DRUG DEVELOPMENT

Keeping the biotechnology of antisense in context

C.A. Stein

There can be no doubt any longer that phosphorothioates, the major antisense oligonucleotide in clinical trials and in use in countless laboratories, induce significant non-sequence-specific effects at both the molecular and supramolecular levels. For example, phosphorothioates bind to heparin-binding proteins with very high affinity[1]. And naked phosphorothioates (i.e., those delivered without a carrier) interact with cell surface proteins, producing biological consequences[2] that may be confused with antisense inhibition.

Other observations on the antisense inhibition of RelA that were thought to originate in an antisense mechanism were shown instead to stem from a combination of non-sequence specificity and from the presence of a G-quartet motif in the sequence[3]. As other non-sequence-specific effects have been observed during the past decade[4], it does not seem credible that all such possible effects have been characterized.

Do these facts prove a case for dismissing antisense therapeutics? Not at all. The non-sequence-specific effects of phosphorothioate oligonucleotides are highly concentration dependent; reduce one, reduce the other. Newer carriers, including cationic lipids, Starburst dendrimers, and cationic porphyrins, can accomplish this as well as (in theory) blocking access of the oligomer to heparin-binding proteins on the cell surface. Chimeric oligomers that reduce the sulfur content while preserving RNase H activity and nuclease resistance are already commonly available. Using the appropriate carriers and controls, biological effects that appear to be a result of an antisense effect begin to emerge with clarity.

Are the proponents of antisense then justified in their optimism? Perhaps, but the question gets sticky. I would contend that antisense "works" in cells, but only in a contextual, contingent framework. By "contextual" I mean that the observation of an antisense effect may not be dependent on sequence alone, but that such an observation occurs within the context of a combination of at least three factors, including both sequence- and non-sequence-specific effects, as well as the nature of the carrier. (The last is only infrequently considered, the unacknowledged assumption being that carriers are not "noticed" by cells. That this is not the case will be amply demonstrated in the near future by experiments employing DNA microarray technology.)

All of these factors may be necessary for the observation of an antisense effect, and none by itself may be sufficient. The proportionate contribution of each has rarely been evaluated in any model system (if it ever has), and the net effect is to produce uncertainty about the "purity" of any observed antisense effect—that is, which effect is directly due to Watson–Crick base-pair hybridization, and which is not. This issue is further complicated by the question of "irrelevant cleavage," a phrase that refers to RNase H-mediated cleavage of nontargeted mRNA[5–7]. Such reactions arise because the length of mRNA–DNA duplex needed to activate the enzyme may be quite short.

To the pharmaceutically inclined, these problems may be irrelevant, and it is certainly of little or no interest to a patient. But to those who cleave mRNA to validate targets, these questions are of far greater concern, and studies to evaluate them further are needed.

There is another conundrum that complicates the interpretation of literature-derived data. It has been a frequent, perhaps even universal, observation (e.g., see refs. 8 and 9) that for every eight or so oligomers tested against any one particular target, only one will be "active." In fact, the ratio of 1 success in 8 tested seems to be the best ratio attained; some researchers report 1 success in 12 or, or even 1 in 15. In this setting, a recent observation of Tu et al.[10] becomes especially curious.

These authors culled 2,026 reports of "successful" antisense inhibition from the biomedical literature. In 1,655 citations (82% of the total) only one antisense oligonucleotide was tested. An additional 248 (12.2%) tested more than two or three. Another 81 (3.9%) evaluated from 4 to 9, and only 42 (2.1%) examined more than 10. Thus, 93.9% of the experiments were "successful" using fewer than three tested oligonucleotides, and the great majority of those used only one.

This occurred despite the large number of careful studies showing that at the very best only one in eight antisense oligonucleotides (12.5%) will be "active." It could be that the published literature represents a selection bias, and that in fact perhaps eight times more than 1,655 (~13,240) unique oligomers were evaluated, the literature reporting only the 12.5% positive results. But such a bias cannot be the only explanation because certain sequence motifs are highly overrepresented in this group of "successful" experiments. Not surprisingly, one such motif is the G-quartet[11], which can be responsible for a mystifying array of non-sequence-specific effects.

Another possibility is that some, if not many, of the 1,655 citations in which only one oligomer was tested do not represent an "antisense" observation but rather combinations of antisense plus non-sequence specificity, or antisense plus cytotoxicity, coupled in some cases with G-quartet effects. But without in-depth evaluation, it may be difficult to sort all this out after the passage of some years, and besides, what are we to make of a field in which more than 90% of the basic observations are open to at least some reasonable criticism?[12]

It is finally the responsibility of both scientists working on antisense, and the journals in which they publish their results, to maintain a balanced outlook with respect to antisense biotechnology. Antisense is not Santa Claus; it really does exist. But the extent to which it exists unambiguously, in addition to questions of its contextual dependency, are aspects of the field that remain ill-defined. A landscape littered with the cinders of antisense biotechnology companies that were once rising stars demonstrates the folly of those who thought otherwise.

C.A. Stein is associate professor of medicine and pharmacology at Columbia University, New York, NY (e-mail: stein@cuccfa.ccc.columbia.edu).

1. Guvakova, M.A., Yakubov, L.A., Vlodavsky, I., Tonkinson, J.L. & Stein, C.A. *J. Biol. Chem*. **270,** 2620–2627 (1995).
2. Rockwell, P., O'Connor, W., King, K., Goldstein, N., Zhang, L.M, & Stein, C.A. *Proc. Natl. Acad. Sci. USA* **94,** 6523– 6528 (1997).
3. Benimetskaya, L., Berton, M., Kolbanovsky, A., Benimetsky, S. & Stein, C.A. *Nucleic Acids Res*. **25,** 2648–2656 (1997).
4. Neckers, L., & Iyer, K. in *Applied Antisense Oligonucleotide Technology* (eds Stein, C.A. & Krieg, A.) 147–159 (Wiley-Liss, New York, NY; 1998).
5. Giles, R., Ruddell, C., Spiller, D., Green, J., & Tidd, D. *Nucleic Acids Res*. **23,** 954–961 (1995).
6. Giles, R., Spiller, D., Green, J., Clark, R., & Tidd, D. *Blood* **86,** 744–754 (1995).
7. Benimetskaya, L., Takle, G., Vilenchik, M., Lebedeva, I., Miller, P., & Stein, C.A. *Nucleic Acids Res*. **26,** 5310–5317 (1998).
8. Dean, N., McKay, R., Condon, T. & Bennett, C. *J. Biol. Chem*. **269,** 16416–16424 (1994).
9. Monia, B., Johnston, J., Ecker, D., Zounes, M., Lima, W. & Freier, S. *J. Biol. Chem*. **267,** 19954–19962 (1992).
10. Tu, G., Cao, Q., Zhou, F. & Israel, Y. *J. Biol. Chem*. **273,** 25125–25131 (1998).
11. Smetsers, T., Boezeman, J., & Mensink, C. *Antisense Nucleic Acid Drug Dev*. **6,** 63– 67 (1996)
12. Monia, B. *Nat. Med*. **5,** 127 (1999).