Drug effects on the fine structure of *Trypanosoma rhodesiense*: Puromycin and its aminonucleoside, Cordycepin and Nucleocidin

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Summary

The effects of four trypanocidal adenine nucleosides on the fine structure of a monomorphic strain of *Trypanosoma rhodesiense* have been examined. All four drugs, Puromycin and its aminonucleoside, Cordycepin and Nucleocidin, induced electron-lucent cytoplasmic clefts in the cytoplasm, which were generally acicular or spindle-shaped, with long axes lying in any direction. The clefts had an intimate relationship with the rough endoplasmic reticulum. The drugs also produced excessive lysosomal vacuolation, and two, Cordycepin and Nucleocidin, caused nucleolar fragmentation and probable segregation, indicative of interference with RNA synthesis. The significance of the lesions is discussed in relation to known properties of adenine nucleoside drugs.

Materials and methods

The strains of *T. rhodesiense* and the methods of mouse infection, drug treatment (intraperitoneal) removal of infected blood and subsequent preparation for light and electron microscopic examination were as described previously (Macadam and Williamson, 1972). The drugs and dosages used were as follows:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Source</th>
<th>CD$_{90}$ (mg/kg)</th>
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<tbody>
<tr>
<td>Puromycin</td>
<td>Lederle Laboratories*</td>
<td>200</td>
</tr>
<tr>
<td>Puromycin aminonucleoside</td>
<td>Lederle Laboratories*</td>
<td>100</td>
</tr>
<tr>
<td>Cordycepin</td>
<td>Original product†</td>
<td>25‡</td>
</tr>
<tr>
<td>Nucleocidin</td>
<td>Lederle Laboratories*</td>
<td>0.1</td>
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* By courtesy of Dr. A. T. Mennie.
† Cunningham, Hutchinson, Manson and Spring (1951); by courtesy of Dr. Norman Cohen.
‡ This is an ED$_{90}$ dose.
complex lysosomes (autophagic vacuoles) were produced (plate 1A). Vacuolated lysosomes occur only occasionally in the normal untreated trypanosome, and to a minor extent compared with the drug-treated organisms.

Lysosomes are morphologically distinct and acid phosphatase activity has been precisely located in them in this strain of *T. rhodesiense* (McLaren and Williamson, unpublished).

**Other changes restricted to Puromycin-treated specimens**

There was a focal excess of rough endoplasmic reticulum, usually closely associated with the lucent "clefts" in the cytoplasm. Variably dense spherical microgranules (up to 33 nm diameter) were present in the clefts and in the general cytoplasmic matrix (plates 1A and B).

**Other changes restricted to Cordycepin-treated specimens**

There were multiple ribosome clumps (up to 0.5 μm in diameter) of apparently aggregated units (plate 3B). The nucleolar material was dispersed throughout the nucleoplasm (plate 4A).

**Other changes restricted to Nucleocidin-treated specimens**

There was very coarse clumping of the chromatin and dispersal of the nucleolar material throughout the nucleoplasm (plate 4B).

**Discussion**

Plates 1-4 show that, with all the nucleoside drugs examined here, the major intracellular lesion was the invariable formation of large cytoplasmic "clefts"; a single identical cleft produced by pentamidine has been described earlier (Macadam and Williamson, 1972) and oxophenarsine and triparsamide also produce clefts (Macadam and Williamson, 1974). The clefts are not sectioning artefacts, as their long axes lie at random, nor are they caused by folding of the pellicle (plate 2B). They strongly resemble those induced in rat jejunal epithelial cells after feeding long chain saturated fatty acids (McKay et al, 1967). The rat cell clefts had one side membrane-bounded with an internal lacework visible at high magnification and containing very fine droplets, which corresponded to the microgranules found in this area in trypanosomes (plates 1A and B). The electron-lucency of the rat cell clefts was attributed to their content of saturated long chain triglyceride which was unable to take up osmic acid; medium chain or unsaturated fatty acids did not produce this effect. None of the clefts described, including those described here in trypanosomes, resembled the characteristic needle-shaped "cholesterol clefts" found in atheromatous arterial walls (Imai and Thomas, 1968).

Puromycin is a specific inhibitor of protein synthesis.

Plate 1B: T. rhodesiense after Puromycin treatment. Cytoplasmic cleft (CC) contiguous with "round cleft" (RC) containing "lipoprotein" granules (LPG) and membrane fragment. Half-empty lysosomes (L) near "round cleft". Secretion granule (SG). Empty lysosome (E). x 78,000.

Plate 2B: *T. rhodesiense* after Cordycepin treatment. Apparent pellicular invagination (IV) produced by folding, compared with cytoplasmic cleft (CC). Lysosomes (L), some of which are evacuated. Secretion granules (SG) near cleft. X 57,000.
Plate 3A: *T. rhodesiense* after Cordycepin treatment. Flagellar reservoir (FR). Numerous secretion granules (SG) and evacuated lysosomes (L) near axially transverse cytoplasmic cleft (CC). "Budding" of mitochondrion (MC). x 54,000.

Plate 3B: *T. rhodesiense* after Cordycepin treatment. Large ribosome aggregate (RA). Small "round cleft" (RC) near evacuated lysosome (L). Minor cleft bounded by flattened secretion granule (FSG). Secretion granules (SG). x 36,000.
Plate 4A: *T. rhodesiense* after Cordycepin treatment. Fragmented nucleolus (NU); light and dark areas indicate probable segregation. Cytoplasmic clefts (CC). Secretion granules (SG). X 24,000.

Plate 4B: *T. rhodesiense* after Nucleocidin treatment. Fragmented nucleolus (NU); light and dark areas indicate probable segregation. Chromatin (C) peripherally marginated. X 45,000.
Puromycin aminonucleoside and Cordycepin are inhibitors of RNA synthesis, and Nucleocidin inhibits DNA, RNA and protein synthesis. None of these activities can be easily linked to the selective uptake and cytoplasmic cleft deposition of radiolabelled palmitic acid which has been demonstrated in T. rhodesiense treated with Cordycepin (WILLIAMSON and MCLAREN, 1974).

If the clefts, which increase progressively with time after drug treatment (WILLIAMSON and MCLAREN, 1974) are regarded as saturated triglyceride deposits, then they could result from any or all of the following: increased triglyceride synthesis or uptake, or decreased lipolysis. Palmitic acid uptake and deposition in T. rhodesiense is known to be accelerated by drug treatment (WILLIAMSON and MCLAREN, 1974) but drug effects on intracellular lipolysis have not yet been examined directly; selective uptake of unhydrolysed saturated triglycerides seems unlikely. T. rhodesiense contains phospholipases and at least three triglyceride lipases (unpublished observations). The location of these is not known, but triglyceride accumulation may be affected by lysosomal lipase activity, as in rat liver (GUJER and WIELAND, 1970). Drug inter-action with lysosomes in cleft formation is suggested by the close association of clefts with vacuolated lysosomes (plates 1A and 2B, 3A and B). Lysosomal activation is also indicated by autophagic vacuole formation (plate 1A), which was especially pronounced in the Puromycin-treated trypanosomes in which lysosome numbers were markedly increased.

Lysosome numbers were increased significantly (approx. 50%) over the normal complement, only by Puromycin and by suramin which does not produce clefts; as all the drugs examined in this series of studies, produced vacuolated lysosomes, the lysosomal aspect of cleft formation will require more detailed study. Approximate estimates from counts per trypanosome section, indicated that the numbers of both secretion granules and empty lysosomes increased with the number of clefts; the possible repair function of the former has been indicated earlier (MACADAM and WILLIAMSON, 1974).

The general association of the cleft with the endoplasmic reticulum (plates 1A and 2, 2A) suggests that local synthesis of triglyceride occurs in this area; according to MCKAY et al (1967), triglycerides are rethesized in the channels of the endoplasmic reticulum. Many of the clefts (e.g. plate 1A) show connection at one or other end with an open membrane channel of this type. Saturated triglyceride, apart from inability to take up osmic acid, is also much less mobile than unsaturated triglyceride; this probably contains unsaturated lipid. The density of some of the microgranules deposited after puromycin treatment (plates 1A and B) indicates that these probably contain unsaturated lipid.

An alternative theory of cleft formation can be based on the observation of MACKENZIE et al (1967) that cellular lipid content depends largely on the chemical nature of the extracellular environment. The appearance of lipid inclusions in T. rhodesiense after nucleoside drug treatment could then be the result of drug-induced free fatty acid mobilization in the mammalian host followed by absorption by the trypanosome. Electron microscop ic study of free fatty acid assimilation (which occurs by free diffusion) and subsequent deposition in heart cells (STEIN and STEIN, 1968) has shown that the route into the cells is from the surface through the cisternae of the endoplasmic reticulum and thence to its lateral sacs where the acids are esterified. This distribution corre-
and disposition such as are produced by Cordycepin in trypanosomes (Williamson and McLaren, 1974), may contribute to the mitochondrial alterations found here.

Acknowledgment
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References