Are PNAs a match for the BBB?

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Scientists at the Mayo Clinic (http://www/mayo.edu) have been granted a patent for their discovery that peptide nucleic acids (PNAs) can cross the blood–brain barrier (BBB) and target DNA in the brain. However, more recent research challenges their conclusions. Welcome to another chapter in the rocky history of antisense technology.

Antisense technology

The idea of antisense technology is simple and is over 20 years old: block the production of a disease-related protein by applying small DNA mimics that bind to the mRNA of the target gene. The pharmaceutical industry greeted the new technology with enthusiasm; several companies were established with the aim to progress antisense molecules into the clinic. However, things turned out to be more difficult than expected, and only a few of those companies remained in business. To date, just one antisense compound is commercially available (Vitravene®; Isis Pharmaceuticals, http://www.isip.com).

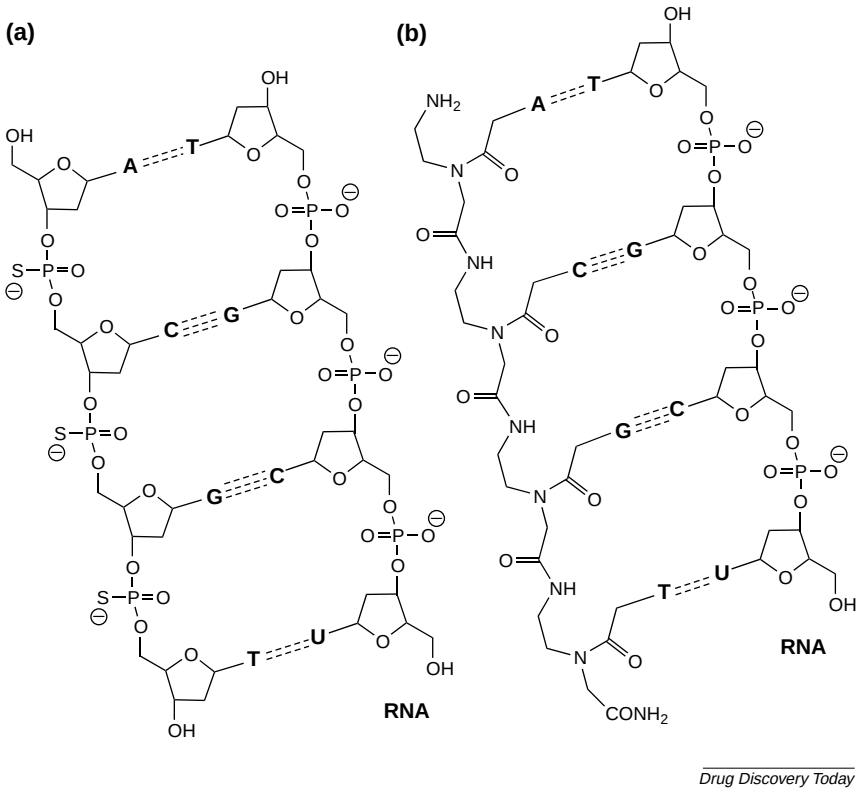

Figure 1. Older and newer generation antisense molecules binding to RNA. **(a)** Phosphorothioate DNA analogue. **(b)** Peptide nucleic acid (PNA). Figure kindly provided by Elliott Richelson (Mayo Clinic; http://www.mayo.edu).

Finding the right backbone

Many problems in the antisense field were a result of issues surrounding the chemical backbone that holds the antisense molecules together. For example, the phosphorothioate backbone of the first generation of antisense compounds is negatively charged, and when these compounds bind in complementary fashion to DNA or mRNA, their binding affinity is decreased because of the opposing electrical charges (Fig. 1).

In the early 1990s, a group of Danish scientists came up with a modified structure for a DNA mimic. 'We wanted to make a backbone that does not have any charge,' says Peter Nielsen at the University of Copenhagen (http://www.ku.dk). Therefore, he and his colleagues connected the nucleobases with peptidal bonds. And indeed, these novel molecules – PNAs – bound more tightly to the target mRNA and proved to be more resistant to enzymatic degradation. However, when *in vitro* tests began, scientists encountered difficulties: although the molecules were not charged, they did not seem to cross the cell membrane. It was therefore doubted that PNAs could affect cellular function.

Surprising results

Despite these reports, Elliott Richelson and colleagues at Mayo decided to use unmodified PNAs for *in vivo* experiments investigating the biological effect of neurotensin. 'We were interested to know what receptor subtype was involved in the anti-nociceptive and hypothermic effects of neurotensin,' recalls Richelson. In their initial experiments, they injected PNAs that were complementary to the neurotensin receptor directly into the brains of rats. Some 24 hours later, they injected neurotensin and observed that they had knocked down the effect of

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neurotensin with respect to hypothermia and antinociception. In the next step, they injected the PNAs intraperitoneally (ip) and found that they could still block the effects of neurotensin that was injected directly into the brain [1].

Richelson concluded that PNAs can cross the BBB: 'There is no other way they could have a biological effect.' A gel mobility shift assay enabled the team to quantify the amount of PNA in the rat brain following ip injection [1]. 'PNAs don't get in in large quantities, but they do get into the brain,' summarizes Richelson.

Scepticism

Based on these findings, the investigators received a patent that covers the use of PNAs administered to an organism to cause a biological effect. 'If it is for real and other people can repeat and confirm the results, that would be very interesting,' agrees Ryszard Kole at the University of North Carolina at Chapel Hill (http://www.unc.edu). But he and many others in the scientific community remain sceptical about whether PNAs really get into the brain.

Nielsen could not find any evidence that PNAs can cross the BBB in mice and has just submitted a research paper with his findings. 'It would be highly surprising if these molecules, from what we know of their properties, would easily cross the blood brain barrier,' he remarks. After all, molecules that can cross the BBB are usually lipophilic, whereas PNAs are hydrophilic compounds. Nielsen is concerned that Richelson has not supported his conclusions by solid molecular biology data. 'The antisense field is full of misinterpretations,' he says, 'where biological effects were interpreted as antisense but later turned out to be activation of the immune system and various other effects on the whole body.'

Mouse models

Kole himself also looked at PNAs in mouse models [3] and saw that the PNAs got into various organs but 'we did not see any effect of the PNAs in the brain'. He is puzzled by Richelson's observations. 'You see their data and can not find that there is anything wrong with them, but our results do not seem to support what they say.'

The only explanation he can think of is that there was a slight difference in what their respective experiments focused on: 'We [used PNAs for modulation of] splicing and therefore the PNAs had to get into the nucleus for us to see their effect. They targetted mRNAs, which are in the cytoplasm, so if PNAs were active only in the cytoplasm, we would have missed it.'

Meanwhile, Richelson and colleagues are taking their work a step further and are studying the effect of PNAs in several disease areas, including Alzheimer's disease [2]. Time will tell whether PNAs can really reach brain cells and reverse disease by knocking down amyloid production – and whether this chapter in the history of antisense technology will end in triumph or disappointment.

References

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