Pharmacokinetics and Distribution of a $^{33}$P-labeled Anti-Human Immunodeficiency Virus Oligonucleotide (AR177) after Single- and Multiple-Dose Intravenous Administration to Rats

THOMAS L. WALLACE, SCOTT A. BAZEMORE, KARSTEN HOLM, PETER M. MARKHAM, J. PAUL SHEA, NILABH CHAUDHARY and PAUL A. COSSUM

Aronex Pharmaceuticals, Inc., The Woodlands, Texas (T.L.W., S.A.B., N.C., P.A.C.), and TSI Mason Laboratories, Inc., Cambridge, Massachusetts (K.H., P.M.M., J.P.S.)

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ABSTRACT

AR177 is a 17-mer oligonucleotide that has anti-human immunodeficiency virus activity in vitro. The disposition of internally labeled $^{33}$P-AR177 was studied after the tail vein injection of single and multiple doses (0.7 mg/kg) to rats. After a single dose, the terminal half-life of AR177 in the blood and plasma was 367 and 271 hr, respectively, significantly longer than values reported for other oligonucleotides. Analysis of the AR177 tissue distribution showed that the majority of the dose was distributed to the liver (40%), bone marrow (17%) and renal cortex (15%) at 8 hr after single dosing. Analysis of the AR177 concentrations in tissues showed that the highest concentrations were achieved in the renal cortex (15.0 $\mu$g-eq/g), liver (7.4 $\mu$g-eq/g), bone marrow (3.9 $\mu$g-eq/g), mesenteric lymph node (3.0 $\mu$g-eq/g) and spleen (2.4 $\mu$g-eq/g) at 8 hr after single dosing. The half-life in these tissues was 9.6, 7.7, 36.8, 10.0 and 30.8 days, respectively. Forty-eight hours after the last of seven i.v. doses given every other day, the concentrations in tissues were as follows: renal cortex, 39.9 $\mu$g-eq/g; liver, 33.9 $\mu$g-eq/g; bone marrow, 12.7 $\mu$g-eq/g; spleen, 9.3 $\mu$g-eq/g; mesenteric lymph node, 5.1 $\mu$g-eq/g. Twenty-one days after administration of the last dose, tissue concentrations were still high, as follows: renal cortex, 18.6 $\mu$g-eq/g; liver, 6.2 $\mu$g-eq/g; bone marrow, 12.5 $\mu$g-eq/g; mesenteric lymph node, 3.9 $\mu$g-eq/g; spleen, 8.1 $\mu$g-eq/g. There was low urinary and fecal excretion (urinary excretion of 12.8% and fecal excretion of 6.0% of the total dose over 21 days) after a single dose. Gel filtration and anion-exchange high-performance liquid chromatography and electrophoretic analysis of the radioactivity in tissues indicated that >90% of the radioactivity represented intact AR177 for at least 7 days after drug dosing. These results demonstrate that AR177 has an extended plasma, blood and tissue half-life, is widely distributed and achieves high concentrations in lymphoid and nonlymphoid tissues in rats.

There are currently a number of drugs approved for the treatment of HIV infection that are either reverse transcriptase or protease inhibitors. To date, monotherapy with any drug has resulted in ultimate therapeutic failure, at least in part because of the development of resistance. Progression of the disease continues to occur in treated patients, with decreases in CD4 $^+$ T lymphocyte concentrations and the onset of opportunistic infections leading to death. There is a consensus among clinical investigators that combinations of drugs from multiple therapeutic classes are required to provide effective treatment of HIV infection (Schmittman and Fauci, 1994; Lange, 1995). Indeed, many studies have shown that combinations of drugs from each therapeutic class provide greater effectiveness than each of the drugs given alone. The utility of this approach is dependent upon the development of anti-HIV drugs whose mechanism of action involves targets other than reverse transcriptase or protease. Among other possible molecular targets is HIV integrase, the enzyme responsible for catalyzing the incorporation of viral DNA into human DNA.

AR177 (T30177; Zintevir) is an oligonucleotide with the sequence 5’-GsTGTTGGGTGGGTGGGsT-3’, where $s$ represents phosphorothioate linkages. AR177 is the most potent inhibitor of integrase described to date, with an IC$_{50}$ in the 30 to 80 nM range (Ojwang et al., 1995; Mazumder et al., 1996). Previous studies have shown that AR177 has antiviral activity against both laboratory and clinical strains of HIV-1 in human lymphocytes and macrophages (Ojwang et al., 1995). AR177 is relatively resistant to serum nucleases (Bishop et al., 1996), is devoid of the cardiovascular toxicity (Wallace et

ABBREVIATIONS: AUC, area under the curve; $C_{min}$, minimum plasma concentration; HIV, human immunodeficiency virus; HPLC, high-pressure liquid chromatography.
seen with total phosphorothioate oligonucleotides in monkeys (Cornish et al., 1993) and does not cause any tissue damage after multiple i.v. doses to monkeys (Wallace et al., 1996b). AR177 is the first integrase inhibitor to enter human testing and is currently in single- and multiple-dose phase I clinical trials. As part of the preclinical assessment of AR177, the pharmacokinetics and tissue distribution were determined for 21 days after single- and multiple-dose administration of $^{33}$P-AR177 to rats. This is the first published report of the pharmacokinetics and distribution of an oligonucleotide after multiple doses.

**Materials and Methods**

**Materials.** Nonradioiodinated AR177 was synthesized on a Milligen 8800 oligonucleotide synthesizer and made into a stock solution at 25 mg/ml in sterile phosphate-buffered saline. AR177 has a molecular weight of 5793 daltons and is a fully neutralized sodium salt. The structure of AR177 was characterized by phosphorus and proton nuclear magnetic resonance spectroscopy, sequencing, base composition, laser desorption mass spectrometry, anion-exchange HPLC and polycrylamide gel electrophoresis. The AR177 preparation was 94% pure, according to HPLC and electrophoretic analysis. All analyses were consistent with the proposed structure.

For HPLC analysis of plasma AR177, Tris was obtained from Fisher, NaBr and NaCl were obtained from Sigma and methanol was purchased from J. T. Baker. The Gen-Pak Fax anion-exchange HPLC column (4.6 x 100 mm; catalogue no. 15490) was purchased from Waters.

**Preparation of $^{33}$P-AR177 dosing solution.** AR177 was internally labeled with $^{33}$P according to the method of Bishop et al. (1996) (5′-GTGGGTGGCTG’GGTGGGTG-3′, where the asterisk indicates the position of labeling). The radiochemical purity was 98% for the single-dose study and 96% for the multiple-dose study. The dosing solution was composed of unlabeled AR177 and $^{33}$P-labeled AR177 formulated in phosphate-buffered saline to a final concentration of 1 mg/ml.

**Single-dose study.** The pharmacokinetics and tissue distribution of $^{33}$P-AR177 were studied in male Sprague-Dawley rats after a single i.v. dose of 0.7 mg/kg. The rats were dosed at 0.7 ml/kg using a 1 mg/ml solution. The single-dose study was performed in two parts, with male rats that were 8 to 20 weeks of age. In the first single-dose study, rats were used for collection of tissues and body fluids up to 4 days after the single dose. These rats (n = 35) had a body weight of 300 ± 12 g (mean ± S.D.) and received a dose of 0.7 mg/kg with a specific activity of 119 μCi/mg. The dose of radioactivity that these rats received was 25.0 ± 2.4 μCi/rat (mean ± S.D.). The single-dose study was extended when it was discovered that the drug had a long half-life after a single dose. In the extended study, male rats were used for collection of tissues and body fluids at days 7, 14 and 21 after a single dose. These rats (n = 9) had a body weight of 255 ± 12 g (mean ± S.D.) and received a dose of 0.7 mg/kg with a specific activity of 101 μCi/mg. The dose of radioactivity that these rats received was 18.1 ± 3.7 μCi/rat (mean ± S.D.). The mean dose of radioactivity for the single-dose study (n = 44 rats) was 24 μCi/rat.

Radioactivity was determined in blood and plasma at 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 18, 24 and 32 hr and 2, 3, 4, 7, 11, 14, 18 and 21 days after the administration of $^{33}$P-AR177. The blood and plasma pharmacokinetic data were derived from six rats at 0.25, 0.5, 1, 2 and 8 hr after dosing, five rats at 4 days and three rats at 1.5, 3, 4, 12 and 18 hr and 1, 1.33, 2, 3, 7, 11, 14, 18 and 21 days after dosing. The blood was collected in EDTA, and the plasma was obtained by low-speed centrifugation of the blood. Aliquots of the blood and plasma samples were reserved for analysis of the radioactivity by HPLC and gel electrophoresis. Blood and plasma were taken from each rat, but a different set of rats was used at each time point. This was done to minimize the number of rats that were used in the study and the amount of blood that was taken from each rat. Radioactivity was determined in tissues at 1, 2, 4, 8, 24, 32 and 48 hr and 4, 7, 14 and 21 days after the administration of $^{33}$P-AR177. These tissues were adrenal, bone marrow, brain, eyes, liver, lungs, renal cortex, renal medulla, mesenteric lymph node, skeletal muscle, skin, spleen, testes and thymus. The tissue pharmacokinetic data were taken from three rats at 1 hr, two rats at 2, 4, 8, 24 and 32 hr and 2, 7, 14 and 21 days and five rats at 4 days. Total radioactivity in tissues was determined in these rats. One additional rat was used to determine the presence of intact $^{33}$P-AR177 in tissues by HPLC and/or gel electrophoresis. Radioactivity was determined in cumulative urine and feces samples at 4, 8 and 24 hr and 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 17, 18, 19, 20 and 21 days after the administration of $^{33}$P-AR177. The urinary and feces pharmacokinetic data were derived from five rats from 4 hr to 4 days of collection and from three rats from 5 days to 21 days of collection. The blood, plasma, urine, feces and tissues were stored frozen until analyzed.

**Multiple-dose study.** The pharmacokinetics and tissue distribution of $^{33}$P-AR177 were studied in male Sprague-Dawley rats after up to seven i.v. doses given every other day at 0.7 mg/kg (specific activity, 20 μCi/mg; 3.8 μCi/rat), using a 1 mg/ml solution. The rats (male) had a body weight of 273 ± 21 g (mean ± S.D.) and were 8 to 9 weeks of age. The body fluids and tissues that were sampled were the same ones described for the single-dose study. Blood and plasma pharmacokinetic data were derived from three rats at 0.25, 1, 2, 4, 8 and 24 hr after dose 1, just before doses 2 to 7 and at 0.25, 0.5, 1, 2, 4, 6 and 8 hr and 1, 2, 3, 7, 14 and 21 days after dose 7. Aliquots of the blood and plasma samples were reserved for analysis by HPLC and gel electrophoresis. Tissue samples were collected from two rats at 48 hr after doses 1, 3 and 7, from two rats at 7 and 14 days after dose 7 and from five rats at 21 days after dose 7. Total radioactivity in tissues was determined in these rats. One additional rat was used to determine the presence of intact AR177 in tissues by HPLC and/or gel electrophoresis. Urine and fecal samples were taken from five rats at each time point up to and including day 21. The values that are presented are the means of these replicate determinations. The blood, plasma, urine, feces and tissues were stored frozen until analyzed.

**Determination of $^{33}$P-AR177-associated radioactivity in tissues, feces and body fluids.** Tissues, feces and blood were homogenized in distilled water (20%, w/v), using a Brinkman Polytron homogenizer, and solubilized with Soluene (Beckman). Plasma, urine and aliquots of homogenized tissue, feces and blood were placed in scintillation cocktail (Packard), and radioactivity was determined in a scintillation counter (Beckman LS6000) using a quench curve.

Calculation of the percentage of the total dose in each organ was based on the actual weight of the excised organ. Calculations of the percentage of the total dose in skeletal muscle, skin and bone marrow were based on these tissues representing 50, 16 and 3% of the total body weight, respectively (Burka et al., 1987).

**Gel electrophoresis.** Tissues were homogenized for 10 sec with 0.5 ml of Nonidet P-40 tissue extraction buffer/g of tissue in a 7-ml Kimble polyethylene vial containing one 4-mm glass bead in a Wig-L-Bug (Crescent Dental Manufacturing Co., Lyons, IL). The Nonidet P-40 tissue extraction buffer consisted of 0.2 M Tris, pH 7.5, 0.1 M EDTA and 3% NP-40. The homogenate was incubated for 5 min at room temperature and then rehomogenized in the Wig-L-Bug. After homogenization, the tissues were incubated at 37°C for 24 hr to extract $^{33}$P-AR177 from the tissues. The homogenate was then transferred to a 2-ml microfuge tube and centrifuged for 10 min at 10,000 × g in an Eppendorf centrifuge. The supernatant fraction was decanted and frozen in microfuge tubes for later analysis.

The supernatant fraction (5 μl) from the tissue extraction or untreated plasma or urine was mixed with sodium dodecyl sulfate sample buffer (5 μl) and formamide (5 μl). The mixture was heated in a boiling water bath for 2 to 3 min. Fifteen microliters were loaded onto the 20% acrylamide/7 M urea gels, and the samples were elec-
trophoresed at 50 mA/gel until the bromphenol blue dye front was near the bottom of the gel. All gels had nonradioactive AR177 run as a standard. This gel electrophoresis system separates intact AR177 from \( n - 1 \) and \( n + 1 \) species. To visualize nonradioactive AR177, the gels were stained in Stains All (Sigma, St. Louis, MO) staining solution for 1 hr and then destained in 50% formamide for approximately 3 hr.

To prepare the gels for the detection of radioactivity, the gels were incubated for 1 hr in 1% glycerol, 50% methanol and 10% glacial acetic acid to help prevent cracking upon drying. The gels were dried on Whatman no. 3 filter paper on a Bio-Rad gel drier.

The dried gels were exposed to the Fuji phosphor-imaging plate for 1 week and were then scanned on the Fujix BAS 1000 PhosphorImager. The limit of sensitivity of the electrophoresis method was approximately 10 cpm/band, when the gels were exposed to the phosphor-imaging plate for 1 week. The images were stored on disk and annotated using the Fuji MacBSW software.

**Anion-exchange HPLC.** Tissues were homogenized for 10 sec with 0.5 ml of Nonidet P-40 tissue extraction buffer/g of tissue in a 7-ml Kimble polyethylene vial containing one 4-mm glass bead in a Wig-L-Bug (Crescent Dental Manufacturing Co.). The homogenate was incubated for 5 min at room temperature and then rehomogenized in the Wig-L-Bug. After homogenization, the tissues were incubated at 37°C for 24 hr to extract \(^{33}\)P-AR177 from the tissues. \(^{33}\)P-AR177 is stable in serum and cells at 37°C for several days (Bishop et al., 1996). The homogenate was then transferred to a 2-ml microfuge tube and centrifuged for 10 min at 14,000 rpm in an Eppendorf centrifuge. The supernatant fraction was decanted and frozen in microfuge tubes for later analysis by gel electrophoresis or HPLC. The recovery of \(^{33}\)P-AR177 spiked into control tissue homogenates was 60 to 70%.

The renal cortex, liver and spleen supernatants were chromatographed on a standard anion-exchange HPLC column (Gen-Pak Fax column, 4.6 mm × 10 cm; Waters). The elution gradient consisted of buffer A (0.1 M Tris, pH 12.0, 20% methanol) to buffer B (0.1 M Tris HCl, pH 12.0, 1.0 M NaBr) over 60 min. The flow rate was 0.5 ml/min. \(^{33}\)P was detected using an on-line radioactivity detector (Packard Radiomatic). The chemiluminescence signal was subtracted as much as possible from the overall signal, using the Radiomatic software, to obtain the true \(^{33}\)P signal. The presence of intact \(^{33}\)P-AR177 in the HPLC-derived material was confirmed by collection of fractions in an ISCO fraction collector, concentration of the fractions by speed vacuuming and electrophoresis in a 20% acrylamide gel, as described above.

**Pharmacokinetic parameters.** Plasma, blood and tissue pharmacokinetic values were calculated using RSTRIP II or PKAnalyst (MicroMath, Salt Lake City, UT). Pharmacokinetic modeling of data from both the single- and multiple-dose studies was performed on the mean values of data from different sets of rats at each time point.

## Results

### Blood and plasma pharmacokinetics after a single i.v. bolus injection of \(^{33}\)P-AR177

After a single i.v. dose of \(^{33}\)P-AR177, the disappearance of drug from blood and plasma (fig. 1) fit a biexponential curve, which yielded an AUC\(_{0-\infty}\) of 24.2 \(\mu g\)-eq/hr/g for blood and 13.5 \(\mu g\)-eq/hr/g for plasma (table 1). The distribution half-lives for blood and plasma were similar (0.21 hr for blood and 0.23 hr for plasma), and the elimination half-lives (\( \beta \)) were long (367 hr for blood and 271 hr for plasma), based on total radioactivity. The long elimination half-life of AR177 and slow clearance (8.7 ml/hr from blood and 15.7 ml/hr from plasma) indicate that there was slow elimination from rats. The 24.9-ml initial volume of distribution in blood suggests that AR177 became rapidly distributed within the vascular compartment after dosing. The blood volumes of distribution (4.6 or 4.1 liters, based upon postdistribution parameters or steady-state conditions, respectively) showed that AR177 became further distributed within the animals over 21 days.

For approximately 1 hr after dosing, the plasma concentration of \(^{33}\)P-AR177 (microgram-equivalents per milliliter) was approximately twice the concentration in blood, indicating limited initial uptake of the drug into blood cells. However, at approximately 1 hr after dosing, the \(^{33}\)P-AR177 plasma/blood concentration ratio began to decrease. Over time, the mean concentration of \(^{33}\)P-AR177 in plasma decreased more rapidly than that observed in whole blood, possibly indicating uptake of AR177 into the formed elements of the blood. This is also illustrated by an approximately 1.4-fold difference between blood (367 hr) and plasma (271 hr) terminal elimination half-lives.

### Blood and plasma pharmacokinetics after multiple i.v. bolus injections of \(^{33}\)P-AR177

During multiple dosing, concentrations of \(^{33}\)P-AR177 in blood and plasma (fig. 2) were measured before doses (\( C_{\text{min}} \)) 2, 3, 4, 5, 6 and 7 and after doses 1 and 7. With each subsequent dose, a steady increase...
in $C_{\text{min}}$ was observed in both the blood and plasma, indicating that there was accumulation of the drug in the blood and plasma. Maximum $C_{\text{min}}$ values of 0.32 mg-eq/g in blood and 0.22 mg-eq/g in plasma were observed before the seventh of seven doses. The observed whole-blood $C_{\text{min}}$ values were consistent with theoretical values predicted from single-dose data (fig. 1).

After the seventh dose, $^{33}\text{P}\text{-AR177}$ concentrations in blood and plasma decreased in a biexponential decay pattern (fig. 2), similar to that observed after the single dose. Derived pharmacokinetic parameters were similar to those generated from the single-dose data. The ratio of the single-dose AUC(0–$\infty$) to the seventh-dose AUC(0–$t$), where $t$ is the dosing interval (0–48 hr), was in the range of one indicating predictable accumulation in blood after multiple dosing.

**AR177 concentrations in tissues after a single i.v. bolus injection of $^{33}\text{P}\text{-AR177}$.** After single-dose administration of $^{33}\text{P}\text{-AR177}$, radioactivity was determined for up to 21 days in 14 tissues and was found to be widely distributed (figs. 3 and 4). Peak concentrations of $^{33}\text{P}\text{-AR177}$-associated radioactivity were found in all tissues at the 8-hr time point after the single dose, except for the testes (4 days) and thymus (32 hr) (table 2). Analysis of the AR177 tissue distribution showed that the majority of the dose distributed to the liver (40%), bone marrow (17%) and renal cortex (15%), at the peak at 8 hr after single dosing. Analysis of the tissue concentration of $^{33}\text{P}\text{-AR177}$ showed that the highest values were achieved in the renal cortex (15.0 µg-eq/g), liver (7.4 µg-eq/g), bone marrow (3.9 µg-eq/g), mesenteric lymph node (3.0 µg-eq/g) and spleen (2.4 µg-eq/g) at 8 hr after single dosing. The half-lives in these tissues were as follows: renal cortex, 231 hr (9.6 days); liver, 185 hr (7.7 days); bone marrow, 883 hr (36.8 days); spleen, 739 hr (30.8 days); mesenteric lymph node, 240 hr (10 days) (table 2).

**AR177 concentrations in tissues after multiple i.v. bolus injections of $^{33}\text{P}\text{-AR177}$.** After multiple dosing, concentrations of $^{33}\text{P}\text{-AR177}$-associated radioactivity in tissues increased steadily (figs. 5 and 6). Peak concentrations of
AR177 were found in all tissues at 48 hr after the seventh (and last) dose, which was the first sampling time after the last injection of $^{33}$P-AR177. After the seventh dose, $^{33}$P-AR177-associated radioactivity in tissues declined slowly over time. Gel electrophoresis of radioactivity in the spleen, kidney and liver at 48 hr after the seventh dose of $^{33}$P-AR177 showed that approximately 90% of the radioactivity represented intact drug (see results below). The total percentage of the original dose decreased by approximately 48 ± 26% (mean ± S.D.) over 21 days in 12 of 14 tissues. These tissues were the adrenals, bone marrow, eyes, liver, lungs, mesenteric lymph node, renal medulla, skin, spleen, testes and thymus. However, in 2 of 14 tissues, the brain and skeletal muscle, the concentration of $^{33}$P-AR177-associated radioactivity continued to rise, going from 0.02 and 0.08 $\mu$g-eq/g, respectively, at 8 hr after dose 7 to 0.08 and 0.11 $\mu$g-eq/g, respectively, at 21 days after dose 7. It could not be determined whether the radioactivity represented intact $^{33}$P-AR177 in the brain and skeletal muscle, because of the low concentration of radioactivity. No differences in the overall tissue distribution pattern were observed after multiple dosing, compared with the single dosing. An average of 83% of
the radioactive dose was recovered from the five rats over the period of 34 days and seven doses.

**Gel electrophoresis of plasma, urine and tissues.** To determine whether radioactivity found in tissues of rats injected i.v. with $^{33}$P-AR177 represented intact AR177, gel electrophoresis was used. Plasma, urine and selected tissues taken from $^{33}$P-AR177-dosed rats, after a single dose of $^{33}$P-AR177, were analyzed by gel electrophoresis, to determine whether the radioactivity represented intact AR177. The results showed that intact AR177 was present in the plasma, urine, liver, kidney and spleen at all time points for which there was sufficient radioactivity for gel analysis, based on co-migration with nonradioactive AR177 standard. Although minor bands could be observed, 90% of the radioactivity in plasma, urine and tissues was found in a band that co-migrated with nonradiolabeled AR177. Data for the liver are shown in figure 7, taken at 2, 8, 24 and 48 hr after a single dose of $^{33}$P-AR177, and are representative of results in plasma, urine and other tissues.

The amount of radioactivity in the intact $^{33}$P-AR177 bands was quantitated from the phosphor-images of the gels, as described in “Materials and Methods.” Figure 8 represents a quantitative analysis and shows the counts per minute per band of intact $^{33}$P-AR177 in the kidney, liver and spleen at 48 hr after doses 1, 3 and 7 and 14 days after dose 7 of $^{33}$P-AR177. There is a close association between the results of the extracted radioactivity in gels (fig. 8) and the results obtained from counting the radioactivity in the same tissues (fig. 5).

**HPLC of plasma, urine and tissues.** In addition to analysis of radioactivity by gel electrophoresis, samples were analyzed by anion-exchange HPLC. It was demonstrated that the radioactivity found in the liver, taken as a representative example at 7 days after dosing, represented intact $^{33}$P-AR177. Radioactivity extracted from the liver (fig. 9A) eluted with the same retention time as a $^{33}$P-AR177 standard (fig. 9B). Integration of the peak from the liver sample indicated that it represented 95 to 100% intact $^{33}$P-AR177. Some chemiluminescence, contributed by endogenous tissue substances, was seen in the void volume of the column (before 5 min) and after the elution of the peak of radioactivity. Thus, based on gel electrophoresis (90% intact) and HPLC (100% intact) analysis, the radioactivity data used to calculate the half-lives of $^{33}$P-AR177 in blood and plasma (table 1) and tissues (table 2) conservatively represent approximately 90% intact drug. This finding is consistent with the unusually long in vitro stability of AR177 in serum that was reported by Bishop et al. (1996).

**Excretion in urine and feces.** The major route of excretion was urinary. In the single-dose experiment, the total amount of drug excreted was 12.8% in the urine and 6.0% in feces (fig. 10) over 21 days after dosing. Gel electrophoresis of the urine demonstrated that approximately 90% of the radioactivity represented intact $^{33}$P-AR177 (data not shown). Gel electrophoresis of the feces was not performed. In the multiple-dose experiment, the total percentage of all seven doses excreted up to 21 days after dose 7 was approximately 30% in the urine and 10% in the feces (data not shown).

**Discussion**

The pharmacokinetics of almost a dozen oligonucleotides have been described in animals such as mice, rats and monkeys (Agrawal et al., 1995), and the pharmacokinetics of two oligonucleotide have been described in humans (Crooke et al., 1994; Zhang et al., 1995d). The half-lives of oligonucleotides vary greatly, depending upon their sequence and modifications. The plasma half-lives of total phosphorothioate oligonucleotides, which have sulfur atoms substituted for at least one oxygen in the internucleotide linkages, have been reported to be the longest, with terminal plasma half-lives of approximately 25 to 55 hr (Cossum et al., 1993, 1994; Zhang et al., 1995a,b,c; Srinivasan and Iversen, 1995). In contrast, the results of the present study show that AR177, a 17-mer oligonucleotide with only two phosphorothioate linkages, has terminal half-lives of 367 hr in blood and 271 hr in plasma, far greater than what has been previously reported for total phosphorothioate or nonphosphorothioate oligonucleotides.

The reason for the low blood and plasma clearance (15.7 and 8.7 ml/hr) and low excretion rate in urine and feces (total...
of 18.8% in urine plus feces after 21 days after a single dose) is unknown but may be due to the tight binding of AR177 to tissues. The evidence for this is derived from observations that were made during the development of the HPLC assay method for AR177. It was observed that AR177 elutes with a salt gradient at approximately physiological pH in the absence of tissues. After the injection of AR177 into rats, however, AR177 is tightly associated with tissue components and cannot be separated during anion-exchange HPLC at physiological pH. It was found to be necessary to raise the pH of the HPLC elution buffer to 12 to dissociate AR177 from tissue components, as reported in “Materials and Methods.”

At pH 12, AR177 completely dissociates from tissues and 100% recovery is obtained. The identity of these components is unknown. Although it is theoretically possible that the slow elimination could be due to tight binding to plasma proteins, the large steady-state volume of distribution argues against this possibility. Thus, binding to tissues may be partially responsible for the long half-life of AR177.

One of the positive features of AR177 that was found in this study was its stability for long periods of time in blood, plasma and tissues. The stability is probably due to the three-dimensional structure of the molecule, in which the drug is folded onto itself by hydrogen-bonding interactions between deoxyguanosine residues. Stabilization is coordinated by a potassium ion in the center of the AR177 molecule (Rando et al., 1995; Bishop et al., 1996). These interactions result in a quartet structure, containing three loops and four segments (Rando et al., 1995; Bishop et al., 1996). As a result of this structure, which is the first to be reported for an oligonucleotide drug, AR177 is highly resistant to nucleases and has a half-life of >4 days in serum in vitro and at least 48 hr intracellularly in vitro (Bishop et al., 1996). Single-base mutations of AR177, with which the molecule could not completely form the quartet structure, produced half-lives of only 3 to 7 min in serum. Although this quartet structure is responsible in large part for the resistance to nucleases, the phosphorothioate linkages at the 3'- and 5'-termini of AR177 also contribute to its resistance to nucleases. This was demonstrated by showing that a total phosphodiester version of AR177 has a shorter serum half-life than that of AR177, although it has a serum half-life much longer than those of nonquartet oligonucleotides (Bishop et al., 1996). Thus, nuclease resistance may be partially responsible for the long half-life of AR177.

Previous studies that have reported the tissue distribution of oligonucleotides have shown that, in general, the highest concentrations can be found in the liver and kidneys (Srinivasan and Iversen, 1995; Zhang et al., 1995a,b,c). In agree-
ment with the results for other oligonucleotides, the concentration and percentage of AR177 dose were highest in the liver and kidney. Importantly for its potential utility as an anti-HIV drug, AR177 was also found in high concentrations in lymphoid tissue, including both the spleen and lymph node. Concentration of AR177 in these tissues is a fortuitous characteristic of this drug, because lymphocytes are the major repositary of HIV and lymphocytes are concentrated in the spleen and lymph nodes.

One question arising from these results is whether the long half-life of AR177 will be reflected by a long duration of antiviral activity. Although no results are yet available from antiviral studies in animals or humans, studies in vitro support the idea that the long half-life of AR177 causes long-lasting antiviral activity. In a study reported by Ojwang et al. (1995), AR177 caused total suppression of HIV-1 production in MT4 cells (a human lymphoid cell line) in vitro for >21 days after washout of the drug and replacement with fresh culture medium. In contrast, resumption of HIV viral synthesis occurred within 2 days after the removal of azidothymidine in the same study.

Another question arising from these results is whether the long residence time of AR177 in tissues will result in toxicity. Although no results are yet available from phase I human studies, studies in cynomolgus monkeys suggest the idea that the long half-life of AR177 is not associated with toxicity. In a study reported by Wallace et al. (1996b), AR177 did not cause any tissue damage or changes in clinical chemistry when it was administered as a bolus i.v. injection at doses up to 40 mg/kg every other day, for a total of 12 doses, to cynomolgus monkeys. In a separate study, AR177 did not cause death, cardiovascular toxicity or alterations in clinical chemistry in cynomolgus monkeys receiving single doses up to 50 mg/kg as a 10-min i.v. infusion (Wallace et al., 1996a), although there was a transient and reversible prolongation of coagulation time at high doses. Taken together, the data indicate that AR177 does not cause the cardiovascular (Corsch et al., 1993; Galbraith et al., 1994) or histopathological (Srinivasan and Iversen, 1995) toxicities that are associated with total phosphorothioate oligonucleotides and it can be administered safely as an i.v. injection.

A third question regarding these findings is whether the blood, plasma and tissue concentrations that were achieved in rats will be sufficient to have an antiviral effect in humans. In vitro results have previously shown that AR177 has an EC50 of approximately 0.2 µM (1.2 µg/ml) against many HIV-1 clinical isolates infected to human peripheral blood mononuclear cells (Ojwang et al., 1995). Assuming that the minimum concentration of AR177 that would be required to have antiviral activity in humans would be approximately the in vitro EC50, and making the assumption that AR177 is pharmacologically available in human blood, plasma and tissues for antiviral activity, the results indicate that therapeutically achievable concentrations of AR177 can be achieved in many tissues after single or multiple dosing. Furthermore, the therapeutic concentration could possibly be sustained for several weeks after single or multiple doses, based on the long tissue half-life reported in this study. In lymphoid tissues, i.e., bone marrow, spleen and mesenteric lymph node, the concentration of AR177 was maintained at approximately 10, 7 and 3 times, respectively, the in vitro EC50 of AR177 at 21 days after the seventh and last dose of 0.7 mg/kg drug in rats. There are indications that AR177 is probably taken up by cells in vivo. The higher concentration of AR177 found in the blood vs. the plasma indicates that the drug is taken up by blood cells. Studies with radiolabeled AR177 have shown that the drug is readily taken up into human cells in vitro (Bishop et al., 1996). Thus, based on the pharmacokinetic results in rats, there is good reason to expect that therapeutic concentrations of AR177 can be achieved, that the therapeutic concentration can be maintained for several weeks in humans and that the drug can be taken up into cells. Based on these findings, it is believed that this drug has the potential to have antiviral activity in humans. However, it is not known whether AR177 is available for antiviral activity in tissues. Clinical anti-HIV data are not yet available.

In conclusion, AR177 exhibits long blood, plasma and tissue half-lives after bolus i.v. administration and distributes widely to tissues in rats. Of particular importance, because of the proposed anti-HIV clinical indication, AR177 is distributed to lymphoid tissues in high concentrations. The pharmacokinetic characteristics of AR177 in rats, along with the low toxicity seen in cynomolgus monkeys, indicate that AR177 has promising characteristics for the treatment of HIV infection in humans. The every other day dosing protocol used in this study is currently being used in a phase I study in humans.

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Send reprint requests to: Dr. Thomas L. Wallace, Aronex Pharmaceuticals,
Inc., 3400 Research Forest Drive, The Woodlands, TX 77381.