EFFECTS OF DAUNORUBUCIN AND DAUNORUBUCIN-BSA CONJUGATES ON TRYPANOSOMES

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Daunorubicin has activity *in vitro* but not *in vivo* against trypanosomes (Williamson et al 1981). Fluorescence microscopy showed that while drug is taken up by the parasite *in vivo*, it is not retained in the cells. Consequently, drug was coupled to protein to prolong exposure and conjugates with a labile drug-protein linkage are indeed active *in vivo* (Williamson et al 1981) : drug is known to be released from such conjugates (Golightly et al, 1983). Daunorubicin has a multiplicity of cellular effects, nuclear changes having been shown in trypanosomes (Williamson et al, 1983). Here the effects on trypanosomes of daunorubicin, daunorubicin-bovine serum albumin coupled by a labile linkage (using glutaraldehyde) (D-BSAG) and by a stable linkage (using succinic anhydride) (D-BSAS) have been investigated to elucidate the mechanism of action of D-BSAG.

A monomorphic strain of *Trypanosoma brucei rhodesiense* was incubated at 37°C for 4 hours at 10^7 cells ml^{-1} in 50/50 inactivated rat serum/Krebs saline-glucose containing drug (free or conjugated). Drug uptake was monitored by fluorescence microscopy and ultrastructural effects by electron microscopy (EM). With daunorubicin, there was an almost immediate diffuse fluorescence throughout the organism with accumulation within the kinetoplast and nucleus occurring by 5 min . From 15 min fluorescence granulation was seen at the anterior end suggesting accumulation in organelles (possibly lysosomes). The two conjugates, particularly D-BSAS, were always taken up to a lesser extent and more slowly than free drug. Whilst diffuse fluorescence was seen in the cytoplasm at 5 min, nuclear accumulation did not occur until 30 min and granulation not until 1 to 2 hours. EM showed that incubation with free daunorubicin and the labile conjugate (D-BSAG) led to nucleolar fragmentation, condensation of chromatin and separation of chromatin from the nuclear membrane. Otherwise, the ultrastructure was normal though the D-BSAG treated organisms showed a higher number of endocytotic vesicles around the flagellar pocket. The stable conjugate (D-BSAS) which is inactive, gave no nuclear or other ultrastructural changes.

The evidence confirms that D-BSAG acts as a delivery system for slow intracellular release of drug which then acts at the nucleus.

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