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SHORT COMMUNICATION

# STUDIES ON THE EFFECT OF *P*-ROSANILINE ON THE KINETOPLAST OF *P*-ROSANILINE RESISTANT *TRYPANOSOMA EVANSI*

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Protozoa of the family Trypanosomatidae are characterized by the presence of a body known as the kinetoplast. A number of workers examined the kinetoplast by electron microscopy and found that it consists of a dense fibrous inclusion (kinetonucleus) and kinetoplast envelope (Inoki et al., 1958; Meyer et al., 1958; Mühlpfordt, 1963; Rudozinska and Vickerman, 1968).

Quite recently, Inoki et al. (1969) observed the effects of *p*-Rosaniline on the kinetoplast by electron microscopy and found that treatment with 10 mg/kg *p*-Rosaniline causes loss of both the kinetonucleus and kinetoplast envelope in a *p*-Rosaniline sensitive strain of *T. gambiense*. However, in a *p*-Rosaniline sensitive strain of *T. evansi* and a *p*-Rosaniline resistant strain of *T. gambiense*, fragmentation of the kinetonucleus regularly occurred, but the envelope membrane remained intact after the dye treatment. These data show that *p*-Rosaniline treatment has a similar effect on the kinetoplasts of the *p*-Rosaniline resistant strain of *T. gambiense* and the *p*-Rosaniline sensitive strain of *T. evansi*.

The present paper describes the effects of *p*-Rosaniline on the kinetoplast of a *p*-Rosaniline resistant strain of *T. evansi*, examined by electron microscopy.

Inoki et al. (1961) reported that a *p*-Rosaniline resistant strain of *T. evansi* could be isolated by the same technique used to obtain the resistant strain of *T. gambiense* (Inoki and Matsushiro, 1959). However, all cells of this resistant strain changed to the akinetoplastic form. Therefore, we used the following method to obtain *p*-Rosaniline resistant *T. evansi* with a kinetoplast. Mice infected with a moderate dose of parasites were treated intraperitoneally with 1.0 mg/kg of *p*-Rosaniline. The parasites were transferred to normal mice 1 hr after the dye treatment. This procedure was repeated increasing the dose by 5 mg/kg at each following passage. When the dose had increased to 30 mg/kg, treatment with *p*-Rosaniline was stopped, because AK forms had increased to as much as 98 percent of the population in mice. The percentage of AK forms decreased to about 5 percent during 20 further passages through normal mice without *p*-Rosaniline treatment. Then, we started to treat the parasites with *p*-Rosaniline again, increasing the dose by 5 mg/kg *p*-Rosaniline at long intervals to avoid inducing a marked increase in AK forms. Finally, after 13 months the dose had been increased up to 85 mg/kg. A large dose than this was so toxic that the mice died within 1 hr. A clone of the parasites was isolated from a mouse treated with this maximum dose,

employing the single cell isolation technique of Inoki (1960). The degree of drug-resistance of the parasites was measured by the AK induction test (Inoki and Matsushiro, 1959).

Table 1 shows that in the clone of *T. evansi* obtained, the rate of appearance of AK forms was 12.1±0.8% by the AK induction test, while in the *p*-Rosaniline sensitive clone, the rate was as high as 28.9±1.3%. The percentage of AK forms was usually about 5% in both the *p*-Rosaniline sensitive and resistant strain of *T. evansi* before the AK induction test. Thus a clone of a *p*-Rosaniline resistant strain of *T. evansi* with a kinetoplast can be obtained by the above technique.

Electron microscopic observations were made on the kinetoplasts in the *p*-Rosaniline resistant clone of *T. evansi* before and after the AK induction test. Electron micrographs were prepared by the technique described by Inoki et al. (1969).

Fig. 1 shows the kinetoplast in the *p*-Rosaniline resistant strain of *T. evansi* before the AK induction test. The typical fibrous structure (kinetonucleus) is seen within a two layered envelope membrane. Fig. 2 shows an electron micrograph of the kinetoplast in the *p*-Rosaniline resistant strain of *T. evansi* after the AK induction test. It is morphologically similar to that shown in Fig. 1 and neither disappearance of the envelope membrane nor fragmentation of the kinetonucleus are observed.

The rate of appearance of AK forms observed by light microsopy is about 12.1% 240 min after the AK induction test and in a few parasites observed by electron microscopy the fibrous structures in the kinetoplast appear to be replaced by fragmented materials, like those seen in the kinetoplast of *T. evansi* AK clone (Inoki et al., 1969). But, in most parasites, the fibrous structures of the kinetoplast persist, as described previously (Fig. 2). The reason why *p*-Rosaniline treatment has different affects on the kinetoplast in *p*-Rosaniline resistant strains of *T. gambiense* and *T. evansi* is unknown.

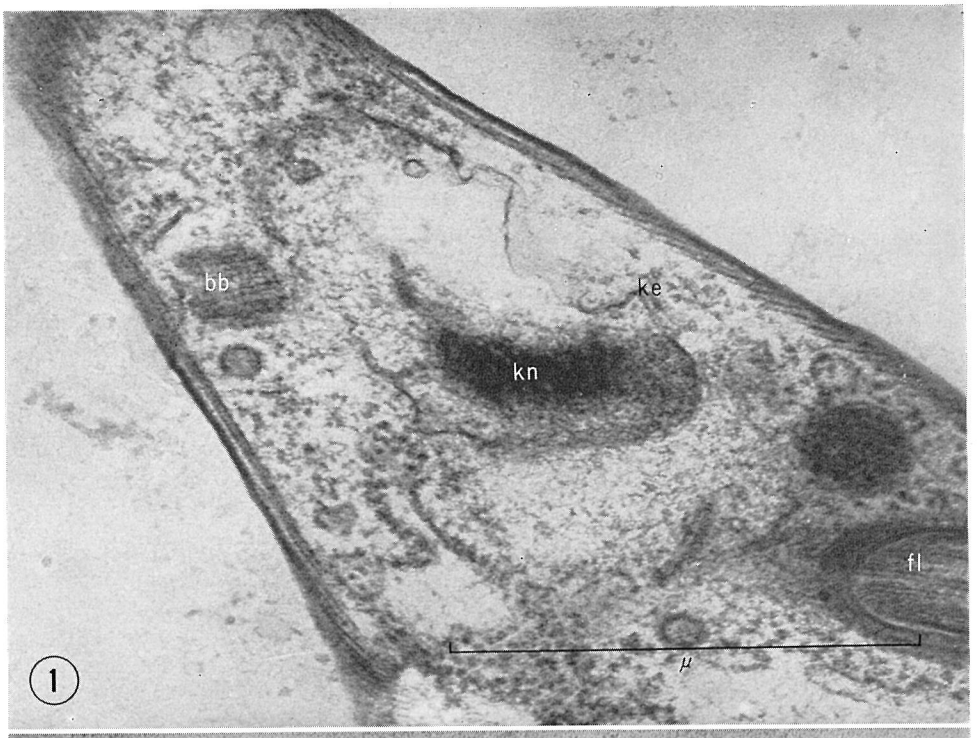
TABLE 1. *Rates of appearance of AK forms in T. evansi before and after the AK induction test*

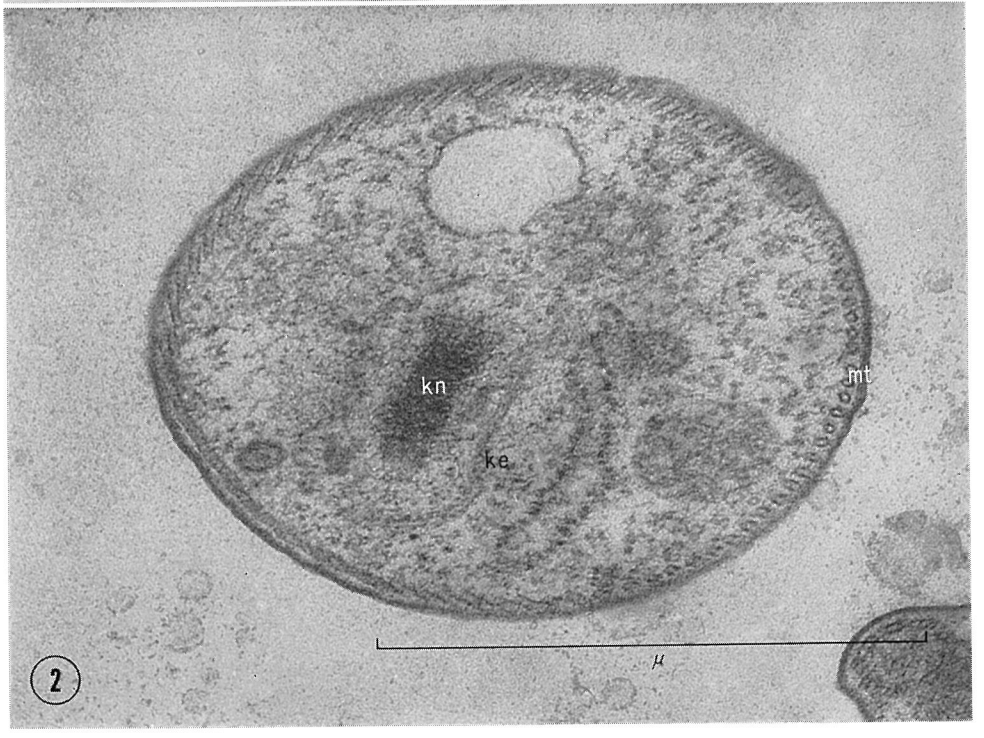
| Parasites | Rate of appearance of AK forms (%) | |
|---|---|---|
| | before AK induction | 240 min. after AK induction |
| T. evansi (R)[a] | 5.2[c]±1.2[d] | 12.1±0.8 |
| T. evansi (S)[b] | 5.0 ±0.4 | 28.9±1.3 |

*a* clone of *T. evansi* treated with p-Rosaniline
*b* *p*-Rosaniline sensitive clone of *T. evansi*
*c* mean
*d* 99% reliability limit

FIGURE 1. *Electron micrograph of the kinetoplast in the p-Rosaniline resistant strain of T. evansi before the AK induction test.*

FIGURE 2. *Electron micrograph of the kinetoplast in the p-Rosaniline resistant strain of T. evansi after the AK induction test.*
*bb, basal body; fl, flagellum; ke, kinetoplast envelope; kn, kinetonucleus; mt, microtubules;*


bb
ke
kn
fl
μ
1


kn
mt
ke
μ
2

There is no apparent morphological difference between the kinetoplasts of *T. gambiense* and *T. evansi*. Ono et al. (1971), however, reported that the kinetoplast envelopes of *T. evansi* AK clone, unlike those of *T. gambiense* after treatment with *p*-Rosaniline, synthesize satellite DNA with the same density as the kinetoplast DNA in *T. evansi* K clone, although they lack a kinetonucleus. If the AK form of *T. evansi* are capable of multiplication differentiated from that of *T. gambiense*, as shown by Inoki (1956) and Inoki et al. (1960), DNA synthesis inside the kinetoplast envelope in the AK forms of *T. evansi* might be related with their duplication ability. This may also be related to the reason why the kinetoplast in the *p*-Rosaniline resistant clone of *T. evansi* was affected differently from the kinetoplast in the *p*-Rosaniline resistant strain of *T. gambiense* by treatment with 10 mg/kg *p*-Rosaniline (AK induction test).

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