Does antisense make sense?

Using antisense oligonucleotides as highly selective pharmaceutical agents to block expression of disease-associated proteins has been a tantalizing promise for more than 20 years. Now that promise has become a reality: Isis Pharmaceuticals (Carlsbad, CA, USA) was granted an approval by the US Food and Drug Administration (FDA) on 24 August 1998, to market fomiversen sodium (Vitravene™), an antisense oligonucleotide intended for use in the treatment of AIDS patients with cytomegalovirus (CMV)-induced retinitis. Does this approval mark the beginning of a new era in which the exquisite specificity of antisense technology will be used to alter the course of many different diseases, or is the approval of Vitravene an anomaly unlikely to be readily repeated?

The concept behind antisense is simplicity itself: an oligonucleotide with sequence complimentary to a region of the mRNA for a targeted protein is introduced into a cell. The oligonucleotide binds to that mRNA through Watson–Crick base pairing to physically block its translation and synthesis of its specified protein. An oligonucleotide only 12 nucleotides in length has sufficient specificity to identify a particular mRNA, blocking, in theory, the synthesis of only the target protein encoded by that mRNA. Instead of being translated, the oligonucleotide–mRNA complex is degraded by RNase H. Potentially, it is an elegantly specific treatment for a host of diseases ranging from inflammation to cancer and from bacterial to viral infections, with little or no side effects.

Tried and failed

Despite the simplicity and promise of the concept, attempts to develop antisense drugs have met with many failures, and many, if not most, drug discovery scientists have discounted the utility of the concept because of the difficulties of getting the simple concept to work in practice. Because of these failures, several companies involved in antisense technology, including Gilead Sciences (Foster City, CA, USA) and Genta (San Diego, CA, USA), have moved their drug discovery efforts to other research areas. Hybridon (Cambridge, MA, USA) is still pursuing antisense programs with its own version of antisense treatment for CMV retinitis (Phase I) and research on an antisense drug for cancer. However, Hybridon had to cancel their program in HIV therapy, despite considerable financial investment, because of dose-related side effects. Even Isis had to drop its first antisense drug, a treatment for human papilloma virus, because the frequency of treatment needed was not consistent with a practical therapeutic.

In spite of the nagging problems that have plagued the use of antisense, Isis Pharmaceuticals was built and continues to be based upon the belief that the antisense technology will eventually work. Consequently, the approval of Vitravene was a particularly momentous event in the life of the young company: not only is it the first drug with a claim for an antisense mechanism to be approved for marketing by the FDA; it is the first product Isis has brought to market, and if it turns out to work through an antisense mechanism, it will provide validation for the therapeutic strategy upon which the entire company is based.

Special case?

Vitravene is administered to patients suffering from CMV infection by intraviteral injection once weekly during the initial stages of treatment and then every two to four weeks during maintenance. The direct injection into the site of infection circumvents many of the problems associated with the systemic administration of antisense therapeutics and was quite probably one of the major reasons that CMV infection was chosen as the first therapeutic target by the young company. Despite this somewhat special case for their first product, 'many other antisense drugs will follow in its footsteps', claims Stanley T. Crooke, the Chairman of the Board and CEO of Isis, who left SmithKline & French to found the company in 1989.

Stringent testing

Isis has several other antisense products in its pipeline that represent a more stringent test for the antisense strategy. In collaboration with Boehringer Ingelheim Pharmaceuticals (Ingelheim, Germany), Isis is testing an antisense drug for down-regulation of the adhesion protein ICAM-1 and its potential for use in the treatment of Crohns' Disease. This drug is currently in a pivotal clinical trial. They also envision that ICAM-1 will be an effective antisense target for the treatment of psoriasis (Phase II completed), rheumatoid arthritis, ulcerative colitis and tissue rejection after transplantation (all in Phase II). Other antisense drugs in various stages of clinical trials target protein kinase Ca (Phase II), c-raf (Phase II), and Ha-ras (Phase I) for the treatment of cancer, and a second-generation antisense product is under investigation for the treatment of CMV retinitis in AIDS patients (Phase I).

Research hurdles

The major problems in developing antisense drugs have been due to the instability of oligonucleotides in a world full of nucleases, and difficulties with delivery of the antisense reagents to the disease site and into the target cell. [For a review of delivery strategies see Miller, K.J. and Das, S.K. (1998) *Pharm. Sci. Tech. Today* 1, 377–386.] A considerable amount of research also had to be

1359-6446/99/$ – see front matter PII: S1359-6446(98)01282-3

invested in finding the right region on each mRNA to hybridize with an antisense oligonucleotide. Originally it was thought that hybridization of an antisense reagent to any portion of the target mRNA would suffice to block translation, but this reasoning was incorrect. Hybridization of an oligonucleotide to some portions of the mRNA sequence have no effect, while other regions provide insufficient inhibition of translation for the desired therapeutic response. In practice, it is necessary to explore dozens of different regions of the target mRNA before a hybridization site is found that allows the antisense oligonucleotide to shut down translation.

Finally, considerable controversy has arisen over the mechanism of action of antisense drugs: most of the oligonucleotides used for the antisense strategy bind nonspecifically to other molecules in the cell and appropriate control experiments were not always performed, particularly in the early days of antisense research, which led to misleading results. Even when appropriate control experiments are conducted, determining the specific mechanism of action can still be a challenge.

Nuclease resistance

Finding a way to increase resistance to nuclease activity was one of the first problems to be dealt with successfully. Substitution of a sulphur atom for one of the oxygen atoms in the phosphate backbone of the oligonucleotide to produce phosphorothioate oligonucleotide provided a molecule with a marked increase in stability in the presence of nuclease activity. Yet the modification still allows the formation of the Watson–Crick base pairs necessary for hybridization with a complimentary region of mRNA.

There is a drawback, however, to the use of phosphorothioate oligonucleotides: they have a tendency to bind nonspecifically to proteins and RNA. Serum albumin, for example, binds phosphorothioate oligonucleotides with an affinity in the range of 150 μM, which Crooke has noted is in a similar range as the binding of aspirin or penicillin to serum albumin [Crooke, S.T. *et al.* (1996) *J. Pharmacol. Exp. Ther.* 277, 923–937].

Specific binding to proteins, dependent upon the nucleotide sequence or the overall structure of the oligonucleotide, has also been observed and presents a more serious problem for the interpretation of antisense experiments. Control experiments usually use an oligonucleotide with a scrambled nucleotide sequence, which may not exhibit the same binding pattern to proteins as the therapeutic oligonucleotide, leading the investigator to conclude that the reagent is acting through an antisense mechanism, when in reality it is altering the activity of a key protein. Because of these complications, a wide range of circumstantial evidence must be collected to support an antisense mechanism of action. Such evidence includes demonstrating the loss of target mRNA on Northern blots in a time-course consistent with the dose–response curve for the activity of the antisense reagent both at the cell level and in the intact organism. Similar experiments can be performed to investigate the correlation of the rank order potency of various antisense agents and the loss of target mRNA and protein both *in vitro* and *in vivo*.

Persistent problem

Getting the antisense drug to the disease site is a problem that persists. Antisense reagents will probably always be limited to parenteral administration – they are not readily absorbed intact when administered orally. Several studies have shown, however, that upon injection by any of a variety of routes, antisense oligonucleotides have a half-life and distribution consistent with their use as therapeutic reagents. Finally, phosphorothioate oligonucleotides are taken up and broadly distributed in a wide variety of cell types in vitro, although the exact mechanism of uptake is unknown.

Remaining questions

In spite of the advances in dealing with the practical problems of antisense oligonucleotides, the approval granted to Isis by the FDA and the extensive data collected by Isis on the mechanism of action of Vitravene, questions remain surrounding the notion that Vitravene proves the case for antisense therapeutics. The administration of Vitravene directly into the eye does not test the general ability of an antisense reagent to reach a disease site upon systemic administration, and the possibility that some other unknown mechanism of action may account for the therapeutic effect still cannot be conclusively ruled out. In fact, it is known that at high doses, Vitravene has non-antisense effects and the compound may actually bind to the CMV coat proteins preventing the cellular uptake of the virus. However, direct binding of Vitravene to the coat proteins is not considered to be a mechanism of action of the drug, because the virus is believed to be transmitted in the eye by direct cell-to-cell contact without the release of free virus.

There may be reservations, but Isis' results with Vitravene are exciting and have succeeded in resurrecting the field of antisense therapeutics. If Vitravene is acting through an antisense mechanism, as Crooke asserts, and if some of the other antisense drugs in Isis' pipeline are also successful in reaching the market, then the antisense strategy, despite its many years of trials and tribulations, may yet pave the way for a new era of highly specific therapies for a wide range of diseases that currently have no specific means of intervention.

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