SHORT REPORT

An antisense Bcr-Abl phosphodiester-tailed methylphosphonate oligonucleotide reduces the growth of chronic myeloid leukaemia patient cells by a non-antisense mechanism

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Summary. The specificity of antisense oligonucleotides targeted to the mRNA breakpoint region of the Bcr-Abl oncogene, found in leukaemic cells from patients with chronic myeloid leukaemia, remains controversial due to non-specific effects. To prevent protein binding of oligonucleotides we designed and tested a methylphosphonate oligonucleotide with an attached 3' soluble phosphodiester tail. Growth of chronic myeloid leukaemia (CML) cell lines BV173, KCL-22 and cells of CML patients tested was inhibited by the b2a2 type antisense Bcr-Abl oligonucleotide and not with controls. Also the growth of control CD34+ cells of two healthy donors, control cell lines and cells from AML patients was only moderately affected or not affected. Bcr-Abl protein studies in combination with growth-determination experiments indicated that the antisense methylphosphonate Bcr-Abl oligonucleotide tested is a potent inhibitor of the growth of CML cells but works in a non-antisense manner.

Keywords: antisense, oligonucleotides, Bcr-Abl, methylphosphonate, apoptosis.

A variety of antisense oligonucleotides targeted to the bcr-abl mRNA breakpoint have been used. Modified oligonucleotides are more stable in serum, but non-sequence specific effects occur (Kirkland et al, 1993; Obrieb et al, 1994; Smetsers et al, 1995). This is probably caused by binding to proteins as a result of the negative charge of the oligonucleotides. Antisense phosphorothioates can bind to protein (for review see Stein, 1995). The uncharged methylphosphonates have been claimed to reduce cell growth by specific antisense effects (Tari et al, 1994). The big disadvantage of these oligos is their insolubility and they need to be delivered to cells in liposomes. In this study we used methylphosphonate oligonucleotides targeted to the Bcr-Abl mRNA breakpoint junctions. We tried to overcome liposomal delivery by adding a charged 3' phosphodiester tail to the oligonucleotides to make them soluble in culture medium.

They were dissolved in sterile IMDM and sterilized by filtration. The sequence of the oligonucleotides is as follows:

\( \text{aB2A2.21MET} : 5' -CTGAAGGCTTTCTCATT-3' \)
\( \text{B2A2.21MET} : 5' -AATAAGGAAGAAGCCCTTCA-3' \)
\( \text{d33A2.24MET} : 5' -GGGCTTTTGAACTCTGCTTAAATC-3' \)

Underlined characters represent methylphosphonate linkages; plain characters represent normal phosphodiester linkages.

CML cell lines culture, flow cytometric analysis and determination of the Bcr-Abl breakpoint type were carried out as described (Smetsers et al, 1994, 1995). Cryopreservation and separation of CD34+ cells were performed as described (Willems et al, 1996). SDS PAGE and immunoblotting were performed as described (Melijerink et al, 1995).

RESULTS

Antisense methylphosphonate oligonucleotides of 15 nucleotides and longer are not soluble in aqueous solutions. We modified the oligonucleotides described by Tari et al (1994). The oligonucleotides used here contain an additional five nucleotide long phosphodiester tail at the 3' end of the oligonucleotide and could be dissolved in culture medium.

MATERIAL AND METHODS

Antisense oligonucleotides were synthesized and purified by the Eurogentec corporation (Eurogentec, Seraing, Belgium).

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The effect of the partial methylphosphonate oligonucleotides on the cell growth of several CML and non-CML cell lines was determined at an oligonucleotide concentration of 10 μM. Fig 1A shows the cell numbers of these cell lines after 6 d in culture. Sense control oligonucleotide B2A2.21MET showed no reduction of cell growth of any of the tested cell lines. Treatment with antisense oligonucleotide αB2A2.21MET resulted in a decrease in cell number of

Fig 1. (A) Relative cell numbers of cell lines after 6 d of treatment with 10 μM of oligonucleotides. (B) Relative cell number of cells of CML patients and purified normal CD34+ cells after 6 d of treatment with 10 μM of oligonucleotides. (C) Relative cell number of cells of AML patients. Horizontal hatching: untreated; oblique hatching: αB1A2.24MET; open bars: B2A2.21MET; solid bars: αB2A2.21MET.

Fig 2A. Flow cytometric analysis of p210\textsuperscript{Bcr-Abl} expression in cell line BV173 after 5 d of treatment with oligonucleotides. AS: αB2A2.21MET; SE: B2A2.21MET; NT: not treated. Upper panel: expression of p210\textsuperscript{Bcr-Abl} relative to actin expression; lower panel: p210\textsuperscript{Bcr-Abl} expression relative to DNA.
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**DISCUSSION**

Oligonucleotide αB2A2.21MET is a growth inhibitor of CML cells. An antisense mechanism, however, is not responsible for this growth inhibition. Many differences exist between the tailed oligonucleotide and liposomal packed full methylphosphonates (Tari et al., 1994). Full methylphosphonates are breakpoint specific and reduce the growth of cell line K562. The use of liposomes, however, is likely to target the oligonucleotides directly to the cytoplasm. This can increase the specificity of the oligonucleotides in an antisense mediated effect. The use of the tailed oligonucleotide αB2A2.21MET has an advantage over the liposome encapsulated oligonucleotide in that it is more easy to...
prepare and it reduces the growth of both breakpoint types of CML cells.

The mechanism of action of this oligonucleotide is not known. The oligonucleotide probably hybridizes to another RNA or some sequences may induce physiological effects. If CML cells are more sensitive to these effects than normal cells the oligonucleotide can induce the specific CML growth reduction that was observed in this study.

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REFERENCES


