INCRETIN-BASED THERAPY IN THE MANAGEMENT OF TYPE 2 DIABETES

RUCSANDRA DĂNCIULESCU MIULESCU [1], MĂDĂLINA MUŞAT [1] and CONSTANTIN IONESCU-TÎRGOVIŞTE [2]

[1]Endocrinology Department, University of Medicine and Pharmacy, "Carol Davila", Bucuresti
[2]Diabetes Department, University of Medicine and Pharmacy, "Carol Davila", Bucuresti
Correspondence Author: Rucsandra Danciulescu Miulescu, rucsandra_m@yahoo.com
fax: 004021/2105575

Received December 11, 2010

Type 2 diabetes is a progressive disease due to reduced beta cell mass, which results in the failure of traditional treatment and the need for more intensive therapeutical measures to maintain a good glycaemic control. Within the last decade there was a preoccupation for developing new classes of drugs to achieve several goals: glycaemic control, weight loss and to preserve residual beta cell mass together with being safe, well tolerated and convenient to use. An important progress in this respect was the development of the incretin-based therapy. Incretins are gut hormones namely glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide/gastric inhibitory peptide (GIP). These 2 incretins have different therapeutic potential. GLP-1 decreases appetite, delays gastric evacuation and inhibits glucagon secretion, while GIP affects minimally gastric emptying and does not affect glucagon secretion. GLP1 is reduced in type 2 diabetes, while GIP is normal. For these reasons only GLP1 was considered for developing a new class of drugs. GLP-1 is rapidly degraded by dipeptidyl peptidase 4 (DPP-4), with a half life of 2 min. Two alternative pathways have been thought to overcome this shortcoming: the development of GLP-1 analogs resistant to DPP-4 named incretin mimetics or the development of DPP-4 inhibitors.
Increased action of GLP-1 in patients with type 2 diabetes mellitus is a valuable therapeutic mean and the evidences support their use in patients in need of glycaemic and weight control together with a minimal risk of hypoglycaemia.

Key words: type 2 diabetes mellitus, incretin-mimetics, dipeptidyl peptidase -4 inhibitors.

INTRODUCTION

Pathogenesis of type 2 diabetes mellitus is due to the decrease of insulin secretion capacity as a consequence of a low beta cell mass in the presence of insulin-resistance. These events progress slowly to end in therapy failure, necessitating a more intensive therapy approach in order to maintain an optimal glycaemic control.

United Kingdom Prospective Diabetes Study (UKPDS) has established that at the time of diagnosis patients with type 2 diabetes mellitus present a reduction of beta cell function with 50%, which further decreases on average of 4% annually[1].

The last decade research was preoccupied on development and license new classes of therapeutic means that cumulate both a hypoglycaemic effect and preservation of the residual beta cell mass together with safety, good tolerability and convenient use. In this respect an important progress in the therapy of type 2 diabetes mellitus has been the development of incretin-based therapy.

Incretins are gut hormones exemplified by glucagon-like peptide (GLP1) and glucose-dependent insulinotropic peptide/gastric inhibitory peptide (GIP). GLP-1 is synthesized by L cells present ubiquitary at the gut level, with a maxim density in the distal ileum and colon, while GIP is produced in K cells from duodenum and proximal

jejunum[2]. Incretin plasma level is reduced during fasting and increases transiently after food ingestion. Incretins stimulate significantly insulin secretion and peptid C level mostly after oral glucose administration *versus* intravenous glucose. This particularity has been named incretin effect [3–5].

Studies showed a significant reduction of GLP1 after a mixed meal in patients with type 2 diabetes mellitus as compared to subjects with normal glucose tolerance. GLP1 secretion was assessed in 54 patients with type 2 diabetes mellitus, 33 subjects with normal glucose tolerance and 15 subjects with impaired glucose tolerance after a mixed meal. GLP-1 secretory response was significantly reduced in diabetics compared to normals. The patients with impaired glucose tolerance had a similar impairment of GLP-1 output as diabetics, though to a lower extend[5].

The therapeutical potential of incretins is different: GLP-1 decreases appetite, delays gastric evacuation and inhibits glucagon secretion, while GIP as no effect o glucagon output and has a minimal effect on gastric emptying; GIP is normal in patients with type 2 diabetes, as opposed to GLP1 whose concentration is reduced. For these reasons only GLP1 has been chosen to develop a new therapeutic class of drugs[6–8].

GLP-1 actions are mediated by a specific receptor present in pancreas, heart, kidney, lungs, thyroid C cells, pituitary, GI tract, peripheral and central nervous system (hypothalamus, hippocampus, cortex)[9]. Activation of the receptor by binding GLP-1is followed by an increase in cAMP and intracellular calcium concentration that stimulates other signaling pathways dependent on proteinkinase A, proteinkinase C, phosphatidyl – inositol 3 kinase (PI-3K), mitogen *activated proteinkinase (MAPK)* [8, 10].

PANCREATIC EFFECTS OF GLP-1

Within beta cells GLP-1 stimulates insulin secretion by stimulation of its gene transcription; the magnitude of this effect is dependent on the plasma glucose level, with a sudden onset at hyperglycaemic levels and an end when normal glycaemic levels are reached [11–17].

In experimental models on rodents and in cell cultures GLP-1 agonists have increased beta cell mass by stimulating cell proliferation via PI3K pathway [18] and by inhibition of apoptosis – mainly by increasing expression of antiapoptotic proteins and reducing expression of caspase 3.

Bcl-2 family comprises of pro- and anti-apoptotic proteins. Bcl-2 and Bcl-XL are anti-apoptotic members present in the mitochondrial membrane, while the pro-apoptotic proteins BAD or BAX reside in the cytosol. The latter act as sensors for stress signals. Cellular stress increases intracellular calcium which activates calcineurin phosphatase to subsequently dephosphorylate BAD and release it from the complex with protein 14-3-37. Now BAD can bind Bcl-2 from mitochondrial membrane, reducing its antiapoptotic function. Binding BAD on mitochondrial membrane induces pore formation and release of cytochrome C and other apoptotic molecules to form apoptosomes in the intermembranar space. Caspase cascade is activated to degrade cell DNA.

GLP-1 stimulates adenylate cyclase to generate cAMP that activates PKA. PKA subsequently phosphorylates BAD and favors its binding to 14-3- protein, thus retaining BAD within the cytosol [19–21].

There are controversies regarding the presence of GLP-1 receptors on the alpha pancreatic cells. Nevertheless GLP-1 administration induces glucagon suppression in a glucose-dependent manner [22, 23].

GLP-1 has also a stimulatory effect on somatostatin secretion in animal models[24].

Tornehave *et al.* have studied GLP-1 receptor expression in pancreatic cells in humans and animal models. Using *in situ* hybridization and double and triple fluorescence microscopy, they showed the presence of GLP-1 receptors in beta cells and ductal cells and the lack of receptor immunoreactivity on alpha and delta cells[25].

Within the pancreatic duct and islet cells GLP-1 can regulate growth and differentiation similar to a growth factor. GLP-1 activation was suggested to induce differentiation of exocrine pancreatic cells into an endocrine-like phenotype, increasing islet cell mass in humans and animal models[26].

EXTRAPANCREATIC EFFECTS OF GLP-1

GLP-1 delays gastric emptying which in turn reduces glycaemic postprandial increase and inhibits gastric secretion. This effect seems to be mediated via central and peripheral neuronal pathways and it is blunted by vagotomy[27, 28].

Chronic pharmacologic administration of GLP-1 agonists induce anorexia and weight loss[29, 30].

In muscle, adipose tissue and liver GLP-1 induces insulin-like effects on glycaemic homeostasis and lipid metabolism. There are still controversies with respect to the expression of GLP-1 receptors in this tissues and it has not been established yet whether GLP-1 acts independently, or its effects are secondary to the alterations in insulin, glucagon and somatostatin secretion. In experimental models GLP-1 administration was followed by an increase of glycogen synthesis and glucose oxidation secondary to activation of PI3K and MAPK pathways in muscle[31, 32]; in hepatocytes GLP-1 increases glycogen and reduces fatty acids synthesis and glucogenesis due to a decrease of phosphoenolpyruvatecarboxikinase and glucose-6-phosphatase[34].

GLP-1 receptors are also expressed in myocardium, endothelial cells and smooth muscle cells in the vessels, both in humans and in rodents. In experimental models GLP-1 has improved cardiac function, insulin sensitivity, myocardial glucose uptake and glycolysis and has reduced the size of myocardial infarction[27 35]. In humans, GLP1 administration has improved left ventricular ejection fraction post myocardial infarction, regardless of the localization of the infarction or the history of diabetes[36]. Ongoing studies will assess cardio protective effect of incretin-based therapy in patients with diabetes mellitus.

In animal models GLP-1 receptors are expressed in the pituitary and the hypothalamus, suggesting GLP-1 involvement in hypothalamo-pituitary axis regulation[37]. Central administration of GLP-1 in animals has increased ACTH secretion and subsequently cortisol and corticosterone secretion[38]. Other studies showed that GLP-1 stimulates the release of TSH and LH[39 40]. The significance of these data in humans is not yet known.

Preclinical studies have revealed that C cells of the thyroid express GLP-1 receptors. In vitro activation of the receptor stimulates calcitonin secretion via cAMP[41] and inhibits postprandial bone resorbtion[42]. In rodents GLP-1 receptor stimulation induces C cell hyperplasia and medullary thyroid carcinoma (MTC) in rodents. Effect of GLP-1 on C cells in humans remain to be established.

GLP-1 is rapidly degraded by dipeptidyl peptidase 4 (DPP-4) that is a ubicuitary serine-peptidase that splits a dipeptidyl from the aminoterminal end of its target peptides. This results in a half life of GLP-1 of approximately 2 minutes. So the only way to use GLP-1 in therapy seemed to be continuous infusion, which is unpractical for the long term therapy of type 2 diabetic patients. Later on have been identified two alternative ways to bypass DPP-4 degradation: development of GLP-1 analogs resistant to DPP-4 (incretin mimetics) and inhibition of DPP4 (DPP-4 inhibitors)[6].

GLP-1 agonists are: exenatide and exenatide-LAR, liraglutide, taspoglutide, albiglutide and DPP inhibitors: sitagliptin, saxagliptin, vildagliptin, alogliptin, linagliptin[43–52].

EFFICACY OF INCRETIN-BASED THERAPY

Exenatide, the first incretin mimetic developed was incorporated into medical practice in 2005 and has been used in the therapy of type 2 diabetes mellitus in Romania since 2008. It has a structural homology of 53% with GLP1 and has a lasting effect of maximum 7 hours. Daily use consists of 2 subcutaneous injections prior to meals. Exenatide is cleared through renal excretion and therefore contraindicated in end stage renal failure[45].

Placebo controlled studies with exenatide added to the current antidiabetic medication have revealed a significant decrease in HbA1c of 1.1% in patients treated with exenatide. There was a significant, progressive, dose-dependent weight loss, without plateau until week 30, with a total reduction of 4.4% of initial weight in patients who participated in an open-labeled extension of the studies[53–56].

Liraglutide is a GLP-1 analogue with 97% homology with GLP1, with one aminoacid substitution and addition of palmitoyl (fatty acid with 16 carbon atoms) to a lysine residue. These alterations in structure result in an increase albumin affinity, increase half-life (up to 12 hours) and sparing of renal clearance[46]. Liraglutide is administered once daily and has no contraindications in hepatic or renal failure[6, 46].

The efficacy of the therapy with liraglutide has been evaluated in randomized placebo controlled trials named LEAD (Liraglutide Effect and Action in Diabetes) that included over 4000 patients with type 2 diabetes mellitus[6].

LEAD 1 compared effects of liraglutide, rosiglitazone or placebo added to glimepiride therapy. The results showed that liraglutide 1.8 mg plus glimepiride have reduced HbA1c with 1.1%, significantly higher than rosiglitazone plus

glimepiride (drop of HbA1c with 0.4%), while glimepiride alone was folllowed by an increase of HbA1c with 0.2%. The reductions in HbA1c were higher in patients with monotherapy prior to enrollment as compared to those wit associated treatment[57].

LEAD 2 compared effects of metformin in association with liraglutide or glimepiride or placebo. Liraglutide 1.8 mg plus metformin have reduced HbA1c with 1%, similar to glimepiride plus metformin and significantly higher than metformin as monotherapy. As in LEAD 1, reductions in HbA1c were higher in patients with monotherapy prior to enrollment as compared to those wit associated treatment[58].

Liraglutide in monotherapy (LEAD 3 study) in naive patients with type 2 diabetes has reduced HbA1c with 1.2-1.6% [6].

LEAD 4 and LEAD 5 studies have assessed effects of triple association liraglutide with metformin and rosiglitazone, or liraglutide in combination with metformin and glimepiride. Results showed a 1.5% reduction of HbA1c with the former combination, and 1.3% with the later[59].

LEAD 6 has compared the efficacy of liraglutide versus exenatide in association with metformin or sulphonylurea. The results had showed a significant reduction of HbA1c in patients treated with liraglutide *versus* exenatide (1.1% *versus* 0.8%)[59].

The positive effects of liraglutide on weight loss observed in preclinical studies has been confirmed in LEAD studies.

In LEAD 1 liraglutide plus glimepiride have reduced body weight with a mean of 0.2 kg, while in LEAD 2 liraglutide plus metformin have dropped weight by 2.8 kg as compared with an average increase with 1 kg in patients treated with glimepiride plus metformin[57, 58].

The LEAD studies have revealed that incretin-mimetics reduce HbA1c with 0.6%-1.5%, drop body weight and have a minimal risk of hypoglycaemia. The drawbacks of this therapy are the need for injections and the GI side effects, which are transient and get better in few weeks from therapy onset.

Inhibitors of DPP-4 have a good biodisponibility and significant antidiabetic effects. They have the advantage of oral administration, but no effect on body weight and have a reduced incidence of hypoglycemia and no GI side effects[60, 61].

Sitagliptin, the first inhibitor of DPP-4 has been approved in the United States in October 2006 and has been in use in Romania since 2008. Sitagliptin in monotherapy has been assessed in a double blind, placebo-controlled study over 24 weeks in 741 type 2 diabetic patients with an average history of 4.4 years since diagnosis. Patients had poorly controlled diabetes, with an average of HbA1c of 8%. 238 patients had been assessed on sitagliptin 100 mg/day, 250 on sitagliptin 200 mg/day and 253 on placebo (n=250). The drop in HbA1c has been statistically significant in patients with sitagliptin *versus* placebo. There were no significant differences in HbA1c reduction in patients on 100 or 200 mg/day[62]. Sitagliptin in association with metformin, sulphonylurea and thiazolidindione have reduced HbA1c with 0.6-0.8% compared with placebo[63–65].

Vildagliptin efficacy has been assessed in several randomized placebo-controlled studies. Ferrannini and all have proved the non-inferiority of vildagliptin *versus* glimepiride in poorly controlled patients previously on metformin. Subjects have received vildagliptin (50 mg bid) or glimepirid (up to 6 mg/day, on average 4.5 mg daily) for 52 weeks[66]. Vildagliptin in association with thiazolidindiones in poorly controlled patients has improved glycaemic profile[67].

In 2009 saxagliptin has been approved by European Medicines Agency for the treatment of type 2 diabetes in adults, in association with metformin, thiazolidindiones and sulphonylurea. The reccommended dosage is 5 mg once daily. Saxagliptin has been assessed in clinical trials that included over 4000 patients with type 2 diabetes. In poorly controlled patients on metformin as monotherapy, association of saxagliptin (2.5 mg, 5 mg or 10 mg) has improved glycaemic control as compared with placebo. The percentage of patients who had attained HbA1c <7% after 24 weeks of treatment was significantly higher in saxagliptin compared to placebo [68]. Association of saxagliptin 2.5 mg or 5 mg/day to gliburide or thiazolidindione has generated a significant reduction of HbA1c and basal glycaemia as compared to placebo[9, 70].

Overall, studies results have showed that DDP-4 inhibitors drop HbA1c with 0.6%-1%, have a neutral effect on body weight and a reduced risk of hypoglycaemia. The advantages of this therapy over incretin-mimetics are oral administration and the lack of GI side effects.

SAFETY OF INCRETIN-BASED THERAPY

The most frequent side effects of GLP-1 agonists are of GI tract: nausea, vomiting, abdominal pain, diarrhea**.** These are transient and get better in few weeks from treatment onset.

Pancreatitis has been reported as a rare adverse event of exenatide, during postmarketing monitoring. An abstract of the first 30 cases of acute pancreatitis in patients treated with exenatide has been published in 2008. The authors have noticed in 90% of patients other predisposing factors for acute pancreatitis. The true relationship between exenatide therapy and incidence of acute pancreatitis is not yet established, as it is well known that patients with type 2 diabetes have an overall increased risk of pancreatitis of 3 fold than the general population[71].

Liraglutide manufacturer suggest a low incidence of acute pancreatitis[46].

Acute pancreatitis has been reported as an adverse event also during therapy with sitagliptin. 88 cases of acute pancreatitis have been recorded by FDA between October 2006 and February 2009.

Due to the available data on acute pancreatitis and the association between these drugs and pancreatic metaplasia and chronic pancreatitis noticed during experimental studies, it is recommended to monitor closely pancreatic function in patients treated with GLP-1 of DPP-4 inhibitors.

Preclinical studies have showed that thyroid C cells express GLP-1 receptors whose activation induces C cell hyperplasia and MTC. Animal models develop spontaneously age-induced nodular hyperplasia of C cells frequently. Sporadic MCT has a frequency of 0.5-1%, in animal models, higher in males secondary to ageing. In humans, MCT represents 3-10% of thyroid cancer and approximately 75% are sporadic, with 25% being part of a multiple endocrine neoplasia or familial MTC, with autosomal dominant inheritance[71]. Familial MCT is due to a mutation of RET proto-oncogene.

In 2009 the Regulatory Committee of FDA has communicated that preclinical toxicological studies with liraglutide have reported that C cell hyperplasia and MTC incidence increase with drug exposure. Single dose administration of liraglutide has increased the frequence of nodular lesions in C cells, but no MTC was reported. The present data suggest that MTC in rodents appears specifically to long acting GLP-1 agonist exposure, probably due to sustained stimulation of GLP-1 receptor. Clinical studies with liraglutide that monitored safety issues have revealed a small number of patients with an increase in calcitonin level during treatment. There have been reported few cases of papillary thyroid carcinoma during clinical development program of liraglutide, but data suggest these cannot be directly related to the GLP-1 agonist. Biological difference in C cells between human and rodents raise doubts about the utility of animal models in the study of human C cells during GLP-1 agonist therapy[71].

Due to DPP-4 expression on the surface of leucocytes, there is a concern regarding potential adverse events of DPP-4 inhibitors on immune system. There are reports of an increased incidence of upper respiratory airways' infections in patients on treatment with DPP-4 inhibitors[71].

It is necessary to perform more long term safety studies to clarify the potential of pancreatic and thyroid impairment secondary to incretin-based therapy.

CONCLUSIONS

To increase action of GLP-1 in patients with type 2 diabetes is a valuable therapeutic tool and can be attained by using either a GLP-1 agonist as a subcutaneous injection or an oral DPP-4 inhibitor.

Studies have shown that incretin-mimetic therapy reduces HbA1c with 0.6%-1.5% on average and also drops weight and presents a lower risk of hypoglycaemia. Disadvantages of this therapy are the use of injections and gastro-intestinal side effects, which are transient and get better in few weeks of the treatment onset.

DPP-4 inhibitors reduce HbA1c with 0.6%-1.% on average, have a neutral effect on body weight and a reduced risk of hypoglicaemia The advantages of this therapy are oral administration and lack of gastroinyestinal side-effects.

Present evidences support the use of incretin-based therapies in patients who need a more efficient glycaemic and weight control with a minimal risk of hypoglycaemia.

REFERENCES

1. UK Prospective Diabetes Study Group. UK prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes, 1995, 44:1249-1258.

2. Fujita Y, Chui JW, King DS, Zhang T, Seufert J, Pownall S, Cheung AT, and Kieffer TJ. Pax6 and Pdx1 are required for production of glucose-dependent insulinotropic polypeptide in proglucagon-expressing L cells. Am J Physiol Endocrinol Metab, 2008, 295**:** E648-E657.
3. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes and cardiovascular disease. JAMA. 2002;287:2414-23.
4. Dunning BE, Gerich JE. The role of a-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endo Rev. 2007;28:253-83.
5. Nauck MA. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab. 1986;63:492-8.
6. Guja C. Eficacitatea si siguranta terapiei bazate pe incretine. Diabetes Care. 2010 Vol 3, No. 2: 124-128.
7. Drucker DJ. Development of glucagon-like peptide-I-based pharmaceuticals as therapeutic agents for the treatment of diabetes. Curr Pharm Des. 2001;7:1399-412.
8. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3:153-65.
9. Estall JL, Drucker DJ. Glucagon and glucagon-like peptide receptors as drug targets. Curr Pharm Des, 2006 12:1731–1750.
10. Kieffer TJ, *et al.*, The glucagon-like peptides. Endocr Rev. 1999;20:876-913.
11. Bode HP, Moormann B, Dabew R, Goke B. Glucagon-like peptide 1 elevates cytosolic calcium in pancreatic ß-cells independently of protein kinase A. Endocrinology, 1999, 140:3919–3927.
12. Skoglund G, Hussain MA, Holz GG. Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. Diabetes, 2000, 49:1156–1164.
13. Holst JJ, *et al.*, The incretin system and its role in type 2 diabetes mellitus. Mol Cell Endocrin. 2009; 297:127-36.
14. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. Proc Natl Acad Sci USA 1987, 84:3434–3438.
15. Holz GG, Kuhtreiber WM, Habener JF.Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1(7–37). Nature, 1993, 361:362–36.
16. Bode HP, Moormann B, Dabew R, Goke B Glucagon-like peptide 1 elevates cytosolic calcium in pancreatic ß-cells independently of protein kinase A. Endocrinology, 1999, 140:3919–3927.
17. Skoglund G, Hussain MA, Holz GG. Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. Diabetes, 200, 49:1156–1164.
18. Buteau J, Roduit R, Susini S, Prentki M. Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells. Diabetologia, 1999, 42:856–864.
19. Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia. 2002;45:1263–1273.
20. Farilla L, Bulotta A, Hirshberg B, Li Calzi S, Khoury N, Noushmehr H, Bertolotto C, Di Mario U, Harlan DM, and Perfetti R. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology. 2003, 144: 5149-5158.
21. Farilla L, Hui H, Bertolotto C, Kang E, Bulotta A, Di Mario U, and Perfetti R. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology, 2002, 143**:** 4397-4408.
22. Fehmann HC, Hering BJ, Wolf MJ, Brandhorst H, Brandhorst D, Bretzel RG, Federlin K, Goke B. The effects of glucagon-like peptide-I (GLP-I) on hormone secretion from isolated human pancreatic islets. Pancreas. 1995;11:196–200.
23. Franklin I, Gromada J, Gjinovci A, Theander S, Wollheim CB. Beta-cell secretory products activate alpha-cell ATP-dependent potassium channels to inhibit glucagon release. Diabetes. 2005;54:1808–1815.
24. Fehmann HC and Habener JF Functional receptors for the insulinotropic hormone glucagon-like peptide-I(7-37) on a somatostatin secreting cell line. FEBS Lett, 1991, 279: 335-340.
25. Ditte Tornehave, Peter Kristensen, John Rømer, Lotte Bjerre Knudsen, R. Scott Heller.Expression of the GLP-1 Receptor in Mouse, Rat, and Human Pancreas Journal of Histochemistry and Cytochemistry, 2008, Volume 56 (9): 841-851.
26. Egan JM, Bulotta A, Hui H, and Perfetti R. GLP-1 receptor agonists are growth and differentiation factors for pancreatic islet beta cells. Diabetes Metab Res Rev, 2003, 19**:** 115-123.
27. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3:153-65.
28. Abbott CR, Monteiro M, Small CJ, Sajedi A, Smith KL, Parkinson JR, Ghatei MA, and Bloom SR. The inhibitory effects of peripheral administration of peptide YY(3–36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res, 2005, 1044**:** 127-131.
29. Flint A, *et al.*, Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest. 1998;101:515-20.
30. Holst JJ. Glucagon-like peptide 1 (GLP-1): an intestinal hormone, signalling nutritional abundance, with an unusual therapeutic potential. Trends Endocrinol Metab. 1999;10:229-35.
31. Acitores A, González N, Sancho V, Valverde I, and Villanueva-Peñacarrillo ML. Cell signalling of glucagon-like peptide-1 action in rat skeletal muscle. J Endocrinol, 2004, 180: 389-398.
32. González N, Acitores A, Sancho V, Valverde I & Villanueva-Peñacarrillo ML. Effect of GLP-1 on glucose transport and its cell signaling in human myocytes. Regulatory Peptides, 2005, 126 203–211.
33. Verónica Sancho, María V Trigo, Nieves González, Isabel Valverde Willy J Malaisse and María L Villanueva-Peñacarrillo. Effects of glucagon-like peptide-1 and exendins on kinase activity, glucose transport and lipid metabolism in adipocytes from normal and type-2 diabetic rats Journal of Molecular Endocrinology, 2005, 35 27- 38.
34. Cool B, Zinker B, Chiou W, Kifle L, Cao N, Perham M, Dickinson R, Adler A, Gagne G, Iyengar R. Identification and characterization of a small molecule

AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metabolism, 2006 3 403–416.
35. Bose AK, Mocanu MM, Carr RD, and Yellon DM. Myocardial ischaemia-reperfusion injury is attenuated by intact glucagon like peptide-1 (GLP-1) in the in vitro rat heart and may involve the p70s6K pathway. Cardiovasc Drugs Ther, 2007, 21: 253-256.
36. Nikolaidis LA, et al. Effects of glucagon-like peptide-1 in patients with acute myocardial infarction and left ventricular dysfunction after successful reperfusion. Circulation. 2004;109:962-5.
37. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience, 1997, 77:257–270.
38. Kinzig KP, D'Alessio DA, Herman JP, Sakai RR, Vahl TP, Figueiredo HF, Murphy EK, Seeley RJ, Figueredo HF. CNS glucagon-like peptide-1 receptors mediate endocrine and anxiety responses to interoceptive and psychogenic stressors. J Neurosci, 2003, 23:6163–6170.
39. Beak SA, Small CJ, Ilovaiskaia I, Hurley JD, Ghatei MA, Bloom SR, and Smith DM. Glucagon-like peptide-1 (GLP-1) releases thyrotropin (TSH): characterization of binding sites for GLP-1 on alpha-TSH cells. Endocrinology, 1996, 137: 4130-4138.
40. Beak SA, Heath MM, Small CJ, Morgan DG, Ghatei MA, Taylor AD, Buckingham JC, Bloom SR, and Smith DM. Glucagon-like peptide-1 stimulates luteinizing hormone-releasing hormone secretion in a rodent hypothalamic neuronal cell line. J Clin Invest, 1998, 101: 1334-1341.
41. Crespel A, De Boisvilliers F, Gros L, and Kervran A. Effects of glucagon and glucagon-like peptide-1-(7-36) amide on C cells from rat thyroid and medullary thyroid carcinoma CA-77 cell line. Endocrinology, 1996, 137: 3674-3680.
42. Clowes JA, Khsla S, Eastell R. Potential role of pancreatic and enteric hormones in regulating bone turnover**.** J Bone Miner Res. 2005, 20(9):1497-506.
43. Mentlein R, *et al.*, Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-l(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem. 1993;214:829-35.
44. Eng J, *et al.*, Isolation and Characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. J Biol Chem. 1992;267:7402-5.
45. Byetta. Summary of Product Characteristics, EMEA, 21 October 2009.
46. Victoza. Summary of Product Characteristics, EMEA, 21 October 2009.
47. Ahrèn B. Emerging dipeptidyl peptidase-4 inhibitors for the treatment of diabetes. Expert Opin Emerg Drugs. 2008;13:593-607.
48. Januvia. Summary of Product Characteristics, EMEA, 21 October 2009.
49. Onglyza. Summary of Product Characteristics, EMEA, 21 October 2009.
50. Galvus/Jalra. Summary of Product Characteristics, EMEA, 21 October 2009.
51. Pratley RE, Gilbert M. Targeting incretins in type 2 diabetes: role of GLP-1 receptor agonists and DPP-4 inhibitors. Rev Diabet Stud. 2008;5:73-94.
52. Tiwari A. Linagliptin, a dipeptidyl peptidase-4 inhibitor for the treatment of type 2 diabetes. Curr Opin Investig Drugs. 2009;10:1091-104.
53. Buse JB, *et al.*, Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients With type 2 diabetes. Diabetes Care. 2004;27:2628-35.
54. DeFronzo RA, *et al.*, Effects of exenatide (exendin-4) on glycemic control over 30 weeks in metformin-treated patients With type 2 diabetes. Diabetes Care. 2005;28:1092-100.
55. Kendall DM, *et al.*, Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients With type 2 diabetes treated with metformin and a sulfonylurea. Diabetes Care. 2005;28:1083-91.
56. Blonde L, *et al.*, Interim analysis of the effects of exenatide treatment on A1C, weight and cardiovascular risk factors over 82 weeks in 314 overweight patients with type 2 diabetes. Diabetes Obes Metab. 2006; 8:436-47.
57. Marre M, *et al.*, Liraglutide, a once-daily human GLP-1 analogue, added to a sulphonylurea over 26 weeks produces greater improvements in glycaemic and weight control compared with adding rosiglitazone or placebo in subjects with Type 2 diabetes (LEAD-1 SU). Diabet Med. 2009;26:268-78.
58. Nauck M, *et al.*, Efficacy and safety comparison of liraglutide, glimepiride, and placebo, all in combination with metformin, in type 2 diabetes. Diabetes Care. 2009;32:84-90.
59. Maria Moța, Emiliana Mocan, Sorina Botescu. Liraglutide efect and action in diabetes (LEAD) The Liraglutide Phase III Development Program. Romanian Journal of Diabetes Nutrition and Metabolic Diseases, 2010, Vol. 17, 1: 21-28
60. Kendall DM, *et al.*, Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use. Am J Med. 2009;122:S37-S50.
61. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet. 2006;368:1696-1705.
62. Aschner P, *et al.*, Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes Care. 2006;29:2632-7.
63. Nauck MA, *et al.*, Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, non-inferiority trial. Diabetes Obes Metab. 2007;9:194-205.
64. Hermansen K, *et al.*, Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, in patients with type 2 diabetes mellitus inadequately controlled on glimepiride alone or on glimepiride and metformin. Diabetes Obes Metab. 2007;9:733-45.
65. Rosenstock J, *et al.*, Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Clin Ther. 2006;28:1556-68.

66. Ferrannini E, *et al.*, Fifty-two-week efficacy and safety of vildagliptin vs. glimepiride in patients with type 2 diabetes mellitus inadequately controlled on metformin monotherapy. Diabetes Obes Metab. 2009;11:157-66.
67. Garber AJ, *et al.*, Vildagliptin in combination with pioglitazone improves glycaemic control in patients with type 2 diabetes failing thiazolidinedione monotherapy: a randomized, placebo-controlled study. Diabetes Obes Metab. 2007;9:166-74.
68. DeFronzo RA, Hissa MN, Garber AJ, Gross JL, Duan R, Ravichandran S, Chen SR. The Efficacy and Safety of Saxagliptin When Added to Metformin Therapy in Patients With Inadequately Controlled Type 2 Diabetes With Metformin Alone Diabetes Care 2009, 32:1649-55.
69. Chacra AR, Tan GH, Apanovitch A, Ravichandran S, List J, Chen R. Saxagliptin added to a submaximal dose of sulphonylurea improves glycaemic control compared with uptitration of sulphonylurea in patients with type 2 diabetes: a randomised controlled trial. Int J Clin Pract. 2009;63(9):1395-406.
70. Priscilla Hollander, Jia Li, Elsie Allen, Roland Chen Saxagliptin Added to a Thiazolidinedione Improves Glycemic Control in Patients with Type 2 Diabetes and Inadequate Control on Thiazolidinedione Alone The Journal of Clinical Endocrinology & Metabolism Vol. 94, No. 12 4810-4819.
71. Drucker D, Sherman S, Grelick F, Bergenstal R, Sherwin R, Buse J. Terapiile pe baza de incretine in diabetul zaharat tip 2: evaluarea riscurilor si beneficiilor. Diabetes Care. 2010 Vol 3, No. 2: 117-123.