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PHARMACOKINETICS AND TISSUE DISTRIBUTION IN RATS OF AN OLIGODEOXYNUCLEOTIDE PHOSPHOROTHIOATE (GEM 91) DEVELOPED AS A THERAPEUTIC AGENT FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE-1

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Abstract—An antisense oligodeoxynucleotide phosphorothioate, namely gene expression modulator 91 (GEM 91), has been demonstrated to have significant anti-human immunodeficiency virus activity in various tissue culture models. The present study was undertaken to determine the pharmacokinetics and tissue distribution of GEM 91 in rats following i.v. bolus administration of ^{35}S-radiolabeled GEM 91. Plasma disappearance curves for GEM 91-derived radioactivity could be described by the sum of two exponentials, with half-lives (mean ± SEM) of 0.95 (± 0.07) and 47.57 (± 14.48) hr. Urinary excretion represented the major pathway of elimination of GEM 91, with 26.67 ± 6.46% (mean ± SD) of the administered dose excreted within 24 hr and 58.12 ± 4.36% over 240 hr after GEM 91 administration. Fecal excretion was a minor pathway of elimination of GEM 91 with 1.4 ± 0.62% (mean ± SD) of the administered dose excreted over 24 hr and 8.54 ± 0.64% over 240 hr. A wide tissue distribution of GEM 91 was observed. During the initial 30 min, the highest levels of tissue radioactivity were found in the kidney, liver, spleen, lungs, and heart. Radioactivity was retained over longer time periods in the kidneys, liver, heart, and intestine. Analyses of the extracted radioactivities from plasma, kidney, and liver by gel electrophoresis showed the presence of both intact GEM 91 and degradative products with smaller molecular weights. Radioactivity in urine was found to be degradative metabolites of GEM 91. Based on the experimental data, pharmacokinetic parameters for GEM 91 in each tissue and biological fluids were calculated using computer-based two-compartmental i.v. bolus or absorption models. This study is important not only in providing the basis for future studies of GEM 91 in humans, but also in understanding the pharmacology and toxicology of antisense oligodeoxynucleotide phosphorothioates, in general.

Key words: antisense oligodeoxynucleotide phosphorothioate; GEM 91; HIV-1; pharmacokinetics; metabolism; tissue disposition

The use of antisense oligodeoxynucleotides targeted to mRNA represents an attempt at specific, genetically based therapy. Antisense oligodeoxynucleotides have been suggested to have potential roles in the treatment of AIDS, cancer, and other diseases [1–8]. Although the replication of HIV‡ could be inhibited by normal phosphodiester oligodeoxynucleotides complementary to HIV RNA [1], due to their sensitivity to nucleases and relative short half-life, the application *in vivo* is limited. Therefore, major efforts have been focused to develop novel oligodeoxynucleotides with modification of phosphate backbones, resulting in compounds such as methylphosphonates, PS-oligodeoxynucleotides, and phosphoramidate analogues. Compared with other oligodeoxynucleotides, PS-oligodeoxynucleotides have several advantages: (1) they are relatively resistant to cleavage by nucleases; (2) they have good aqueous solubility; (3) they hybridize efficiently with target RNA with relatively high specificity; (4) they are relatively efficiently taken up by cells; and (5) the widely used method of automated oligonucleotide synthesis using phosphoramidites can be adapted to provide a convenient and relatively cost-effective means of synthesis.

Several reports have been published on the development of PS-oligodeoxynucleotides as potential anti-AIDS therapeutic agents. Although extensive studies on chemical and molecular mechanisms of oligodeoxynucleotides have demonstrated the potential value of this novel therapeutic strategy, little is known about the pharmacokinetics and

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‡ Abbreviations: HIV, human immunodeficiency virus; HIV-1, human immunodeficiency virus type-1; GEM 91, gene expression modulator 91; PS-oligodeoxynucleotide, oligodeoxynucleotide phosphorothioate; and CPG, controlled pore glass.

metabolism of these compounds *in vivo*. Recently, several preliminary studies have been published [9–14]. In a previous study [9], a 20-mer PS-oligodeoxynucleotide was administered i.v. and i.p. to mice. Approximately 30% of the administered dose was excreted in the urine over the first 24 hr with accumulation preferentially in the liver and kidney. Plasma half-lives were about 1 hr ($T_{1/2\alpha}$) and 40 hr ($T_{1/2\beta}$), respectively. Similar results have been reported in subsequent studies [10, 13, 14].

In continuation of our studies in developing antisense oligodeoxynucleotides as therapeutic agents for AIDS, we have designed GEM 91, which has been shown to have significant anti-HIV effects. The rationale for AIDS chemotherapy with GEM 91 is based on the use of antisense oligodeoxynucleotides to specifically inhibit the expression of HIV-1 [15]. The target of GEM 91 has been found to be conserved among various HIV-1 isolates. GEM 91 is 56% G + C rich, water soluble, and relatively stable under physiological conditions. GEM 91 binds to a complementary RNA target under physiological conditions, with the melting temperature of the duplex being approximately 56°. The antiviral activity of GEM 91 has been tested in several models, including acutely and chronically infected CEM cells, long-term cultures mimicking *in vivo* conditions, human peripheral blood lymphocytes and macrophages, and isolates from HIV-1-infected patients [16–19].

The purpose of the present study is to provide a comprehensive analysis of the kinetics and tissue distribution of GEM 91 in rats following i.v. bolus administration of ^{35}S-labeled GEM 91. Analyses of kinetics and disposition of this PS-oligodeoxynucleotide were carried out for each tissue and biological fluids. This is important not only in providing the basis for future studies of GEM 91 in humans, but also in understanding the pharmacology and toxicology of antisense PS-oligodeoxynucleotides, in general.

MATERIALS AND METHODS

Chemicals and radiochemicals

Synthesis of oligodeoxynucleotide. GEM 91 (5′-CTC-TCG-CAC-CCA-TCT-CTC-TCC-TTC-T-3′), a 25-mer PS-oligodeoxynucleotide, was synthesized, purified, and analyzed by the same methods as previously described [20].

^{35}S-Labeled PS-oligodeoxynucleotide. To obtain ^{35}S-labeled GEM 91, synthesis was carried out in two steps. The first 19 nucleotides of the sequence of GEM 91 (from 3′-end) were assembled using the β-cyanoethylphosphoramidite approach [21] and the last six nucleotides were assembled using the H-phosphonate approach [22]. CPG support-bound oligodeoxynucleotide (30 mg of CPG; approximately 1 μM) containing five H-phosphonate linkages was oxidized with $^{35}S_8$ (4 mCi, 1 Ci/mg, Amersham; 1 Ci = 37 GBq) in 60 mL carbon disulfide : pyridine : triethylamine (10 : 10 : 1). The oxidation reaction was performed at room temperature for 1 hr with occasional shaking. Then 2, 5, and 200 μL of 5% unlabeled sulfur ($^{35}S_8$) in the same solvent mixture was added every 30 min to complete the oxidation. The solution was removed, and the CPG support was washed with carbon disulfide : pyridine : triethylamine (10 : 10 : 1) (3 × 500 μL) and with acetonitrile (3 × 700 μL). The product was deprotected in concentrated ammonium hydroxide (55°, 14 hr) and evaporated. The resultant product was purified by polyacrylamide gel electrophoresis (20% polyacrylamide containing 7 M urea). The desired band was excised under UV shadowing, and the PS-oligodeoxynucleotide was extracted from the gel and desalted with a Sep-Pak C18 cartridge (Waters) and a Sephadex G-15 column. The yield was 20 A_{260} units (600 μg; sp. act. 1 μCi/μg). Other chemicals and reagents used in the present study were of the highest grade available.

Animals

Male Sprague–Dawley rats (100–120 g, Harlan Laboratories, Indianapolis, IN) were used in the study. The animals were fed with commercial diet and water *ad lib.* for 1 week prior to the study. Fifty-one animals were used in the present study for 17 time points (3 animals for each time point), i.e. 5, 10, 15, 30, 60, and 90 min, 2, 3, 6, 12, 18, 24, 48, 72, 120, 168, and 240 hr. Blood and tissue samples were collected from each animal. Complete collection of urine and feces was carried out for groups designated for time points of 12 hr and longer, using rat metabolism cages.

Kinetic and tissue distribution study

Animals were dosed via a single bolus i.v. injection into a tail vein at a dose of 30 mg/kg. Unlabeled and ^{35}S-labeled GEM 91 were dissolved in physiological saline (0.9% NaCl) in a concentration of 10 mg/mL (with final specific activity of ^{35}S-labeled GEM 91 of 2.78 μCi/mg). Doses were based on the pretreatment body weight and rounded to the nearest 0.01 mL. After injection, each animal was placed in a metabolism cage and fed with commercial diet and water *ad lib.* Total voided urine was collected, and each metabolism cage was then washed following the collection intervals. Total excreted feces was collected from each animal at various time points, and feces samples were homogenized prior to quantitation of radioactivity. Blood samples were collected in heparinized tubes from animals at the various time points. Plasma was separated by centrifugation.

Animals were euthanized by exsanguination under sodium pentobarbital anesthesia. Following euthanasia, the tissues were collected from each animal. All tissues/organs were trimmed of extraneous fat or connective tissue, emptied and cleaned of all contents, individually weighed, and the weights recorded.

Total radioactivity measurements

The total radioactivities in tissues and body fluids were determined by liquid scintillation spectrometry (LS 6000TA, Beckman, Irvine, CA) with automatic quenching curve adjustment. In brief, biological fluids (plasma, 50–100 μL; urine, 50–100 μL) were mixed with 6 mL of scintillation solvent (Beckman Ready-Safe biodegradable liquid scintillation fluid, Beckman) to determine total radioactivity. Feces

were ground and weighed prior to being homogenized in a 9-fold volume of 0.9% NaCl saline. Following their removal, tissues were immediately blotted on Whatman No. 1 filter paper and weighed prior to being homogenized in 0.9% NaCl saline (3–5 mL/g of wet weight). An aliquot of the homogenate (100 μL) was mixed with solubilizer (TS-2; RPI, Mt. Prospect, IL) and then with scintillation solvent (6 mL) to permit quantitation of total radioactivity. To obtain the best counting efficiency and minimize quenching of radioactivity by colored materials in tissues and fecal extracts, a bleaching procedure was used to remove any color, using a 7% (w/w) solution of benzoyl peroxide in toluene.

Gel electrophoresis of PS-oligodeoxynucleotides from biological samples

Plasma and homogenized tissues were treated with proteinase K (2 mg/mL) in extraction buffer (0.5% SDS/10 mM NaCl/20 mM Tris–HCl, pH 7.6/110 mM EDTA) for 2 hr at 37°. The samples were then extracted twice with phenol:chloroform (1:1, v/v) and once with chloroform. After ethanol precipitation, the oligodeoxynucleotides were analyzed by electrophoresis in 20% polyacrylamide gels containing 7 M urea. The gels were fixed in 10% acetic acid/10% methanol solution before autoradiography.

Data analysis

The concentrations of GEM 91-derived radioactivity are expressed as GEM 91 equivalents in either μg/mL of biological fluids (e.g. plasma) or μg/g of tissue/organ. The results are expressed as means ± SD.

The pharmacokinetic parameters of GEM 91 were estimated by using the NLIN procedure of SAS programs [23–25]. Functions consisting of the sum of one-, two-, or three-exponential components

$$(C_t = \sum_{i=1}^{n} A_i e^{-K_i t}, n = 1,2,3)$$

were fitted to data by a least squares method, where C_t is the concentration at time point t; A_i is the concentration coefficient; and K_i is the elimination rate coefficient. Selections of models were based on comparison of Akaike's Information Criterion (AIC) and standard errors (SE) of estimated parameters. One-, two-, and three-compartmental models of i.v. bolus injection or first order absorption administration models were tested to fit for each tissue/biological fluid. For example, it was shown that a two-compartmental model of i.v. bolus injection best fit the plasma concentration–time curve and that a two-compartmental model of a first order absorption administration model best fit the kidney concentration–time curve. The area under the curve (AUC) was calculated from

$$\sum_{i=1}^{n} A_i/K_i.$$

Elimination $T_{1/2}$ values of GEM 91 were calculated

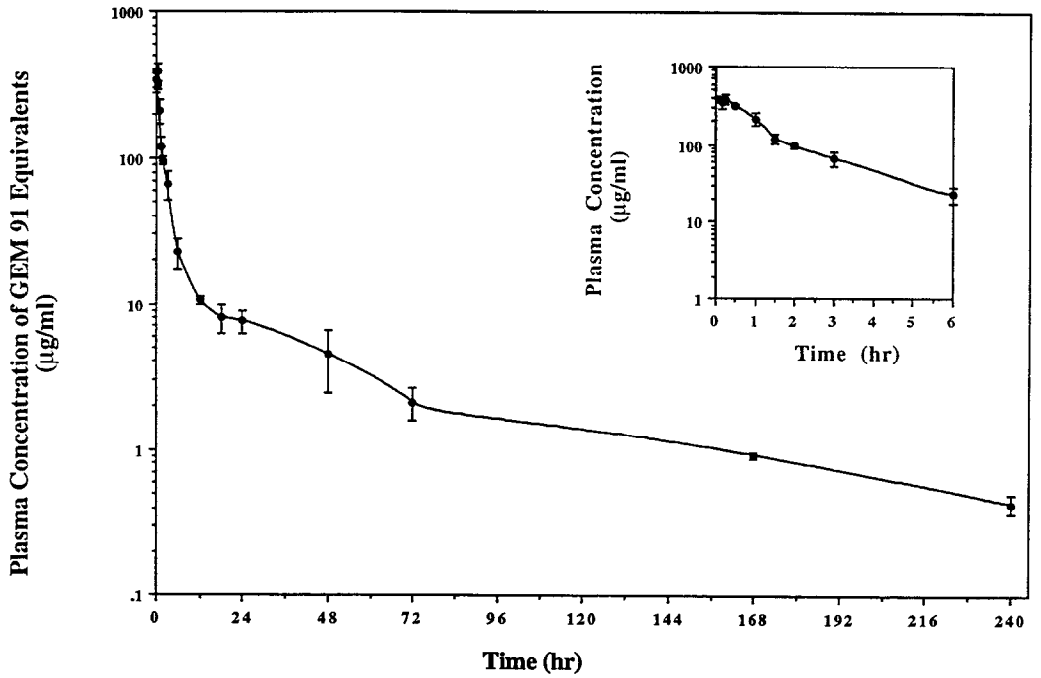

Fig. 1. Plasma concentration–time course of GEM 91-derived radioactivity. Plasma concentrations are expressed as micrograms of GEM 91 equivalents per milliliter (mean ± SD) after i.v. bolus administration of ^{35}S-labeled GEM 91 into rats at a dose of 30 mg/kg. Three animals were used for each time point. Plasma concentration was based on the quantitation of radioactivity. Pharmacokinetic analysis revealed that plasma disappearance curves for GEM 91-derived radioactivity could be described by the sum of two exponentials, with half-lives of 0.95 and 47.57 hr (see Table 1). *Insert:* expanded time course over the initial 6 hr.

Table 1. Pharmacokinetic parameters for GEM 91 in various tissues (two-compartmental i.v. bolus model)*

Tissue	C_{max} (μg/mL or μg/g)	$T_{1/2\alpha}$ (hr)	$T_{1/2\beta}$ (hr)	AUC [(μg/mL)·hr]	MRT† (hr)	CL [mL/(kg·hr)]
Plasma	418.40	0.95 ± 0.07	47.57 ± 14.48	1432.51	42.59	20.94
Liver	78.19	24.48 ± 19.28	97.16 ± 75.61	6538.18	116.36	4.59
Spleen	67.35	2.32 ± 0.54	136.72 ± 66.5	2389.02	182.00	12.56
Lung	48.36	4.36 ± 1.62	101.69 ± 42.43	2178.61	132.53	13.77
Adrenal	66.24	2.34 ± 0.06	76.98 ± 0.10	1879.03	101.24	15.97
Heart	44.48	5.17 ± 1.26	119.32 ± 59.74	1591.96	143.76	18.84
Small intestine	39.73	14.21 ± 3.13	118.44 ± 57.75	2263.93	129.93	13.25
Large intestine	35.90	11.48 ± 5.39	112.56 ± 54.08	2129.02	133.61	14.09
Pancreas	38.34	3.29 ± 1.57	97.35 ± 41.24	1445.03	127.55	20.76
Stomach	40.67	2.401 ± 0.74	96.62 ± 25.46	1913.95	132.63	15.67
Thymus	11.27	4.33 ± 0.76	198.05 ± 113.14	1188.42	275.09	25.24
Eye	7.24	22.47 ± 16.94	319.46 ± 572.73	1430.24	417.72	20.98
Skeletal muscle	9.31	8.50 ± 2.92	245.12 ± 150.27	1244.45	333.51	24.11
Stomach contents	3.67	126.63 ± 22.91	234.97 ± 1540.00	1091.05	313.45	27.50

* Values are means (±SEM) based on the experimental data from 51 rats following administration of ^{35}S-labeled GEM 91. Concentrations in various tissues were based on the quantitation of radioactivity.
† MRT = mean residue time.

from $0.693/K_i$. The clearance rate (CL) of GEM 91 was calculated by dividing the dose by the AUC [24].

RESULTS

Kinetics of GEM 91 in plasma

Figure 1 illustrates the plasma concentration–time course of GEM 91 equivalents after i.v. bolus administration of radiolabeled GEM 91 into rats. Pharmacokinetic analysis revealed that plasma disappearance curves for GEM 91-derived radioactivity could be described by the sum of two exponentials, with half-lives of 0.95 (± 0.07) and 47.57 (± 14.48) hr (see Table 1). The chemical form of radioactivity in plasma was further evaluated by gel electrophoresis, demonstrating the presence of both intact GEM 91 as well as metabolites with smaller molecular weights (Fig. 2).

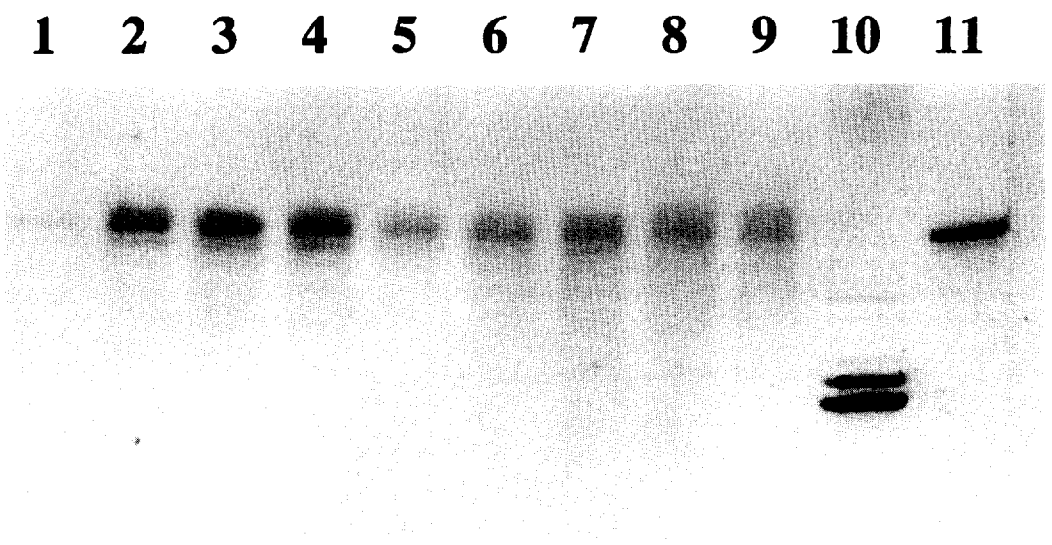

Fig. 2. Gel electrophoresis of plasma GEM 91-derived radioactivity. Plasma samples were treated with proteinase K (mg/mL) in extraction buffer (see text) for 2 hr at 37°. The samples were then extracted twice with phenol:chloroform (1:1, v/v) and once with chloroform. After ethanol precipitation, the oligodeoxynucleotides were analyzed by polyacrylamide gel electrophoresis. This figure shows the representative autoradiography for plasma samples. Lane 1 and 11: ^{35}S-labeled GEM 91 standard; lane 2: 5 min; lane 3: 15 min; lane 4: 30 min; lane 5: 2 hr; lane 6: 6 hr; lane 7: 12 hr; lane 8: 18 hr; and lane 9: 24 hr. [To enhance the sensitivity of detection in electrophoresis, a larger volume (600–1200 μL) of plasma sample was used for time points longer than 2 hr.] Lane 10: ^{35}S-labeled molecular markers (15–21 mer oligodeoxynucleotides).

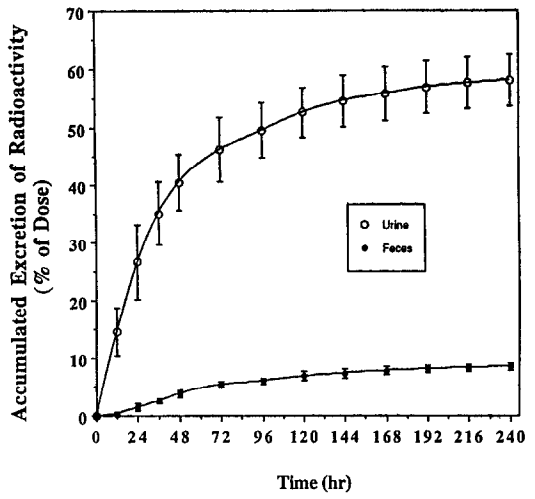

Fig. 3. Cumulative urinary and fecal excretion of GEM 91-derived radioactivity. Urinary and fecal excretions of GEM 91-derived radioactivity are expressed as means ± SD of the cumulative percentage of administered dose excreted over time. ^{35}S-Labeled and unlabeled GEM 91 was administered by i.v. bolus injection into rats at a dose of 30 mg/kg. Total excretion was based on the quantitation of radioactivity in urine and feces.

Cumulative urinary and fecal excretion

For the study of total urinary and fecal excretion of radioactivity following i.v. bolus administration of GEM 91, complete urine and feces collections were carried out for the groups of animals designated for time points 12, 24, 48, 72, 120, 168, and 240 hr. Figure 3 shows the cumulative excretion of urinary and fecal radioactivity over 240 hr following administration of radiolabeled GEM 91. Urinary excretion represented the major pathway of elimination of GEM 91. Rapid excretion of radioactivity was observed for the first 48 hr following GEM 91 administration, with 26.67 ± 6.46% of the administered dose excreted within 24 hr, 40.48 ± 4.94% within 48 hr, and 58.12 ± 4.36% over 240 hr. The radioactivity in urine was present as degradative products of GEM 91 with smaller molecular weights (data not shown). Fecal excretion was a minor pathway of elimination of GEM 91 with 1.4 ± 0.62% of the administered dose excreted over 24 hr and 8.54 ± 0.64% over 240 hr. The radioactivity in feces was present as degradative products of GEM 91 with smaller molecular weights (data not shown).

Tissue distribution

Figure 4 illustrates the GEM 91-equivalent concentrations of radioactivity in tissues after i.v. bolus administration of radiolabeled GEM 91 into rats. The results indicate that GEM 91 had a wide tissue distribution.

Kidney. The highest tissue concentration of GEM 91 was detected in kidneys within 15 min following administration of GEM 91, peaked between 6 and 18 hr, and decreased thereafter (Fig. 4A). The concentration of radioactivity in kidney remained 10- to 100-fold higher than that of plasma throughout the rest of the experimental period. Gel electrophoretic analysis demonstrated that both intact and metabolic forms of GEM 91 were present in the kidneys (Fig. 5).

Enterohepatic system. Throughout the experimental period a high concentration of radioactivity persisted in the liver, with the highest concentration occurring between 15 and 30 min (Fig. 4B). Gel electrophoretic analysis indicated that both intact and metabolic forms of GEM 91 were present in the liver (Fig. 6). A high concentration of radioactivity was also observed in the intestinal tract, especially in the small intestine (Fig. 4B). The highest concentrations were observed between 15 min and 3 hr. Radioactivity was detected in the large intestine throughout the experimental period, the concentrations being similar to those observed in the small intestine (Fig. 4B). As illustrated in Fig. 4B, radioactivity was detected in the stomach and pancreas throughout the experimental period, the concentrations being lower than those observed in the small intestine.

Spleen. Radioactivity in the spleen was observed throughout the experimental period, with the highest concentration between 15 and 30 min with decreased levels thereafter (Fig. 4A). This pattern was similar to the concentration–time course observed in plasma.

Heart. Radioactivity in the heart was detected within 5 min, with the highest concentrations observed between 15 and 30 min (Fig. 4A). The concentration in the heart then decreased and was similar to the corresponding level in plasma throughout the remainder of the experimental period.

Lungs. Throughout the experimental period, radioactivity was detected in the lungs, with concentrations being higher than that observed in plasma 18 hr after dosing (Fig. 4A).

Bone marrow. Relatively high concentrations of GEM 91 were observed in the bone marrow (Fig. 4A). After 6 hr, the concentrations in bone marrow were markedly higher than those observed in plasma.

Nervous system. Limited amounts of radioactivity were observed in the brain (Fig. 4C). Radioactivity in the eyes was detected within 15 min and remained present at a lower concentration throughout the experimental period.

Thyroid. High concentrations of GEM 91 were observed in the thyroid with the highest concentrations observed between 15 and 60 min (Fig. 4C). The concentration of radioactivity in thyroid remained 10- to 20-fold higher than that of plasma throughout the rest of the experimental period.

Other tissues. Relatively high concentrations of GEM 91 were observed in the adrenal (Fig. 4C). Low concentrations of radioactivity in skeletal muscle were observed throughout the experimental period (Fig. 4D). Radioactivity was also detected in skin, thymus, testes, and fat, with the concentrations being the same or lower than the corresponding plasma level (Fig. 4C and D).

Metabolism and stability of GEM 91 in vivo

The chemical forms of radioactivity were examined by polyacrylamide gel electrophoresis. As illustrated in Fig. 2, both intact GEM 91 and its metabolites

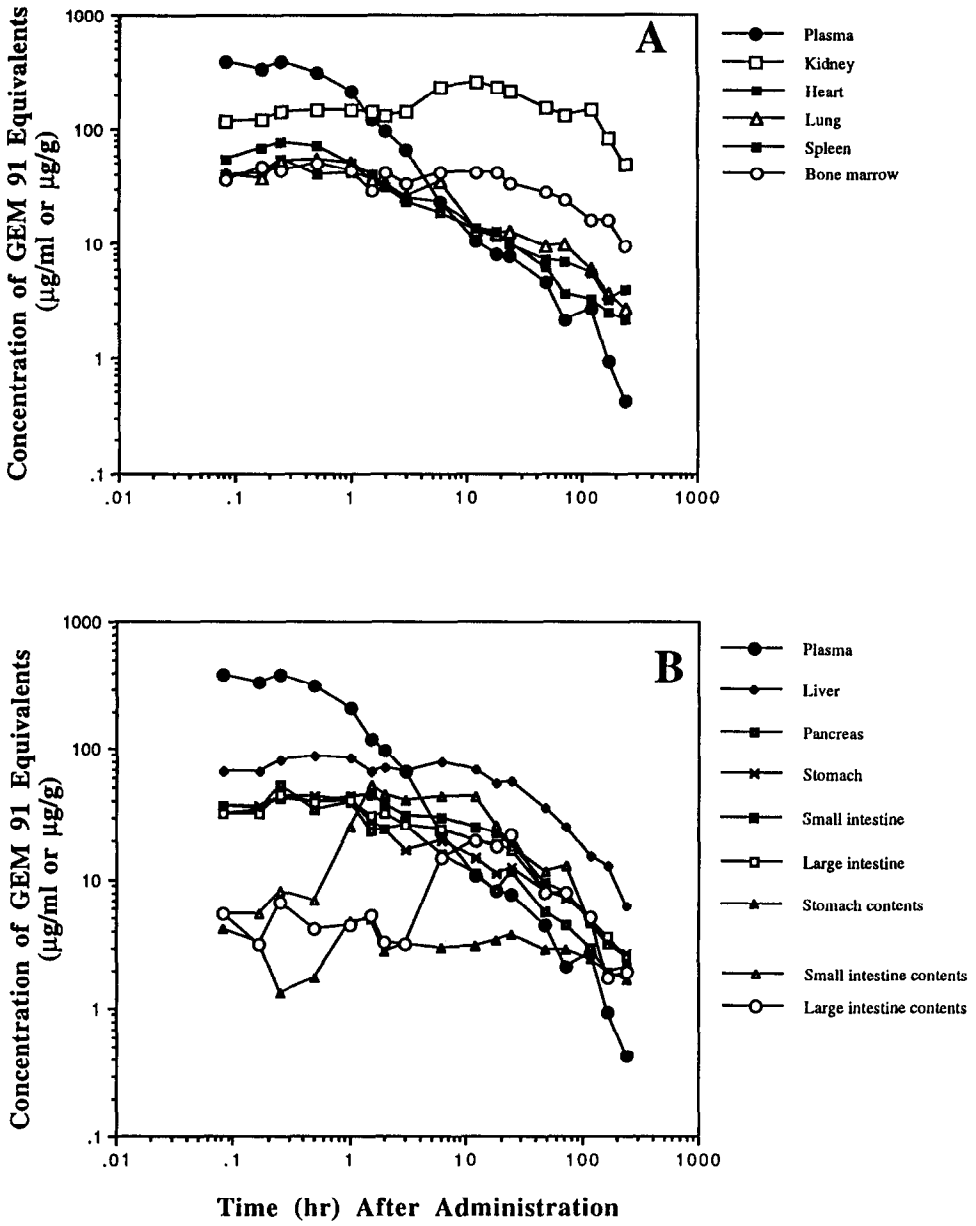

Fig. 4.

with smaller molecular weights were detected in plasma, with the presence of mainly intact form within 2 hr after dosing. Intact GEM 91 was still detectable in plasma up to 24 hr, indicating that GEM 91 is relatively stable *in vivo*. Intact GEM 91 was retained in several tissues for a longer period. Extensive metabolism of GEM 91 was also observed. As illustrated in Figs 5 and 6, degradative products of GEM 91 were observed within 3 hr following administration of GEM 91.

Pharmacokinetic analysis

Based on the experimental data, pharmacokinetic parameters for GEM 91 in each tissue and biological fluids were calculated using computer-based two-compartmental i.v. bolus or absorption models. Table 1 summarizes the results using a two-compartmental i.v. bolus model to fit the data for selected tissues/biological fluids, including plasma, liver, spleen, lung, heart, adrenal, small intestine, large intestine, pancreas, stomach, thymus, eye, and skeletal muscle. Statistical analysis indicated that this model best fit the experimental data. Table 2 summarizes the results using a two-compartmental (first order absorption) model to fit the data for selected tissues/biological fluids, including kidney, bone marrow, contents of small and large intestine, skin, and thyroid. Statistical analysis indicated that this model best fit the experimental data for these biological samples. Due to large variations and low

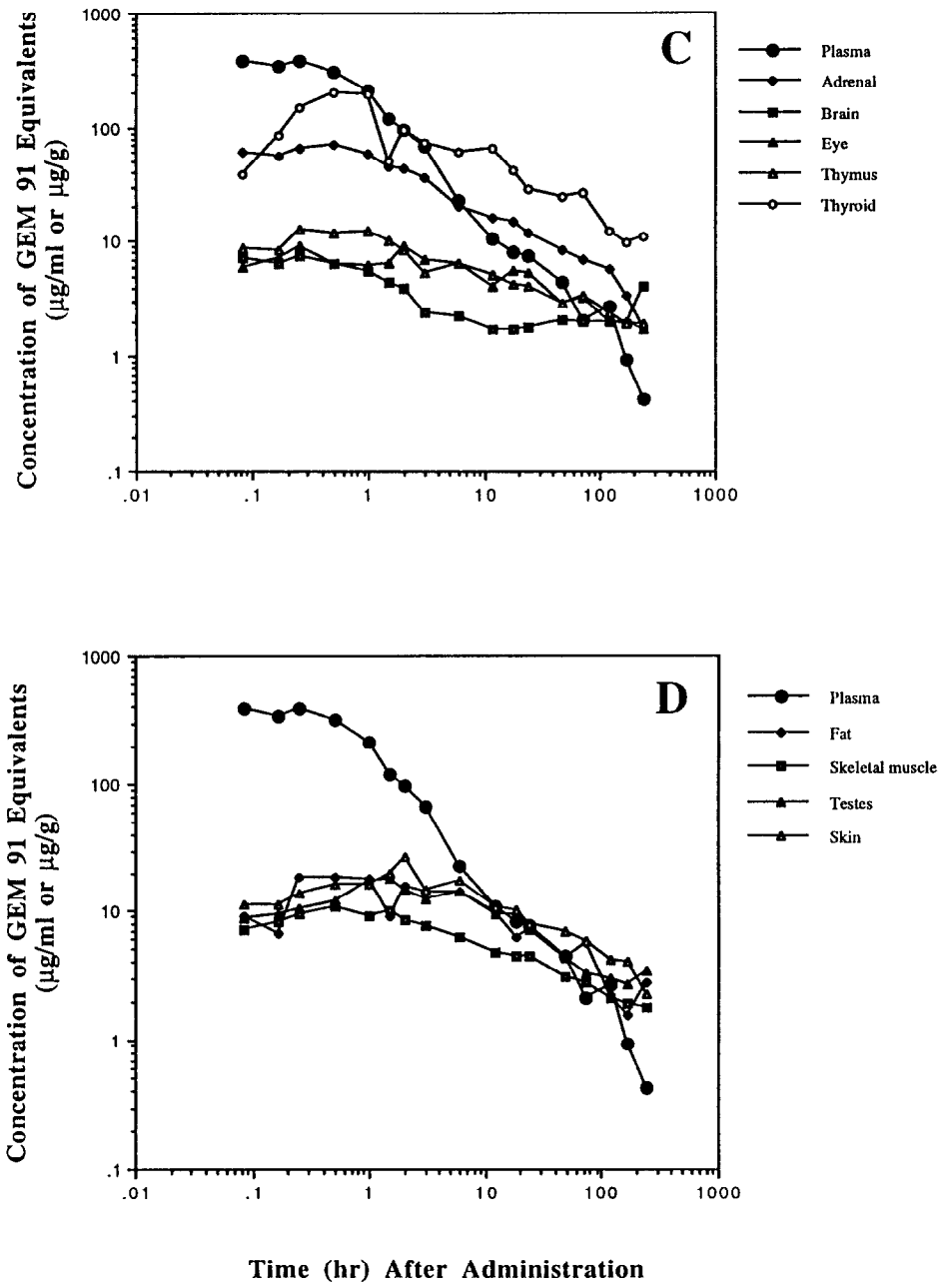

Fig. 4. Tissue concentration–time course of GEM 91-derived radioactivity. Mean tissue concentrations are expressed as micrograms of GEM 91 equivalents per gram of tissue/organ after i.v. bolus administration of ^{35}S-labeled GEM 91 into rats at a dose of 30 mg/kg. Three animals were used for each time point. Tissue concentration was based on the quantitation of radioactivity. To show the comparison of tissue concentrations and corresponding plasma levels, a plasma concentration–time curve was plotted in each panel. (A) Kidney, heart, lung, spleen, and bone marrow. (B) Liver, pancreas, stomach, small intestine, large intestine, and contents of stomach, small intestine and large intestine. (C) Adrenal, brain, eye, thymus, and thyroid. (D) Fat, skeletal muscle, testes, and skin.

concentrations of GEM 91 equivalents in several tissues/biological samples including brain and fat, we were unable to develop satisfactory models for these tissues.

DISCUSSION

This is the first report of a pharmacokinetic study of GEM 91 in rats. This antisense PS-

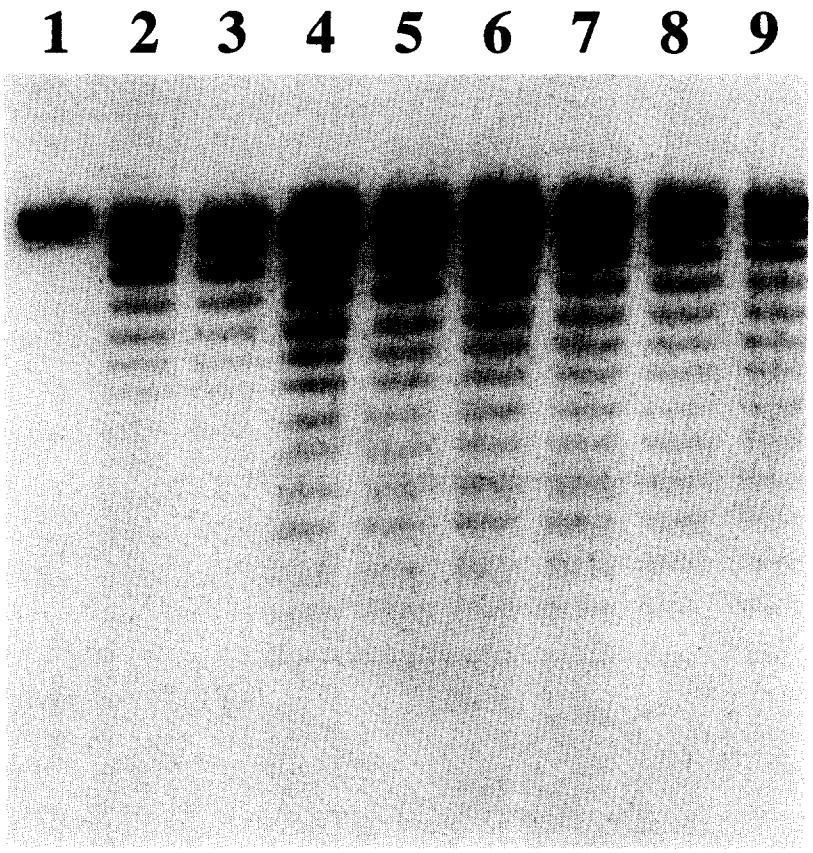

Fig. 5. Gel electrophoresis of GEM 91-derived radioactivity in kidneys. Homogenate (200 μL) was treated with proteinase K (2 mg/mL) in extraction buffer (see text) for 2 hr at 37°. The samples were then extracted twice with phenol:chloroform (1:1, v/v) and once with chloroform. After ethanol precipitation, the oligodeoxynucleotides were analyzed by polyacrylamide gel electrophoresis. This figure shows the representative autoradiography for kidney samples. Lanes 1: ^{35}S-labeled GEM 91 standard; lanes 2 and 3: 3 hr (two animals); lanes 4 and 5: 6 hr (two animals); lanes 6 and 7: 12 hr (two animals); and lanes 8 and 9: 18 hr (two animals).

oligodeoxynucleotide has been demonstrated to have significant anti-HIV activity [15,18,19]. Kinetic and tissue distribution analyses of GEM 91 were conducted in rats following i.v. bolus administration of ^{35}S-radiolabeled GEM 91 at a dose of 30 mg/kg. Plasma disappearance curves for GEM 91-derived radioactivity could be described by the sum of two exponentials, with half-lives of 0.95 (± 0.07) and 47.57 (± 14.48) hr. Urinary excretion represented the major pathway of elimination of GEM 91 with fecal excretion being a minor pathway for elimination. A wide tissue distribution of GEM 91 was observed. Based on the experimental data, pharmacokinetic parameters for GEM 91 in each tissue and biological fluid were calculated using computer-based two-compartmental i.v. bolus or absorption models. The analysis of distribution and excretion of GEM 91 should be useful not only in future studies of GEM 91 in humans, but also important in understanding the pharmacology and toxicology of this class of antisense oligodeoxynucleotides.

Compared with the extensive molecular and biochemical studies of antisense oligodeoxynucleotides, relatively little published information exists on pharmacokinetics of antisense oligodeoxynucleotides. In 1991, we described a pharmacokinetic study of a 20-mer PS-oligodeoxynucleotide in mice [9]. More recently, we reported studies on pharmacokinetics and tissue distribution of several end-modified PS-oligodeoxynucleotides in mice [12]. Significant differences in biostability of these PS-oligodeoxynucleotides were observed. Iversen [10] also studied the pharmacokinetics of a single injection of phosphorothioates of different lengths in rats. The plasma concentration as a function of time appeared to follow a two-compartment model. The initial distribution half-time ($T_{1/2\alpha}$), which represented transit out of the plasma, was 23 min, with the elimination half-life ($T_{1/2\beta}$) being 33.9 hr. They suggested that the plasma clearance of oligodeoxynucleotide was not affected by oligomer length (21-, 27-, and 40-mer) or base composition. Recently, Iversen *et al.* [13] reported a study of 27-mer PS-oligodeoxynucleotide. Based on limited animal data, they estimated the plasma clearance of this PS-oligodeoxynucleotide with $T_{1/2\alpha}$ and $T_{1/2\beta}$

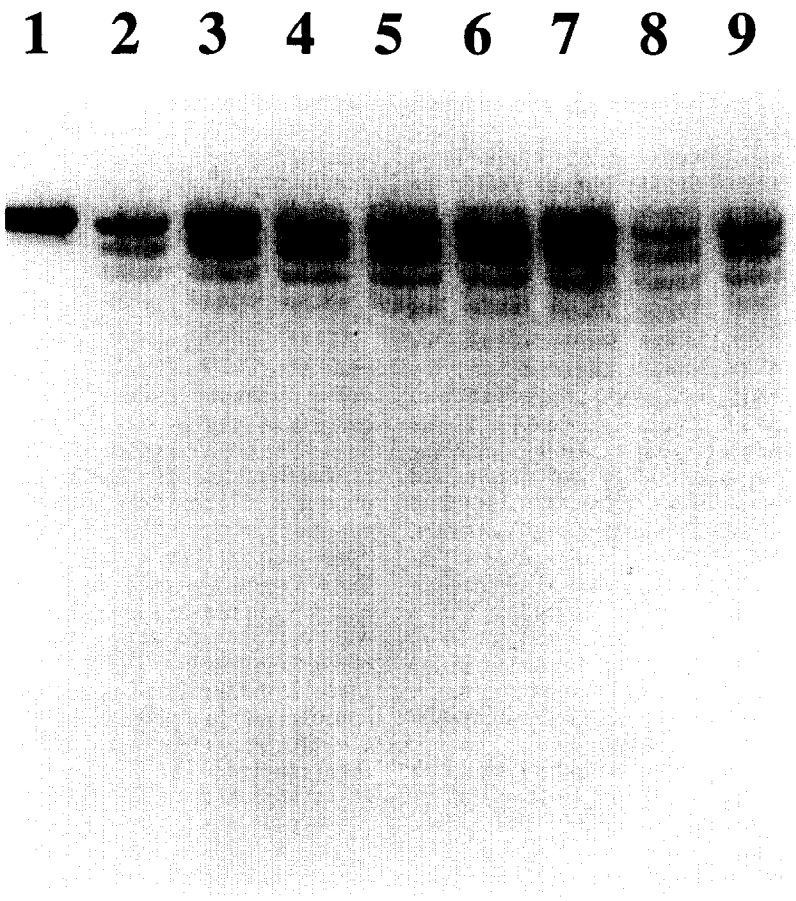

Fig. 6. Gel electrophoresis of GEM 91-derived radioactivity in liver. Homogenate (200 μL) was treated with proteinase K (2 mg/mL) in extraction buffer (see text) for 2 hr at 37°. The samples were then extracted twice with phenol : chloroform (1 : 1, v/v) and once with chloroform. After ethanol precipitation, the oligodeoxynucleotides were analyzed by polyacrylamide gel electrophoresis. This figure shows the representative autoradiography for liver samples. Lane 1: ^{35}S-labeled GEM 91 standard; lanes 2 and 3: 3 hr (two animals); lanes 4 and 5: 6 hr (two animals); lanes 6 and 7: 12 hr (two animals); and lanes 8 and 9: 18 hr (two animals).

Table 2. Pharmacokinetic parameters for GEM 91 in various tissues (two-compartmental absorption model)*

Tissue	Absorption $T_{1/2}$ (hr)	$T_{1/2\alpha}$ (hr)	$T_{1/2\beta}$ (hr)	AUC [(μg/mL)·hr]	MRT† (hr)	CL [mL/(kg·hr)]
Kidney	5.27 ± 69.2	7.73 ± 2.20	115.32 ± 42.35	41705.81	168.04	0.72
Bone marrow	0.01 ± 0.59	29.21 ± 3.21	141.18 ± 5.82	7056.19	194.74	4.25
Large intestine contents	2.14 ± 17.12	1.31 ± 10.68	103.94 ± 28.29	2350.79	154.02	12.76
Small intestine contents	0.51 ± 0.07	10.95 ± 2.97	83.38 ± 34.20	2617.36	97.04	11.46
Skin	0.58 ± 0.48	4.94 ± 4.24	134.36 ± 51.26	1777.34	181.79	16.88
Thyroid	0.15 ± 0.10	0.70 ± 0.44	91.30 ± 32.48	7024.83	127.72	4.27

* Values are means (±SEM) based on the experimental data from 51 rats following administration of ^{35}S-labeled GEM 91. Concentrations in various tissues were based on the quantitation of radioactivity.
† MRT = mean residue time.

being 20–25 min and 27–41 hr, respectively. More recently, Sands *et al.* [14] compared the biodistribution and metabolism of phosphodiester and PS-oligodeoxynucleotide, demonstrating that PS-oligodeoxynucleotides are more stable than phosphodiester *in vivo*.

The present study represents a detailed study on pharmacokinetics and tissue disposition of GEM 91, a PS-oligodeoxynucleotide, which has been demonstrated to have anti-HIV activity in several *in vitro* models. In contrast to earlier studies, the present study emphasized the use of a larger sample

size with a longer observation period, and comprehensive tissue collection. In addition, since we obtained data on 24 tissues/biological samples for each animal, comprehensive pharmacokinetic modeling was possible.

Pharmacokinetic analysis of GEM 91 in plasma is in general agreement with our preliminary studies of other PS-oligodeoxynucleotides [9], which suggested that PS-oligodeoxynucleotides had prolonged elimination half-lives and wide tissue distribution, based on radioactivity levels. In the present study, with a larger sample size, we demonstrated that GEM 91 has a relatively short mean distribution half-life (0.95 hr) and a prolonged mean elimination half-life of 47.57 hr, following i.v. bolus administration of ^{35}S-labeled GEM 91. Initially, GEM 91 was distributed mainly in highly perfused tissues including the liver, kidneys, heart, lungs and spleen. The prolonged elimination phase reflected retention of GEM 91 (and its metabolites) in several tissues, especially the kidney, liver, and intestine. As reported earlier [15], the IC_{50} of GEM 91 for anti-HIV activity *in vitro* is approximately 0.2 μM (2–3 μg/mL). In the present study with the administered dose of 30 mg/kg in rats, a maximum concentration of GEM 91-derived radioactivity was estimated to be 418 μg/mL and greater than 3 μg/mL within 48 hr after administration of GEM 91.

In the present study, urinary excretion represents the major route of elimination of GEM 91-derived radioactivity. Urinary excretion of GEM 91-derived radioactivity mainly occurred in the first 48 hr (approximately 40%). Over the subsequent 8-day period, 3–5% of the administered dose was excreted into urine in each 24-hr period. Further statistical analysis (correlation and regression) indicated that the pattern of urinary excretion of GEM 91-derived radioactivity corresponded to the changing plasma level of GEM 91-derived radioactivity. Fecal excretion was less than 10% of the administered dose over 10 days following administration of GEM 91. These results on urinary and fecal excretion of GEM 91-derived radioactivity were in agreement with that observed with other PS-oligodeoxynucleotides [9,11,13,14]. Recently, Cossum *et al.* [26] reported a pharmacokinetic study of a ^{14}C-labeled PS-oligodeoxynucleotide (ISIS 2105) in rats following i.v. administration. They found that the radioactivity in expired air accounted for 51% of the administered dose over a 10-day period and urinary and fecal radioactivity only accounted for 15 and 5% of the administered dose, respectively [26]. It is not clear whether the reported results [26] may represent an extensive metabolism of the examined PS-oligodeoxynucleotide, which was ^{14}C-labeled at the carbon-2 position of the thymine ring. One possibility is that thymine was removed from the ^{14}C-labeled PS-oligodeoxynucleotide (ISIS 2105) by a glycosidic bond cleavage without prior hydrolysis of the internucleotide linkage [26]. It is worth noting that these differences of results from various pharmacokinetic and metabolic studies might be explained by variations in species, sequences and labeling of oligonucleotides, as well as analytical methods.

Since relatively high concentrations of GEM 91-derived radioactivity were observed in liver and the intestinal tract, but not in feces, an enterohepatic circulation of the PS-oligodeoxynucleotide was suggested. Both intact and metabolic forms of GEM 91 were found in the liver, intestinal tract and intraluminal contents. Further studies are needed to determine the biliary excretion, transport of the PS-oligodeoxynucleotide across the intestinal wall, chemical forms at each site of the enterohepatic system, and lastly, the significance of the enterohepatic circulation on the pharmacology and toxicology of PS-oligodeoxynucleotides.

Following administration of GEM 91, there was wide distribution of radioactivity in various tissues. GEM 91-derived radioactivity in the kidneys was consistently higher than the corresponding plasma level over the experimental period. Persistence of radioactivity in the kidney may be important in terms of potential toxicity. Relatively high concentrations of GEM 91-derived radioactivity in bone marrow, heart, lung, spleen, adrenal, and thyroid were observed. In particular, consistently higher levels were observed in these tissues compared with the corresponding plasma levels during the elimination phase of GEM 91. As shown in Tables 1 and 2, for most tissues and biological fluids, the half-lives of the elimination phase of GEM 91-derived radioactivity were significantly longer than that observed in plasma. This may indicate an accumulation of PS-oligodeoxynucleotide (and possibly its metabolites) in those tissues possibly related to both therapeutic and toxic effects [27]. Further studies are needed to determine: (1) the mechanism(s) of transport and distribution of GEM 91 (and its metabolites); (2) protein binding of GEM 91 in plasma and tissues; and (3) the application of GEM 91 kinetics in experimental animals to humans.

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