Transcellular Transport of Fluorescein Dextran through an Arterial Endothelial Cell Monolayer

Ryoichi Hashida, Chie Anamizu, Yasuko Yagyu-Mizuno, Shoji Ohkuma and Tatsuya Takano

Department of Microbiology & Molecular Pathology, Teikyo University, Sagamiko, Kanagawa 199-01, Research Development Corporation of Japan, Koishikawa, Bunkyo, Tokyo 112 Japan

ABSTRACT. Transcellular transport of fluorescein dextran (FD) of various molecular weights (4K, 10K, 20K, 70K and 150K daltons) through porcine arterial endothelial cells cultured on a type I collagen gel supported by a dacron sheet was studied and compared with the transport of low density lipoprotein labeled with rhodamine B (RB-LDL) described previously (Hashida et al., Cell Struct. Funct. 11, 31-42, 1986).

The rate of FD transport through the monolayer depended on the size of the FD. FD transport was not temperature-dependent and was not a saturable process. Our findings show that FD transport differs from RB-LDL transport which is temperature- and dose-dependent. The mechanism of the transport of FD is compared with that of RB-LDL.

Vascular endothelial cells act in the regulation of the passage of fluid and macromolecules from the blood to the vascular wall. Macromolecules have been shown to cross the endothelial cell monolayer by such different transcellular transport mechanisms as vesicular transport (transcytosis) and transport through transcellular channels and/or intercellular spaces (junctional transport) (10-13).

In a previous report, we described the development of an in vitro trans-endothelial transport model: Endothelial cells were cultured as a monolayer on a type I collagen gel supported by a dacron sheet, after which transport through the monolayer of low density lipoprotein labeled with rhodamine B (RB-LDL) was examined (5). The results suggest that RB-LDL is transported in transcytotic vesicles by a temperature- and energy-dependent process.

Transport via intercellular junctions also is well known. Simionescu et al. have reported that the rates of transport such electron dense tracers as hemeundecapeptide, horseradish peroxidase and ferritin through capillary venular junctions depend on their molecular size (9, 13). Dextran labeled with fluorescein isothiocyanate (FD) appears to be a good tracer for the study of the vascular permeability of various tissues in situ (1, 4, 6-8, 14, 15) because FDs with a wide range of molecular weights can be obtained and can be measured quantitatively.

We now have studied the trans-endothelial transport of FDs of various molecular weights in our in vitro model and found that the rate of FD transport through the

Abbreviations used: FD, dextran labeled with fluorescein isothiocyanate; RB-LDL, low density lipoprotein labeled with rhodamine B isothiocyanate.
intercellular junctions varies depending on the molecular weight of the FD molecule.

MATERIALS AND METHODS

Preparation of the model for measuring trans-endothelial transport. The model was prepared as described elsewhere (5). A dacron sheet (50 and 75 deniers; Nakao Filter, Osaka) was placed in a culture dish. Acid-soluble type I collagen that had been dissolved in 1 mM acetic acid then neutralized with Hank's buffered salt solution was gelated on the sheet at room temperature after which it was sterilized by UV irradiation overnight.

Endothelial cells from porcine arterial wall (2-3 passages) cultured in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% fetal calf serum (Flow Lab., McLean, VA) were seeded on the collagen gel (8.0 × 10⁵/dish). Confluent cell monolayers that formed within 2-3 days were used as the experimental material.

Fluorescein isothiocyanate-labeled dextran (FD). FDs in the molecular weight range of 4K, 10K, 20K, 70K and 150K daltons were purchased from Sigma Chem. (St Louis, MO). The glucose residues in the different-size molecules were labeled with almost the same amount of fluorescein isothiocyanate molecules (4 mole per 1,000 mole glucose residues).

Detection of FD transport through the endothelial cell monolayer. As described elsewhere (5), the endothelial cell monolayer on a collagen gel supported by a dacron sheet was placed in the middle of a chamber made in this laboratory. Both compartments of the chamber were filled with Dulbecco's modified Eagle's minimum essential medium (phenol red free; Gibco Lab., Grand Island, NY) supplemented with 10% fetal calf serum, then the same medium was perfused through the lower chamber with a peristatic mini-pump. An appropriate amount of FD was introduced into the upper chamber, after which the fluorescence intensity in the lower chamber was monitored with a fluorescence spectrophotometer (excitation, 495 nm; emmission, 550 nm).

RESULTS

FDs of various sizes were prepared for use in our study of transcellular transport. The Sepharose 4B gel filtration patterns of these FDs are shown in Fig. 1. Sharp peaks corresponding to the molecular weights of 4K, 10K, 20K, 70K and 150K daltons were recovered, and the fractions representing these peaks were used in the experiments described.

The transport rates of the different-size FD samples through the endothelial monolayer were examined. All sizes of FD passed freely through a collagen gel supported by a dacron sheet without an endothelial monolayer at about the rate of 30%/h (Fig. 2). The 4K-dalton FD passed most rapidly (about 4%/h) through the endothelial monolayer. The permeation rates of the FD fractions decreased with the increase in molecular weight, the permeation rates of the FDs of 10K and 20K being approximately 1%/h, and those of the FDs of 70K and 150K less than 1%/h (Fig. 2).

Data for the time-courses of the transport of the FD samples through the endothelial cell monolayer are shown in Fig. 3. The penetration of FD was linear for at least 2h, 4K FD having a rate of approximately 3.3%/h, and 10K, 20K, 70K and 150K FD respective rates of 1.8%/h, 1.5%/h, 0.6%/h and 0.4%/h.

We next measured the passage of the 4K and 70K FDs at 0°C and 37°C to determine whether transport is temperature-dependent. The rates for the 40K and 70K FD
Fluorescein Dextran Transport through Endothelial

Fig. 1. Sepharose 4B gel chromatography of various sizes of FD (4K, 10K, 20K, 70K and 150K). FD solutions (0.1 mM, 500 µl) were applied to a column (1.5 × 50 cm) equilibrated with 0.15 M NaCl. The effluent was collected in fractions of 1.0 ml/tube at a flow rate of about 10 ml/h. The ordinate shows the % of recovery of the total FD applied. The arrow indicates the void volume.

Fig. 2. Relation between the molecular weight of FD and its rate of transport through an endothelial monolayer. A 1 ml sample of FD (5 µM) was introduced into the upper compartment, then the FD transported through the membrane at 37°C to the lower compartment was monitored. Dacron sheet with gelated collagen only (○); endothelial monolayer on a dacron sheet with gelated collagen (●). The transport of FD is shown in pmole per cm² membrane per h (left) and as the % of recovery per h of the amount of FD applied (right).
were similar at both temperatures (Fig. 4), evidence that the transport of both sizes of FD through the endothelial monolayer does not depend on temperature.

The dose-response curves for the transport of FDs of various sizes (Fig. 5) were linear and non-saturable in the range of 5 μM to 100 μM. The order of the transport rates of the FD preparations was dependent on molecular weight.

---

**Fig. 3.** Time courses of FD transport. FD samples (50 μM) with molecular weights of 4K (○), 10K (●), 20K (■), 70K (▲) and 150K (▼) were introduced into the upper compartment, and their passage through the membrane was studied for 2 h at 37°C. The transport of FD is shown in nmole per cm² membrane per h (left) and as the % of recovery per h of the amount of FD applied (right).

**Fig. 4.** Temperature-dependence of FD transport. FD samples (50 μM) of 4K and 70K were introduced into the upper compartment. Incubation took place at 0°C (open columns) and 37°C (stipled columns). The passage of FD is shown as nmole per cm² membrane per h (left) and as the % of recovery per h of the amount of FD applied (right).
DISCUSSION

In a previous study (5), we developed a new in vitro trans-endothelial system to study the transport of macromolecules. This system should prove useful in quantitative studies made to determine the mechanisms of transport of molecules across vascular endothelial cells. The state of confluence of the cultured cells and the integrity of their cell junctions in the cultured endothelial monolayer were confirmed by scanning and transmission electron microscopy and by measurements of electrical resistance. In this system, RB-LDL was shown to be transported in an energy-dependent manner.

We now have examined the transport of FD, which differs from that of RB-LDL as shown in Table 1. The transport of 4K and 70K FD does not depend on temper-

![Graph showing the relation between the concentration of FD and its transport per h at 37°C.]

**TABLE 1.**

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>FD</th>
<th>0°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4K</td>
<td></td>
<td>3.3</td>
<td>3.3</td>
</tr>
<tr>
<td>10K</td>
<td></td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>20K</td>
<td></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>70K</td>
<td></td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>150K</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>RB-LDL</th>
<th>0°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000K–1,500K</td>
<td>0.0</td>
<td>0.5–0.7</td>
<td></td>
</tr>
</tbody>
</table>

Transport rate (±/h) | Temperature-independent | Temperature-dependent
Dose-response | Unsaturable | Saturable
ature, and the dose-response curves show that the transport of FDs of various sizes is non-saturable up to 100 μM FD. In contrast, RB-LDL transport is temperature-dependent and saturable at approximately 200 μg/ml (about 0.2 μM).

We concluded from the results of our previous study that RB-LDL is transported in transcytotic vesicles (not through cellular junctions or by endocytosis and exocytosis via a lysosomal system) because its transport was temperature- and energy-dependent and saturable. In contrast, FD probably passes through the intercellular junctions of the endothelial monolayer.

We also examined the effects of certain chemicals on FD transport through the endothelial monolayer. Treatment of the cells with cholchicine or cytochalasin B, both known to dissociate cytoskeleton, might cause separation of the tight junctions of cultured cells. Both compounds promptly increased the passage of 4K and 70K FD; 10 μM cholchicine causing 3.2-fold and 7.1-fold increases, and 5 μg/ml cytochalasin B causing 6.1-fold and 22-fold increases. Removal of ionized calcium from the medium with ethylenediaminetetraacetic acid (2.5 mM) also induced separation of the junctions, as well as a 15-fold increase in the transport of the 4K FD and a 22-fold increase in that of 70K (Hashida et al., unpublished data). Our findings are consistent with those of Bowman et al. (3) and Baetscher et al. (4). Destruction of the cellular junctions by these chemicals enhanced the passage of FD, which indicates that FD is transported through the monolayer via the intercellular spaces. Vasoactive mediators such as histamine and serotonin had no effect on FD transport in this system (data not shown).

The rate of transport of FD through the endothelial monolayer depended on the molecular weight of FD used in this in vitro system. Results were consistent with histological findings in situ. Simionescu et al. have reported some effects of molecular weight on intercellular transport by based on the use of histological methods and electron dense probes (9, 13). The rate of transport of hemeundecapeptide (1.9K) was the highest. Only about 50% of the horseradish peroxidase (40K) passed through the open junctions of the venular endothelium of mouse diaphragm. Very little of the hemoglobin (68K) passed through and almost no (<5%) ferritin (450K). Rather than indicating a difference between endothelial cells from different organs, Simionescu et al.'s findings were consistent with our findings of differences in the rates of passage of FDs of various sizes through a cultured arterial endothelial monolayer.

In studying the function of the blood brain barrier, Tervo et al. injected FDs of various molecular weights (3K-150K), and found that the capillaries of the cerebral cortex were impermeable to all the intravenous tracer substances used (14). Measurements of transport rates of FD in vivo compared with those found in our in vitro system may help widen our understanding of the construction and integrity of the cellular junctions of the endothelial monolayer, which seem to vary with the organ from which the cells are derived.

This type of in vitro study should help to clarify the complicated transendothelial transport of various substances under physiological as well as pathological conditions.

REFERENCES

Fluorescein Dextran Transport through Endothelial


(Received for publication, July 15, 1986)