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# In vitro activity of the trypanocidal diamidine DAPI on animal-infective *Trypanosoma brucei brucei*

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## Summary

A mammalian feeder layer system for the continuous cultivation of infective bloodstream forms of *Trypanosoma brucei brucei* has been used for investigating the antitrypanosomal activity of the aromatic diamidine DAPI. The drug was active at concentrations which can be reached under physiological conditions. The minimum effective concentration was 0.05 $\mu$g/ml. Minimum exposure times required for antitrypanosomal activity were dependent on the drug concentrations. Furthermore, DAPI was found to cause toxic side effects on bovine fibroblast feeder layer cells at high drug concentrations (10 $\mu$g/ml), however, at low concentrations (1 $\mu$g/ml), the drug acted selectively on trypanosomes. Both the reproducibility and the high sensitivity to drugs of the system make this assay a valuable technique for chemotherapeutic studies on trypanosomes.

**Key words:** *Trypanosoma brucei brucei;* mammalian feeder layer system; DAPI; trypanocidal in vitro activity.

## Introduction

Resistance has been reported to most of the small number of trypanocides in current use. Often resistance to one compound is accompanied by cross resistance to others (Williamson, 1980). In the case of bovine trypanosomiasis, where chemotherapy is based on the three compounds Berenil, Samorin, and Homidium Bromide, the "sanative pair concept", a combination of prophylaxis and therapy (Whiteside, 1962), has so far helped to reduce resistance to an

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economically acceptable level. However, drug resistance is more problematical for other livestock. Camel trypanosomiasis, for instance, could be therapeutically controlled only with suramin, when Antrycide was removed from the market between 1976 and 1983. For swine trypanosomiasis, no curative drug exists at all (Njogu, 1980). Even in cattle the risk seems to increase. Recently *Trypanosoma congolense* strains, isolated in Kenya, have been reported to show resistance against various trypanocidal drugs (Gitatha, 1981; D. Rottcher, personal communication).

Although new therapeutic compounds are urgently needed, the small and uncertain market for trypanocidal drugs as well as the increasing financial risk of developing new drugs, discourages significant involvement of the pharmaceutical industry (Bauer, 1980). Thus, a great part of the research on novel trypanocides is carried out in non-commercial laboratories, where new compounds are usually synthesized in small amounts and under high labour input. Therefore, in vitro techniques, which require only minute amounts of substance for drug screening, become of considerable importance.

In fact, since Yorke et al. (1929) introduced a method which allowed the maintenance of African trypanosomes of the *brucei*-subgroup in vitro at 37° C for 24 h, the chemotherapeutic activity of potentially trypanocidal compounds has been tested in this or similar systems (reviewed by Hawking, 1963). It should be noted, however, that all such systems failed in one way or another to produce data for the standard drugs which were concordant with in vivo findings.

We have evaluated the suitability of an in vitro system for use in chemotherapeutic studies, namely that of Hirumi et al. (1977a, b), which allowed for the first time the continuous cultivation of animal-infective bloodstream forms of *T. b. brucei* in the presence of bovine feeder layer cells. The aromatic diamidine DAPI was chosen as a model substance. DAPI has been reported to be active in mice infected with *T. b. gambiense*, *T. b. rhodesiense*, and *T. congolense* (Dann et al., 1970). The strong fluorescence of DAPI makes this drug easily and precisely detectable in body fluids and tissues (Kaliwoda, 1976; Kratzer et al., 1984). DAPI has also been under intensive investigation with regards to its therapeutic activity in rodents and cattle (Fink et al., unpublished results). In this paper we have compared the in vitro activity of DAPI to that found in an in vivo situation.

## Materials and Methods

*Trypanosomes:* Animal-infective bloodstream forms of *T. b. brucei* TC 221 propagated in vitro in the presence of a feeder layer of bovine fibroblasts (Hirumi et al., 1977a, b) were used in this study. *Trypanosoma b. brucei* TC 221 has been cloned in vitro from stock S 427 (Hirumi et al., 1980). Prior to selection for experiments, the size distribution of each batch of in vitro-propagated trypanosomes was examined by a Coulter Counter Model $Z_B I$ (Coulter Electronics Ltd., U.K.; aperture size: 70 $\mu$m, aperture current: 1/4, amplification: 2, base channel threshold: 10, window width: 50).

Only trypanosome populations, consisting predominantly of long slender forms, whose distribution curve showed a maximum peak between 25–35 cubic microns, were chosen for experiments. Such trypanosomes were suspended to a concentration of $10^5$/ml in fresh HEPES (25 mM)-buffered RPMI-1640 medium (GIBCO Bio-Cult, U.K.) supplemented with 20% (v/v) heat-inactivated (56°C, 30 min) fetal bovine serum (Flow, U.K.). Plastic tissue culture flasks (25 cm² T-type; Falcon Products, USA, or Costar, USA) containing a confluent feeder layer of bovine fibroblasts received 6 ml of this cell suspension. Starting with such a density, trypanosome populations could be kept in a logarithmic growth phase for at least 24 h (population doubling time: $8.9 \pm 2.0$ h).

*Feeder layer cells:* Lines of fibroblasts derived either from bovine blood or fetal bovine tissues were initiated and maintained as described by Hirumi (1979). Feeder layer cells were cultured in 25 cm² T-type flasks in 4 ml RPMI-1640 medium supplemented with 20% (v/v) heat-inactivated fetal bovine serum. For experiments, $0.8–1.2 \times 10^5$ cells/ml, whose viability had been assayed by eosin staining, were seeded into flasks (4 ml/flask) three days prior to trypanosome inoculation. Such cultures were fed daily with fresh medium and reached confluency by day 3.

*DAPI:* The compound, 6-amidino-2-(4-amidinophenyl)indol, SN 102/198 (batch No. 297/115) was a gift of Prof. Dr. O. Dann, Institute for Food Chemistry and Pharmacy, University of Erlangen/Nuremberg (FRG). The compound was obtained as the bilactate salt, containing 55% active base. DAPI was pure as assayed by thin layer chromotography (Kratzer, 1982). All concentrations referred to were calculated for the active base. A stock solution of 1 mg/ml was prepared by dissolving DAPI in autoclaved triple-distilled water, and sterilized by passing through an 0.2 $\mu$m Acrodisc syringe filter unit (Gelman, USA). From this solution required concentrations were obtained by dilution with sterile triple distilled water. Aqueous DAPI solutions were added to culture medium in a 1:100 (v/v) ratio. In this manner, osmolarity of the drug-containing medium was not altered by more than 0.8%, which had no influence on cultures.

*Experimental design:* For determining the minimum effective concentration (MEC) of DAPI, trypanosomes were inoculated into flasks 4 to 6 h prior to drug addition. At the beginning of drug exposure as well as after various drug exposure periods, trypanosome numbers were determined by haemacytometer counting, using improved Neubauer haemacytometer chambers, 0.1 mm deep (American Opticals Co., USA). Only motile trypanosomes were counted, and dividing stages were counted as one individual. When the drug exposure period exceeded 24 h, trypanosome numbers were reduced by half by replacing 3 ml of medium containing the appropriate drug concentration. This maintained the trypanosomes for another 24-h period in the logarithmic growth phase. In drug exposure periods exceeding 48 h trypanosomes within each experimental group were pooled, counted, and inoculated for further drug exposure at a density of $10^5$ trypanosomes/ml into new flasks containing confluent, 3-day-old feeder layers. Each drug concentration was tested in quadruplicate. For the determination of the minimum exposure time (MET) required to express antitrypanosomal activity in vitro, trypanosomes were exposed to DAPI for various periods of time. After the respective drug exposure periods, trypanosomes were pooled within each experimental group and washed twice with fresh, drug-free medium. After washing, trypanosome suspensions were diluted to a density of $10^5$ trypanosomes/ml and placed into flasks with fresh feeder layers after which drug exposure was either continued or discontinued. Washed trypanosomes of each pool were also used to test infectivity for mice. Each drug concentration as well as each exposure period were tested in triplicate.

*Infectivity test:* Adult male Balb/c mice, weighing 20–25 g, were used for infectivity tests. Each animal received $10^5$ trypanosomes intraperitoneally. For each trypanosome sample to be tested, four mice were inoculated. Tail blood from each inoculated animal was examined for parasitaemia every other day for 30 days as described by Lumsden et al. (1973). Animals, which survived over 30 days postinoculation, were sacrificed and examined for splenomegaly.

# Results

## Minimum effective concentration (MEC)

The relation between different concentrations of DAPI and their antitrypanosomal effects on growth of trypanosome populations during a continuous exposure for 48 h is shown in Fig. 1. Control trypanosomes increased in numbers continuously for 40 h. Parasites exposed to 0.1 $\mu$g/ml showed similar growth characteristics. Exposure to 1.0 $\mu$g/ml resulted in the decrease of trypanosome populations after a period of 11 h. Trypanosomes exposed to 10 $\mu$g/ml were no longer detectable after 40 h, and those exposed to 100 $\mu$g/ml had disappeared within 18 h. In the latter group precipitates of DAPI were observed in the medium, indicating that the actual amount of dissolved drug was less than 100 $\mu$g/ml.

Feeder layers in all groups were examined microscopically after a drug exposure period of 42 h. The number of vacuoles in fibroblasts of the 10 $\mu$g/ml group was increased, and at a higher concentration of drug, the feeder layers were disintegrated and the fibroblasts were partly or totally detached from the flasks.

When trypanosomes were maintained for 48 h without changing the medium, control trypanosomes increased in numbers during the first 40 h, but after 48 h showed low motility. Drug effects observed after 48 h were, therefore, not reliable because of the effect of deteriorating cultivation conditions. However, evaluation of DAPI-induced inhibitory effects on population growth after 24 h was unequivocal. After this exposure period, growth dynamics of trypanosome populations were already significantly different for each drug concentration. Only at a concentration of 0.1 $\mu$g/ml was trypanosome multiplication not inhibited by DAPI.

In view of the above observations, DAPI concentrations without detectable side effects on the feeder layers were chosen for further investigations. As described in Materials and Methods, trypanosomes were cultivated after the respective drug-exposure periods under drug-free cultivation conditions, in order to find out whether drug effects were as effective in vitro as they are in vivo.

Trypanosomes exposed to 1 $\mu$g/ml decreased in number during 24-h exposure. After a period of 48 h no trypanosomes could be detected, regardless of whether drug exposure was stopped or continued after 24 h. Similarly, a 24-h exposure to 0.1 $\mu$g/ml caused irreversible trypanocidal effects, although no adverse effects could be detected at this concentration after 24 h of exposure (Fig. 1), all trypanosomes died during the following 72 h post-exposure in the absence of drug. Whereas trypanosomes proliferated at a mean population doubling time ($t_D$) of 8.7 h during the 24-h exposure, the $t_D$ increased to 18.7 h for the following 24 h post-exposure. Trypanosome populations were reduced by 50% within the second 24-h period, when exposed to the same concentration for 48 h.
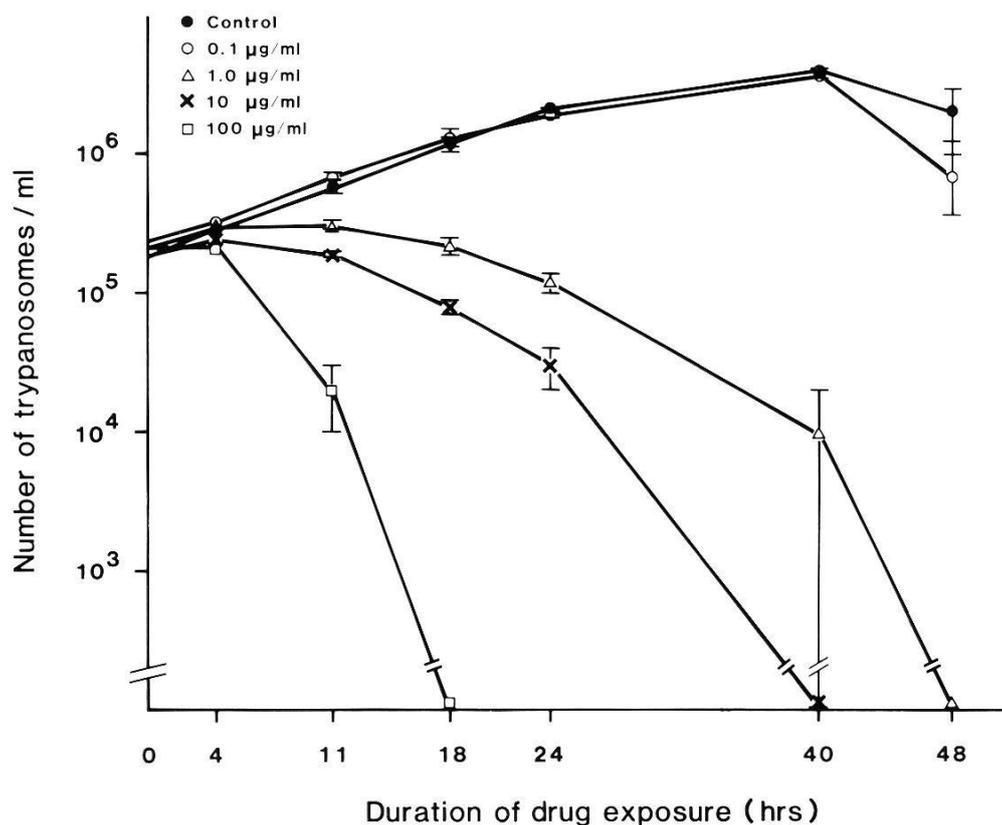
Fig. 1. Growth curves of *T. b. brucei* populations in vitro during continuous exposure to DAPI (for details see Materials and Methods). The data represent mean trypanosome numbers from 4 flasks.

Exposure to 0.1 μg/ml for 24 h resulted in the loss of infectivity for mice. The parasites did not regain their infectivity during the post-exposure cultivation period. Trypanosomes exposed to 0.05 μg/ml for 24 h were also irreversibly damaged. Such trypanosomes died after 48 h post-exposure, although the number of parasites increased 5.6 times during 24 h of drug exposure plus the first 24 h post-exposure. On the other hand, exposure to 0.01 μg/ml for 24 h had no demonstrable effect on the parasites. During 144 h post-exposure, their $t_D$ was within the range of 6.8–11.0 h.

Mice inoculated with trypanosomes exposed to 0.05 μg/ml for 24 h remained negative throughout 30 days post-inoculation, regardless of whether they were inoculated with trypanosomes after 24-h exposure or 24 h post-exposure. However, all mice inoculated with the trypanosomes exposed to 0.01 μg/ml developed infections. Mean survival times of the infected mice did not significantly differ from those infected with unexposed trypanosomes. Thus, 0.05 μg/ml was determined as the MEC of DAPI for *T. b. brucei* TC 221 in culture.

*Minimum exposure times (MET).* In order to find out whether or not a dose-time relationship exists for DAPI, METs required for DAPI to express trypanocidal in vitro activity have been established for the concentrations 0.1 μg/ml, 1.0 μg/ml, and 10.0 μg/ml.

Table 1. Growth of *T. b. brucei* populations after exposure to 0.1 μg DAPI/ml

| Group | Duration of in vitro culture (h) | | | | |
|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 |
| | x | x ± sd | x ± sd | x ± sd | x ± sd |
| A ......... | 1.0 | 12.4 ± 0.75 | 23.9 ± 4.96 | 2.4 ± 0.10 | 13.0 ± 1.87 |
| B ......... | 1.0 | 9.3 ± 1.10 | 10.2 ± 3.05 | 0.2 ± 0.06 | 1.0 ± 0.06 |
| C ......... | 1.0 | 12.3 ± 1.10 | 4.9 ± 1.93 | – | – |
| Control .... | 1.0 | 14.7 ± 1.53 | 29.6 ± 5.73 | 5.3 ± 1.15 | 25.9 ± 2.29 |

Trypanosomes were observed over a period of 96 h after exposure to 0.1 μg DAPI/ml for 1 h (A), 2 h (B), and 4 h (C). Each parasite population in a culture flask was reduced by 50% by replacing a half of the culture medium with the same volume of fresh medium after 24 h and 72 h. After 48 h, trypanosomes of each group were pooled and inoculated in a density of $1.0 \times 10^5$/ml into new flasks (for details see Materials and Methods). Trypanosomes were no longer detectable in group C after post-exposure periods of 72 h and 96 h.
x = Mean number of trypanosomes $\times 10^5$/ml ± standard deviation (n = 3).

Trypanosomes were exposed to 0.1 μg/ml for 1 h (group A), 2 h (group B), and 4 h (group C) (Table 1). During the drug exposure periods trypanosome populations increased in numbers in all groups. The $t_D$ of control trypanosomes was 8.8 h during the entire post-exposure observation period. For group A a $t_D$ of 11.2 h has been calculated for the same period, which is not significantly different from the control $t_D$, which was $8.9 \pm 2.0$ h for the controls of all experiments of this study. Growth patterns in groups B and C were similar to those of the controls during the first 24 h post-exposure. However, after the first 24 h in drug-free medium propagation in group B was reduced, and in group C trypanosome numbers decreased. After 72 h post-exposure, all group C trypanosomes had died, whereas group B populations were reduced to one fifth. Thereafter, group B trypanosomes began to multiply again.

When inoculated with trypanosomes after drug exposure all mice in group A, one in group B, and none in group C developed infections. Trypanosomes of all groups were 100% infective for mice after 48 h post-exposure. Whereas group A trypanosomes showed no response to the drug exposure at all, in groups B and C infectivity for mice was lost after drug exposure. Trypanosomes of groups B and C regained their infectivity after 48 h post-exposure, although only group B trypanosomes recovered from the drug damage, when maintained post-exposure in vitro.

When trypanosomes were exposed to 1.0 μg/ml for 20 min (group D), 40 min (group E), and 60 min (group F) (Table 2), propagation of group D trypanosomes was not significantly different from control trypanosomes for the first 24 h post-exposure. During the following 48 h, however, group D trypanosomes

Table 2. Growth of *T. b. brucei* population after exposure to 1 µg DAPI/ml

| Group | Duration of in vitro culture (h) | | | | |
|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 |
| | x | x ± sd | x ± sd | x ± sd | x ± sd |
| D ......... | 1.0 | 8.2 ± 0.5 | 7.9 ± 1.0 | 0.09 ± 0.08 | 0.1 ± 0.06 |
| E .......... | 1.0 | 6.7 ± 0.5 | 6.1 ± 0.5 | 0.02 ± 0.01 | – |
| F .......... | 1.0 | 5.5 ± 0.3 | 3.8 ± 0.6 | 0.02 ± 0.01 | – |
| Control .... | 1.0 | 8.0 ± 0.6 | 19.2 ± 1.5 | 7.7 ± 2.5 | 12.6 ± 2.1 |

Trypanosomes were observed over a period of 96 h after exposure to 1 µg DAPI/ml for 20 min (D), 40 min (E), and 60 min (F). Each parasite population in a culture flask was reduced by 50% by replacing a half of the culture medium with the same volume of fresh medium after 24 h and 72 h. After 48 h, trypanosomes of each group were pooled and inoculated in a density of $1.0 \times 10^5$/ml into new flasks (for details see Materials and Methods). Trypanosomes were no longer detectable in the groups E and F after a post-exposure period of 96 h.
x = Mean number of trypanosomes $\times 10^5$/ml ± standard deviation (n = 3).

decreased in numbers, and 96 h post-exposure trypanosome populations increased again. The trypanosomes of groups E and F also multiplied for the first 24 h post-exposure, but decreased thereafter in numbers, and, contrary to group D, were no longer detectable after 96 h post-exposure.

Trypanosomes of group D were 100% infective for mice after drug exposure. Those of group E infected only 2 out of 4 animals, whose parasitaemias became detectable as late as 23 days post-inoculation. Group F trypanosomes were not infective for mice. After a post-exposure period of 48 h, however, trypanosomes of the groups D and E were no longer infective, whereas 1 out of 4 mice became infected with group F trypanosomes.

Finally, the effects of exposure to a concentration of 10 µg/ml for 10 min (group G), 20 min (group H), and 30 min (group I) were investigated (Table 3). Trypanosomes of all drug-exposed groups were able to increase in numbers up to 48 h post-exposure, but decreased in number during the following 24 h. Whereas trypanosomes of the groups H and I were no longer detectable 96 h post-exposure, those of group G began to multiply again.

After drug exposure, group G trypanosomes were 100% infective for mice, whereas trypanosomes of the groups H and I failed to establish infections. Also after 48 h post-exposure, trypanosomes of the groups H and I were non infective for mice. In only 2 out of 4 mice, inoculated with group G trypanosomes 48 h post-exposure, parasites were detectable, however, splenomegaly was detected in all mice of this group.

METs required to became irreversibly trypanocidal in vitro were 4 h for 0.1 µg/ml, 40 min for 1.0 µg/ml, and 20 min for 10 µg/ml. Results obtained

Table 3. Growth of *T. b. brucei* populations after exposure to 10 μg DAPI/ml

| Group | Duration of in vitro culture (h) | | | | |
|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 |
| | x | x ± sd | x ± sd | x ± sd | x ± sd |
| G ......... | 1.0 | 6.6 ± 1.3 | 15.3 ± 2.2 | 0.2 ± 0.05 | 0.4 ± 0.2 |
| H ......... | 1.0 | 3.8 ± 0.2 | 11.5 ± 2.7 | 0.07 ± 0.03 | – |
| I ......... | 1.0 | 4.7 ± 1.2 | 9.0 ± 1.7 | 0.02 ± 0.01 | – |
| Control .... | 1.0 | 6.2 ± 0.5 | 14.6 ± 1.4 | 9.4 ± 2.1 | 19.8 ± 3.7 |

Trypanosomes were observed over a period of 96 h after exposure to 10 μg DAPI/ml for 10 min (G), 20 min (H), and 30 min (I). Each parasite population in a culture flask was reduced by 50% by replacing a half of the culture medium with the same volume of fresh medium after 24 h and 72 h. After 48 h, trypanosomes of each group were pooled and inoculated in a density of $1.0 \times 10^5$/ml into new flasks (for details, see Materials and Methods). Trypanosomes were no longer detectable in the groups H and I after a post-exposure period of 96 h.
x = Mean number of trypanosomes $\times 10^5$/ml ± standard deviation (n = 3).

from the infectivity tests were, however, inconsistent, and were, therefore, of little conclusive value.

*Drug effects on feeder layers.* As described above, DAPI was found to have some effect on feeder layer cells in concentrations of 10 μg/ml and 100 μg/ml. Therefore, experiments have been carried out to determine whether or not DAPI also showed detectable effects on feeder layers at lower yet completely trypanocidal concentrations.

Fibroblasts were exposed to DAPI concentrations of 10 μg/ml, 1.0 μg/ml, and 0.1 μg/ml for 72 h, immediately after seeding into flasks (Table 4). Thus, non-confluent fibroblasts were exposed to the drug during a period of actual propagation. In such a phase of highly active metabolism, any cytotoxic effects caused by DAPI should become readily apparent. However, an inhibition in cell propagation could be detected only in the 10 μg/ml group. Thus, DAPI seems to act on trypanosomes selectively at 0.1 μg/ml as well as at 1 μg/ml.

## Discussion

Many different cell-lines of bovine fibroblasts at various passage levels, ranging from passage 3 up to passage 24, were used as feeder layers in the experiments described here. Standardizations of the feeder layer cells, regarding their density and age, made prior to drug testing appeared to be sufficient to obtain reproducible results for in vitro drug assays.

The antitrypanosomal activity of DAPI was demonstrated to be concentration dependent, with a MEC of 0.05 μg/ml. Kratzer (1982) reported serum

Table 4. Growth of bovine fibroblast feeder layer cells during 72 h of exposure to DAPI

| Concentration ($\mu$g/ml) | Total number of cells per flask | Percentage of viable cells |
|---|---|---|
| Control ......... | 10.3* ± 0.9 | 72.8 ± 8.7 |
| 0.1 ............. | 9.8 ± 1.8 | 78.6 ± 21.4 |
| 1.0 ............. | 11.0 ± 1.0 | 75.5 ± 5.5 |
| 10.0 ............ | 4.3 ± 1.1 | 60.5 ± 16.3 |

Bovine fibroblasts were seeded into flasks at a density of $0.9 \times 10^5$/ml and immediately exposed to DAPI. The total exposure time was 72 h. After drug exposure, cells were trypsinized and counted after eosin staining in order to examine cell viability (for details see Materials and Methods).
* Mean number of cells $\times 10^5$ per flask; n = 4.

levels of DAPI, between 0.05–10 $\mu$g/ml in mice receiving curative doses. Thus, the in vitro trypanocidal activity of DAPI found in the present work is in accordance with in vivo results, and effectively illustrates the high sensitivity of the applied in vitro technique.

The precise control of exposure time and actual drug concentration is a substantial advantage of any in vitro screening assay compared to in vivo models. In the case of DAPI it was demonstrated that the drug needed a certain exposure period to produce detectable antitrypanosomal activity, even at a concentration of 100 $\mu$g/ml. The METs required for drug induced damage on trypanosomes were inversely related to the applied concentrations of DAPI.

Trypanosome populations which were exposed to low trypanocidal concentrations, such as 0.1 $\mu$g/ml for 24 h and were maintained thereafter in culture for additional 96 h, were able to increase in numbers 4–5 times before they finally died off. Assuming that the individual trypanosomes acted like the population as a whole, it could be concluded that within the time of survival, depots of essential substance, which could no longer be produced after drug exposure, were consumed by the trypanosomes. In addition, it is possible that drug induced damage was aggravated with every division of the parasites until they reached a lethal level.

Trypanocidal effects in vitro were accompanied by the loss of infectivity for mice, even if the trypanosomes did not show any detrimental effects at the time of inoculation. However, when trypanosomes were exposed to the drug for short periods (e.g. 1 h) results of the infectivity tests became contradictory. Reversibility of drug effects increased obviously with decreasing exposure time. Since those trypanosomes were exposed to DAPI for a considerably shorter time than their population doubling time, such results might be a reflection of different drug susceptibilities within the cell cycle. The bisbenzimidazole Hoechst 33342, for example, with a similar mode of action than DAPI has a significantly higher toxicity for Chinese hamster ovary cells in G2 + M phase than in S phase (Wie-

zorek, 1984). Synchronized trypanosome cultures would probably provide a tool for answering this question, but such cultures are not available at present.

Although the presence of mammalian feeder layer cells might hamper many applications of the system for continuous cultivation of bloodstream forms of *T. b. brucei*, especially with regard to drug assays, they provide an opportunity to examine possible side effects of drugs on bovine cells. DAPI could be shown to be toxic for the feeder layer cells in high concentrations, while low, yet trypanocidal concentrations such as 0.1 $\mu$g/ml and 1 $\mu$g/ml, had no detectable effect on the fibroblasts. These findings confirm the work of Hawking and Smiles (1941), who reported, that diamidines are selectively taken up by trypanosomes, but only to barely detectable levels by mammalian tissue cells. More recently, Lydon et al. (1980) have found that the uptake of DAPI in low concentrations by mammalian cells is of reversible nature and does not affect viability of these cells. However, since the function of the feeder layer for the propagation of trypanosomes, be it structural, regulatory, or multiplication inducing, is not known, the possibility can not be exluded, that a drug like DAPI interferes with this function, and thus, causes indirectly trypanocidal effects on the parasites.

Diamidines like DAPI or Berenil are divalent cations under physiological conditions and are, therefore, able to interact with numerous anionic cell structures and enzyme systems (Kaliwoda, 1976). DAPI has been shown to inhibit arginine-specific esteroproteases (Tidwell et al., 1978; Lonsdale-Eccles, personal communication). This, however, seems to be of little importance for its antitrypanosomal activity, since 4'6-bis(2'-imidazolinyl-4H,SH)-2-phenylindol (DIPI, SN 192/188), which has no protease inhibitory activity (O. Dann, personal communication), is three times more active against *T. b. brucei* in vitro than DAPI (Borowy et al., 1985). Compared to DAPI, DIPI has been found to have a higher binding affinity to polydeoxynucleotides (Chandra and Mildner, 1979).

The most relevant antitrypanosomal activity of DAPI seems to be caused by its specific binding affinity to DNA, rich in adenine-thymine base pairs (Williamson and Fennel, 1975). Kinetoplast DNA (kDNA) of trypanosomes contains large domains especially enriched with such base pair repeats. At a concentration of 1 $\mu$g DAPI/ml, DAPI was found to inhibit DNA synthesis by 85% (Borowy, unpublished results) and it is assumed that the synthesis of both kDNA as well as nuclear DNA is affected. If diamidines, like DAPI, interfere also with nuclear DNA, they should be therapeutically active against *Plasmodium* sp. and *Babesia* sp., which possess a higher percentage of adenine-thymidine base pairs in their nuclear DNA than trypanosomes (Borst and Fairlamb, 1976). Indeed, therapeutic action of DAPI on experimental murine malaria has been reported (Steiger et al., 1980), and the aromatic diamidine Berenil is in veterinary use as a babesiacidal drug.

In conclusion, we have examined a mammalian feeder layer system (Hiru-

mi et al., 1977a, b) to see whether it would prove useful in testing the trypanocidal effects of potential drugs against *T. b. brucei* organisms. The system, presently the most widely used in vitro technique for continuous propagation of bloodstream *T. b. brucei*, was demonstrated to be highly sensitive and reproducible. Thus, the mammalian feeder layer system might become an important tool in the search for new trypanocidal drugs as well as for the elucidation of modes of drug action on trypanosomes.

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