Phosphorothioate Oligodeoxynucleotides Distribute Similarly in Class A Scavenger Receptor Knockout and Wild-Type Mice

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Accepted for publication October 6, 1999 This paper is available online at http://www.jpet.org

ABSTRACT
It has been suggested that binding of phosphorothioate oligodeoxynucleotides (P=S ODNs) to macrophage scavenger receptors (SR-AI/II) is the primary mechanism of P=S ODN uptake into cells in vivo. To address the role of scavenger receptors in P=S ODN distribution in vivo, several pharmacokinetic and pharmacological parameters were compared in tissues from scavenger receptor knockout mice (SR-A−/−) and their wild-type counterparts after i.v. administration of 5- and 20-mg/kg doses of P=S ODN. With an antibody that recognizes P=S ODN, no differences in cellular distribution or staining intensity in livers, kidneys, lungs, or spleens taken from SR-A−/− versus wild-type mice could be detected at the histological level. There were no significant differences in P=S ODN concentrations in these organs as measured by capillary gel electrophoresis as well, although the concentration of P=S ODN in isolated Kupffer cells from livers of SR-A−/− mice was 25% lower than that in Kupffer cells from wild-type mice. Furthermore, a P=S ODN targeting murine A-raf reduced A-raf RNA levels to a similar extent in livers from SR-A−/− (92.8%) and wild-type (88.3%) mice. Finally, in vitro P=S ODN uptake studies in peritoneal macrophages from SR-A−/− versus wild-type mice indicate that other high- and low-affinity uptake mechanisms predominate. Taken as a whole, our data suggest that, although there may be some contribution to P=S ODN uptake by the SR-AI/II receptor, this mechanism alone cannot account for the bulk of P=S ODN distribution into tissues and cells in vivo, including macrophages.

Phosphorothioate oligodeoxynucleotides (P=S ODNs) can be designed to hybridize to specific mRNAs and inhibit gene expression through an antisense mechanism (Crooke, 1998). Antisense P=S ODNs have been shown to reduce target RNA and protein levels both in vitro (Bennett et al., 1992; Monia et al., 1992; Dean et al., 1994) and in vivo (Dean and McKay, 1994; Monia and Dean, 1998) and are being developed as therapeutic agents against various diseases. Pharmacokinetic studies in animals indicate that P=S ODNs are rapidly cleared from plasma and distribute to various organs after parenteral delivery, with major sites of accumulation including liver, kidney, spleen, and bone marrow (Agrawal et al., 1991; Cossum et al., 1994). Using histological techniques and capillary gel electrophoretic (CGE) analysis, we and others have demonstrated that P=S ODNs are taken up by cells within these organs (Rappaport et al., 1995; Butler et al., 1997; Graham et al., 1998). However, the mechanisms underlying P=S ODN uptake on the cellular level are not well characterized.

It has been suggested that scavenger receptors mediate the primary mechanism of P=S ODN uptake in the liver, kidney, and spleen in vivo (Sawai et al., 1996; Bijsterbosch et al., 1997; Steward et al., 1998). There are several different types or classes of scavenger receptors in mammalian tissues, but the best characterized and the one most relevant to P=S ODN uptake is the macrophage scavenger receptor SR-AI/II. Immunohistochemical studies on the distribution of SR-AI/II indicate that it is localized primarily on macrophages and sinusoidal endothelial cells in the liver (Naito et al., 1991; Hughes et al., 1995), and Kupffer cells and endothelial cells in the liver accumulate high concentrations of P=S ODN.

SR-AI/II was originally identified on the basis of its ability to cause massive lipid accumulation in macrophages through internalization of chemically modified LDL (Goldstein et al., 1979; Brown et al., 1980). However, the physiological role of SR-AI/II has still not been established, and the prevailing view holds that this receptor’s main function is to facilitate the clearance of microbial pathogens, senescent cells, or altered plasma proteins by phagocytic cells (Krieger and Herz, 1994; Terpstra et al., 1997). SR-AI/II exhibits an exceptionally broad ligand specificity in that various anionic proteins,
carbohydrates, lipids, and polynucleotides bind to it (Steinbrecher, 1999). The receptor-ligand interaction is predominantly electrostatic, but there are conformational constraints, as evidenced by the fact that polyinosinic acid (poly(I)) and polyguanylic acid are good ligands, whereas polycytidylic acid is not.

Previous studies demonstrated that the hepatic uptake of P-S ODN in vivo was inhibited by ligands of SR-AI/II such as poly(I) and dextran sulfate (Bijsterbosch et al., 1997; Stewart et al., 1998). Based on the results of these experiments, it was suggested that the scavenger receptor was the predominant route for P-S ODN uptake and that scavenger receptor-mediated uptake might impair the therapeutical ability of P-S ODN by shuttling and sequestration into lysosomes. However, the use of compounds like poly(I) as competitors makes it difficult to conclude that P-S ODNs are subject to internalization by SR-AI/II, because these compounds also bind to other receptors such as macrosialin/CD68 and scavenger receptor expressed by endothelial cells (Steinbrecher, 1999).

Important insights into the function of SRA-/− have recently been obtained from mice in which the SR-AI/II gene has been inactivated through targeted gene disruption (Suzuki et al., 1997). Thus, to directly address the role of SR-AI/II in P-S ODN uptake in vivo, we compared several pharmacokinetic and pharmacological parameters in SR-A-/− mice and their wild-type counterparts. Specifically, histological localization, intact P-S ODN concentrations, and antisense activity of P-S ODN in tissues were characterized in wild-type versus SR-A-/− mice after i.v. administration. Additionally, the uptake of a P-S ODN in peritoneal macrophages from wild type versus SR-A-/− was measured in vitro in the presence and absence of polyanionic competitors. Our data suggest that, although there may be a small contribution to P-S ODN uptake by the SR-AI/II receptor, this mechanism alone cannot account for the bulk of P-S ODN distribution into tissues and cells in vivo, including macrophages.

Materials and Methods

Oligonucleotide Synthesis. Several different P-S ODNs were used in the experiments described herein (Table 1), depending on the purpose of the experiments and availability of radioactively labeled compounds. Nonetheless, comparisons between experiments can be made because many studies have demonstrated that, as a class, P-S ODNs behave similarly after parenteral administration in terms of bulk tissue distribution and whole-organ uptake (Agrawal et al., 1995; Crooke et al., 1996; Geary et al., 1997). Also, the doses used in the in vivo experiments, 5 or 20 mg/kg, were in the range typically used to obtain antisense reduction of target RNA in mice (Dean and McKay, 1994; Bennett et al., 1997; Monia and Dean, 1998). All the oligonucleotides used in these experiments were fully modified P-S ODNs. They were synthesized at Isis Pharmaceuticals on a Milligen 8800 DNA synthesizer by the phosphoramidite method (Iyer et al., 1990). All compounds were purified with reversed-phase HPLC and determined to be greater than 85% full-length by capillary gel electrophoresis (CGE).

Animals. All experiments involving animals were approved by the Institutional Animal Care and Use Committee. SR-AI/II knockout mice were generated as previously reported (Suzuki et al., 1997). In brief, a targeting vector was introduced into exon 4 for disruption of the SR-AI/II gene. SR-AI/II knockout animals have only minimal phenotypic abnormalities, and the homozygotes appear healthy and breed normally. Brother-sister mating of homozygous mutants was done, and absence of the scavenger receptor in tissues from SR-A−/− mice was verified with a scavenger receptor antibody (clone 2F8; Serotech, Raleigh, NC). Either wild-type SR-AI/II mice or Institute of Cancer Research (ICR) mice from Harlan Laboratories (Indianapolis, IN) were used as controls. Mice were fed ad libitum and kept under controlled conditions.

Immunohistochemistry. Wild-type or SR-A−/− mice were injected i.v. via tail vein with either 5 or 20 mg/kg of ISIS 2105 in saline or saline alone (n = 4/group). Isis 2105 was used for these studies because the P-S ODN-specific antibody used for immunostaining was generated against it (Butler et al., 1997).

At 30 min, 4 h, or 24 h after injection, the mice were sacrificed with CO2 inhalation, and the tissues were immediately removed and placed in 10% neutral buffered formalin overnight. The tissues were paraffin embedded, and sections were cut, deparaffinized, and stained as described previously (Butler et al., 1997). Briefly, endogenous peroxidase activity was quenched with 0.3% H2O2; the sections were then rinsed in PBS and treated with 20 µg/ml proteinase K for 20 min. After blocking with normal donkey serum (Jackson Laboratories, West Grove, PA), the sections were incubated for 1 h with the anti-ISIS 2105 monoclonal or anti-SR-AI/II antibody (clone 2F8; Serotech). The sections were then rinsed in PBS and then incubated with donkey anti-mouse peroxidase or donkey anti-rat peroxidase (Jackson Laboratories, respectively). 3,3′-Diaminobenzidine (Sigma Chemical Company, St. Louis, MO) was used to visualize the peroxidase activity, and the sections were counterstained with hematoxylin and mounted in a permanent mounting media. Tissues from saline-injected animals were used as controls and treated the same as tissues from P-S ODN-injected animals.

Cellular Digestion and Organic Extraction for CGE Analysis. SR-A−/− and wild-type mice were injected i.v. with either 20 mg/kg ISIS 2105 (n = 7/group), ISIS 1082 (n = 4/group), or ISIS 3082 (n = 4/group). Animals were sacrificed 24 h after injections, and pieces of the kidney, liver, spleen, and lung were immediately removed and frozen on dry ice. The tissues were digested with proteinase K extraction solution, as described previously (Crooke et al., 1996; Graham et al., 1998). Samples were incubated for 2 h at 55°C to digest proteins after the addition of 30 pmol of an internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standard (T27) with the absorbance and migration time of the internal standa...
suspended in 200 μl of 50 mM sodium phosphate, 0.1 mM EDTA, pH 7.8, 8.3 μl of β-mercaptoethanol, and 5 Ci/g tritiated water (DuPont-NCI, Boston, MA) and heated for 6 h at 90°C to facilitate C 8 proton exchange. Total RNA was purified with the RNeasy method (Qiagen). Equal amounts of liver RNA (15 μg) from each animal were run on 1% agarose gel containing formaldehyde. After transfer onto a nylon membrane (Hybond; Amersham, Arlington Heights, IL), the blot was probed for A-raf with the previously described probe (Cioffi et al., 1997). The 473-bp cDNA was radiolabeled with [32P]dATP by a RadPrime DNA-labeling system (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. The blot was prehybridized for 30 min at 68°C in hybridization buffer (Rapid-hyb; Amersham), and the hybridization was carried out at 68°C for 2 h with the denatured [α-32P]dATP-A-raf cDNA. The membrane was washed for 15 min at room temperature with 2× standard saline citrate/0.1% SDS and then washed for 30 min at 60°C with 0.1× standard saline citrate/0.1% SDS. RNA transcript normalization was performed with glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and quantified with a Molecular Dynamics PhosphorImager (Sunnyvale, CA).

Tritium Labeling Procedure. Tritiated ISIS 1570 and ISIS 2302 were prepared with a previously described technique (Graham et al., 1993). Briefly, 12 to 24 mg of HPLC-purified P=S ODN was suspended in 200 μl of 50 mM sodium phosphate, 0.1 mM EDTA, pH 7.8, 8.3 μl of β-mercaptoethanol, and 5 Ci/g tritiated water (DuPont-NEN, Boston, MA) and heated for 6 h at 90°C to facilitate C 8 proton exchange. Subsequently, repetitive lyophilization and Sephadex G-10 (Pharmacia, Piscataway, NJ) column chromatography were performed to eliminate unincorporated tritium. Radiopurity, as assessed by anion exchange HPLC, was greater than 90% with this procedure, with a specific activity of 3.98 × 109 dpm/μmol.

Assays with Cultured Macrophages. Resident peritoneal macrophages were obtained from wild-type mice or SR-AI/II knockout mice by peritoneal lavage with ice-cold Ca2+−free Dulbecco’s PBS, as previously described (Lougheed et al., 1997). Cells were suspended in α-modified Eagle’s medium (MEM) (Canadian Life, Mississauga, Ontario, Canada) with 10% fetal bovine serum (Hyclone; Intermedico, Markham, Ontario, Canada) and plated in 12-well plastic culture plates at a density of 1 × 105/well. Adherent macrophages were cultured overnight in a humidified CO2 incubator and then washed with serum-free MEM. Radiolabeled P=S ODNs were added to the cells in α-MEM supplemented with 2.5 mg/ml of lipoprotein-deficient serum to minimize cytotoxicity, either in the presence or absence of various competitors. Poly(I) and polycytidyl acid were from Sigma. Acetyl low-density lipoprotein (Ac-LDL), oxidized LDL (Ox-LDL), and maleylated BSA (maleBSA) were prepared as previously described (Haberland and Fogelman, 1985; Lougheed et al., 1991). After 5 h incubation at 37°C, the media were removed, and cells were washed twice with Dulbecco’s PBS and then scraped from the plates. Cell-associated radioactivity was determined by scintillation counting, and cell protein content was determined by Lowry assay.

Statistics. Concentration data are expressed as the mean ± S.E. Data were evaluated by an unpaired Student’s t test on the matching tissues from wild-type versus knockout mice. Statistical significance was set at P < .05.

Results

Immunohistochemistry. We have previously characterized a monoclonal antibody that specifically recognizes exogenous P=S ODN (Butler et al., 1997). Using this antibody, we immunostained liver, kidney, lung, and spleen from SR-A/−/− versus wild-type mice at 30 min, 4 h, and 24 h after injection of 5 and 20 mg/kg of ISIS 2105. In wild-type animals, P=S ODN immunoreactivity can be detected in sinusoidal endothelial cells, Kupffer cells, and hepatocytes in liver (Fig. 1) at 30 min after injection at both doses. By 24 h, the intensity and the distribution of the immunohistochemical signal appears different than at 30 min, in that the sinusoidal cells are less intensely stained. This agrees with CGE analysis of fractionated cells from liver, showing that the concentration of P=S ODN is highest in endothelial cells at 30 min after i.v. injection and in Kupffer cells at 24 h after injection (Graham et al., 1998). Nonetheless, the localization remains the same in both wild-type and SR-A/−/− mice, with no visible differences in the relative staining of the various cell types. It appears that, at least at the microscopic level, sinusoidal endothelial cells, Kupffer cells, and hepatocytes take up P=S ODN in the SR-A/−/− mice to a similar extent as in the wild-type mice. Kidney, lung, and spleen were also stained with the antibody, and once again, no difference in the cellular distribution or the intensity of staining could be found between wild-type and knockout mice at any of the time points or doses studied (data not shown).

Total Organ Distribution of P=S ODN. In that immunohistochemistry is semiquantitative, the amount of intact ISIS 2105 in liver, kidney, lung, and spleen of knockout and wild-type mice was more precisely determined by CGE analysis 24 h after i.v. administration of a 20-mg/kg dose. As shown in Fig. 2, the amount of oligonucleotide detected varied as a function of organ, with the highest concentration of drug found in kidney, followed by liver, and the lowest amounts detected in spleen and lung. Little or no differences in organ concentrations were noted when intact ISIS 2105 was compared in the organs of wild-type and SR-A/−/− mice. Kidney, spleen, and lung oligonucleotide levels were essentially the same, and only in the liver was a 22% decrease noted in SR-A/−/− versus wild-type mice (3.6 ± 0.45 μM in SR-A/−/− mice versus 4.6 ± 0.31 μM in wild-type mice); however, this difference was not significant (P = .08). These same trends were also found when organ distribution was compared with two similar-length heterosequences, ISIS 3082 and ISIS 1082, again with no significant differences in P=S ODN concentrations in matching organs from wild versus knockout mice (data not shown). These results suggest that distribution into whole organs of knockout mice was independent of P=S ODN sequence and was essentially the same as that of wild-type mice.

Distribution of ISIS 2105 in Isolated Kupffer Cells. As described above, scavenger receptors have been localized by various means on macrophages and/or liver Kupffer cells. With established liver perfusion and cell isolation tech-
niques, the amount of ISIS 2105 was quantitated by CGE analysis in purified Kupffer cells derived from knockout and wild-type mice 24 h after a 20-mg/kg i.v. bolus. The data in Fig. 3 indicate that there was 25% difference ($P = .05$) in the amount of oligonucleotide within these cells, with wild type containing $9.9 \times 10^7$ molecules/cell, compared with $7.1 \times 10^7$ molecules/cell.

Fig. 1. Immunostaining of livers from wild-type (a, c, e) and SR-A$^{-/-}$ (b, d, f, h) mice with 2E1 antibody as described in Materials and Methods. 3,3'-Diaminobenzidine was used to visualize immunostaining (brown), and hematoxylin was used as a counterstain (blue). a and b, livers from saline-injected animals stained with 2E1 antibody. c and d, representative livers from mice 30 min after a 5-mg/kg injection of ISIS 2105. e and f, livers from mice 30 min after a 20-mg/kg injection of ISIS 2105. g and h, livers from mice 24 h after a 20-mg/kg injection of ISIS 2105. Original magnification, 400×.
Antisense-Mediated RNA Reduction in Livers in SR-A<sup>2</sup>/<sup>2</sup> Versus Wild-Type Mice. The above studies suggest that the tissue distribution of P<sup>5</sup>S ODN in SR-A<sup>2</sup>/<sup>2</sup> and wild-type mice is essentially similar. To verify that there was also no effect of SR-AI/II gene disruption on the pharmacodynamics of P<sup>5</sup>S ODN, we compared the effect of a P<sup>5</sup>S ODN targeting the protein kinase A<sup>-</sup>raf (ISIS 9064) in livers from wild-type versus knockout mice. A<sup>-</sup>raf is expressed in many tissues, including liver (Storm et al., 1990), and the A<sup>-</sup>raf antisense, ISIS 9064, has previously been shown to specifically reduce murine A<sup>-</sup>raf but not other members of the raf kinase family (Cioffi et al., 1997).

Mice were injected with either saline or 20 mg/kg of ISIS 9064 in saline every day for 1 week, and the animals were sacrificed 24 h after the last dose. RNA was isolated from liver and analyzed via Northern blot. As can be seen in Fig. 4, there was a significant (P< .0001) reduction of the murine A<sup>-</sup>raf RNA in the livers from P<sup>5</sup>S ODN-treated knockout relative to untreated knockout mice and in treated wild-type mice relative to untreated wild-type mice. However, the level of RNA reduction was similar in the wild-type (88.3 ± 5.7%) and knockout (92.8 ± 1.6%) mice, and there was no significant difference in this decrease between the two treated groups (P = .47). Thus, the absence of SR-AI/II did not affect the ability of an antisense P<sup>5</sup>S ODN to reduce target RNA in the liver. Results from experiments done with lower doses of the P<sup>5</sup>S ODN produced less of a reduction in RNA, but it was again the same in wild-type versus knockout mice, and control P<sup>5</sup>S ODN failed to reduce A<sup>-</sup>raf mRNA expression in liver (data not shown).
In Vitro Uptake of P=S ODN into Peritoneal Macrophages. Based on a comparison of uptake of tritiated ISIS 1570 and ISIS 2302 into peritoneal macrophages from SR-A−/− versus wild-type mice as a function of concentration, both high-affinity/low-capacity and low-affinity/high-capacity uptake mechanisms for P=S ODN appear to exist. The results of the double-reciprocal plots from two experiments indicate that the $K_m$ values of high- and low-affinity uptake pathways are similar in wild-type and knockout mice (Table 2). The data indicate that there was about a 50% decrease in the high-affinity component and very little difference in the low-affinity component between knockout and wild-type mice. The fact that there is only a 50% difference in the high-affinity uptake suggests that other high-affinity receptor-based mechanisms exist.

Competition experiments in macrophages from knockout mice were performed at a low P=S ODN concentration to characterize the second high-affinity second uptake pathway. Previous studies have shown that Ac-LDL binding is reduced by 80% in SR-A−/− mice (Suzuki et al., 1997), and as shown in Fig. 5, Ac-LDLs have little effect as competitors on P=S ODN binding to SR-A−/− macrophages. Interestingly, P=S ODN binding is partly inhibited by poly(I) and malBSA but not by oxidized Ox-LDL. This is in agreement with the work of Takakura et al. (1999) who found that although Ac-LDL and Ox-LDL failed to inhibit uptake of pDNA in SR-A−/− macrophages, poly(I) and malBSA did inhibit uptake. The difference in the high-affinity uptake is probably not due to other scavenger receptors such as macrosialin/CD68, MARCO, or CD36, because Ac-LDL and Ox-LDL are ligands for these receptors as well. Thus, the inhibition by poly(I) and malBSA suggests another unknown class of receptors, but even these cannot account for all of the uptake, even at low concentrations of P=S ODN, because inhibition never exceeded 50%.

Discussion

Research on the significance of various receptors and mechanisms involved in P=S ODN uptake into cells has produced seemingly contradictory results from different laboratories. Part of the confusion is due to the increasingly apparent differences in the mechanism of P=S ODN uptake into cells in vitro versus in vivo. Whereas cationic lipids or other vectors seem to be required for antisense activity in transformed cells in vitro, P=S ODN uptake into cells and antisense activity are observed in tissues in vivo without formulations. It is generally agreed that P=S ODNs as a class appear to behave similarly in terms of organ distribution after parenteral administration, with kidney and liver accumulating the highest concentrations of P=S ODN, followed by bone marrow, spleen, and other tissues (Agrawal et al., 1995; Crooke et al., 1996; Geary et al., 1997). However, at increasing concentrations, the accumulation of P=S ODN by kidney and liver begins to level off, and P=S ODN accumulation in other organs increases. This result has been interpreted by some as evidence that saturation in the kidney and liver may in part result from receptor-mediated uptake of P=S ODN, although it is also possible that it occurs because of saturation of P=S ODN binding to tissue matrix.

The scavenger receptors SR-AI/II and Mac-1 have both been suggested by several authors to play a predominant role in P=S ODN uptake in vivo (Benimetskaya et al., 1995; Bijsterbosch et al., 1997; Steward et al., 1998). However, the importance of such receptors is limited immediately by the fact that they have more restricted distribution compared with that of P=S ODN after parenteral administration. Thus, uptake via the scavenger receptor or Mac-1 cannot explain the presence of P=S ODN in the proximal tubules of the kidney and hepatocytes, which clearly accumulate P=S ODN. Nonetheless, it is possible that, at least in macrophages and liver endothelial cells, the predominant mechanism of uptake is via the scavenger receptor.

Important insights into the function of SR-AI/II have been obtained by other investigators who used SR-A−/− mice (Fraser et al., 1993; Suzuki et al., 1997), and we therefore used the SR-A−/− mouse to more directly address the relative importance of the SR-AI/II in P=S ODN uptake. Specifically, we compared the histological localization of P=S ODN, CGE analysis of P=S ODN concentration in tissues, measurement of target RNA reduction by P=S ODN in vivo, and P=S ODN uptake by macrophages in vitro in wild-type versus SR-A−/− mice.

Previous work indicates that by 24 h, only 7% of P=S ODN in liver is present in hepatocytes, with the bulk of the injected P=S ODN in Kupffer cells and endothelial cells (Graham et al., 1998) after a 10-mg/kg dose. If indeed binding to SR-AI/II

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<td>Kinetic analysis of P=S ODN binding to isolated mouse peritoneal macrophages</td>
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<th>$V_{max}$ (pmol/mg)</th>
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Fig. 5. Competition for the uptake of ISIS 1570 into macrophages from SR-A−/− mice. Macrophages from SR-A−/− mice were incubated with 2 μM ISIS 1570 in the presence of indicated competitors, as described in the Materials and Methods. Uptake is expressed as percentage relative to uptake in the absence of competitors. Each point represents the mean of duplicate experiments. Poly(C), polycytidylic acid.
is the predominant mechanism of uptake of P-S ODN in liver endothelial and Kupffer cells, a difference in the intensity and/or distribution of PS ODN immunostaining should be observable at the histological level in the livers from wild-type versus SR-A−/− mice at this time point. However, after i.v. administration of both 5- and 20-μg/kg doses, no detectable differences in the localization of P-S ODN among the liver cell types were observed, at any of the time points studied.

It is possible that there are subtle differences in distribution of P-S ODN between wild-type and SR-A−/− mice that cannot be detected by immunostaining. Indeed, a reduction in P-S ODN concentration in total liver (22%) and isolated Kupffer (25%) cells from knockout mice was detected relative to wild type at 24 h after a 20-μg/kg dose. Overall, however, CGE results with three different P-S ODN revealed no major differences in P-S ODN concentrations in whole liver, kidney, spleen, and lung in SR-A−/− mice compared with controls. Also, using a P-S ODN that targets murine Araf, we found that the level of target RNA reduction in the liver was essentially the same in both knockout and wild-type mice. Finally, in macrophages isolated from wild-type versus knockout mice, the SR-A/II receptor accounted for about half the high-affinity uptake of P-S ODN at low concentrations, and there were no differences in uptake at higher concentrations.

There are several possible reasons why our experiments demonstrated little effect of SR-AI/II deletion on P-S ODN distribution in vivo. First, because SR-AI/II expression is normally restricted to macrophages and liver endothelial cells, it is possible that other uptake mechanisms that are more widely distributed predominate. Second, we administered relatively high concentrations of P-S ODN to animals, and this would have increased the proportion of P-S ODN that was internalized by low-affinity, high-capacity mechanisms. These low-affinity pathways, which may include receptor-mediated, absorptive, and fluid-phase endocytosis, evidently are active in a broad range of tissues and probably account for the bulk of P-S ODN uptake in vivo. P-S ODNs bind to many different serum and matrix proteins (Benimetskaya et al., 1995), and it seems likely that there are many proteins that play a role in P-S ODN transport, binding, and uptake. As mentioned above, many cells that accumulate P-S ODN, like the proximal tubules of the kidney and hepatocytes, do not express either SR-AI/II or Mac-1, the two most commonly cited putative P-S ODN binding proteins.

Our results appear to disagree with those of some investigators. Stewart et al. (1998) and Bistehborst et al. (1997) reported significant changes in P-S ODN uptake in liver and spleen when competitive inhibitors of scavenger receptors were coadministered with radioactive P-S ODN in vivo. Both groups found between a 40 and 60% decrease in radioactivity in liver with low doses (0.06 and 1 mg/kg, respectively) of P-S ODN and a 10-fold excess of dextran sulfate or poly(I).

The discrepancy might partly be because of the much lower doses of P-S ODN used by these investigators, thereby favoring high-affinity uptake pathways. Also, both of these groups relied on radioactivity measurements and did not determine the integrity of the material in the tissues. Finally, inhibition by poly(I) and dextran sulfate cannot be taken as definitive evidence of specific binding to SR-AI/II, because several other receptors and uptake mechanisms are also inhibited by these compounds. In support of this, our in vitro experiments demonstrated that poly(I) partially inhibits the uptake of P-S ODN even in macrophages from SR-AI/II knockout mice. Our findings do support those of Takakura et al. (1999), who found that Ac-LDL and Ox-LDL failed to inhibit P-DNA binding to peritoneal macrophages from SR-A−/− mice. Also, they could not detect any major differences in organ distribution even after a low dose (1 mg/kg) of a radioactively labeled P-DNA at 10 min after injection of SR-A−/− mice versus wild-type mice.

In conclusion, our in vitro and in vivo results comparing P-S ODN uptake in SR-A−/− versus wild-type mice do not support a major role for the SR-AI in P-S ODN uptake, even in tissues where expression is high.

References


