ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1999, p. 985–987 Vol. 43, No. 4
0066-4804/99/$04.00+0

The Anti-Influenza Virus Drug Rimantadine Has Trypanocidal Activity

JOHN M. KELLY,[1]* MICHAEL A. MILES,[1] AND ANITA C. SKINNER[2]†

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT,[1] and Division of Virology, National Institute for Medical Research, The Ridgeway, London NW7 1AA,[2] United Kingdom

Received 24 November 1998/Accepted 27 January 1999

We report here that bloodstream forms of the African trypanosome, *Trypanosoma brucei*, are sensitive to the anti-influenza virus drug rimantadine (50% inhibitory concentration of 1.26 μg ml^{-1} at pH 7.4). The activity is pH dependent and is consistent with a mechanism involving inhibition of the ability to regulate internal pH. Rimantadine is also toxic to the trypanosomatid parasites *Trypanosoma cruzi* and *Leishmania major*.

In recent years there has been a major resurgence of African sleeping sickness, with an estimated 300,000 people affected (21). In humans, the disease is caused by infection with the tsetse fly-transmitted protozoan parasites *Trypanosoma brucei gambiense* (western and central Africa) and *Trypanosoma brucei rhodesiense* (eastern and southern Africa). Untreated, sleeping sickness is fatal. Current chemotherapeutic regimes are unsatisfactory (7, 15); they require hospitalization and are expensive, can fail to eradicate parasitemia, and often produce toxic side effects. For example, melarsoprol is the only effective drug for the advanced stage of sleeping sickness, which occurs once parasites have invaded the central nervous system. However, melarsoprol treatment can cause arsenic encepalopathy and results in 5 to 10% patient mortality. Consequently, the development of new trypanocidal drugs is a major priority of the World Health Organization (WHO).

In the course of work aimed at expressing modified forms of the viral M2 protein in trypanosomes, we noticed that the anti-influenza virus agent rimantadine (α-methyl-1-adamantane methylamine hydrochloride) was highly effective at killing bloodstream forms of *T. brucei* in vitro. Overnight incubation in medium containing more than 5 μg ml^{-1} resulted in the death of all cells in the culture. Rimantidine is an amide derivative of amantadine, and both drugs are licensed for the treatment and prophylaxis of influenza A (5). To establish the extent of this activity, bloodstream form *T. brucei* (strain 427) was cultured in the presence of rimantadine or amantadine, and the concentrations of the drugs which inhibited growth by 50% (IC_{50}) and 90% (IC_{90}) were determined. Both compounds were found to have trypanocidal activity, with rimantadine being more effective (Table 1). In these experiments, the cells were incubated for 3 days at 37°C in 4-ml volumes of modified Iscove's medium (pH 7.4) (10) in 25-cm^3 flasks. The density of untreated cultures increased from 1×10^5 cells ml^{-1} to 4×10^6 cells ml^{-1} under these conditions. We next investigated the effect of pH on rimantadine activity and found that above neutral it had increased toxicity (Fig. 1A and Table 1). For example, 2 μg of rimantadine ml^{-1} inhibited cell growth by 70% at pH 7.4 (the normal pH of blood), whereas at pH 7.0, *T. brucei* grew at a similar rate with or without the drug (Fig. 1A). At higher drug concentrations (20 μg ml^{-1}) rimantadine was toxic at all pH levels tested (Fig. 1A). Amantadine was also found to exhibit activity, but only at concentrations of the drug that are not physiologically attainable. As with rimantadine, the trypanocidal effect was pH dependent and was enhanced in an alkaline environment (Table 1).

We then determined whether rimantadine was active against the trypanosmatid parasites *Trypanosoma cruzi* (which causes Chagas' disease, or American trypanosomiasis) and *Leishmania major* (a causative agent of cutaneous leishmaniasis). The *T. cruzi* and *L. major* growth inhibition experiments were carried out in Nunclon 24 well plates in 2 ml of growth medium. In the case of *T. cruzi* (CL Brener clone) (3), the initial inoculum was 2×10^5 epimastigotes ml^{-1}. After 5 days of incubation at 28°C in RPMI medium (13) in the absence of drug, the cell density increased to 2×10^7 ml^{-1}. With *L. major* (strain 5ASKH), 2×10^5 promastigotes ml^{-1} were incubated in minimal essential medium (6) at 24°C. Under these conditions, the growth rate was similar to that of *T. cruzi*. Both parasites were found to be susceptible to rimantadine, although to a lesser extent than *T. brucei* (Fig. 1). At pH 7.8, the IC_{50}s for *T. cruzi* epimastigotes and *L. major* promastigotes were 6.0 and 10.5 μg ml^{-1} respectively. The response of *T. cruzi* cells to rimantadine treatment was characterized by swelling and loss of typical epimastigote morphology, including the flagellum. *T. cruzi* and *L. major* also exhibited pH-dependent sensitivity to rimantadine (Fig. 1B and C). In an alkaline environment they were more susceptible, whereas at an acidic pH they were

TABLE 1. Susceptibilities of the cultured bloodstream form of *T. brucei* to rimantadine and amantadine

Drug	pH of medium	IC_{50} (μg ml^{-1})	IC_{90} (μg ml^{-1})
Rimantadine	7.4	1.26 ± 0.05	2.50 ± 0.30
	7.6	0.98 ± 0.10	1.65 ± 0.19
Amantadine	7.4	>20	>20
	7.6	13.8 ± 1.7	19.1 ± 0.5

[a] IC_{50} and IC_{90}s were obtained from plots and are expressed relative to the growth of untreated cells at the corresponding pH (Fig. 1). Values shown are the means of three experiments ± standard deviations from the mean.

* Corresponding author. Mailing address: Department of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel St., London WC1E 7HT, United Kingdom. Phone: 44 171 927 2330. Fax: 44 171 636 8739. E-mail: j.m.kelly@lshtm.ac.uk.

† Present address: Eastman Dental Institute, University College London, London WC1X 8LD, United Kingdom.

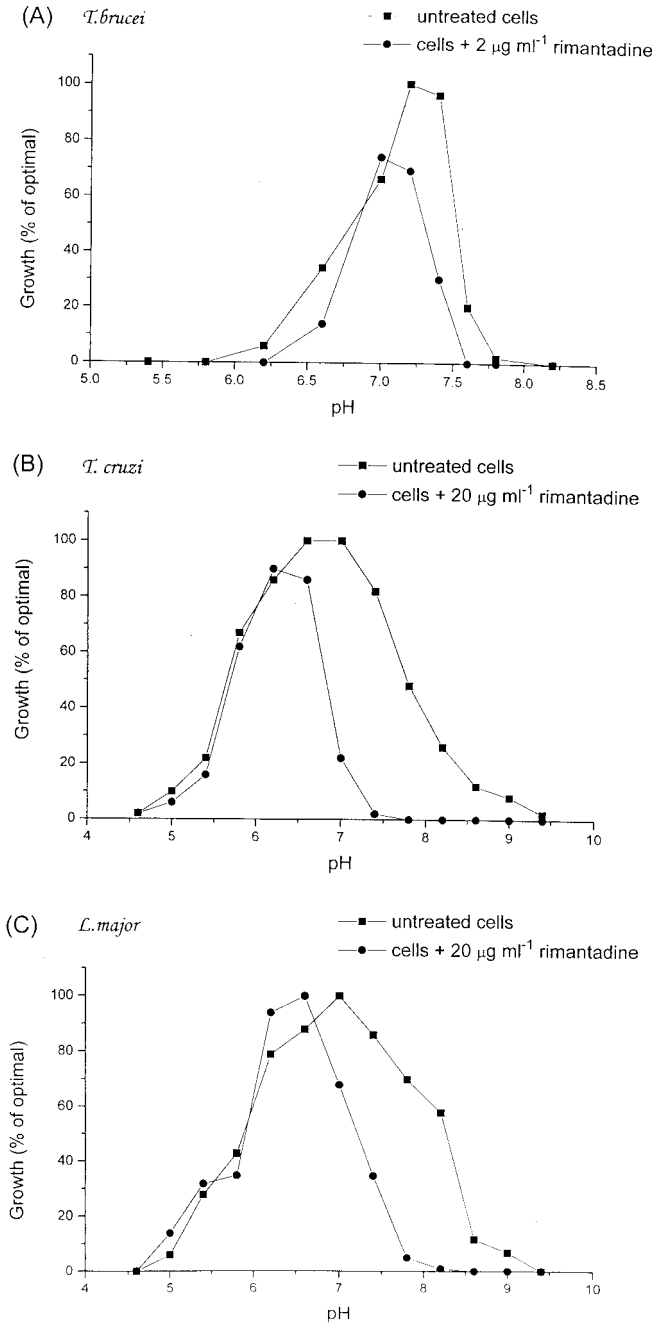

FIG. 1. Susceptibility of bloodstream form *T. brucei* (A), *T. cruzi* epimastigotes (B), and *L. major* promastigotes (C) to rimantadine at different pHs. Parasites were cultured as described in the text in the presence or absence of 2 μg of rimantadine ml^{-1} (A) or 20 μg of rimantadine ml^{-1} (B, C). In the case of *T. brucei*, treatment with 20 μg of rimantadine ml^{-1} resulted in total cell death at each pH tested. Values are expressed as a percentage of growth obtained at optimal pH in the absence of drug.

largely refractory. Consistent with this, rimantadine treatment (20 μg ml^{-1}) of an *L. major*-infected human macrophage cell line (THP-1) had no effect on parasitemia (data not shown). In mammalian cells the intracellular amastigote form of the parasite resides in the acidic phagolysosme compartment. We also examined the effect of amantadine on *T. cruzi*. Under normal culture conditions (pH 7.4), there was no significant growth inhibitory effect at concentrations below 20 μg ml^{-1}. However, greater toxicity was observed at higher pH levels, and at pH 8.4 the IC_{50} was 12 μg ml^{-1} compared with 1.8 μg ml^{-1} for rimantadine under the same conditions.

In influenza A virus-infected cells the target of both rimantadine and amantadine is the viral ion channel protein M2 (8, 16). In its tetrameric form, M2 functions by translocating protons into the virus interior (4, 17, 18), an acidification process which facilitates virus uncoating (19). The drugs bind to an amino acid(s) within the NH_2-terminal portion of the M2 transmembrane region, leading to blockage of the proton channel, probably as a result of conformational changes (17). In trypanosomatids, rimantadine toxicity is associated with a reduced ability to tolerate an alkaline environment (Fig. 1). Therefore, one possibility, by analogy with the situation in influenza virus-infected cells, is that rimantadine blocks a transmembrane proton pump which acts to maintain intracellular pH. Proton-translocating, membrane-localized ATPases (H^+-ATPases) have been identified as the primary mechanism for the maintenance of intracellular pH homeostasis in trypanosomatids (22). The use of genetic (11) and biochemical (2) approaches should make it possible to test if these proton pumps are the target for rimantadine and to investigate the precise mechanism of action.

Rimantadine has many desirable properties as a chemotherapeutic agent. It is inexpensive, can be taken orally, produces fewer side affects than amantadine (9), and readily crosses the blood brain barrier. In addition, the pharmacokinetics have been extensively investigated (20); it is well absorbed from the gastrointestinal tract and in humans has a plasma half-life of 24 to 36 h (1). Serum levels above 1 μg ml^{-1} have been reported (14). The results presented here suggest that rimantadine may have potential as a drug against African sleeping sickness, particularly if it can be administered in combination with agents which elevate blood pH. Furthermore, the considerable difference between the susceptibilities of *T. brucei* to amantadine and rimantadine (Table 1) suggests that the trypanocidal effects of other aminoadamantane derivatives (12) warrant investigation.

This work was funded by the Leverhulme Trust.

REFERENCES

1. **Anderson, E. L., L. P. Van Voris, J. Bartram, H. E. Hoffman, and R. B. Belshe.** 1987. Pharmacokinetics of a single dose of rimantadine in young adults and children. Antimicrob. Agents Chemother. **31:**1140–1142.
2. **Benchimol, F., W. De Souza, N. Vanderheyden, L. Zhong, H.-G. Lu, S. N. J. Moreno, and R. Docampo.** 1998. Functional expression of a vacuolar-type H^+-ATPase in the plasma membrane and intracellular vacuoles of *Trypanosoma cruzi*. Biochem. J. **332:**695–702.
3. **Cano, M. I., A. Gruber, M. Vasquez, A. Cortes, M. J. Levin, A. Gonzalez, W. Degrave, E. Rondinelli, B. Zingales, J. L. Ramirez, C. Alonso, J. M. Requena, and J. Franco da Silveira.** 1995. Molecular karyotype of clone CL Brener chosen for the *Trypanosoma cruzi* genome project. Mol. Biochem. Parasitol. **71:**273–278.
4. **Chizhmakov, I. V., F. M. Geraghty, D. C. Ogden, A. Hayhurst, M. Antoniou, and A. J. Hay.** 1996. Selective proton permeability and pH regulation of the influenza virus M2 channel expressed in mouse erythroleukaemia cells. J. Physiol. **494:**329–336.
5. **Ellsworth, A. J., D. M. Witt, D. C. Dugdale, and L. M. Oliver.** 1998. Mosby's medical drug reference. Mosby-Year Book Inc., St. Louis, Mo.
6. **Evans, D. A.** 1993. In vitro cultivation and biological cloning of *Leishmania*, p. 29–41. *In* J. E. Hyde (ed.), Protocols in molecular parasitology. Humana Press Inc., Totowa, N.J.
7. **Gompel, A. V., and T. Vervoort.** 1997. Chemotherapy for leishmaniasis and trypanosomiasis. Curr. Opin. Infect. Dis. **10:**469–474.
8. **Hay, A. J.** 1992. The action of adamantanamines against influenza A viruses: inhibition of the M2 ion channel protein. Semin. Virol. **3:**21–30.
9. **Hayden, F. G., J. M. Gwaltney, R. L. Van de Castle, K. F. Adams, and B. Giordani.** 1981. Comparative toxicity of amantadine hydrochloride and rimantadine hydrochloride in healthy adults. Antimicrob. Agents Chemother. **19:**226–233.
10. **Hirumi, H., and K. Hirumi.** 1989. Continuous culture of *Trypanosoma brucei*

blood stream forms in a medium containing a low concentration of serum protein without feeder cell layers. J. Parasitol. **75:**985–989.
11. **Kelly, J. M., M. C. Taylor, G. Rudenko, and P. A. Blundell.** 1995. Transfection of the African and American trypanosomes, p. 349–359. *In* J. A. Nickoloff (ed.), Electroporation protocols for microorganisms. Humana Press Inc., Totowa, N.J.
12. **Kolocouris, N., A. Kolocouris, G. B. Foscolos, G. Fytas, J. Neyts, E. Padalko, J. Balzarini, R. Snoeck, G. Andrie, and E. De Clercq.** 1986. Synthesis and antiviral activity of some new aminoadamantane derivatives. J. Med. Chem. **39:**3307–3318.
13. **Miles, M. A.** 1993. Culturing and biological cloning of *Trypanosoma cruzi*, p. 15–28. *In* J. E. Hyde (ed.), Protocols in molecular parasitology. Humana Press Inc., Totowa, N.J.
14. **Patriarca, P. A., N. A. Kater, A. P. Kendal, D. J. Bregman, J. D. Smith, and R. K. Sikes.** 1984. Safety of prolonged administration of rimantadine hydrochloride in the prophylaxis of influenza A virus infections in nursing homes. Antimicrob. Agents Chemother. **26:**101–103.
15. **Pepin, J., and F. Milford.** 1994. The treatment of human African trypanosomiasis. Adv. Parasitol. **33:**1–47.
16. **Pinto, L. H., L. J. Holsinger, and R. A. Lamb.** 1992. Influenza virus M2 protein has ion channel activity. Cell **69:**517–528.
17. **Pinto, L. H., and R. A. Lamb.** 1995. Understanding the mechanism of action of the anti-influenza virus drug amantadine. Trends Microbiol. **3:**271.
18. **Sugrue, R. J., and A. J. Hay.** 1991. Structural characteristics of the M2 protein of influenza A viruses: evidence that it forms a tetrameric channel. Virology **180:**617–624.
19. **Wharton, S. A., R. B. Belshe, J. J. Skehel, and A. J. Hay.** 1994. The virion M2 protein in influenza virus uncoating: specific reduction in the rate of membrane fusion between virus and liposomes by amantadine. J. Gen. Virol. **75:** 945–948.
20. **Wills, R. J., D. A. Farolino, N. Choma, and N. Keigher.** 1987. Rimantadine pharmacokinetics after single and multiple doses. Antimicrob. Agents Chemother. **31:**826–828.
21. **World Health Organization.** 1994. Control of tropical diseases: sleeping sickness. WHO, Geneva, Switzerland.
22. **Zilberstein, D., and M. Shapira.** 1994. The role of pH and temperature in the development of *Leishmania* parasites. Annu. Rev. Microbiol. **48:**449–470.