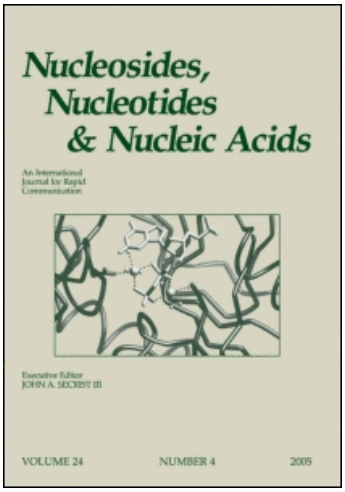

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Janet M. Leeds[a]; Richard S. Geary[a]; Scott P. Henry[a]; Jo Glover[a]; William Shanahan[a]; Jon Fitchett[a]; Todd Burckin[a]; LoAnne Tmong[a]; Arthur A. Levin[a]

[a] Isis Pharmaceuticals, Carlsbad, CA

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PHARMACOKINETIC PROPERTIES OF PHOSPHOROTHIOATE OLIGONUCLEOTIDES.

Janet M. Leeds, Richard S. Geary, Scott P. Henry, Jo Glover, William Shanahan, Jon Fitchett, Todd Burckin, LoAnne Truong, and Arthur A. Levin. Isis Pharmaceuticals, 2292 Faraday Ave., Carlsbad, CA 92008.

ABSTRACT. The pharmacokinetic parameters determined for different phosphorothioate oligonucleotides were compared. The data suggest that phosphorothioate pharmacokinetics are primarily determined by chemical class. The pharmacokinetics are consistent across species, show dose-dependency, and are independent of sequence.

Introduction. Preclinical pharmacokinetics and safety studies of phosphorothioate oligonucleotides have enabled five of these compounds to enter clinical studies. As a consequence there is now a substantial database of pharmacokinetic parameters for phosphorothioate oligonucleotides. By comparing the results across species for several of the phosphorothioate oligonucleotides several key conclusions have emerged. The pharmacokinetics of phosphorothioate oligonucleotides are primarily determined by chemical class, and for the most part, this chemical class is handled similarly across species. The pharmacokinetics show consistent and predictable dose-dependency. The metabolism appears to be independent of nucleotide sequence and identical in all species examined. Thus, as clinical trials move forward, studies in animals appear to be predictive of the behavior of phosphorothioate oligonucleotides in humans.

Methods. Plasma concentrations of phosphorothioate oligonucleotides were determined using gel-filled capillary electrophoresis as described [1]. Tissues were initially extracted using the procedure described by Cossum et. al. [2]; after addition of an internal standard this initial extract was further processed identically to plasma. Plasma $t_{1/2}$ values were estimated using log linear regression for plasma concentration time profiles. Area under the plasma concentration curve (AUC) was calculated using the linear trapezoidal rule and

Table 1. Comparison of Pharmacokinetic Parameters Calculated after Intravenous Infusion of ISIS 2302 to Cynomolgus Monkeys and Human Subjects over the Same Dose Range

Species	Route	Dose (mg/kg)	$t_{1/2}$ (hours)	$AUC_{0\rightarrow\infty}$ ($\mu g \bullet hr \bullet ml^{-1}$)	Vss ($ml \bullet kg^{-1}$)	CL ($ml \bullet hr^{-1} \bullet kg^{-1}$)
Monkeys						
	Bolus IV	0.2	0.22	0.37 ± 0.07	171.4	540.5
		1.0	0.29	16.2 ± 5.1	25.8	61.7
		2.0	0.47	21.4 ± 1.4	63.4	93.5
		4.0	0.68	74.6	51.5	53.5
		5.0	0.82	120.8 ± 17.8	49.0	41.4
	2-Hour IV Infusion	1.0	0.46	9.67 ± 0.19	71.4 ± 7.7	103.4 ± 2.1
		4.0	0.95	77.5 ± 2.9	69.2 ± 11.7	51.6 ± 1.9
Human	2-Hour IV Infusion	0.5	0.91	4.1 ± 0.7	155.4 ± 13.6	124.2 ± 28.8
		1.0	0.79	8.44 ± 0.94	143.5 ± 15.26	119.5 ± 13.9
		2.0	0.88	30.4 ± 1.9	97.5 ± 7.1	76.8 ± 7.2

extrapolated to infinity by dividing the last plasma concentration (C_{last}) by the terminal elimination rate constant.

Results and Discussion. The plasma oligonucleotide concentrations over time were determined after administration to cynomolgus monkeys over a dose range of 0.2-5.0 mg/kg of ISIS 2302. Complete plasma oligonucleotide concentrations profiles were collected from human subjects during and after a two-hour intravenous infusion of 0.5, 1.0, and 2.0 mg/kg of this same phosphorothioate oligonucleotide. The pharmacokinetic parameters (Table 1) illustrate the dose dependence of the pharmacokinetics for cynomolgus monkeys and humans. With increasing dose over a 25-fold range, the estimated elimination $t_{1/2}$ increased in monkeys; this was not evident in humans over a 4-fold dose range. With increasing dose in both monkeys and humans, clearance decreased and AUC values increased. In general, the pharmacokinetic parameters at a given dose are quite similar in the cynomolgus monkey and humans.

In the monkey the relative distribution of phosphorothioate oligonucleotides to tissues is consistent, regardless of the route of administration or sequence, but does show a dose-dependency, as is seen with plasma pharmacokinetics. The highest concentration of phosphorothioate oligonucleotides is consistently found in the kidney cortex (Table 2 data), followed by kidney medulla, liver, spleen and lymph nodes. When the total

Table 2. Concentrations of Phosphorothioate Oligonucleotide in Target Tissues at the End of One Month Toxicology Studies in Cynomolgus Monkeys

Tissue	ISIS 5132/CGP 69846A[1] Daily IV 1 mg/kg (28[2])	ISIS 2302[1] Daily IV 2 mg/kg (56[2])	ISIS 2302[1] QOD, SC 4 mg/kg (56[2])	ISIS 2302[1] QOD, SC 20mg/kg(280[2])	Ratio[3] Concentrations 20/4 mg/kg
Kidney Cortex	83.3 ± 19.9	269.1 ± 56.1	222.5 ± 65.7	1102.9 ± 168.1	5.0
Kidney Medulla	35.5 ± 11.3	43.8 ± 21.2	114.0 ± 52.6	412.5 ± 302.3	3.6
Liver	32.1 ± 6.8	113.4 ± 22.0	109.7 ± 33.5	318.7 ± 78.3	2.9
Spleen	11.2 ± 9.0	24.8 ± 11.8	15.6 ± 11.7	40.9 ± 19.7	2.6
Axial Lymph	0.62 ± 0.31	12.6 ± 5.8	2.9 ± 2.5	61.6 ± 28.8	21.2
Inguinal Lymph	Not anal.	31.5 ± 15.3	48.7 ± 15.4	82.4 ± 33.7	1.7

[1] Oligonucleotide concentration units are μg/ml; [2] Total dose administered over 28-days; [3] Ratio of concentrations of tissues from 20 mg/kg group divided by concentrations of tissues from 4 mg/kg group

administered dose was the same the concentration of full length ISIS 2302 in each tissue at the end of a one-month toxicology study was equivalent, whether the oligonucleotide was administered by intravenous infusion or subcutaneous injection (see Table 2, Daily IV, 2 mg/kg, and every other day-QOD, subcutaneous-SC-4 mg/kg). Given the same total dose, the tissue concentrations of ISIS 5132/CGP 69846A at the end of a one-month study were similar to ISIS 2302 concentrations, confirming the sequence independence of the tissue distribution. As the dose is increased the concentration of oligonucleotide in liver, kidney and spleen plateaus, suggesting these tissues become saturated. This apparent saturation of liver and kidney with oligonucleotide may in part be responsible for the non-linearity seen in the plasma pharmacokinetics.

The profiles of metabolites seen by gel-filled capillary electrophoresis analysis of plasma compared to tissues are quite different. In plasma the predominant peak at most time points is the full length oligonucleotide, with progressively smaller quantities of progressively shorter oligonucleotides (Figure 1). In tissues, at early time points the predominant species is the full length oligonucleotide, while at later time points the n-1, n-2, as well as a slower migrating species are often present at levels equivalent to the full length oligonucleotide (Figure 2). In addition, the quantity of smaller oligonucleotides is greater than that seen in plasma extracts. From samples analyzed at similar time points

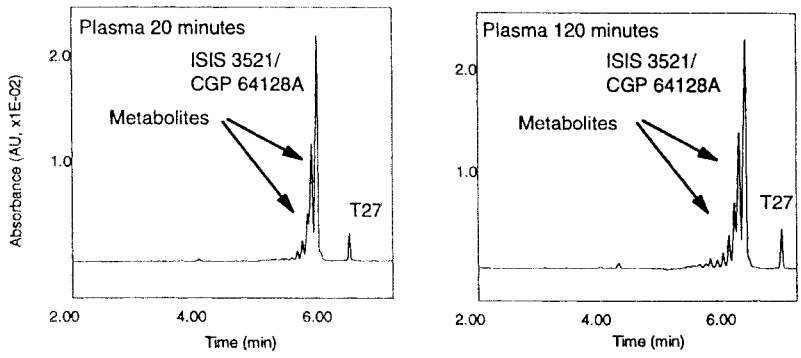

Figure 1. Comparison of Metabolite Profiles by Capillary Gel Electrophoresis in Plasma at 20 and 120 Minute Time Points

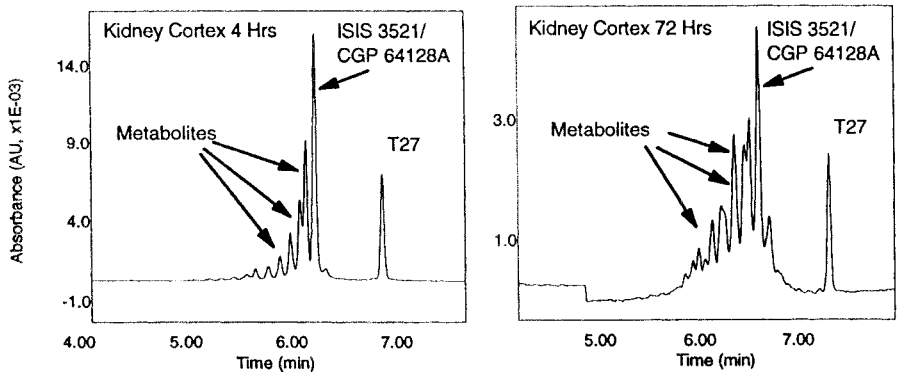

Figure 2. Comparison of Metabolite Profiles by Capillary Gel Electrophoresis in Tissues at 4 Hour and 72 Hour Time Points

it appears that the differences seen in plasma and tissue extracts are likely due to differences in metabolism and clearance.

In summary, phosphorothioate oligonucleotides pharmacokinetics appear to be determined primarily by their chemistry. Evidence of dose-dependent pharmacokinetics was observed in both plasma and tissues. The pharmacokinetics, however, are reproducible, consistent across species, and independent of sequence.

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