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The Anti Trypanosomal Potential of Vanadium: Its Effect on the Lipid Profile of *Trypanosoma congolense in Vitro*

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Abstract

In search for new therapeutic agents against Human African Trypanosomiasis, an in vitro investigation of the impact of vanadium on the lipid profile of *T. congolense*, a causative agent of nagana in animals, was carried out. Purified bloodstream forms of the parasites were incubated with graded concentration (1.0×10^{-1}M to 1.0×10^{-8}M) of V_2O_5 and the effect on total lipid, total polar, total neutral and fractions of both polar and neutral lipid components studied separately. Data from this investigation reveal that vanadium, as its oxide; bring about a reduction in the total lipid content of the parasite by about 48.94% to 52.20% while the total polar and total neutral lipids suffered reduction by about 56% to 60% and 40% to 47% respectively. The effects of the metal on the polar and neutral lipid fractions show greater variation. This reduced level of total and fractional lipid components may pose a challenge to the parasite in terms of membrane structure and function as well as general metabolic activity. Therefore, vanadium may be a basis for a potent chemotherapeutic agent useful against *Trypanosoma species*, the pathogens of trypanosomiasis.

Keywords: Lipid Profile, *T. congolense,* Trypanosomiasis, and Vanadium

Introduction

Various strains of Trypanosoma have been reported to cause much ill health in man and his animals. The disease condition caused by these flagellates is called trypanosomiasis. Trypanosomiasis is diverse in name and causative agents; surra is caused by *T. evansi;* gall by *T. theileri*; souma and acute nagana by *T. vivax*; peracute by *T. simiae;* and nagana caused by *T. congolense* (Hunt, 2002). Thirty six Sub-Saharan countries are generally considered endemic to this disease (WHO, 2008), putting a minimum of 50, 000, 000 people at risk (Hunt, 2007).

Therefore a search for effective chemotherapeutic agents is increasingly imperative.

This work was therefore designed to evaluate the effect of vanadium, as V_2O_5, on the lipid profile of *T. congolense*. Reports have revealed that several metals or their complexes exhibit diverse effects, to various degrees, on the activities of some protozoans (Shuaibu *et al.*, 2001; Urquiola *et al.,* 2006, Benitez *et al.,* 2009a and Benitez *et al.,* 2009b). Vanadium, as one of such metals have been reported to indicate potential anti-trypanocidal activity (Urquola, *et al.,* 2006) interfere with biosynthesis of cholesterol and other biomolecules (Lagerkvist and Okarsson, 2007); have increasing therapeutic uses in diabetes (Mukherjee, *et al.,* 2004; Martiny, *et al.,* 2006) and influenced biosynthesis of lipids in fungus (Anekwe, 1976) and *T. vivax* (Anekwe and Dubal, 983). Vanadium compounds have also been shown to inhibit, markedly, the growth of human tumour colony formation (Faneca, *et al.,* 2009; Mukherjee, *et al.,* 2004; Evangelou, 2002; Anke, *et al.,* 2005; Brownsey and Dong, 2005) and exhibit anti *Leshmania spp.* activity (Silveira, *et al.,* 1999). These reports justify the choice of the metal to undertake an *in vitro* study of its effect against lipid profile of *T. congolense* as an attempt in the search for viable trypanocidal agents.

Materials and Methods

Materials

Male and female wistar strain rats 100-120g body weight as well as, strains of *T. congolense* were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, while laboratory chow were obtained from Nigerian Veterinary Research Institute (NVRI), Vom feed mills. All chemicals were of analytical grade and were obtained from BDH Chemicals Ltd, Poole England except sodium dihydrogen phosphate, DEAE cellulose (type DE52) and silicic acid (60-200 mesh) which were obtained from Sigma Chemical Company, U.S.A. Others like potassium dichromate and sodium citrate were obtained from May and Baker Ltd, Dagenham, England while Kieselgel 60G (silica gel 60G) and Ca_2H_2O were obtained from Machererey Nagal and Duren, Germany.

Growing and Separation of Trypanosomes

Bloodstream *T. congolense* rat adapted strains were grown by inoculating the rats intraperitoneally with host blood and fed with laboratory chow. The parasitemia was monitored by microscopy. Ten rats were used for growing the parasites.

At peak parasitemia (12-14days), the rats were exsanguinated under ether anesthesia with 3.8% sodium citrate as anticoagulant and the blood pull together. The parasites were separated from the whole blood using the method of Lanham and Godfrey (1970).

Incubation of Trypanosomes

For interaction with the metals, 1ml of the buffered trypanosomes was transferred into nine test tubes and to each of the first eight test tubes was added different but equal volume of graded concentrations of V_2O_5 solution and the ninth test tube was used as a control. This set up was done in triplicates. They were then incubated with shaking at room temperature for one hour on Orbital Shaker (Gallenhamp) at the end of which the reaction was stopped by the addition of chloroform: methanol (2:1v/v).

Extraction/Analysis of Lipids

Lipid extraction was carried out in all cases as described by Onwuliri and Anekwe (1993). Briefly, 3.75ml of chloroform : methanol (2:1v/v) was added to the wet paste of the parasites in a conical flask and the material shaken at room temperature for six hours. At the end, the sample was centrifuged for 15minutes at 5000g and the supernatant decanted and kept. The residue was re-suspended in 4.75ml chloroform : methanol : water (2:1:0.8v/v/v), shaken and centrifuged for 15minutes at 5000g. The supernatant was decanted and combined with the previous one. To the combined supernatant, 2.5ml of each of chloroform and water were added and the mixture centrifuged. The lower chloroform phase was withdrawn for purification. Purification of the lipid extract was done as descried by Anekwe and Dubal (1971) by passing the mixture through a column of sephadex G25 and eluted with chloroform : methanol : water (190:10:1v/v/v) at a flow rate of 7.5ml/minute. The purified lipids were dried under vacuum and weighed. The weighed lipids were re-dissolved in chloroform : methanol (2:1v/v) and separated into neutral and polar fractions using silicic acid column chromatography. Identification and quantification of the lipids on thin layer chromatography was done as described by Beach, *et al* (1979).

Results

100mg of *T. congolense* was found to contain about 18.17mg total lipid. However, after treatment with vanadium, there was a general decrease in the total lipid content ranging from 48.94% to 52.20% depending on the concentration of vanadium applied (Table 1).

Table 1: Effect of V_2O_5 on Total Lipid of 100mg *T. congolense*

Molar Concentration of Salt	V_2O_5	
	Average Lipid Weight (mg)	% Lipid Extract
$1x10^{-1}$	08.8034±0.003	48.45
$1x10^{-2}$	08.8978±0.010	48.97
$1x10^{-3}$	09.0850±0.001	50.00
$1x10^{-4}$	08.9814±0.002	49.43
$1x10^{-5}$	08.8742±0.002	48.84
$1x10^{-6}$	08.6870±0.001	47.81
$1x10^{-7}$	08.6853±0.002	47.80
$1x10^{-8}$	09.2776±0.003	51.06
Control	18.1700 ±0.001	100.00

Values are mean ± SD; n=3

Figure 1 shows the decreasing effect of vanadium on the polar and neutral lipid fractions. Neutral lipids suffered a decrease of 40% to 47% while the polar fractions decreased by about 56% to 60%. In the controls, the polar lipids made about 48% while the neutral lipids made about 30% of the total lipid extract of the parasites.

Figure 1: Effects of exposure of polar and neutral lipids of *T.congolense* to increasing V_2O_5 concentration

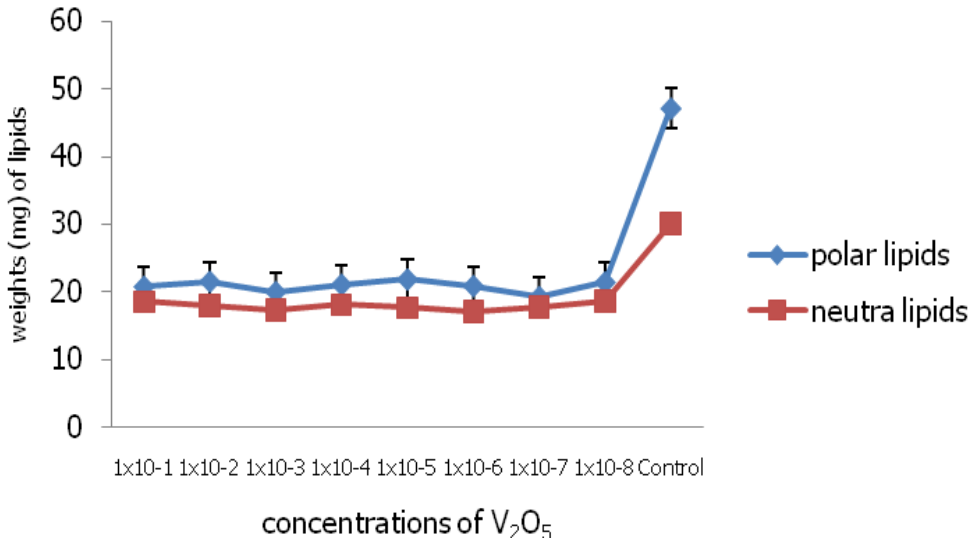


Figure 2: Results of TLC of polar lipid fractions of 100mg *T.congolense* incubated with V_2O_5

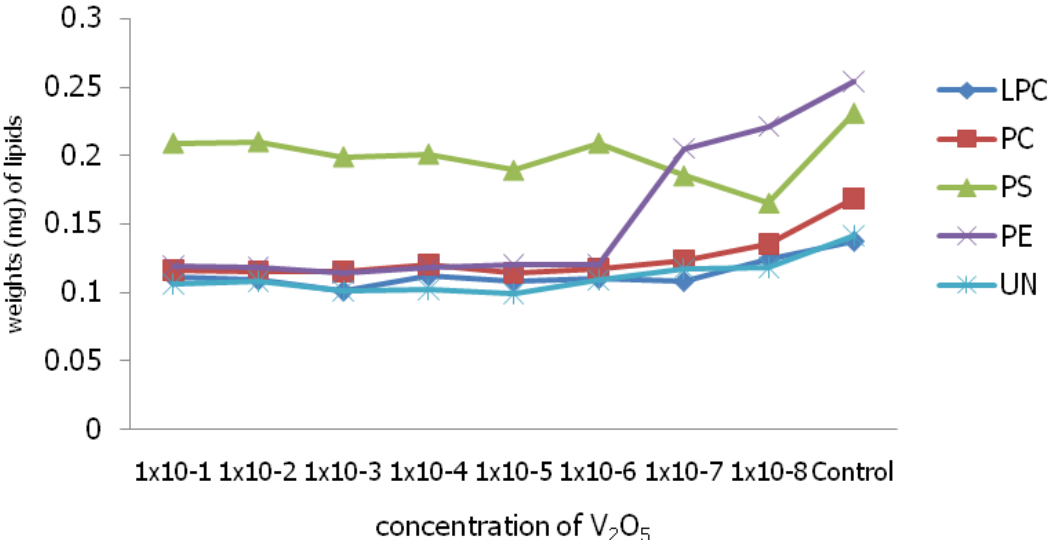
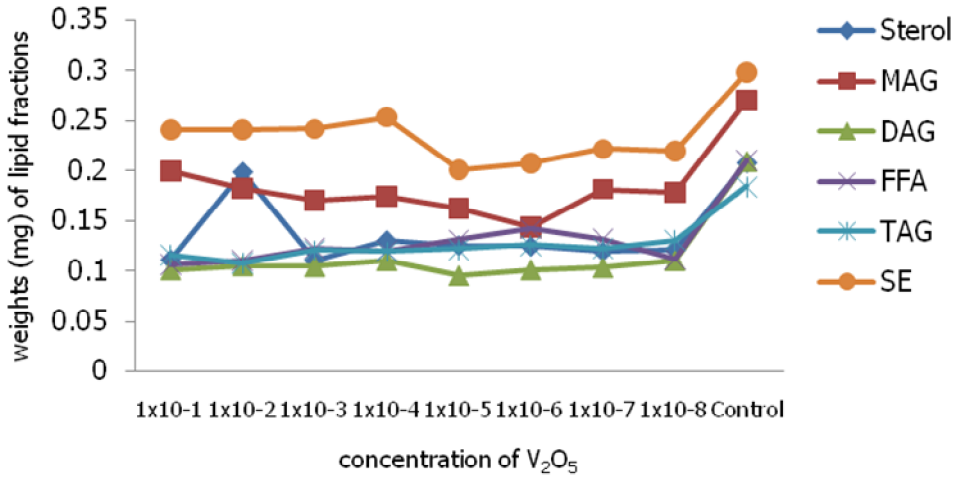


Figure 3: Results of TLC of neutral lipids of *T.congolense* incubated with V_2O_5



The effect of the treatment is also evident on the various polar and neutral lipid fractions. Figure 2 depicts the influence of the metal on the polar lipids. This effect reveals the reduction as follows: Lipophosphatidyl choline (LPC) by 29% to 14%; Phosphatidyl choline (PC) by 18%; Pophosphatidyl serine (PS) by 33%; Phosphatidyl ethanolamine (PE) by 48% to 10%; while the Unknown (UN) by about 1.0%.

The neutral lipid fractions, as shown in Figure 3, equally suffered decrease to diverse extents due to the treatment with the metal. Sterol was reduced by about 48%; Monoacyl glycerol (MAG) by about 18%; Diacyl glycerol (DAG) by 53%; Free Fatty Acids (FFA) by 52% to 5%; Triacyl glycerol (TAG) by 44% to 11% and Sterol Esters (SE) by 14% to 5%.

Discussion

The results of this work reveal lipid composition of 100mg buffered *T. congolense* to be 18.19mg (18.19%). This report agrees with those obtained for other Trypanosoma species. Meyer and Holz (1966) obtained a value of 16% for the monogenic trypanosomatids; Dixon and Williamson (1970) reported a range of 9.15 – 17.20% for the lipid content of mammalian trypanosomes; Oliviera, *et al.,* (1977) reported a value of 15% for the lipid content of the epimastigote form of *T. cruzi* in the lag phase of growth. Anekwe and Egbuna (1983) reported a value of 13% - 15% for lipid content of *T. vivax*.

The post vanadium incubation analysis showed a reduction in the level of lipids in the parasites This agrees with the findings of Curran (1954) that vanadium decreased the incorporation of labeled acetate into cholesterol; Anekwe (1981) that vanadium depressed lipid biosynthesis in rat liver; Egbuna and Anekwe (1982) that the total lipids of *T. vivax* incubated in the presence of vanadium suffered 13% inhibitions.

The influence of the metal on the fractional components (polar and neutral) of the lipids agrees with reports in literature. Anekwe (1976) reported that vanadium inhibited the profile of some major neutral and polar lipids in some parasites. Also, Egbuna and Anekwe (1982) reported the following reductions in neutral lipid contents: Sterol (11%), Diacyl glycerol (71%), Free fatty acids (50%), Triacyl glycerol (33%), Monoacyl glycerol (0%); and in polar lipid contents: Lipophosphatidyl choline (77%), Phosphotidyl choline (67%), phosphatidyl ethanolamine (negligible), and phosphotidyl serine (0%).

The effect of vanadium on the lipid profile could be explained on the basis of reports of this transition metal inhibiting the actions of some metabolic enzymes. Evangelou (2002) reported that vanadium inhibits tyrosine phosphatase; Waters, Gardner and Coffin (1973) reported decrease in the specific activities of acid phosphatase, a lysosomal indicator enzyme on treatment with vanadium; Brownsey and Dong (1995) reported the inhibition of cyclic AMP-dependent phosphorylation of Kemptide by about 50% upon incubation of freshly prepared fat-pad supernatant with vanadyl-gluthatione.

Lagerkvist and Oskarson (2007) reported that although the absorption of vanadium from the gastrointestinal tract is poor, its metabolic effects include interference with the biosynthesis of cysteine, cholesterol, phospholipid and serotin in animals. Mukherirjee (2004) also reported that salts of vanadium interfere with an essential array of enzymatic systems such as different ATPase, protein kinases, ribonucleases and phosphotases. Additional information reveals that complexes of vanadium exhibit anti trypanocidal activity which electrophoretic analysis suggests that the mechanism of action could include DNA interaction (Benitez et al., 2009a and b). Also, the anti-trypanocidal activity of the metal has been linked to its lipophilicity (Evangelou, 2002).

It is therefore possible that the effect of the metal on the lipid profile may become a reason for the parasites' subsequent low level of activity, non pathogenicity and even death. This may be a basis for the preparation of potent chemotherapeutic agents against, not only, *T.congolense,* but all species of Trypanosoma.

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