Galactosylated poly(L-lysine) as a hepatotropic carrier of 9-β-D-arabinofuranosyladenine 5′-monophosphate

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D-Galactopyranosyl residues were coupled to poly(L-lysine) and the antiviral agents arabinofuranosyladenine 5′-monophosphate (ara-AMP) and acyclovir were conjugated with this glycosylated polymer. In mice the ara-AMP conjugate accomplished a selective drug delivery to liver cells.

Arabinofuranosyl AMP *Acyclovir* *Drug targeting* *Antiviral chemotherapy* *Galactosylation* *Poly(L-lysine)* *Lactosamination* *Albumin*

1. INTRODUCTION

Albumin after conjugation with D-galactopyranosyl residues binds to Ashwell's receptor for galactosyl terminating glycoproteins [1,2] and specifically penetrates into liver cells [3]. Galactosylated albumin has been successfully used in mouse as a hepatotropic vector of 9-β-D-arabinofuranosyladenine 5′-monophosphate (ara-AMP) [4–6] and of 9-(2-hydroxyethoxymethyl)guanine (acyclovir) (Fiume et al., unpublished), two antiviral agents employed in the treatment of chronic hepatitis B (review [7]). The present experiments were undertaken to ascertain whether poly(L-lysine) after coupling to D-galactosyl residues can also accomplish a selective delivery of ara-AMP and acyclovir (ACV) to the hepatic cells. Poly(L-lysine) has been used by several different authors as a drug carrier in experimental cancer chemotherapy (review [8]).

2. MATERIALS AND METHODS

2-Imino-2-methoxyethyl 1-thioglycoside of D-galactose was prepared and covalently attached to poly(L-lysine) (M_r 47000) (Sigma) according to Lee et al. [9]. The resulting complex (Gal-poly(L-lysine)) had 320 μg 2-iminoethyl-1-thioglycoside of galactose per mg derivative (about one-third of the lysine residues were substituted). Ara-AMP was coupled to poly(L-lysine) or to Gal-poly(L-lysine) using 1-ethyl-3-(dimethylaminopropyl)carbodiimide (ECDI). 290 mg ara-AMP and 80 mg polymer were dissolved in 1.2 ml H_2O and the pH was adjusted to 7.5 with 10 N NaOH. After the addition of 290 mg ECDI the mixture was incubated for 24 h at 45°C and then dialyzed against 0.9% NaCl. Precipitated material was removed by centrifugation and the weight ratio (w/w) ara-AMP/poly(L-lysine) was determined by calculating the ara-AMP concentration from the extinction at 260 nm ($E^{1\%}$ = 420) and measuring the polymer according to Gornall et al. [10]. The ratios were 0.60 and 0.18 for poly(L-lysine)-ara-AMP, and Gal-poly(L-lysine)-ara-AMP, respectively.

Conjugation with ACV was performed by converting the drug into its monophosphate form (ACV-MP). ACV was phosphorylated by adding phosphorus oxychloride to a cold (−2°C) suspension of the drug in triethylphosphate [11,12]. ACV-MP was purified by chromatography on a Dowex-1-formate column eluted with a formic acid gradient (0–4 M). It was found to be essentially pure with a ratio of base ($E^{1\%}$ = 529 at

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255 nm in 0.1 M HCl) to phosphate [13] of 1:1. Thin-layer chromatography performed on precoated silica gel plates (60 F-254; Merck) with *n*-propanol-concentrated ammonium hydroxide-water (20:20:3, v/v) showed only one spot with a R_f of 0.51. ACV-MP was coupled to Gal-poly(L-lysine) with the same procedure used for ara-AMP. The weight ratio (w/w) ACV-MP/Gal-poly(L-lysine) was 0.11.

Coupling of D-galactosyl residues to human serum albumin (HSA) was obtained by reductive lactosamination of lysine residues [14]. The molar ratio lactose/HSA was 30; ara-AMP and ACV-MP were coupled to lactosaminated HSA (L-HSA) as previously described [6]; the molar ratios of drug/carrier were 13 and 10, respectively.

3. RESULTS AND DISCUSSION

Gal-poly(L-lysine) interacted with the hepatic receptor for galactosyl terminating glycoproteins. As shown in table 1, it inhibited the disappearance of ^{14}C-labelled asialofetuin from the blood of mice. Fetuin is a glycoprotein which, following desialylation and consequent exposure of galactosyl residues, is removed from the blood selectively by the liver after being bound by this receptor [2]. For reasons unknown, Gal-poly(L-lysine) when conjugated with ara-AMP or ACV-MP inhibited AF clearance to a significantly higher extent than the nucleotide-free polymer.

Table 1

Effect of poly(L-lysine), Gal-poly(L-lysine) and conjugates of Gal-poly(L-lysine) with ara-AMP or ACV-MP on plasma disappearance of [^{14}C]asialofetuin (AF)

Compound injected with [^{14}C]AF	
None	5506 ± 752
Poly(L-lysine)	5308 ± 300
Gal-poly(L-lysine)	13268 ± 145
Gal-poly(L-lysine)-ara-AMP	20056 ± 642
Gal-poly(L-lysine)-ACV-MP	16732 ± 342

Fetuin was enzymatically desialylated and labelled with [^{14}C]formaldehyde as described [4]. Swiss female mice (28–30 g) were injected i.v. with 2 μg/g body wt [^{14}C]AF (4.9×10^6 dpm/mg). The compounds were administered i.v. simultaneously with [^{14}C]AF at 2 μg/g. In all cases the volume injected was 10 μl/g. After 10 min mice were bled from retroorbital plexus under ether anaesthesia and the radioactivity of plasma was measured. Each entry (dpm/200 μl) represents the mean value ± SE of results from 7 animals. The difference between the results from mice injected with Gal-poly(L-lysine) or with the drug conjugates was statistically significant as evaluated by Student's *t*-test

Table 2 shows the effect of free and coupled drugs on [*methyl*-^{3}H]thymidine incorporation into DNA in liver, intestine and bone marrow of mice with Ectromelia virus hepatitis. In these animals thymidine incorporation in liver is almost certainly due to virus DNA synthesis [4]. Free ara-AMP injected at the dose of 7.5 μg/g, which corresponds to that administered to patiens with chronic hepatitis B [7], inhibited DNA synthesis in the three organs. Poly(L-lysine)-ara-AMP produced significant inhibition in liver and intestine; Gal-poly(L-lysine)-ara-AMP and L-HSA-ara-AMP inhibited DNA synthesis only in liver. The results with free ara-AMP and with L-HSA-ara-AMP confirmed previous data [4–6]. ACV and ACV-MP were not active at the dose of 5 μg/g body wt. ACV significantly inhibited DNA synthesis in liver and intestine at the dose of 10 μg/g which corresponds to that used in patients with chronic hepatitis B [7]. ACV-MP coupled to Gal-poly(L-lysine), tested at 1.1 and 2.2 μg ACV/g, was ineffective in the three organs; higher amounts of this complex could not be injected because of its toxicity (see below). ACV-MP conjugated with L-HSA inhibited DNA synthesis in liver at the dose of only 0.6 μg/g without producing significant inhibition in intestine and bone marrow. In conclusion Gal-poly(L-lysine) accomplished a liver targeting of ara-AMP but not of ACV-MP. The reason why ACV-MP was active only when coupled to L-HSA may depend on the different bonds between the drug and the two carriers. In the conjugate with Gal-poly(L-lysine) the drug is linked by a phosphoamide bond, while its bond(s) with L-HSA have not been identified as yet.

Gal-poly(L-lysine) possesses some advantages over L-HSA as a hepatotropic carrier of ara-AMP: (i) it is obtained by a synthetic method; (ii) it is available in a wide range of molecular sizes; (iii) it contains a large number of functional groups to which the drug can be coupled. On the other hand,

Table 2

Effect of free and coupled ara-AMP and ACV on [*methyl*-^{3}H]thymidine incorporation into DNA of liver, intestine and bone marrow of Ectromelia virus-infected mice

Compound	Dose (μg/g)	Ara-AMP or ACV administered (μg/g)	Inhibition of [*methyl*-^{3}H]thymidine incorporation (%)		
			Liver	Intestine	Bone marrow
Ara-AMP	7.5	7.5	49(S)[a]	61(S)	34(S)
Poly(L-lysine)-ara-AMP	8	3.0	55(S)	40(S)	16(NS)[a]
	12	4.5	57(S)	50(S)	11(NS)
Gal-poly(L-lysine)-ara-AMP	10	1.5	29(S)	0	0
	20	3.0	51(S)	2	0
	20[b]	3.0	25(S)	0	0
L-HSA-ara-AMP	50	3.2	50(S)	6(NS)	9(NS)
ACV	5	5	0	7	0
	10	10	42(S)	38(S)	15(NS)
	100	100	65(S)	75(S)	59(S)
ACV-MP	6.8	5	15(NS)	23(NS)	20(NS)
Gal-poly(L-lysine)-ACV-MP	10	1.1	0	3	5
	20	2.2	11(NS)	11(NS)	13(NS)
L-HSA-ACV-MP	25	0.6	40(S)	6(NS)	0
	50	1.2	55(S)	16(NS)	7(NS)

[a] Results were statistically evaluated by means of Student's *t*-test. The difference was considered statistically significant (S) or not significant (NS) for $P<$ or >0.05, respectively

[b] In this experiment the conjugate was injected 2 h before [*methyl*-^{3}H]thymidine

Experiments were performed as described [5]; when free or coupled ACV was administered, the injection of 9-erythro-(2-hydroxyl-3-nonyl)adenine was omitted. Compounds were injected 1 h before [*methyl*-^{3}H]thymidine. In control animals the mean values of dpm/100 μg DNA ranged in the different experiments from 14177 to 24692 in liver, from 19141 to 24121 in intestine and from 16967 to 21289 in bone marrow

L-HSA and its conjugates with ara-AMP do not display any recognizable sign of toxicity even at high doses [6] whereas poly(L-lysine) is rather toxic [8,15]. Glycosylation reduces this toxicity; in fact we found that the LD_{50} of the poly(L-lysine) used in these experiments increased from 20 to 40 μg/g body wt after coupling to galactosyl residues. Further experiments will be undertaken to elucidate whether by substituting a higher number of ϵ-NH_2 groups, the toxicity of Gal-poly(L-lysine) can be decreased to levels compatible with clinical use.

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