Using Nucleases to Probe the Effects of Heterocyclic Diamidine Binding on DNA Structure

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Heterocyclic diamidines have been shown to be effective as antiparasitic agents. Previously the sequence and topological specificity of the binding of the compounds DB 75, DB 293 and DB 818 was examined using restriction enzyme activity assays. These agents were shown to actually enhance the cleavage of DNA by certain restriction enzymes, e.g., Mlu I. These results suggest that the binding of these compounds may distort the structure of DNA. It is hypothesize that Heterocyclic diamidine would alter the structure of the DNA which would affect the reactions of mung bean nuclease and bal31 nuclease. Furthermore, heterocyclic diamidine might bind at the DNA distortion or they might be the cause of the DNA alteration. Therefore, if they are producing the distortion they might enhance the activity of mung bean and bal31. On the other hand, if they are binding at the distortions that are already in the DNA structure, then they would inhibit the effects of mung bean and bal31. Mung bean nuclease and Bal 31 nuclease are specific for activity at locations of DNA distortion. If the heterocyclic diamidines are indeed producing distortions, they could alter the activity of these nucleases. Further, analysis of the DNA fragments produced by nuclease reaction in the presence of these compounds could help locate the sites of distortion. In the experiments, circular phiX174RF DNA will be digested by either mung bean nuclease or Bal 31 nuclease in the presence of increasing concentrations of each of the diamidine. Reaction products will be separated on agarose electrophoresis gels for analysis. The results will be compared with those from nuclease digestion in the presence of a diamidine of DNA previously digested with Mlu I. Such comparison should help localize the site of drug-induced distortion. The results of these experiments will further our understanding of how these agents work and help in designing more effective agents.