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Essentials of radionanotargeting using oligodeoxynucleotides

Review Article

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Summary

Antisense oligomers may be used for carrying radiation source into a specific location inside a tumour cell. Effects of radioactive labeled oligos may be exerted both via direct antisense inhibition and radiation. This radionanotargeting approach may provide several benefits to conventional treatment modalities, and radiation is minimized in adjacent tissue. In addition, a combination of radiation and antisense activity of oligodeoxynucleotide may result in synergistic interaction, as there are two different treatment modalities hitting a single mechanism of action. We have previously shown that oligonucleotide therapy is effective with internally labeled oligodeoxynucleotide phosphorothioates P-32, P-33 and S-35. Here, we review our results and discuss the role of radionanotargeting. We refer to our previous results of a large selection of radionuclides; we have calculated *in vivo* subcellular tissue distribution for oligodeoxynucleotide phosphorothioates using decay characteristics of ten β- and Auger-emitting radionuclides. The absorbed nuclear doses of these radiolabelled oligonucleotides were estimated in different cellular dimensions using the subcellular biodistribution data. These results indicate that Auger-emitter isotopes do not give higher absorbed cell nuclear doses than the isotopes suitable for internal labeling of oligo phosphorothioates. The best isotopes for subcellular targeting were P-33 and S-35 giving smallest variation of nuclear dose in the cell dimensions we studied (nuclear diameter 6-16 μm, cellular diameter 12-20 μm). Therefore, we conclude that radionanotargeting by oligonucleotides may provide synergistic interaction and should be carried on with short range β-emitters suitable for internal labelling of oligonucleotides unless relative biological effectiveness of Auger-emitters could be remarkably improved. Further preclinical evaluation of radionanotargeting based on radio-oligos should be continued.

I. Introduction

Radionanotargeting may provide several benefits over conventional treatment modalities. Theoretically high subcellular concentration is achievable due to an accumulation effect. Radiation is minimized in adjacent tissue. In addition, a combination of radiation and antisense activity of oligodeoxynucleotide may result in synergistic interaction.

Antisense oligodeoxynucleotides (oligos) are designed against the most specific target, a DNA/ RNA sequence. The primary target for ionizing radiation is nuclear DNA, and the radiotoxicity of Auger electron emitters is mainly due to low energy electrons with short ranges (1-10 nm). Antisense techniques have also limitations, e.g. limited uptake and unspecific binding (Kairemo et al. 1999a).

Antisense oligos have been studied *in vivo* with regards to their pharmacokinetics, pharmacological and toxicological properties. The pharmacokinetics of various oligos in animal models have already been determined (Agrawal et al.1991, Sands et al.1994). The selection of

an appropriate nucleotide sequence for oligos is of importance in inducing translation arrest. However, the therapeutic possibilities of radiolabeled antisense oligodeoxynucleotides are very much dependent on the characteristics of radiation.

The aim of this article is to demonstrate the importance of radionuclides in radionanotargeting. We also discuss a label optimization procedure based on cellular data, and discuss the suitability of several radionuclides for labeling oligos. This review article updates our research programs focused on developing nanotargeting with radiolabelled oligodeoxynucleotides planned up to the first in human trial.

II. State of the art

We have previously shown using the biodistribution data of oligonucleotide phosphorothioates in a xenograft model that this type of therapy can theoretically be given with P-32 and P-33 (Kairemo et al. 1996a). We have extended the analysis to several more isotopes, e.g. beta and Auger emitting radionuclides, and we calculated tumor and organ doses, and in vivo subcellular tissue distribution for oligodeoxynucleotide phosphorothioates (Kairemo et al. 1998).

We estimated the dosimetric properties of oligonucleotides at the cellular level, that could be predicted from existing data on the characterization of phosphorolabeled oligos. Predetermined situations differed from each other by nuclear and cell sizes. This made it possible to assess relative radiation exposures in these variable cellular dimensions. We used 10 radionuclides, P-32, S-35, Cr-51, Ga-67, In-111, In-114m, I-123, I-125, I-131, Tl-201, with different physical properties in our calculations. Four predetermined cellular dimensions were used, even though in reality cell and nuclear sizes may vary in a particular tumor type (Kairemo et al. 1996b). We chose to keep mathematics simple to get more understandable results. Calculations can give a recommendable source for the labeling of an oligo, and thus allow proper selection of the optimal label. This requires estimation of benefits of one radionuclide over others among different isotopes in several cell models with different cell dimensions. The accumulated dose from internally administrated radionuclides has been estimated in our calculations as described earlier (Kairemo et al. 1996a, b, 1998, 1999a).

In addition to the tumor dosimetry it is equally important to consider the dosimetry in the normal tissue for the optimization of the radiation therapy. For that reason we have made dose calculations using published biodistribution data of oligonucleotides in the mouse with a 15-mer In-111-labeled oligonucleotide sequence coupled with diethylenetriamine pentaacetate-isothiocyanate (Dewanjee et al.1994). All 15-21-mer oligos gave almost identical liver and kidney distributions in mice as well as in rats with the 25-mer oligodeoxynucleotide phosphorothioate GEM91 (Zhang et al. 1995) and the 27-mer oligonucleotide phosphorothioate (Iversen et al. 1994). The pharmakokinetics of the compounds were not expected to change for phosphorus or sulfur labeling. The activity concentrations were calculated using the estimated ratios of relative organ weights to whole animal weight; for liver, kidneys, bone marrow and tumor these were 9, 2, 3 and 5%, respectively (Cossum et al. 1993, Iversen et al. 1994). The cumulative concentration, i.e., the area under the time-concentration curve, was estimated either from the published time-concentration data assuming mono-exponential elimination after the maximum uptake, or using published biological half-lives and areas under the time-concentration curve. **Table I** shows all the oligonucleotides used in our calculations. The results for the estimated organ doses are presented in **Table II**. There is only a slight difference between the doses of P-33 and S-35 whereas the doses of P-32 are approximately 10-fold higher due to the greater disintegration energy.

The absorbed dose distribution does not merely describe the biological response of the cell or tissue to radiation (Kairemo et al.1999a). With an externally delivered radiation dose in radiotherapy of cancer it is known that total dose and dose rate (fraction size and interval) and total treatment time are the most important external factors affecting radiation reaction of healthy tissue and tumor response. Even if the biokinetics and activity distribution of two tracers labeled with different radionuclides are similar, the differences in physical dose distribution and decay time lead to differences in dose-rate distribution which can be biologically significant.

III. Discussion

The ideal dose in external radiation therapy has traditionally been defined as a dose which gives as many cures as possible before exponential increase in complications. The dose is always depending on the nature of the complications. Of course, the worst complication of radiotherapy is tumor recurrence. Enhanced local control is obtained when radiotherapy is followed by or administered simultaneously with adjuvant chemotherapy in locally advanced cancer. Combination treatment is based on attempting to increase the therapeutic index. External radiation may be replaced by oligonucleotide radiotherapy which is highly specific minimizing the radiation effects to normal tissue and dramatically reducing complications (Kairemo et al. 1999a). Therapeutic index is increased as dose limiting late side-effects are not occurring or occur only minimally.

In order to improve antisense oligonucleotide efficiency, chemical modifications have been developed, and improvement of oligonucleotide uptake has been achieved with different systems of vector development including liposomes (neutral, cationic, immunolipo-somes), nanoparticles, or covalent attachment to a carrier (Lefbrevre-d´Hellencourt et al. 1995). Polyalkyl-cyanoacrylate nanoparticles have been introduced as polymeric carriers of oncogene-targeting antisense DNA (Schwab et al. 1994). Our aim was to optimize radiation

exposure and to select the radiation source that provides the highest amount of radiation to the subcellular target (nucleus) and to diminish the radiation in the surroundings.

The envisioned therapeutic use of radiolabeled antisense oligos is based on the assumption that an appropriate amount of radiation is delivered to a targeted location of specific sequence of cellular DNA or RNA causing local damage. In addition, the radiotherapeutic effect may be enhanced by antisense mediated inhibition of gene function. Tumor-specific activity will be obtained by hitting appropriate targets, such as anti-oncogenes or tumor-suppressor genes, e.g. p53-mutations. The number of targets, may be increased in many ways, e.g. by inductive manipulation or gene transfer, and thus the efficacy of nucleotide radiotherapy can be improved. Targets with different cellular locations have been described, such as mRNA translation sites, pre-mRNA splicing sites, or the DNA molecules themselves. Use of antisense oligos to inactivate genes has still several difficulties and requires improvements: these include delivery of the oligo into cells and entry to an appropriate intracellular compartment, nonsequence specificity, optimizing pharmacokinetic properties and designing new and better oligo backbones. Radionanotargeting has a limited range from the radiation source resulting in a rapid dose fall-off effect while avoiding damage to surrounding tissues. However, applications remain local and systems for successful systemic administration need to be established.

Our approach has focused on optimizing the type of label for oligos using Auger-emitting radionuclides by calculating subcellular dose distribution. We show that, for subcellular targeting, the S-35 and P-32 internal labels give the lowest variation in estimated absorbed nuclear doses using our cell model of given dimensions (nuclear diameter 6-16 μm, cellular diameter 12-20 μm). The doses vary considerably using Auger-emitting isotopes depending on cellular dimensions; however, in small cells Auger-emitting isotopes may give a high dose (Kairemo et al. 1998). In tumors, cell dimensions may vary and, therefore, the above mentioned Auger-emitting isotopes should be applied only when nuclear target circumstances are well characterized. The high energy β-emitter P-32 gives a nuclear dose closest to uniform distribution in cell sizes; however, this is due to high energy.

Table I. List of used oligos in our calculations

Name of the oligo	Size, mer	Reference
c-myc antisense	15	Dewanjee, 1994
Anti-ICAM-1, ISIS 3082	20	Crooke, 1996
Anti-ICAM-1, ISIS 9045	20	Crooke, 1996
Anti-HPV, ISIS 2105	21	Crooke, 1996
Anti-HPV, ISIS 2911	20	Crooke, 1995
Anti-HIV-1	25	Iversen, 1994
Anti-HIV-1	27	Iversen, 1994
Peptide nucleic acid (PNA)	15	Mardirossian, 1997

Table II . Calculated organ doses for different internally labelled oligomers in mouse models. The percentage organ doses refer to those obtained in tumor models.

Oligomer	**Initial activity (% of injected dose)**	**Biological half-life, *Tb (hrs)**	**Liver dose (S35) Gy/MBq**	**Kidney dose (S35) Gy/MBq**	**Reference**
Peptide nucleic acid, 15-mer	0.19, liver 1.45, kidney	5.1, liver 4.8, kidney	0.003 (0.078%)	0.010 (0.79%)	Mardirossian, 1997
c-myc, antisense, 15 mer	6.95, liver 5.15, kidney	178.2, liver 170.7, kidney	0.40 (100%)	1.30 (100%)	Dewanjee, 1994
ISIS 3082, 20-mer	18.0 liver 25.0, kidney	62.0, liver 112.0, kidney	0.40 (90%)	4.0 (320%)	Crooke, 1996
ISIS 9045, 20-mer	45.0, liver 12.0, kidney	>1000, liver >1000, kidney	30 (7620%)	35.0 (2710%)	Crooke, 1996
ISIS 2105, 21-mer	18.0, liver 25.0, kidney	62.0, liver 112.0, kidney	0.40 (90)	4.0 (320%)	Crooke, 1996
c-myc, antisense, 15-mer	11.0, tumor	194.0, tumor	1.0, tumor (100%, tumor)		Dewanjee, 1994

We have previously found (Kairemo et al. 1996a,b) that P-32 labeled oligos destroy non-target cells because of their long range. This is not the case when the β-emitters, P-33 and S-35, were used which are optimal when targets are smaller than 300 μm in diameter (Kairemo et al. 1996a).

Preliminary studies using I-125 labeled oligos for mammalian cell lines suggested that oligonucleotides delivered with liposomes give a lower nuclear dose than DNA-incorporated I-125-UdR (Sedelnikova et al. 1998). However, our calculations indicate that there are several more optimal radionuclides than I-125 (Kairemo et al. 1999b). For example, S-35 and P-32 doses seem to concentrate more efficiently around the nucleus than I-125, which may be of practical importance in delivering the effective doses to the nuclear target. It should be emphasized that the behavior of the radiation at small distances is crucial. It would be important in oligoradiotherapy to achieve the highest possible uptake in the target cell and minimal radiation toxicity to surrounding cells. I-125 and other radiolabels should not be used unless better specificity is achieved.

It is possible to use a mixture of radioisotopes to ensure a complete coverage of targets in more than one locations, e.g. targeting nuclear related and cellular RNA at the same time. In addition, modern imaging technique allows visual control over kinetic events. Sometimes the target is dense, e.g. in nucleus or it can be diffusely spread around the cellular area. Dual labeling with P-32 and S-35 may provide therapeutic benefits when treating smaller and larger targets simultaneously (Kairemo et al. 1999b). P-33 and S-35 have some benefits over P-32, since the organ doses remain smaller and thus the therapeutic index may be wider. Critical organ exposure remains 10-fold lower with P-33 and S-35 than with P-32. Moreover, P-33 and S-35 concentrate more efficiently around the target than P-32, which could be of practical importance for the delivery of effective doses to the tumor. It can be addressed that the behavior of the radiation at small distances is crucial. Therefore, the radionuclides P-33 or S-35 are more suitable than P-32 for cell destruction at short distances. This was also clearly demonstrated with calculations of tumor doses for 1 g, 1 mg and 1 μg tumor masses (Kairemo et al. 1996b). The microscropic tumors cannot be treated using P-32. Instead, with P-33 or S-35 tumor doses up to 7.5-fold for 1 mg tumor mass and up to 50-fold for 1 μg tumor mass are achieved. Tumors larger than 1 g could be treated with any of these radionuclide labeled oligos. Oligos are carrying the radioactivity source inside the cell and finally achieve close contact with target RNA macromolecules.

Nanotargeting means a specific way of targeting small molecules at nanometer scale by antibodies or antisense oligonucleotides. Isotopes in oligonucleotide phosphorotioates are located in S or P atoms. Emitted radiation of isotopes affects structures close to the binding site of oligo. Antisense oligonucleotides serve as vehicles for radioisotopes enhancing targeted efficacy (**Figure 1**).

Currently, RNA expressions in selecting the target gene are being able to detect by cDNA microarrays in a single experiment screening thousands of genes. Thus the likelihood of the selected gene being the most important target is going to be high. This provides an excellent opportunity of modulating tumor to be more sensitive for chemotherapeutic agents and radiation. For example, use of the p53 as an early model for this approach. Transcriptional activation of genes by p53 may coordinately shut down cell cycle progression and induce a battery of genes involved in DNA repair. DNA damage induces p53 accumulation. Cells lacking p53 are resistant to other forms of apoptotic induction, such as that caused by chemotherapeutic agents and radiation. Tumors that have lost p53, are no longer able to respond to adverse growth conditions by initiating apoptosis. Docetaxel and irinotecan are examples of new efficacious drugs in variety of tumor types with new mode of action: prevention of depolymerization of tubulin and specific DNA topoisomerase I inhibition, respectively. These drugs have a favorable interaction with radiation, and presumably highly usable in radionanotargeting approach.

When obstacles have been resolved in antisense approach, including administration, delivery, uptake, accumulation in the target, binding to the receptor molecule, effective time, oligonucleotide radionanotargeting may be provide a new option for radiation therapy. The possible cellular targets have been presented in **Table III**. Successful radionanotargeting with oligos should result in a therapy where metastases are treated while irradiation is low in normal tissue except close surroundings of tumor. On the contrary, when using external radiotherapy, metastases are not located in the field but are left outside, while healthy normal tissue is also radiated. Radionanotargeting with oligos may many applications, e.g. in treatment of metastases in lymph nodes, locally advanced cancer, adjuvant therapy before and after the surgical operation with intention of either making tumors smaller or treating remnant tumors. In addition, true interactions between chemotherapeutic drugs and gene expressions are continuously being defined, which may provide an opportunity to resensitize tumor cells for already acquired resistance.

Our project of developing clinical therapy option based on radionanotargeting with oligos has now been partially completed: the first section which consists of an estimation and rationale for the selection of label. The second section is underway consists both of in vitro and in vivo experiments challenging our current conclusions, and the third section will be the first in human trial.

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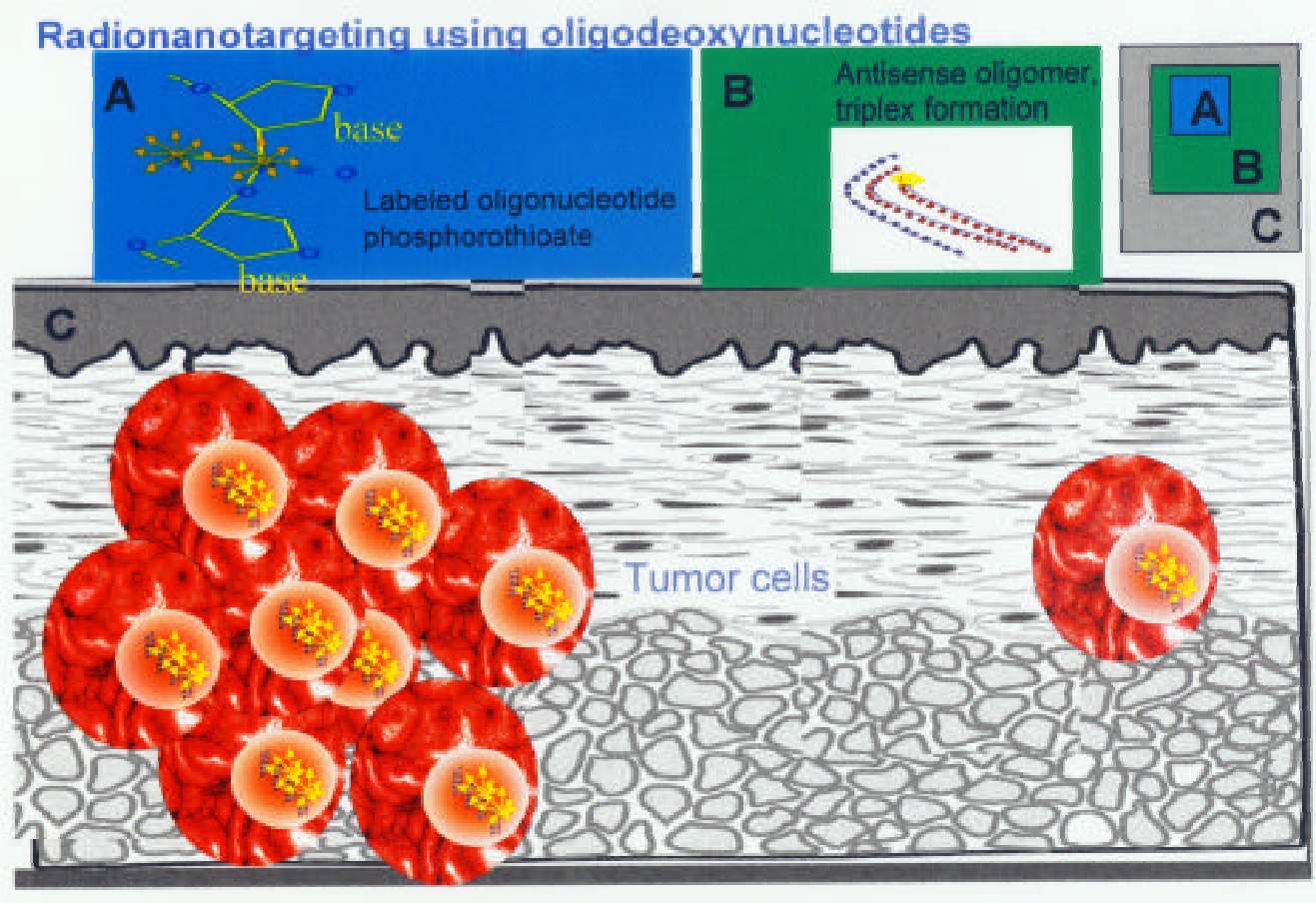

Figure 1. Schematic representation of radionanotargeting principles using oligos.

Table III. Schematic presentation of main characteristics in different cellular targets.

Factor	**Outer membrane receptor**	**RNA**	**Nuclear**
accessibility	easy	moderate	difficult
target	stable	transient	stable
induction	possible, slow	usual, fast	no
efficacy	low	moderate	high
exposure for surrounding cells	high	moderate	low

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