

Impact of Glucagon-like Peptide-1 Receptor Agonists on Intestinal Epithelial Cell Barrier

Original Paper

Takizawa Y.¹✉, Kato A.¹, Onsui A.¹, Kanatanai S.¹, Ishimura A.², Kurita T.¹, Nakajima T.¹

¹Division of Clinical Pharmaceutics, Department of Pharmaceutical Sciences, Nihon Pharmaceutical University, Saitama, Japan

²Division of Practical Pharmaceutical Sciences, Department of Pharmaceutical Sciences, Nihon Pharmaceutical University, Saitama, Japan

Received 30 January, 2024, accepted 21 May, 2024

Abstract While many types of diabetes medications are currently available, orally administered formulations of glucagon-like peptide-1 (GLP-1) receptor agonists have recently been launched. Therefore, gastrointestinal epithelial cells will be increasingly exposed to GLP-1 receptor agonists; however, their effects on these cells remain unclear. The present study attempted to clarify the effects of GLP-1 receptor agonists on intestinal epithelial barrier functions. Semaglutide (5, 50, and 500 ng/mL) and dulaglutide (15, 150, and 1500 ng/mL) were selected as GLP-1 receptor agonists and applied to the Caco-2 cell line. Changes in mRNA and protein expression levels of epithelial cell barrier regulators due to exposure to GLP-1 receptor agonists were examined by real-time RT-PCR and Western blotting. Neither semaglutide nor dulaglutide changed the growth rate or ratio of Caco-2 cells. Furthermore, they did not significantly affect the mRNA expression levels of membrane proteins involved in epithelial cell barrier functions. However, dulaglutide increased the protein expression levels of these membrane proteins in a concentration-dependent manner, whereas semaglutide did not. Only dulaglutide enhanced epithelial cell barrier functions. Since various gastrointestinal symptoms develop in patients with diabetes and epithelial cell barrier functions may be compromised, medicines that promote barrier function, such as dulaglutide, may effectively attenuate these changes. However, their mechanisms of action remain unknown; therefore, further studies are warranted.

Keywords GLP-1 receptor agonist – intestinal epithelial barrier – dulaglutide – semaglutide – Caco-2

INTRODUCTION

The number of patients with diabetes worldwide has increased to 537 million, with a 3.6-fold increase occurring in the last 20 years. The prevalence of diabetes has reached 10.5%, affecting 1 out of 10 adults, and the global diabetes population is predicted to increase to 783 million by 2045 (IDF Diabetes Atlas 10th edition). Common reasons for the increased prevalence of type 2 diabetes include a lack of exercise and physical inactivity and greater numbers of overweight and obese individuals.

Diabetes is commonly associated with gastrointestinal symptoms, such as nausea, vomiting, diarrhea, abdominal pain, and constipation (Bytzer et al., 1996; Maleki et al. 2000), and affects most parts of the gastrointestinal tract. Gastric motility is impaired in the stomach of diabetics, with delayed gastric emptying and gastroparesis being reported (Camilleri, 2007; Intagliata & Koch, 2007). Furthermore, it was reported that diabetes mellitus is associated with structural changes in the connective tissue matrix and in the muscles in the wall of intestine and colon and further causes biomechanical remodeling (Zhao et al., 2017). Although

the diabetes-induced dysfunctions in the small and large intestines are becoming clearer (Horváth et al., 2015), the effects of diabetes on the functions of the small intestine and colon have yet to be clarified in detail. The prevalence of constipation and diarrhea is high in patients with diabetes. A previous study suggested that the intestinal transit time was slower in animal models of diabetes, leading to bacterial overgrowth and, ultimately, diarrhea (Sellin & Hart, 1992), while other studies reported an accelerated intestinal transit time in some models (Rosa-e-Silva et al., 1996) and the induction of oxidative stress (Yarandi & Srinivasan, 2014). The colonic transit time is often increased, and constipation is a common symptom in patients with diabetes mellitus (Imaeda et al., 1998).

Due to the overwhelming number of patients with diabetes, the development of antidiabetic drugs is still actively conducted. Some traditional diabetes medicines include biguanides, α -glucosidase inhibitors, and thiazolidinediones, while others are relatively new, such as dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and sodium-glucose cotransporter 2 (SGLT2) inhibitors, which were launched after 2000.

* E-mail: y-takizawa@nichiyaku.ac.jp

Although the main route of administration of GLP-1 receptor agonists is subcutaneous, orally administered GLP-1 receptor agonists (Ozempic, semaglutide, Novo Nordisk Pharma Ltd.) were approved in the United States in 2019 and in Japan in 2020. Since GLP-1 receptor agonists have been shown to not only improve blood glucose levels but also reduce body weight (Okamoto et al., 2021), they are used as diet medications in the United States and may be taken by non-diabetic patients. Therefore, the use of orally administered formulations of GLP-1 receptor agonists will continue to increase in the future.

GLP-1 receptor agonists exert inhibitory effects on oxidative stress-induced disorders, such as renal ischemia-reperfusion injury (Zheng et al., 2019; He et al., 2020; Tiba et al., 2023); therefore, they may also be used to treat digestive diseases caused by diabetes-induced oxidative stress. On the other hand, the administration of GLP-1 receptor agonists for weight loss has been shown to delay gastric emptying in non-diabetic patients (Klein & Hobai, 2023), and GLP-1 receptor agonists were found to be cytotoxic at high concentrations in a human neuroblastoma cell line (Salles et al., 2023). These findings indicate that GLP-1 receptor agonists exert negative effects on the gastrointestinal tract and, thus, may promote digestive disorders associated with diabetes. Therefore, the effects of GLP-1 receptor agonists on the gastrointestinal tract are controversial. Since these effects were observed from a vascular approach after subcutaneous administration, limited information is currently available on their effects from an apical approach of gastrointestinal epithelial cells (Takizawa et al., 2022). The bioavailability of orally administered formulations of GLP-1 receptor agonists is less than 1% (Overgaard et al., 2021), indicating that the majority of an ingested GLP-1 receptor agonist will remain in the gastrointestinal tract and continue to be exposed to gastrointestinal epithelial cells. Therefore, the effects of GLP-1 receptor agonists on gastrointestinal epithelial cells need to be clarified in order to confirm their safety and efficacy. The present study investigated the effects of GLP-1 receptor agonists on gastrointestinal epithelial cells, particularly barrier functions.

MATERIALS AND METHODS

Medicines and reagents

Ozempic® (semaglutide, M.W.: 4113.6, Novo Nordisk) and Trulicity® (dulaglutide, M.W.: 63,000, Eli Lilly) were used as GLP-1 receptor agonists. All other reagents were of an analytical grade or higher.

Cell culture

The Caco-2 cell line was obtained from the Riken Cell Bank (Ibaraki, Japan) and kept in a humidified incubator at 37°C with 5% CO₂. Cells were maintained in DMEM-High glucose (Fujifilm Wako, 044-29765, Osaka, Japan) supplemented with 10% fetal

bovine serum (SELBORNE, FBS-04, Tasmania, Australia), 0.1 mM non-essential amino acids (Fujifilm Wako, 139-15651, Osaka, Japan), 2 mM L-glutamine (Fujifilm Wako, 073-05391, Osaka, Japan), 100 U/mL of penicillin, 100 µg/ml of streptomycin, and 250 ng/mL of amphotericin B (Fujifilm Wako, 161-23181, Osaka, Japan) and were used between passages 13–18.

Proliferation assay

In the growth assay, cells were seeded on a 96-well culture plate (1.0×10^4 cells/well) and cultured for 24 h to allow for adherence. Thereafter, the culture medium was replaced with a fresh medium with/without each concentration of semaglutide and dulaglutide and incubated for 96 h.

To assess cell proliferation, relative cell numbers were measured using crystal violet staining for adherent cells. Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min, stained with a 0.04% crystal violet aqueous solution for 30 min, and dissolved in a 1% SDS solution. Cell viability was estimated by measuring absorbance on a microplate reader (SpectraMax iD5, Molecular Devices, LLC., CA, USA) at a wavelength of 560 nm.

Cell viability assay

In the cell viability assay, cells were seeded on a 96-well culture plate (1.0×10^4 cells/well) and cultured to confluency (approximately 96 h). Cells were then treated with each concentration of semaglutide and dulaglutide and incubated for 24 h.

To assess cell viability, relative cell numbers were measured using crystal violet staining for adherent cells. Cells were fixed with 4% paraformaldehyde in PBS for 10 min, stained with a 0.04% crystal violet aqueous solution for 30 min, and dissolved in a 1% SDS solution. Cell viability was estimated by measuring absorbance on a microplate reader (SpectraMax iD5, Molecular Devices, LLC., CA, USA) at a wavelength of 560 nm.

Western blotting

Cells were seeded on 12-well plates (1.0×10^5 cells/well), incubated until confluent, treated with each concentration of semaglutide and dulaglutide, and then incubated for 48 and 96 h. Cells were lysed with Laemmli Sample Buffer (Sigma Aldrich Co., Ltd., Tokyo, Japan). Proteins were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked in 5% skim milk in Tris-buffered Saline with Tween 20 (TBST) at room temperature for 1 h and incubated with primary antibodies (GLP-1 receptor: GLP1R Rabbit pAb, A13990, ABclonal, P-gp: P-Glycoprotein antibody [C219], GTX23364, GeneTex, Claudin-4: Claudin 4 Polyclonal Antibody, bs-2790R, Bioss Antibodies, Claudin-7: Claudin 7 Polyclonal Antibody, bs-8482R, Bioss Antibodies, MUC1: MUC1 Rabbit pAb, A0333, ABclonal, MUC2: MUC2 Rabbit

mAb, A4767, ABclonal, β -actin: Beta Actin Mouse Monoclonal antibody, 66009-1-Ig, ProteinTech) at 4°C overnight. After washing three times in TBST, PVDF membranes were incubated with a secondary antibody solution at room temperature for 1 h. PVDF membranes were reacted with a chemiluminescent reagent (Pierce ECL Western Blotting Substrate, 32106, Thermo Scientific), and their protein bands were visualized on ChemiDoc touch (Bio-Rad). Each protein expression level was normalized by β -actin.

RNA extraction and cDNA synthesis

Cells were seeded on 12-well plates (1.0×10^5 cells/well), incubated until confluent, treated with each concentration of semaglutide and dulaglutide, and then incubated for 48 and 96 h. Total RNA isolation from Caco-2 cells were performed using TRIzol reagent (Thermo Fisher Scientific K.K, 15596026, Tokyo, Japan) following the manufacturer's instruction. To obtain cDNA, 1 μ g of RNA was subjected to reverse transcription using the ReverTra Ace qPCR RT kit (TOYOBO Co., Ltd., Tokyo, Japan).

Real-time RT-PCR

Real-time RT-PCR was performed using THUNDERBIRD SYBR qPCR Mix (TOYOBO Co., Ltd., Tokyo, Japan) on CFX Maestro (Bio-Rad Laboratories, Inc., CA, USA). The specificity of the primers was theoretically tested by the BLAST database. The sequences of all primers are shown in Table 1. Each experiment was performed in duplicate and repeated for each condition tested. The mRNA levels of all genes were normalized using the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. All quantification were performed three times independently.

Statistical analysis

All results are expressed as the mean $\pm$ standard deviation (S.D.). The significance of differences between groups was analyzed using Tukey's test; $P < 0.05$ was considered to be significant.

RESULTS

Effects of GLP-1 receptor agonists on GLP-1 receptors in Caco-2 cells

Caco-2 cells were exposed to 5, 50, and 500 ng/mL and 15, 150, and 1500 ng/mL of semaglutide and dulaglutide, respectively, and neither affected the proliferation rate or ratio of Caco-2 cells (Fig. 1a, 1b). Furthermore, confluent Caco-2 cells were exposed to semaglutide and dulaglutide in order to assess their cytotoxicities, and no effects were noted for either (Fig. 1c, 1d). On the other hand, the protein expression level of the GLP-1 receptor was not significantly changed by semaglutide, whereas dulaglutide exerted significant concentration- and time-dependent effects (Fig. 1e, 1f).

Effects of GLP-1 receptor agonists on mRNA expression levels of epithelial cell barrier factors in Caco-2 cells

The present study examined the effects of GLP-1 receptor agonists (exposure for 48 and 96 h) on the mRNA expression levels of epithelial cell barrier factors in Caco-2 cells. The mRNA expression level of ABCB1, which codes P-glycoprotein (P-gp), was not significantly changed by the 48- or 96-h exposure to semaglutide and dulaglutide (Fig. 2a, 2b). Although the mRNA expression levels of Claudin-4 and Claudin-7, components of tight junctions (TJ), were not also significantly affected by the 48- or 96-h exposure to semaglutide and dulaglutide (Fig. 2c, 2d, 2e, 2f), slight decreases were observed in a semaglutide concentration-dependent manner. In contrast, slight increases were noted in a dulaglutide concentration-dependent manner after the 96-h exposure.

Effects of GLP-1 receptor agonists on protein expression levels of epithelial cell barrier factors in Caco-2 cells

Next, the effects of GLP-1 receptor agonists (exposure for 48 and 96 h) on the protein expression levels of epithelial cell

Table 1 Sequences of primers used for real-time RT-PCR

Gene	Sequence (5'-3')		Amplicon Size	GenBank ID
ABCB1	Forward	CAGAGGGGATGGTCAGTGTT	87 bp	AF016535.1
	Reverse	CCTGACTCACCACCAATG		
Claudin-4	Forward	CATCTCCTCTGTTCGGGTA	91 bp	NM_001305.5
	Reverse	AAGGCCTCAGCCATACTCCT		
Claudin-7	Forward	CCCTCCACCTTTTGTGGCC	116 bp	NM_001307.6
	Reverse	GCACAGGGAGTAGGATACGC		
GAPDH	Forward	ACCAGGGCTGCTTTAACTCTG	104 bp	NM_001256799.3
	Reverse	TGGGTGGAATCATATTGGAACAT		

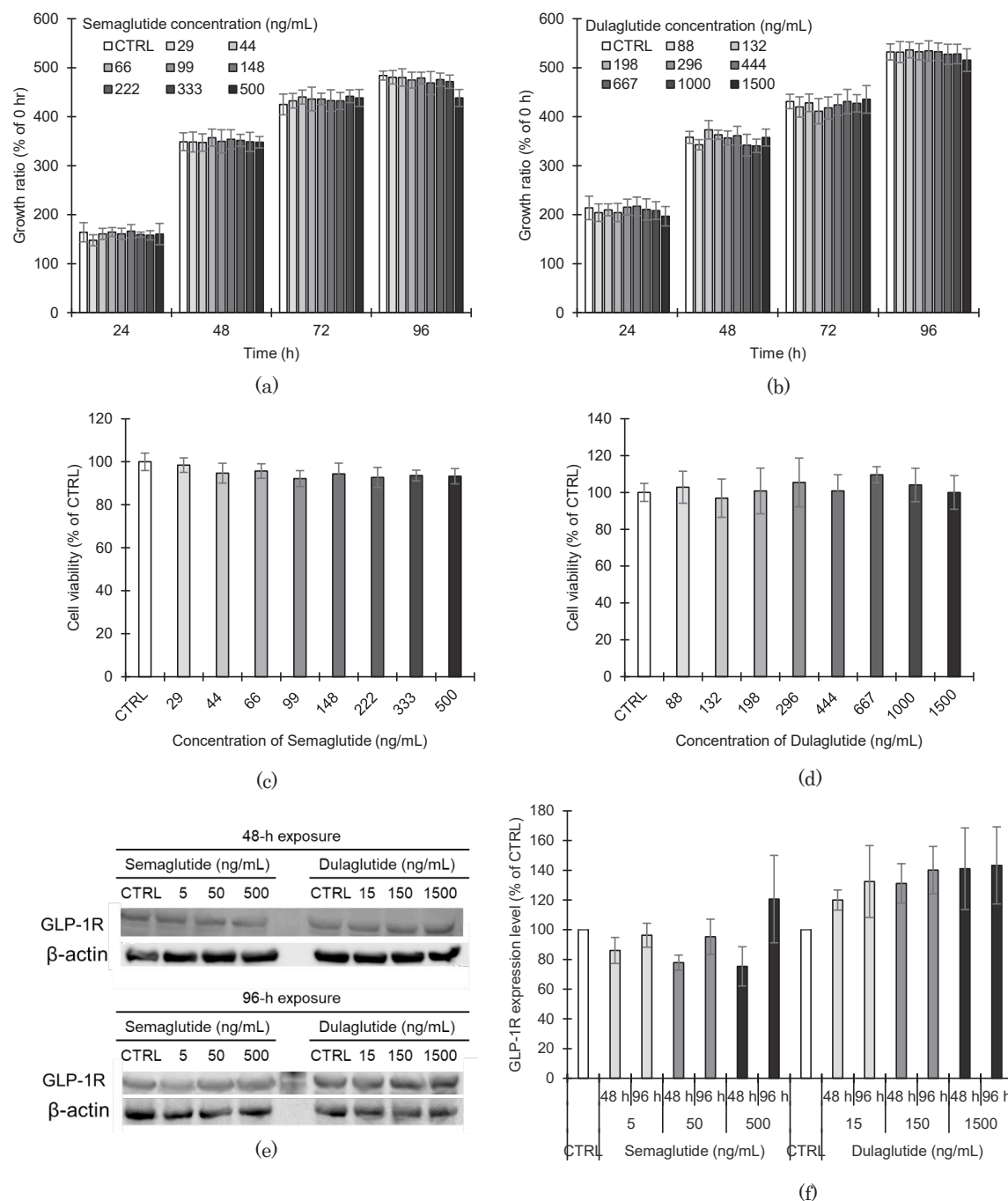


Figure 1. Effects of semaglutide and dulaglutide on Caco-2 cells. (a, b) Cell proliferation, (c, d) cell viability, and (e, f) GLP-1 receptor protein expression. Data represent means and S.D. (n = 3-6 for each condition). *P < 0.05 vs. the control (CTRL) condition

barrier factors in Caco-2 cells were examined, and their typical bands are shown in Figs. 3a and 3b. The protein expression levels of MUC1 and MUC2, components of unstirred water layer (UWL), were decreased by the 48-h exposure to semaglutide (Fig. 3i), whereas those of other proteins were not. Furthermore, the 96-h exposure to semaglutide did not

affect the expression levels of any of the proteins examined (Fig. 3). In contrast, in cells exposed to dulaglutide, significant concentration- and exposure time-dependent increases were noted in the protein expression levels of P-gp, Claudin-4, Claudin-7, MUC1, and MUC2 (Fig. 3).

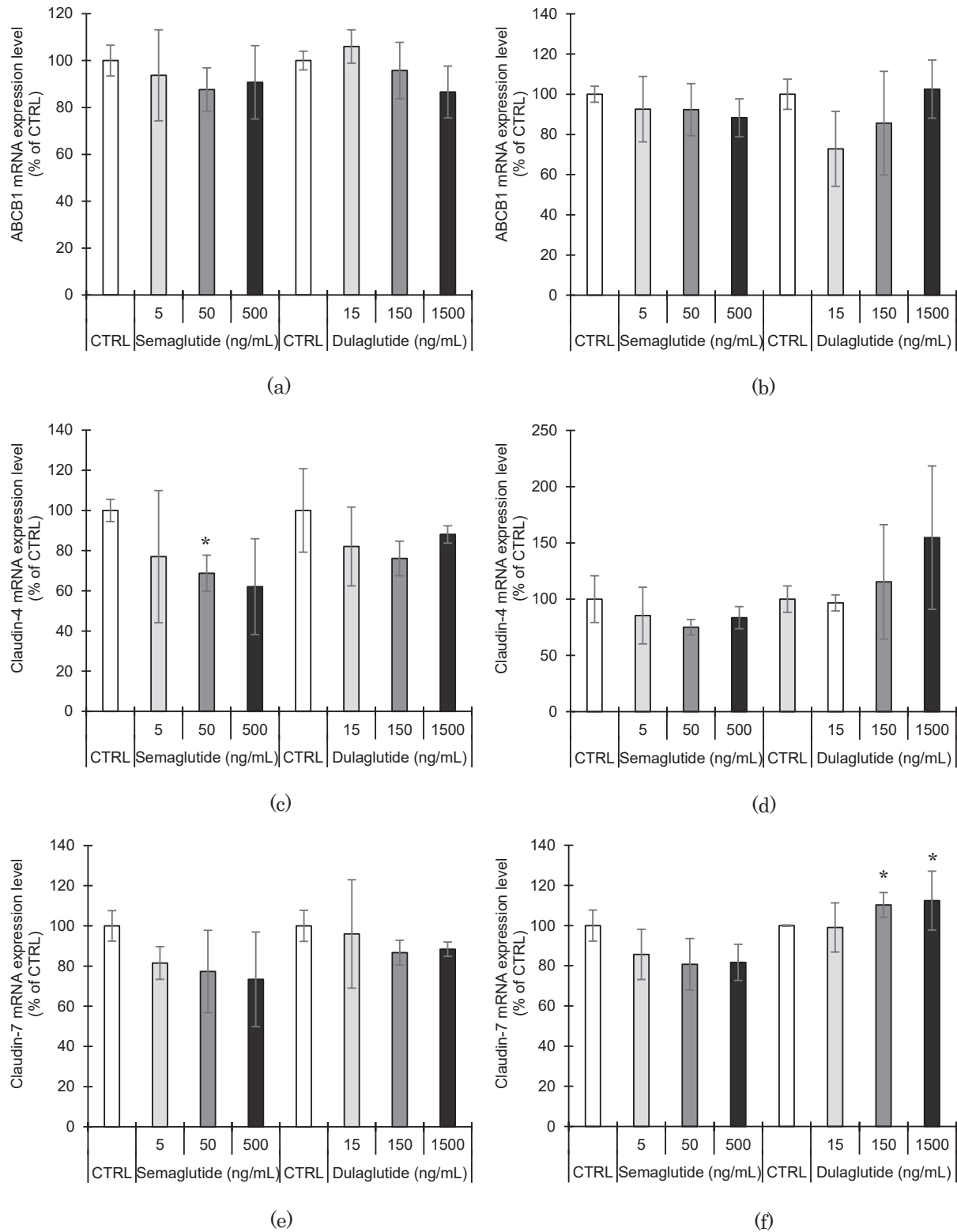


Figure 2. Effects of semaglutide and dulaglutide on mRNA expression levels of epithelial barrier function-related factors in Caco-2 cells. (a, b): ABCB1, (c, d): Claudin-4, and (e, f): Claudin-7. Exposure to a GLP-1 receptor agonist for 48 h (a, c, e) and 96 h (b, d, f). Data represent means and S.D. ($n = 3$ for each condition).

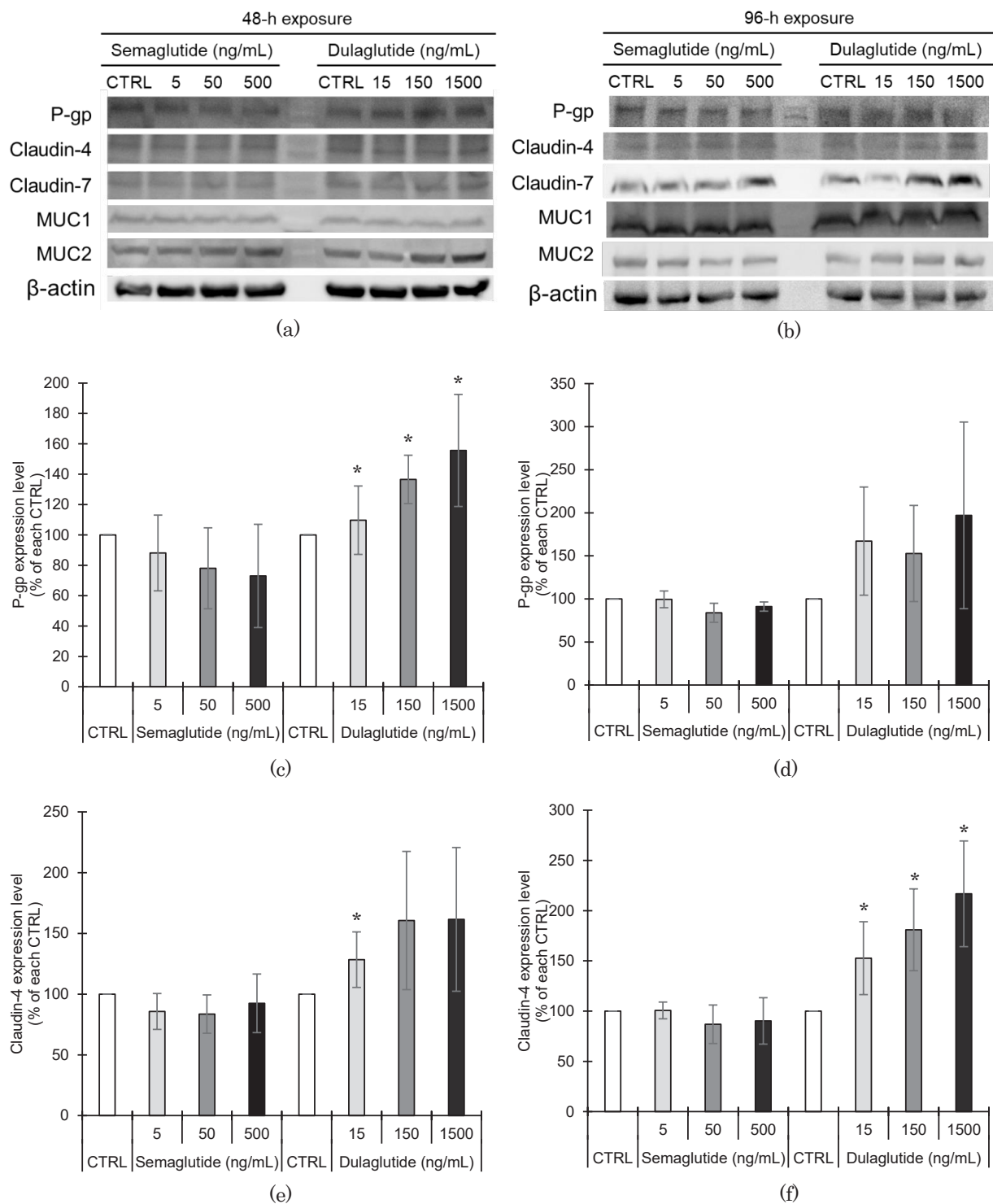
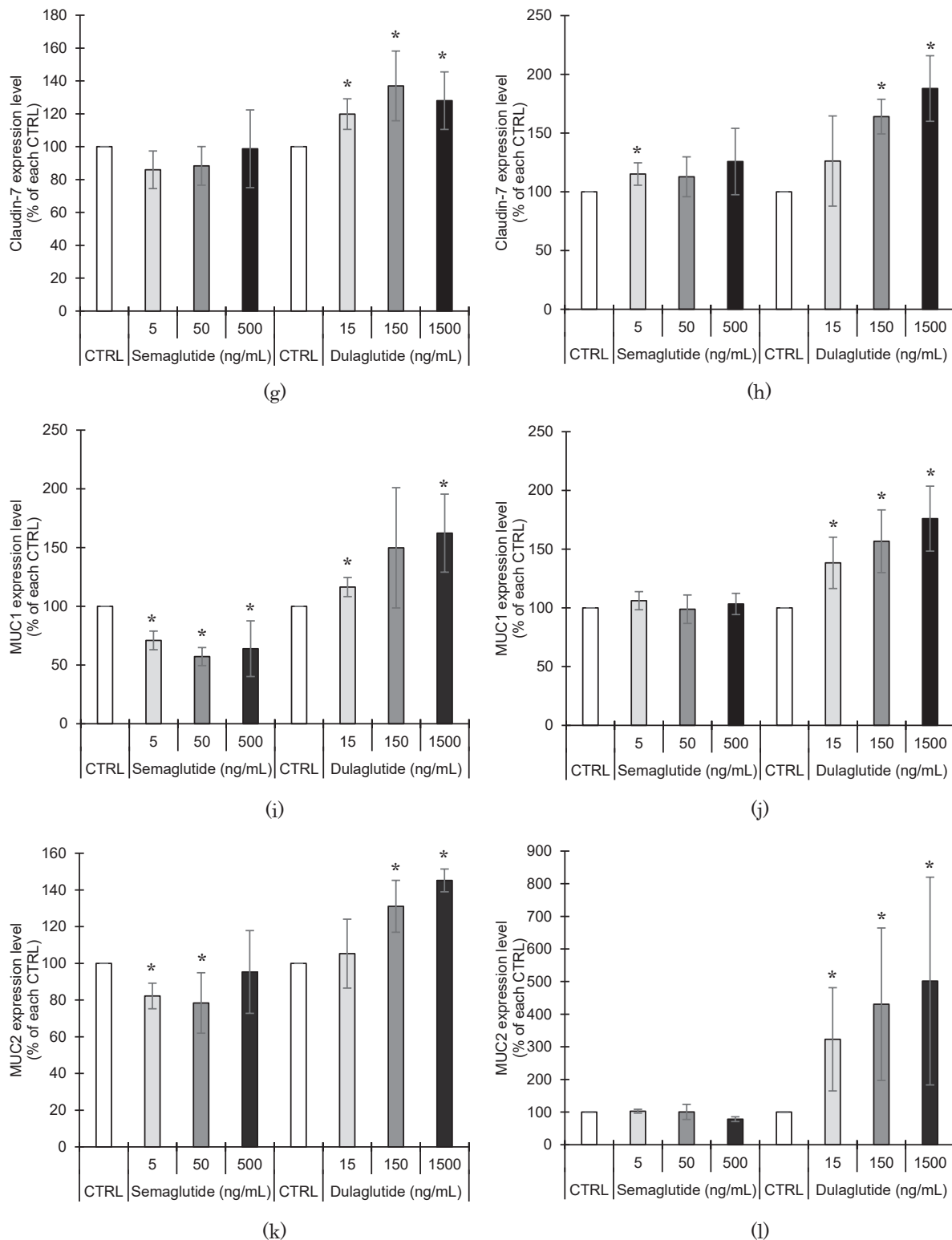


Figure 3. Effects of semaglutide and dulaglutide on protein expression levels of epithelial barrier function-related factors in Caco-2 cells. (a, b): Typical bands for Western blotting, (c, d): P-gp, (e, f): Claudin-4, (g, h): Claudin-7, (i, j): MUC1, and (k, l): MUC2. Exposure to a GLP-1 receptor agonist for 48 h (a, c, e, g, i, k) and 96 h (b, d, f, h, j, l). Data represent means and S.D. ($n = 3$ for each condition). * $P < 0.05$ vs. the control (CTRL) condition.



Continued Figure 3. Effects of semaglutide and dulaglutide on protein expression levels of epithelial barrier function-related factors in Caco-2 cells. (a, b): Typical bands for Western blotting, (c, d): P-gp, (e, f): Claudin-4, (g, h): Claudin-7, (i, j): MUC1, and (k, l): MUC2. Exposure to a GLP-1 receptor agonist for 48 h (a, c, e, g, i, k) and 96 h (b, d, f, h, j, l). Data represent means and S.D. (n = 3 for each condition). *P < 0.05 vs. the control (CTRL) condition.

DISCUSSION

In this study, the Caco-2 cell line, an intestinal epithelial model of small intestinal epithelial cell-like functions, was used. Semaglutide and dulaglutide concentrations were set to 5, 50, and 500 ng/mL and 15, 150, and 1500 ng/mL, respectively, based on steady-state plasma concentrations during subcutaneous administration (Interview Form of Ozempic® and Trulicity®). Neither semaglutide nor dulaglutide affected the proliferation rate or ratio and cell viability of Caco-2 cells (Fig. 1). We previously reported that liraglutide, another GLP-1 receptor agonist, did not affect the proliferation of Caco-2 cells (Takizawa et al., 2022). Collectively, these findings and the present results indicate that the oral administration of GLP-1 receptor agonists is safe at *in vitro* cell proliferation and viability assay levels, even if unabsorbed medicine is exposed to gastrointestinal epithelial cells for a prolonged period of time.

On the other hand, the protein expression level of the GLP-1 receptor was not significantly changed by semaglutide, whereas dulaglutide exerted significant concentration- and time-dependent increase effects (Fig. 1E, 1F). There is currently no evidence to support increases in the expression of GLP-1 receptors in intestinal epithelial cells (Caco-2) following their exposure to GLP-1 receptor agonists. Interestingly, only dulaglutide exerted significant effects. Therefore, the mechanisms responsible for this change in expression may provide useful information for the effective use of GLP-1 receptor agonists.

Since the GLP-1 receptor is a 7-transmembrane G protein-coupled receptor (Goñke & Conlon, 1988; Brubaker & Drucker, 2002), the present results also suggest that dulaglutide changes the expression of other membrane proteins, such as drug transporters.

Intestinal epithelial cells directly interact with the external environment; therefore, various barrier functions exist, which may be broadly classified into two types: biological and physical. Biological barriers include ABC transporters, such as P-gp, which excrete foreign substances taken into cells (Suzuki, 1999; Watanabe et al., 2013), and metabolic enzymes, such as CYP (Canaparo et al., 2007). On the other hand, physical barriers include TJ in intercellular spaces and UWL on the cell surface, which are formed by membrane proteins, such as members of the Claudin family and MUC family (Madara et al., 1993; Chiou, 1994).

The effects of GLP-1 receptor agonists on the mRNA expression levels of these factors in Caco-2 cells were examined (Fig. 2). No significant changes were detected in the mRNA expression levels of each epithelial barrier function regulator upon exposure to semaglutide or dulaglutide, suggesting that the luminal approach of GLP-1 receptor agonists did not affect the regulatory signals for the expression of various factors in these cells.

Although no significant changes were observed in the mRNA expression levels of each factor, protein expression levels

were examined under the same conditions as those for mRNA. It was observed that fluctuations in protein expression of epithelial cell barrier factor by GLP-1 receptor agonist exposure were greater than fluctuations in their mRNA expression. In particular, dulaglutide significantly increased the protein expression levels of many epithelial cell barrier factors in concentration- and exposure time-dependent manner (Fig. 3).

We previously reported that liraglutide did not affect MUC1 or Claudin-4 protein expression levels but increased P-gp protein expression levels in concentration- and exposure time-dependent manners (Takizawa et al., 2022). Collectively, these findings and the present results demonstrated that the effects of GLP-1 receptor agonists on membrane proteins differed depending on the type of GLP-1 receptor agonist. On the other hand, the protein expression levels of AMP-activated protein kinase α and phosphatidylinositol-5-phosphate 4-kinase type 2 beta, which function as intracellular ATP and GTP sensors, were not significantly changed by semaglutide or dulaglutide (data not shown). These results confirmed that dulaglutide did not induce the expression of all proteins. On the other hand, there are reports that GLP-1 receptor agonists alter the expression of proteins such as transporters and enzymes (Zhang et al., 2021; Wang et al., 2023), but the detailed mechanism is not clear. Therefore, further investigation is required, including the mechanistic theory of the phenomenon observed in this study.

In the present study, dulaglutide was used at a higher concentration (ng/mL) than semaglutide, whereas that of semaglutide was higher when considered in terms of mol concentration (nmol/L). Since both concentrations were above the EC_{50} for GLP-1R (Assessment report of Ozempic® and Trulicity®), the effects of dulaglutide do not appear to be mediated by GLP-1 receptors. Moreover, semaglutide did not induce the expression of these proteins, suggesting that dulaglutide-mediated changes were not due to a common target with semaglutide. The mechanism by which dulaglutide increases the expression of these proteins is unknown. Although the expression of proteins on the cell surface that function as defense factors increased across the board, the presence of dulaglutide, the molecular weight of which is very high (M.W.: 63,000), on the cell surface caused the strong recognition of foreign substances by cells, resulting in a temporary increase in the expression of these epithelial barrier factors. Liraglutide (M.W.: 3751.26), a GLP-1 receptor agonist with a smaller molecular weight than that of semaglutide (M.W.: 4113.6), induced the protein expression of P-gp in Caco-2 cells at high concentrations and exerted protective effects against external oxidative stress (Takizawa et al., 2022). These findings suggest that the induction of membrane proteins related to epithelial cell barrier functions is due to a defense mechanism at the cell surface.

On the other hand, previous studies reported a decrease in P-gp expression in the liver and significant increases in P-gp mRNA and protein levels in the small intestine in a type 2 diabetic

mellitus model rat (Yao et al., 2020; Gwak et al., 2020). If there is a mixture of the up-regulation of P-gp due to pathological conditions and that due to therapeutic agents, an additive effect may occur. Therefore, further studies are needed to elucidate the mechanisms underlying the up-regulation of P-gp by GLP-1 receptor agonists, such as dulaglutide, which will provide important information for the appropriate use of concomitant medicines that are P-gp substrates.

In addition, the protein expression levels of TJ components, such as Claudin-1, Occludin, and ZO-1, were found to be decreased in the colon of a type 2 diabetic mellitus model mouse (Rehman et al., 2022). Furthermore, Occludin mRNA levels in the small intestine were higher, whereas MUC2, MUC12, MUC20, and MUC21 mRNA levels were significantly lower in patients with type 1 diabetes than those in a normal group (Lo Conte et al., 2023). Moreover, since inflammation of the gastrointestinal tract during diabetes is an important factor in its pathogenesis, some studies have attempted to control the disease by amplifying MUC in the gastrointestinal tract (Eliuz Tipici et al., 2023). Therefore, the increases observed in MUC levels by dulaglutide may suppress disease progression due to diabetes; however, further studies are needed.

CONCLUSIONS

In this study, semaglutide has a relatively small effect on epithelial cells barrier, while dulaglutide was shown to have the potential to improve epithelial cell barrier function. Since various gastrointestinal symptoms develop during diabetes and epithelial cell barrier functions change, medicines that

promote barrier functions, such as dulaglutide, may be effective treatments. However, their mechanisms of action remain unknown; therefore, further detailed studies are warranted.

DECLARATIONS

Funding

Not applicable

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author Contributions

Y.T. and A.I. designed the research. Y.T., A.K., A.O., and S.K. performed the research. Y.T., A.I., T.K., and T.N. analyzed data. Y.T. wrote the manuscript.

Acknowledgments

The authors thank Miss Miho Ishii, Miss Mariya Kawagoe, and Miss Yuka Nagai for their technical assistance.

References

- [1] IDF Diabetes Atlas 10th edition. The International Diabetes Federation (IDF). URL: <https://diabetesatlas.org/data/en/>
- [2] Bytzer P, Talley NJ, Leemon M, Young LJ, Jones MP, Horowitz M. Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15,000 adults. *Arch Intern Med.* 2001;161:1989–1996.
- [3] Maleki D, Locke GR, 3rd, Camilleri M, Zinsmeister AR, Yawn BP, Leibson C, Melton LJ, 3rd. Gastrointestinal tract symptoms among persons with diabetes mellitus in the community. *Arch Intern Med.* 2000;160:2808–2816.
- [4] Camilleri M. Clinical practice. Diabetic gastroparesis. *N Engl J Med.* 2007;356:820–829.
- [5] Intagliata N, Koch KL. Gastroparesis in type 2 diabetes mellitus: prevalence, etiology, diagnosis, and treatment. *Curr Gastroenterol Rep.* 2007;9:270–279.
- [6] Zhao M, Liao D, Zhao J. Diabetes-induced mechanophysiological changes in the small intestine and colon. *World J Diabetes.* 2017;8(6):249–269.
- [7] Horváth VJ, Putz Z, Izbéki F, Körei AE, Gerő L, Lengyel C, Kempler P, Várkonyi T. Diabetes-related dysfunction of the small intestine and the colon: focus on motility. *Curr Diab Rep.* 2015;15(11):94.
- [8] Sellin JH, Hart R. Glucose malabsorption associated with rapid intestinal transit. *Am J Gastroenterol.* 1992;87:584–589.
- [9] Rosa-e-Silva L, Troncon LE, Oliveira RB, Foss MC, Braga FJ, Gallo Júnior L. Rapid distal small bowel transit associated with sympathetic denervation in type I diabetes mellitus. *Gut.* 1996;39:748–756.
- [10] Yarandi SS, Srinivasan S. Diabetic gastrointestinal motility disorders and the role of enteric nervous system: current status and future directions. *Neurogastroenterol Motil.* 2014;26:611–624.
- [11] Imaeda K, Takano H, Koshita M, Yamamoto Y, Joh T, Suzuki H. Electrical properties of colonic smooth muscle in spontaneously non-insulin-dependent diabetic rats. *J Smooth Muscle Res.* 1998;34:1–11.
- [12] Okamoto A, Yokokawa H, Nagamine T, Fukuda H, Hisaoka T, Naito T. Efficacy and safety of semaglutide in glycemic control, body weight management, lipid profiles and other biomarkers among obese type 2 diabetes patients initiated or switched to semaglutide from other GLP-1 receptor agonists. *J Diabetes Metab Disord.* 2021;20(2):2121–2128.
- [13] Zheng C, Zhou M, Sun J, Xiong H, Peng P, Gu Z, Deng Y. The protective effects of Liraglutide on AD-like neurodegeneration

- induced by oxidative stress in human neuroblastoma SH-SY5Y cells. *Chem Biol Interact.* 2019;310:108688.
- [14] He W, Wang H, Zhao C, Tian X, Li L, Wang H. Role of Liraglutide in brain repair promotion through Sirt1-mediated mitochondrial improvement in stroke. *J Cell Physiol.* 2020;235(3):2986–3001.
- [15] Tiba AT, Qassam H, Hadi NR. Semaglutide in renal ischemia-reperfusion injury in mice. *J Med Life.* 2023;16(2):317–324.
- [16] Klein SR, Hobai IA. Semaglutide, delayed gastric emptying, and intraoperative pulmonary aspiration: a case report. *Can J Anaesth.* 2023;70(8):1394–1396.
- [17] Salles GN, Calió ML, Hölscher C, Pacheco-Soares C, Porcionatto M, Lobo AO. Neuroprotective and restorative properties of the GLP-1/GIP dual agonist DA-JC1 compared with a GLP-1 single agonist in Alzheimer's disease. *Neuropharmacology.* 2020;162:107813.
- [18] Takizawa Y, Oguri J, Uno M, Onsu A, Ishimura A, Kurita T, Nakajima T. Effects of a GLP-1 Receptor Agonist on Gastrointestinal Epithelial Cells. *Sch Acad J Pharm.* 2022;11(4):60–66.
- [19] Overgaard RV, Navarria A, Ingwersen SH, Bækdal TA, Kildemoes RJ. Clinical Pharmacokinetics of Oral Semaglutide: Analyses of Data from Clinical Pharmacology Trials. *Clin Pharmacokinet.* 2021;60(10):1335–1348.
- [20] Ozempic® Subcutaneous Injection 2mg Interview Form, Edition 5 (prepared May 2022)
- [21] Trulicity® Subcutaneous Injection ATEOS, Interview Form, Edition 12 (prepared February 2023)
- [22] Go'ke R, Conlon JM. Receptors for glucagon-like peptide-1(7–36) amide on rat insulinoma-derived cells. *J Endocrinol.* 1988;116:357–362.
- [23] Brubaker PL, Drucker DJ. Structure-function of the glucagon receptor family of G protein-coupled receptors: the glucagon, GIP, GLP-1, and GLP-2 receptors. *Recept Channels.* 2002;8(3-4):179–188.
- [24] Suzuki H. Analysis of xenobiotic detoxification system mediated by efflux transporters. *Yakugaku Zasshi.* 1999;119(11):822–834.
- [25] Watanabe T, Maeda K, Nakai C, Sugiyama Y. Investigation of the effect of the uneven distribution of CYP3A4 and P-glycoprotein in the intestine on the barrier function against xenobiotics: a simulation study. *J Pharm Sci.* 2013;102(9):3196–3204.
- [26] Canaparo R, Finnström N, Serpe L, Nordmark A, Muntoni E, Eandi M, Rane A, Zara GP. Expression of CYP3A isoforms and P-glycoprotein in human stomach, jejunum and ileum. *Clin Exp Pharmacol Physiol.* 2007;34(11):1138–1144.
- [27] Madara JL, Carlson S, Anderson JM. ZO-1 maintains its spatial distribution but dissociates from junctional fibrils during tight junction regulation. *Am J Physiol.* 1993;264(5 Pt 1):C1096–C1101.
- [28] Chiou WL. Effect of 'unstirred' water layer in the intestine on the rate and extent of absorption after oral administration. *Biopharm Drug Dispos.* 1994;15(8):709–717.
- [29] Zhang Z, Du Z, Liu Q, Wu T, Tang Q, Zhang J, Huang C, Huang Y, Li R, Li Y, Zhao Y, Zhang G, Zhou J, Huang H, Fang Z, He J. Glucagon-like peptide 1 analogue prevents cholesterol gallstone formation by modulating intestinal farnesoid X receptor activity. *Metabolism.* 2021;118:154728.
- [30] Wang ZJ, Li XR, Chai SF, Li WR, Li S, Hou M, Li JL, Ye YC, Cai HY, Hölscher C, Wu MN. Semaglutide ameliorates cognition and glucose metabolism dysfunction in the 3xTg mouse model of Alzheimer's disease via the GLP-1R/SIRT1/GLUT4 pathway. *Neuropharmacology.* 2023;240:109716.
- [31] Assessment report, Ozempic®. European Medicines Agency. EMA/CHMP/715701/2017
- [32] Assessment report, Trulicity®. European Medicines Agency. EMA/CHMP/524604/2014
- [33] Yao H, Gu J, Shan Y, Wang Y, Chen X, Sun D, Guo Y. Type 2 diabetes mellitus decreases systemic exposure of clopidogrel active metabolite through upregulation of P-glycoprotein in rats. *Biochem Pharmacol.* 2020;180:114142.
- [34] Gwak EH, Yoo HY, Kim SH. Effects of Diabetes Mellitus on the Disposition of Tofacitinib, a Janus Kinase Inhibitor, in Rats. *Biomol Ther (Seoul).* 2020;28(4):361–369.
- [35] Rehman AU, Siddiqui NZ, Farooqui NA, Alam G, Gul A, Ahmad B, Asim M, Khan AI, Xin Y, Zexu W, Song Ju H, Xin W, Lei S, Wang L. Morchella esculenta mushroom polysaccharide attenuates diabetes and modulates intestinal permeability and gut microbiota in a type 2 diabetic mice model. *Front Nutr.* 2022;9:984695.
- [36] Lo Conte M, Cosorich I, Ferrarese R, Antonini Cencicchio M, Nobili A, Palmieri V, Massimino L, Lamparelli LA, Liang W, Riba M, Devecchi E, Bolla AM, Pedone E, Scavini M, Bosi E, Fasano A, Ungaro F, Diana J, Mancini N, Falcone M. Alterations of the intestinal mucus layer correlate with dysbiosis and immune dysregulation in human Type 1 Diabetes. *EBioMedicine.* 2023;91:104567.
- [37] Eliuz Tipici B, Coskunpinar E, Altunkanat D, Cagatay P, Omer B, Palanduz S, Satman I, Aral F. Lactobacillus GG is associated with mucin genes expressions in type 2 diabetes mellitus: a randomized, placebo-controlled trial. *Eur J Nutr.* 2023;62(5):2155–2164.