Review

A Novel Strategy for a Drug Delivery System Using a Claudin Modulator

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Received June 1, 2006

With the continued progress in genomic drug discovery, the high-throughput production of drug candidates has become possible, and thus today there are a number of candidates that are extremely effective both in cell-free and in cell models. However, a drug delivery system suitable for the high-throughput production has yet to be fully developed. In tissues, the tight junction (TJ) plays a pivotal role as both a barrier to restrict various substances and in intra-tissue maintenance. Claudin, a *ca.* 23 kDa transmembrane protein with four transmembrane domains, is responsible for the TJ functions. Interestingly, for each of the 24 members of the claudin family, expression profiles and exact barrier functions differ. Therefore, claudin may be a potential target for use as a drug delivery system *via* a paracellular route. The C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE) is known to modulate the barrier function of claudin. We found that C-CPE is a potent absorption-enhancer and that this enhancing activity is 400-fold greater than clinically used enhancers. The enhancing activity examined in this study involved an interaction between C-CPE and claudin-4. These findings indicate that claudin might be a novel target for a drug delivery system. In the current review, we describe about background and data on our research about claudin modulator, and we also discuss the possibility of the use of the claudin family in a new approach for developing a drug delivery system.

Key words claudin; *Clostridium perfringens* enterotoxin; tight junction; drug delivery

INTRODUCTION

Recent progress in human genomic information has provided dramatic changes ranging from accidental to theoretical developments within the realm of drug discovery. Identification of lead compounds that can act as target proteins is proving to be easier through the use of combinatorial chemistry and high-throughput screening. This allows for more efficiently selecting compounds with clinically effective pharmacological activity. However, a large number of promising drug candidates are never developed as clinical drugs due to unacceptable pharmacokinetic properties.[1,2] On average, only 1 out of 9000—10000 compounds that are selected as potential candidates make it to the market. Potentially, there are a large number of the developmental drug candidates that could be very effective in therapy but end up being dropped during development due to problems related to low penetration of biological barriers. Epithelial cells surround mammalian organs and tissues and epithelial cell sheets constitute the principal barrier to cellular uptake and transport. For absorption of a drug into systemic fluid to occur, it must pass across epithelial cell sheets *via* epidermal and mucosal absorption. Likewise, for transfer of a drug from the systemic fluid into a target tissue it also must pass through endothelial cell sheets. Thus, passage across epithelial and endothelial cells is a major determinant of the pharmacokinetic properties of a drug. Routes for passing through these cell sheets can be classified into transcellular and paracellular routes (Fig. 1).[3—7] Drug delivery systems have been developed that allow for compounds to cross the cell sheets *via* both of these transcellular and paracellular routes.

Transcellular uptake of drug is mainly mediated *via* receptor- and transporter-dependent routes. Several receptors are expressed on the cell surface, some of which are responsible for the internalization of nutrients, such as folate, vitamin B_{12} and transferrin. For example, folate receptors are expressed to a greater extent in many human cancers as compared to normal cells. Therefore, by targeting the folate receptors it may be possible to develop a tumor-targeting drug delivery system.[8] Asialoglycoprotein receptors are expressed on the membranes of mammalian hepatocytes, and the asialoglycoprotein receptors have been used to target the liver.[9] On the contrary, many different drug transporters are expressed in various tissues, including intestinal and hepatic epithelial cells and brain capillary endothelial cells.[10—13] Some of these transporters are involved in the transportation of drugs, and can be used to determine the pharmacokinetic characteristics of a drug with regard to its intestinal absorption, tissue distribution, and elimination. To optimize the pharmacokinetic profiles of various drugs, attention needs to be paid to the drug delivery system with regard to the transcellular route and types of receptors and transporters that will be involved. While there are a number of receptors and transporters that are available for drug delivery, each drug needs to be modified in order for recognition of a drug by receptors

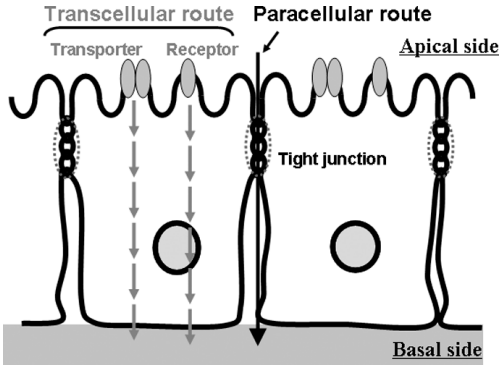

Fig. 1. Schematic Showing the Transport Route within the Epithelia

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and transporters to occur. Therefore, when attempting to maximize efficiency, it is necessary to try and develop a delivery system for each and every drug in order that they can specifically target tissues and organs.

The tight junction (TJ) is found in the intercellular space between epithelial cells, and acts as a barrier that prevents substances from leaking across epithelial cells. The paracellular route of drug delivery works *via* the intercellular space and is mediated by alterations of the TJ barriers in epithelial cells. As a way of developing absorption-enhancers in the intestine, the previous attempts that tried to increase the paracellular transport by loosening the TJ were made. However, unacceptable side effects were induced by most of the absorption-enhancers that were tried.[14,15] Enhancers of absorption are classified as Ca^{2+} chelators or surfactants.[14] Ca^{2+} chelators result in the disruption of adherent junctions, while surfactants cause exfoliation of the intestinal epithelium and irreversible compromise of the TJ barrier.[14] Sodium caprate is the only clinically used enhancer that works *via* the paracellular route. Sodium caprate increases paracellular permeability in TJs *via* a phospholipase C-dependent pathway.[16] Due to the modes of action of the paracellular route enhancers, these agents exhibit low tissue-specificity and high toxicity. In spite of the problems associated with the paracellular route, opening of the TJs by enhancers is an attractive method that might be suitable for high-throughput development of drug candidates. If a method to open TJs could be developed, this might be useful for various drug candidates without having to make further modifications of the drugs.

In previous experiments, we have focused on the possibility of a paracellular route as a method for drug delivery, and have found that a constituent of the TJs might be a promising candidate for use as a drug delivery system.[17] In the current review, we present information on the claudin modulator and on possible pathways for future research.

WHY PARACELLULAR ROUTE?

Differences exist between the drug delivery systems that have been designed for transcellular and paracellular routes. Since a drug has to be recognized by the transporter or receptor in the target tissue, the drug delivery system for each drug has to be prepared and modified for every target tissue within the transcellular route. Therefore, information about the structure-activity relationship of the drug that is to be used as well as the expression profiles of the receptors and transporters needs to be considered. Moreover, in some cases, a drug must be modified not only so that it can be recognized by receptors and transporters but also to ensure that its pharmaceutical activity can be maintained. Since the experimental approach for the development of a drug delivery system *via* the transcellular route has a limited efficacy, there has been a wide development of *in vitro* screening systems for transporters, and methods for rational predictions and extrapolations of a drug's disposition *in vivo* based on *in vitro* data.[5] In spite of these efforts, over 90% of promising candidates for brain diseases are never applied as a pharmaceutical therapy since the candidates have such low efficacy with regard to penetration of the blood-brain barrier. While it is true that the transcellular route is an ideal system for drug delivery, developing such a system for use as a pharmaceutical therapy will take a long time to be completed, as the mechanisms for the uptake of drugs into epithelial or endothelial cells, intracellular trafficking of drugs, and release of drugs on the basal side all have to be examined. Considering the number of various candidates with pharmaceutical activity that have been discovered, there is a great need to develop a drug delivery system that uses a different route.

Over the past 40 years, various investigations of absorption enhancers have been undertaken.[18—20] In Japan, Denmark and Sweden the absorption enhancer, sodium caprate, has been used in drug formulation. One strategy that has been used for absorption enhancers is to try and modulate the TJ barriers in the mucosal epithelia, after the opening of the TJs.[16, 21] The method that is used to open the TJs is applicable for any type of drug and can be utilized without having to modify the drug that is being tested. Therefore, this method is suitable for high-throughput drug discovery. However, the application of the absorption enhancers during the development of new drugs has not been fully adopted simply because the enhancers have low tissue-specificity and can cause the influx of substances other than the test compound into a target tissue. As a result, our group has been examining the utility and possibility of employing a drug delivery system that uses the paracellular route and which is based on the highly efficient drug discoveries that have been made using genomic, proteomic, and metabolomic information. After analyzing the problems associated with absorption-enhancers we drew the following conclusions: The limitations associated with absorption-enhancer use does not necessarily indicate that there will also be a limitation for drug delivery through the paracellular route. In fact, the present limitations are due to factors related to Barriology. Most of the previous investigations on absorption-enhancers were performed before 1998, a time for which key components of the TJ barrier function had yet to be identified. Since 1999, dramatic progress has been made in understanding the constitutive and functional aspects of TJs. New discoveries have documented variations of the barrier permeability of TJs for the epithelium and endothelium of different tissues and these variations are dependent upon physiological requirements.[3] Additionally, a molecular-based mechanism for these variations has been proposed.[22] Based on all of the new data that is currently available, the likelihood of being able to develop a paracellular route for drug delivery appears to have a much greater chance of succeeding at the present.

WHY CLAUDIN?

In order to develop a novel strategy for drug delivery through the paracellular route, the target molecule needs to be carefully considered. Since this molecule will play an essential role in the tissue-specific barrier function of TJs, we decided to focus our attention on members of the claudin family. TJs can be considered to be "complex biochemical machinery", composed of junctional adhesion molecules, occludin, and claudins (Fig. 2). The junctional adhesion molecule is a junction-associated membrane protein. However the junctional adhesion molecule's involvement with the TJ remains unclear at the present time.[23] Occludin is a four-transmembrane protein with a molecular mass of 65 kDa, and is the first molecule expected to be a pivotal constituent within

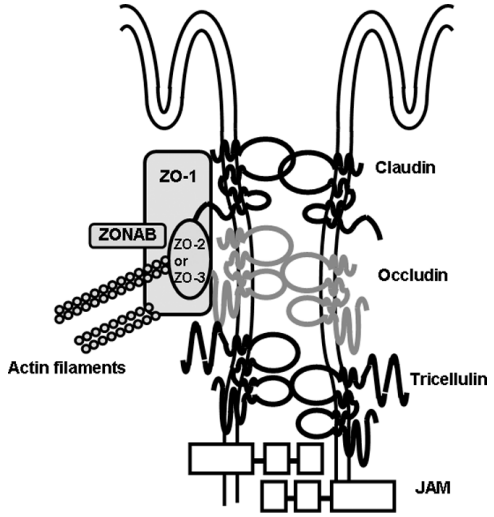

Fig. 2. A Model of Protein Interactions That Occur at Tight Junctions

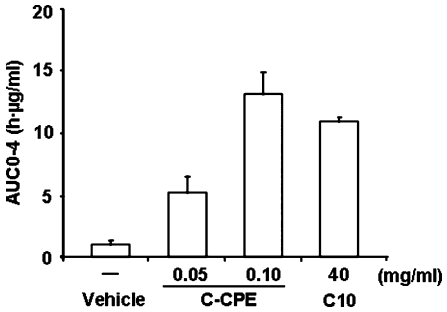

Fig. 3. Effect of C-CPE on Jejunal Absorption in Rats

Rat jejunum was treated with FD-4 (10 mg/ml) in the presence of vehicle C-CPE or C10. The FD-4 levels in plasma collected from the carotid artery were determined, and the AUC_{0-4h} was calculated. Data are means±S.E. ($n=4$).

the TJ barrier.[24—28] However, a knockdown of occludin does not result in the loss of the TJ barrier.[29] Tsukita *et al.* reported that claudin is a major constituent of the TJ.[22,30,31] Claudin has a tetra transmembrane domain and a molecular mass of *ca.* 23 kDa, with the family being composed of 24 members.[22] Within the TJs, the claudin family consists of forms that have both hetero and homo strands. There are "kissing points" within the TJs, where the distance between adjacent cells is essentially zero and intercellular space is obliterated. Expression profiles for the claudin family members differ from tissue to tissue, resulting in the formation of tissue-specific characteristics of the TJ barrier. For example, claudin-1 and claudin-5 deficient mice lose their epidermal and blood-brain barriers, respectively.[32,33] Claudin-11 plays a role in the blood-testis barrier.[34] Thus, the only molecule that has been definitively determined by molecular based research to have a role in the TJ barriers is claudin. Moreover, the formation of the hetero and homo strands by the 24 members of the claudin family provides ideal characteristics for the production and maintenance of numerous types of tissue-specific TJ barriers that are required by the different tissues.[30] Epithelial cells surround tissues and organs, and are responsible for the partition of every tissue and organ. In addition to being an essential component involved in the TJ barriers of epithelial cells, the claudin family is also pivotal in intra tissue maintenance.

Since claudin plays such an essential role in the formation of the barriers involved with the movement between cells and tissues, we attempted to develop a novel method of modulating the members of the claudin family that would allow for tissue-specific delivery of a drug *via* a paracellular route.

CLAUDIN IS A PROMISING MOLECULE FOR DRUG DELIVERY SYSTEM

In the next step, we searched for an inhibitor or modulator of claudin and discovered that the C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE) is capable of modulating claudin-4.[35] *Clostridium perfringens* enterotoxin (CPE) is a single polypeptide with a molecular mass of approximately 35 kDa, and which can cause food poisoning in humans.[36] CPE has a unique mechanism of action in which there is formation of large, sodium dodecyl sulfate-resistant complexes that involve TJ proteins. These complexes induce alterations of the plasma membrane permeability in the host's intestinal epithelial cells, leading to cell death and epithelial desquamation.[37] The functional domains of CPE can be separated into a receptor-binding region (C-terminal of CPE, C-CPE) and cytotoxic region (N-terminal of CPE). A receptor on the cell membrane for CPE was identified by Katahira *et al.* in 1997, which was prior to the first cloning of claudin.[38] In 1999, Tsukita's research group observed that there was significant sequence similarity for claudin-1 and -2 and the CPE receptor previously reported by Katahira *et al.* This CPE receptor was identical to claudin-4. Moreover, they revealed that C-CPE not only bound to claudin-4 but also disrupted the barrier-function of TJs formed by claudin-4.[35] Based on these findings, we decided to use C-CPE as a model of claudin modulator and to investigate whether claudin could be used for the development of a drug delivery system.

In the first step of our analysis, we evaluated the ability of C-CPE to deliver drug by examining the absorption-enhancing effects of C-CPE in rat jejunum. Using fluorescein isothiocyanate-dextran, (FD-4), as the model drug, we performed an *in situ* loop assay in the rat jejunum. FD-4 has a molecular mass of 4000 Da and is not absorbed *via* the transcellular route but by the paracellular route in epithelium. Therefore, FD-4 can be used as an indicator of passage through a paracellular route.[39] As shown in Fig. 3, there was a dose-dependent C-CPE enhanced absorption of FD-4, and this enhanced activity of C-CPE was 400-fold over that observed for sodium caprate (C10), which is routinely used clinically as an enhancer.[17] There was no mucosal toxicity (*i.e.*, leakage of lactose dehydrogenase from intestinal mucosa and histological evaluations) observed with the C-CPE (0.1 mg/ml) treatments. However, with C10 treatments (40 mg/ml), leakage of lactose dehydrogenase was induced and histological damage was observed in the rat jejunum. Thus, C-CPE is both a safe and a potent enhancer.[17] To evaluate size-dependent limitations of the absorption-enhancing effects of C-CPE, we investigated the absorption of fluorescein-isothiocyanate dextrans with various molecular masses. As seen in Fig. 4, a remarkable absorption was observed by C-CPE treatments using dextrans with molecular masses of up to 10000 Da (16.4-fold and 15.0-fold for FD-4 and -10, respectively).

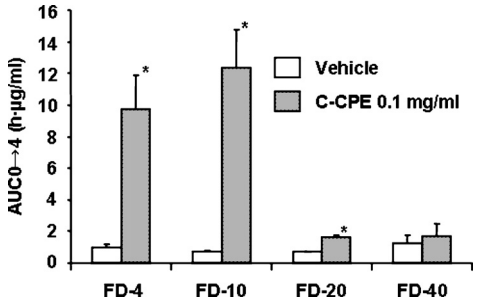

Fig. 4. Dependence of the Enhancement of Absorption by C-CPE on the Molecular Mass of Dextran

Rat jejunum was treated with FD-4, -10, -20, or -40 (10 mg/ml) in the presence of vehicle or C-CPE (0.1 mg/ml). FD levels in plasma collected from the carotid artery were determined, and the $AUC_{0-4\,h}$ was calculated. Data are means±S.E. ($n=4$). * Significant difference from the vehicle-treated group ($p<0.05$).

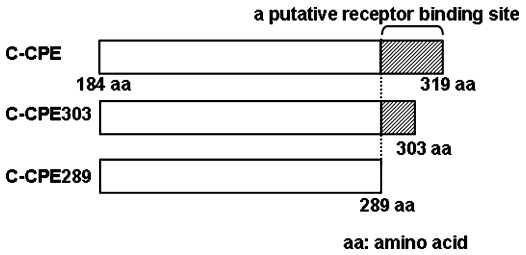

Fig. 5. Schematic Diagram of C-CPE, C-CPE289 and C-CPE303

A putative receptor-binding region for C-CPE is located on its C-terminus and is shown here as the slashed area.[42]

Stokes radius for the FD-4, FD-10, FD-20, and FD-40 were calculated to be 1.4, 2.3, 3.3, and 4.5 nm, respectively. Under physiological conditions, absorption-enhancer epithelial cell treatments resulted in an opening of the cavity of the TJs from 0.5 nm up to 1.5 nm.[40,41] Using histofluorescent observation, we also found that FD-4 was absorbed across epithelial sheets. These data suggest that C-CPE enhances the absorption of dextran by modulation (opening) of the TJs.

C-CPE is a C-terminal fragment composed of the CPE amino acids 184 to 319.[38] The receptor binding region of CPE has been reported to be in the C-terminal 30 amino acids region of CPE.[42] These previous reports indicate that the receptor binding region is identical between CPE and C-CPE. To clarify the involvement of claudin in C-CPE's absorption-enhancing effect, we prepared all of and part of the binding region of CPE-deleted mutant C-CPE289 and C-CPE303, respectively (Fig. 5). C-CPE had absorption-enhancing activity at 0.1 mg/ml, but C-CPE289 and C-CPE303 did not exhibit any absorption-enhancing activity even at 0.2 mg/ml (Fig. 6). Treatment of the Caco-2 monolayer cells with C-CPE resulted in a decrease in the transepithelial electric resistance value, which is an index of the TJ barrier. When the binding region was deleted, a loss of the reducing activity of the transepithelial electric resistance values was observed.[43] To evaluate the interactions between claudin-4 and the mutant C-CPEs, we performed a pull down assay using rat jejunum lysates and Caco-2 lysates. Pull down of claudin-4 was achieved by addition of C-CPE in the rat jejunum and Caco-2 lysates, but not by the addition of C-CPE289 or C-CPE303.[17,43] These results indicate that the interaction of C-CPE with claudin-4 is an essential part of the absorption-enhancing activity of C-CPE.

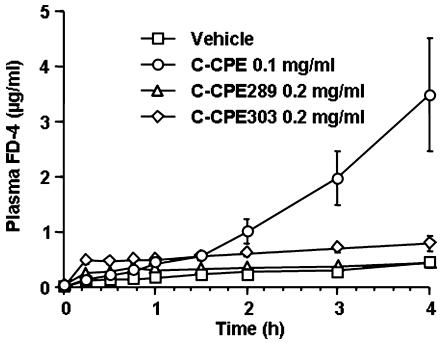

Fig. 6. Effect of C-CPE, C-CPE289, and C-CPE303 on Absorption

Rat jejunum was treated with FD-4 (10 mg/ml) in the presence of C-CPE, C-CPE289, or C-CPE303 (0.1 or 0.2 mg/ml). FD-4 levels in plasma collected from the carotid artery were determined at the indicated points. Data are means±S.E. ($n=4$).

Interestingly, the enhancing effect of C-CPE was observed in rat jejunum but not in rat colon, and in contrast, the enhancing effect of C10 was observed in both rat jejunum and colon.[17] Although the mechanism of tissue-specific absorption-enhancing effects of C-CPE has yet to be proven, the specificity observed for claudin might suggest that it is indeed a tissue-specific enhancer through the paracellular route. Thus, we propose that modulation of TJs through the targeting claudins could be a novel strategy for the use of a paracellular route of drug delivery.

PREPARATION OF A CLAUDIN-TARGETING MOLECULE

As described above, a claudin modulator could be a promising candidate for the development of a new drug delivery system. There are 24 member of the claudin family, but CPE can only bind to some of the family members, including claudin-3, -4, -6, -7, -8 and -14.[44] This also indicates that C-CPE will bind to these same claudin members. Claudin-1 and -5 are possible targets for the development of transepidermal and trans-blood-brain-barrier drug delivery systems, respectively.[32,33] However, these reports indicate that C-CPE in and by itself is insufficient as a drug delivery system. Therefore, the properties of C-CPE need to be modified in order to ensure the claudin modulator will have a narrow claudin inhibitory range and that the claudin modulator will be able to regulate the claudin-1 or -5-barrier function. Within this context, it is very important to be able to know whether or not the claudin-binding domain and TJ barrier disruptions are associated or if they are separated. Hanna *et al.* reported that deletion of the receptor binding region of CPE attenuated cytotoxicity of CPE.[42] We found that mutant C-CPEs without properties that allow binding to claudin-4 were not capable of modulating the TJ barrier.[17,43,45] Binding of C-CPE to claudin is, at least, a determinant factor. Thus, we hypothesized that various type of claudin modulators could be produced by utilizing C-CPE as a prototype of the claudin modulator. We pursued this by determining the functional residues required for binding of C-CPE to claudin-4 and prepared a screening system for claudin modulators. Since then, we have been investigating key residues that take part in the interactions between claudin-4 and C-CPE by using site-directed mutagenesis. In current ongoing experiments, we have discovered that there are some amino acid

residues that are indeed involved in the association of C-CPE with claudin-4. Data on these functional residues of C-CPE will be submitted for publication in the near future.

To prepare the screening system for the claudin modulators, we introduced a phage display system developed by Dr. Tsutsumi (NIBI, Japan). This system uses a protein synthesis inhibitory factor (PSIF) as a reporter protein. Ligand molecule, which is fused to PSIF, is displayed on the coat of the phage and is then released into a culture medium of *E. coli*, as the phagemid vector has a signal sequence for the transfer of protein into periplasm. Therefore, this allows for screening of a ligand molecule for a target molecule by determining the affinity for the target molecule. Subsequently this is followed by an assay for cytotoxicity against the target molecule-expressing cell in order to confirm binding of the ligand molecule to the target on the membrane. To check whether C-CPE can be used as a claudin-4-targeting molecule, we prepared C-CPE-fused PSIF (C-CPE-PSIF). C-CPE-PSIF is toxic to the human breast cancer cell line MCF-7 cells, endogenous claudin-4-expressing cells, but does not affect mouse fibroblast cells, claudin-negative cells.[46] Pretreatment of MCF-7 cells with C-CPE attenuated C-CPE-PSIF-induced cytotoxicity, indicating that C-CPE-PSIF interacts with the cells *via* its C-CPE-domain. Pretreatment with bovine serum albumin had no effect. C-CPE is known to bind to claudin-4 *via* its C-terminal 30 amino acids.[17] To investigate whether interaction of C-CPE-PSIF with claudin-4 on the cell membrane is essential for the cytotoxicity of C-CPE-PSIF to occur, we prepared a deleted mutant C-CPE-PSIF without the claudin-4-binding regions. Deletion of the C-terminal 30 amino acids of C-CPE attenuated the cytotoxicity of C-CPE-PSIF.[46] We also checked the cytotoxicity of C-CPE-PSIF in claudin-expressing L cells, and found C-CPE-PSIF to be toxic to claudin-4-expressing L cells, but not to claudin-1, -2, -5-expressing L cells (Fig. 7). These data are in agreement with previous reports on the claudin-specific cytotoxicity of CPE.[44] Thus, these results indicate that the C-CPE region of C-CPE-PSIF associates with claudin-4 on the cell membrane. Therefore, the targeting property of C-CPE-PSIF reflects the targeting property of C-CPE. We have also confirmed that C-CPE-PSIF-displayed phage interact with claudin-4 proteins (unpublished data).

Overall, in this set of studies, we have been able to prepare a C-CPE-PSIF library with random mutations that bind residues of C-CPE to claudin-4. In addition, we have been able to screen various types of claudin modulators using this C-CPE-PSIF-displayed phage library.

FUTURE STUDIES

Claudin forms hetero- and homo-strands on the membrane, and strand pairs between adjacent cells.[47] Claudin is composed of 24 family members, which allows for various combinations of the claudin strands to be formed. These variations of the claudin strands are hypothesized to play pivotal roles in the maintenance of intra-tissue specific circumstances.[22] For example, overexpression of claudin-11 or -15 in MDCK II cells results in elevation of the TJ barriers, while overexpression in LLC-PK1 cells results in the reduction of TJ barriers.[48,49] Claudin-3 forms strands with claudin-1 or -2, but when claudin-1 and -2 are combined, they do not form strands.[50] Claudin-5 is expressed in many tissues, such as liver, kidney, lung, etc., but claudin-5 is responsible for only the blood-brain TJ barriers.[33] The claudin system is the only system that can explain how various compartmental areas in the body are maintained.

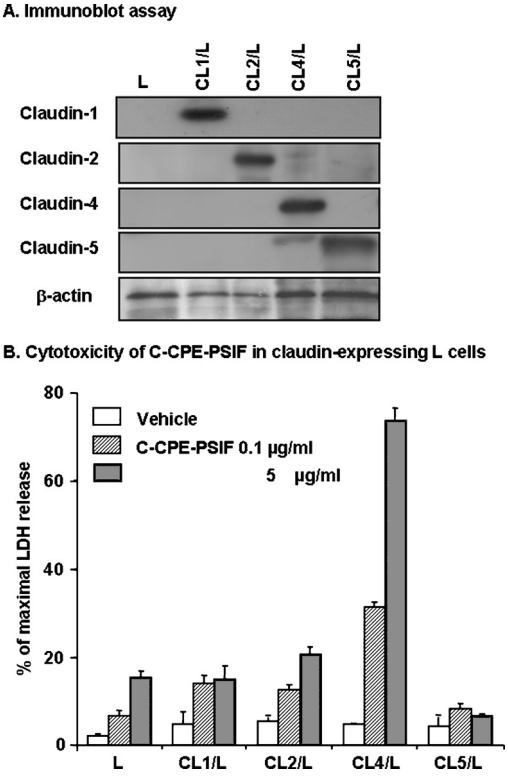

Fig. 7. Involvement of Claudin-4 in the Cytotoxicity of C-CPE-PSIF

(A) Immunoblot analysis of claudin-expressing L cells. The expression profiles for claudin family members were determined by immunoblotting. (B) Cytotoxicity of C-CPE-PSIF in claudin-expressing L cells. L cells expressing claudins were treated for 36 h with the indicated concentrations of C-CPE-PSIF, after which LDH release was assessed. Results represent the means±S.D. ($n=3$).

Thus, since the claudin system has a pivotal role in tissue-specific barrier-functions, this makes it an ideal target for the development of a drug delivery system using the paracellular route. Previous approaches for absorption-enhancement that have used Ca^{2+} chelators or sodium caprate are not based on Barriology. However, our proposed approach for a drug delivery using a claudin modulator is based on the Barriology strategy. If we can design a way to freely modulate the functions of the claudin system, this will allow us to deliver drugs to a target tissue *via* a paracellular route. The specific opening or closing of TJs may be a potent drug delivery system that could be applied for various types of drugs. Over 90% of drugs that are thought to be effective against brain disease have never been used as pharmaceutical therapies for the simple reason that they have a very low penetration of rate of the blood-brain-barrier. With development of a claudin-5 modulator, this could potentially open the way to successfully treat over 2 million patients with brain disease.

However, the major problem present in the past with systems that attempted to use the modulation of the TJ barriers for drug delivery has been the influx of other substances that occur along with the administered drug. Similar to the past systems, our proposed strategy using a claudin modulator would also be subject to this problem. Therefore, in the fu-

ture, we will need to determine if there is an influx of other substances besides the administered drug and additionally, we will need to investigate if there are any resulting side effects associated with this type of drug administration. But, by using a claudin modulator approach, it may be possible to achieve a much greater specificity since the system uses a molecular-based method to enhance absorption or delivery of a drug. Additionally, since the inhibitory effects of a claudin modulator will be specific for only the targeted claudin system, this also would decrease the likelihood of side effects. Knockout mice studies have revealed that deficiencies in claudin can result in an influx of substances only up to a molecular size of 1000 Da and which are tissue-specific.[32,33] Taken together, a claudin modulator-based drug delivery system would have a much more unique drug administration strategy compared to the other delivery methods. With regard to side effects, compared to other systems, such as those that use chelators and sodium caprate, a claudin modulator-induced influx of substances other than the administered drugs would most likely be dramatically reduced due to the specificity of the modulator. Further investigation of a claudin modulator drug delivery system using C-CPE as a prototype needs to be performed in order that it can be used clinically in the future.

In this review, we have presented the relevant history on the development of a drug delivery system that employs the use of modulation of the claudin system. Other approaches have been reported on the use of TJ modulators. Wong and Gumbiner found that modulation of occludin when using a peptide that corresponds to the portion of the extracellular domain of occludin, which is a TJ protein, resulted in the reduction of the TJ barrier in epithelial cells.[28] Very recently, tricellulin was identified as playing a pivotal role in the barrier function of a tricellular junction located at a tricellular space among three epithelial cells.[51] A modulator of tricellulin may also be useful as a drug delivery system *via* a paracellular route. In Japan, at the beginning of this century, Drs. Tsukita and Furuse made new breakthroughs concerning our knowledge of Barriology. With constitutive information on TJs now accumulating, further data on the functional and mechanical aspects of TJ barriers will undoubtedly be forthcoming. It is our hope that in the years to come, we will be able to develop a drug delivery strategy based on these original tenets of barriology that have been discovered and reported in Japan.

Acknowledgements We thank Drs. Y. Horiguchi and Y. Tsutsumi for providing us with the C-CPE-expressing plasmid and PSIF-expression system, respectively. We also thank Dr. H. Mizuguchi for his continuous encouragement, and his helpful comments and discussion. And finally, we would like to thank all of the members of our laboratory for their assistance with this research. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, SHISEIDO Grant for Scientific Research, and TAKEDA Science Foundation.

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