Gut-Derived Incretin Hormones and New Therapeutic Approaches

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This is the first of a series of articles on presentations at the American Diabetes Association Annual Meeting, Orlando, Florida, 4–8 June 2004, addressing an important theme of the meeting: new therapeutic approaches based on gut-derived incretin hormones.

Physiology of glucagon-like peptide 1 action

Alan Cherrington (Nashville, TN) described a series of studies of peripheral and hepatic arterial and portal vein flow measurement and sampling that allowed for the assessment of hepatic glucose balance and effects of glucagon-like peptide (GLP)-1. In a study comparing peripheral with portal venous glucose delivery during hyperglycemia (glucose increased from 75 to 150 mg/dl), with somatostatin to suppress endogenous insulin and glucagon and administration of glucagon to replace basal levels and insulin to levels approximately four times greater than basal, there was a similar increase in the hepatic glucose load, from ~25 to 50 mg $\cdot kg^{-1} \cdot min^{-1}$, but portal glucose delivery doubled hepatic glucose uptake. Net hepatic glucose uptake remained considerably greater with intraportal glucose delivery when the hepatic glucose load was varied from 50 to 75 to 100 mg $\cdot kg^{-1} \cdot min^{-1}$. Thus, Cherrington suggested, hepatic glucose entry depends on insulin and glucose levels and a "portal signal." Portal glucose delivery, then, can be said to "activate the liver," which may be in part the mechanism of increased hepatic glucose uptake following oral glucose delivery. When measuring hepatic sinusoidal insulin, as levels increase, the net hepatic glucose uptake increases but there is further uptake with activation of the portal glucose signal. In further studies varying peripheral and portal vein glucose delivery and maintaining hyperinsulinemia, a fivefold increase in hepatic glucose uptake was demonstrated, as portal glucose delivery ranged from nil to 100%. Conversely, when glucose was infused peripherally versus in the portal vein at 2.5 mg $\cdot kg^{-1} \cdot min^{-1}$, arterial glucose levels were identical but there was a rapid drop from hepatic glucose output to uptake with portal delivery, whereas a much slower fall was seen in hepatic glucose output occurring with peripheral delivery, with consequent lack of increased peripheral glucose uptake following intraportal administration. Peripheral glucose administration led to most glucose uptake being peripheral. Thus, whole-body glucose clearance did not change with the method of glucose administration, but the portal signal appeared to instruct the liver to take up more, and muscle to take up less, glucose. This is not due to lower peripheral insulin levels, as these tended to be somewhat higher after portal glucose administration, suggesting alternative mechanisms, potentially including the incretin effect and neurally mediated metabolic signals.

Addressing the incretin effect, GLP-1 levels increased from basal levels of ~15 pmol/l to arterial levels of 30 pmol/l and to 60 pmol/l in the portal vein, leading Cherrington to note that levels above ~100 pmol/l should be considered nonphysiological. In a study administering glucose at a rate of 4 mg $\cdot kg^{-1} \cdot min^{-1}$ via the portal vein, as well as peripheral glucose to increase plasma glucose levels to 160 mg/dl, intraportal GLP-1 increased arterial and portal GLP-1 to ~40 and 85 pmol/l and tripled hepatic glucose uptake, in part because of greater increase in insulin and suppression of glucagon, but peripheral GLP-1 in a similar study had a lesser effect in increasing hepatic glucose uptake, suggesting this to be an important direct influence "without the mediation of a change in [insulin and glucagon] secretion." In a presentation at the American Diabetes Association Annual Meeting on this topic by Cherrington's group, Dardevet et al. (abstract 1410) assessed the potential direct effects of GLP-1 on the liver, showing in somatostatin-, insulin-, and glucagon-infused dogs (thus not allowing GLP-1 to change insulin or glucagon levels) that peripherally administering the peptide increased hepatic glucose uptake 60%, while administration either via the portal vein or hepatic artery let to a 150% increase in hepatic glucose uptake, suggesting the effect to be in the liver rather than specifically requiring GLP-1 levels to increase in the portal venous system. Ionut et al. (abstract 1412) similarly showed a glucose-lowering effect of portal vein GLP-1 infusion.

Cherrington concluded that the portal vein and liver may contain nutrient- and hormone-sensing mechanisms that help coordinate the disposition of ingested nutrients. Portal vein glucose delivery generates a signal that augments the role of the liver and limits muscle glucose disposition, with portal vein GLP-1 infusion studies suggesting an important direct hepatic effect of the hormone, which mediates at least part of this process. In response to questions, he suggested that the molecular mechanism of glucose sensing may involve glucokinase and GLUT2, noted that the effect of hepatic denervation includes decreased response to portal and increased response to peripheral glucose delivery, and suggested that GLP-1 produces similar augmentation of hepatic response with and without somatostatin, suggesting that this experimental method did not artifactually alter the results of his studies.

In a fascinating study related to this topic presented at the meeting, Li and Drucker (abstract 1) studied mice not expressing the GLP-1 receptor, showing that superimposing transgenic expression of the receptor restricted to pancreatic β- and ductal cells via the PDX-1 gene allowed restoration of normal fasting glucose and response to intraperitoneal glucose with and without administration of exendin-4, a natural peptide with >50% sequence homology to GLP-1, but with greater potency and longer duration of action, produced in the saliva of the Gila monster. This study suggests that although the stomach, nervous system, and liver also express the GLP-1 receptor, ac-

tion at these sites may not be required for the peptide's systemic glycemic effect.

Potential effects of GLP-1 in type 2 diabetes

Michael A. Nauck (bad Laukenberg em Nacht, Germany) discussed GLP-1 and glucose-dependent insulinotropic peptide (GIP) in type 2 diabetes. GLP-1 is produced by L-cells in the distal small bowel, while GIP comes from K-cells in proximal small intestine. Classically, the incretin effect has been demonstrated by comparing effects of intravenous versus oral glucose administered to produce similar plasma glucose curves and hence "glycemic stimulus." Under such conditions, oral glucose produces a threefold higher insulin and C-peptide response. In persons with type 2 diabetes, however, the incretin effect is attenuated or even completely lost. One explanation would be that the relative secretion of hormones mediating the incretin effect is decreased. Indeed, comparing persons with and without type 2 diabetes, differences in GLP-1 appear ~1 h after oral glucose (1), while GIP may either be hypo- or hypersecreted (2) and therefore was not felt to explain the lack of incretin effect in type 2 diabetes. Studies comparing the effects of GIP and GLP-1 in diabetic and nondiabetic persons show, however, that the response to GIP is decreased while that to GLP-1 is similar in diabetic and nondiabetic persons (3), suggesting that the lack of GIP effect may indeed be important in type 2 diabetes. Comparing bolus injections of GLP-1 and GIP, persons with type 2 diabetes do show response to GIP as to GLP-1, but longer periods of infusion of GIP fail to increase insulin levels in the fashion seen with GLP-1 (4), implying that the mechanism is complex. In a study of GIP infusion during hyperglycemia, approximately half of a group of relatives of persons with type 2 diabetes had attenuation of insulin response. Although this might seem a likely candidate as an early marker of type 2 diabetes, there was no difference in glucose tolerance between responders and nonresponders either at baseline or at 4-year follow-up, further indicating the intricacy of these effects (5).

Effects of GLP-1 in normalizing glucose may go beyond the acute insulin-stimulatory action of the peptide to effects increasing β-cell mass, decreasing glucagon, decreasing food intake leading to reduction in body weight, and decelerating gastric emptying. Although Nauck stated that there is no evidence of effect on insulin sensitivity, the studies discussed by Cherrington suggest the possibility that the peptide changes hepatic versus peripheral partitioning of glucose metabolism. Initial studies of persons with type 2 diabetes showed that subcutaneous GLP-1 injection stimulated insulin and inhibited glucagon secretion, decreased gastric emptying, and, with repeated injections, normalized plasma glucose (6). Nauck described a study of 34 persons with type 2 diabetes who received a 6-week intravenous infusion of GLP-1 or saline and had baseline HbA_{1c} of 9.4%. Glucagon levels were suppressed, insulin and C-peptide levels were increased, fasting and mean glucose levels were decreased by 77 and 99 mg/dl, respectively, HbA_{1c} was reduced by 1.3%, and there was a weight loss of 4 lb (7). Below a blood glucose of 120 mg/dl, the insulin secretory and glucagon suppressive effects lessened, with levels of both hormones returning to the basal range. In a study from Nauck's group, Fehse et al. (abstract 351) compared 13 persons with type 2 diabetes, using insulin infusion to attain euglycemia with and without a 5-h exenatide infusion, and 12 healthy control subjects. Both the first- and second-phase insulin response to intravenous glucose increased two- to fourfold with the GLP-1 analog to the range seen in the nondiabetic group, and glucose disposal was normalized. However, GLP-1 infusion increases total GLP-1 to a greater extent than it affects the biologically active molecule and therefore might not be appropriate for therapy (8), suggesting the importance of development of incretin-mimetic agents (vide ifra). Nauck concluded that the incretin effect is reduced in persons with type 2 diabetes, explained by both abnormal secretion and decreased response, and that the use of natural GLP-1 is limited by its rapid degradation and elimination. GLP-1 derivatives such as exenatide may therefore be better suited to diabetes therapy.

Long-acting GLP-1 agonist preparations

Laurie Baggio (Toronto, Canada) described albumin-bound GLP-1 agonist delivery systems. She stated that GLP-1 is derived from a larger precursor, stimulates insulin gene expression and glucose-mediated insulin secretion, improves insulin sensitivity, and slows gastric emptying, either via a central nervous system (CNS) effect or by activation of vagal afferent fibers. An important question, she noted, is whether albumin-bound GLP-1 will be able to access CNS sites. GLP-1 also increases β-cell mass via stimulation of neogenesis and inhibition of apoptosis, again leading to the question of whether larger albumin-bound complexes will be able to access β-cells to produce trophic effects.

GLP-1 action controls fasting and postprandial insulin release in a glucose-dependent manner. GLP-1 is rapidly inactivated by dipeptidyl peptidase (DPP)-IV, limiting its therapeutic potential, although as discussed above therapeutic effects can be shown when the peptide is administered by continuous infusion. The development of long-acting safe and efficacious GLP-1 receptor agonists, such as exenatide, allows consideration of practical therapeutic trials. In phase 3 studies completed by Amylin and Lilly, two injections daily are required for effect, with a 1-year open-label study showing a 1.2% decrease in HbA_{1c}, an 8-lb weight loss, mild to moderate nausea, and hypoglycemia in patients also receiving sulfonylureas.

A novel alternative approach involves linking GLP-1 with albumin. Albumin is a 67-kDa protein with 19-day half-life in humans, and albumin-binding of GLP-1 slows its absorption following subcutaneous administration, resulting in steady blood concentrations. Liraglutide is a compound developed by Novo Nordisk that has completed phase 2 clinical trials. The compound has 97% homology with GLP-1, is released slowly from the injection site, and has a fatty acid binding moiety leading to albumin binding (9). After a single injection the half-life of liraglutide is 11–15 h, with a decrease in glucagon, an increase in insulin, and lowering of 24-h glucose profiles in persons with type 2 diabetes following once-daily administration (10). After 12 weeks of administration, HbA_{1c} levels decrease to a similar extent to that seen with sulfonylureas but with weight loss rather than weight gain (11). Baggio noted that an important question for study is whether liraglutide reaches the same CNS binding sites as GLP-1. Liraglutide reduces blood glucose in a glucose-dependent manner, without effect in mice not expressing the GLP-1

receptor. When injected twice daily in a mouse model, the agent stimulates islet proliferation, again mimicking biological actions of GLP-1. Studies reported at the meeting suggested that liraglutide will be an effective therapeutic agent. Nauck et al. (abstract 356) administered 2 mg liraglutide s.c. daily versus placebo for 5 weeks in 144 persons with type 2 diabetes receiving 1 g metformin twice daily, leading to a 1.1% fall in HbA_{1c}. Compared with metformin plus glimepiride, patients treated with metformin plus liraglutide had a 2.9-kg greater weight decrease and a 22-mg/dl greater fall in fasting blood glucose. In an obese rat model using candy feeds, Knudsen et al. (abstract 1408) reported that over 12 weeks, body weight decreased with liraglutide in association with a decrease in candy, but not chow, intake but did not show significant change with the DPP-IV inhibitor LAF237.

Two newer products, CJC-1131 and Albugon, exhibit covalent binding to albumin. CJC-1131, which is currently in phase 2 clinical trials, was developed by the research company ConjuChem based on their DAC (drug affinity construct) technology, leading to decreased clearance. The drug is not prebound to albumin before injection but binds covalently to endogenous albumin. A modification of the second COOH-terminal amino acid from L- to D-alanine renders the peptide resistant to DPP-IV, while not blocking binding to the GLP-1 receptors or reducing activation of GLP-1 receptor–dependent signal transduction pathways in vitro. After a single injection, the half-life is ~10 days. In CJC-1131–treated persons with type 2 diabetes, Baggio showed evidence of a dose-response reduction in a seven-point glycemic profile, with evidence of body weight reduction in animal models and in the human studies. In a study presented at the meeting, Guivarc'h et al. (abstract 535) reported efficacy of CJC-1131 administered daily to 22 persons with type 2 diabetes for 14–20 days, showing a dose-dependent lowering of fasting and postprandial glucose levels and a weight decrease. Wen et al. (abstract 634) found no evidence of immunogenicity of CJC-1131 after administration of two doses separated by 6 weeks.

Albugon is a molecule covalently bound to albumin produced by the company Human Genome Sciences, exhibiting similar effects to those seen with exendin-4, although with less potent in vitro activation of the GLP-1 receptor. After intravenous or subcutaneous injection, albugon has a half-life of 11 h in mice and 2–4 days in cynomolgus monkeys. In the mouse model, it mimics the biological action of GLP-1 in decreasing glucose and glucagon and increasing insulin levels, and the molecule can be shown to act via the pancreatic GLP-1 receptor. Although less potent than exendin-4, there is definite effect in inhibiting gastric emptying in the mouse model, with no effect seen in mice lacking the GLP-1 receptor. "These studies," Baggio stated, "demonstrate that these larger albumin-bound molecules are able to access the neuronal pathways." Intraperitoneal albugon reduced food intake in the model, again without effect in mice lacking the GLP-1 receptor, with both albugon and exendin-4 increasing brainstem c-Fos in a similar fashion targeting the area postrema, further suggesting exhibition of at least some CNS effects similar to those of GLP-1. In response to a question regarding antibody production, Baggio noted that this has been shown in some patients treated with exendin-4, although not appearing to affect activity, and has not been shown at this point with either liraglutide or CJC-1131.

Neural effects of GLP-1

Tracy Ann Perry (Baltimore, MD) discussed neural actions of incretin peptides. GLP-1 receptor expression has been well demonstrated in rodent and human brain, with circulating GLP-1 entering the brain primarily in the periventricular areas, although to a lesser extent throughout the brain. In addition, GLP-1 is synthesized in the nucleus solitarius of the brainstem. CNS effects of GLP-1 include regulation of food intake and body weight consistent with receptor expression in the brain, and CNS GLP-1 signaling appears to be due, to large extent, to centrally synthesized GLP-1. GLP-1 is also a mediator of multiple stress responses. Infused into the lateral ventricle, it potentiates the release of corticotropin-releasing hormone. Perry noted that the central nucleus of the amygdala, which acts as a "fear center," and the hippocampus, playing a role in memory, also show response to GLP-1, with infusion into the amygdala leading to anxiety response and paraventricular infusion suppressing food intake. GLP-1 agonists increase heart rate and blood pressure. Furthermore, GLP-1 exhibits antiapoptotic and neurotrophic effects in neuronal cells, similar to its trophic actions in β-cells.

Perry hypothesized that GLP-1 and GLP-1 agonists may induce neuronal differentiation. In vitro, pheochromocytoma-derived PC12 cells express a functional GLP-1 receptor, increasing intracellular cAMP upon activation. The effects of GLP-1 are similar to those of nerve growth factor (NGF), while exendin-4 had somewhat different morphologic effects, with the combination of exendin-4 and nerve growth factor leading to an amplified effect. In an ibotenic acid–induced partial basal nucleus lesion in vivo, cholinesterase transferase activity was restored by local GLP-1 infusion, therefore providing evidence of benefit in a neurocytotoxic lesion. A series of GLP-1 analogs based on combined properties of GLP-1 and exendin-4 are being developed, some of which may exhibit neurotrophic activity. The peptides also protect against apoptosis in cultured hippocampal neurons. If these studies are confirmed, GLP-1 and exendin-4 may be considered neuroprotective, perhaps involving protein kinase C and cAMP-dependent protein kinase A pathway activation. In an oxidative insult model in cultured hippocampal neurons, both exendin-4 and GLP-1 showed concentration-dependent protective effects, with decreased amyloid β protein 1-40 levels, leading Perry to suggest that the agent be explored as a treatment for Alzheimer's disease. There is also evidence of a neuroprotective effect in a middle cerebral artery ligation stroke model.

Perry further discussed protective effects of GLP-1 and exendin-4 in the peripheral nervous system. In pyridoxine-induced sensory neuropathy model, pyridoxine alone led to weight loss, while GLP-1 infusion (but not with the receptor antagonist exendin-4 (9-39) protected against the pyridoxine-induced functional impairment, as shown by measurement of inclined screen climbing performance. Sciatic nerve morphology showed loss of large-diameter fibers and increase in small-diameter fibers, with both abnormalities improved by GLP-1 and by exendin-4 injection. The degree of degeneration was quite marked with pyridoxine alone but diminished by GLP-1 and by exendin-4.

In a study presented at the meeting,

Anini et al. (abstract 320) assessed the effects on food intake of exendin-4 in mice, showing decreased food intake at doses lower than those decreasing gastric emptying, with capsaicin, which disrupts sensory C-type neural pathways, partially decreasing the effect of low exendin-4 doses, suggesting a neural afferent mechanism as well as possible CNS effects at higher doses. Young et al. (abstract 1344) noted that the area postrema of the brain stem lacks a blood-brain barrier, responds to glucose, as well as to peptides including GLP-1, insulin, amylin, and cholecystokinin, and has vagal output, potentially controlling secretion of these hormones. In a rat model ablating this area, euglycemic clamps following arginine infusion were associated with the doubling of lactate and insulin levels, confirming its role in the control of insulin secretion.

Clinical studies of exenatide

Mack et al. (abstract 1717) administered exenatide (the designation for synthetic exendin-4 used as a pharmacologic agent) to high-fat–fed rats, showing dose-related weight loss over a 28-day period, which at the highest dose was approximately twice as great as that with sibutramine. Hiles et al. (abstract 1585) administered exenatide to mice and rats for 2 years at levels 6, 25, and 90 times higher (per kilogram body weight) than the maximal human dose and showed no increased frequency of islet cell hyperplasia, adenoma, or carcinoma.

In a human study, Calara et al. (abstract 508) reported that absorption of exenatide was similar after subcutaneous administration in the arm, thigh, and abdomen in 25 persons with type 2 diabetes. Poon et al. (abstract 588) reported a study of 156 metformin- or diet-treated persons with type 2 diabetes given placebo or 2.5, 5, 7.5, or 10 μg exenatide twice daily for 28 days. HbA_{1c} decreased by 0.04, 0.27, 0.37, and 0.49%, respectively, from the baseline average of 7.5%, with weight loss of 0.8, 0.7, 1.4, and 1.8 kg.

Ralph DeFronzo (San Antonio, TX) reported, at a presentation of late-breaking studies and in a poster (abstract 6-LB) the effects of exenatide treatment for 30 weeks in 336 persons with type 2 diabetes receiving metformin. He noted that initial dose-response studies using the agent in persons with type 2 diabetes showed effects in increasing insulin and decreasing glucagon levels with a consequent decrease in blood glucose (12). In the present study, 113 patients received placebo and the initial dosage for active treatment was 5 μg twice daily; after 4 weeks, 110 of the active treatment patients continuing this dose and the remaining 113 participants increased the dose to 10 μg twice daily. The study was completed by 79, 82, and 82% of participants, respectively. From baseline, HbA_{1c} levels of 8.2% decreased 0.5 and 0.9% in the 5- and 10-μg treatment groups at 30 weeks, with 13, 32, and 46% of patients assigned to placebo, 5 μg, and 10 μg, respectively, achieving HbA_{1c} <7%. A meal tolerance test showed lowering of postprandial glucose excursions as well as of fasting glucose in a dose-response fashion, with an earlier insulin peak and a lower proinsulin-to-insulin ratio in the treatment groups. From a baseline BMI of 34 kg/m^2, body weight decreased 0.3, 1.6, and 2.8 kg, respectively. Nausea occurred in 23, 36, and 45% of patients, occurring principally during the first 2 months, with severe symptoms in 2, 3, and 4% leading to withdrawal of 0, 1, and 2% of patients. Diarrhea occurred in 8, 12, and 16% and vomiting in 4, 11, and 12% of the respective groups. Hypoglycemia occurred in 5% of participants in each group. In a 1-year open-label extension of the study, HbA_{1c} was stable in previously treated patients and decreased by 1.1% in those previously receiving placebo. Overall, with treatment, HbA_{1c} decreased from 8.1 to 7% and weight decreased from 102 to 98 kg at 30 weeks, with further weight loss at 52 weeks, and multivariate analysis showed that the weight loss only explained a portion of the improvement in glycemia. Kendall et al. (abstract 10-LB) reported a similar 30-week study of 733 persons receiving sulfonylureas plus metformin, with HbA_{1c} decreasing from 8.5% by 0.8 and 0.5% with the 10- and 5-μg twice-daily exenatide doses, respectively, with a 1.6-kg weight loss in both groups. Hypoglycemia occurred in 13% of those receiving placebo but in 19 and 28% of patients receiving the 5- and 10-μg twice-daily exenatide doses. Buse et al. (abstract 352) reported a final similar 30-week trial of 377 persons with type 2 diabetes receiving sulfonylureas alone, with a baseline HbA_{1c} 8.6%, showing 0.5 and 0.9% reduction in HbA_{1c} with 5- and 10-μg twice-daily exenatide doses and a significant 1.6-kg weight loss in the 10-μg twice-daily dose group.

Clinical studies of DPP-IV inhibitors

Bo Ahren (Lund, Sweden) discussed a 1-year study of the DPP-IV inhibitor LAF237 in patients with type 2 diabetes treated with metformin. He noted that GLP-1 is rapidly eliminated from plasma, with $t_{1/2}$ of 1.5 min, because of inactivation by DPP-IV, a cell surface serine peptidase expressed by endothelial cells and rapidly inactivating biologically active peptides with serine or alanine as the second NH_2-terminal peptide. The active form of DPP-IV exists as a dimmer (13,14). Thus, >80% of total circulating GLP-1 is inactive due to DPP-IV action (8). Animal models not expressing the enzyme have been studied and show increased GLP-1 action (15). Ahrén reviewed his initial studies of DPP-IV inhibition in humans (16). The new DPP-IV antagonist LAF237 is similar to the earlier-studied compound valine pirolidide, which lacks action on P450 enzymes and has high enzyme affinity and reversible action. Single-dose efficacy studies in persons with type 2 diabetes, as well as 4-week studies, show decreased fasting, prandial, and 24-h glucose levels, with a 20-mg/dl decrease in fasting glucose, decreased glucagon, increased GLP-1, and no change in insulin levels despite lower glucose levels (17).

Ahren presented (see also abstract 354 and abstract 7-LB) a 1-year study of 107 metformin-treated patients with type 2 diabetes, 56 of whom received 50 mg LAF237 daily plus metformin at a mean dose of 1.8 g daily and 51 of whom received metformin with placebo. The baseline HbA_{1c} was 7.7% with LAF237 and 7.8% with placebo. At 3 months, HbA_{1c} was 0.7% lower and fasting and mean prandial glucose decreased 22 and 40 mg/dl. Forty-two LAF237 and 29 placebo patients were followed for 12 months, with HbA_{1c} increasing to 8.4% with placebo but remaining stable at 7.1% with LAF237, an overall 0.5% decrease vs. 0.6% increase in HbA_{1c}, with 41 vs. 10% reaching HbA_{1c} <7%. Body weight decreased similarly by 0.2 kg in both groups. In 12 patients treated with 100 mg LAF237 daily, HbA_{1c} decreased 0.8% at 12 weeks, compatible with a dose-related effect. Meal tolerance tests showed somewhat lower fasting and greater low-

ering of postprandial glucose excursion, with increased prandial insulin response. No change was reported in homeostasis model insulin resistance or in lipids. Mild hypoglycemia was seen in three of the patients in the active treatment group, without other adverse events described. Thus, Ahren characterized the DPP-IV inhibitor as a well-tolerated weight-neutral and effective agent achieving glucose lowering comparable to GLP-1 agonists while starting from a somewhat lower baseline level of glycemia. Pratley and Galbreath (abstract 355) reported a 12-week study of 72 persons with type 2 diabetes not receiving other pharmacologic therapy who were treated with 25 mg LAF237 twice daily. The fall in HbA_{1c}, adjusted for the response of 28 patients receiving placebo, was 0.6%, with evidence of a greater fall at higher baseline HbA_{1c} levels at 0.7 and 1.2% for baseline HbA_{1c} 7–8 and 8–9.5%, respectively. Ten percent of treated patients had one or more hypoglycemic episode.

Ahrens was asked whether, as the duration of action of LAF237 appears to be ~12 h, it might be appropriate to use the agent twice daily and noted that this is an important area for future study with this agent. Addressing the lack of weight loss with the agent, he pointed out that DPP-IV inhibitors do not achieve the same level or duration of GLP-1 action seen with injected GLP-1 agonists, suggesting that more potent agents might have additional effects. He was asked about whether other biologically active peptides regulated by DPP-IV might affect blood pressure and stated that mice not expressing the enzyme do not exhibit such abnormality, suggesting safety in this regard.

A number of additional reports were presented at the meeting addressing aspects of DPP-IV inhibition. Bose et al. (abstract 2) studied potential cardioprotective effects of GLP-1 in an ischemia-reperfusion model with or without administration of GLP-1, showing a decrease in infarct size both in vivo and in vitro in isolated perfused rat hearts, suggesting that the effect is not mediated by increased insulin secretion. Curiously, in vivo pretreatment with valine pyrolidide, which inhibits DPP-IV to prevent GLP-1 breakdown, blocked the cardioprotective effect, suggesting either a need for short duration of GLP-1 effect or an adverse consequence of DPP-IV inhibition unrelated to its degradation of GLP-1. Leiting et al. (abstract 6) and Lankas et al. (abstract 7) noted that DPP-IV is present as a surface marker on activated immune cells (where it is referred to as CD26). Other peptidases, including QPP, DPP8, and DPP9, may also be involved in immune regulation, and in an in vitro T-cell activation model, they showed that the nonselective inhibitor Val-boro-Pro and a DPP8/9 inhibitor inhibited proliferation, whereas there was no T-cell effect in their model of specific DPP-IV inhibition. Other adverse effects of DPP8/9 inhibition were, in rats, alopecia, thrombocytopenia, anemia, splenomegaly, and death and, in dogs, bloody diarrhea and emesis, while QPP inhibition lowered reticulocyte counts in rats and had no effects in dogs. Masur et al. (abstract 49-LB) showed that in vitro migration of stimulated human CD8 T-cells was reduced by ~40% by coincubation with GLP-1, suggesting physiologic effects on gut and islet immune function but also raising the possibility of untoward effects with exogenous administration.

Larsen et al. (abstract 1413) showed evidence of efficacy of another orally administered DPP-IV inhibitor, NN7201, which lowered postload glucose and increasing GLP-1 and GIP levels in a minipig model of mild insulin-deficient diabetes. Ahren and Hughes (abstract 1406) studied the effect of DPP-IV inhibition with valine-pyrrolidide in mice administered intravenous glucose alone or with GLP-1, GIP, pituitary adenylate cyclase–activating polypeptide, or gastrin-releasing peptide and showed that the insulin response to these agents was increased by 80, 40, 75, and 25%, respectively. Thus, the effects of DPP-IV inhibitors may be not limited solely to actions on GLP-1. Weber et al. (abstract 633-P) studied the Merck DPP-IV inhibitor MK-0431 in animal models and showed similar action in decreasing postglucose load glycemia in insulin-resistant models. Petrov et al., from the same group, used the DPP-IV inhibitor (2S,3S)-isoleucyl thiazolidide, showing delayed progression of hyperglycemia and development of insulin deficiency in female Zucker diabetic fatty rats fed a high-fat diet, thereby suggesting prevention of "β-cell exhaustion." Zhang et al. (abstract 58-LB) studied an insulin-deficient mouse type 2 diabetic model, showing that a 3-month period of treatment with this agent improved glycemia, triglycerides, and free fatty acid levels, with histological evidence of increases in insulin-positive islet cells, suggesting that this approach "may have disease-modifying capacity in the treatment of type 2 diabetes."

In a human study, Heins et al. (abstract 539) reported effects of the DPP-IV inhibitor P93/01, developed by the company Probiodrug. Following a 240-mg oral dose in 16 persons with mild type 2 diabetes, in 8 who had HbA_{1c} <6%, GLP-1 and GIP increased, without a change in glycemia, while those with HbA_{1c} >6% also had a fall in postmeal glycemia. Herman et al. (abstract 353) administered 25 or 200 mg MK-0431 to 56 persons with type 2 diabetes not receiving other pharmacologic treatment and showed a doubling of GLP-1 levels and a >20% increase in insulin with both doses and 22 and 26% decreases in the blood glucose increment following oral glucose and placebo, respectively.

Comparison of GLP-1 with other pharmacologic agents in diabetes

Zander et al. (abstract 1422) compared glycemic effects of pioglitazone and GLP-1 in eight persons with type 2 diabetes, showing a decrease in fasting plasma glucose from 13.5 mmol/l with neither drug to 11.7 mmol/l with GLP-1, which was administered by continuous subcutaneous infusion, to 11.5 mmol/l with 30 mg pioglitazone daily and to 9.5 mmol/l with both agents. Insulin levels were higher and glucagon levels lower with GLP-1 compared with pioglitazone, and sensation of appetite was reduced with GLP-1 alone or in combination. Although preliminary, the study suggests that thiazolidinedione–GLP-1 combinations offer an additional promising treatment approach.

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