Comparative DNA Binding Studies with Clofazimine and B1912

To The Editor:

B1912 is a phenazine quinoneimine that was synthesized by Barry and co-workers (1) and subsequently recommended as an analog of clofazimine (B663) for possible screening of antileprosy activity in man (1,7). The compound was active against *Mycobacterium leprae* in the mouse foot pad assay (6) as well as inhibiting the growth of *M. lepraemurium* (3). Like clofazimine, B1912 will accumulate in the tissues of rodents (2,3) particularly in the skin, when added in powder form to the diet. It has been found in this laboratory that at a concentration of 0.05% by weight in the diet, rapid skin pigmentation will occur in the mouse in a three to four day period.

Following the observation that clofazimine will form complexes with DNA from various sources, including human (4), it was of interest to compare the relative interaction of clofazimine versus B1912 with this important macromolecule. The degree of interaction was measured with a spectrophotometric assay (5) of the amount of upfield red shift that took place in the spectrum of clofazimine or B1912 following complexing to DNA. The upfield red shift resulted from the interaction of the heterocyclic phenazine quinonoidal ring with the nucleotide bases of the DNA strand. The assays were carried out in 0.01 M tris-HCl buffer, ph 7.0, at 22°C with DMSO added at 10% (v/v) to maintain the compounds in solution. Clofazimine or B1912 was added at a final concentration 2×10^{-6} M and dialyzed DNA was present at 250 μg per ml. The upfield red shifts were measured in a Cary 14 recording spectrophotometer (5).

It was found that, like clofazimine, B1912 interacted with DNA's from various sources with upfield red shifts and hypochromic spectral changes in the absorption peak at

482 nm. The degree of interaction was dependent upon the G + C content of the DNA strand. The upfield red shifts were significantly greater than those found with clofazimine. The extent of the increased interaction was calculated from the ratio B1912/B663 as measured from the upfield peak-to-peak shift in nanometers. For human DNA the B1912/B663 ratio was 4, for bovine DNA the B1912/B663 ratio was 5, and for *M. lysodeikticus* DNA the B1912/B663 ratio was 5.7. When the DNA was replaced by the synthetic polyribonucleotide strand, poly G, it was found that B1912 interacted to produce an upfield red shift of 50 nm and a calculated B1912/B663 ratio of 4.1. These data indicate that B1912 undergoes a 4- to 5.7-fold greater degree of spectrally identified interaction with DNA than does clofazimine.

A structural basis to this increased interaction has been found from the fact that B669, a derivative of B1912 in which the 7-position chloro substituent has been removed ([1]), interacted with DNA to produce upfield red shifts that were comparable to those found with clofazimine. Evidently the 7-chloro substituent on the phenazine ring has an activating or enhancing effect on the binding of B1912 to DNA. By contrast when the chloro groups were located on phenyl and anilino substituent sites, such as in clofazimine, there was no significant enhancement of upfield red shifts. B1912 has also been found to interact with transfer RNA.

The interaction of B1912 with DNA would also account for the finding in this laboratory that B1912 has strong antimitotic effects in tissue culture. These effects were seen at 2 to 3μg per ml concentrations in monolayer growth of human epithelial cells. Such concentrations represent levels that are attainable in human serum. These data thus have implications for the testing of B1912 in human volunteers.

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REFERENCES

1. Barry, V. C. Synthetic phenazine derivatives and mycobacterial disease: a twenty year investigation. Sci. Proc. Roy. Dublin Soc. Ser. A3 (1969) 153-170.
2. Barry, V. C., Belton, J. G., Blake, P., Byrne, J., Conalty, M. L. and Jina, A. G. Antimycobacterial activity and tissue distribution of B1912 compared with that of its analogue, clofazimine (B663). Ann. Microbiol. Inst. Pasteur **124B** (1973) 193-203.
3. Chang, Y. T. Effect of B1912, a new riminophenazine derivative, in murine leprosy. Int. J. Lepr. **38** (1970) 417-421.
4. Morrison, N. E. and Marley, G. M. The mode of action of clofazimine: DNA binding studies. Int. J. Lepr. **44** (1976) 133-134.
5. Morrison, N. E. and Marley, G. M. Clofazimine binding studies with deoxyribonucleic acid. Int. J. Lepr. **44** (1976) 475-481.
6. Shepard, C. C., Walker, L. L., Van Landingham, R. M. and Redus, M. A. Comparison of B1912 and clofazimine (B663) in *Mycobacterium leprae* infections. Proc. Soc. Exp. Biol. Med. **137** (1971) 728-729.
7. Shepard, C. C., Ellard, G. A., Levy, L., Opromolla, V. A., Pattyn, S. R., Peters J. H., Rees, R. J. W. and Waters, M. F. R. Experimental chemotherapy in leprosy. Bull. WHO **53** (1976) 425-433.

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