Trypanosoma congolense: The Use Of 4,6-Diamidino-2-Phenylindole (DAPI) in the Akinetoplast Induction Sensitivity Test

Harrison CHITAMBO1,2 and Akira ARAKAWA1
1Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, 1-1 Gakuen-cho, Sakai City, Osaka 593, Japan and 2The University of Zambia, Faculty of Veterinary Medicine, Department of Disease Control, P. O. Box 32379, Lusaka, Zambia

(Received 3 September 1991/Accepted 10 March 1992)


KEY WORDS: Ak-induction, DAPI, trypanosome-sensitivity.

African animal trypanosomiasis control is being seriously affected by the emergence of trypanosome strains resistant to almost all the commonly used trypanocides [5, 19, 21]. Under the field conditions, the incidence of such strains is difficult to assess using the present drug screening methods which normally use mice and/or in vitro assays. Results obtained from the conventional use of mouse infection and treatment method may not correspond with the actual curative doses for cattle [18], and an observation period of more than 30 days is necessary to confirm curative doses. While, T. vivax which is not infective to rodents can not be screened using mice. The present quantitative assays which use in vitro radioisotope or growth inhibition techniques, are also inappropriate for wide-scale application because of the high expertise and the complicated logistics involved in distinguishing drug-resistant from drug-susceptible strains following incubation in the presence of drugs [9, 16].

African trypanosome has a kinetoplast which contains genetic material, kDNA [12–15]. Certain compounds including diminazene aceturate (Berenil, Hoechst) or isometamidium chloride (Samorin, May and Baker) are known to induce akinetoplasmic trypomastigotes, Ak-forms [3, 8, 13, 15]. The rate of the Ak-form induction in trypanosomes following drug exposure has an inverse correlation with the sensitivities to the drug. The more resistant the strain is to the drug, the higher the Ak-rate is. The more susceptible strain is to the drug, the higher the rate is. Thus, drug sensitivities in African trypanosomes can easily be established using the Ak-rates [3, 8].

In this study, we tried to determine whether 4,6-diamidino-2-phenylindole (DAPI), a synthetic trypanocide which readily binds DNA to give rise to a fluorescent DNA-DAPI complex [10], could be of any staining value for rapid detection of the Ak-form.

Three clonal populations of Trypanosoma congolense, derived from two field isolates found in Zambia [2] were used. The cloning was carried out according to the established methods [11]. The Berenil-resistant (BR) clone was derived from the protozoan isolated in Chipata and it had minimal curative dose (MCD) values of 45 mg/kg Berenil and 1 mg/kg Samorin. The Berenil-Samorin-susceptible (BSS) clone and the Samorin-resistant (SR) clone were derived from the protozoan isolated in Chisamba. The BSS-clone had MCD values of 7 mg/kg Berenil and 0.5 mg/kg Samorin and it was maintained under drug-free condition. The SR-clone was derived from a sub-population of BSS-clone by administration of repeated sub-curative Samorin dose to infected mice. After six months, it became resistant to Samorin with a new MCD value of 16 mg/kg but it remained susceptible to 7 mg/kg Berenil.

Four groups of twenty ddY mice weighing 20–30 g each were chosen and intraperitoneally (i.p.) inoculated with each of the trypanosome clones. In the Berenil-treatment groups, one group was inoculated with BR-clone and the other with BSS-clone. In the Samorin-treatment groups, one group was inoculated with SR-clone and the other with BSS-clone. The infected mice were monitored daily for parasitemia. At ≥ 5 × 10³ trypanosomes/ml of blood (6 to 7 days post-inoculation) the treatments were commenced. Three subgroups of five mice each in each of the Berenil-treatment groups were treated (i.p.) with 3.5, 14.0, or 45.0 mg/kg Berenil. Similarly, three subgroups of five mice each in each of the Samorin-treatment groups were treated with a 0.5, 4.0, or 16.0 mg/kg Samorin. Five mice inoculated only with distilled water in each group, served as control. Berenil and Samorin solutions were prepared by dissolving the required quantities of each compound in sterile distilled water just before use.

Just before treatment (0 hr) and 4, 10, 24 and 48 hr post-treatment, wet blood film preparation from each mouse was examined for parasitemia to determine the efficacy of the drug doses used. At 10 hr post-treatment, ten thin blood smear slides for Ak-counts were also prepared from the peripheral blood of each treated mouse [3]. Aparasitemic mice were monitored for at least 8 weeks for any relapses before being declared as permanently cured. Five of the slides from each mouse were fixed in methyl alcohol for 10 min, dried and hydrolyzed at 60°C in 1 N HCl for 3 min, washed and stained for 45 min with 5% Giemsa solution. Ak-induction rates at 10 hr after the treatment for each mouse were derived by determining the percentage of the counts of Ak-forms in a total count of at least 500 trypanosomes per slide, and the final group average of the Ak-rates (n=5) was thus established. The slides were examined using a light microscope (Nikon UFX II A System). The other five slides from each mouse, were fixed in methyl alcohol for 10 min, air dried and hydrolyzed in 1 N HCl for 3 min as before. After washing, the slides were stained for 8 min in 4.6 diamidino-2-phenylindole (DAPI) staining solution (1 μg DAPI / ml distilled water). This staining was followed
by a two minute-double staining with DAPI solution containing one percent mercaptoethanol before rinsing by dipping in clean water for one to two seconds. The air dried slides were examined directly without coverslips or oil immersion, using a fluorescent microscope (Nikon, Japan) with a BV 400 nM filter at × 400 magnification. The Ak-rates were again estimated as before, and photographs were taken at a × 1,000 magnification.

The Ak-forms were clearly distinguished from the intact blood stream-forms both in the Giemsa stained (Fig. 1A and 1B) and, in the DAPI stained (Fig. 1C and 1D) slides. It was also possible to accurately predict the dose efficacies in 98% of the treated mice from the Ak-rates. Consistent correlations between Ak-rates and dose efficacies, with respect to trypanosome sensitivities, were observed in both the Berenil- (Tables 1 and 2) and Samorin- (Tables 3 and 4) treatment groups. In general, all mice at 0 h and all the non-treated control mice had Ak-rates of 0.0 to 0.1%. In contrast, in the treatment

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>10 h AK Value (%)</th>
<th>Dose (mg/kg)</th>
<th>10 h AK Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>0.1±0</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>3.5</td>
<td>25.7±2</td>
<td>26.8±2</td>
<td>NE (0/5)</td>
</tr>
<tr>
<td>14.0</td>
<td>36.0±2</td>
<td>37.0±2</td>
<td>TE (0/5)</td>
</tr>
<tr>
<td>45.0</td>
<td>67.3±2</td>
<td>72.0±0.5</td>
<td>PE (5/5)</td>
</tr>
</tbody>
</table>

a) Doses with different designation (d, e, f & g) had significantly different AK-values, P<0.01 (Student t-test).

b) Mean±Standard deviation (n=5).

c) NA, not applicable; NE, no effect on clearance of parasitemia; TE, temporal clearance of parasitemia resulting in relapse; PE, permanently effective cure with no relapse seen. Numbers in parentheses mean mice per total mice treated.
THE USE OF DAPI IN AKI-TEST 775
detection of Ak-forms. It facilitated a quick and easy slide processing which substantially reduced the overall time from about 60 min in Giemsa staining to only about 20 min in DAPI staining. Under a fluorescent dark field (Fig. 1C), a trypanosome stained with DAPI showed a more distinct kinetoplast and nucleus than those in Giemsa-stained one even at low magnification (× 400). In the Giemsa-stained slides, the kinetoplast could be clearly detected only at high magnification (× 1,000) using an oil immersion objective lens. This makes DAPI staining more convenient when protozoans are in low parasitemia and a sample of large size has to be examined. Some trypanosomes contain chromophil-like cytoplasmic granules which stains similar to the kinetoplast in Giemsa staining. HCl-hydrolysis helps to remove such granules and free RNA [Ono, personal communication]. The HCl-treatment was also essential for reducing the non-specific back ground effects and for enhancing the fluorescence of trypanosomes in the DAPI fluorescence microscopy.

The use of DAPI staining in the Ak-induction test is also economically feasible for wide-scale application because this stain makes fluorescent microscopic observation possible without use of conventional reagents such as buffers, mono-clonal antibodies, conjugates and others. Above all, only one milligram of DAPI prepares enough staining solution for more than one thousand slides. Moreover, the DAPI solution has economical advantage in that it can be successfully stored for three weeks or more under refrigeration (4°C) and in the dark.

ACKNOWLEDGEMENTS. This work was supported in part by financial assistance from the Japanese Ministry of Education, Science and Culture (Mombusho) under a Ph. D. Scholarship grant. We are grateful to Drs. T. Fukata, K. Fukui, K. Kubo, and E. Baba for assisting the study.

REFERENCES