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Determination of antisense phosphorothioate oligonucleotides and catabolites in biological fluids and tissue extracts using anion-exchange high-performance liquid chromatography and capillary gel electrophoresis

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Abstract

Chemically modified phosphorothioate oligodeoxynucleotides (ODNs) have become critical tools for research in the fields of gene expression and experimental therapeutics. Bioanalytical assays were developed that utilized fast anion-exchange high-performance liquid chromatography (HPLC) and capillary gel electrophoresis (CGE) for the determination of 20-mer ODNs in biological fluids (plasma and urine) and tissues. A 20 mer ODN in the antisense orientation directed against DNA methyltransferase (denoted as MT-AS) was studied as the model ODN. The anion-exchange HPLC method employed a short column packed with non-porous polymer support and a ternary gradient elution with 2 *M* lithium bromide containing 30% formamide. Analysis of the MT-AS is accomplished within 5 min with a detection limit of approximately 3 ng on-column at 267 nm. For plasma and urine, samples were diluted with Nonidet P-40 in 0.9% NaCl and directly injected onto the column, resulting in 100% recovery. For tissue homogenates, a protein kinase K digestion and phenol–chloroform extraction were used, with an average recovery of about 50%. Since the HPLC assay cannot provide one-base separation, biological samples were also processed by an anion-exchange solid-phase extraction and a CGE method to characterize MT-AS and its catabolites of 15–20-mer, species most relevant to biological activity. One base separation, under an electric field of 400 V/cm at room temperature, was achieved for a mixture of 15–20-mer with about 50 pg injected. Assay validation studies revealed that the combined HPLC–CGE methods are accurate, reproducible and specific for the determination of MT-AS and its catabolites in biological fluids and tissue homogenates, and can be used for the pharmacokinetic characterization of MT-AS.

Keywords: Phosphorothioates; Oligodeoxynucleotides

1. Introduction

Chemically modified oligodeoxynucleotides (ODNs), such as antisense phosphorothioates or methylphosphonates have become critical tools for research in the fields of gene expression and experimental therapeutics. Antisense ODNs are able to

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alter gene expression by inhibiting transcription or translation via sequence-specific binding of either DNA or mRNA, respectively [1]. These specific interactions enable production of unique proteins to cease, and lead to a clearer understanding of the gene and function of the encoded protein. Inhibition of certain proteins serves as therapeutic targets. ODNs are generally between 15 to 50 bases in length and show much more resistance to exonucleases than unmodified DNA. The chemical properties of a modified DNA molecule can be quite different from its phosphodiester counterpart and consequently, optimization and modification of analytical methods used for their measurement may be required [2]. Currently, biological analysis of antisense ODNs includes radiochemical [3,4], hybridization [5] and chromatographic [6,7] methods. These methods suffer from one or more problems, such as inability to distinguish degradation products or catabolites, lack of sensitivity and inconvenience. Moreover, a radiolabeled ODN has physicochemical properties that differ from the unmodified ODN, which may result in a different disposition in vivo. Bourque and Cohen [8,9] have developed an alternative method for the determination of antisense ODNs in human plasma and urine using anion-exchange high-performance liquid chromatography (HPLC)–capillary gel electrophoresis (CGE). As currently used, the HPLC method provides a measure of total ODN, whereas CGE can resolve individual ODNs. Recently, Leeds et al. [10] reported a CGE assay for quantitation of phosphorothioate oligonucleotides in human plasma. However, none of the existing HPLC and/or CGE assays have been applied to the measurement of antisense ODNs in tissue samples.

Monia et al. [11] found that phosphorothioate ODNs of between 17- and 20-mer have adequate binding affinity to mRNA to produce biological activity. Thus, a parent 20-mer antisense ODN will produce shorter chain nucleoside catabolites that are likely to be active. Therefore, it is critical to develop bioanalytical techniques to identify and quantitate parent ODNs and their therapeutically relevant catabolites in biological fluids and tissues. To achieve this goal, the current analytical methods modified and extended Bourque and Cohen's [9] approach to quantify antisense ODNs in plasma and further applied them to urine and tissue samples. One essential difference was the use of solid-phase extraction to prepare biological samples for CGE rather than collecting HPLC eluents.

The new analytical methods were validated and applied to a pharmacokinetic and tissue distribution study of an antisense ODN (referred to as MT-AS), designed to inhibit DNA methyltransferase, an enzyme that methylates cytosine on DNA. Unabated DNA methylation, leading to a state of hypermethylation, has been implicated in the inactivation of tumor suppressor genes [12]. Thus, inhibition of this enzyme can lead to a normal pattern of DNA methylation and activation of tumor suppressor genes. The goals of the investigation were to establish bioanalytical techniques, independent of radiochemical and molecular biological methods, to characterize the disposition of ODNs in both plasma and tissue.

2. Experimental

2.1. Chemicals and reagents

The antisense ODN directed against DNA methyltransferase was a 20-mer phosphorothioate ODN (referred to as MT-AS) and was provided by Hybridon (Worcester, MA, USA). It was supplied with 2–3% of a 19-mer impurity. The 15–19-mer ODNs based on the MT-AS sequence were synthesized in-house. Lithium bromide was obtained from Fluke (Ronkonkoma, NY, USA). Phenol was obtained from Amresco (Solon, OH, USA). Nonidet P-40, formamide, chloroform and proteinase K and other chemicals were obtained from Sigma (St. Louis, MO, USA). Solid-phase cartridges with anion-exchange membranes (Ultrafree-MC-DEAE) were purchased from Millipore (Bedford, MA, USA). HPLC water was deionized distilled water filtered through a Millipore Milli-Q ultra pure water system. The resistance of the water was more than 18.0 $M\Omega/cm^3$. All other reagents were of the highest grade available.

2.2. Instrumentation and equipment

The liquid chromatographic system (Hewlett-Packard Series 1050, Palo Alto, CA, USA) consisted of

a gradient pump, an autoinjector and a variable-wavelength UV detector. Separations were performed with a 20×1 mm I.D. guard column from Upchurch Scientific (Oak Harbor, WA, USA), which was hand-packed with spherical 13 μm Nucleopak PA-100 support (Dionex Chromatography, Sunnyvale, CA, USA). Titanium frits (2 μm pores) were placed on both ends of the column.

CGE was performed on a Model 3850 capillary electropherograph (ISCO, Lincoln, NE, USA) that consisted of a 30-kV, 300 μA direct current high voltage power supply, a lock-in sample compartment and a fixed wavelength UV detector. Signals were detected at 267 nm and collected on an integrator (Hewlett-Packard model 3396A).

2.3. Chromatography

2.3.1. Mobile phase

The composition of the mobile phase was developed to separate MT-AS and catabolites from endogenous substances under ambient conditions. The ternary solvent system consisted of eluent A: 25 m*M* Tris, 1 m*M* EDTA, pH 7.0; eluent B: 25 m*M* Tris, 1 m*M* EDTA, 2 *M* LiBr, pH 7.0; eluent C: formamide. The initial mobile phase consisted of 60% A, 10% B and 30% C at a flow-rate of 1 ml/min for 2.5 min and then changed to 30% A, 40% B and 30% C over 1.5 min. The flow-rate was then increased to 1.5 ml/min while the composition was returned to the initial conditions over 1 min. After 1 min, the flow-rate was returned to 1 ml/min and the next sample was injected.

2.4. Sample preparation

Plasma and urine samples were diluted with 1% Nonidet P-40 in 0.9% NaCl (1:3–5, v/v) and were injected directly onto the HPLC system. Tissue samples (0.1 g/ml) were homogenized at 12 000 rpm for 1 min in lysis buffer (10 m*M* Tris, 10 m*M* NaCl, 3 m*M* $MgCl_2$ in 1% Nonidet P-40, pH 7.5) over ice. Each homogenate sample (180 μl) was incubated with 18 μl of 10 mg/ml proteinase K at 37°C for 2 h and then extracted with 220 μl of ice-cold phenol–chloroform (50:50, v/v). Samples were then centrifuged at 14 000 *g* for 15 min and the supernatant (aqueous phase) was removed and extracted again with 150 μl of chloroform. After centrifugation at 14 000 rpm for 10 min at 4°C, 50 μl of the supernatant were injected onto the column.

2.5. Assay validation

Stock solutions of MT-AS were prepared in mobile phase component A and the final concentration was determined spectrophotometrically. Standards were stored at 4°C for two weeks without significant degradation. For analysis, standards were added to biological samples at either a 1:10 or 1:5 ratio and processed along with samples. Absolute recovery of the analyte was determined by comparing the ratio of the peak area of the sample to the peak area of the standard. The precision of the analysis was evaluated in terms of within-day ($n=3$) and between-day ($n=3$) variability and expressed as the percentage coefficient of variation (percentage C.V.). Accuracy was measured as the difference between the actual to predicted concentrations divided by the actual concentration (×100). The limit of quantitation was determined based on an acceptable precision, accuracy and confidence interval [13].

2.6. Electrophoresis

2.6.1. Gel-filled capillaries

The preparation of gel-filled capillary columns was similar to that previously reported [7,8]. Briefly, fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 75 μm, outer diameter of 375 μm, effective length of 30 cm and total length of 50 cm was treated sequentially with 1 *M* KOH, HPLC water, 0.03 *M* HCl, HPLC water, and then was derivatized with 2% 3-methacryloxypropyl trimethoxysilane in methanol for more than 3 h. After flushing with methanol and water, the tubing was filled with a degassed solution of 18% polymerizing linear acrylamide in 30% (v/v) formamide media containing 7 *M* urea, 0.1 *M* Tris–boric acid–EDTA (TBE) buffer (pH 8.3). Polymerization was initiated by ammonium persulfate–N,N,N′,N′-tetramethylethylenediamine (TEMED) chemistry and achieved under a pressure of 400 bar using an isocratic LC pump to prevent the formation of air bubbles [14]. An electric field of 500 V/cm,

producing a current of 5–10 μA, was used for the analyses.

2.7. Sample preparation

The tissue homogenates (1 ml) or urine samples (100–200 μl) were centrifuged at 14 000 *g* for 10 min at 4°C, and then 60 μl of the supernatant were removed and diluted with 340 μl of loading buffer (30% formamide, 70% 25 m*M* Tris, 1 m*M* EDTA, pH 7.0). Plasma (50 μl) samples were directly diluted with 350 μl of the loading buffer without centrifugation. The solid-phase anion-exchange cartridge was activated with 400 μl of the elution buffer (30% formamide, 70% 25 m*M* Tris, 1 m*M* EDTA, 2 *M* LiBr, pH 7.0) and 400 μl of the loading buffer. In order to prevent a potential cartridge blockage due to heavy loading, the prepared sample (plasma, urine or tissue) was split into three aliquots (100/100/200 μl) and then loaded sequentially onto the cartridge, followed by centrifugation at 8000 *g* for 2–4 min at 4°C. The cartridge was first washed with 100 μl of the washing buffer (50% 25 m*M* Tris, 1 m*M* EDTA, pH 7.0; 20% 25 m*M* Tris, 1 m*M* EDTA, 2 *M* LiBr, pH 7.0; 30% formamide) and then eluted with 60 μl of the elution buffer. The column effluent was dialyzed on a 0.025-μm membrane (Millipore, Bedford, MA, USA) for 20 min over deionized water and then injected electrokinetically into a gel-filled capillary.

2.8. Percentage determination and verification

Standard mixtures of 15–20-mer ODNs were prepared in Tris–EDTA (TE) buffer and were added to plasma, urine and tissues. Identification of individual ODNs in unknown samples was based on the comparison of retention times obtained from the standard mixtures and was verified by "standard addition". For this latter analysis, a single ODN standard was added to a standard mixture (i.e., 15–20-mer) and the increase in peak height was measured to confirm the identity of the individual ODN. The relative percentage of each individual ODN present in plasma and tissue samples was calculated as:

$$i\text{th mer (\%)} = \text{peak height of } i\text{th mer} / \text{peak height of total mers} \times 100$$

The relative recovery of total ODN from plasma, urine and tissues was determined by comparison of peak heights of known amounts of individual ODNs to the corresponding peak height obtained in TE buffer.

2.9. Pharmacokinetic studies

The HPLC–CGE method was applied to a pharmacokinetic and tissue distribution study of MT-AS. Nude mice (NIH-nu/nusf), aged four–five weeks and weighing 20–30 g, bearing a human small lung carcinoma (NCI-H446) were administered different doses of MT-AS (10, 30, 100 and 300 mg/kg body weight) as an i.v. bolus. Plasma, urine and tissue samples were collected from 5 min to 48 h after dosing and then stored at −80°C until analyzed.

3. Results and discussion

3.1. Chromatography

Fig. 1 Fig. 2 illustrate representative HPLC chromatograms from plasma and tissue homogenates. The 15–20-mer ODN mixtures appear as one peak. There was no endogenous interference in plasma and urine (data not shown). In contrast, chromatograms from tissue showed (see Fig. 2) a large peak from the extraction solvent on the front of the ODN peak, yet resolution was adequate to enable measurement of total ODN. The plasma and tissue sample preparation methods were convenient and reproducible. There was no evidence of ODN degradation during sample preparation. It was also found that MT-AS was stable in phosphate buffered saline (PBS) and TBE at room temperature for at least four days. It degraded slowly at 25°C in nude mouse plasma, with an in vitro half life of 106 h. Incubation of tissue homogenates with proteinase K was used to digest proteins that could potentially degrade ODNs, such as endo- and exonucleases, and also proteins able to bind ODNs. These combined actions of proteinase K increased the extraction recovery as

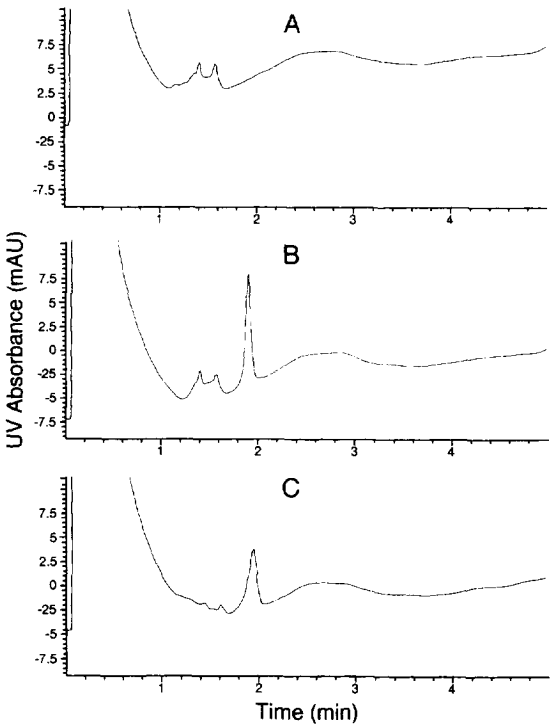

Fig. 1. HPLC chromatograms of antisense oligodeoxynucleotide MT-AS in nude mouse plasma: (A) blank plasma; (B) plasma spiked with 10 μg/ml of MT-AS; (C) plasma sample taken 8 h following an i.v. bolus dose of 300 mg/kg of MT-AS.

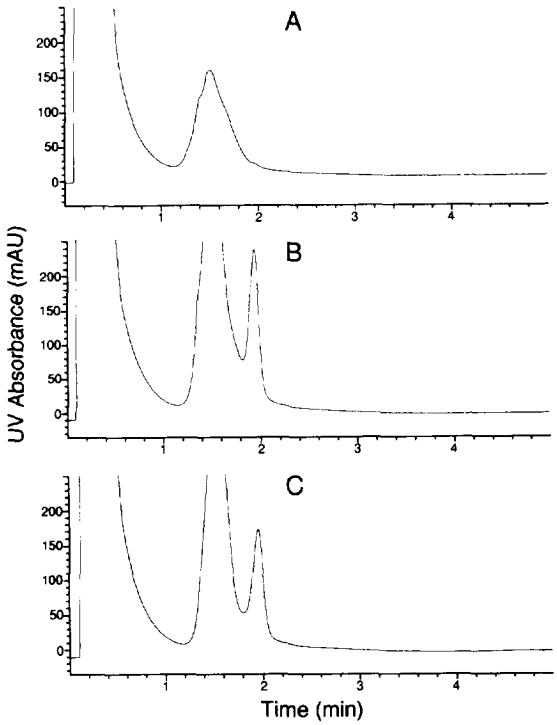

Fig. 2. HPLC chromatograms of antisense oligodeoxynucleotide MT-AS in kidney: (A) blank kidney; (B) kidney spiked with 150 μg/g of MT-AS; (C) kidney sample taken 8 h following an i.v. bolus dose of 300 mg/kg of MT-AS.

well as extended the life of the analytical column. The combination of the short column and the mobile-phase program resulted in a narrow peak of total ODN at approximately 2 min, and enabled a sample run time of 5 min.

The HPLC assay validation data are summarized in Table 1. For plasma and urine in which samples were diluted with Nonidet P-40 and injected directly, recovery was close to 100%. Nonidet P-40 is a non-ionic detergent that is used to solubilize plasma proteins and increase the life of a column. Standard curves were linear over a MT-AS concentration range of 0.21 to 13.3 μg/ml, with coefficient of determination (r^2) values of greater than 0.99. Precision was examined within-day in plasma, urine and all tissues, and between-day in plasma and liver. These media were used for between-day validation because of their greater availability. In addition, the method of extraction from liver homogenate is analogous to that for other tissue homogenates and should be indicative of the reproducibility of the technique, although not necessarily of the actual percentage recovery. Percentages of coefficient of variation (%C.V.) ranged from 1.6 to 15.8% in plasma and urine, and from 1.9 to 13.4% in liver homogenates. The limit of quantitation was 200 ng/ml in plasma, 160 ng/ml in urine and 600 ng/ml in tissues. Extraction recovery for brain, heart, kidney and muscle ranged from approximately 50 to 85%, suggesting a fraction of MT-AS is possibly trapped in tissue debris and bound to proteins or DNA. Recoveries in liver, lung, spleen and tumor showed a pronounced concentration-dependence, varying from low values of about 12% to high values of 75%. These variations may be due to differences in tissue concentrations of endonucleases and exonucleases and differences in protein-binding concentrations amongst tissues. Since % recoveries increased with increasing MT-AS concentrations, saturation of either degradative nucleases or protein-binding

Table 1
Validation of HPLC assay of antisense oligodeoxynucleotides (MT-AS) in plasma, urine and tissue homogenates of nude mice

Sample	Added concentration (μg/ml)	Extraction recovery (%)	Accuracy (%)	Precision (%C.V.)	
				Within-day	Between-day
Plasma	0.476[a]		13.68	15.80	8.72
	2.38	94[b]	5.91	7.68	5.65
	9.524	94.5[c]	7.65	2.67	8.27
Urine	3	87.0	−13.10	1.61	ND[d]
	60	97.30	−2.60	2.78	ND
Liver	8	49.0	14.14	12.42	13.40
	30	69.30	4.11	3.89	3.83
	600	76.45	5.78	1.91	3.05
Tumor	8	0.00		0.00	ND
	30	24.97	19.24	1.93	ND
	600	60.82	5.40	2.02	ND
Lung	8	12.38	2.53	0.44	ND
	30	28.74	7.53	3.39	ND
	600	63.51	4.47	8.47	ND
Spleen	8	19.60	26.30	6.20	ND
	30	39.10	−1.60	3.46	ND
	600	72.40	9.50	2.70	ND
Kidney	8	55.64	0.72	1.64	ND
	30	56.80	17.80	8.57	ND
	600	64.50	4.84	1.46	ND
Muscle	8	57.84	5.15	7.42	ND
	30	62.92	5.49	4.35	ND
	600	64.92	5.63	2.05	ND
Brain	8	44.56	2.40	4.36	ND
	30	51.92	13.81	1.99	ND
	600	48.79	13.76	1.84	ND
Heart	8	85.70	30.30	16.90	ND
	30	87.10	8.30	2.60	ND
	600	82.30	−0.08	2.27	ND

[a]Three samples were processed at each concentration.
[b]At a concentration of 3 μg/ml.
[c]At a concentration of 60 μg/ml.
[d]Not determined.

would lead to greater recoveries at 'saturating' MT-AS concentrations. Standard curves in tissue homogenates were linear from 6 to 1000 μg/g with r^2 values greater than 0.99. In order to compensate for decreasing recovery at lower concentrations in tumor, lung, brain and spleen samples, two linear calibration curves were constructed for these tissues and resulted in r^2 values of greater than 0.99.

3.2. Electrophoresis

One-base separation of 15–20-mer ODNs was readily achieved over run times of 60 to 70 min (see Figs. 3 and 4). Bourque and Cohen [9] were able to attain similar separations in 25 min with a 9-cm effective column length. However, many commercial systems require a minimum column configuration of

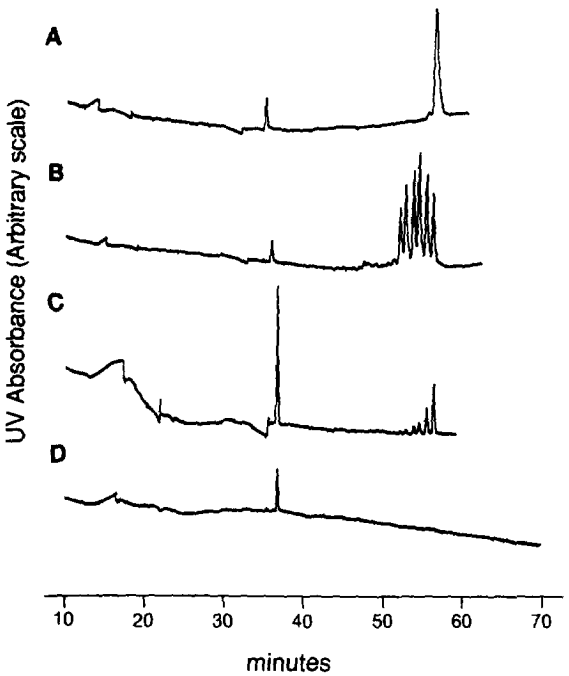

Fig. 3. CGE electropherograms of an individual antisense oligodeoxynucleotide of 15–20-mer in nude mouse plasma: (A) plasma spiked with MT-AS; (B) plasma spiked with 15–20-mer; (C) plasma sample taken 4 h following an i.v. bolus dose of 300 mg/kg of MT-AS; (D) blank plasma.

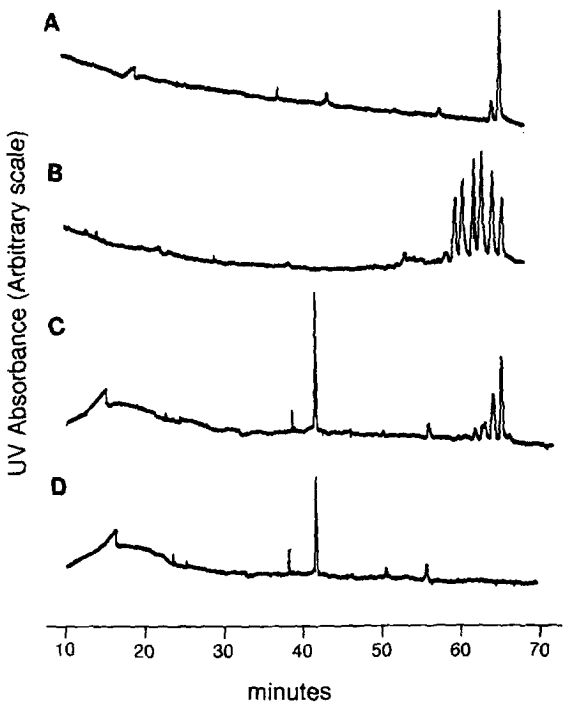

Fig. 4. CGE electropherograms of an individual antisense oligodeoxynucleotide of 15–20-mer in nude mouse kidney: (A) kidney spiked with MT-AS; (B) kidney spiked with 15–20-mer; (C) kidney sample taken 4 h following an i.v. bolus dose of 300 mg/kg of MT-AS; (D) blank kidney.

25 cm. The retention time and peak intensity varied significantly between different columns and only a limited number of biological samples could be processed with one column due to instability of the gel bed. Therefore, CGE is time-intensive, yet offered superior resolving power than HPLC for the qualitative determination of MT-AS and its catabolites.

A solid-phase extraction method was developed in conjunction with the CGE analyses. An alternative procedure is to collect eluting fractions from the HPLC analysis and then introduce these samples onto the CGE system [9], although sample dilution could be a problem. Nonetheless, the solid-phase extraction method resulted in adequate recovery (>60% in plasma) and afforded sufficient sensitivity for pharmacokinetic analyses. The potential problem with CGE analyses is incomplete desalting, which can cause strong solvent resistance of ODNs injected by an electrokinetic method [15]. In order to improve the injection efficiency, an extended time of 30–900 s was used for injections at 5 kV. The relative peak height, band width and separation efficiency did not appear to be adversely affected by an increase in the duration of the injection.

The variability of the CGE analyses in TE buffer is shown in Table 2. For each ODN, the %C.V. was 15% or less. Since the CGE assay was used to reflect the relative percentages of parent drug and catabolites present in biological samples, it was also important to determine the extraction efficiency for each ODN. As shown in Table 3, the relative percentages of ODN (15–20-mer) recovered from biological media did not change significantly compared to those obtained from the original stock mixtures prepared in TE buffer. The %C.V.s ($n=3$) were less than 10% in plasma and 15% in most

Table 2
Within- and between-day variability of the capillary gel electrophoresis assay for the determination of the percentage composition of 15–20-mer of MT-AS in buffer

Analysis		20-mer	19-mer	18-mer	17-mer	16-mer	15-mer
Within-day	% Mean	30.28	14.82	17.04	16.35	12.48	9.03
($n=3$)	SD[a]	2.51	1.41	0.73	1.72	0.86	0.58
	%C.V.[b]	8.29	9.49	4.31	10.52	6.89	6.44
Between-day	% Mean	22.66	13.82	19.05	17.52	15.31	11.63
($n=6$)	SD	3.46	1.24	0.67	1.42	1.84	1.18
	%C.V.	15.25	9.00	3.54	8.12	12.05	10.16

[a]SD = standard deviation.
[b]%C.V. = percentage coefficient of variation.

tissues. Plasma samples analyzed by CGE are shown in Fig. 3, in which the parent 20-mer MT-AS and its catabolites (15–19-mer) are separated. There were no compounds present in plasma that were found to interfere with the analysis (Fig. 3D). The electropherogram of the spiked plasma (Fig. 3A) showed only about 3–4% of 19-mer, which was close to the expected purity of the drug, and further supports the finding that the extraction procedure did not cause significant degradation. Following a 300 mg/kg i.v. bolus dose of MT-AS to a nude mouse, the relative percentages of ODNs in plasma were 50% parent drug, 25% 19-mer, 11% 18-mer, 8% 17-mer, 4% 16-mer and 4% 15-mer (Fig. 3C). The CGE method was also applied to tissues samples (see Fig. 4). Analysis of the kidney sample collected from the same animal as used in Fig. 3C, revealed the presence of 53% parent MT-AS, 29% 19-mer, 11% 18-mer and 7% 17-mer.

3.3. Pharmacokinetic studies

Fig. 5 shows the total (15–20-mer) plasma concentration–time profile of MT-AS in nude mice bearing subcutaneous human lung carcinomas (NCI-H446), following i.v. bolus administration of 300 mg/kg doses of MT-AS. Following a single injection, MT-AS was rapidly distributed into tissues and cleared from blood, with an elimination half-life of about 4 h. Urinary excretion of total MT-AS, as determined by HPLC, was less than 10% of the administered dose in 48 h. Based on CGE analysis, about 50% of the total MT-AS was the 20-mer parent drug, with the remainder degraded to 15–19-

Table 3
Percentage composition of 15–20-mer MT-AS added to biological samples ($n=3$), as determined by a capillary gel electrophoresis (CGE) assay

	20-mer		19-mer		18-mer		17-mer		16-mer		15-mer	
	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)	Mean (%)	C.V. (%)
In TE buffer[a]	13.5		16.0		22.6		18.6		16.7		12.5	
In plasma	13.5	9.3	17.8	3.6	22.2	3.6	18.9	2.6	16.5	8.8	11.1	7.4
Kidney	12.9	3.8	16.1	2.4	20.8	0.5	19.7	1.3	16.8	0.7	13.6	2.4
Liver	11.2	15.8	14.8	6.6	20.4	3.0	20.2	5.0	18.5	6.2	14.9	7.0
Tumor	11.4	14.3	13.0	4.1	19.0	3.0	18.6	1.7	19.6	2.2	18.4	0.3
Urine	10.6	26.7	11.6	17.3	17.4	10.7	19.6	1.3	20.8	7.6	20.0	5.1
Lung	13.9	4.3	14.4	2.0	20.8	2.3	19.0	2.4	17.7	3.4	14.1	1.7
Spleen	8.5	10.0	12.5	13.0	20.2	5.1	20.8	2.8	20.4	4.6	17.6	2.2
Muscle	13.3	1.4	16.6	0.7	21.6	0.9	18.9	0.5	16.8	1.0	12.9	1.4

[a]Single injection.

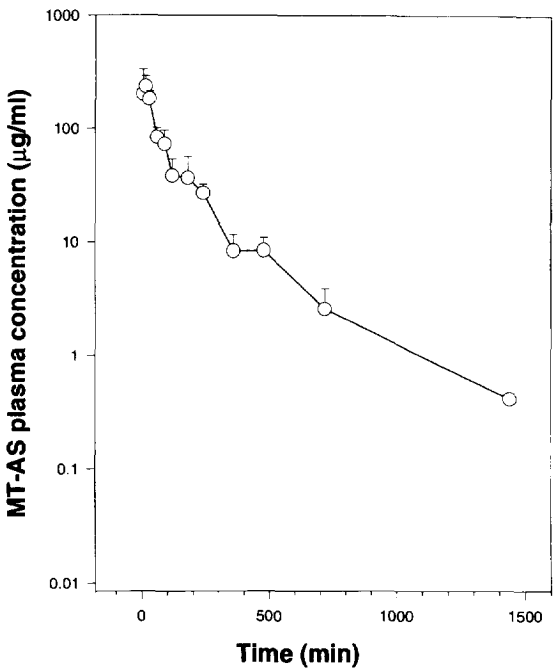

Fig. 5. Total MT-AS oligonucleotides (i.e. 15–20-mer), as measured by HPLC, plasma concentration–time profile following i.v. bolus doses of 300 mg/kg of MT-AS to nude mice bearing subcutaneous human lung carcinoma (NCI-H446).

mer ODNs. A preliminary analysis for the pharmacokinetics and tissue distribution of MT-AS was reported elsewhere [16].

4. Conclusions

Two analytical methods, HPLC and CGE, have been developed and validated for the measurement of ODNs in plasma, urine and tissue samples. The HPLC assay provides a rapid and reproducible determination of total 15–20-mer ODNs, in this case MT-AS. This measurement is most indicative of ODNs possessing biological activity. The time-consuming, yet reproducible, CGE assay permits measurement of individual ODNs, parent ODNs and catabolites, and thus provides analyte-specific data. Each technique can be used independently, provided that analytical standards are included in both the HPLC and CGE assays. In our application of the methods, since standards were not incorporated into the CGE method, the methods are used in tandem to provide individual ODN concentrations. This application of the methods will facilitate a higher throughput of samples via HPLC and, when desired, can be followed by CGE to obtain tissue distribution data on specific ODNs.

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