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THE MODE OF ACTION OF CLOFAZIMINE

DNA BINDING STUDIES

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Clofazimine is a phenazine imino-quinone derivative used for the treatment of sulfone-resistant leprosy. Its mode of action is not clearly defined. This report is concerned with a study of clofazimine binding to DNA. A spectrophotometric assay has been developed which permits binding studies with clofazimine in aqueous medium with control of pH and conformity to Beer's law. The highly lipophilic clofazimine molecule has a calculated octanol/water partition coefficient or Log P value of +7.48. This lipophilic property imposes limitations on binding studies that can be carried out in aqueous media.

Clofazimine binds rapidly to all DNA's studied, including human and mycobacterial, with typical upfield red shifts and hypochromic displacement of the clofazimine absorption peak in the red region of the spectrum. The magnitude of the upfield red shift is sufficient to be seen as a visible change. The degree of drug interaction, as measured by the upfield shift, is dependent on DNA concentration and the G + C content of the DNA strand. Clofazimine interacts to the same extent with native or denatured DNA strands.

Through the use of synthetic polynucleotide strands it has been possible to localize clofazimine binding to the guanine region of the strand. Clofazimine binds to both double and single-stranded polymers, thus base-pairing does not appear to be essential. The upfield red shifts have been found to occur with the double-stranded duplex, poly dG.poly dC as well as the single-stranded poly dG and poly G. Little interaction occurred with poly dA, poly dT, poly dC, poly A or poly U. Clofazimine will interact with purified yeast tRNA that has been stripped of amino acids. This interaction is presumably at the G + C base-paired regions of the tRNA tertiary structure. Clofazimine did not interact with hyaluronic acid, chondroitin sulfate, glycogen or serum albumin with upfield red shifts that were characteristic of guanine containing strands.

The presence of a concentration-dependent isosbestic point in the clofazimine poly G upfield spectral shifts is consistent with the view that a single interaction site is predominant on the poly G strand. This site is likely to be the 2-amino group on the guanine ring participating in H-bonding to the clofazimine molecule.

It was instructive to find that clofazimine or B.1912 did not displace methyl green complexed to the bovine DNA strand. On the other hand, intercalators such as ethidium bromide, lucanthone, quinacrine or chloroquine all displace large amounts of methyl green under the conditions of assay used. Since methyl green is complexed along the phosphodiester bridge of the DNA backbone, its displacement arises from distortion and stretching of the DNA coiled helix due to insertion of intercalative molecules between the base pairs. The methyl green displacement data did not indicate any intercalative function arising out of the clofazimine DNA interaction. This argument receives further support from space-filling molecular models of clofazimine which show the chlorophenyl substituent attached to the phenazine ring N is sterically out of plane with respect to the flat planarity of the phenazine ring itself. This substituent sterically prevents intercalation from taking place between the base pairs.

Two further important properties arising from clofazimine DNA interaction have been assessed. It has been found that whereas clofazimine will complex with *E. coli* DNA, the nature of the interaction is such that it does not involve the activity of the excision repair enzyme, DNA polymerase I or pol I. Deficient *polA1* mutants did not show any increased sensitivity to clofazimine as compared to the parent strain. Secondly it has been found that after exposing populations of *Salmonella typhimurium* mutants to clofazimine no increases occurred in back-mutation rates. It is concluded that although clofazimine will interact with DNA it does not function as a bacterial mutagen which is consistent with the fact that the interaction does not involve excision repair activity.

In summary, evidence has been presented to show that clofazimine will bind to DNA with primary binding sites located on the guanine base. Base-pairing was unessential. It is likely that the drug binds to DNA at those guanine bases which are in juxtaposition to the narrow groove region of the DNA backbone thus permitting both primary and secondary site binding of the drug molecule. Clofazimine binding in the narrow groove of mycobacterial DNA will undoubtedly block template function of the strand and result in growth inhibition. No evidence was found to support an intercalative mechanism for binding. Clofazimine itself does not exhibit mutagenic activity. It is feasible that the increased G + C content of mycobacterial DNA as compared to human DNA provides a quantitative basis of increased clofazimine interaction with mycobacterial DNA that could explain the selective inhibition of intracellular mycobacteria growing in human cells. Furthermore, the highly lipophilic nature of the clofazimine molecule is likely to be an important property in preventing cytotoxic effects from occurring in mammalian cells.