -dose of Cotrimoxazole-group were significantly ($P < 0.05$) lower than 11.73±0.86 of the 75 % -dose of Cotrimoxazole-group and 11.30±1.01 of the 75 % -dose of Cotrimoxazole in Antivirt®-group but there was no significant difference ($P > 0.05$) in mean parasitemia (11.73±0.86) of the group of 75 % -dose of Cotrimoxazole and 11.30±1.01 of the 75 %-dose of Cotrimoxazole in Antivirt®-group. Mean parasitemia of the trypanosome-infected groups of mice treated with Cotrimoxazole and Cotrimoxazole- Antivirt® are as shown on Table 1.

© 2020 Global Journals

Mean WBC was highest in the group infected and treated with 100 %-dose of Cotrimoxazole in Antivirt® when compared with the other infected groups. The mean WBC of the group of infected/treated with 100 %-dose of Cotrimoxazole was comparable to that of the group of infected/untreated, that of the group of infected/treated with 75 %-dose of Cotrimoxazole and that of the group of infected/treated with 75 %-dose of Cotrimoxazole in Antivirt® (Table 2).

Table 1: Parasitaemia (x106/L) in Trypanosome-infected mice treated with different doses of Cotrimoxazole and Cotrimoxazole in Antivirt®

S/N	Infected/Untreated	Infected/Treated			
		Cotri		Cotri-MSAMS	
		100%	75%	100%	75%
1	15.85	6.31	12.59	0.00	12.59
2	12.59	5.01	10.00	0.00	10.00
3	12.59	6.30	12.59	0.00	12.59
4	10.00		10.00		10.00
MEAN±SE	12.76±1.20^{c}	5.87±0.43^{b}	11.73±0.86^{c}	0.00±0.00^{a}	11.30±1.01^{c}

Table 2: Total WBC (X103/µL) of Trypanosome-infected mice treated with different doses of Cotrimoxazole and Cotrimoxazole in Antivirt®

S/N	Infected/Untreated	Infected/Treated			
		Cotri		Cotri-MSAMS	
		100%	75%	100%	75%
1	1.50	1.30	1.50	2.90	1.28
2	1.39	1.56	1.77	2.78	1.56
3	1.94	2.01	1.89	2.89	1.39
4	1.17		10.00		1.00
MEAN±SE	1.50±0.16^{b}	1.62±0.21b	1.72±0.11^{ab}	2.86±0.38a	1.31±0

IV. Discussion

Significant (P≤ 0.05) reduction of parasitemia from 12.76±1.20 to 5.87±0.43 (54 % infection-reduction) in trypanosome-infected mice treated with 100 % antibacterial-dose of Cotrimoxazole is evidence that the drug has anti-trypa mosomal effect. Cotrimoxazole is known to inhibit synthesis of Folic acid and trypanosomes need the vitamin for replication. However, 54 % infection clearance is much lower than the 95 % required for treatments to be effective. This failure to achieve enough level of clearance to terminate trypanosome infections may be reason Cotrimoxazole is not yet being recommended for treatment of trypanosomosis.

When the drug was stabilized with Antivirt®, the 100 %-dose completely and significantly (P≤0.05) cleared the parasitemia (00.00±0.00). AMS is a stabilizing agent and a Nanomedicine. As stabilizing agent it prolongs time medicines remain at high concentration in blood of treated animals while as a Nanomedicine, it enhances delivery of drugs to targets. Both prolongation of time of high bioavailability of drugs and delivering them to effect-targets enhance efficacy of drugs. So, the Antivirt® may have enhanced ability of Cotrimoxazole to inhibit synthesis of Folic acid and so terminated the trypanosome infections.

As a silicate AMS also enhances immune response. So, the increase in WBC count of the mice suggests that the Antivirt® may, in addition to improving efficacy of Cotrimoxazole, have enhanced immune response of the mice. Synergy of the enhanced immune response of patients and enhanced efficacy of medicines may be responsible for the zero parasitemia achieved.

In earlier studies of effects of the Antivirt® on antimicrobial drugs, it made 75 %-dose more effective than recommended (100%) doses of drugs but in this study its effects on 100 %-dose of Cotrimoxazole were the best.

Dose used as 100 % in this study is the dose recommended for treatment of bacterial infections (not for trypanosomosis). That the 100 %-dose of Cotrimoxazole without the Antivirt® was able to reduce the trypanosome infection, significantly, suggests that if the dose is increased it may lead to cure of the infection

© 2020 Global Journals

even without the Antivirt®. Failure to determine correct dose of Cotrimoxazole for treatment of trypanosomosis may be reason the drug has not been recommended for treatment of the zoonotic/tropical disease. That dose, used as 100 % may be 75 % of dose of the drug needed for treatment of trypanosomosis in absence of the Antivirt®. However, reducing doses by stabilizing drugs with the Antivirt® reduces side effects to enhance immune responses and also reduces cost of drug formulations.

Trypanosomosis is a very serious disease of both man and animals in the tropics and the causative agents, very often, develop resistance against existing drugs. So, there is need to constantly research for new drugs. Since the Antivirt® made Cotrimoxazole achieve total clearance of the trypanosome-infections it may cure the disease and may also make development of resistance against the new therapy difficult by achieving total clearance of infections.

References Références Referencias

1. Barret, M.P.; Burchmore, R.J.S.; Stich, A.; Lazzari, J.O.; Frasch, A.C.; Cazullo, J.J.; Krishna, S. (2003): The trypanosomiases. The Lancet 362:1469-1480.
2. Rashid, A.; Rasheed, K. and Hussain, A. (2008): Trypanosomiasis in dogs: A case report. Iranian J. Arthropod-Born Dis. 2 (2): 48-51
3. Samdi, S.M.; Abenga,J.N.; Attahir, A.; Haruna, M.K.; Wayo, B.M.; Fajinmi, A.O.; Sumayin, H.M.; Usman, A.O;. Hussaina, J.Z.; Muhammad, H.; Yarnap, J.E.; Ovbagbedia, R.P. and Abdullah, R.A. (2010): Impact of trypanosomosis on food security. Security in Nigeria: A review. Int. J. Anim. Vet. Adv., 2(2): 47-50.
4. Soulsby, E.J.I. (1982): Helminthes, Arthropods and Protozoa of domesticated animals, 7th edition, Balliere Tindall London Pp. 203 – 206.
5. Urquhart, G.M.; Amour, J.; Duncan, J.L.; Dunn, A.M.; Jennings, F.W. (2002): Veterinary Parasitology, 2nd Edition. Blackwell Science co. UK. Pp 217.
6. Abenga, J.N; Enwozor, F.N.C; Lawani, F.A.G; Ezebuiro, C; Sule J; David, K.M (2002): Prevalence of trypanosomosis in trade cattle at slaughter in Kaduna, Nigeria Journal of Parasitology, 23: 107-110.
7. Anene, B.M.; Ogbuanya, C.E.; Mbah, E.S. and Ezeokonkwo, R.C. (1999): 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: 123-128.
8. Anene, B.M.; Ezeokonkwo, R.C.; Mmesirionye, T.I.; Tettey, J.N.A.; Brock, J.M.; Barret, M.P. and Deckoning, H.P. (2006): A Diminazene-Resistant strain of Trypanosoma brucei isolated from a dog in cross resistant to Pentamidium in experimentally infected albino rats. Parasitology, 132: 127-133.
9. World Health Organization (1998): Control and surveillance of African trypanosomiasis. Report of WHO Expert Committee, World Health Organization Technical Report series 881.
10. Taylor, K. and Authie, E. (2004): Pathogenesis of Animal trypanosomosis. In: The Trypanosomiasis. Edited by: Maudlin, I.; Holmes, P.H. and Mills, M.A. CAB International. UK. Pp. 331-353.
11. Ezeokonkwo, R.C.; Ezeh, I.O.; Onunkwo, J.I.; Obi, P.O.; Onyenwe, I.W. and Agu, W.E (2010): Comparative haematological study of single and mixed infections of mongrel dogs with Trypanosoma congolense and Trypanosoma brucei. Journal of Veterinary Parasitology, 173: 48-54.
12. Mattioli, R.C., Feldmann, G., Hendrickx, W., Wint, J., Jannin, J., Slingenbergh, J., (2004). Tsetse and trypanosomiasis intervention policies supporting sustainable animalagricultural development. Food, Agr. Environ. Food Agr Environ 2, 310-314.
13. Delespaux, V., Geysen, D., Van den Bossche, P., Geerts, S., 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 24, 236- 242.
14. Sones, K., 2001. Pharmaceutical companies: partners or enemies? ICPTV Newsletter 3, 19-21.
15. Delespaux, V., de Koning, H.P., 2007. Drugs and drug resistance in African trypanosomiasis. Drug Resist. Update 10, 30-50
16. WHO (2014): Guidelines on Post-Exposure Prophylaxis for HIV and the Use of Co-Trimoxazole Prophylaxis for HIV-Related Infections Among Adults, Adolescents and Children: Recommendations for a Public Health Approach: December 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: World Health Organization; 2014 Dec. 5, Available from: https://www.ncbi.nlm.nih.gov/books/NBK298965/
17. Gleckman R, Blagg N, Joubert DW. Trimethoprim: mechanisms of action, antimicrobial activity, bacterial resistance, pharmacokinetics, adverse reactions, and therapeutic indications. Pharmacotherapy 1981; 1:14.
18. Wormser, GP; Keusch, GT; Heel, RC (1982). "Co-trimoxazole (trimethoprim-sulfamethoxazole): an updated review of its antibacterial activity and clinical efficacy". Drugs. 24 (6): 459–518. doi:10.2165/00003495-198224060-00002. PMID 6759092.
19. Kalkut G. Sulfonamides and trimethoprim. Cancer Invest 1998; 16:612
20. Hong YL, Hossler PA, Calhoun DH, Meshnick SR (1995). Inhibition of recombinant Pneumocystis

© 2020 Global Journals

carinii dihydropteroate synthetase by sulfa drugs. Antimicrob Agents Chemother 1995; 39:1756.
21. Pattishall KH, Acar J, Burchall JJ, et al. Two distinct types of trimethoprim-resistant dihydrofolate reductase specified by R-plasmids of different compatibility groups. J Biol Chem 1977; 252:2319.
22. Darrell J.H.; Garrod, L.P.; and Waterworth, P.M. (1968): Trimethoprim: laboratory and clinical studies. J Clin Pathol. 1968; 21202- 209.
23. Wilcke, J. R. (1988). Therapeutic application of sulfadiazine/trimethoprim in dogs and cats: a review. Companion animal practice (USA).
24. Böhni E (1969). "Chemotherapeutic activity of the combination of trimethoprim and sulfamethoxazole in infections of mice". Postgrad Med J. 45 (Suppl): Suppl: 18–21. PMID 4902845.
25. Wormser, GP; Keusch, GT; Heel, RC (December 1982). "Co-trimoxazole (trimethoprim-sulfamethoxazole): an updated review of its antibacterial activity and clinical efficacy". Drugs. 24 (6): 459–518. doi: 10.2165/00003495-198224060-00002. PMID 6759092.
26. Bartlett JG Medical Management of HIV Infection. Baltimore, Md Johns Hopkins University1999.
27. Keystone, J. S., & Kozarsky, P. (2000). Isospora belli, Sarcocystis species, Blastocystis hominis, and Cyclospora. Mandell, Douglas & Bennett's principles and practice of infectious diseases, 2, 2915-2920.
28. Sones, K., 2001. Pharmaceutical companies: partners or enemies ? ICPTV Newsletter 3, 19-21.
29. Vanderbilt Report (2012): Technical Information: VEEGUM – The versatile ingredient for Pharmaceutical Formulations. R.T. Vanderbilt Company Bulletin No. 91R. R.T. Vanderbilt Company Inc., Norwalk.
30. Brent, W., Gunderson Gigi, H., Ross, K.H.I. and John, C.R. (2001): What Do We Really Know about Antibiotics Pharmacodynamics? Pharmcotherapy, 21, 28-31. https://doi.org/10.1592/phco.21.18.302S.33905.
31. Suni, L., Hiroaki, H., Megumi, M., Hidenori, M., Aoko, K.T., Ying, C., Kozo, U., Masayasu, K., Yasumitsu, N. and Takemi, O.T. (2014): Immunostimulation by Silica Particles and the Development of Autoimmune Dysregulation. In TechOpen, London.
32. Lee, S., Hayashi, H., Maeda, M., Matsuzaki, H., Kumagai-Tkei, N. et al., (2014): Immunostimulation by Silica particles and the development of autoimmune dysregultion. Immune Response Activation, Guy Huynh Thien Duc, IntechOpen, Doi 10.57772/57544.
33. Ezeibe, M.C.O., Okafor, U.C., Okoroafor, O.N., Eze, J.I., Ngene, A.A. et al., (2011): Effect of Aluminum Magnesium Silicate on anticoccidial activity of sulphadimidine. Tropical Veterinarian 29:41-44.

© 2020 Global Journals