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A CONJUGATE OF LACTOSAMINATED POLY-L-LYSINE WITH ADENINE ARABINOSIDE MONOPHOSPHATE, ADMINISTERED TO MICE BY INTRAMUSCULAR ROUTE, ACCOMPLISHES A SELECTIVE DELIVERY OF THE DRUG TO THE LIVER

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Abstract—A conjugate of the antiviral agent adenine arabinoside monophosphate (ara-AMP) with a low molecular mass lactosaminated poly-L-lysine, administered to mice by i.m. route, selectively delivers the drug to the liver. In mice the conjugate is devoid of acute toxicity even at high dose (1.3 mg/g) and injected i.m. for 20 days does not induce antibodies. Moreover it is highly soluble in water; this means that a pharmacologically active dose may be administered in a small volume compatible with the i.m. route. Compared to the similar ara-AMP complex with lactosaminated albumin which must be injected intravenously, the present conjugate might assure a better compliance of patients with hepatitis B virus infection for a long lasting, liver targeted antiviral treatment.

The aim of our laboratory is to increase the chemotherapeutic index of the antiviral drugs used in treatment of chronic hepatitis B. Our approach has been to conjugate the drugs to asialofetuin [1, 2], L-SA† [3, 4] or galactosylated poly-L-lysine [5] in order to deliver them selectively to hepatocytes via Ashwell's receptor which recognises galactosyl terminating glycopeptides [6].

Experiments in mice [1–6] and rats [7] have demonstrated a selective entry of the conjugates into liver cells where the drugs are then released. Preliminary clinical studies in patients with chronic HBV infection [8, 9] showed that the antiviral agent ara-AMP conjugated with L-SA inhibited virus growth when administered at a daily dose three to six times lower than that of the free drug.

L-SA conjugates have to be given intravenously because by other routes they induce antibody production even if prepared with homologous albumin [10]. A hepatotropic complex injectable by i.m. route would improve the compliance of patients in long lasting antiviral treatments.

We report here experiments in which we substituted practically all the ε-amino groups of a low molecular mass poly-L-lysine (average number of lysine residues = 19) with galactosyl residues and with ara-AMP. We found that this conjugate, administered i.m. to mice, selectively delivered the drug to the liver and did not induce antibodies. In contrast to the galactosylated poly-L-lysine conjugate previously used [5], which was prepared with a high molecular mass homopolymer and had less than 50% of ε-amino groups substituted, the new complex did not display any recognisable sign of acute toxicity even at high doses.

We also conjugated ACV (another antiviral agent) with low molecular mass galactosylated poly-L-lysine. Like the conjugate with ara-AMP, this complex, injected i.m. to mice, selectively delivered the drug to the liver.

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† Abbreviations: ara-AMP, adenine arabinoside monophosphate; L-SA, lactosaminated serum albumin; HBV, hepatitis B virus; ACV, acyclovir; ACVMP, ACV monophosphate; AUFS, absorbance units (per) full scale (deflection); ara-AMPIm, ara-AMP imidazolide; HSA, human serum albumin.

MATERIALS AND METHODS

Preparation of galactosylated poly-L-lysine and its drug conjugates

Poly-L-lysine · HBr with a molecular mass ranging from 1000 to 4000 Da (Sigma Chemical Co., St Louis, MO, U.S.A.) was gel chromatographed on a Bio-Gel P-2 eluted with NH_4HCO_3 0.2 M in order to remove the small polymers. Polymers eluted in the void volume were lyophilized and used in the preparation of all the compounds employed in these experiments (Table 1).

In all the conjugates the galactosyl residues were bound to ε-amino groups via reductive lactosamination in the presence of cyanoborohydride [11].

[^{3}H]*Poly-L-lysine (compound 1)*. Labelling of poly-L-lysine was performed by using [^{3}H]formaldehyde (100 mCi/mmol) (NEN, Boston, MA, U.S.A.) according to the method of Jentoft and Dearborn [12]. The reaction mixture contained 44 μCi [^{3}H]-formaldehyde per mL. [^{3}H]Poly-L-lysine was isolated from the reaction mixture by gel filtration on a Bio-Gel P-2 column eluted with NH_4HCO_3 0.2 M and was subsequently lyophilized.

[^{14}C]*Lac-poly-L-lysine (compound 2)*. As reported above, lactose was coupled to poly-L-lysine by reductive amination with $NaBH_3CN$ [11]. To 20 mg of polymer dissolved in 2 mL of boric acid/borax buffer 0.1 M pH 8.5, 80 mg α-lactose (containing 50 μCi [D-glucose-1-^{14}C]lactose; Amersham International, Amersham, U.K.) and 50 mg $NaBH_3CN$ were added. The mixture was incubated at 37° for 48 hr. [^{14}C]Lac-poly-L-lysine was isolated by gel filtration on Bio-Gel P2 as described for compound 1. Lactose content was determined by the phenol/sulphuric acid method of Dubois *et al.* [13] using a galactose standard and was related to the dry weight of the compound.

[^{14}C]*Lac-poly-L-lysine–ara-AMP (compound 3)*. ara-AMP was coupled via its imidazole (ara-AMPIm) [14]. This procedure is more efficient than that employing carbodiimides and avoids the unwanted side reactions caused by these reagents. The imidazole of ara-AMP was synthesized according to Lohrmann and Orgel [15]. To 50 mg poly-L-lysine dissolved in 1 mL $NaHCO_3/Na_2CO_3$ buffer 0.1 M pH 9.5, 75 mg of ara-AMPIm was added and the pH was adjusted to 9.5. The mixture was incubated at 37° for 48 hr. Conjugate was isolated from reaction mixture as described for compound 1. Drug content was determined spectrophotometrically and was related to the dry weight of the conjugate. Afterwards, the conjugate was lactosaminated using radioactive lactose as described for compound 2.

Lac-poly-L-lysine–ara-AMP (compound 4). It was prepared as described for compound 3, but in much larger amounts and using non radioactive lactose.

Lac-[^{3}H]*poly-L-lysine–ara-AMP (compound 5)*. This conjugate was prepared in order to obtain a radioactive antigen with a high specific activity, for the detection of antibodies. A conjugate poly-L-lysine–ara-AMP was prepared as described for compound 3 and was subsequently labelled with [^{3}H]formaldehyde (100 mCi/mmol) according to Jentoft and Dearborn [12]. The reaction mixture contained 2800 μCi [^{3}H]formaldehyde per mL. The labelled conjugate was then lactosaminated as described above.

Lac-poly-L-lysine–ara-[^{3}H]*AMP (compound 6)*. In order to prepare this complex, radioactive in the drug moiety, 1-ethyl-3-(dimethyl aminopropyl) carbodiimide was used as coupling agent. Conjugation was not performed via ara-AMPIm because a marked loss of tritium occurred during the conversion of the labelled drug to its imidazolide. Poly-L-lysine was first lactosaminated as described above (compound 2), but the reaction was performed in boric acid/borax buffer 0.1 M pH 8 and was prolonged for 24 hr only, so that about 2/3 of the lysine residues were substituted by lactose. Coupling of ara-[^{3}H-adenine]AMP (22 μCi/mg) (Amersham) was carried out according to the method described previously [5]. In this conjugate lactosamination was performed first in order to reduce the number of free NH_2 groups during the coupling reaction via carbodiimide, thus decreasing the possibility of poly-L-lysine condensation.

Lac-poly-L-lysine–[^{3}H]*ACVMP (compound 7)*. Tritiated [^{3}H]ACVMP (3.4 μCi/mg) was obtained by phosphorylation [16] of the primary OH group of [^{3}H]ACV (labelled in position 2 of the lateral chain) (NEN). Conjugate was prepared as described for compound 3, but the time of incubation of poly-L-lysine with [^{3}H]ACVMP imidazole was reduced to 18 hr only.

Determination of average molecular masses of conjugates

They were determined by permeation chromatography using HPLC equipment (Waters) with two Protein-Pak columns (125 and 300 SW) connected in series. Conjugate (80 μg) (compounds 4 or 6) (see Table 1) was dissolved in 20 μL of the mobile phase (125 mM Na_2SO_4 + 2 mM $NaH_2PO_4 \cdot H_2O$, to pH 6.0 with 0.1 N NaOH, filtered and degassed) and chromatographed with the following conditions. Flow rate: 0.9 mL/min; detection: UV at 260 nm, 0.1 absorbance unit full scale (AUFS). Columns were calibrated with aprotinin (M_r 6500); ribonuclease A (RNAse A) (M_r 13,700) and HSA (M_r 69,000). Mass-average molecular mass and number-average molecular mass were determined using the GPC 745/745 B Waters Software.

Animals and i.m. injections

Female Swiss mice were obtained from Nossan Laboratories (Correzzana Milano, Italy) and maintained under standard environmental conditions. They were given a commercial diet and tap water *ad libitum*. Animals weighing 28–30 g were used in the experiments.

Intramuscular injections were performed into the back muscles of the hind legs. Compounds, dissolved in saline (NaCl 0.9%), were injected in a volume of 10 μL/animal using a 25 μL Hamilton microsyringe.

Stability of the bonds between ara-AMP and Lac-poly-L-lysine in mouse plasma

Lac-poly-L-lysine–ara-AMP (compound 4) was incubated in fresh heparinized mouse plasma (20 μg/mL) at 37°. At different intervals (0, 1 and 4 hr) conjugated ara-AMP was measured by a procedure [17] which involves the splitting of the phosphoamide bonds [18] between the drug and the carrier in an acidic medium; the released drug was measured by using the HPLC method of McCann *et al.* [19].

Organ distribution of the conjugates

At different times after i.m. injection of the radioactive conjugates (see Fig. 2) mice were bled from the retro-orbital plexus under ether anaesthesia, and liver, spleen, kidneys, a tract of intestine (8 cm long starting from pylorus) and, in some experiments, brain were rapidly removed and homogenized in 4 vol. (w/v) of water. The total radioactivity of plasma and homogenates was measured. The radioactive contribution given by the plasma trapped in the organs was calculated [20] and subtracted.

Digestion of [^{14}C]*Lac-poly-L-lysine–ara-AMP in liver*

Three or six hours after i.m. injection of the conjugate (24 μg/g), four mice (two animals for each time) were killed and livers were pooled and homogenized with 4 vol. of cold water; 5 vol. of cold 1.2 M perchloric acid were added and after centrifugation, the supernatants were neutralized

Table 1. Characteristics of poly-L-lysine conjugates

Compounds	Lactose (μg) Compound (mg)	Drug (μg) Compound (mg)	% ε-NH_2, groups substituted by Lactose	Drug	dpm μg
1 [^{3}H]poly-L-lysine	0	0	0	0	1200
2 [^{14}C]Lac-poly-L-lysine	721	0	92	0	860
3 [^{14}C]Lac-poly-L-lysine–ara-AMP	540	206	72	28	500
4 Lac-poly-L-lysine–ara-AMP	493	240	66	33	0
5 Lac-[^{3}H]poly-L-lysine–ara-AMP	573	147	72	20	36,000
6 Lac-poly-L-lysine–ara-[^{3}H]AMP	613	176	73	22	7500
7 Lac-poly-L-lysine–[^{3}H]ACVMP	638	81	80	12	440

In each compound lactose and drug content were determined as described in Materials and Methods and were related to 1 mg of conjugate. Lactose and drug share was subtracted from the dry weight of the conjugates to obtain their poly-L-lysine content. The percentage of substituted amino groups was then determined considering that 1 mg of poly-L-lysine contains 7.8 μ equivalent ε-amino groups and that the M_r of lactose, ara-AMP and ACVMP were 360, 347.2 and 305.1, respectively.

with KOH. After 30 min in the cold, $KClO_4$ was removed by centrifugation and supernatants were lyophilized. The dry material was redissolved with 2 mL H_2O; the solution was clarified by centrifugation and 1 mL was chromatographed on a 1.6 × 92 cm Bio-Gel P-2 column equilibrated and eluted with NH_4HCO_3 0.2 M.

As a control, 140 μg of conjugate was added to 10 mL of liver homogenate obtained from two untreated mice; this sample was then processed in the same way.

Immunogenicity of Lac-poly-L-lysine–ara-AMP

Twelve mice received the conjugate (compound No 4) i.m. for 5 days a week for 4 consecutive weeks (single daily dose = 700 μg/animal). One week after the last injection the animals were bled from retro-orbital plexus under ether anaesthesia. Antibodies were measured in 50 μL of serum, in triplicate, with the ammonium sulphate method according to Minden and Farr [21]. The conjugate No. 5 (see Table 1) was used as antigen.

RESULTS

Physico-chemical characteristics of conjugates

The lactose and ara-AMP (or ACVMP) content of the different conjugates as well as the percentages of the ε-NH_2 groups of poly-L-lysine substituted by the sugar and by the drug molecules are reported in Table 1.

Figure 1 shows the gel permeation chromatography of Lac-poly-L-lysine–ara-AMP (compound 4). The conjugate had a mass-average molecular mass of 11,183 and a number-average molecular mass of 9286 with a polydispersity index of 1.2. Since lactose and ara-AMP contributed to the mass of this conjugate by 49.3 and 24%, respectively, it can be calculated that in this complex the poly-L-lysine had a number-average molecular mass of 2479 (corresponding to 19 lysine residues) and a mass-average molecular mass of 2986 (corresponding to 23 lysine residues).

In this conjugate only one to two free amino groups per conjugate molecule were detected using the trinitro benzene-sulphonic acid method [22]. This fits with the calculations reported in Table 1 according to which in this complex, as well as in the other conjugates, practically all the ε-amino groups

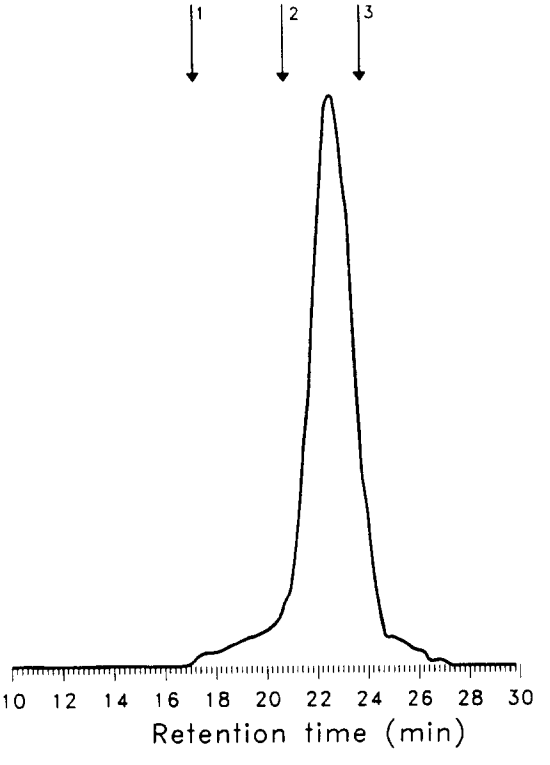

Fig. 1. Gel permeation chromatography of Lac-poly-L-lysine–ara-AMP conjugate using two Protein-Pak columns (125 and 300 SW) (Waters) connected in series. (For conditions of chromatography see Materials and Methods.) The arrows 1, 2 and 3 indicate the retention times of HSA, RNAse A and aprotinin, respectively.

Table 2. Radioactivity in plasma (% of injected dpm/mL) of mice administered with poly-L-lysine, its conjugates and free drugs

Exp. No. Compounds	Time (hr) 0.2	0.5	1	2	3	4
1 [^{3}H]poly-L-lysine			2.0	1.2	1.2	1.0
2 [^{14}C]Lac-poly-L-lysine			0.8	0.3	0.2	0.1
3 [^{14}C]Lac-poly-L-lysine		2.4	0.6	0.1	0	
4 ara-[^{3}H]AMP			3.2	2.7	3.1	2.6
5 [^{14}C]Lac-poly-L-lysine–ara-AMP			1.4	0.4	0.2	0.2
6 Lac-poly-L-lysine–ara-[^{3}H]AMP			1.0	0.2	0.2	0.1
7 [^{3}H]ACVMP	3.0	0.5	0.3	0.2		
8 Lac-poly-L-lysine–[^{3}H]ACVMP		3.8	1.8	0.6	0.2	

The compounds were injected i.m. except in experiment No. 3 in which [^{14}C]Lac-poly-L-lysine was administered i.v. The plasma was obtained from mice used in the experiments reported in Fig. 2.

of poly-L-lysine were substituted by lactose and by the drug.

Lac-poly-L-lysine–ara-[^{3}H]AMP (compound 6) had a mass-average molecular mass of 12,827 and a number-average molecular mass of 11,929 with a polydispersity index of 1.07. The slightly higher average molecular masses of this complex were probably due to a partial oligomerization of poly-L-lysine molecules caused by carbodiimide during the preparation of this conjugate (see Materials and Methods).

Lac-poly-L-lysine–ara-AMP (compound 4) demonstrated a good solubility since it dissolved easily in saline (NaCl 0.9%) at 400 mg/mL.

Stability of the bonds between ara-AMP and Lac-poly-L-lysine in mouse plasma

When Lac-poly-L-lysine–ara-AMP (compound 4) was incubated in mouse plasma only 3 and 16% of the coupled drug was released from the carrier after 1 and 4 hr, respectively.

Organ distribution of the conjugates

Table 2 shows the levels of radioactivity in mouse plasma after administration of poly-L-lysine, its conjugates and free drugs.

Organ distribution of these compounds is reported in Fig. 2. After i.m. injection of unconjugated drugs and non-lactosaminated poly-L-lysine the amounts of radioactivity in liver, spleen, intestine and brain were similar (Fig. 2A, D and G). On the contrary administration of [^{14}C]Lac-poly-L-lysine, given either i.m. or i.v. resulted in its accumulation in liver (Fig. 2B and C). A similar result was obtained after i.m. injection of Lac-poly-L-lysine conjugates of ara-AMP or ACVMP, labelled either in lactose or in the drug (Fig. 2E, F and H). In mice administered with [^{14}C]Lac-poly-L-lysine–ara-AMP the values of radioactivity in liver were rising during the first 4 hr. On the contrary, after administration of Lac-poly-L-lysine–ara-[^{3}H]AMP, radioactivity in liver reached a steady level after the first hour. Given the good stability in mouse plasma of the bonds between ara-AMP and lysine ε-NH_2 groups, the lower values of radioactivity observed in the liver of mice treated with Lac-poly-L-lysine–ara-[^{3}H]AMP were probably due to a partial release of ara-[^{3}H]AMP (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 was observed in rats injected with L-SA–ara-AMP conjugate [23].

In spite of liver targeting of conjugated ara-[^{3}H]-AMP, comparable percentages of the injected radioactivity were found in liver of mice after administration of the same dose (5 μg/g) of either free or coupled drug (Fig. 2D and F). This result can be explained by the large amounts of the Lac-poly-L-lysine complexes which were eliminated by the kidney (see below). The dose of ara-AMP (5 μg/g) used in these experiments was that at which the free drug inhibited virus DNA synthesis in liver of Ectromelia virus infected mice [4] and virus growth in patients with chronic type B hepatitis [8, 9].

One hour after administration of the conjugates, the levels of radioactivity in kidney were similar (for [^{14}C]Lac-poly-L-lysine–ara-AMP) or 1.5–2 times higher (for the conjugates labelled in the drug moiety) than those measured in liver. After 3–4 hr the values of radioactivity in kidney were comparable to those of liver (compare the data of Figs 2 and 3). The high amounts of radioactivity detected in kidney are explained by the finding that several low molecular mass peptides easily filter through the glomeruli and are then endocytosed by the proximal tubular cells of kidney [24]. Penetration in these cells should not decrease the chemotherapeutic index of Lac-poly-L-lysine conjugates of antiviral agents since these drugs are not particularly toxic for renal cells.

Digestion of [^{14}C]Lac-poly-L-lysine–ara-AMP in liver

In previous experiments it was found that in liver cells the bonds between ara-AMP and the ε-amino groups of galactosylated poly-L-lysine are rapidly broken down with release of the drug in a pharmacologically active form [5, 18].

To ascertain whether the poly-L-lysine backbone

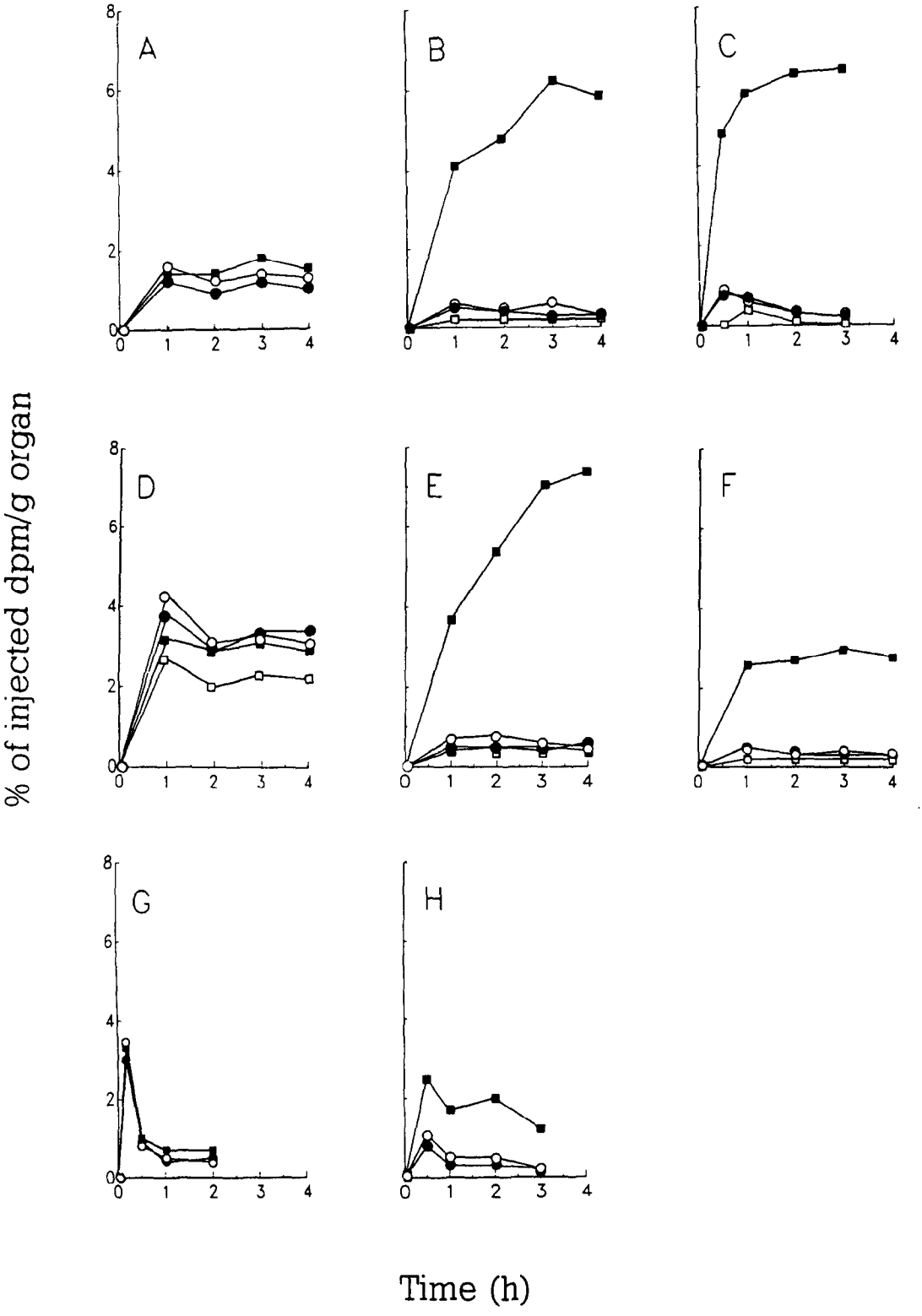

Fig. 2. Distribution of radioactivity in liver (■), spleen (●), intestine (○) and brain (□) of mice injected i.m. with: (A) [^{3}H]poly-L-lysine (24 μg/g), (B) [^{14}C]Lac-poly-L-lysine (24 μg/g), (C) [^{14}C]Lac-poly-L-lysine (24 μg/g i.v.), (D) ara-[^{3}H]AMP (5 μg/g), (E) [^{14}C]Lac-poly-L-lysine–ara-AMP (24 μg/g), (F) Lac-poly-L-lysine–ara-[^{3}H]AMP (28 μg/g corresponding to 5 μg/g of ara-[^{3}H]AMP), (G) [^{3}H]ACVMP (4 μg/g), (H) Lac-poly-L-lysine–[^{3}H]ACVMP (50 μg/g corresponding to 4 μg/g of [^{3}H]ACVMP). All the compounds were injected i.m. except [^{14}C]Lac-poly-L-lysine which in the experiments of frame C was administered intravenously in a volume of 0.1 mL/animal. Each entry represents the mean value of results from two to three animals. SE ranged from 0.1 to 2% of mean values.

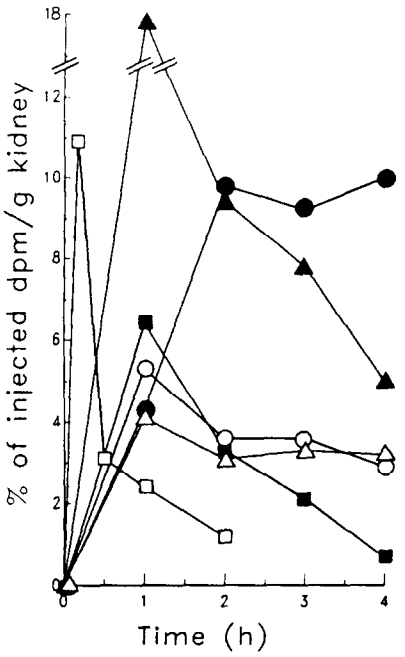

Fig. 3. Radioactivity in kidney of mice injected i.m. with [^{3}H]poly-L-lysine (24 μg/g) (▲), ara-[^{3}H]AMP (5 μg/g) (△), [^{14}C]Lac-poly-L-lysine–ara-AMP (24 μg/g) (●), Lac-poly-L-lysine–ara[^{3}H]AMP (28 μg/g corresponding to 5 μg/g of ara-[^{3}H]AMP) (○), [^{3}H]ACVMP (4 μg/g) (□), Lac-poly-L-lysine–ara-[^{3}H]ACVMP (50 μg/g corresponding to 4 μg/g of [^{3}H]ACVMP) (■). The kidneys were obtained from mice used in experiments reported in Fig. 2.

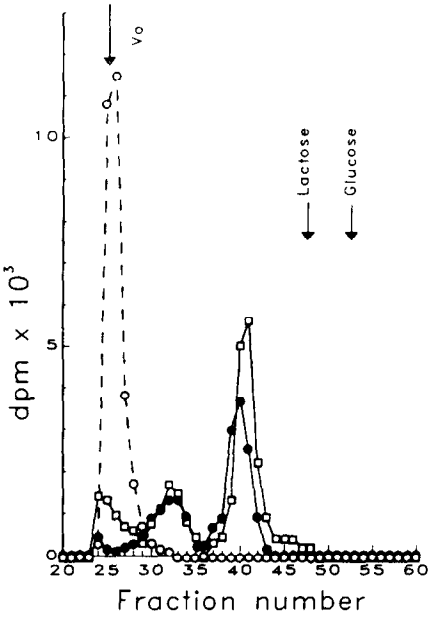

Fig. 4. Bio-Gel P-2 chromatography of acid-soluble materials of liver from mice injected i.m. with [^{14}C]Lac-poly-L-lysine–ara-AMP. Animals were killed 3 (□) or 6 (●) hr after administration of the conjugate. The symbol (○) corresponds to the acid-soluble materials of a liver homogenate from two untreated mice to which the conjugate was added (14 μg/mL) immediately before the precipitation with perchloric acid (see Materials and Methods).

of the conjugates is also cleaved in hepatic cells, the acid-soluble materials from liver of mice treated i.m. with [^{14}C]Lac-poly-L-lysine–ara-AMP were chromatographed on a Bio-Gel P-2 column (exclusion limit = 1800 Da). The chromatographic profiles (Fig. 4) show radioactive fragments retained in the gel but with a molecular mass higher than that of free lactose, indicating that the heavy substitution of the ε-amino groups did not hinder the cleavage of poly-L-lysine.

Immunogenicity of Lac-poly-L-lysine–ara-AMP

When 50 μL of serum from five untreated mice was tested according to Minden and Farr [21] (see Materials and Methods) the dpm precipitated was 78 ± 11 (mean value ± SE). In the presence of 10 μL of a rat antiserum, obtained in previous experiments [25], which recognises both ara-A (938 pmol ara-A bound by 1 mL serum) and ara-AMP conjugated with lactosaminated albumin, the precipitated dpm was 1599 ± 21. In the presence of the sera from the 12 mice treated i.m. with Lac-poly-L-lysine–ara-AMP for 20 days (see Materials and Methods), the dpm precipitated ranged from 41 ± 5 to 80 ± 6. This result demonstrates that none of the treated mice produced antibodies in amounts detectable by our assay, the limit of sensitivity being about 0.5 μg IgG/mL serum.

Toxicity

Lac-poly-L-lysine–ara-AMP dissolved in saline (NaCl 0.9%) was administered to mice at the dose of 1.3 mg/g by i.v. or s.c. route (five animals in each group). The volume injected was 0.4 mL/animal. The conjugate did not cause any recognisable sign of acute toxicity. Body weight increase was equal to that of control mice. The injected dose was about 50 times higher than that used in the experiments of organ distribution of conjugates in mice. The LD_{50} of the poly-L-lysine used for preparing this conjugate, administered i.v. as salt of HCl to mice, was between 30 and 60 μg/g.

Two mice treated i.m. with the conjugate administered at 24 μg/g with the same schedule used for immunization (5 days a week for 4 consecutive weeks) were killed 24 hr after the last injection. No ultrastructural changes were observed in either parenchymal or sinusoidal cells of the liver. Two mice and two male Wistar rats (200–210 g) which received 120 μg/g of the conjugate i.m. also showed no ultrastructural changes in liver cells 24 hr after the injection.

DISCUSSION

It was previously observed that high molecular mass poly-L-lysines after attachment of galactosyl or mannosyl residues accomplish a selective delivery of

drugs to hepatocytes [5] and to macrophages [26, 27] respectively. In the experiments reported here it was found that a low molecular mass poly-L-lysine, bearing a small number of galactosyl residues, is also selectively taken up by the liver and that it can accomplish a hepatic targeting of drugs when administered i.m.

Lac-poly-L-lysine–ara-AMP conjugate, given by repeated i.m. injections to mice, did not induce antibody production. The homogeneity of chemical groups exposed can account for this non-immunogenicity. Also high molecular mass poly-L-lysines, when their ε-amino groups are heavily substituted, do not induce antibodies [28, 29].

In contrast to poly-L-lysines [30, 31], the conjugate, even at high doses, did not display acute toxicity. The toxic effects of poly-L-lysines are associated with their polycationic character and are probably caused by the binding to the electronegative charges present on cell membranes. Coupling of lactose molecules by reductive amination [11] preserves the cationic character of lysine ε-amino groups, but the bound lactose together with the coupled ara-AMP (which is negatively charged) are expected to hinder the binding of conjugate to the electronegative groups of cell membrane.

The conjugate was found to be very soluble in water; this should allow the administration of a pharmacologically active dose in a small volume well compatible with the i.m. route.

The small molecular mass of the conjugate can in part contribute to its characteristics (high solubility, bioresorbability, non-immunogenicity, absence of acute toxicity).

In conclusion, the properties of the Lac-poly-L-lysine–ara-AMP complex described here make it a candidate drug for an i.m., liver targeted antiviral treatment in chronic HBV infection.

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