Iron chelation excludes protein synthesis inhibition in the tetracycline management of African trypanosomosis

Justine T. EKANEM*, Titilayo O. JOHNSON, Iyabo S. ADENIRAN and Valeelat OKEOLA

Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Received 12 December 2003

MS/No BKM/2003/057, © 2004 Nigerian Society for Experimental Biology. All rights reserved.

Abstract

Ribonucleotide reductase, an iron requiring enzyme necessary in the production of deoxyribonucleotides required for replication in cell division and proliferation is induced during the S phase of the cell cycle. We have compared the trypanocidal properties of four antibiotics that show bactericidal activities by destabilizing ribosome-mRNA complex to inhibit protein synthesis. Tetracycline and oxytetracycline that have iron chelating properties extended the lifespan of trypanosome infected rats from 6 and 5 days of control to 15 and 12 days respectively while chloramphenicol and streptomycin that have no iron chelating properties could not extend the lifespan of infected rats. We confirm our earlier report that iron chelation plays a prominent role in the tetracycline management of African trypanosomosis.

Key words: Tetracycline, iron chelation, *T. brucei*, growth inhibition

*Author to whom all correspondence should be addressed.

E-mail: jtekanem@scientist.com , **Tel:** 08033880310

INTRODUCTION

Trypanosoma brucei, the causative agent of African trypanosomiasis divides and proliferates by binary fission in the process of establishing an infection in the host. Parasitaemia has been shown to correlate with the severity of the disease¹.

A major requirement of rapidly dividing cells including trypanosomes is the intracellular availability of unbound iron required by ribonucleotide reductase^{2,3} in the synthesis of deoxyribonucleotides (dNTPs) from ribonucleotides (NTPs). RNR is responsible for the production of all the four dNTPs required for replication, a necessary step in cell division and proliferation⁴⁻⁷. Iron chelation inhibits ribonucleotide reductase⁸⁻¹⁰. The enzyme has been suggested as an enzyme of promise for controlling African trypanosomiasis¹¹.

Ribonucleotide reductase in itself is a cell cycle dependent enzyme¹². Its activity peaks at the synthetic (S) phase of the cell cycle and falls at other phases. The protein of the enzyme is synthesized from its gene by induction during the S phase¹². Many antibiotics with bactericidal properties exert their effects by inhibiting the induction of proteins or specific enzymes from their genes¹³.

We have earlier reported that tetracycline^{14,15} and oxytetracycline¹⁶ can be useful in the clinical management of African trypanosomiasis based on earlier reports that these antibiotics have iron chelating properties¹¹. They are however well documented protein synthesis inhibitors that destabilize ribosome-mRNA complex during protein synthesis^{13,18}. Other antibiotics that also destabilize the complex are chloramphenicol and streptomycin^{13,18}. They however do not have iron chelating properties¹⁸.

In this report we have investigated the possibility of protein synthesis inhibition contributing to the possible management of African trypanosomiasis by tetracycline.

MATERIALS AND METHODS

Federe strain of African trypanosomes, *Trypanosoma brucei*, was obtained from the

Veterinary and Livestock Studies Department, Nigeria Institute for Trypanosomiasis Research (VLS – NITR), Vom near Jos in Plateau State of Nigeria. The parasite was injected into the rats intraperitoneally and maintained by repeated passages into other rats.

Antimicrobial drugs

Four antimicrobial drugs were used to treat the experimental rats after infection. Injectable form of oxytetracycline HCl was a product of Haemexmedical Limited, Teviot Street, London, U.K, streptomycin sulphate B.P was a product of RIKA Pharma GmbH, Hamburg, Germany and chloramphenicol B.P was a product of Greenfield International Ltd. London- England. Tetracycline was a product of Sigma Chemical Company, England.

Inoculation of parasite

The tail of an infected rat was cleaned with a damp cloth and tip of the tail was cut with a clean pair of scissors. Then the blood was extruded into about 0.5ml normal saline. The extruded blood and saline solution was swirled to mix and drawn into a 1ml syringe. A drop of the solution was placed on a microscope slide and observed under the light microscope to ascertain the presence of the parasite in the solution. Inoculation into the peritoneal cavity of an uninfected rat was carried out when parasite suspension contained 3 or 4 trypanosomes per view at x100 magnification.

Parasite count

Parasitaemia was determined by counting the number of trypanosomes per view under the light microscope at x100 magnification from a thin blood smear obtained from the tip of the tail of an infected rat. The Parasitaemia was recorded at 12 hour interval until the rats died.

Administration of drugs

Tetracycline, oxytetracycline-HCl, streptomycin and chloramphenicol were all injected intraperitoneally into the animals twice daily at various concentrations as recommended by manufacturers taking the body weights of the rats into consideration. The administration

started on the first day of sighting parasite in the blood and was continued until the rats died.

Tetracycline:

50 mg of tetracycline mixed with a small quantity of water was made up to 100ml. Volumes corresponding to 10 mg/Kg rat weight were administered to the rats.

Chloramphenicol: 500mg of streptomycin was dissolved in 10ml of distilled water. Volumes corresponding to 40mg/kg body weights were administered to the rats.

Streptomycin: 5mg of streptomycin was dissolved in 10ml of distilled water. Volumes corresponding to 15mg/kg and 30mg/kg body weight of the animals were administered to the rats.

Oxytetracycline-HCl: The injectable solution was obtained. The solution contained 50mg of oxytetracycline-HCl per ml. Volumes

corresponding to 40mg/Kg body weights were administered to the rats.

Control: Control rats (n=3) were infected with the parasite *Trypanosoma brucei* and allowed to develop without treatment. The parasitaemia was recorded until the death of the animals. Another group of three uninfected rats was also treated with the antimicrobial drugs for 21 days without infection to ascertain survival of treated animal in presence of the drugs.

RESULTS

Treatment with the Drugs

The parasitaemia of untreated rats increased infinitely and the rats died on day 5. The uninfected rats survived the treatments for 21 days. The treatment of *Trypanosoma brucei* infected rats immediately parasite was first sighted in the blood with chloramphenicol is shown in Fig 1.

Fig 1. Treatment with Chloramphenicol. Parasitaemia of *T. brucei* infected rats treated twice daily with chloramphenicol as the infection progressed until death. Treatment of rats started on the day parasite was first sighted in the blood with 40 mg/kg body weight dosage of rats. Control rats were infected but not treated. The parasite count was made at 12 hour interval. Each point is an average of count from three rats.

Chloramphenicol when administered at 40mg/kg body weight did not extend the life span of the infected rats considerably more than the control group since the animals died on day 6 which is a day after death was recorded for the control group. A lower parasitaemia was however observed during the duration of the infection before day 6 that the animals died.

The results obtained for the treatment of infected rats when parasite was first sighted in the rats using streptomycin are shown in Fig. 2. At 15mg/kg dosage, steady increase in parasitaemia

was recorded and death ultimately on day 5 as in the control. At double the dosage, 30 mg/Kg body weight, there was steady increase in parasitaemia until day 4 when the rats died. Thus, there was no extension in the life span of the animals beyond that of the control. Parasitaemia was generally lower than those of the control in both cases.

Oxytetracycline-HCl extended the lifespan from 5 to 12 days as seen in Fig. 3 and tetracycline did not only extend the lifespan to 15 days but also cleared parasitaemia by the 13th day (Fig. 4).

Fig 2. Treatment with streptomycin. Parasitaemia of *T. brucei* infected rats treated with Streptomycin twice daily as the infection progressed until death. Treatment of rats started on the day parasite was first sighted in the blood with 15 mg/Kg and 30 mg/Kg body weight of rats. Control rats were infected but not treated. The parasite count was made at 12-hour interval. Each point is an average of count from three rats.

Fig 3. Treatment with oxytetracycline-HCl. Parasitaemia of *T. brucei* infected rats treated with oxytetracycline-HCl twice daily as the infection progressed until death. Treatment of rats started on the day parasite was first sighted in the blood with 40 mg/Kg body weight of rats. Control rats were infected but not treated. The parasite count was made at 12-hour interval. Each point is an average of count from three rats.

Fig 4. Treatment with tetracycline. Parasitaemia of *T. brucei* infected rats treated with tetracycline twice daily as the infection progressed until death. Treatment of rats started on the day parasite was first sighted in the blood with 10 mg/Kg body weight of rats. Control rats were infected but not treated. The parasite count was made at 12-hour interval. Each point is an average of count from three rats.

DISCUSSION AND CONCLUSION

On invasion of a mammalian system, trypanosomes proliferate rapidly through binary cell division to establish its population in the infected host¹⁹. Toxins are released into the mammalian system by the trypanosomes²⁰ and this eventually kills their host. Generally, trypanosomosis lowers the resistance of infected host to other diseases and worse still, it leads to sub-optimal immune responses after vaccination^{21,22}.

Iron plays a pivotal role in the synthesis of DNA in trypanosomal system through the enzyme ribonucleotide reductase^{23,24}. If a biological system is starved of iron, the enzyme ribonucleotide reductase becomes inactivated²⁵⁻²⁷. Previous studies have revealed that growth of cells even those of trypanosomes can be successfully inhibited by depriving the cells of iron²⁷. Consequently, iron-chelation has thus been adopted as an attractive and possible strategy for new anti-parasite drugs and treatment of sleeping sickness^{11,28}. Ribonucleotide reductase is a cell cycle induced enzyme¹² that is equally dependent on continuous availability of recyclable iron².

The antibiotics used in this study are all established protein synthesis inhibitors and in addition tetracycline and oxytetracycline are reported iron chelators¹⁷ that we have earlier reported as possible candidates for clinical management of African trypanosomosis¹⁴⁻¹⁵. Chloramphenicol and streptomycin do not chelate iron¹⁸.

Chloramphenicol and streptomycin have been shown in this study to be ineffective against the protozoan parasite *Trypanosoma brucei* (Figs. 1 and 2). Their ineffectiveness could be attributed to their inability to chelate iron. In fact, chloramphenicol has an untoward effect of increasing serum iron²⁹. Tetracycline and oxytetracycline notably extended the lifespan of *T. brucei* infected rats (Figs 3 and 4).

The results suggest that protein synthesis inhibition contributes minimally to reduction in parasitaemia observed at the early stages of infection and do not play a part in the extension of the lifespan of infected rats as observed in the treatment with the tetracycline group. We confirm our earlier result that iron chelation plays a prominent role in the suggested tetracycline management of African sleeping sickness.

REFERENCES

1. **Murray, M., Morrison, W. Y. and White-Kiv, D. V. (1988)** Host susceptibility to African trypanosomosis, trypanotolerance: *Adv. Parasitol.* **21**:52-57.
2. **Thelander, L., Graslund, A. and Thelander (1983)** Continual presence of oxygen and iron required for mammalian ribonucleotide reduction: Possible regulation mechanisms. *Biochem. Biophys. Res. Comm.* **110**:859-865.
3. **Stubbe J. (2003)** Di-iron tyrosyl radical ribonucleotide reductases. *Curr. Op. Chem. Biol.* **7**:183-188
4. **Reichard P. (1998)** Interactions between deoxyribonucleotide and DNA synthesis. *Ann. Rev. Biochem.* **57**:349-374
5. **Chabes A. and Thelander L. (2003)** DNA building blocks at the foundation of better survival. *Cell Cycle* **2**:172 173
6. **Burton T. R., Kashour T., Wright J. A. and Amara F. M. (2003)** Cellular signaling pathways affect the function of ribonucleotide reductase mRNA binding proteins: mRNA stabilization, drug resistance, and malignancy (Review). *Int. J. Oncol.* **22**:21-31
7. **Dormeyer, M., Reckenfelderbaumer, N., Ludemann, H. and Krauth-Siegel, R. L. (2001)** Trypanothine dependent synthesis of Deoxyribonucleotides by *Trypanosoma brucei* Ribonucleotide Reductase. *J. Biol. Chem.* **276**:10602-10606.
8. **Cooper, C. E., Lynagh, G. R., Hoyes, K. P., Hider, R. C., Cammack, R. and**

- Porter, J. B. (1996).** The relationship of intracellular iron chelation to the inhibition and regeneration of human ribonucleotide reductase. *J. Biol. Chem.* **271**:20291-20299.
9. **Nyholm, S., Mann, G. J., Johannaon, a. G., Bergerson, R. J. Graslund, A. and Thelander, L. (1993)** Role of ribonucleotide reductase in inhibition of mammalian all growth by potent iron chelators. *J. Biol Chem.* **268**: 26200-26205
 10. **Romeo, A. M., Christen, L., Niles, E. G. and Kosman, D. J. (2001)** Intracellular chelation of iron by Bipyridyl inhibits DNA virus replication - Ribonucleotide reductase maturation as a probe of intracellular iron pools. *J. Biol. Chem.* **276**:243301-24308.
 11. **Ekanem, J. T. (2001)** Ribonucleotide reductase as target for drug discovery against African sleeping sickness. *NISEB Journal* **1**:287-292
 12. **Thelander, L. and Reichand, P. (1997)** Ribonucleotide reductase. *Annu. Rev. biochem.* **48**:133-158.
 13. **Aharonowitz, Y. and Cohen, G. (1981)** The microbial production of pharmaceuticals. *Sci. Am.* **245**:106-118.
 14. **Ekanem, J. T., Johnson T. O. and Obaleye, J. A. (2002)** Tetracycline: possible cheap drug in the management of African sleeping sickness. *NISEB Journal* **2**:83-87.
 15. **Johnson T. O. and Ekanem J. T. (2003)** Effect of tetracycline on late-stage African trypanosomiasis in rats. *Biokemistri* **14**:51-56
 16. **Ekanem J. T. and Adeniran I. S. (2003)** Oxytetracycline in the possible management of African trypanosomosis. *Biokemistri* **15**:67-75
 17. **Grenier, D., Huot, M. and Mayrand, D. (2000)** Iron- chelating activity of Teracyclines and its impact on the susceptibility of *Actinobacillus antinomycetemcomitans* to these Antibiotics. *Antimicrob. Agents Chemother.* **44**:763-766
 18. **Sande, M. A. and Mandell, G. L. (1980)** Antimicrobial agents: The aminoglycosdides, Tetracyclines and Chloramphenicol. In Goodman and Gilman, S. *The Pharmacological basis of therapeutics*, 6th edition. Macmillan Publishing Co., Inc., New York.
 19. **Vickerman, K. (1985)** Developmental cycles and Biology of pathogenic trypanosomes. *Brit Med. Bull.* **4**:105-144.
 20. **Ekanem, J. T., Akanji, M. A. and Odutuga, A. A. (1996)** Extracellualr proteins of *Trypanosoma brucei* origin lyse erythrocytes of rats *in vitro*. *Biokemistri.* **6**:21-29.
 21. **Twinamasiko, E. K. and Kakaire, D. W. (1994)** The impact of the antibody response to rinderpest vaccination under field conditions. *Bull. Anim. Hlth. Prod. Afri.* **42**.
 22. **Okuna, N. (2001)** African animal trypanosomosis: Nagana pp. 90-97. In J.K. Mukiibi (ed) *Livestock Disease and Pest. Agriculture in Uganda*, Vol. iv. National Agricultural Research Organisation. Fountain Publishers/CTA/NARD.
 23. **Hofer, A., Schmidt, P. P., Graslund, A. and Thelander, L. (1997)** Cloning and Characterization of the R1 and R2 subunits of ribonucleotide reductase from *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. USA.* **94**:6959-6964.
 24. **Hofer, A. Ekanem, J. T. and Thelander, L. (1998)** The Allosteric regulation of *Trypanosoma brucei* Ribonucleotide reductase studied *in vitro* and *in vivo* *J. Biol. Chem.* **273**:34098-34104.
 25. **Chabes, A., Domkin, V., Larsson, G., Liu, A., Grashund, A., Wijmenga, S. and Thelander, L. (2000)** Yeast ribonucleotide reductase has heterodimeric iron-radical containing subunit. *Proct. Nat. Acad. Sci., USA.* **97**:2447-2479.
 26. **Chitamber, C. R., Wereley, J. P., Herman, T., Antholine, W. E. and O'Brien, W. J. (2000)** Cellular adaptation of down-regulated iron transport into lymphoid leukaemia cells: effects on the expression of the gene for ribonucleotide reductase. *Biochem. J.* **345**:681-685.

27. **Fast, B. Kremp, K., Boshart, M. and Steverding, D. (1999)** Iron-dependent regulation of transferrin receptor expression in *Trypanosoma brucei*. *Biochem. J.* **342**:691-696.
28. **Breidbach, T., Scory, S., Krauth-Siegel, R.L. and Steverding, D. (2002)** Growth inhibition of bloodstream forms of *Trypanosoma brucei* by the iron chelator deferoxamine. *Int. J. of Parasitology.* **32**:473-479.
29. **Aiello, S. E., and Mays, A., (1998)** The Merck Veterinary Manual, 8th ed. Merck & Co. Inc. Whitehouse Station, N. J. USA.