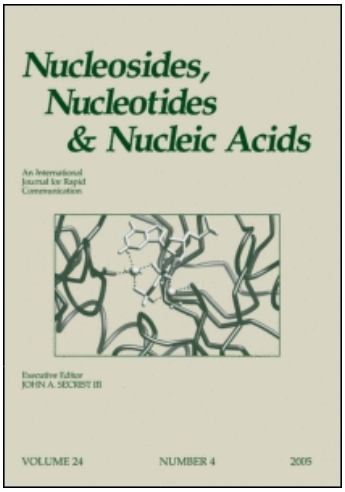

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In Vivo Imaging and Pharmacokinetics of Oligonucleotides

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IN VIVO IMAGING AND PHARMACOKINETICS OF OLIGONUCLEOTIDES

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Important efforts have been done in the last ten years to evaluate oligonucleotides (ONs) as potential therapeutic agents. Despite the simplicity of drug design due to the Watson-Crick rules, ONs are poor drug candidates because of their lack of stability, cell penetration and bioavailability. To circumvent these disadvantages, numerous chemical modifications have been proposed, but their evaluation in animals is painstaking. We present here a set of methods allowing to follow *in vivo* the pharmacokinetics and biodistribution of fluorine-18 oligonucleotides, injected in living primates, with Positron Emission Tomography (PET). The radioactivity associated with an ON in three different chemistries allowed us to image quantitatively and describe the whole body pharmacokinetics. Together with metabolic analysis of plasmatic samples this method provide a full description of any ON.

Methods : Three different chemistries (Phosphodiester : PO, Phosphorothioate : PS, 2'-O-methyl RNA : 2'-Om) of an 18-mer with the same sequence 5' - AGAATACAGGGTCCAAT-3', without any complementarity to known mammalian RNA sequences, were labelled on their 3' end with an 18-fluorine synthon following a previously described method (2). The influence of the labelling on the ability of the ON to hybridise to a complementary sequence was demonstrated in a competitive hybridization assay (3). Dynamic whole body acquisition with PET (Siemens EXACT HR+ camera) was performed in baboons during 160 min in 20 sequential acquisition at 8 min intervals.

Attenuation was measured with an ^{68}Ge transmission scan. Each baboon (5 to 24 Kg) received an *i.v.* injection of 1.72 ± 0.76 mCi (SRA 457 ± 255 mCi/μmole ^{18}F-[ON]). Organs were defined on trans-axial slides using both the transmission images, and the first and tenth emission images. Since PET is a quantitative technique that measures absolute concentrations of radioactivity, radioactivity *vs.* time curves were derived from the regions of interest and corrected for the injected dose.

Metabolism of the ONs was studied by cetylpyridinium bromide selective precipitation and by HPLC analysis of plasma from sequential blood samples drawn during the PET acquisition.

Results: The addition of the synthon at the 3' end of the ONs has no significant effect on the capacity of hybridization of the three species of ONs, although there were differences in the affinities for the complementary target (2'-Om > PO > PS) (1).

In the liver, the PO follows an accumulation (0.063%ID/cc at 5 min) and elimination (0.036%ID/cc at 60min) kinetic with a peak at 20 min (0.079%ID/cc), suggesting hepatic metabolisation of the ON. The 2'-Om is rapidly eliminated from the organ (0.055%ID/cc and 0.023%ID/cc at 5 and 60 min respectively), while the PS is highly retained in the liver (0.066%ID/cc and 0.1%ID/cc at 5 and 60 min.). Kinetics are similar for the spleen. In the heart, the radioactivity corresponds mostly to the blood flow, with a faster elimination for the PO compared to the PS and the 2'-Om (0.062 ; 0.085 ; 0.1%ID/cc at 5 min respectively and 0.0026 ; 0.028 ; 0.022%ID/cc at 60 min). The brain shows no significant differences between PO, 2'-Om and PS (0.0054 ; 0.0074 ; 0.005%ID/cc at 5 min and 0.002 ; 0.0042 ; 0.003%ID/cc). In the brain and muscle the uptake is very low compared to liver and kidneys . Lungs also show an important non specific retention for the phosphorothioate. Finally in the kidneys, the PO shows a rapid accumulation of the radioactivity (0.272%DI/cc at 10 min) and a slow decrease (0.044%ID/cc) corresponding to the renal dialysis through the bladder. On the contrary, the 2'-Om and the PS show continuous accumulation of the radioactivity (0.273%ID/cc and 0.195%ID/c at 80 min) Selective precipitation of the ONs by the CPB methods and radio-HPLC analysis show a rapid breakdown of the PO (5 min) in contrast with the PS and the 2'O-m which are recovered intact in the plasma samples one hour after injection. The 2'-Om is particularly stable since it was found intact in urine samples more than one hour after injection.

Conclusion : This set of technologies offers a powerful method to assess the pharmacokinetics and the biodistribution of any oligonucleotide, and can thus provide essential information for drug administration and evaluation of new chemical modifications of ON. It can also be used to study the efficacy of different vectors designed to improve the bioaviability and cellular penetration of oligonucleotides. In addition these results suggest that oligonucleotides could represent good candidates for *in vivo* imaging of nucleic acids.

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REFERENCES

1 Tavitian B. *et al Nature Medicine*, **1998**, *4*(4), 467-471.

2. Dollé F. *et al, J. Label. Compounds Radiopharm.* **1997**, *39*, 319-330.

3. Deverre JR *et al., Nucleic Acid Res.* **1997**, *25*, 1584-3589.