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Permeability of macromolecules in cultured endothelial and tumor cell layers on microporous membranes

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Summary

The permeation characteristics of macromolecules were studied in layers of bovine aortic endothelial cells (EC) and human colon adenocarcinoma SW-1116 cells (SW-1116) cultured on fibronectin-coated polycarbonate membranes. EC monolayer and SW-1116 layers which consisted of two to four cell layers at confluency were considered as models for vascular wall and tumor tissue, respectively. The studies using [^{14}C]sucrose (Mw: 342), [^{14}C]inulin (Mw: 5000), and [^{14}C]dextran (T-70) (Mw: 70000) showed that both cell layers functioned as size-selective barriers and the permeability coefficients for SW-1116 were larger than those for EC by a factor of 1.5. Permeation and association with the cell layers were evaluated for radiolabeled charged macromolecules with the same molecular weight (70000), including derivatives of dextran and bovine serum albumin (BSA) and macromolecular prodrugs of mitomycin C (MMC), MMC-dextran conjugates (MMCD). Both anionic and cationic macromolecules had lower permeability coefficients in both cell layers compared with neutral dextran. On the other hand, neutral and anionic macromolecules showed almost no interaction with the cell layers whereas cationic macromolecules were significantly associated with both of the cell layers. These findings suggest that cationic macromolecules would exhibit low permeability across the cell layers because of electrostatic interaction with the negative charges of the cells, while the permeation of anionic macromolecules might be restricted by electric repulsion. The present study, thus, clarified the permeability characteristics of macromolecules at the cellular level.

Introduction

In recent years, various macromolecular drug carrier systems have been developed in order to control the pharmacokinetic properties of drugs. In our series of investigations, we have studied the disposition characteristics of polymeric prodrugs of mitomycin C (MMC), MMC-dextran conjugates (MMCD), and other macromolecular compounds in several experimental systems including in vivo, organ perfusion and in vitro methods (Matsumoto et al., 1986; Takakura et al., 1987, 1990; Nakane et al., 1988; Sato et al., 1989; Nishida et al., 1990, 1991). We have clarified the

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relationships between their disposition characteristics and the physicochemical properties, such as molecular weight and electric charge.

More recently, cell culture systems have been employed as useful tools to study drug disposition characteristics in vitro (Audus et al., 1990). Various kinds of cultured endothelial and epithelial cells grown on microporous membranes have been used for the assessment of transport and metabolic characteristics of specific biological barriers.

In the present study, we examined the in vitro permeability characteristics of macromolecules using cultured endothelial and tumor cell layers on microporous membranes. We selected bovine aortic endothelial cells (EC) and a tumor cell line, human colon adenocarcinoma SW-1116 cells (SW-1116), as a model of endothelial and tumor cells, respectively. The purpose of this study was to clarify the effects of physicochemical characteristics of macromolecules on the vascular permeability characteristics and the transport properties in the tumor tissue at the cellular level.

Materials and Methods

Chemicals

Dextran with an average molecular weight of about 70000 (T-70) was purchased from Pharmacia, Uppsala, Sweden. Bovine serum albumin (BSA; fraction V) was obtained from Armour Pharmaceutical Co., U.K. MMC was kindly supplied by Kyowa Hakko Kogyo (Tokyo, Japan). Indium chloride ($^{111}InCl_3$) was supplied by Nihon Mediphysics, Takarazuka, Japan. γ-Amino[U-^{14}C]butyric acid (3.7 MBq/ml), [(U)-^{14}C]sucrose (0.11 MBq/mmol) and [*methoxy*-^{14}C]inulin (0.35 MBq/g) were obtained from New England Nuclear (Boston, MA). Potassium [^{14}C]cyanide (30 MBq/mg) and [^{3}H]inulin (66 GBq/mmol) were purchased from Amersham, Japan (Tokyo, Japan). All other products were reagent grade products that were obtained commercially.

[*carboxy*-^{14}C]Dextran(T-70) was prepared using potassium [^{14}C]cyanide according to the method of Isbell et al. (1957) with slight modifications. Diethylaminoethyl-dextran (DEAED) (DEAED(T-70)), carboxymethyl-dextran (CMD) (CMD(T-70)), and cationized BSA (cBSA) were synthesized according to the methods reported previously (Takakura et al., 1990). [^{14}C]-DEAED(T-70) and [^{14}C]CMD(T-70) were synthesized from [*carboxy*-^{14}C]dextran(T-70) in the same manner as for the unlabeled molecules. BSA and cBSA were labeled with ^{111}In using a bifunctional chelating agent, diethylenetriaminepentaacetic acid anhydride (Dojindo Labs, Kumamoto, Japan) according to the method of Hnatowich et al. (1982). [^{14}C]$MMCD_{cat}$ (T-70) and [^{14}C]$MMCD_{an}$ (T-70) were synthesized as reported previously (Matsumoto et al., 1986).

Cell culture

Bovine aorta was purchased from a local slaughterhouse and transported to the laboratory in Hank's balanced salt solution (HBSS) at 0–4°C. In the laboratory, the aorta was transected longitudinally and its inner side was washed several times with sterilized Ca^{2+}, Mg^{2+}-free phosphate buffer. Then it was gently scraped once with a slide glass. Endothelial cells (EC) on the slide glass were released in culture medium, RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco Laboratories, Grand Island, NY) and transferred to a T-25 culture flask. EC were cultured in RPMI 1640 supplemented with 10% FBS at 37°C in a 95% air-5% CO_2 humidified atmosphere. SW-1116 cells (SW-1116), an epithelial-like human colon carcinoma cell line (Leibovitz et al., 1976) were cultured under the same conditions.

Transport experiment

EC from two to 10 passages and SW-1116 were detached from the flask by treatment with 0.25% trypsin and 0.02% EDTA in HBSS and seeded on fibronectin-coated (10 $\mu g/cm^2$) microporous polycarbonate membranes (3.0 μm pore size, 6.5 mm diameter) fixed to cylinders (Transwell™, Costar, Cambridge, MA). The seeding densities were 7.0×10^3 and 7.0×10^4 cells/cm^2 for EC and SW-1116, respectively, and the culture medium was changed every other day. The EC and SW-1116 reached confluency at 7 and 8 days

after seeding, respectively, and the confluent cell layers were used for transport experiments.

Prior to the experiments, cell layers were washed three times with HBSS buffer (HBSS containing 1% BSA, 20 mM Hepes, pH 7.4). Then the cylinder with the cell layer was placed in the well of a 24-well tissue culture plate. To avoid effects of hydrostatic pressure, 0.13 and 0.7

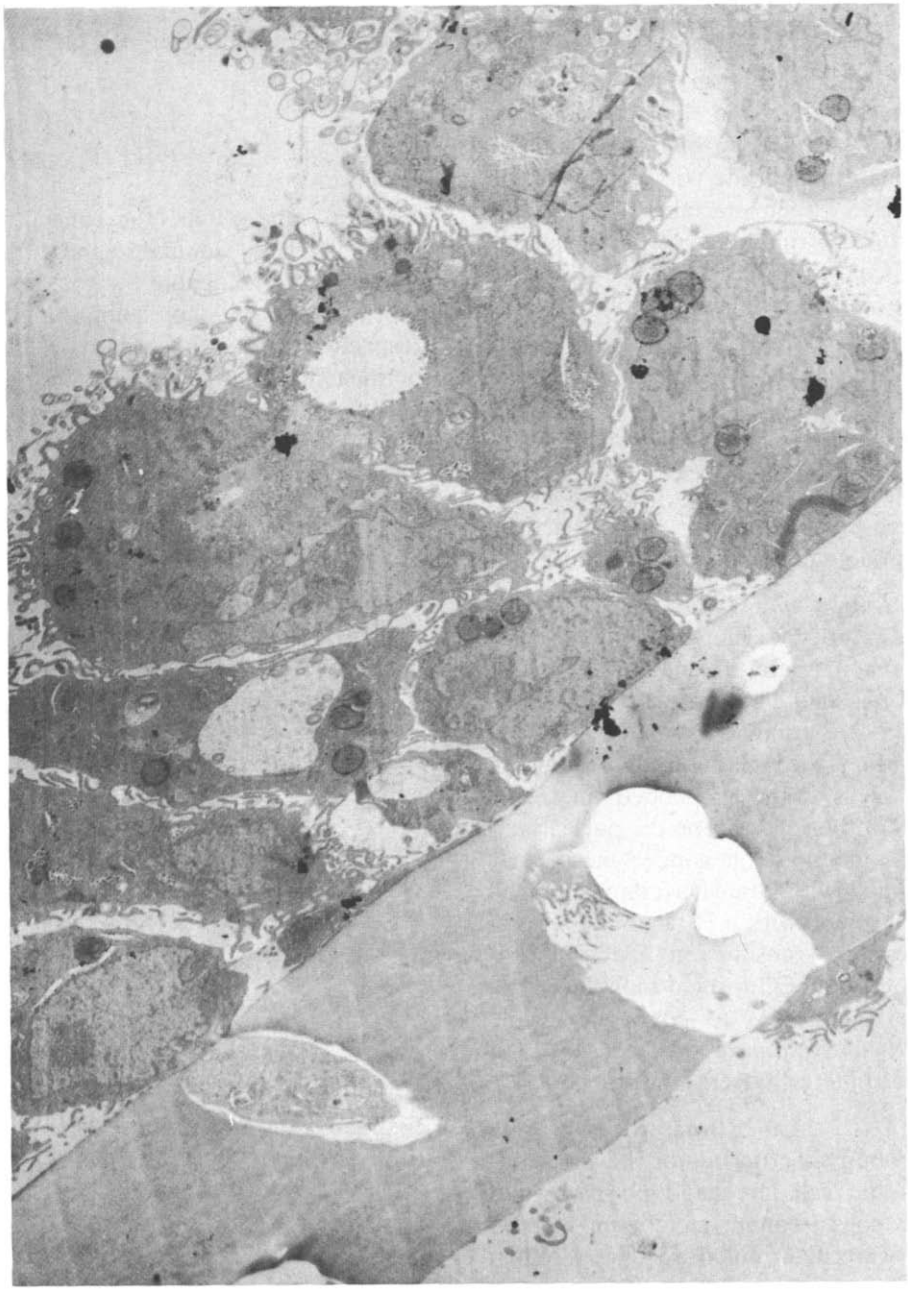

Fig. 1. Transmission electron micrograph of day 8 culture of SW-1116 cells grown on polycarbonate membrane. Magnification, ×3600.

ml of HBSS buffer were added to the luminal and abluminal sides, respectively. The experiment was performed at 37°C for 2 h. After addition of a solution containing ^{14}C-labeled or ^{111}In-labeled test compound and [^{3}H]inulin (reference) to the luminal side, a 10 μl aliquot of HBSS buffer was removed from the abluminal side at various times after addition. An aliquot (10 μl) of HBSS buffer was added back to the same side to maintain a constant volume. At the end of the experiment, the polycarbonate membrane with cell layer was rinsed with HBSS buffer and taken off from the cylinder for the determination of the remaining radioactivity. Control experiments were carried out using the fibronectin-coated polycarbonate membrane without the cell layer to measure the permeability across the polycarbonate membrane only. ^{14}C and ^{3}H radioactivity was measured in a liquid scintillation system (LSC-900, Alloca Co, Tokyo) after adding of 5 ml of scintillation medium (Clear-sol 1, Nacalai Tesque, Kyoto, Japan) to each sample. The ^{111}In radioactivity was counted in a gamma scintillation counter.

Transmission electron microscopy

Cells on polycarbonate membranes were fixed for 2 h at room temperature in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, and then post-fixed with 1% osmium tetroxide for 2 h. After dehydration through a graded series of ethanol, the cells were embedded in resin. Cross-sections of the cell layer on the polycarbonate membrane mounted on copper mesh grids were post-stained with uranyl acetate and lead citrate, and examined with a Hitachi H-500 electron microscope. Transmission electron microscopy showed that EC formed monolayers on polycarbonate membranes at confluency while confluent SW-1116 preparations consisted of double to quadruple cell layers (Fig. 1).

Data analysis

The permeability coefficients of the test compound across the cell layer and polycarbonate membrane or polycarbonate membrane without the cell layer were determined as follows. When the test compound is transported between the luminal and abluminal compartments solely by diffusion across the cell layer and polycarbonate membrane, the transport rate of the test compound to the abluminal compartment is expressed as follows:

$$dC_a/dtV_a = PA(C_l - C_a) \quad (1)$$

and the integrated form of Eqn 1 is as follows.

$$C_aV_a = V_aX_0/(V_l + V_a) \times [1 - \exp\{-PA(1/V_a + 1/V_l)t\}] \quad (2)$$

where C_a and C_l are the concentrations in the abluminal and luminal compartments, respectively, V_a and V_l denote the volumes in abluminal (0.7 ml) and luminal compartments (0.13 ml), respectively, t is time (min), P represents the permeability coefficient, A is the surface area of the membrane (0.33 cm^2) and X_0 corresponds to the dose of the test compound applied to the luminal compartment at time 0. The penetration profile was fitted to Eqn 2 using the nonlinear least-squares regression program MULTI (Yamaoka et al., 1981) to obtain the permeability coefficient of the test compound (P).

The permeability coefficient in the cell layer alone was calculated from the following relationship:

$$1/P_c = 1/P_{c+m} - 1/P_m \quad (3)$$

where P_c, P_m and P_{c+m} are the permeability coefficients in the cell layer alone, polycarbonate membrane without cell layer and polycarbonate membrane plus cell layer, respectively.

To describe the effect of molecular size on the permeability across a cell monolayer, we used the model and the following equation according to the cylindrical pore theory of Landis and Pappenheimer (1963). If there is a kind of pore of radius r_p on the cell layer, the permeability-to-diffusion coefficient ratio of the test solute with a Stokes-Einstein radius r_e is described by this model as:

$$P_c/D_{37} = A_p/(A\,dx)(1-a)^2 \times (1 - 2.104a + 2.089a^3 - 0.9481a^5 - 1.372a^6) \quad (4)$$

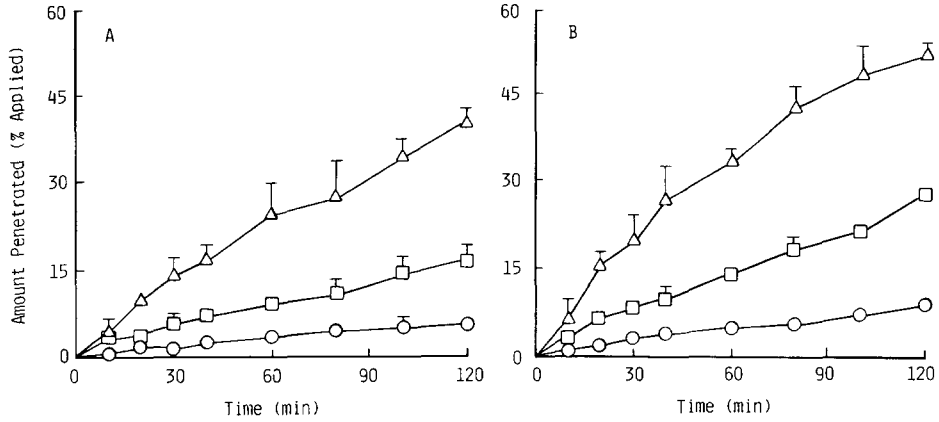

Fig. 2. Transport of [^{14}C]sucrose (△), [^{14}C]inulin (□) and [^{14}C]dextran(T-70) (○) across EC monolayers (A) and SW-1116 layers (B). Results are expressed as means ± S.D. of at least four experiments.

where $a = r_e/r_p$, when $r_e < r_p$ and $a = 0$, when $r_e \geqq r_p$. D_{37} is the diffusion coefficient of the test compound at 37°C, A_p denotes the total pore area on the cell layer, and A and dx are the surface area and thickness of the cell layer, respectively. The permeability coefficients, diffusion coefficients and Stokes-Einstein radii of molecules were fitted to Eqn 4 using MULTI to obtain the values for the pore radius (r_p) and the total pore area divided by the pore length (A_p/dx).

Results

Permeation of molecules with different sizes across EC and SW-1116 layers

Fig. 2 shows the transport of neutral molecules with various molecular weights across EC and SW-1116 layers. The amount penetrating through the cell layers decreased significantly with increase in molecular weight. SW-1116 layers were more permeable to these neutral molecules than EC monolayers. Permeability coefficients of the molecules for polycarbonate membrane, EC and SW layers are summarized in Table 1. Permeability coefficients for both cell layers (P_{ec} and P_{sw}) were remarkably decreased compared with those for polycarbonate membrane (P_m). The ratio of the permeability coefficient to free diffusion coefficient in water at 37°C (D_{37}) decreased with increase in molecular weight in both cell layers, indicating restriction of the diffusion of the molecules across the cell layers. In addition, permeability coefficients for EC monolayers were lower than those for SW-1116 layers in all molecules by a factor of about 1.5.

Table 2 summarizes the parameters calculated

TABLE 1

Permeability coefficients of solutes with different macromolecular weights for EC monolayers and SW-1116 layers

Compound	Mw	Permeability coefficient (cm/h)						
		Membrane	EC monolayer			SW-1116 layer		
		P_m	P_{ec}	$P_{ec\text{-}in}$	P_{ec}/D_{37} (cm^{-1})	P_{sw}	$P_{sw\text{-}in}$	P_{sw}/D_{37} (cm^{-1})
[^{14}C]Sucrose	342	0.2806	0.2383	0.1242	9.272	0.4143	0.2101	16.440
[^{14}C]Inulin	5000	0.1421	0.0727	0.1300	8.078	0.1114	0.1951	12.378
[^{14}C]Dextran(T-70)	70000	0.0725	0.0185	0.1372	4.895	0.0254	0.2031	6.721

P_m, permeability coefficient for polycarbonate membrane; P_{ec}, permeability coefficient for EC monolayer; $P_{ec\text{-}in}$, permeability coefficient of [^{3}H]inulin for EC monolayer; P_{sw}, permeability coefficient for SW-1116 layer; $P_{sw\text{-}in}$, permeability coefficient of [^{3}H]inulin for SW-1116 layer; D_{37}, diffusion coefficient in water at 37°C (cm^2/h).

TABLE 2

Parameters for EC monolayers and SW-1116 layers calculated based on the cylindrical pore theory

Cell layer	A_p/dx (cm)	R_p (nm)
EC monolayer	3.45	21.7
SW-1116 layer	6.20	16.1

A_p, total pore area of cell layer; dx, thickness of cell layer; R_p, pore radius.

according to the cylindrical pore theory in the permeation experiments using molecules with different molecular weights. The value of the total pore area divided by the pore length (A_p/dx) for SW-1116 layers was about 2-fold greater than that for EC monolayers. On the other hand, the pore radius (r_p) for EC monolayers was almost identical to that for SW-1116 layers.

Transport of charged macromolecules across EC and SW-1116 layers

Figs 3–5 illustrate the penetration amount-time curves of [^{14}C]dextran(T-70), [^{14}C]CMD(T-70), [^{14}C]DEAED(T-70), [^{14}C]MMCD$_{an}$(T-70), [^{14}C]MMCD$_{cat}$(T-70), [^{111}In]BSA, and [^{111}In]-cBSA across EC and SW-1116 layers. The transport rates of anionic [^{14}C]CMD(T-70) and [^{14}C]-

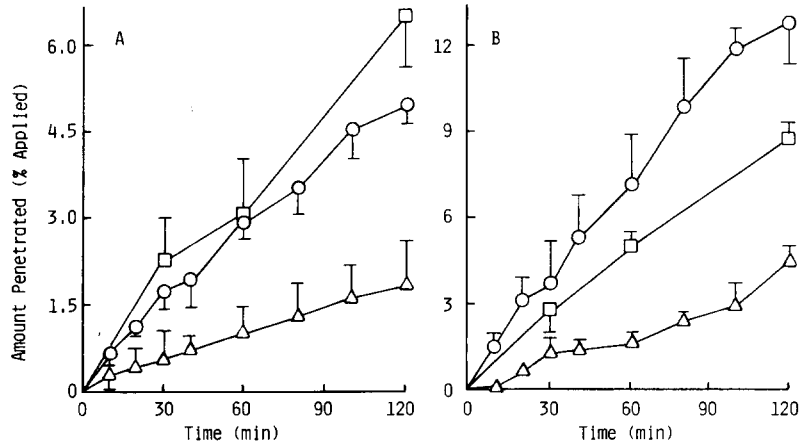

Fig. 3. Transport of [^{14}C]dextran(T-70) 20 mg/ml (○), [^{14}C]CMD(T-70) 20 mg/ml (△), and [^{14}C]MMCD$_{an}$(T-70) 20 mg/ml (□) across EC monolayers (A) and SW-1116 layers (B). Results are expressed as means ± S.D. of at least four experiments.

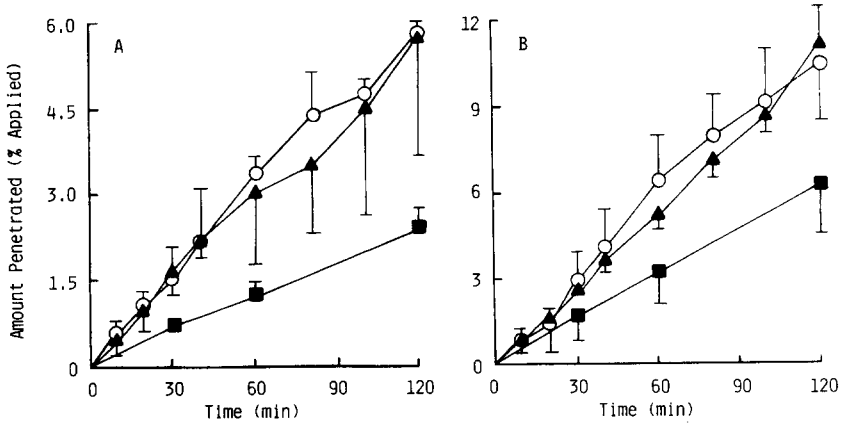

Fig. 4. Transport of [^{14}C]dextran(T-70) (○), [^{14}C]DEAED(T-70) (▲), and [^{14}C]MMCD$_{cat}$(T-70) (■) across EC monolayers (A) and SW-1116 layers (B). Results are expressed as means ± S.D. of at least four experiments.

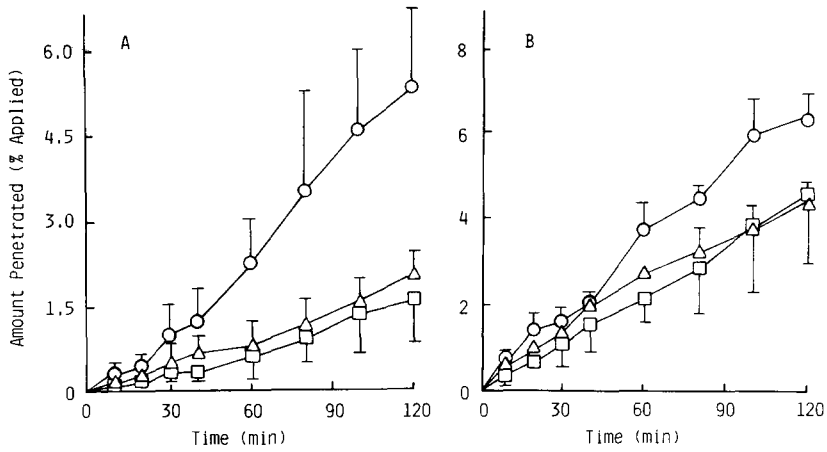

Fig. 5. Transport of [^{111}In]BSA (○), [^{111}In]cBSA 0.2 mg/ml (△), and [^{111}In]cBSA 2 mg/ml (□) across EC monolayers (A) and SW-1116 layers (B). Results are expressed as means ± S.D. of at least four experiments.

$MMCD_{an}$(T-70) across both cell layers were slower than those of neutral [^{14}C]dextran(T-70) except for [^{14}C]$MMCD_{an}$(T-70) in EC monolayers (Fig. 3). Concerning cationic dextran derivatives, [^{14}C]-$MMCD_{cat}$(T-70) showed restricted permeation across both cell layers while the permeabilities of [^{14}C]DEAED(T-70) were similar to those of neutral [^{14}C]dextran(T-70) (Fig. 4). [^{111}In]cBSA exhibited lower permeabilities across EC and SW-1116 layers compared with [^{111}In]BSA (Fig. 5). No significant difference was observed between the permeabilities of [^{111}In]cBSA at different concentrations applied to the luminal side.

The permeability coefficients of all macromolecules for both EC and SW-1116 layers are summarized in Table 3, together with those of

TABLE 3

Permeability coefficients of macromolecules for EC monolayers and SW-1116 layers

Compound	Concentration (mg/ml)	Permeability coefficient ($\times 10^{-3}$) (cm/h)						
		Membrane	EC monolayer			SW-1116 layer		
		P_m	P_{ec}	$P_{ec\text{-}in}$	$P_{ec}/P_{ec\text{-}in}$	P_{sw}	$P_{sw\text{-}in}$	$P_{sw}/P_{sw\text{-}in}$
[^{14}C]Dextran(T-70)	2	72.5	14.6	111.6	0.1332	35.5	124.4	0.2796
[^{14}C]DEAED(T-70)	2	77.6	13.7	110.0	0.1255	31.1	171.8	0.1847 [a]
[^{14}C]$MMCD_{cat}$(T-70)	2	54.5	5.5 [a]	113.3	0.0490 [a]	17.6	166.3	0.1054 [a]
[^{14}C]Dextran(T-70)	20	72.5	12.7	117.2	0.1077	50.3	213.2	0.2333
[^{14}C]CMD(T-70)	20	79.9	5.1 [b]	137.5	0.0364 [b]	10.0 [b]	151.2	0.0683 [b]
[^{14}C]$MMCD_{an}$(T-70)	20	76.5	16.3	135.6	0.1214	24.4 [b]	141.5	0.1736
[^{111}In]BSA		81.2	12.0	93.2	0.1256	16.3	89.3	0.1818
[^{111}In]cBSA	0.2	71.2	3.1 [c]	84.3	0.0346 [c]	10.6	105.2	0.0981 [c]
[^{111}In]cBSA	2	65.5	4.0 [c]	82.1	0.0493 [c]	10.8 [c]	104.3	0.1062 [c]

[a,b,c] Significantly different ($p < 0.05$) from the corresponding values of dextran(T-70) 2 mg/ml, dextran(T-70) 20 mg/ml and BSA, respectively.

P_{ec}, permeability coefficient for EC monolayer; $P_{ec\text{-}in}$, permeability coefficient of [^{3}H]inulin for EC monolayer; P_{sw}, permeability coefficient for SW-1116 layer; $P_{sw\text{-}in}$, permeability coefficient of [^{3}H]inulin for SW-1116 layer.

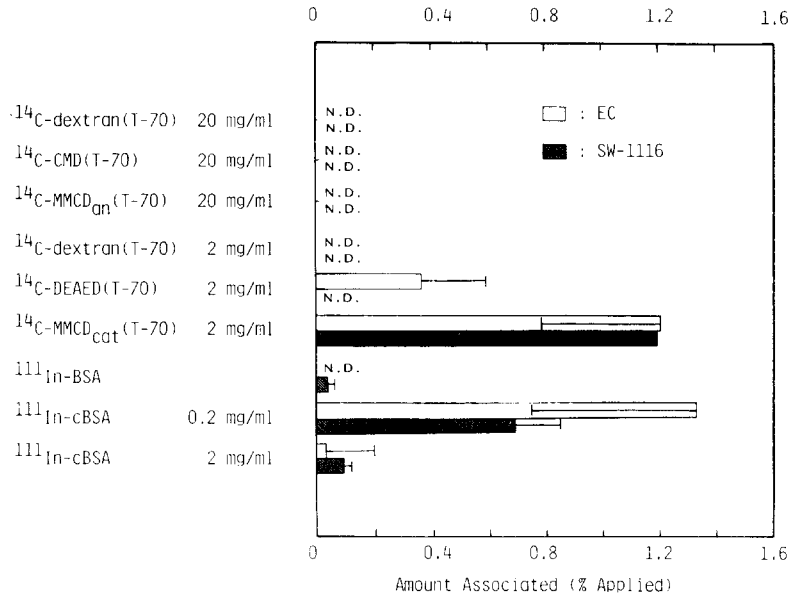

Fig. 6. Interaction of radiolabeled macromolecules with EC monolayers and SW-1116 layers. Associated amount of the molecules after 2 h transport experiment was corrected by subtraction of the amount of [^{3}H]inulin. Results are expressed as means ± S.D. of at least four experiments. N.D., not detectable.

[^{3}H]inulin and the ratios of both values. Both anionic and cationic macromolecules had lower values than those of neutral dextran. No significant differences were observed for [^{14}C]-DEAED(T-70) and [^{14}C]MMCD$_{an}$(T-70) in EC monolayers.

Interaction of macromolecules with EC and SW-1116 layers

Fig. 6 shows the amount of macromolecules associated with both cell layers during the transport experiment. Remarkable accumulation of radioactivity on the cell layers was observed with cationic macromolecules. In contrast, anionic and neutral molecules displayed no significant interaction with both cell layers.

Discussion

In the present study, we used two types of cultured cell layers, EC monolayers and SW-1116 layers, to study the effect of physicochemical properties of macromolecules on their transport across cell layers. Our particular interest was in the elucidation of the role of the electric charge of macromolecules, since previous studies have demonstrated the importance of electric charge in determining their disposition characteristics. In general, macromolecules which are not recognized by any special cellular transport system penetrate the cell layers across the intercellular pathway; i.e., the permeation would correspond to a junctional transport phenomenon. In this sense, transcellular transport is unlikely to contribute to the permeation of the present macromolecules and the following discussion will be focused on the intercellular transport.

Prior to the study of the effect of electric charge, we examined the influence of molecular weight on the permeability of cell layers and found that both cell layers acted as size-selective barriers (Fig. 2 and Table 1). Barrier function based on the molecular weight of solutes has been reported for cultured endothelial (Hashida et al., 1986; Siflinger-Birnboim et al., 1987; Albelda et al., 1988; Wolfe and Borchardt, 1988) and epithelial (Madara et al., 1988; Cho et al., 1989; Hidalgo et al., 1989) cell monolayers. The permeability coefficient of [^{111}In]BSA for EC

monolayers observed in this study (12.0×10^{-3} cm/h) was comparable to the values reported by Casnocha et al. (1989) in human umbilical vein EC monolayers (17.3×10^{3} cm/h) and by Albelda et al. (1988) in bovine fetal aortic EC monolayers (20.2×10^{3} cm/h). Thus, EC monolayers used in this study were shown to be adequate for examining the permeability of macromolecules.

SW-1116 layers were more permeable to neutral molecules at any molecular weight than EC monolayers (Fig. 2 and Table 1). The higher permeability of SW-1116 layers was explained by the larger value of A_p/dx (Table 2), suggesting larger total pore area or smaller cell layer thickness. It was likely that SW-1116 layers had a much higher A_p value, since electron microscopy showed that the thickness of SW-1116 layers was rather larger and the cell junction was considerably loose (Fig. 1).

Monolayers of a human colon carcinoma cell line, Caco-2 cells, have been most extensively used as a model system of intestinal epithelium since they develop morphological and functional characteristics of polarized epithelium (Audus et al., 1990). The permeability for SW-1116 layers determined in this study was much larger than that for Caco-2 cell monolayers (Hidalgo et al., 1989). In contrast to Caco-2 cells, SW-1116 did not form monolayers but double to quadruple cell layers without tight intercellular junctions (Fig. 1) although it had also originated from human colon carcinoma cell lines (Leibovitz et al., 1976). Therefore, SW-1116 layers appeared to show characteristics of neoplastic tissue rather than those of epithelium. The transport process of macromolecules across SW-1116 layers would correspond to that in a well-perfused viable region of the tumor tissue.

In this study, we used several charged macromolecules with molecular weights of about 70000 and examined their permeabilities across EC and SW-1116 cell layers and their association with the cells in order to clarify the role of molecular charge. Significant charge effects were expected since it is generally known that the negative charges of the glycocalyx are randomly scattered on the cell surface. As a whole, both anionic and cationic macromolecules exhibited lower permeability coefficients in both cell layers compared with neutral dextran (Table 1). On the other hand, with respect to cellular association, neutral and anionic macromolecules showed almost no interaction with the cell layers whereas cationic macromolecules were significantly associated with both layers (Fig. 6). These results suggest that cationic macromolecules would exhibit low permeability across the cell layers due to electrostatic interaction with the negative charges of the cells while the permeation of anionic macromolecules might be restricted by electric repulsion.

The degrees of the charge effect on permeability coefficients varied with compounds and the effects were not clear for anionic [^{14}C]MMCD$_{an}$-(T-70) and cationic [^{14}C]DEAED(T-70) (Table 3). In addition, no significant interaction was observed for [^{14}C]DEAED(T-70) in SW-1116 layers. Although the reason for these results remains to be elucidated, a difference in electric charge densities and elctrochemical structures of the charged macromolecules might be involved.

For anionic macromolecules, conflicting results have been reported on their permeabilities. Areenkul (1969) and Haraldsson et al. (1982) examined the transcapillary exchange of neutral dextran and anionic sulfated dextran in isolated perfused rabbit ear and rat hindquarter, respectively, and found that the reflection coefficient of sulfated dextran was considerably higher than that of dextran. Lower permeability of anionic dextran compared with neutral dextran was reported in cultured mouse brain microvascular endothelium (Sahagum et al., 1990). These reports suggest the presence of a charge barrier to anionic macromolecules in the blood vessel or EC monolayer and are in agreement with our findings.

On the other hand, no significant difference was observed between permeabilities of anionic carboxymethyl dextran and neutral dextran in the cultured bovine fetal aortic EC monolayers (Albelda et al., 1988). Negatively charged dextrans were considerably less restricted than neutral dextrans of corresponding size in isolated rat lung (Pietra et al., 1982). The osmotic reflection coeffi-

cient of negative lactate dehydrogenase isoenzymes was reported to be lower than that of the neutral or positively charged type (Perry et al., 1983). Differences in the molecular properties of anionic molecules and also experimental systems may lead to variation in results. It would be interesting if similar results were obtained in bovine aortic EC monolayers and human colon adenocarcinoma cell layers in this study.

Facilitated transport of cBSA and cationized IgG across the blood-brain barrier has been demonstrated in rats in vivo, isolated bovine brain microvessels, and cultured bovine brain capillary EC monolayers (Smith and Borchardt, 1989; Triguero et al., 1989). Based on these findings, cationic macromolecules are considered to be useful carriers for targeting of peptide drugs to the brain (Kumagai et al., 1987; Pardridge et al., 1987). In the present study, cationization of macromolecules did not enhance but restricted their permeation across the cell layers. The discrepancy might be explained by the difference in cell types and experimental systems.

The present investigation, thus, elucidated the permeability and association characteristics of macromolecules across aortic EC monolayers and human colon adenocarcinoma cell layers on microporous membranes. These findings should provide important information about the development of macromolecular prodrugs.

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