Use of Nuclease Digestions to Prove DNA Structural Alterations Produced by N-Methylpyridyl Porphin Binding

Edilmary Urdaneta* & Stephen Winkle

Porphins have been one of the most study compounds in biochemistry since they tend to bind and to alter DNA structure. In particular, the para-N-methyl pyridyl isomer has been shown to stabilize quadraplex structures in telomeric DNA. The binding of N-methyl-pyridyl porphins to DNA was previously studied using restriction enzyme activity assays. With certain restriction enzymes, e.g., Mlu I, these compounds produced enhanced enzyme cleavage rather than the expected inhibited cleavage. These results suggest that the binding of these compounds may distort the structure of DNA. Mung bean nuclease and Bal 31 nuclease are specific for activity at locations of DNA distortion. If the porphins are indeed producing distortions, they could alter the activity of these nucleases. Consequently, the hypothesis proposes for this experiment is to determine whether the N-methyl pyridyl porphins, specially porphins 1,2, and 3, are indeed producing distortions of the DNA when they interact with specific enzymes such as Mung bean nuclease, Mlu 1 and Bal 31 nuclease.

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 either the ortho, meta or para N-methyl pyridyl porphine. Moreover, the concentration that would be used for the porphins P1, P2, and P3 are going to at 1:100 ratio while the concentration of DNA will be 1:10 ratio. The 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 porphine] 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 how they help in the improvement of treatments in medicine.