J. Pharm. Pharmacol. 1998, 50 (Supplement): 110

Synthesis of anthraquinone–oligodeoxynucleotide conjugates as inhibitors of gene transcription

V. GIBSON, R. J. ANDERSON, J. A. HARTLEY* AND D. CAIRNS

*Institute of Pharmacy and Pharmacy Practice, School of Health Sciences, University of Sunderland, Sunderland, and *Department of Biochemistry, University College London, London*

We have shown recently that anthraquinone - oligodeoxynucleotide (AQ-ODN) conjugates may be synthesized in good yield (Gibson et al 1996a) and that these agents can form sequence specific triple helices with double helical DNA (Gibson et al 1996 b, Gibson et al 1997). In an attempt to investigate whether these agents can inhibit gene transcription, a number of AQs were synthesized and linked to a 13 mer ODN 5'-CTTTTTCCTTCTC-3' (or with methylcytosine [^{Me}C] instead of cytosine) which is complementary to a homopurine region located downstream of the promoter site of the β lactamase gene in *E. coli* and the presence or absence of transcription products assayed (Duval-Valentin et al 1992). Free ODN was used as a control in each case. The target DNA, cut from plasmid pBR322, was incubated with RNA polymerase, RNasin, dithiothreitol and bovine serum albumin in a transcription buffer (Tris HCl 40 mM pH 7, $MgCl_2$, spermidine and NaCl), and the presence of full length mRNA monitored over time for concentrations of conjugate ranging from 0.1 to 100 μ molar. The two most effective inhibitors, **TFO13** and **TFO14** were then investigated in time-course experiments of between 0.5 and 60 minutes.

All AQ-ODN complexes inhibited gene transcription by between 25 and 50% at a concentration of 0.1 μM, whereas free ODN showed negligible inhibition of transcription up to a concentration of 10 μ M. This confirms previous footprinting studies in which we demonstrated that free ODNs form very weak triple helices (Gibson et al 1996 a).

When the binding of **TFO10** and **11** was compared to **TFO12** and **13** it was found that the presence of 5-methylcytosine in the ODN does have a stabilising effect on triplex formation, presumably due to the higher pKa of 5-methylcytosine.

TFO15, which contains the 1,8-disubstituted AQ exhibited less inhibition than the 1- substituted or 1,5-disubstitited AQs. This supports a previous study (Gibson et al 1996 a) where the 1,8 -disubstituted AQ showed weaker intercalative binding to triplex DNA. The 1- and 1,5 AQs showed similar levels of transcription inhibition, with the 1-substituted compound (**TFO13**) being the most active. This leads us to conclude that, once linked to an ODN, a second cationic sidechain on the AQ has little effect in stabilising a triple helix.

The timecourse experiments demonstrated that **TFO12**, the free ODN with ^{Me}C, inhibited transcription, compared to control, for a period of 20 minutes. **TFO13** and **14,** however, produced a significant reduction in mRNA production for up to 60 minutes. This suggests that AQ-ODN conjugates can act to inhibit transcription of the β lactamase gene in *E. coli* at μ molar levels, for periods of up to 60 minutes.

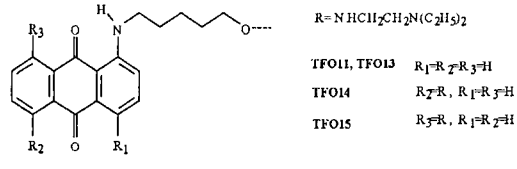
5'-CTTTTTCCTTCTC-3' = **TFO10**

5'-AQ-CTTTTTCCTTCTC-3' = **TFO11**

5'-^{Me}CTTTTT^{Me}C^{Me}CTT^{Me}CTC-3' = **TFO12**

5'-AQ-^{Me}CTTTTT^{Me}C^{Me}CTT^{Me}CTC-3' = **TFO13, TFO14, TFO15**

Where AQ is



Duval-Valentin, G., Thuong, N.T., Helene, C. Proc. Nat. Acad. Sci. USA, (1992), 89, 504-508.

Gibson,V., Anderson, R.J., Brown, J.R., Hartley, J.A., Cassidy, S.A., Fox, K. R., and Cairns, D. Pharm. Sci. (1996 a), 2, 49-53

Gibson, V., Anderson, R. J., Brown, J.R., Mackay, S.P. and Cairns, D. Pharm. Sci. (1996 b), 2, 545-548

Gibson, V., Anderson, R. J., Cairns, D.and Mackay, S. P. Pharm. Sci. (1997), 3, 569-572