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The combination of metformin and a dipeptidyl peptidase IV inhibitor prevents 5-fluorouracil-induced reduction of small intestine weight

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Abstract

Glucagon-like peptide 2 (GLP-2), which has intestinotrophic effects, is secreted from L-cells in the intestine in response to nutrient ingestion and is degraded by dipeptidyl peptidase IV (DPPIV). In this report, we show that biguanides promote GLP-2 release. Plasma GLP-2 levels were significantly increased by 1.4- to 1.6-fold in fasted F344 rats 1 h after oral meformin (300 mg/kg), phenformin (30 and 100 mg/kg) and buformin (100 mg/kg) treatment. In addition, metformin administration (300 mg/kg, p.o.) significantly elevated plasma GLP-2 in fasted CD-1 mice by about 2.0-fold 1 and 3 h after the treatment. Metformin and/or valine-pyrrolidide, a DPPIV inhibitor, was orally given (300 and 30 mg/kg, respectively, p.o., b.i.d., 3 days) to BALB/c mice treated with 5-fluorouracil (5-FU; 60 mg/kg, s.i.d.), which induces gastrointestinal damage leading to a reduction of small intestine wet weight. Metformin and valine-pyrrolidide co-administration prevented the 5-FU-induced reduction of wet weight of the small intestine, whereas metformin or valine-pyrrolidide alone had no effect. These results suggest that GLP-2 is co-secreted with GLP-1 flollowing biguanide stimulation, and that the combination of metformin with a DPPIV inhibitor might a useful oral treatment for gastrointestinal damage, based on GLP-2 actions.

Keywords: Biguanide; Glucagon-like peptide 2; Dipeptidyl peptidase IV; Intestine

1. Introduction

Glucagon-like peptide 1 (GLP-1) and GLP-2 are products of the proglucagon gene, which is differentially processed in different tissues to produce either glucagon and the major proglucagon fragment or GLP-1 and GLP-2. Both GLP-1 and GLP-2 are synthesized in and released from enteroendocrine L-cells in the distal intestine, and degraded by dipeptidyl peptidase IV (DPPIV or CD26, EC 3.4.14.5) (Drucker, 1998). While GLP-1 is mainly involved in glucose metabolism, GLP-2 has several actions on the intestine: stimulation of mucosal growth of the small and large intestine, inhibition of apoptosis of enterocytes and crypt cells, stimulation of enterocyte glucose transport and glucose transporter 2 (GLUT2) expression, and inhibition of gastric emptying and gastric acid secretion (Drucker, 1999b, 2002a,b).

GLP-2 and GLP-1 are co-secreted from L-cells in response to ingestion of nutrients, particularly carbohydrate and fat (Sigalet, 2001). GLP-2 and GLP-1 secretion is mediated by direct nutrient stimulation of L-cells, and by indirect actions of enteroendocrine and neural inputs, including glucose-dependent insulinotropic peptide, gastrin-releasing peptide, and the vagus nerve (Roberge et al., 1996; Rocca and Brubaker, 1999; Burrin et al., 2003). In addition, plasma GLP-2 levels were found to be elevated in the setting of intestinal injury (Drucker, 2002a). However, secretory mechanisms of GLP-2, as well as of GLP-1, remain to be determined. We recently found that biguanides, including metformin, enhance GLP-1 secretion in rats, independently of nutrient stimulation (Yasuda et al., 2002).

Because GLP-1 and GLP-2 are co-secreted from L-cells after nutrient intake stimulation, we were interested to know whether this is also the case after biguanide treatment. If so, there could be a novel application of biguanide agents for the treatment of gastrointestinal disturbance, based on the physiological actions of GLP-2 on intestinal tissues. In this

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report, we demonstrate that oral administration of biguanides stimulates GLP-2 secretion in rats and mice. Furthermore, we show that the combination of metformin with a DPPIV inhibitor is effective for alleviating intestinal change in mice treated with 5-fluorouracil, a chemotherapeutic drug, which causes gastrointestinal damage as a result of intestinal epithelial dysfunction caused by injury to intestinal mucosal villi (Hirata and Horie, 1999).

2. Materials and methods

2.1. Chemicals

Metformin (1,1-dimethylbiguanide) hydrochloride, phenformin (phenethylbiguanide) hydrochloride, and 5-fluorouracil (5-FU) were purchased from Sigma (St. Louis, MO). Buformin (butylbiguanide) hydrochloride (Dibetos® B) was obtained from Japan Galen (Saitama, Japan). Valine-pyrrolidide hydrochloride, a DPPIV inhibitor (Ahrén et al., 2000), was synthesized in our laboratories. Methyl cellulose 400cP (MC) was purchased from Wako (Osaka, Japan). Human GLP-2 was purchased from Bachem (Bubendorf, Switzerland). The biguanides, valine-pyrrolidide and 5-FU were suspended in 0.5% MC, and human GLP-2 was dissolved in saline (Otsuka Pharmaceutical, Tokyo, Japan) before administration.

2.2. Animals

Seven-week-old male F344/Jcl rats were purchased from Japan Clea (Tokyo, Japan). Seven-week-old female Crj:CD-1 (CD-1) mice and five-week-old male BALB/cAnCrj (BALB/c) mice were obtained from Charles River Japan (Tokyo, Japan). The rats and mice were provided with a commercial diet (MF; Oriental Yeast, Tokyo, Japan) and water ad libitum and were kept under conventional conditions with controlled temperature, humidity and lighting (22 ± 2 °C, 55 ± 5% and a 12-h light/dark cycle with lights on at 07:00 a.m.). The animals were acclimated at least for a week. All procedures were conducted according to the Eisai Animal Care Committee's guideline.

2.3. Plasma GLP-2 determination

Plasma rat and mouse immunoreactive GLP-2 levels were measured with a rat GLP-2 EIA kit (Yanaihara Institute, Fujinomiya, Japan).

2.4. Effects of biguanides on plasma GLP-2 levels in rats

Nine-week-old male F344/Jcl rats were used. Food was withheld for 18 h, and then the rats were given metformin (30, 100 or 300 mg/kg) or the vehicle orally via a gastric tube at 10:00 a.m. ($n = 5$ per group). Blood (200 μl) was taken from the caudal vein with a heparinized capillary tube 0 and 1 h after the administration, and subjected to the measurement of plasma GLP-2 levels.

In addition, we examined the effects of phenformin and buformin on plasma GLP-2 levels in F344/Jcl rats. Rats were treated orally with phenformin (30 or 100 mg/kg), buformin (30 or 100 mg/kg) or the vehicle via a gastric tube at 10:00 a.m. after 18-h fasting ($n = 5$ per group). Blood (200 μl) was taken from the caudal vein with a heparinized capillary tube 0 and 1 h after the administration, and subjected to the measurement of plasma GLP-2 levels. Although no acidosis was observed up to 300 mg/kg of metformin, or 100 mg/kg of phenformin or buformin, both phenformin and buformin caused a severe increase of plasma lactic acid and death at 300 mg/kg in a preliminary experiment (data not shown). Therefore, we excluded this dose of phenformin and buformin in this study.

2.5. Determination of plasma GLP-2 level changes in mice after metformin administration

Eight-week-old female CD-1 mice were fasted for 18 h, and then mice were given metformin (300 mg/kg) or the vehicle orally via a gastric tube at 10:00 a.m. ($n = 5$ per group). Blood (50 μl) was taken from the caudal vein with a heparinized capillary tube 0, 1 and 3 h after the administration, and subjected to the measurement of plasma GLP-2 levels.

2.6. Effects of GLP-2 on small intestine weights of mice treated with 5-FU

Eight-week-old BALB/c mice were used. The mice were orally given 5-FU (60 mg/kg) or the vehicle once daily at 08:00–09:00 a.m. for 3 days. 5-FU-treated mice were given GLP-2 (20 μg) or saline s.c. twice a day at 08:00–09:00 a.m. and 04:00–05:00 p.m. for 3 days ($n = 5$). The dose of human GLP-2 was based on the report of Hartmann et al. (2000), who found that plasma GLP-2 concentrations were elevated for 4–8 h after s.c. injection (40 μg) in rats. On Day 4, mice were killed by cervical dislocation after fasting for 18 h and the entire small intestines (the pylorus to ileocecal junction) were removed. The tissues were washed with saline, and the wet weight was determined.

2.7. Effects of metformin and/or DPPIV inhibitor on small intestine weight of mice treated with 5-FU

Six-week-old BALB/c mice were employed. They were orally treated with 5-FU (60 mg/kg) or the vehicle once daily at 08:00–09:00 a.m. for 3 days. 5-FU-treated mice were given metformin alone (300 mg/kg) or valine-pyrrolidide alone (30 mg/kg), or a mixture of metformin (300 mg/kg) and valine-pyrrolidide (30 mg/kg), or the vehicle via a gastric tube twice a day at 08:00–09:00 a.m. and 04:00–05:00 p.m. for 3 days ($n = 6$). The procedures for sampling the small intestines were described above.

2.8. Statistical analysis

Data are expressed as means ± S.E.M. Statistical analysis of differences between two groups was conducted by use of the *F*-test, followed by two-tailed unpaired Student's *t*-test or Mann–Whitney's *U*-test if appropriate. For multiple comparison, a one-way analysis of variance (ANOVA) was carried out, followed by Sheffé's test as a post hoc test (Statcel, OMS Publishing, Tokorozawa, Japan). We considered a p value less than 0.05 to be statistically significant.

3. Results

3.1. Increase of plasma GLP-2 after oral biguanide treatment of rats

Fig. 1A shows the increase of plasma GLP-2 in male F344/Jcl rats 1 h after the oral administration of metformin under fasting conditions. The basal plasma GLP-2 concentration of fasting rats was 1.59 ± 0.04 ng/ml. A significant increase of plasma GLP-2 was observed in the 300 mg/kg metformin-treated group versus all three other

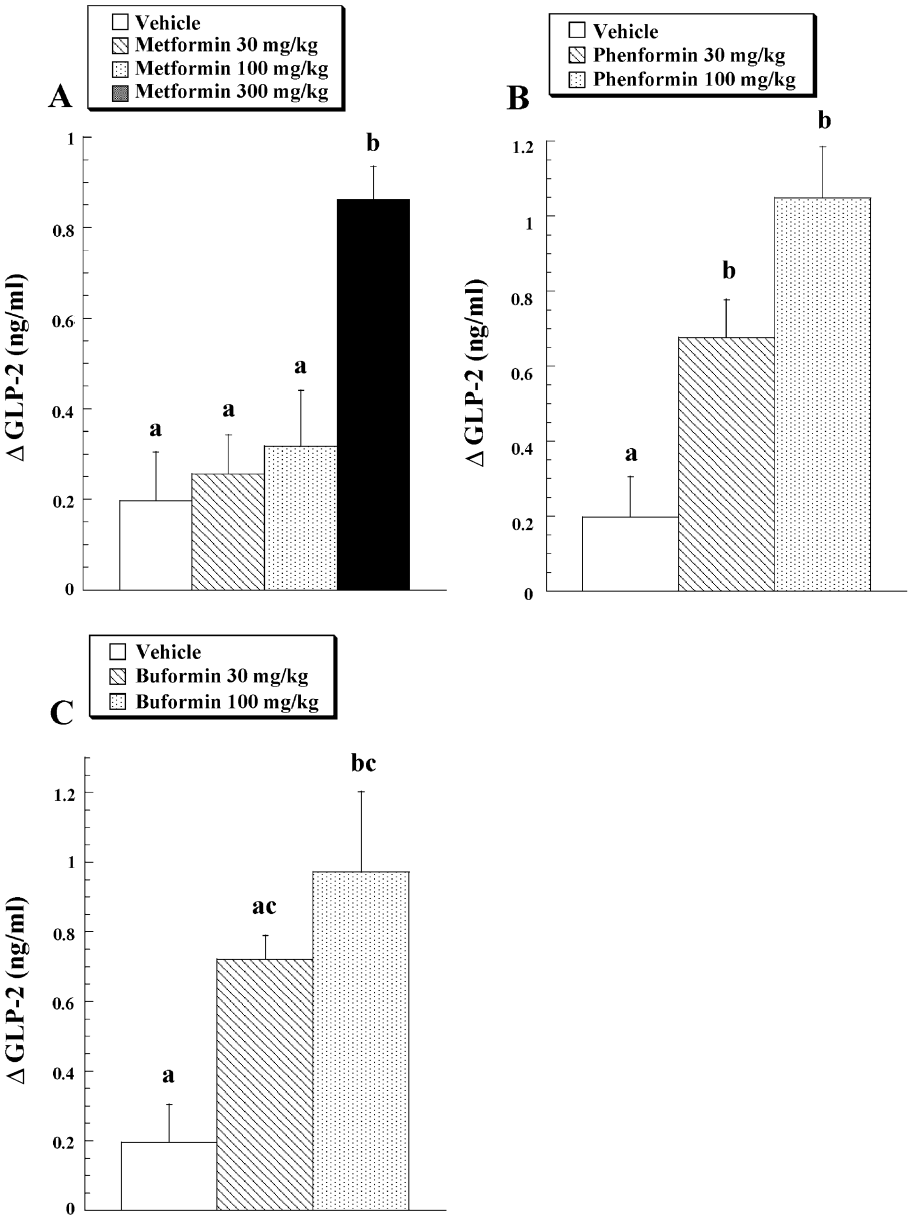

Fig. 1. Increases of plasma GLP-2 levels in 9-week-old male F344/Jcl rats treated with metformin (A), phenformin (B) or buformin (C) 1 h after biguanide treatment. Rats were orally given metformin (30, 100 or 300 mg/kg), buformin (30 or 100 mg/kg), phenformin (30 or 100 mg/kg) or vehicle after fasting for 18 h. Values are expressed as means ± S.E.M. Mean values were compared using one-way ANOVA, followed by Scheffé's test. Bars with the same letter are not statistically significantly different by Scheffé's test ($p > 0.05$). $N = 5$ in each group.

groups ($p = 0.0025$ versus the vehicle group, $p = 0.0057$ versus the 30 mg/kg-treated group, and $p = 0.0130$ versus the 100 mg/kg-treated group). The increase was to a level about 1.5-fold higher than that at 0 h.

A significant elevation of plasma GLP-2 was also induced by phenformin treatment at 30 mg/kg ($p = 0.0409$ versus the vehicle-treated group) and at 100 mg/kg ($p = 0.0009$ versus the vehicle-treated group) (Fig. 2B). Furthermore, buformin treatment at 100 mg/kg resulted in a significant increase of plasma GLP-2, compared with the vehicle-treated group ($p = 0.0122$) (Fig. 2C). There was a trend toward an increase of GLP-2 at 30 mg/kg ($p = 0.0890$ between the vehicle and 30 mg/kg-treated group). The increase of plasma GLP-2 concentration by phenformin and buformin was clearly dose-dependent. The order of efficacy to increase plasma GLP-2 levels appeared to be phenformin ≥ buformin>metformin.

3.2. Changes of plasma GLP-2 level after oral metformin treatment in mice

In order to confirm biguanide-mediated GLP-2 secretion in mice, we treated fasting female CD-1 mice with metformin at 300 mg/kg, and determined plasma GLP-2 at 0, 1 and 3 h. At 1 h, plasma GLP-2 levels of the metformin-treated mice were significantly increased by about twofold, in comparison with the control mice ($p = 0.0090$) (Fig. 2). The high concentration of plasma GLP-2 was maintained at least until 3 h after the treatment ($p = 0.0090$ versus the control group).

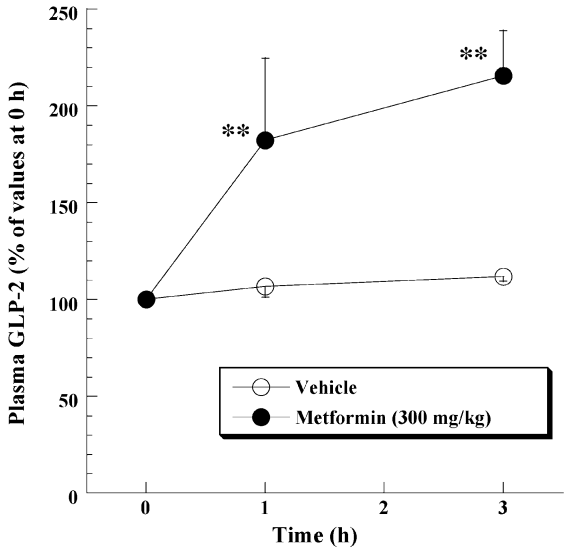

Fig. 2. Changes of plasma GLP-2 levels in 8-week-old female CD-1 mice treated with metformin (●) or vehicle (○). Mice were orally given metformin (300 mg/kg) after fasting for 18 h. Significant increases of plasma GLP-2 by about twofold occurred in the group given metformin treatment (**$p < 0.01$). Data are shown as means ± S.E.M. $N = 5$ per group.

Table 1
Effect of GLP-2 treatment on small intestine wet weight of BALB/c mice treated with 5-fluorouracil

Groups	5-Fluorouracil (mg/kg)	Body weight (g)	Small intestine wet weight (g)
Normal	0	23.3 ± 0.3	0.804 ± 0.014[a]
Control	60	22.3 ± 0.4	0.614 ± 0.007
GLP-2 (20 μg)	60	22.0 ± 0.3	0.672 ± 0.013[b,c]

Data are expressed as means ± S.E.M. $n = 5$ in each group.
[a] $p < 0.001$ versus the control group.
[b] $p < 0.05$ versus the control group.
[c] $p < 0.001$ versus the normal group.

3.3. Effects of GLP-2 treatment on small intestine weight of mice treated with 5-FU

Table 1 shows the effects of exogenous GLP-2 injection on the reduction of small intestine weight in mice treated with 5-FU. 5-FU treatment at 60 mg/kg for 3 days significantly reduced the small intestine wet weight ($p < 0.0001$ between the normal and control groups). GLP-2 administration caused a significant improvement of the intestine weight reduction ($p = 0.0141$ between the control and GLP-2-treated groups).

3.4. Effects of metformin and/or DPPIV inhibitor treatment on small intestine weight of mice treated with 5-FU

Table 2 shows the small intestine wet weight of 5-FU-loaded mice treated with metformin and/or valine-pyrrolidide. Three-day treatment with 5-FU at 60 mg/kg resulted in a significant reduction of small intestine weight, as in the experiment using GLP-2 ($p = 0.0062$ between the normal and control groups). Neither metformin nor valine-pyrrolidide alone significantly affected small intestine weight, compared with the control group. On the other hand, the metformin and valine-pyrrolidide co-administered group showed a significant increase of small intestine wet weight,

Table 2
Effect of the combination of metformin with valine-pyrrolidide, a dipeptidyl peptidase IV inhibitor, on small intestine wet weight of BALB/c mice concomitantly treated with 5-fluorouracil

Groups	5-Fluorouracil (mg/kg)	Body weight (g)	Small intestine wet weight (g)
Normal	0	20.2 ± 0.3	0.700 ± 0.009[a]
Control	60	19.5 ± 0.3	0.622 ± 0.005
Metformin (300 mg/kg)	60	20.1 ± 0.3	0.642 ± 0.017
Valine-pyrrolidide (30 mg/kg)	60	20.3 ± 0.3	0.637 ± 0.015
Metformin (300 mg/kg) plus valine-pyrrolidide (30 mg/kg)	60	20.1 ± 0.4	0.693 ± 0.015[b]

Data are expressed as means ± S.E.M. $n = 6$ per group.
[a] $p < 0.01$ versus the control group.
[b] $p < 0.05$ versus the control group.

in comparison with the control group ($p = 0.0139$), almost reaching the normal range.

4. Discussion

We recently discovered that biguanides enhance GLP-1 secretion upon to fasted rats (Yasuda et al., 2002). It is known that GLP-1 and GLP-2 are co-secreted from L-cells in response to food intake. Therefore, we investigated whether biguanides induce GLP-2, as well as GLP-1, secretion in fasting rats. We found that oral treatment of biguanides triggers GLP-2 secretion in rats and mice, independently of nutrient stimulation. This finding is the same as in the case of GLP-1. It is thought that GLP-1 and GLP-2 are secreted synchronously and equimolar amounts (Ørskov et al., 1986; Hartmann et al., 1995), because of their common precursor and their co-localization in secretory granules (Varndell et al., 1985). Further, Hansen et al. (2000) reported that somatostatin restrains secretion of both GLP-1 and GLP-2 from isolated porcine ileum. Taking these results together with ours, it seems likely that secretion of GLP-1 and GLP-2 is commonly regulated.

GLP-2 is an intestinotrophic peptide, secreted in response to intestinal injury (Drucker, 1999). Exogenous administration of GLP-2 promotes growth of the small and large intestinal epithelium in part via stimulation of crypt cell proliferation and inhibition of crypt and enterocyte apoptosis, leading to an increase in mucosal surface area (Tsai et al., 1997). These physiological effects of GLP-2 were found to cause a reduction in epithelial damage, augmentation of endogenous adaptation or prevention of intestinal atrophy in the following animal models: indomethacin-induced enteritis (Boushey et al., 1999), vascular ischemia–reperfusion-induced intestinal injury (Prasad et al., 2000), dextran sulfate-induced colitis (Drucker et al., 1999), chemotherapy-induced intestinal damage (Boushey et al., 2001), intestinal resection (Scott et al., 1998; Ljungmann et al., 2001), and total parenteral nutrition (TPN) (Chance et al., 1997, 2000). Clinical studies indicate that GLP-2 may enhance energy absorption and reduce fluid loss in subjects with short bowel syndrome, suggesting that GLP-2 functions as a key regulator of mucosal integrity, permeability, and nutrient absorption in humans (Jeppesen et al., 2001). Hence, GLP-2 may be therapeutically useful in diseases characterized by injury or dysfunction of the gastrointestinal epithelium.

Chemotherapeutic agents produce cytoablative actions on rapidly proliferating cells via induction of cell cycle arrest and/or cellular apoptosis. The actions are not specific to tumors, but also affect normal cells in the bone marrow and intestinal crypt. 5-FU has been used in the treatment of cancers, but often causes diarrhea, mucositis and ulceration. 5-FU treatment of rats and mice causes diarrhea with appearance of apoptotic cells in the small intestine and decrease of the height of villi (Tamaki et al., 2003), and reduces the wet weight of the small intestines with the appearance of intestinal leukocyte infiltration and epithelial barrier dysfunction (Hirata and Horie, 1999). It was suggested that the side effects of anticancer drugs could be prevented by inhibiting apoptosis in the gastrointestinal tract (Tamaki et al., 2003).

We demonstrated that exogenous GLP-2 administration ameliorated the decrease of small intestine weight induced by 5-FU. This result may reflect the intestinotrophic effects of GLP-2. The reduction of small intestine wet weight was also diminished by a prostaglandin E_1 derivative, which is associated with improvement of intestinal damage, in 5-FU-treated rats (Hirata and Horie, 1999). Boushey et al. (2001) reported that GLP-2 reduces chemotherapy-associated mortality, bacteremia, epithelial injury and crypt apoptosis in mice, using 5-FU and irinotecan as chemotherapeutic agents, and suggested that GLP-2 would be useful for attenuation of chemotherapy-induced intestinal mucositis. Our findings support the possible therapeutic value of GLP-2 to treat intestinal disturbances caused by chemotherapy.

Next, we examined the effect of the combination of metformin with a DPPIV inhibitor on the model, because GLP-2 is degraded by DPPIV after release. In this experiment, only the co-administration group showed a significantly lessened reduction of the wet weight of the small intestines. The metformin-treated group showed no effect, even though metformin increases plasma GLP-2 levels. However, it should be noted that we measured total GLP-2. It is, thus, thought that the metformin alone was ineffective because of rapid degradation of active GLP-2 by DPPIV, while inhibition of DPPIV resulted in sustained availability of active GLP-2, leading to the alleviation of the small intestinal weight loss in the combination group. Two-week treatment with metformin and valine-pyrrolidide (300 and 30 mg/kg, respectively, b.i.d.) in female CD-1 mice resulted in significant increases of the wet weights of the small and large intestines (data not shown). This observation supports the above speculation.

The L-cells of the gastrointestinal tract contain peptide YY (PYY), which exists in two forms: PYY(1–36) and PYY(3–36). The latter is generated by the action of DPPIV (Batterham and Bloom, 2003). There were studies hinting a trophic effect of PYY on epithelia (Goodlad et al., 1987; Voisin et al., 1993). Gomez et al. (1995) showed that PYY(1–36) treatment increases weight and DNA content of the bowels of rats and mice. Furthermore, Chance et al. (1996) demonstrated that co-infusion of PYY with TPN results in significant preservation of wet weights and elevated protein contents in the small intestine and colon. It is, thus, very interesting to examine whether biguanides increase plasma PYY level, as well as GLP-2 and GLP-1. PYY(1–36) may also contribute to the intestinotrophic effect in the combination of metformin with the DPPIV inhibitor.

Teduglutide (ALX-0600), a GLP-2 analog, is being developed as a candidate drug for the treatment of gastrointestinal diseases, including short bowel disease

(Sigalet, 2001). In addition, this analog is expected to have potential value for treating mucositis associated with cancer chemotherapy and inflammatory bowel disease, based on preclinical studies (Tavakkolizadeh et al., 2000; Kouris et al., 2001). However, teduglutide might have shortcomings in chronic clinical use, i.e., the need for s.c. injection, and possible antigenicity. Combination therapy with metformin and an orally effective DPPIV inhibitor may be a useful oral alternative for the treatment of gastrointestinal diseases.

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