African Journal of Biotechnology Vol. 2 (11), pp. 474-476, November 2003
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315

Short communication

In vitro activity of commercial formulation and active principle of trypanocidal drugs against bloodstream forms of *Trypanosoma brucei gambiense*

Clarisse Lekane Likeufack[1], Lisette Kohagne Tongue[1], and Philippe Truc[1,2] *

[1]Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Department of Research and Control of Human African Trypanosomiasis, BP 288, Yaounde, Cameroon.
[2]Institut de Recherche pour le Développement, IRD, Research Unit 35 BP 1857, Yaounde, Cameroon.

Accepted 14 October 2003

The *in vitro* trypanocidal activities of 4 commercial formulations Ornidyl®, Pentamidine isethionate®, Germanin® and Lampit® and their corresponding active principles (Dl-α-difluoromethylornithine, pentamidine isethionate, suramine and 5-nitrofuran) were compared against *Trypanosoma brucei gambiense*. Differences of minimum inhibitory concentration (MIC) were observed between Ornidyl® and Dl-α-difluoromethylornithine and between Lampit® and 5-nitrofuran. For RO 15 strain and the comparison of Ornidyl®/ DFMO, the MIC when using the commercial drug was more than twice the MIC value obtained with the active principle. For all 3 trypanosome strains, MICs were identical for Lampit® and 5-nitrofuran but the MIC with the commercial formulation was twice the MIC obtained with the active principle. The active principles, rather than commercial formulations, should be used for standardization of *in vitro* assay protocols.

Key words: *In vitro* activity, trypanocidal drugs, commercial formulations, *Trypanosoma brucei gambiense*.

INTRODUCTION

Several *in vitro* methods have been developed for studying the drug sensitivity of *Trypanosoma brucei gambiense*, the agent of the chronic form of Human African Trypanosomiasis (HAT) or sleeping sickness. The chemotherapy of HAT is based on few drugs (W.H.O., 1998). Cases of treatment failure have been reported in Central Africa (Ollivier and Legros, 2001), and drug-resistant parasites are spreading. For example, in Angola and Sudan, up to 30% of treatment failures has been reported by the World Health Organization (W.H.O.) in 2003.

Within this context, Drug Resistance Network funded by W.H.O. is initiating mapping of drug efficiency against HAT in 9 Central African countries; Cameroon, Gabon, Central African Republic, Chad, Angola, Uganda, Republic of Congo, Democratic Republic of Congo, and Equatorial Guinea. Before starting this work, it is necessary to standardize the *in vitro* drug sensitivity assay so that results obtained from different laboratories can be compared. Unlike in *in vitro* studies on other human parasites, *Plasmodium falciparum* for instance,

*Corresponding author. Mailing address: OCEAC, BP 288, Yaounde, Cameroon. Phone: + 237 984 60 57. Fax: + 237 220 18 54. E-mail: truc@iccnet.cm.

commercial drugs instead of active principles have been mainly used for minimum inhibitory concentration (MIC) or IC_{50} determination for HAT studies (Kaminsky and Brun, 1993). The active principle is a pure compound, and its use is probably more reliable than commercial formulations, especially for long-term or multicentric *in vitro* studies. For drugs obtained from commercial sources, storage and interference of excipients could influence the *in vitro* activity.

In the present study, a comparison of the use of commercial drugs versus active principles for *in vitro* drug sensitivity assay on *T. b. gambiense* is described. In our knowledge, this is the first study about such a comparison for Human African Trypanosomiasis. We tested 3 reference strains (STIB 894, STIB 891, RO15) exposed to Ornidyl®, Pentamidine isethionate®, Germanin® and Lampit® versus their corresponding active principles Dl-α-difluoromethylornithine (DFMO), pentamidine isethionate sodium salt, suramine sodium salt and 5-nitrofuran, respectively.

MATERIALS AND METHODS

Reference strains of *T. b. gambiense* STIB 894, STIB 891, RO15 were isolated in Omugo, northwest Uganda and kindly provided by the Swiss Tropical Institute in Basel, Switzerland (Matovu et al. 2001). Parasites were cultured at 37°C in a 5% CO_2 incubator. The medium used was a 1:1 mixture of RPMI 1640 and MEM, supplemented with 15% heat-inactivated human serum and 5% heat-inactivated fetal calf serum. Additional supplements were 1% 2-mercaptoethanol, 1% L-glutamine, and 1% of a mixture containing 1 mM sodium pyruvate, 0.5 mM hypoxanthine, 0.05 mM bathocuproine sulfonate, 1.5 mM L-cysteine, as previously described (Hirumi and Hirumi, 1994). Cultures were monitored daily with medium change in order to maintain the trypanosomes in the exponential growth phase.

Ornidyl® and Pentamidine Isethionate BP® were kindly provided by Aventis (Antony, France). Germanin®, Lampit®, and 5-nitrofuran were provided by Bayer (Wuppertal, Germany). Dl-α-difluoromethylornithine (DFMO), pentamidine isethionate sodium salt, and suramine sodium salt were purchased from Sigma Chemical Co. (St. Louis, MO). Ornidyl® (eflornithine hydrochloride) was provided in 200 mg/ml aqueous solution. Stock solutions of Lampit® and 5-nitrofuran were prepared in 10 mg/ml DMSO, and further dilutions were prepared in sterile water. The final concentration of DMSO was < 0.1 %. All other drugs were dissolved in sterile water. All drugs and their corresponding active principles have the same molecular weight (MW), except for DFMO (MW 219) and Ornidyl® (MW 237).

The long-term viability assay was used to determine the MIC for both the active principle and the corresponding commercial formulation in the same 24-well plates (Kaminsky and Brun, 1993). The trypanosomes (10^5 parasites/ml) were exposed for 10 days to twofold serial drug dilutions ranging from 0.462 to 7.39 µg/ml DFMO and 0.5 to 8 µg/ml Ornidyl®, 0.2 to 3.2 µg/ml for both suramine sodium salt and Germanin®, 0.0005 to 0.008 µg/ml for both Pentamidine isethionate® and pentamidine isethionate sodium salt, and 0.625 to 10 µg/ml for both Lampit® and 5-nitrofuran. Cultures were monitored and evaluated daily with appropriate medium replacement and addition of fresh drug or active principle every 48 h. The MIC was defined as the lowest concentration at which no trypanosomes of normal morphology and motility could be detected microscopically. Each test was repeated 6 times for each comparison of drug pair.

RESULTS AND DISCUSSION

The MIC was identical at each repetition of test (6) for a given strain and a given active principle or commercial drug. Results are summarized in Table 1. For the 3 trypanosome strains, MICs of two drug pairs, Germanin®/suramine sodium salt and Pentamidine isethionate®/pentamidine isethionate sodium salt, were similar. Therefore, the purity of the drug powder and active principle seemed to be identical. For these two drug pairs, the sodium salt forms and commercial drug/active principle have identical molecular weights.

For the comparison of Ornidyl®/DFMO, the MICs obtained for both STIB 894 and STIB 891 strains were 4 and 3.69 µg/ml, respectively. Using Ornidyl®, Matovu et al. (2001) found similar results within experimental error. For both STIB 894 and STIB 891 strains, the difference of molecular weight between DFMO (MW 219) and Ornidyl® (MW 237), which is due to the presence of a water molecule in the latter, could explain the slight difference observed when MIC was expressed in µg/ml but the MIC was identical (16.9 µM) when expressed as molar concentrations. However, for RO 15 strain, the MIC when using the commercial drug (4 µg/ml = 16.9 µM) was more than twice the MIC value obtained with the active principle (1.85 µg/ml = 8.45 µM). For this strain, Matovu et al. (2001) observed a similar MIC using Ornidyl® within experimental error. Despite the absence of excipients mentioned by the manufacturer and the high solubility of both commercial formulation and active principle in water, the possible reasons underlying the discordant result were not found. Commercial drug is formulated to deliver a stable and reliably absorbed compound. This may well require additives.

For all 3 trypanosome strains, MICs were identical for Lampit® (2.5 µg/ml) and 5-nitrofuran (1.25 µg/ml). Again, the MIC with the commercial formulation was twice the MIC obtained with the active principle. The active principle 5-nitrofuran was provided as micro-fine granules, while Lampit® is tablet. When we dissolved them in DMSO, a slight precipitation was observed with Lampit® but not with 5-nitrofuran. The solubility of Lampit® and 5-nitrofuran seemed to be different. Therefore, 5-nitrofuran may have a higher solubility than Lampit®, which could explain a higher *in vitro* trypanocidal activity. However, there is need to use sensitive and accurate methods (e.g. high pressure liquid chromatography) to investigate this problem of solubility.

Several hypotheses could explain the differences in MIC when using active principle and commercial formulation, such as solubility and interference of

Table 1. *In vitro* activity of trypanocidal drugs.

Strain	MIC (µg/ml)*							
	Ornidyl®	DFMO	Germanin®	suramine	Pentamidine®	pentamidine	Lampit®	5-nitrofuran
STIB 894	4	3.69	0.8	0.8	0.001	0.001	2.5	1.25
STIB 891	4	3.69	0.8	0.8	0.002	0.002	2.5	1.25
R0 15	4	1.85	0.8	0.8	0.001	0.001	2.5	1.25

*Minimum inhibitory concentration observed at each of 6 independent experiments. DFMO, Dl-α-difluoromethylornithine.

excipients in the commercial formulations with the components of culture medium, such as serum. As far as we know, there is no previous study on the comparison of *in vitro* trypanocidal activities between different pharmaceutical formulations and their corresponding active principles. Although MIC determination was considered for many years as a reliable technique, these results must be confirmed using a more accurate technique such as 3H-hypoxanthine incorporation assay (Kaminsky and Brun, 1993). These preliminary results suggest that standardization of *in vitro* assay protocols is required and should be based on the use of active principles to ensure drug quality and purity that permits rationale and therefore to allow comparison of MIC between laboratories.

ACKNOWLEDGMENTS

This work was supported by World Health Organization (CDS/CPE), Geneva, Switzerland, and by the Institut de Recherche pour le Développement (IRD), France. We thank Dr L. K. Basco (IRD/OCEAC), Yaounde, Cameroon for critical reading of the manuscript.

REFERENCES

Hirumi H, Hirumi K (1994). Axenic culture of african trypanosome bloodstream forms. Parasitol. Today 10: 80-84.

Kaminsky R, Brun R (1993). *In vitro* assays to determine drug sensitivities of African trypanosomes: a review. Acta Trop. 54: 279-289.

Kaminsky R, Zweygarth E (1989). Feeder layer-free *in vitro* assay for screening antitrypanosomal compounds against *Trypanosoma brucei brucei* and *T. b. evansi*. Antimicrob. Agents Chemother. 33: 881-885.

Matovu E, Enyaru JCK, Legros D, Schmid C, Seeback T, Kaminsky R (2001). Melarsoprol refractory *T. b. gambiense* from Omugo, north-western Uganda. Trop. Med. Int. Health 6: 407-411.

Ollivier G, Legros D (2001). Trypanosomiase humaine africaine : historique de la thérapeutique et de ses échecs. Trop. Med. Int. Health 6: 855-863.

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