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Effect of electric charge on the hepatic uptake of macromolecules in the rat liver

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Summary

The effect of electric charge on the hepatic uptake of macromolecules was studied employing model macromolecules of identical molecular weight but differing in electrical charge. Rat liver was perfused using the single-pass method and model macromolecules were momentarily introduced into this system from the portal side. Outflow dilution patterns of model macromolecules were evaluated using statistical moment analysis. Anionic macromolecules were almost completely recovered in the outflow but cationic species showed a remarkable degree of hepatic uptake during the single passage. The values of the apparent retention volume (V) of cationic macromolecules were greater than that of human serum albumin, indicating reversible interaction with tissue. Inhibition of hepatic uptake was observed among cationic macromolecules in the perfused liver. In the in vitro binding experiment with isolated rat hepatocytes, the association of cationic macromolecules occurred in a saturable manner and no significant difference was observed between incubation at 4 and 37 °C. These results suggested that cationic macromolecules were adsorbed onto the hepatocyte surface by the effect of an electrostatic force. Thus, the electric charge of macromolecules was concluded as playing an important role in their hepatic uptake and is considered to offer a promising approach to controlling the pharmacokinetical characteristics of macromolecules.

Introduction

Recently, various macromolecular drug carrier systems have been developed in order to control the in vivo behavior of drugs. However, their application has often been limited by their hepatic accumulation and degradation. Nevertheless, systematic information on the properties of disposition of the carrier macromolecules is available only in sparse amounts.

In our series of investigations, we have examined the physicochemical, pharmacokinetic and pharmacological characteristics of polymeric prodrugs of mitomycin C (MMC), i.e., MMC-dextran conjugates bearing cationic (MMC-D_{cat}) and anionic (MMC-D_{an}) charges (Kojima et al., 1980; Takakura et al., 1987a,b; Nakane et al., 1988). The study of the in vivo disposition of MMC-D_{cat} in rats and mice demonstrated the considerable de-

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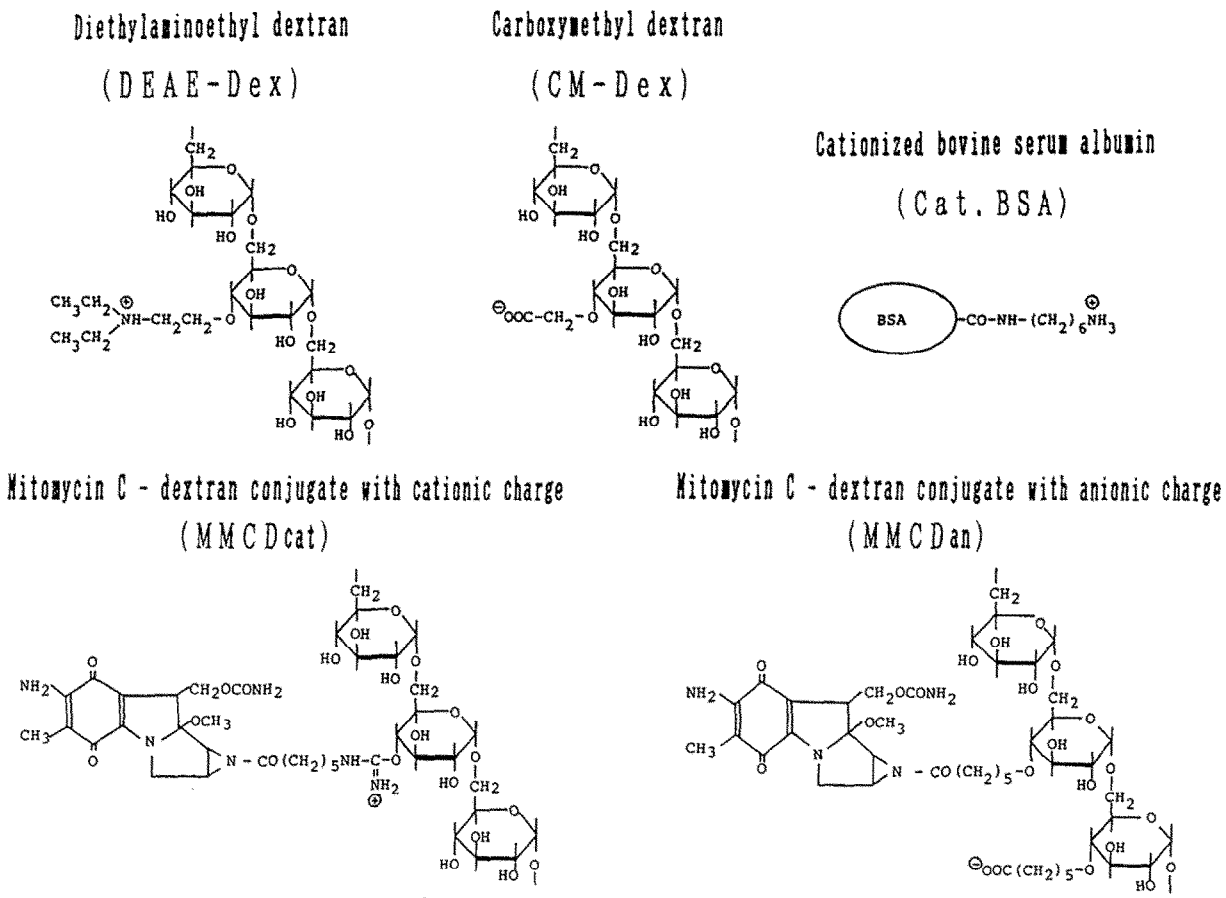

Fig. 1. Chemical structures of model macromolecules.

gree of its accumulation in the liver, while MMC-D_{an} showed a slow rate of plasma clearance and accumulated in very small amounts in the liver (Takakura et al., 1987a,b). In liver perfusion experiments, hepatic extraction of MMC-D_{cat} was considerable and increased with increasing molecular weight (Sato et al., 1989). In contrast, liver uptake of MMC-D_{an} was not observed during the single passage, irrespective of the molecular weight. Consequently, the hepatic disposition of MMC-D was suggested to be affected by its net electric charge.

The negative charges of the glycocalyx are randomly scattered throughout the cell surface membrane, including hepatocytes (Ghitescu and Fixman, 1984). If hepatic uptake of MMC-D_{cat} can be attributed to cell surface adsorption due to electrostatic forces, then a similar phenomenon should be generally observed in other polycationic macromolecules. Consequently, it is of interest to examine the interaction of cationic macromolecules with the liver and to explore the general features of their fate in vivo. In the present paper, we have investigated the behavior of model macromolecules of almost identical molecular weight but possessing different electrical charges using perfused rat liver and isolated hepatocytes.

Materials and Methods

Animals

In all animal experiments, male Wistar rats (190–210 g) were used and had free access to standard rat foods and water.

Chemicals

Dextran of average molecular weight 70000 (T-70), Polybuffer exchanger 94 resin, Polybuffer 96 elution buffer, Sephadex G-200, DEAE-Sephadex A-50 anion exchanger, and CM-Sephadex C-50 cation exchanger were obtained from Pharmacia (Uppsala, Sweden). Collagenase (type I) was obtained from Sigma (St Louis, U.S.A.). Diethylenetriaminepentaacetic acid (DTPA) anhydride was obtained from Dojindo Labs (Kumamoto, Japan). $K^{14}CN$ (30 MBq/mg) was supplied by Amersham Japan (Tokyo, Japan). $^{111}InCl_3$ (74 MBq/ml) was kindly supplied by Nihon Mediphysics Co. (Takarazuka, Japan). ^{131}I-labeled HSA (spec. act. 37 MBq/ml) was purchased from Daiichi Radioisotope (Tokyo, Japan). All other chemicals were reagent grade products obtained commercially.

Syntheses of model macromolecules

Diethylaminoethyl-dextran (DEAE-dextran) was synthesized according to the method of Mckerman et al. (1960). Carboxymethyl-dextran (CM-dextran) was synthesized according to Peterson et al. (1956). Cationized BSA was synthesized by covalent coupling of hexamethylenediamine to BSA (Pardridge et al., 1987). The product was purified by chromatofocussing using Polybuffer exchanger 94 resin and the Polybuffer 96 elution buffer system. The major protein peak eluted in the region of $pI > 9.0$.

Radiolabeling of model macromolecules

[*carboxyl*-^{14}C]Dextran (T-70) was prepared as described by Isbell et al. (1957) with slight modifications. Briefly, 0.02 mmol of dextran (T-70), sodium bicarbonate and sodium hydroxide were dissolved in 10 ml of distilled water and frozen in a glass tube. $K^{14}CN$ (0.02 mmol) was then added and the tube was sealed in a flame. The mixture was thawed and stored at 45 °C for 24 h, followed by heating for 7 h at 50 °C in a stream of air to effect hydrolysis. The product was purified by gel filtration using a Sephadex G-25 column and concentrated by ultrafiltration. The specific activity of [*carboxyl*-^{14}C]dextran (T-70) was approx. 11 kBq/mg. Radiolabeled DEAE-dextran and CM-dextran were synthesized from ^{14}C-labeled dextran (T-70) in the same manner as for unlabeled compounds.

Cationized BSA was labeled with ^{111}In using the bifunctional chelating agent DTPA anhydride according to the method of Hnatowich et al. (1985). Cationized BSA (10 mg) was dissolved in 1 ml of 0.1 M Hepes buffer (pH 7.0) and an equimolar amount of DTPA anhydride in 10 μl of DMSO was added. The mixture was stirred for 30 min at room temperature and purified by gel filtration on Sephadex G-25 to separate free DTPA. To 50 μl of 1 M sodium acetate buffer (pH 6.0), 50 μl of $^{111}InCl_3$ solution was added and then 100 μl of DTPA coupled protein solution. At 30 min after addition, the mixture was purified and concentrated by ultrafiltration.

Molecular size estimation

A Sephadex G-200 column (1.7 × 65 cm) was employed for molecular size estimation. A sample (2 mg) dissolved in 1 ml of 0.5 M NaCl solution was applied on a column and eluted with 0.5 M NaCl solution. The modified macromolecules were of almost the same size as the original dextran (T-70) and BSA.

Molecular charge

The net charges on the macromolecules were estimated via a batch method using DEAE-Sephadex A-50 anion exchanger and CM-Sephadex C-50 cation exchanger, as described by Roos et al. (1984). Their pK_a values were determined by the acid-base titration method. The adsorption ratios of model macromolecules to CM-Sephadex and DEAE-Sephadex and pK_a values are summarized in Table 1.

TABLE 1

Physicochemical characteristics of model macromolecules

Compounds	pK_a	Adsorption at pH 7.2 (%)	
		CM-Sephadex	DEAE-Sephadex
DEAE-dextran	8.7–8.9	69.4	0
CM-dextran	3.2–3.3	0	99.6
MMC-D_{cat}(T-70)	n.d.	97.1	0
MMC-D_{an}(T-70)	n.d.	0	38.3
Cationized BSA	9.0–9.4	94.7	0

n.d., not detectable.

Assay

The radioactivity of ^{14}C in the venous outflow, bile samples, and incubation medium was measured using a liquid scintillation counter (LSC-5000, Beckman, Tokyo, Japan). Those of ^{131}I and ^{111}In were counted using a well NaI scintillation counter (ARC-500, Aloka, Tokyo, Japan). The concentration of MMC-D_{cat} (T-70) was determined spectrophotometrically.

Liver perfusion

The operative procedure for in situ rat liver perfusion has previously been described (Nishida et al., 1989). The perfusate was circulated using a peristaltic pump at an average flow rate of 12.45 ml/min. Macromolecule (10 mg/ml) dissolved in Krebs-Ringer bicarbonate buffer (0.133 ml) was introduced into the portal vein using a six-position rotary valve injector. Venous outflow samples were collected into the previously weighed tubes at intervals of 1–3 s for 1 min. The sample volume was calculated from the gain in weight of the tube, assuming the density of the outflow perfusate to be 1.0. The time point of sampling was calculated from each sample volume assuming a constant flow rate. In the study of inhibition by successive application, the interval between the first and second injections was 15 min.

Pharmacokinetic analysis of outflow patterns

Moment analysis has been carried out previously on the hepatic disposition of small molecules (Nishida et al., 1989). In the current paper, the same theoretical treatment was employed for the analysis of venous outflow of macromolecules with a slight modification in terminology. The detailed theoretical background of this analysis is described in a previous article (Kakutani et al., 1985).

The statistical moment parameters for the outflow pattern are defined as follows;

$$\mathrm{AUC} = \int_0^\infty C \, \mathrm{d}t$$

$$\bar{t} = \int_0^\infty tC \, \mathrm{d}t / \mathrm{AUC}$$

where t is time and C represents the concentration of compounds normalized with respect to the injection dose. AUC and $\bar{t}$ denote the area under the concentration-time curve and mean transit time of the drug through the liver, respectively. The moment parameters are calculated by integration numerically using a linear trapezoidal formula and extrapolation to infinite time based on a single-exponential equation (Yamaoka et al., 1978; Kakutani et al., 1985).

The disposition parameters representing reversible and irreversible processes in the hepatic disposition of macromolecules are calculated from the moment parameters. The methods of derivation of the disposition parameters are summarized as follows;

$$V = Q \cdot \bar{t}/F \quad t_{ret} = \bar{t}/F$$

$$F = \mathrm{AUC} \cdot Q \quad E = 1 - F$$

$$k_{irr} = E/\bar{t} \quad \mathrm{CL}_{int} = k_{irr} \cdot V$$

where V is the apparent retention volume, reflecting reversible interaction. t_{ret} is the retention time, F corresponds to the recovery ratio, E denotes the extraction ratio, k_{irr} is the first-order irreversible elimination rate constant, CL_{int} refers to the intrinsic clearance, and Q represents the perfusion rate. These parameters can be divided into three groups; i.e., parameters representing reversible (V and t_{ret}) and irreversible (E, F, k_{irr}) processes and both (CL_{int}).

Isolation of liver cells

Parenchymal cells were isolated according to the method of Seglen (1976) with minor modifications. In brief, rat liver was perfused with Ca^{2+}, Mg^{2+}-free Hanks' balanced salt solution (HBSS) for 10 min and then with HBSS containing 5 mM $CaCl_2$ and 0.05% (w/v) collagenase for 10–20 min. The perfusion rate was 30 ml/min. Parenchymal cells were obtained from a total cell suspension as described by Horiuchi et al. (1985). The viability of cells was assessed by the trypan blue exclusion test.

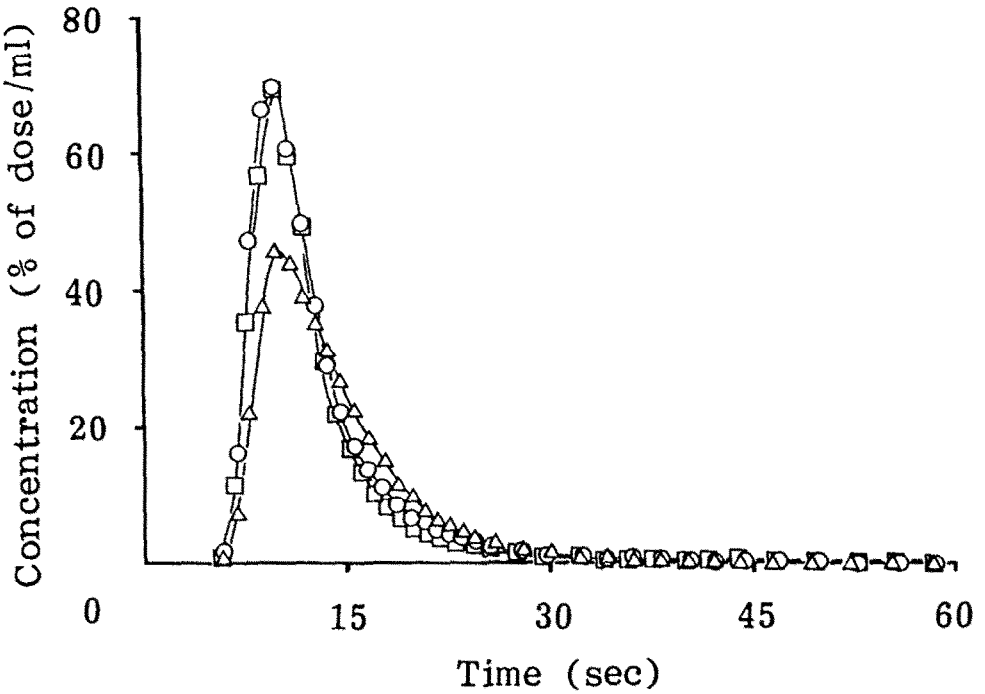

Fig. 2. Typical outflow patterns obtained for [^{14}C]dextran(T-70) (○), [^{14}C]CM-dextran (□), and [^{14}C]DEAE-dextran (△) via liver perfusion.

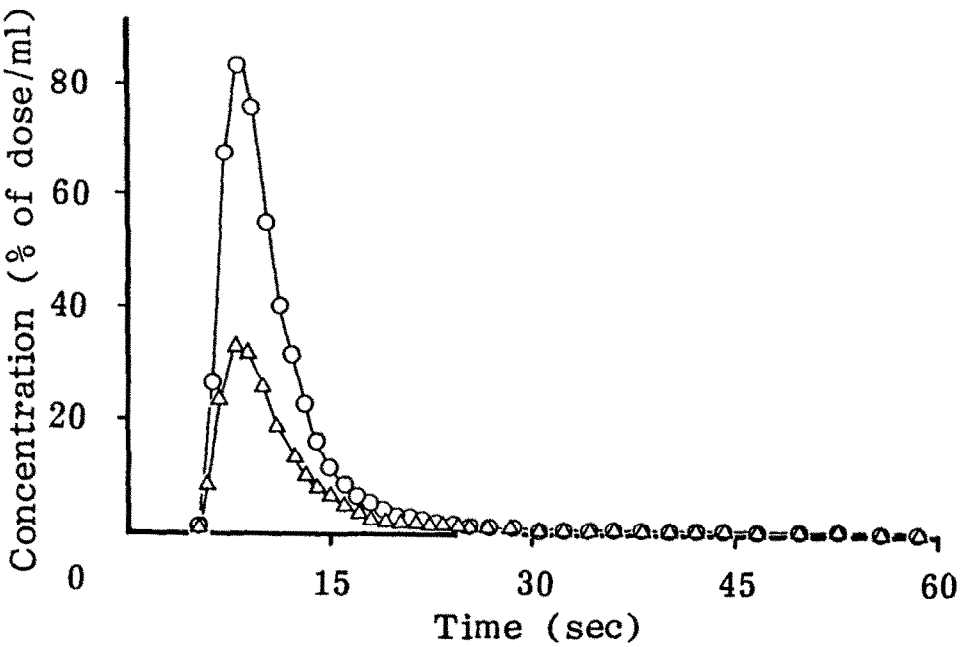

Fig. 3. Typical outflow patterns for [^{131}I]HSA (○) and [^{111}In] cationized BSA (△) in experiments on liver perfusion.

In vitro cellular association of model macromolecules

Isolated liver cells (10^6 cells/ml) were incubated in 10 mM Hepes-buffered HBSS (pH 7.2) containing model macromolecules (3 ml). After an appropriate time, cells were separated from the medium by centrifugation at 3000 rpm for 10 min. The concentration of cationic macromolecules remaining in the supernatant was determined by measuring the radioactivity or absorbance, and the amount of macromolecules associated with cells was calculated as a percentage of that applied. 15 min before or after application of MMC-D_{cat} (T-70), association inhibitors (unlabeled DEAE-dextran and cationized BSA) were added to the incubation medium. The results were compared with those of simultaneous administration. The effects of inhibitors were compared with respect to the amount of MMC-D_{cat} (T-70) associated with cells. The mode of inhibition was studied by simultaneous application of 0.1 and 1.0 mg/ml DEAE-dextran against MMC-D_{cat} (T-70) of various concentrations. An acid-wash experiment was carried out by replacing the incubation medium with acidified buffer (pH 5.0) for parenchymal cells preincubated for 15 min with MMC-D_{cat} (T-70) or [^{14}C]DEAE-dextran (Dunn et al., 1984). After 15 min, the amount of [^{14}C]DEAE-dextran or MMC-D_{cat}(T-70) dissociated was measured.

Results

Liver perfusion of model macromolecules

Figs 2 and 3 illustrate typical outflow concentration-time curves of [^{14}C]dextran(T-70), [^{14}C]DEAE-dextran, [^{14}C]CM-dextran, [^{131}I]HSA, and [^{111}In] cationized BSA. The outflow pattern of [^{14}C]DEAE-dextran showed a diminution in peak concentration in comparison with that of [^{14}C]dextran. Similarly, the peak value of [^{111}In] cationized BSA was markedly lower than that of [^{131}I]HSA. In contrast, [^{14}C]CM-dextran demonstrated almost the same outflow pattern as that of [^{14}C]dextran.

The moments and disposition parameters for these results are summarized in Table 2 together with those of MMC-D(T-70) for comparison (Sato et al., 1989). Cationic macromolecules such as [^{14}C]DEAE-dextran, MMC-D_{cat}(T-70) and [^{111}In] cationized BSA display higher values for V of 0.256, 0.387, and 0.423 ml/g liver, respectively, than did HSA (0.184 ml/g liver). MMC-D_{cat}(T-70) and [^{111}In] cationized BSA resulted in high values for the extraction ratio of 0.429 and 0.506, respectively. On the other hand, anionic macromolecules such as [^{14}C]CM-dextran and MMC-D_{an}(T-70) showed almost 100% recovery in outflow.

Macromolecules were undetectable in bile during the course of the study except for MMC-

TABLE 2

Moments and disposition parameters for model macromolecules in single-pass rat liver perfusion system

	N	Moment parameter		Disposition parameter				
		AUC (% dose s ml^{-1})	$\bar{t}$ (s)	V (ml g^{-1})	t_{ret} (min)	E (%)	k_{irr} (min^{-1})	CL_{int} (ml min^{-1} g^{-1})
[^{14}C]Dextran	4	469.7 ± 23.7	7.71 ± 2.14	0.191	0.131	2.38	0.214	0.037
[^{14}C]DEAE-dextran	4	418.6 ± 14.4	8.62 ± 1.70	0.256	0.164	12.26	0.869	0.215
[^{14}C]CM-dextran	4	459.0 ± 14.2	9.08 ± 1.93	0.230	0.159	3.46	0.204	0.052
MMC-D_{cat}(T-70)	4	285.5 ± 24.4	9.33 ± 2.71	0.387	0.278	42.91	2.832	1.057
MMC-D_{an}(T-70)	4	507.3 ± 13.7	9.21 ± 2.51	0.222	0.152	0	–	0
[^{131}I]HSA	6	482.4 ± 18.5	7.37 ± 1.24	0.184	0.123	0	–	0
[^{111}In] cationized BSA	4	228.7 ± 13.1	7.95 ± 1.07	0.423	0.268	50.60	3.865	1.610

Values are means ± S.D. The results for MMC-D_{cat}(T-70) and MMC-D_{an}(T-70) were taken from a previous report (Sato et al., 1989).

TABLE 3

Moments and disposition parameters for [^{14}C]DEAE-dextran in the liver perfusion experiment after preperfusion of other cationic macromolecules

Preperfusion [a]	N	Moment parameter		Disposition parameter				
		AUC (% dose s ml^{-1})	$\bar{t}$ (s)	V (ml g^{-1})	t_{ret} (min)	E (%)	k_{irr} (min^{-1})	CL_{int} (ml min^{-1} g^{-1})
None	4	418.6 ± 14.4	8.62 ± 1.70	0.256	0.164	12.26	0.869	0.215
MMC-D_{cat}(T-70)	3	455.2 ± 20.0	7.18 ± 0.54	0.192	0.118	4.73	0.392	0.075
Cationized BSA	3	433.9 ± 10.3	7.34 ± 0.75	0.193	0.133	8.06	0.674	0.129

Values are means ± S.D. [a] Cationic macromolecules (MMC-D_{cat}(T-70) or cationic BSA) were momentarily applied 15 min before the application of [^{14}C]DEAE-dextran.

D_{cat}(T-70) for which 0.59% of the dose was recovered in bile (Sato et al., 1989).

Effect of preperfusion of MMC-D_{cat}(T-70) and cationic BSA on the hepatic uptake of DEAE-dextran

The moment and disposition parameters for [^{14}C]DEAE-dextran applied after the momentary pretreatment of MMC-D_{cat}(T-70) or cationized BSA and successive 15 min perfusion, are listed in Table 3. The hepatic uptake of [^{14}C]DEAE-dextran was altered by pretreatment of MMC-D_{cat}(T-70) and cationized BSA and E, k_{irr}, and CL_{int} were significantly decreased ($p < 0.05$).

In vitro cellular interaction of model macromolecules

Fig. 4 demonstrates plots of percent association vs time for DEAE-dextran and cationized BSA at 4 and 37°C in the case of hepatic parenchymal cells together with those of MMC-D_{cat}(T-70) (Nakane et al., 1988). MMC-D_{cat} and cationized BSA underwent rapid association with parenchymal cells, followed by a constant level of association being maintained during the course of incubation. No significant difference was observed between incubations at 4 and 37°C. Acid washing resulted in 5–10% of the cationic macromolecules initially associated with the parenchymal cells undergoing dissociation at both 4 and 37°C.

Fig. 5 shows the concentration effect in the cases of MMC-D_{cat}, DEAE-dextran, and cationized BSA on association with parenchymal cells. Cellular association was compared after 15 min incubation. At low concentrations, the extent of cellular association increased in proportion to the concentration of cationic macromolecule, however, saturation occurred at higher concentrations. A linear relationship was observed to exist between the reciprocal of the amount of compounds associated with the cells and that of their concentrations remaining in the incubation medium. This suggested that the interaction between ca-

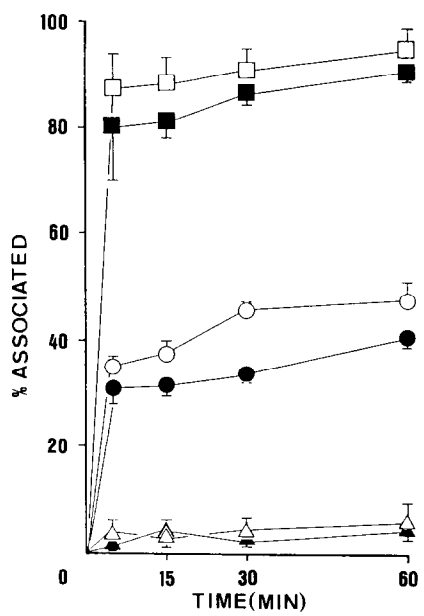

Fig. 4. In vitro association vs time curves of cationic model macromolecules with parenchymal cells at 37°C (○, △, □) and 4°C (●, ▲, ■). (○) MMC-D_{cat}(T-70); (△) [^{14}C]DEAE-dextran; (□) [^{111}In] cationized BSA. Results are expressed as means of four experiments, with vertical bars indicating the S.D.

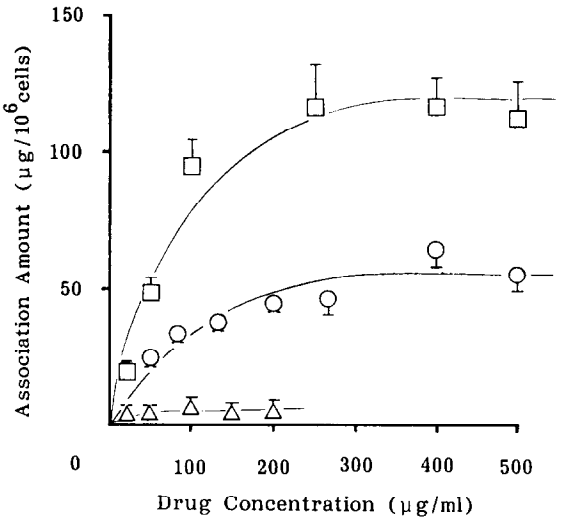

Fig. 5. Effect of concentration of cationic model macromolecule on its association with parenchymal cells. (○) MMC-D_{cat}(T-70); (△) [^{14}C]DEAE-dextran; (□) [^{111}In] cationized BSA. Incubation was carried out at 37 °C for 15 min. Results are expressed as means of four experiments, vertical bars indicating the S.D.

tionic macromolecules and the cells behaved in conformity with the Langmuir adsorption isotherm:

$$X = X_\infty \cdot K \cdot C/(1 + K \cdot C)$$

$$1/X = (1/X_\infty \cdot K) \cdot (1/C) + 1/X_\infty$$

where X represents the amount associated in the case of a cationic macromolecule, X_∞ is the maximum amount associated for cationic macromolecules, K is a constant expressing the strength of binding, and C denotes the concentration of cationic macromolecule in the medium after incubation. X_∞ and K were evaluated according to a least-squares method and yielded the results summarized in Table 4. The X_∞ values of MMC-D_{cat}(T-70) and cationized BSA were greater than that of DEAE-dextran. Anionic macromolecules displayed no significant interaction under these conditions.

TABLE 4

Maximum amount associated with parenchymal cells (X_∞) and the constant in the Langmuir adsorption isotherm (K) for cationic macromolecules

Compounds	Maximum amount associated (X_∞) (μg/10^6 cells)	K (ml/μg)
MMC-D_{cat}(T-70)	56.90	0.0405
[^{14}C]DEAE-dextran	1.73	0.0044
[^{111}In] cationized BSA	128.60	0.0664

Mutual inhibition of cellular interaction was evident among cationic model macromolecules at 37 °C (Table 5). The percentage of cellular association for MMC-D_{cat}(T-70) after 15 min incubation was found to decrease in the cases of preincubation or simultaneous administration with DEAE-dextran or cationized BSA. In contrast, cellular association of MMC-D_{cat}(T-70) was

TABLE 5

Effect of addition of DEAE-dextran and cationic BSA on the association of MMC-D_{cat}(T-70) with rat liver parenchymal cells at 37 °C

	Concentration (mg/ml)	Without inhibitor (control)	Association (%) of MMC-D_{cat}(T-70) [a]		
			Inhibitor → MMC-D_{cat} [b]	Simultaneous application of inhibitor	MMC-D_{cat} → inhibitor [c]
DEAE-dextran	0.1	100	75.2	74.8	92.3
	1.0	100	59.8	61.4	76.2
Cationized BSA	0.1	100	71.2	62.5	100.0
	1.0	100	32.6	29.2	89.2

[a] Association (%) of MMC-D_{cat}(T-70) was measured in the presence of inhibitors at a concentration of 0.1 mg/ml and compared to that of the control without inhibitors.

[b] Preincubation with inhibitors was for 15 min and MMC-D_{cat}(T-70) was incubated for 15 min.

[c] After 15 min incubation with MMC-D_{cat}(T-70), inhibitors were added and values of association (%) of MMC-D_{cat}(T-70) were determined after an additional 15 min incubation.

scarcely affected by successive incubation (15 min) with 10-fold greater amounts of other cationic macromolecules. Similar results were obtained at 4°C. On investigating the inhibition of MMC-D_{cat}(T-70) association by simultaneous application of DEAE-dextran, competitive inhibition was confirmed as existent based on a Lineweaver Burk plot for a concentration range of MMC-D_{cat}(T-70) similar to that examined in Fig. 5.

Discussion

The liver is the predominant organ which determines the in vivo systemic disposition of colloidal drug carrier systems such as macromolecular and particulate carriers. However, little is known about the relationship between the physicochemical properties and disposition of carriers. In the present study, the hepatic disposition of model macromolecular carriers bearing different electrical charges was examined in perfused rat liver. The macromolecules tested here included dextran and albumin, both of which possess biocompatibility and have been utilized as macromolecular carriers of various drugs (Molteni, 1979; Hurwits et al., 1980; Kojima et al., 1980; Larsen and Johansen, 1985). Dextran with an average molecular weight of 70000 was selected in order to facilitate comparison with albumin derivatives on the basis of equal molecular weight (not size).

The perfusion of isolated liver provides an appropriate tool for describing the characteristics of hepatic transport, since experiments can be performed independently of the influence of other organ system. Model macromolecules were introduced momentarily into the portal side and the outflow curves resulting were analyzed according to statistical moment theory. A concrete advantage of the present approach is that the assessment of the hepatic disposition of macromolecules can be achieved by its division into reversible and irreversible processes. Furthermore, the irreversible process of elimination can be evaluated with respect to both rate and extent. The reversible process of disposition mainly represents the weak interaction with the liver cell surface and the irreversible type generally corresponds to the combination of internalization and metabolic degradation. Strong binding to the liver cell surface coupled with a slow rate of dissociation may also be regarded as an irreversible disposition process, since the period of time involved in liver perfusion was short (1 min).

In the perfusion experiment, the V values for cationic model macromolecules were greater than that for HSA (vascular reference substance) and the ratio of the V values for cationic model macromolecules to that of HSA ranged between 139 and 230%, suggesting the existence of a reversible distribution process between the perfusate and the tissue. In contrast, the retention volumes of anionic and electrically neutral macromolecules were similar to that of HSA.

During the single passage of 1.33 mg of [^{111}In] cationized BSA, MMC-D_{cat}(T-70), and [^{14}C] DEAE-dextran through the liver (~ 8 g), 0.0841, 0.0713, and 0.0201 mg were extracted per g liver, respectively. These amounts (but not the extraction ratio) appear rather large on comparison with the values for maximum uptake reported previously for ligands such as immunoglobulin A, asialo-orosomucoid, epidermal growth factor, insulin, and glucagon, which undergo endocytosis via a receptor-mediated mechanism (Finck et al., 1985; Sato et al., 1988).

In in vivo experiments on rats (Nakane et al., 1988), the association of MMC-D_{cat} was demonstrated to occur with both parenchymal and nonparenchymal cells depending on the surface area ratio of the cell types (73:27, %/%) (Dalen et al., 1981). In contrast to other organs where the capillary presents a substantial barrier between the vascular and interstitial spaces, the liver possesses discontinuous endothelial capillaries, this form of structure allowing free contact between the hepatocyte surface and substances circulating in the plasma. This anatomical feature of the liver explains the apparent specific interaction of cationic macromolecules with the surface of liver cells.

In such experiments, [^{14}C]DEAE-dextran showed a lower degree of hepatic extraction than did [^{111}In] cationized BSA and MMC-D_{cat} (T-70), in good agreement with the absorption ratio on CM-Sephadex and the pK_a value. Thus, the electrical charge density and electrochemical structure

of cationic macromolecules are reflected in the hepatic extraction of macromolecules.

In a previous study (Sato et al., 1989), repeated applications of MMC-D_{cat} resulted in hepatic uptake decreasing in a stepwise manner, suggestive of the existence of a saturable and irreversible association process. In this study, the same type of inhibition was observed between [^{14}C]DEAE-dextran and the other cationic macromolecules on successive administration. Corresponding results were also obtained during the investigation of in vitro cellular association of MMC-D_{cat}(T-70) as shown in Table 5.

From the results discussed above, cationic macromolecules have been demonstrated to undergo strong, irreversible, and high-capacity interactions with liver cells during the single passage of the liver. The finding that the extent of in vitro association of cationic macromolecules with hepatocytes is similar at both 4 and 37 °C suggested that biological processes such as internalization and metabolism do not play a major role in such phenomena. In addition, the amount associated vs dose relationship was shown to behave in accordance with the Langmuir adsorption isotherm and competitive inhibition was observed between cationic macromolecules. Acid washing induced dissociation of adsorbed cationic macromolecules at neither 4 nor 37 °C. These data suggest that the actual state of interaction observed in these phenomena represents a type of irreversible association on the plasma membrane surface with a strong force. Our previous results showed that MMC-D_{cat} associated with the plasma membrane of Ehrlich ascites carcinoma cells as a result of an electrostatic force (Matsumoto et al., 1986), the interaction between cationic macromolecules and hepatocytes appearing to be similar.

Following adsorption on the membrane surface, cationic macromolecules are believed to become internalized, metabolized and/or excreted into the bile. However, the rate of internalization appears to be so slow compared with the total amount associated that most of the observed association can be attributed to cell surface adsorption as occurs in the case of Ehrlich ascites carcinoma cells. ^{14}C labeling to yield [^{14}C]DEAE-dextran results in stability and practically no metabolic degradation occurs. Cationized BSA labeled with ^{111}In is accumulated in the liver by exchange of ^{111}In with iron-binding protein (Brown et al., 1987) after intracellular degradation. Therefore, internalization and successive metabolic degradation were undetectable in the perfusate and bile studied here.

The uptake of anionic and electrically neutral macromolecules by the liver was found not to occur in this experimental system. These molecular species are considered to be incorporated into the liver via a mechanism of fluid-phase endocytosis (Takakura et al., 1987a; Nakane et al., 1988), however, this process was not observed during the indicator dilution experiment due to its slow rate. Assessment of the slow process of uptake of anionic model macromolecules by the liver may be achieved by carrying out liver perfusion with the recirculation or constant infusion mode of drug administration.

In the present investigation, the relationship between the electrical characteristics and the hepatic uptake of macromolecules has been elucidated. These findings should provide important information concerning the development of macromolecular prodrugs; for example, cationic macromolecules should prove useful as a targeting device to the liver and anionic macromolecules should be advantageous for retaining the drug in the blood circulation or, in the case of a target site other than the liver, through systemic administration. However, it should be ensured that the specificity of the hepatic interaction for cationic macromolecules is lower than that for ligands incorporated via receptor-mediated endocytosis, since cationic compounds can also interact with other organs (Becker and Green, 1960; Farquhar, 1978).

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