mately eight times higher than that for the HUVEC monolayer, whereas only two times higher SPC of D-4 was noted. Therefore, these SPC and TER results indicate that, compared to the HPMC monolayer, the HUVEC monolayer is less permeable to middle-to-high molecular weight solutes but their permeabilities to smaller molecules are similar.

Clinical data usually obtained from PETs reflect overall mass transfer area coefficients, the inverse parameter of which is represented as overall mass transport resistance: $R_{overall} = 1/K_{overall}A_{overall} = R_{meso} + R_{endo} = 1/K_{meso}A_{meso} + 1/K_{endo}A_{endo}$ (20). Since each resistance is represented as an inverse of the product of surface area and SPC, the contribution of R_{meso} to $R_{overall}$ may increase when vascular surface area (A_{endo}) increases. For example, a two-times increase in vascular surface area (A_{endo}) gives a solute transport resistance of the mesothelium (R_{meso}) equal to a 4-kD solute. On the other hand, solute transport resistance (R_{meso}) for 10 kDa would be less influenced by the same increment. If SPTs using at least two marker solutes with widely varying molecular weights are available, the differences in solute transport profiles between HPMCs and HUVECs might enable an educated guess as to which layer might be deteriorated.

SOLUTE TRANSPORT AND INTERCELLULAR LOCALIZATION OF TJPs UNDER OXIDATIVE CONDITIONS

It has been suggested that repeated exposure to fresh peritoneal dialysate induces an oxidative stress to peritoneal resident cells, particularly in long-term peritoneal dialysis. In the present study, we employed exogenous supplementation of H_2O_2 to assess the effect of ROS production and its effects on TJP organization. According to Makino *et al.*'s recent study, exogenous H_2O_2 is reduced in a stepwise manner mainly by glutathione when present in low concentrations, and by catalases when present in high concentrations (21). The crossover point is assumed to be around 0.1 mmol/L. In the present study, we employed supplementation of exogenous H_2O_2 at concentrations ranging from 0.01 to 1.0 mmol/L for the DCFH-DA study, and at a concentration of 0.1 mmol/L for the other experiments (TER, SPT, TJP staining, and GSH). Based on a previous report (22), it can be supposed that the actual intracellular concentrations are probably between one-seventh and one-tenth of the supplemented concentrations.

It has been widely reported that TER and/or solute permeability reflect a well-formed intercellular junction in various types of cells (3–9). The intercellular space between epithelial and endothelial cells is bridged by a set of specialized structures, namely, TJs or ZO, zonula adherens, desmosomes, and gap junctions. Tight junctions, the most apical structures of the junctional complex, act as a diffusion barrier by controlling the passage of ions, water, and other molecules as well as by maintaining the polarity of cells. Therefore, localization of TJPs is a key factor in comprehending solute transport through a cell layer. Loss of occludin localization was more prominent in HPMCs (Figure 7); this may be evidence enough to speculate that occludin plays a role in the barrier function for electrolyte mobility. A unique extracellular loop of occludin, which contains approximately 60% tyrosine and glycine residues, may partially participate in charge selectivity. Claudins, which are also TJP members, may contribute to the diversity of permeability across different cell types because there are 20 types of claudins expressing differently on each cell type (23). It is speculated that both number and type of TJPs differ between HPMCs and HUVECs, and their expressions along the lateral region might be attributed to the integrity of the extracellular matrices.

INTRACELLULAR PRODUCTION OF ROS

Oxidative stress is usually balanced by the oxidant and antioxidant capabilities of a cell. Scavenging of various types of oxidants occurs largely through H_2O_2 generation, followed by reduction of H_2O_2 to H_2O by an antioxidative enzyme, various peroxides, and catalases, resulting in an intracellular H_2O_2 concentration of <1 μmol/L (approximately 1 – 700 nmol/L) under physiological status (22).

DCFH-DA was used in our experiment to detect the intracellular production of ROS because DCFH-DA incorporated into the cells is immediately de-acetylated to DCFH by esterase, a membrane enzyme. This form of DCFH can remain inside the cell. When ROS exist in proximity to this compound, DCFH is oxidized to DCF, which is a fluorescein form. Almost all ROS can oxidize DCFH despite the different reactivities among the ROS, which are as follows (24):

$$\cdot OH > ONOO^- \gg ROO\cdot \gg H_2O_2,\ NO > {}^-OCl,\ O_2 > {}^1O_2$$

In our experimental conditions, both $\cdot OH$ and H_2O_2 were the major ROS detected by fluorescein intensity and resulted from the imbalance between the intracellular antioxidative activity and the excess amount of exogenous H_2O_2. While some exogenous H_2O_2 that passes through the membrane is reduced to H_2O by GSH and/or catalase, the remaining H_2O_2 would contribute to $\cdot OH$ generation by the gain of electrons from reduced-type

transient metal ions such as Fe^{2+} and Cu^{+}. The latter reaction, known as the Fenton reaction, is the first-order reaction until adequate amounts of reduced-type transient metal ions exist. As seen in Figure 8, there were significant dose-dependent increases in DCF intensity in both HPMC and HUVEC monolayers. In particular, the HPMC monolayer exhibited a stronger intensity than the HUVEC monolayer when exposed to a concentration of up to 1.0 mmol/L H_2O_2. The difference in the intensity between the HPMC and HUVEC monolayers reveals that the antioxidative capability of HUVECs is much stronger than that of HPMCs. This finding may be important in terms of the primary host defense capacity of HPMCs.

AMOUNT OF INTRACELLULAR GSH AND GSSG

These results help us understand the reasons for the differences in the amounts of antioxidants between the two monolayers. Glutathione (a tri-peptide: L-γ-glutamyl-L-cysteinyl-glycine) is the most abundant intracellular antioxidant responsible for regulating the oxidation–reduction balance.

The GSSG reductase–DTNB recycling method, which was employed in this study, is generally considered a specific, sensitive, rapid, and reliable method (19). Under oxidative stress, GSH is oxidized to GSSG, which is subsequently reduced to GSH by glutathione reductase to maintain a constant intracellular GSH concentration. Under an excess load of oxidative stress, such as 24-hour exposure to 0.1 mmol/L H_2O_2, which was applied in our experiment, the balance between GSH and GSSG shifted toward the oxidation state, as shown in Figure 9. Our results clearly indicate that the amount of GSH per milligram protein as well as total glutathione amount (GSH + GSSG) are significantly higher in HUVECs than in HPMCs.

Maintaining the integrity of the peritoneal mesothelial layer would contribute to the preservation of natural host defense, which plays an important role in susceptibility to foreign bodies (25). In their *in vitro* study, Shostak *et al.* demonstrated D-glucose-induced H_2O_2 production in rat peritoneal mesothelial cells (26). Lee *et al.* (18) have reported that a high concentration of glucose (100 mmol/L) causes DCFH-sensitive ROS generation in HPMCs; the intensity of the ROS corresponded to that of the supplemented 0.1 mmol/L H_2O_2.

Our basic *in vitro* results reasonably support the results of the *in vivo* preclinical study with respect to the use of antioxidative agents. Breborowicz *et al.* and Tager *et al.* demonstrated the effect of L-2-oxothiazolidine carboxylic acid, a glutathione precursor, on glucose-induced dysfunction of mesothelial cells (27,28). Based on these results, antioxidative treatment of the mesothelium to maintain peritoneal integrity may be important to prevent not only a hyperpermeable state but also epithelial-to-mesenchymal transition.

CONCLUSION

The HUVEC monolayer, which is less permeable to middle-to-high molecular weight solutes, is more tolerant against ROS stress than the HPMC monolayer in terms of maintaining solute transport characteristics. Availability of intracellular GSH is important to maintain the integrity of the peritoneum, particularly the mesothelium.

DISCLOSURE

I, Takashi Horiuchi, do not have any financial support or relationships with pharmaceutical companies or other entities such as employment contracts, consultancy, advisory boards, speaker bureaus, membership of Board of Directors, stock ownership.

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