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Inducing a change in the pharmacokinetics and biodistribution of poly-L-lysine in rats by complexation with heparin

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Abstract

The aim of our study was to induce changes in the plasma elimination half-life ($t_{½\ elim}$), rate and extent of urinary excretion, and biodistribution of a model macromolecule, poly-L-lysine, in rats following complexation with heparin. Male Sprague-Dawley rats were dosed intravenously with either unfractionated [^{3}H]heparin, FITC-labelled poly-L-lysine, or an [^{3}H]heparin:FITC-labelled poly-L-lysine complex. Serum and blood concentration vs time and urinary excretion profiles were determined as well as the resulting patterns of biodistribution to liver, spleen, kidney, and muscle tissue. While the mean values for the total body clearance of poly-L-lysine and the complex were not significantly different, the volume of distribution and the half-life associated with elimination from the serum were increased greater than 2-fold for the complex compared with free poly-L-lysine. The rate and extent of elimination in the urine followed the relative rank order; heparin > poly-L-lysine > heparin:poly-L-lysine complex. Thirty minutes following intravenous administration, there was significantly more tissue deposition/uptake of the complex in the liver, kidney, and muscle, but not the spleen, when compared with poly-L-lysine administered alone. Complexation of heparin to poly-L-lysine effectively increased the fraction of an administered dose of poly-L-lysine that was deposited in liver, kidney, and muscle tissue. Due to the macromolecular complex being nontoxic and uncharged, potentially it might serve as a suitable carrier for both conventional and peptidic drugs to increase drug distribution to liver, kidney, or muscle tissue.

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Introduction

Targeting of drug substances to particular tissues or cells still remains a high priority in the pharmaceutical sciences and the practice of medicine. This is especially true for drug substances such as cancer chemotherapeutic agents with their associated high level of toxicity to normal cells. While attempts to optimally deliver therapeutic agents in the correct temporal fashion and directly to the intended target have been intensely investigated for over 30 years, newer strategies have emerged. Monoclonal antibodies (Cao & Suresh 2000; Zhang & Pardridge 2001) and gene therapy using viral and non-viral vectors (Castro et al 2001; Takeda & Miyagoe-Suzuki 2001; Yang et al 2001) are appropriate examples of newer strategies. A somewhat older strategy has been to modify a drug substance by chemically attaching various functional groups or complexing the drug substance with some other carrier molecule, which will direct the drug to a specific set of cells or certain tissues.

Drug complexes are loosely categorized into three groups depending on whether the so-called acceptor component is a metal ion or an organic molecule (Martin 1993). The third class, inclusion/occlusion compounds, involve the entrapment of one compound in the molecular framework of another. An organic molecule that has been used in the past to alter the absorption of drug substances by cultured cells is poly-L-lysine (Persiani & Shen 1989; Ekrami & Shen 1995). This same molecule has been used to direct select anticancer drugs in cell culture (Cortes et al 1993) and in-vivo (Morimoto et al 1984; Di Stefano et al 2001). Poly-L-lysine is a polycationic molecule

formed by repeating L-lysine amino acid groups to form a polypeptide with various degrees of polymerization and molecular weights. When poly-L-lysine is complexed to another drug substance having an overall opposite electrostatic charge, the resulting organic molecular complex may be referred to as a charge transfer complex (Martin 1993). No covalent bonds are involved; instead, one molecule polarizes the other, resulting in a type of ionic interaction or charge transfer and hence the name charge transfer complex.

The commercial drug heparin has been used universally as an anticoagulant for over 60 years. Heparin is a highly sulfated, polydisperse, linear polysaccharide and polyanion with an average molecular mass of 10 000 to 15 000 Da (Edens et al 1992; Desai & Linhardt 1995). The pharmacokinetics and biodistribution of unfractionated as well as fractionated heparin have been studied by many groups and in many animal species (Schaefer et al 1980; Watanabe et al 1982; Boneu et al 1990). In the rat, parenteral administration of unfractionated heparin is generally observed to disappear from the serum by a first-order (monoexponential) process.

When poly-L-lysine is allowed to form a complex in the presence of excess heparin, the resulting poly-L-lysine:heparin complex thus formed is charge neutral (Bleiberg et al 1981) and non-cytotoxic (Shen & Ryser 1981; Morad et al 1984; Ryser et al 1986). The physicochemical properties of a poly-L-lysine:heparin complex have been extensively investigated (Bleiberg et al 1981; Shen & Ryser 1981, 1983; Morad et al 1984; Ryser et al 1986; Mulloy et al 1996). The purpose of this study was to advance previous in-vitro work on heparin:poly-L-lysine complexes (Shen & Ryser 1981, 1983; Morad et al 1984). We have evaluated what effect heparin would exert on the overall pharmacokinetics and biodistribution of poly-L-lysine following its intravenous administration as a complex to rats.

Materials and Methods

[^{3}H(G)]Heparin, sodium salt (sp. act. = 0.2 mCi mg^{-1}; biological act. = 135 U mg^{-1}) was obtained from NEN Life Sciences Products, Inc. (Boston, MA). ScintiSafe liquid scintillation solution was obtained from Fisher Scientific (St Louis, MO). Solvable tissue digest solution was obtained from Packard Instruments Co. (Meriden, CT). FITC-labelled poly-L-lysine.HBr with a molecular weight of 15–30 kDa (degree of substitution = 0.003–0.01 mol FITC per mol lysine monomer; average MW = 26 300) and heparin sodium salt derived from porcine intestinal mucosa (181 USP units mg^{-1}) were used as received and purchased from Sigma (St Louis. MO). Pre-cannulated (external jugular vein), male Sprague-Dawley rats (251 ± 7 g) were purchased from Charles River, Inc. (Wilmington, MA). Relative fluorescent units (RFUs) were determined using a model 'f-max' microplate fluorescence reader manufactured by Molecular Devices Corporation (Sunnyvale, CA).

Administration of heparin, poly-L-lysine, and the heparin:poly-L-lysine complex

The [^{3}H]heparin solution (1.4 mg/0.25 kg body weight in 200 μL; 280 μCi; equivalent to approximately 190 U) was administered intravenously to each of four rats. We investigated the pharmacokinetics and biodistribution of heparin in this study to confirm our results with previously published findings. Blood samples (200 μL) were obtained from each rat receiving heparin at pre-determined time points up to and including 32-min post dosing, and placed directly into 20-mL glass scintillation vials. Into each vial was placed 1 mL of a 2:1 mixture of ethanol:Solvable. The mixture was immediately swirled and the vials then incubated at 55 °C for 1 h with gentle swirling using an orbit shaker. After the incubation period, the vials were removed from the incubator and allowed to reach room temperature. To each vial was added 200 μL 30% w/w hydrogen peroxide in two equal portions, allowing gas evolution to subside between additions. The scintillation solution (ScintiSafe 30% w/w) was then added to bring the total volume to 15 mL. The vials were then capped and shaken vigorously by hand after which time 0.5 mL 0.5 M HCl solution was added, the vials recapped and again shaken by hand. The samples were then equilibrated at 8 °C for 17–24 h before counting in duplicate for 10 min at 2 sigma error using a Beckman Instruments (Fullerton, CA) model LS 6500 liquid scintillation counter. Following data output, the observed counts of each blood sample were corrected for counts associated with blank blood samples (time t = 0 min or pre-injection blood) and the concentration of heparin in the blood calculated based on the specific activity associated with the dosing solution. The total volume of blood collected throughout the kinetic study for each compound did not exceed 15% of the total blood volume, assuming a normal total blood volume of approximately 16 mL for a 250-g rat (Lindstedt & Schaeffer 2002).

Administration of poly-L-lysine was conducted in a similar fashion to that of [^{3}H]heparin. Since the length of blood collections for administration of either poly-L-lysine or the complex did not exceed a time period greater than 15 min, no additional unlabelled heparin was used to maintain cannula patency for these compounds. Four rats were each individually administered a dose of 500 μg/0.25 kg body weight of FITC-labelled poly-L-lysine by intravenous injection. This dose was selected based on the value of the LD50 for poly-L-lysine in rats (de Vries et al 1953). Blood samples (400 μL) were collected as above and immediately centrifuged at 10 000 rev min^{-1}, the serum (≈ 200 μL) obtained by removing the coagulated or clumped mass of erythrocytes, and the serum samples placed directly into the wells of a 96-well microtitre plate. The relative fluorescent units (RFUs) associated with each serum sample were compared with a set of standards prepared by adding known amounts of FITC-labelled poly-L-lysine into pooled blank serum which had previously been collected from three separate additional rats. To determine whether collection of serum by centrifugation and removal of the coagulated mass

resulted in loss of poly-L-lysine, blank whole blood was mixed with the same amounts of FITC-labelled poly-L-lysine as used above for blank serum. All samples were analysed by fluorescence spectroscopy at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Administration of the heparin:poly-L-lysine complex was similar to that for poly-L-lysine. Two solutions were prepared and then combined so that the final dosing volume (200 μL as above for [^{3}H]heparin and FITC-labelled poly-L-lysine) would contain a ratio of [^{3}H]heparin to FITC-labelled poly-L-lysine of 10:1. Solution number 1 was prepared by dissolving 5 mg FITC-labelled poly-L-lysine in 1 mL sterile water for injection, USP. Solution number 2 consisted of 14 mg (2.8 mCi) [^{3}H]heparin and 36 mg unlabelled heparin dissolved in the same volume (1 mL) of sterile water for injection. The two solutions were then combined and a bolus dose (500 μg poly-L-lysine + 1.4 mg tritiated heparin/0.25 kg body weight in 200 μL) of the [^{3}H]heparin:FITC-poly-L-lysine solution was administered to each of eight rats by intravenous administration (four rats in each of two groups). Blood samples (200 μL) obtained from the four rats contained in group 1 were processed and counted for radioactivity as described above following intravenous administration of [^{3}H]heparin alone. Blood samples (400 μL) collected from the four rats in group 2 were treated as described above following the administration of FITC-labelled poly-L-lysine alone. The calibration curve for detection of FITC-labelled poly-L-lysine contained in the [^{3}H]heparin:FITC-poly-L-lysine complex was prepared with known concentrations of the original [^{3}H]heparin:FITC-poly-L-lysine dosing solution added to pooled blank rat serum used above. Similar to the analysis described above for FITC-labelled poly-L-lysine alone, blank whole blood was mixed with the same concentrations of the original [^{3}H]heparin:FITC-poly-L-lysine dosing solution to determine whether collection of serum by centrifugation and removal of the coagulated mass resulted in loss of FITC-labelled poly-L-lysine by this step during sample processing. The serum samples were then analysed by fluorescence spectroscopy as described above following administration of FITC-poly-L-lysine alone.

All animal treatment procedures were in accordance with the Institutional Animal Care and Use Committee (IACUC) at the University of Missouri-Kansas City. The Laboratory Animal Center at the University of Missouri-Kansas City is approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Urinary excretion studies

Each of four rats in three separate groups were administered either 1.4 mg/200 μL [^{3}H]heparin alone, 1 mg/200 μL FITC-labelled poly-L-lysine alone, or the [^{3}H]heparin:FITC-poly-L-lysine complex prepared such that 200 μL of the dosing solution contained 10 mg heparin (1.4 mg of which was radiolabelled (280 μCi)) and 1 mg FITC-poly-L-lysine. In the urinary excretion studies, a 1 mg dose FITC-labelled poly-L-lysine was selected when administered alone or as the complex, compared with 500 μg in the serum concentration–time studies, due to concerns about adequate detection of the FITC-poly-L-lysine in a larger volume of a biological matrix (urine). All animals were then placed in individual metabolism cages and provided with unrestricted access to food and water. Urine was collected on ice at various times throughout the 70-h study depending on urine flow. The volume of urine collected over any given time interval was calculated by placing the specimen into a tarred glass vial and weighing the vials using an analytical balance.

Collected urine fractions from all rats were first centrifuged at 5000 rev min^{-1} for 5 min to remove any food particles and the supernatants immediately frozen on dry ice and stored at –20 °C while protected from light. At the time of assay, urine specimens were thawed, thoroughly mixed with a vortex mixer for 1 min, and then 200 μL of each sample from rats dosed with either FITC-poly-L-lysine alone or the [^{3}H]heparin:FITC-poly-L-lysine complex placed directly into the wells of a 96-well microtitre plate. Two calibration curves were required for this experiment, namely, known concentrations of the FITC-labelled poly-L-lysine and the solution containing the [^{3}H]heparin:FITC-poly-L-lysine complex, each added into blank urine obtained from rats described above that were used for harvesting blank blood and serum. The samples were then analysed by fluorescence spectroscopy as described above and the concentration of FITC-labelled poly-L-lysine determined by comparison with the appropriate calibration curve. Urine samples collected from rats administered the [^{3}H]heparin:FITC-poly-L-lysine complex were analysed for radioactivity similarly to rats administered [^{3}H]heparin alone. This was accomplished by simply combining 0.5 mL urine with 14.5 mL ScintiSafe scintillation solution and then counting the sample. As was performed for blood samples containing [^{3}H]heparin dosed either alone or as part of the complex, the observed counts of each urine sample were corrected for counts associated with blank urine and the concentration of heparin in the urine then calculated based on the specific activity of [^{3}H]heparin contained in the dosing solution.

Biodistribution study

This experiment was conducted to determine the tissue localization of [^{3}H]heparin, FITC-labelled poly-L-lysine, and the [^{3}H]heparin:FITC-poly-L-lysine complex at one time point following intravenous administration. Similar to the urinary excretion studies described above, a 1.0 mg test dose of FITC-labelled poly-L-lysine alone, or when contained in the [^{3}H]heparin:FITC-poly-L-lysine complex, was selected for adequate detection of the fluorescent label. The dose of [^{3}H]heparin administered was 1.4 mg (280 μCi) to correspond with the dose administered in both the blood concentration–time and urinary excretion studies. Briefly, solutions were prepared as described

above and a 200-μL dosing volume administered by intravenous injection to each of four rats in three separate groups. Four additional rats comprised a fourth group and received the same dose of the [^{3}H]heparin:FITC-poly-L-lysine complex so that a second method of detection could be utilized to evaluate the extent of tissue localization. Thirty minutes following the administered dose, all rats were killed with an overdose of pentobarbital sodium and the following organs obtained; liver, spleen, kidney, and a representative section of skeletal muscle (gastrocnemius). All tissue specimens were immediately placed in tarred vials to obtain a wet weight of the tissue sample.

Retrieved tissue specimens were processed in two different ways. Tissues from the four rats injected with [^{3}H]heparin and from four rats in one of the two groups that were injected with the [^{3}H]heparin:FITC-poly-L-lysine complex were not homogenized, but rather, a small piece of tissue ($\approx$ 500 mg) was placed into a 20-mL glass scintillation vial. Samples were then processed in a similar fashion to blood samples obtained following intravenous administration of either [^{3}H]heparin or the [^{3}H]heparin:FITC-poly-L-lysine complex (when monitored using the tritium label) using the 2:1 ethanol:tissue solubilizer solution and hydrogen peroxide to decolorize the digestate. Scintillation solution was added to the tissue digests to bring the total volume of each vial to 15 mL and the vials then counted for radioactivity. As was performed for blood and urine samples containing [^{3}H]heparin, the observed counts of each tissue sample were corrected for counts associated with tissue obtained from control (non-injected) rats and the concentration of heparin in each tissue then calculated based on the specific activity of [^{3}H]heparin contained in the dosing solution.

Before the analysis of FITC-labelled poly-L-lysine in tissue specimens, 2 mL deionized water was added to retrieved spleens and kidneys, and 3 mL water was added to the livers and the samples of muscle tissue. Tissue specimens that contained either FITC-labelled poly-L-lysine or the [^{3}H]heparin:FITC-poly-L-lysine complex (when followed using the FITC label) were then homogenized with an Ultra-Turrax tissue homogenizer (Tekmar Co., Cincinnati, OH). Mechanical homogenization was not necessary for tissue samples obtained from heparin-injected rats described above, since those tissues were chemically digested. Next, tissue homogenates were centrifuged at 40 000 rev min^{-1} for 60 min, the supernatants collected, and 20 μL of each sample placed in a 200-μL well of a 96-well microtitre plate. To each sample in a well was added an additional 180 μL deionized water and the plate gently swirled to mix the contents of each well. As with the serum concentration–time and urinary excretion studies, two separate calibration curves were prepared. This was accomplished by combining known amounts or volumes of either FITC-labelled poly-L-lysine or the [^{3}H]heparin:FITC-poly-L-lysine solution containing the complex, respectively, with the individual organs retrieved from control animals (non-injected rats used previously for collection of blank blood, serum, and urine). The preparations representing the standards were processed in the same manner as the tissue specimens containing the unknown amounts of either FITC-labelled poly-L-lysine or the [^{3}H]heparin:FITC-poly-L-lysine complex. The samples were then analysed by fluorescence spectroscopy and the concentration of FITC-labelled poly-L-lysine (either alone or when associated with the complex) in an individual tissue determined by comparison with the appropriate standard curve.

Pharmacokinetic and statistical analysis

Serum concentration–time data following intravenous administration of either heparin, poly-L-lysine, or the heparin:poly-L-lysine complex were fitted to a one-compartment model using standard equations (Gibaldi & Perrier 1982). As indicated in the footnote to Table 1, the apparent volume of distribution (Vd) for the complex was calculated by dividing the dose of FITC-labelled poly-L-lysine contained in the complex (0.5 mg; i.e. 200 μL heparin:poly-L-lysine dosing solution which contained 5 mg poly-L-lysine in 2 mL) by the serum concentration of the complex at time t = 0 min obtained by extrapolation of the 'best-fit' line (Figure 1). It is important to note that the profile depicted in Figure 1 for the disappearance of the heparin:poly-L-lysine complex from the serum was determined by following the concentration of the FITC-labelled poly-L-lysine associated with the complex and not the disappearance of the tritium label contained in the complex, since the heparin:poly-L-lysine dosing solution contained uncomplexed tritiated heparin.

Table 1 Pertinent pharmacokinetic parameters following intravenous administration of [^{3}H]heparin, FITC-labelled poly-L-lysine, and a [^{3}H]heparin:FITC-poly-L-lysine complex to Sprague-Dawley rats.

Pharmacokinetic parameter	[^{3}H]Heparin	FITC-poly-L-lysine	[^{3}H]Heparin:FITC-poly-L-lysine complex
k_d (min^{-1})	0.0654 ± 0.0056	0.466 ± 0.031*$^{\#}$	0.211 ± 0.007*
$t_{1/2elim}$ (min)	10.6 ± 0.87	1.49 ± 0.10*$^{\#}$	3.30 ± 0.12*
Vd (mL)	123 ± 19.8	32.8 ± 3.5*$^{\#}$	71.5 ± 6.7*†
Cl (mL min^{-1})	8.0 ± 0.9	15.3 ± 1.9*	15.1 ± 1.3*

*$P < 0.05$ compared with the same parameter for heparin. $^{\#}P < 0.05$ compared with the same parameter for the heparin:poly-L-lysine complex. †The apparent Vd for the complex was calculated using a dose of 0.5 mg for the poly-L-lysine/the serum concentration at time t = 0 min for the complex in Figure 1.

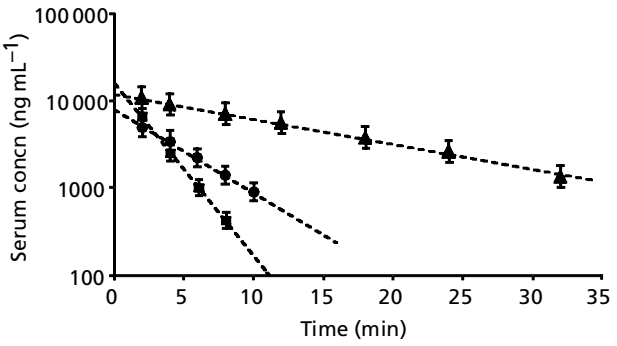

Figure 1 Serum concentration–time profiles following intravenous administration of [^{3}H]heparin (▲) (r = − 0.9991), FITC-labelled poly-L-lysine (■) (r = − 0.9984), and the [^{3}H]heparin:FITC-poly-L-lysine complex (●) (r = − 0.9957). The profile shown for the disappearance of the complex was determined by measuring the concentration of the FITC-labelled poly-L-lysine in serum. All symbols represent the mean value ± s.d. of four rats. Dashed lines through the symbols represent a mathematical fit of the data using a least squares regression analysis.

All comparisons of mean values were performed using a standard one-way analysis of variance. Post hoc testing utilized the method of Scheffé to identify significantly different mean values and were deemed significant if $P < 0.05$.

Results

Elimination of test compounds from the blood/serum

As can be noted from Figure 1, the serum concentration–time profiles for each compound over the time intervals evaluated followed first-order elimination. As listed in Table 1, the mean values ± s.d. of the elimination half-life, $t_{½elim}$, for [^{3}H]heparin, FITC-labelled poly-L-lysine, and the [^{3}H]heparin:FITC-poly-L-lysine complex following intravenous injection were 10.6 ± 0.87, 1.49 ± 0.10, and 3.30 ± 0.12 min, respectively. The mean $t_{½elim}$ for heparin was significantly ($P < 0.05$) greater than the corresponding values determined for poly-L-lysine and the heparin: poly-L-lysine complex. More importantly, the $t_{½elim}$ for the complex was approximately 2.2-fold ($P < 0.05$) the corresponding value determined following administration of poly-L-lysine alone. Estimated volumes of distribution, Vd, for [^{3}H]heparin, FITC-labelled poly-L-lysine, and the [^{3}H]heparin:FITC-poly-L-lysine complex were 123 ± 19.8, 32.8 ± 3.5, and 71.5 ± 6.7 mL, respectively (Table 1). The mean value of Vd for the complex was significantly ($P < 0.05$) greater (2.2-times) than the corresponding mean value for poly-L-lysine administered alone (Table 1). Lastly, the mean values determined for the total body clearance, Cl, for [^{3}H]heparin, FITC-poly-L-lysine, and the [^{3}H]heparin:FITC-poly-L-lysine complex were 8.0 ± 0.9, 15.3 ± 1.9, and 15.1 ± 1.3 mL min^{-1}, respectively (Table 1). There was no significant difference in the mean values of the Cl for the heparin:poly-L-lysine complex and poly-L-lysine (Table 1).

It should be noted that concentration–time profiles for elimination of the complex analysed by disappearance of the tritium label from blood and the FITC-labelled poly-L-lysine from serum resulted in profiles that were not superimposable. The data in Figure 1 for the disappearance of the [^{3}H]heparin:FITC-poly-L-lysine complex were based on detection of the FITC-labelled poly-L-lysine contained in the complex and not the disappearance of radioactivity associated with [^{3}H]heparin contained in the complex. The disappearance of FITC-labelled poly-L-lysine contained in the complex was used to follow the elimination kinetics associated with the heparin:poly-L-lysine complex because it was reported previously that poly-L-lysine bound to heparin in a stochiometric weight ratio of 1 to 1.25 (Morad et al 1984). Thus, when the complex was administered as a solution in which the ratio of heparin to poly-L-lysine was 10:1, all of the poly-L-lysine was present in the complexed state, whereas, in contrast, only a portion of the [^{3}H]heparin formed a complex with poly-L-lysine. Using the dosing solution for the complex in the urinary excretion studies as an example, 1.4 mg [^{3}H]heparin was homogeneously mixed with 8.6 mg unlabelled heparin and combined with 1 mg poly-L-lysine. Furthermore, using the stochiometric weight ratio of 1:1.25 for the poly-L-lysine:heparin complex (Morad et al 1984), only approximately 14% or 0.175 mg of the 1.25 mg complexed heparin would be radiolabelled if it was assumed that poly-L-lysine exhibited no preferential affinity for labelled vs unlabelled heparin in the complex. With residual uncomplexed [^{3}H]heparin in the dosing solution, monitoring the appearance of tritium in the urine alone would not necessarily reflect the urinary excretion kinetics associated with the heparin:poly-L-lysine complex.

Urinary excretion profiles

The rate and extent of elimination of the test compounds in the urine followed the relative rank order: [^{3}H]heparin > FITC-poly-L-lysine > [^{3}H]heparin:FITC-poly-L-lysine complex (Figure 2). The cumulative amount of poly-L-lysine and the heparin:poly-L-lysine complex in the urine over the 70-h experiment was adequately described by a first-order equation. The amount of poly-L-lysine and the heparin:poly-L-lysine complex excreted at time $t = \infty$ ($A_{e\infty}$) was 711 and 451 μg, respectively. In contrast, a biexponential equation best described the cumulative amount of heparin excreted into the urine as a function of time. It can be noted in Figure 2 that the cumulative amount of heparin excreted in the urine after 1 h was approximately 80% of the value of $A_{e\infty}$ (730 μg). As was the case for serum concentration–time profiles for the heparin:poly-L-lysine complex, excretion of the complex into urine evaluated by both monitoring the appearance of the tritium label associated with the complex and the FITC-labelled poly-L-lysine contained in the complex did not result in identical profiles. Thus, similar to the rationale described above, the urinary excretion profile for the

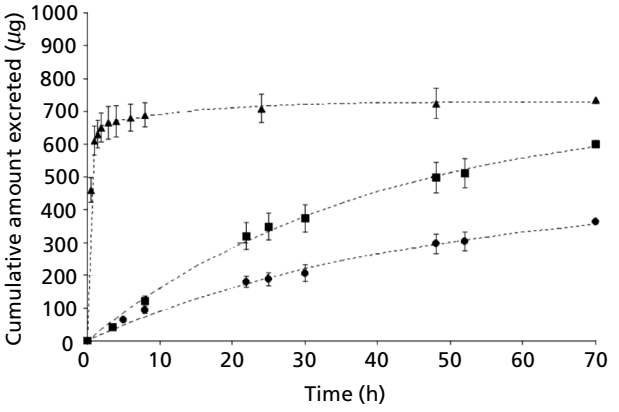

Figure 2 Urinary excretion profiles following intravenous administration of [^{3}H]heparin (▲), FITC-labelled poly-L-lysine (■), and the [^{3}H]heparin:FITC-poly-L-lysine complex (●). The profile shown for the complex was determined by measuring the urinary excretion of the FITC-labelled poly-L-lysine. All symbols represent the mean value ± s.d. of four rats. Dashed lines through the symbols represent a mathematical fit of the data using a monoexponential (poly-L-lysine alone and the complex) or a biexponential (heparin) equation.

heparin:poly-L-lysine complex depicted in Figure 2 represents urinary elimination based on the appearance of FITC-labelled poly-L-lysine contained in the complex and not the tritium label associated with the complex.

Tissue deposition following intravenous administration

The tissue distribution of [^{3}H]heparin, FITC-poly-L-lysine, and the [^{3}H]heparin:FITC-poly-L-lysine complex in various tissues 30 min following intravenous administration is shown in Figure 3. It can be noted that there was significantly ($P < 0.05$) more tissue deposition/uptake of the complex in the liver, kidney, and muscle, but not the spleen, when compared with poly-L-lysine administered alone. The ((percent of the dose)/(g kidney tissue)) following administration of the complex was significantly ($P < 0.05$) greater than the corresponding mean values determined for poly-L-lysine alone or heparin alone and the mean value for the concentration of poly-L-lysine in kidney tissue was significantly ($P < 0.05$) greater than the corresponding mean value determined for heparin alone (Figure 3). In general, poly-L-lysine had greater affinity for kidney tissue, whether dosed alone or as the complex, than did heparin; however, heparin dosed alone appeared to distribute more to the liver than either poly-L-lysine or the heparin:poly-L-lysine complex. As with the serum concentration–time and urinary excretion studies, the extent and pattern of tissue distribution for the [^{3}H]heparin:FITC-poly-L-lysine complex was not identical for each assay method, namely, detection of [^{3}H]heparin vs the FITC-labelled poly-L-lysine associated with the complex. However, it should be noted that the biodistribution data for the heparin:poly-L-lysine complex shown in Figure 3 represents tissue localization based on detection of the FITC-labelled poly-L-lysine contained in the complex.

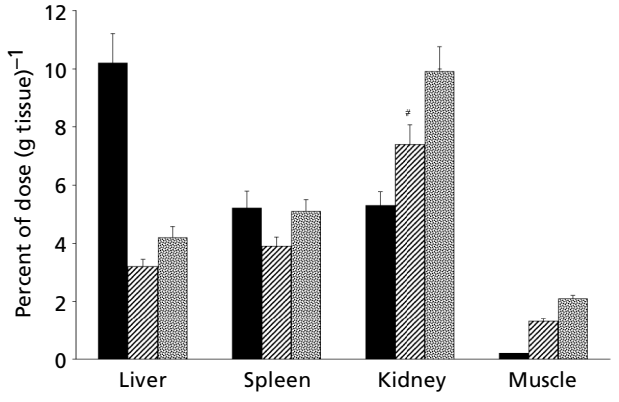

Figure 3 The tissue distribution of [^{3}H]heparin (■), FITC-labelled poly-L-lysine (▨), and the [^{3}H]heparin:FITC-poly-L-lysine complex (▩) 30 min following an intravenous injection. The data shown for the complex was determined by assaying the supernatant of the homogenized tissues for FITC-labelled poly-L-lysine. All bars represent the mean value ± s.d. of four rats. *$P < 0.05$, compared with corresponding mean values for either poly-L-lysine or the complex. #$P < 0.05$, compared with the corresponding mean value for the complex.

Discussion

This study has demonstrated that it was possible to increase the fraction of an intravenously administered dose of poly-L-lysine that was deposited/distributed to liver, kidney, and muscle tissue by forming a complex with heparin. While much earlier research had been conducted with poly-L-lysine:heparin complexes in-vitro and in cell culture (Shen & Ryser 1981, 1983; Morad et al 1984; Ryser et al 1986) it was not until 1995 (Ekrami et al 1995) that the biodistribution of a polylysine:heparin complex was evaluated in mice. Ekrami et al (1995) investigated the distribution of tyramine-derivatized, 62 kDa, ^{125}I-labelled poly-D-lysine administered alone or as a [^{125}I]poly-D-lysine:heparin complex, in which a 2-fold excess of heparin was used to neutralize most of the positive charge of poly-D-lysine (the complex retained an overall net negative charge), determined in the blood, kidney, lung, and liver tissue of mice 3 h following an intravenous injection. Unfortunately, the time course associated with the elimination of the [^{125}I]poly-D-lysine:heparin complex from both the blood and into the urine was not characterized.

To understand better the biodistribution of a polylysine:heparin complex, it was useful to examine the biodistribution of the free polylysine. Our results could be compared and contrasted with the findings of Ekrami et al (1995). In this study, a similar trend was observed for deposition of a neutral poly-L-lysine:heparin complex in liver tissue of rats 30 min following an intravenous injection as was observed by Ekrami et al (1995) with a negatively-charged poly-D-lysine:heparin complex 3 h after

intravenous administration in mice. That is, a greater percent of the administered dose of the complex was localized in the liver compared with free polylysine. However, in this study, we observed two major differences in the biodistribution of free and heparin-complexed poly-L-lysine. When the percent of the administered dose present in a particular tissue was normalized for tissue weight (percent of the dose/g tissue), our findings demonstrated that more of the free and heparin-complexed poly-L-lysine were detected in kidney than in the liver, whereas Ekrami et al (1995) showed a greater overall localization of free and heparin-complexed poly-D-lysine in liver compared with kidney. Secondly, Ekrami et al (1995) demonstrated that distribution of poly-D-lysine to the kidney was virtually abolished when it was complexed to heparin, whereas this study demonstrated a larger percent of the administered dose of the complex was localized in kidney tissue relative to free poly-L-lysine. These differences in the tissue distribution of polylysine when complexed to heparin may be due to overall ionic charge associated with the complex (this study = neutral; Ekrami et al (1995) = negative), molecular weight of the polylysine used (this study = poly-L-lysine$_{26\,kDa}$; Ekrami et al (1995) = poly-D-lysine$_{62\,kDa}$), polylysine isomer type (this study = poly-L-lysine or L-isomer; Ekrami et al (1995) = poly-D-lysine or D-isomer), and time of tissue sampling following intravenous administration of either free polylysine or the complex (this study = 0.5 h; Ekrami et al (1995) = 3 h).

One unique physical property of positively-charged polymers is their strong binding to negative charges which are normally present at the surface of mammalian cells as well as their subsequent transport into cells by non-specific adsorptive endocytosis (Ryser et al 1986). This may prove advantageous for certain therapeutic macromolecules/proteins because cationization generally offers a convenient means of altering their biodistribution and transport properties. It has been demonstrated that cationized proteins have a shorter half-life and a higher organ uptake and penetration compared with native proteins (Bergmann et al 1984; Triguero et al 1991). However, using poly-L-lysine as an agent for cationization reveals two serious limitations. The first is its charge-related in-vitro and in-vivo toxicity and the second is its lack of specificity in its cellular interactions and, hence, its unsuitability for drug targeting (Ryser et al 1986). On the other hand, it has been demonstrated that the charge-related toxicity of poly-L-lysine is totally negated in the presence of a 10-fold excess of heparin, a natural anionic polymer (Morad et al 1984). As such, a poly-L-lysine:heparin complex might potentially serve as a drug carrier for other macromolecular or conventional drug substances (Ryser et al 1986). Shen and Ryser (1981, 1983) demonstrated that methotrexate (MTX)-poly-L-lysine:heparin complexes were cytotoxic to Chinese hamster ovary (CHO) PRO^{-3} cells and to M5076 tumour cells, two lines that are resistant to free MTX because of defective drug transport. The MTX-poly-L-lysine:heparin complexes were transported into cells despite their lack of free positive charge and suggested a rate of transport that was different from those of either free poly-L-lysine or free heparin and which further suggested a receptor-mediated endocytotic process (Morad et al 1984). More recently, Di Stefano et al (2001) coupled 5-fluoro 2N-deoxyuridine (FUdR) to lactosaminated poly-L-lysine and demonstrated increased uptake of the conjugate by hepatic cells with subsequent release in high enough amounts so as to be pharmacologically active on neoplastic cells infiltrating the livers of mice.

Even without the advantage of heparin to neutralize the positive charge of poly-L-lysine and thereby eliminate its charge-related toxicity, it was demonstrated over 20 years ago that poly-L-lysine could enhance the cellular uptake of proteins. Ryser (1968, 1970) and Ryser & Hancock (1965) demonstrated that addition of positively-charged proteins or polyamino acids to a medium containing radiolabelled albumin enhanced the cellular penetration of albumin into sarcoma S 180 monolayers. Others used these important findings to increase the cellular penetration of various genes (Han & Yeom 2000; Schwarzenberger et al 2001; Suh et al 2001; Zhong et al 2001), viral nucleic acids (Koch et al 1966), diphtheria toxin (Moehring & Moehring 1968), and other biologically-active macromolecules into various avian and mammalian cells. This same procedure was effective in increasing infection of plant cell protoplasts with tobacco mosaic virus RNA (Takebe & Otsuki 1969). Shen & Ryser (1978) showed that conjugation of poly-L-lysine to albumin and horseradish peroxidase enhanced membrane transport of each protein into cultured L-929 fibroblasts approximately 11- and 200-fold, respectively. In the future, the delivery of biologically-active protein and peptide drugs may potentially be enhanced by attaching the drug to a poly-L-lysine:heparin complex and taking advantage of the specific cellular uptake of a macromolecular drug carrier. In addition to targeting drugs to a specific tissue, a poly-L-lysine:heparin complex may offer a platform for drug delivery with improved tissue selectivity and a heparin-mediated decrease in the charge-related toxicity that is typically associated with polycationic carriers.

Conclusion

We have demonstrated in rats that the blood and serum concentration–time profiles, rate and extent of urinary excretion, and tissue distribution 30 min after an intravenous injection of poly-L-lysine may be altered by complexing it with heparin. Complexation of heparin to poly-L-lysine could effectively increase the fraction of an administered dose of poly-L-lysine that was deposited in liver, kidney, and muscle tissue. Since the macromolecular complex was nontoxic and uncharged, it might potentially serve as a suitable carrier for both conventional and peptidic drugs to increase drug distribution to liver, kidney, or muscle tissue.

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