

Supplemental Materials

The causes of the inoculum effect are still unclear to us. We initially suspected that drug depletion might have occurred at the higher inoculum, as trypanosomes are known to accumulate diamidines at markedly higher intracellular concentrations than the extracellular drug concentration in culture media. Mathis *et al.* (1) reported an intracellular concentration of approximately 4 mM when *T. b. brucei* s427 cells were incubated with 7.5 μ M DB829 for 8 h. Considering an individual trypanosome volume of 30 femtoliter and an intracellular drug concentration of 4 mM, a maximum of 3% drug depletion would be expected when DB829 was added to 2 ml of culture medium (200 nM starting drug concentration) containing an initial parasite density of 5×10^4 trypanosomes/ml. Drug depletion could reach 60% at a parasite density of 10^6 trypanosomes/ml. However, at 2,000 nM starting drug concentration, DB829 depletion would be negligible (<6%) even at 10^6 trypanosomes/ml density. Therefore drug depletion could partially contribute to the inoculum effect at lower drug concentrations, but other reasons must also exist. One might be quorum sensing of the trypanosomes leading to down-regulation of the P2 aminopurine transporter at higher trypanosome densities.

1. **Mathis, A. M., A. S. Bridges, M. A. Ismail, A. Kumar, I. Francesconi, M. Anbazhagan, Q. Hu, F. A. Tanious, T. Wenzler, J. Saulter, W. D. Wilson, R. Brun, D. W. Boykin, R. R. Tidwell, and J. E. Hall.** 2007. Diphenyl furans and aza analogs: effects of structural modification on in vitro activity, DNA binding, and accumulation and distribution in trypanosomes. *Antimicrob Agents Chemother* **51**:2801-2810.