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SELECTIVE DELIVERY TO THE LIVER OF ANTIVIRAL NUCLEOSIDE ANALOGS COUPLED TO A HIGH MOLECULAR MASS LACTOSAMINATED POLY-L-LYSINE AND ADMINISTERED TO MICE BY INTRAMUSCULAR ROUTE

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Abstract—In order to obtain hepatotropic conjugates of antiviral drugs suitable for intramuscular administration, three nucleoside analogs (adenine arabinoside monophosphate, ribavirin and azidothymidine) were coupled to a high molecular mass lactosaminated poly-L-lysine. The conjugates had a high molar ratio drug/conjugate and after intramuscular administration to mice, were selectively taken up by the liver and eliminated by the kidney only in minute quantities. The high molar ratio and low renal elimination are important properties not possessed by conjugates previously prepared by using a small molecular mass lactosaminated poly-L-lysine. The conjugate with adenine arabinoside monophosphate (ara-AMP) was found to be devoid of acute toxicity for mice and in spite of its high molecular dimension (M_n = *ca.* 72,500) did not induce antibodies in this animal after repeated intramuscular injections. This conjugate could have two advantages over a similar complex of ara-AMP with lactosaminated human albumin currently under clinical trials for the treatment of chronic type B hepatitis which must be injected intravenously: it might provide better patient compliance since it is injectable intramuscularly and could introduce larger amounts of ara-AMP into hepatocytes due to its higher drug/carrier molar ratio.

Key words: antiviral chemotherapy; drug targeting; lactosaminated-poly-L-lysine; asialoglycoprotein receptor; adenine arabinoside monophosphate; hepatitis B

In order to reduce the neurotoxic side effects of adenine arabinoside monophosphate (ara-AMP†) in the treatment of chronic hepatitis caused by B virus (HBV) [1], the drug was coupled to lactosaminated human serum albumin (L-HSA) [2–5]. L-HSA is a neoglycoprotein which binds to Ashwell's receptor for galactosyl terminating glycoproteins and specifically enters hepatocytes where it is digested in lysosomes [6, 7]. In HBV-infected patients [8, 9], ara-AMP coupled to L-HSA inhibited hepadnavirus replication at a dose (1.5 mg/kg) three to six times lower than the free drug. In a clinical study lasting 28 days, L-HSA-ara-AMP exerted strong antiviral activity without producing either the neurotoxic side effects which usually appear 3–4 weeks after administration of the free drug [10] or other adverse reactions (unpublished results).

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† Abbreviations: ara-AMP, adenine arabinoside monophosphate; HBV, hepatitis B virus; HSA, human serum albumin; L-HSA, lactosaminated HSA; L-HSA-ara-AMP, conjugate of L-HSA with ara-AMP; AZT, 3-azido-3 deoxythymidine; AZTMP, AZT monophosphate; RIBV, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin); RIBVMP, RIBV monophosphate; Lac-poly-L-lysine, lactosaminated poly-L-lysine; M_n, number-average molecular mass; M_w, mass-average molecular mass; LD_{50}, lethal dose 50%; IgG, immunoglobulin G.

L-HSA-ara-AMP must be given intravenously otherwise it induces antibodies [11]. A complex suitable for i.m. injection would assure better patient compliance. In addition to the properties required for a liver-targeted drug–carrier complex, a hepatotropic conjugate injectable i.m. must have high solubility and a heavy drug load in order that the pharmacologically active dose can be administered in a small volume. Poly-L-lysine with galactosyl residues, which binds to Ashwell's receptors and is selectively internalized by hepatocytes [12], may be an appropriate carrier for such a conjugate since it contains a large number of amino groups to which the drug can be coupled. In previous experiments [13] we conjugated ara-AMP with a low molecular mass lactosaminated poly-L-lysine (Lac-poly-L-lysine). We used a polymer with a small mass to facilitate the coupling of the drug and reduce the risk of acute toxicity and immunogenicity. The conjugate had a high drug load (240 μg ara-AMP per mg conjugate), was very soluble in water and when administered i.m. to mice was selectively taken up by the liver. It was devoid of acute toxicity and after repeated i.m. injections did not induce antibodies. However this conjugate had two drawbacks which were a consequence of its small dimension (M_n = *ca.* 9,300): the number of drug molecules carried by a single conjugate molecule was low (approx. 6) and, after i.m. injection, the

conjugate was elmininated by the kidney in large quantities. These were important disadvantages given that liver uptake of galactosyl terminating peptides is much slower in patients with chronic hepatitis B infection than in healthy humans or in laboratory animals, probably because of a diminished number of receptor molecules [14–16]. Consequently, an even greater renal loss of the conjugate can be expected in these patients. Moreover, with the low drug/conjugate molar ratio, the amount of ara-AMP introduced into hepatocytes might be too small to exert adequate antiviral activity even when the receptor is saturated by the conjugate.

By using drastic reaction conditions we have now succeeded in coupling ara-AMP to a large number of ε-amino groups of high molecular mass Lac-poly-L-lysine. This conjugate (M_n = *ca.* 72,500) displayed the same properties (selective uptake by liver after i.m. injection, absence of acute toxicity and of immunogenicity) as the previous complex but had the advantage of having a much higher drug/conjugate molar ratio (equal to 69) and of being eliminated by the kidney only in minute quantities.

Under the same conditions, high molecular mass Lac-poly-L-lysine was conjugated with AZT and Ribavirin (RIBV), two antiviral nucleoside analogs. These conjugates, injected i.m. to mice, were also selectively taken up by the liver.

MATERIALS AND METHODS

Preparation of conjugates. Conjugates were prepared using a poly-L-lysine·HBr with a molecular mass of 28,000–42,000 Da and a polymerization degree of 135–203 (Sigma Chemical Co., St. Louis, MO, U.S.A.). As in the preparation of low molecular mass poly-L-lysine conjugates [13], drugs were coupled via the imidazolides of their phosphoric esters [17] and lactose was linked by reductive amination in the presence of $NaBH_3CN$ [18]. However, the procedure used previously was modified: the pH, temperature, length of reaction time as well as the imidazolide concentration were all increased in the drug conjugation step. Moreover, lactosamination was performed before drug coupling, since at high pH, high molecular mass poly-L-lysine would precipitate unless a part of the ε-NH_2 groups were substituted with lactose residues.

Lac-poly-L-lysine-ara-AMP. Reductive lactosamination of ε-NH_2 groups was carried out by dissolving 200 mg poly-L-lysine in 20 mL 0.4 M potassium phosphate buffer, pH 7, together with 800 mg α-lactose and 500 mg $NaBH_3CN$. After incubation at 37° for 24 hr, the pH was raised to 8 with 5 M KOH and the solution left at 37° for a further 6 hr. Lac-poly-L-lysine was diafiltered with 0.9% NaCl and concentrated to 100 mg/mL. Lactose was measured by the phenol–sulphuric acid method of Dubois *et al.* [19] using galactose as a standard; poly-L-lysine was determined by measuring the nitrogen according to Kjeldahl. Two millilitres of Lac-poly-L-lysine solution (200 mg) was diluted with 2 mL 1 M sodium carbonate buffer, pH 11. Eight hundred milligrams of ara-AMP imidazolide, synthesized according to Lohrman and Orgel [20], was dissolved and the pH re-adjusted to 11 with 5 M NaOH. After incubation at 50° for 96 hr, the conjugate was diafiltered with 0.9% NaCl. Chemical characterization of the complex was performed by assaying the coupled drug spectrophotometrically and measuring lactose as described. The interference of ara-AMP in the colorimetric analysis was subtracted. The poly-L-lysine content of conjugate was calculated from the amount of lactose, the lactose/poly-L-lysine weight ratio having been determined before drug coupling (see above). This was possible because the bond between the sugar and lysine ε-NH_2 groups did not break down during drug conjugation, as we verified experimentally. The conjugate was concentrated in saline (0.9% NaCl) to 150 mg/mL and lyophilized after freezing to approx. −80°. The concentration of conjugate was calculated without taking account of contraions. Prior to use, Lac-poly-L-lysine-ara-AMP was dissolved with water at a concentration of 150 mg/mL. It dissolved easily provided the freezing was rapid. When necessary, the conjugate was diluted with 0.9% NaCl.

Lac-[^{3}H]poly-L-lysine-ara-AMP. Prior to coupling with ara-AMP, Lac-poly-L-lysine was labelled with [^{3}H]formaldehyde (NEN, Boston, MA, U.S.A.) according to Jentoft and Dearbon [21]. The reaction mixture contained 78 μCi [^{3}H]formaldehyde/mL. After diafiltration with 0.9% NaCl Lac-[^{3}H]poly-L-lysine was conjugated with ara-AMP as described above.

Lac-[^{3}H]poly-L-lysine-RIBVMP. Ribavirin (ICN Pharmaceuticals, Irvine, CA, U.S.A.) was first phosphorylated according to Allen *et al.* [22]. The pyridinium salt of the phosphorylated derivative was then converted to the imidazolide [20], which was coupled to Lac-[^{3}H]poly-L-lysine as described for the conjugate with ara-AMP. In this conjugate coupled RIBVMP was assayed by measuring the organic phosphate according to Ames [23]. Due to the strong interference of RIBV with the colorimetric assay of sugar, we could not measure lactose as described for Lac-poly-L-lysine-ara-AMP; Lac-[^{3}H]-poly-L-lysine content of the conjugate was thus determined by counting the radioactivity.

Lac-poly-L-lysine-[^{14}C]AZTMP. 3′-Azido-3-[2-^{14}C]deoxythymidine([^{14}C]AZT) (Moravek Biochemicals, Brea, CA, U.S.A.) was phosphorylated according to Yoshikawa *et al.* [24]. The pyridinium salt of the phosphorylated derivative was converted to its imidazolide [20] and subsequently coupled to Lac-poly-L-lysine. Coupling was performed as described for Lac-poly-L-lysine-ara-AMP, but in the reaction medium the ratio of the amount of drug imidazolide to that of the polymer was 2.7 instead of 4. The chemical characterization of this conjugate was performed as described for Lac-poly-L-lysine-ara-AMP.

Determination of average molecular mass of conjugates. The average molecular mass of conjugates were determined by permeation chromatography on HPLC as previously described [13]. Columns were calibrated with aprotinin, ribonuclease A, HSA and bovine IgG. M_n and M_w [25] were determined using the GPC 745/745 B Waters Software.

Animals and conjugate administration. Female Swiss mice weighing 28–30 g and Wistar male rats

Table 1. Characteristics of conjugates

Conjugate	Lactose (μg) / Conjugate (mg)	Drug (μg) / Conjugate (mg)	% ε-NH_2 groups substituted by lactose	% ε-NH_2 groups substituted by drug	dpm / μg
Lac-poly-L-lysine-ara-AMP*	380–385	314–337	46–48	38–43	—
Lac-[^{3}H]poly-L-lysine-ara-AMP	380	337	48	44	2900
Lac-[^{3}H]poly-L-lysine-RIBVMP	382	323	48	45	2500
Lac-poly-L-lysine-[^{14}C]AZTMP	412	243	43	26	460

In each conjugate lactose, drug and poly-L-lysine content was determined as described in Materials and Methods. The percentages of substituted amino groups were calculated given that 1 mg of poly-L-lysine contains 7.8 μmol of lysine residues and that the M_r of lactose, ara-AMP, RIBVMP and AZTMP were 360, 347.2, 324.2 and 347.2, respectively.

* Several preparations of this conjugate were obtained. The range of values of bound lactose and ara-AMP in the different preparations are given.

of 200–220 g were used. Animals were injected into the back muscles of the hind legs. Conjugates dissolved in 0.9% NaCl were injected in a volume of 10 or 50 μL/animal in mice or rats, respectively, using Hamilton microsyringes. Intravenous injections were performed into a tail vein of mice and the dorsal vein of the rat penis; conjugates were inoculated in a volume of 10 μL/g and 1 μL/g in mice and rats, respectively. The doses administered are reported in the Results. The primary eye irritation study was performed on six New Zealand white male rabbits 2–3 months old, weighing 2.5–3 kg. One hundred microlitres of a solution of Lac-poly-L-lysine-ara-AMP in 0.9% NaCl (150 mg/mL), sterilized through a 0.45 μm filter, was placed in the conjunctival sac of the right eye of each animal. The lids were then gently held together for approx. 3–4 sec in order to prevent loss of the solution. The left eye served as a control. Eyes were observed 1, 2, 24, 48 and 72 hr after conjugate instillation.

Stability of the bond between drug and Lac-poly-L-lysine in blood. Lac-poly-L-lysine-ara-AMP and Lac-poly-L-lysine-[^{14}C]AZTMP were incubated (100 μg/mL) at 37° in sodium phosphate buffer 0.15 M, pH 7.3 or in fresh heparinized human or mouse blood. At different time intervals (0, 1, 2, 3 and 6 hr) blood cells were removed by centrifugation and 200 μL of plasma or buffer was gel filtered through a PD 10 column (Pharmacia Uppsala, Sweden), equilibrated with 0.45 M NaCl. Conjugated drugs were measured in the non-retarded fraction: ara-AMP by the HPLC method of Fiume *et al.* [26] and [^{14}C]AZTMP by counting the radioactivity.

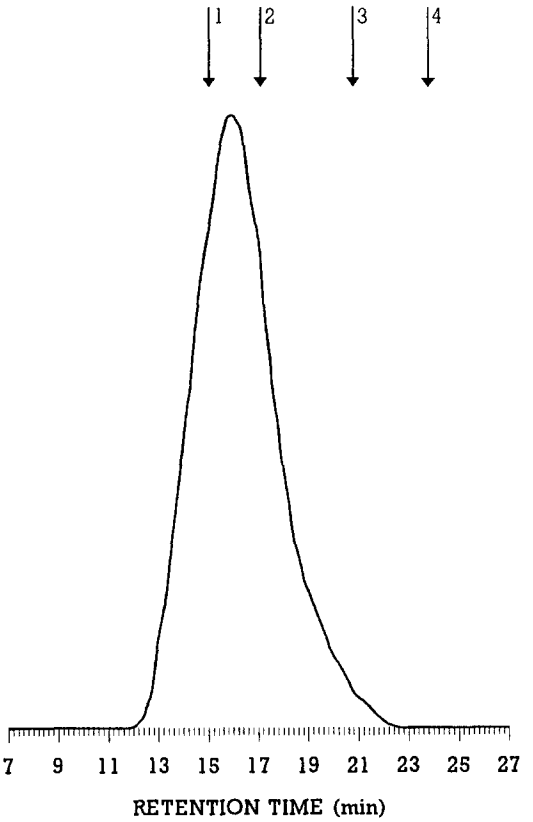

Fig. 1. Gel permeation chromatography of Lac-poly-L-lysine-ara-AMP. Arrows 1, 2, 3 and 4 indicate the retention times of bovine IgG (M_r 158,000), HSA (M_r 69,000), RNAse A (M_r 13,700) and aprotinin (M_r 6,500), respectively.

RESULTS

Chemical characteristics of conjugates are reported in Table 1. Lac-poly-L-lysine-ara-AMP had a drug/conjugate weight ratio of 0.33. The complex dissolved easily in saline (0.9% NaCl) at 150 mg/mL. Assuming that ara-AMP, when conjugated to Lac-poly-L-lysine, is active in HBV-infected patients at the same daily dose as when conjugated to L-HSA (1.5 mg/kg) [8, 9] the amount of Lac-poly-L-lysine-ara-AMP required for a 70 kg patient would be approx. 2 mL.

Figure 1 shows the gel permeation chromatography of Lac-poly-L-lysine-ara-AMP. The conjugate had an M_n = *ca.* 72,500 and an M_w = *ca.* 140,000. These values, 30 and 47 times higher than those of the previous low molecular mass Lac-poly-L-lysine-ara-AMP complex [13], indicate that the majority of

Table 2. Percentages of drug released from lac-poly-L-lysine conjugates incubated at 37° in buffer or in human or mouse blood

Time (hr)	Lac-poly-L-lysine-ara-AMP			Lac-poly-L-lysine-[^{14}C]AZTMP		
	Buffer*	Human blood	Mouse blood	Buffer	Human blood	Mouse blood
1	—	7	11	—	0	1
2	—	8	23	—	0	1
3	3	6	28	1	0	1
6	2	4	55	1	1	2

Data were obtained as described in Materials and Methods.
* 0.15 M sodium phosphate, pH 7.3.

conjugate molecules had dimensions which hindered the passage through the renal glomeruli [27]. The drug/conjugate molar ratio calculated using the M_n was 69. It was 10 and 6–7 times higher than those of low molecular mass Lac-poly-L-lysine-ara-AMP [13] and L-HSA-ara-AMP [8], respectively.

Table 2 shows the percentages of drug released from Lac-poly-L-lysine-ara-AMP and Lac-poly-L-lysine-[^{14}C]AZTMP when the conjugates were incubated at 37° for 1–6 hr in buffer and in mouse or human whole blood. The bond between the drugs and the carrier was stable in buffer. A small release of ara-AMP occurred in human blood but was not time dependent. On the contrary a progressive splitting of the bond occurred in mouse blood and more than 50% of ara-AMP was released after 6 hr. [^{14}C]AZTMP was not released from Lac-poly-L-lysine in blood of either humans or mice. The observation that the linkage between ara-AMP and Lac-poly-L-lysine was stable in buffer and human blood suggests that its cleavage in mouse blood was due to an enzymatic activity. The stability of the drug–carrier bond in human blood is an important requisite in view of a possible clinical use of these conjugates.

Organ distribution of radioactivity after administration of labelled conjugates and free [^{14}C]AZTMP is shown in Fig. 2. Conjugates of ara-AMP and RIBVMP were radioactive in the carrier whereas the conjugate of AZTMP was labelled in the drug moiety. An ara-AMP conjugate labelled in the drug could not be prepared because the radioactive ara-AMP obtained from Amersham International (Amersham, U.K.) was labelled with tritium in the adenine ring and a marked loss of this isotope occurred during the conversion of ara-[2, 8 ^{3}H]AMP to its imidazolide [13].

After i.m. administration of the conjugates labelled in the carrier (Fig. 2A, C), radioactivity was high in liver and low in spleen, intestine and kidney. The percentages of injected dpm recovered in kidneys were 10–20 times lower than those measured after i.m. administration of the complexes prepared with low molecular mass poly-L-lysine [13]. Since renal accumulation of proteins is a consequence of their glomerular filtration [27], the present result indicates that, as expected, only small amounts of the high molecular mass conjugates passed through the glomeruli, at least after i.m. administration (see below).

In mice i.m. injected with Lac-poly-L-lysine-[^{14}C]AZTMP, organ radioactivity was due to the drug as well as to its metabolites. In liver radioactivity was from 2.5 to 6 times higher than in kidneys, spleen and intestine (Fig. 2D). The difference between the amount of radioactivity in liver and in other organs was less marked than that in animals administered with the conjugates labelled in the carrier (Fig. 2A, C). Since the bond between [^{14}C]-AZTMP and Lac-poly-L-lysine is stable in mouse blood (Table 2), this result was probably due to a partial release of the drug (and/or its metabolites) from liver cells into the bloodstream after the intracellular cleavage of the drug–carrier bond. A similar release of the drug from hepatic cells in the bloodstream was observed after administration of other drug/carrier hepatotropic conjugates [28, 29].

When free [^{14}C]AZTMP was injected i.m. into mice the radioactivity was equally distributed in liver, spleen and intestine, with higher values in kidneys (Fig. 2E). The rate of accumulation and decline of radioactivity after administration of free or coupled [^{14}C]AZTMP was different. After injection of the free drug, radioactivity accumulated in tissues within the first 15 min and then rapidly declined; after injection of the conjugated drug radioactivity in liver increased up to 4–5 hr. At 1–2 hr, the amounts of radioactivity in liver were higher in animals injected with conjugated [^{14}C]AZTMP at the dose of 2 μg/g than in those administered the free drug at 5 μg/g.

In mice injected i.v. with Lac-[^{3}H]poly-L-lysine-ara-AMP (Fig. 2B) the conjugate rapidly accumulated in liver. In these animals the values of radioactivity in kidneys were higher than in those which received the same conjugate i.m. (Fig. 2A). This can be explained as follows: (i) approx. 30% of conjugate molecules had a molecular mass lower than that of HSA; (ii) a direct relationship exists between plasma concentration and glomerular filtration of small proteins [27]; (iii) the concentrations of conjugate in plasma which were constantly low (less than 0.9 μg/mL) following i.m. administration reached high levels after intravenous injection (104 μg/mL at 3 min).

Tolerability and immunogenicity experiments

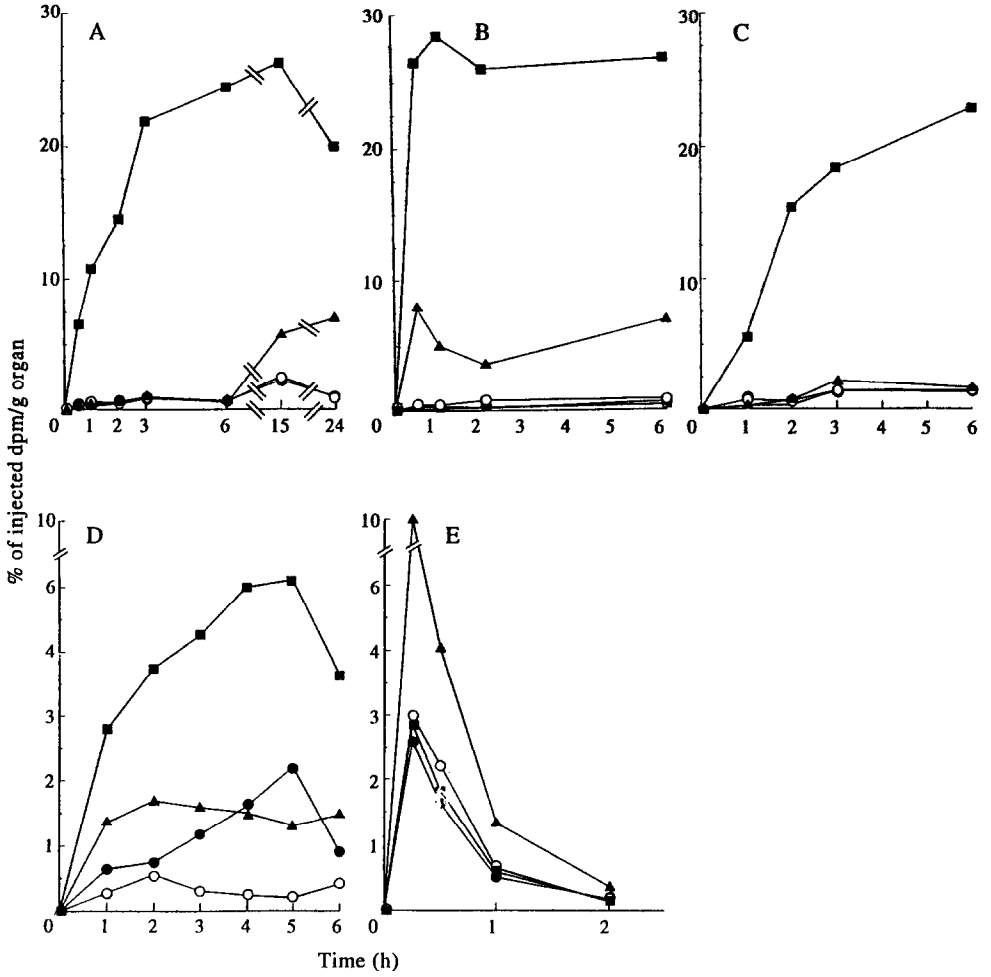

Fig. 2. Distribution of radioactivity in liver (■), kidney (▲), spleen (●) and intestine (○) of mice injected i.m. with: (A) Lac-[^{3}H]poly-L-lysine-ara-AMP (5.9 μg/g corresponding to 2 μg/g of ara-AMP); (B) Lac-[^{3}H]poly-L-lysine-ara-AMP (5.9 μg/g); (C) Lac-[^{3}H]poly-L-lysine-RIBVMP (6.2 μg/g corresponding to 2 μg/g RIBVMP); (D) Lac-poly-L-lysine-[^{14}C]AZTMP (8.2 μg/g corresponding to 2 μg/g of [^{14}C]AZTMP); (E) [^{14}C]AZTMP (5 μg/g). All compounds were injected i.m. except Lac-[^{3}H]poly-L-lysine-ara-AMP, which, in experiment B, was administered i.v. Free and conjugated [^{14}C]-AZTMP had the same specific activity (2100 dpm/μg). The experimental procedure followed was as described in [13]. Since the conjugates were not precipitated by either trichloroacetic or perchloric acid, only total (acid soluble + insoluble) radioactivity was measured. Each entry represents the mean value of results from two to four animals. SE ranged from 1 to 5% of mean values.

were performed using Lac-poly-L-lysine-ara-AMP. Administration of a single bolus intravenous injection of 1.5 g of conjugate per kg (the highest dose tested) in five mice resulted in no mortality; 1.5 g of Lac-poly-L-lysine-ara-AMP contained 480 mg of drug (see Table 1), a dose 300 times higher than that at which ara-AMP, when conjugated to L-HSA, inhibits virus growth in HBV-infected patients [8, 9]. In mice the LD_{50} of poly-L-lysine used for preparing the conjugate, given i.v. as salt of HCl, was between 15 and 30 mg/kg. To study whether Lac-poly-L-lysine-ara-AMP dissolved in saline at a concentration of 150 mg/mL damages tissues at the site of administration, a primary eye irritation experiment was performed by placing 0.1 mL of the solution in the conjunctival sac of six rabbits (see Materials and Methods). No eye changes were observed in any of the animals.

Semithin sections of liver from mice and rats which had received Lac-poly-L-lysine-ara-AMP administered with different schedules (see Table 3) were observed under light microscope. No changes were found in either parenchymal or sinusoidal liver cells in any of the animals. Accumulation into secondary lysosomes of incompletely digested molecules (disaccharides and peptides) which cannot cross lysosomal membrane resulted in a rapid swelling of these organelles which under a light microscope appeared as cytoplasmic vacuoles [30, 31]. Such vacuoles were observed in hepatic

Table 3. Schedules of administration of Lac-poly-L-lysine-ara-AMP to mice and rats for the microscopic study of liver cells

Animal	Daily dose (μg/g)	Route of injection	Days of administration
Mice	6	i.m.	20
	30	i.v.	1
	60	i.v.	1
Rats	6	i.m.	7
	30	i.m.	7
	60	i.v.	1

Animals were killed 24 hr after the last injection. Liver samples were fixed and semithin sections were stained as described in [32].

cells of mice and rats 24 hr after a single administration of L-HSA-ara-AMP at doses 5–10 times higher than that active in HBV-infected patients [32]. The absence of vacuoles in liver cells of mice and rats after administration of high doses of Lac-poly-L-lysine-ara-AMP gave indirect evidence of a rapid digestion of this conjugate into products able to cross the lysosomal membrane.

To study the immunogenicity of Lac-poly-L-lysine-ara-AMP, 24 mice received the conjugate 5 days per week for four consecutive weeks (single daily dose = 200 μg/animal). Twelve mice were injected i.m. while the others received i.v. administration. A week after the final injection, the mice were bled and antibodies against the conjugate measured as described in [13]. None of the animals produced antibodies in amounts detectable by our assay (sensitivity approx. 0.5 μg IgG/mL serum).

DISCUSSION

We prepared hepatotropic conjugates of antiviral nucleoside analogs, injectable i.m., by using a high molecular mass Lac-poly-L-lysine as carrier. These conjugates had two advantages over similar complexes made previously by using a small molecular mass Lac-poly-L-lysine [13]; they had a 10-fold greater drug/conjugate molar ratio and were lost through the kidney only in minute quantities. The higher molar ratio should assure penetration of greater amounts of drug into liver cells when the Ashwell's receptor is saturated by the conjugate.

The conjugate with ara-AMP was found to be highly soluble, which would allow the dose of ara-AMP active in HBV-infected patients to be injected in a small volume, compatible with the i.m. route. This conjugate administered i.m. to woodchuck carriers of woodchuck hepatitis virus at the daily dose of 4.2 mg/kg (equal to 1.5 mg/kg ara-AMP) strongly inhibited virus replication, indicating that the drug was released in liver in a pharmacologically active form [33]. Lac-poly-L-lysine-ara-AMP was devoid of acute toxicity for mice and repeated administration by i.m. injection did not induce antibodies in this animal. The loss in toxicity of poly-L-lysine obtained by coupling to lactose and ara-AMP required a heavy substitution of ε-amino groups. In fact a complex prepared in previous experiments using a poly-L-lysine of a similar mass in which less than 50% of ε-NH_2 groups were substituted by galactose and ara-AMP maintained the high toxicity of the homopolymer [12]. The finding that this conjugate did not induce antibodies in spite of its high molecular dimension was in agreement with the results of Levine [34]. This author conjugated a poly-L-lysine constituted by more than 500 lysine residues with benzylpenicillin; the lightly coupled conjugates were immunogenic while heavily conjugated polymers did not induce antibodies even if administered in complete Freund's adjuvant. Exhaustive succinylation of lightly coupled conjugates converted them to non-antigens.

In conclusion, the Lac-poly-L-lysine-ara-AMP complex fulfilled the criteria required for a hepatotropic drug–carrier conjugate injectable i.m. In addition to being injectable i.m., it could have another advantage over the L-HSA-ara-AMP conjugate currently under clinical trials for the treatment of chronic type B hepatitis. Small amounts of L-HSA-ara-AMP are still present in the bloodstream of patients 24 hr after administration of the minimally effective daily dose of this conjugate [9]. Under these conditions, a higher concentration of ara-AMP within hepatocytes cannot be reached by increasing the dosage of L-HSA-ara-AMP; it could be obtained, however, by administering a conjugate with a higher drug/conjugate molar ratio such as the Lac-poly-L-lysine-ara-AMP complex, which has a molar ratio 6–7 times higher than that of L-HSA-ara-AMP.

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