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Biopharmaceutical Aspects of Lixisenatide for the Treatment of Type II Diabetes

Deslyna Taruli Manurung[1], Yulistiani[2,3]
[1]Master of Clinical Pharmacy Program, [2]Clinical Pharmacy Department,
Faculty of Pharmacy Airlangga University, Surabaya, Indonesia,
[3]Clinical Pharmacy Division, Airlangga Teaching Hospital, Surabaya, Indonesia

Abstract
Diabetes Mellitus (DM) is a metabolic disease (mostly hereditary) as a result of insufficient effective insulin, either due to pancreatic beta cell dysfunction or glucose uptake in peripheral tissues, or both (DMT2), or lack of absolute insulin (DMT1), with signs of hyperglycemia and glucosuria, accompanied by acute clinical symptoms (polyuria, polydipsia, weight loss), and/or chronic or sometimes asymptomatic symptoms. Primary disorder lies in the metabolism of carbohydrates, and secondary to the metabolism of fats and proteins. Epidemiologically, it is estimated that in 2030 the prevalence of DM in Indonesia reaches 21.3 million people. Based on the results of Basic Health Research (Riskesdas) in 2007, it was found that the proportion of causes of death due to DM in the age group of 45-54 years in urban areas was second ranked (14.7%), and in rural areas sixth ranked (5.8%). According to Diabetes International Diabetes (IDF) Diabetes Atlas data, Indonesia is the seventh country with DM population in the world in 2013, after China, India, the United States, Brazil, Russia and Mexico. One of the DMT2 therapies under development includes Lixisenatide, the Glucagon Like Peptide (GLP-1) receptor agonist group. GLP-1 works by stimulating insulin secretion and lowering glucose levels, having a lower risk of hypoglycemia than the Sulfonylurea group. This article will review biopharmaceutical aspects of Lixisenatide used in DMT2 therapy. Lixisenatide was approved by the FDA in July 2016. The review results show that Lixisenatide can be used either monotherapy or add-on, by subcutaneous injection once daily.

Keywords: *Diabetes Melitus Type 2, GLP-1, Lixisenatide*

I. INTRODUCTION

Insulin is synthesized and secreted from β cells in the Langerhans Island of the pancreas. The normal pancreas has about 1 million cells, which contain about 2-3% of the mass of the gland. The main types of Langerhans island cells are β cells that produce insulin, α cells that secrete glucagon, δ cells that produce somatostatin, and PP cells that produce pancreatic polypeptides. The β cell is the most numerous cell type and is mainly present in the core of the island of Langerhans, while the α and δ cells are found in the periphery. Langerhans island cells interact with each other through direct contact with the substances it produces (such as glucagon stimulates insulin secretion and somatostatin inhibits insulin and glucagon). In the picture below, the blood flow in the Langerhans island cell is centrifugally arranged so that different cell types are supplied in sequence β → α → δ. Insulin also has an autocrine effect (self-regulating) that converts insulin transcription and glucokinase gene into β cells. The Langerhans island cell as a whole is innervated by autonomic and peptidergic nerve fibers. Inactivity of parasympathetic nerve fibers from the vagus stimulates insulin release, whereas sympathetic nerve fibers inhibit insulin and stimulate glucagon secretion. Other pancreatic nerves contain peptides such as vasoactive intestinal peptide (VIP), which stimulates the release of all Langerhans island cell hormones, and Y neuropeptides (NPYs) that inhibit insulin secretion. The importance of the overall role of neuropeptides in controlling the secretion of Langerhans island cells remains unclear. The insulin molecule consists of a polypeptide chain, which is connected to a disulfide bridge. A chain contains 1 amino acid and B chain contains 30 amino acids.

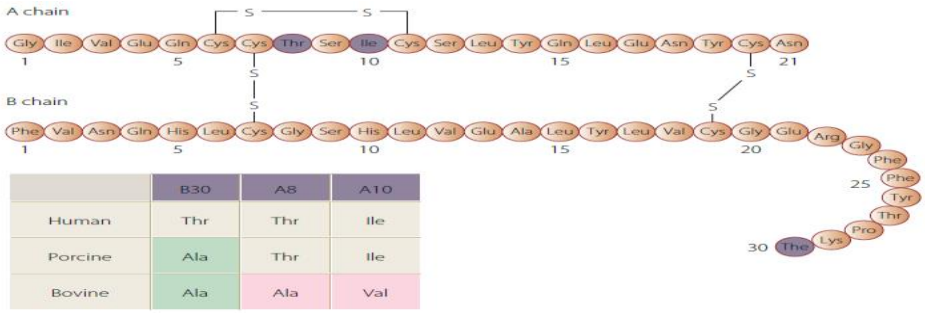

Figure I.1

The primary structure (amino acid sequence) of human insulin. There are differences in human insulin with pigs and cows (Bilous & Donelly, 2010)

Insulin and glucagon are produced in the pancreas by the cells within Langerhans Island. The β cells play a 70-90% role in producing insulin and amylin, while α cells produce glucagon. The main function of insulin is to reduce blood glucose levels. Glucagon, along with counter-regulatory hormones such as growth hormone, cortisol and epinephrine, increase blood glucose levels. Although blood glucose levels vary, the action of insulin and glucagon are opposite, along with the counter-regulatory hormone, usually maintaining fasting levels between 79-99 mg/dL (4.4-5.5 mmol/L) (Sease & Shealy, 2016).

Effect of Incretin: When nutrition enters the stomach and intestines, the incretin hormone is released, and stimulates insulin secretion. This incretin effect is mediated by two hormones, glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). GLP-1 is secreted by L cells from the ileum and especially the colon, while GIP is secreted by K cells. GLP-1 secretion is caused by endocrine and nerve signals that begin when the nutrients enter the digestive tract. A few minutes later, levels of GLP-1 will increase rapidly. The release of glucose-dependent insulin and dipeptidyl peptidase-4 (DPP-4) enzyme bind to GLP-1 rapidly become inactive metabolites. The effects of decreased glucose levels from GLP-1 include glucagon suppression, slowing gastric emptying and prolonging satiety (Sease & Shealy, 2016).

II. DIABETES MELLITUS

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia due to insulin secretion defects, or because of the work of insulin or because of both (ADA, 2016). Another opinion describes DM is a group of chronic metabolic disorders characterized by hyperglycemia that can lead to long-term microvascular and neuropathic complications. These complications suggest that diabetes is a major cause of (a) new cases of blindness in adults, (b) the final stage of kidney disease, and (c) nontraumatic lower extremity amputation. Macrovascular complications (coronary artery disease, peripheral vascular disease, and stroke) may also be associated with DM (Sease & Shealy, 2016). Insulin resistance is a major factor that differentiates DMT2 from other types of diabetes. Insulin resistance can be experienced several years before the diagnosis of DM is established. Insulin resistance occurs in adipose tissue, skeletal muscle and liver. Insulin resistance in the liver is a double threat because the liver becomes non responsive to insulin in glucose absorption, and glucose production in the liver after meals does not stop, and is higher during fasting and after meals (Sease & Shealy, 2016).

III. MANAGEMENT THERAPY OF DMT2

Specific treatment given to patients with DMT2 is determined based on clinical decisions regarding the balance of β-cell disorders and insulin resistance in certain cases. Patients who are overweight and obese tend to be insulin-resistant, meaning that metformin as an insulin sensitizer becomes the first logical choice. Lean patients generally experience a substantial β-cell failure, so sulfonylurea that works to stimulate insulin secretion tends to be effective when administered. The function of β cells decreases by about 4% per year so that sulfonylurea becomes less effective in relieving the disease. Approximately 50% of patients with DMT2 require insulin within 6 years after diagnosis, although there is still a growing choice of new therapies in combination therapy (Bilous & Donelly, 2010). The key to DMT2 therapy is diet and other modifications of lifestyle, such as diligent exercise and smoking cessation. The main goal of therapy is to lose weight obese patients and improve glycemic control. In addition, therapy is performed to reduce risk factors for cardiovascular disease (CVD) such as hyperlipidemia and hypertension, which contribute to 70-80% of deaths from DMT2 (Bilous & Donelly, 2010).

Table III.1 Glucose-lowering drugs available in the US and Europe as guidelines for the selection of therapy in patients with DMT2 (ADA, 2016)

CLASS	DRUGS	CELLULAR MECHANISM	PRIMARY PHYSIOLOGICAL ACTION(S)	ADVANTAGES	DISADVANTAGES	COST
Biguanides	Metformin	Activates AMP-kinase	↓ Hepatic glucose production	• Extensive experience • No hypoglycemia • ↓ CVD events	• GI side effects (diarrhea, abdominal cramping) • Vitamin B12 deficiency • Contraindications: CKD, acidosis, hypoxia, dehydration, etc • Lactic acidosis risk (rare)	Low
Sulfonylureas	2nd generation: Glyburide/ Glibenclamide, Glipizide, Gliclazide, Glimepirid	Closes K-ATP channels on β-cells plasma membranes	↑ Insulin secretion	• Extensive experience • ↓ Micro-vaskular risk	• Hypoglycemia • ↑ Weight	Low
Meglitinides (glinides)	Repaglinide, Nateglinide	Closes K-ATP channels on β-cells plasma membranes	↑ Insulin secretion	• ↓ PPG excursions • Dosing flexibility	• Hypoglycemia • ↑ Weight • Frequent dosing schedule	Moderate
TZDs	Plioglitazone, Rosiglitazone	Activate the nuclear transcription factor PPAR-γ	↑ Insulin sensitivity	• No hypoglycemia • Durability • ↑ HDL-C • ↓ Triglycerides (pioglitazone)	• ↑ Weight • Edema/heart failure • Bone fractures • ↑ LDL-C (rosiglitazone)	Low

CLASS	DRUGS	CELLULAR MECHANISM	PRIMARY PHYSIOLOGICAL ACTION(S)	ADVANTAGES	DISADVANTAGES	COST
α-Glucosidase inhibitors	Acarbose, Miglitol	Inhibits intestinal α- glucosidase	Slows internal carbohydrate digestion/ absorption	• No hypoglycemia • ↓ PPG excursions • Non systemic	• Generally modest A1C efficacy • GI side effects (flatulence, diarrhea)	Low to moderate
DPP-4 inhibitors	Sitagliptin, Vildagliptin, Saxagliptin, Linagliptin, Alogliptin	Inhibits DPP-4 activity, increasing postprandial active incretin (GLP-1, GIP) concentration	↑ Insulin secretion (glucose dependent) ↓ Glucagon secretion (glucose dependent)	• No hypoglycemia • Well tolerated	• Angioedema/urticarial and other immune-mediated dermatological effects • ? Acute pancreatitis	High
Bile acid sequestrants	Colesevelam	Binds bie acids in intestinal tract, increasing hepatic bile acid production	• ? ↓ Hepatic glucose production • ? ↑ Incretins levels	• No hypoglycemia • ↓ LDL-C	• Generally modest A1C efficacy • Constipation • ↑ Triglycerides • May ↓ absorption of other medications	High
Dopamine-2 agonists	Bromocriptine (quick release)	Activates dopaminergic receptors	• Modulates hypothalamic regulation of metabolism • ↑Insulin sensitivy	• No hypoglycemia • ? ↓ CVD events (Cycloset Safety Trial)	• Generally modest A1C efficacy • Dizziness/syncope • Nausea, fatigue, rhinitis	High

CLASS	DRUGS	CELLULAR MECHANISM	PRIMARY PHYSIOLOGICAL ACTION(S)	ADVANTAGES	DISADVANTAGES	COST
SGLT2 inhibitors	Canagliflozin, Dapagliflozin, Empagliflozin	Inhibits SGLT2 in the proximal nephron	Blocks glucose reabsorption by the kidney, increasing glucosuria	• No hypoglycemia • ↓ Weight • ↓ Blood Pressure • Effective at all stages of T2DM • Associated with lower CVD event rate and mortality in patients with CVD	• Genitourinary infections • Polyuria • Volume depletion/ hypotension/dizziness • ↑ LDL-C • ↑ Creatinine (transient) • DKA, urinary tract infections leading to urosepsis, pyelonephritis	High
GLP-1 receptor agonists	Exenatide, Exenatide extended release, Liraglutide, Albiglutide, Lixisenatide, Dulaglutide	Activates GLP-1 receptors	• ↑Insulin secretion (glucose dependent) • ↓Glucagon secretion (glucose dependent) • Slows gastric emptying • ↑ Satiety	• No hypoglycemia • ↓ Weight • ↓ PPG excursions • ↓ Some cardiovascular risk factors	• GI side effects (nausea/ vomiting/diarrhea) • ↑ Heart Rate • ? Acute pancreatitis • C-cell hyperplasia/medullary thyroid in animals • Injectable • Training requirements	High
Amylin mimetics	Pramlintide	Activates amylin receptors	• ↓Glucagon secretion • Slows gastric emptying • ↑ Satiety	• ↓ Weight • ↓ PPG excursions	• Generally modest A1C efficacy • GI side effects (nausea/vomiting) • Hypoglycemia unless insulin dose is simultaneously reduced • Injectable • Frequent dosing schedule • Training requirements	High

CLASS	DRUGS	CELLULAR MECHANISM	PRIMARY PHYSIOLOGICAL ACTION(S)	ADVANTAGES	DISADVANTAGES	COST
Insulins	• Rapid-acting analogs : Lispro, Aspart, Glulisine, Inhaled insulin • Short-acting : Human Regular • Intermediate-acting : Human NPH • Basal insulin analogs : Glargine, Detemir, Degludec • Premixed (several types)	Activates insulin receptors	• ↑ Glucose disposal • ↓ Hepatic glucose production • Supresses ketogenesis	• Nearly universal response • The oretically unlimited efficacy • ↓ Microvascular risk	• Hypoglycemia • Weight gain • ? Mitogenis effects • Training requirements • Patient reluctance • Injectable (except inhaled insulin) • Pulmonary toxicity (inhaled insulin)	Moderate to high

A. GLP-1 RECEPTOR AGONISTS

Oral glucose provokes a threefold to fourfold higher insulin response than an equivalent dose of glucose given intravenously. This is because the oral glucose causes a release of gut hormones (incretin), principally glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP 1), that amplify the glucose-induced insulin release. **Incretin effect** of GLP-1 secretion (but not GIP 1 secretion) is reduced in patients with type 2 diabetes dan when GLP-1 infused in patients with type 2 diabetes, it stimulates insulin secretion and lowers glucose levels. GLP-1, unlike the sulfonylureas, has only a modest insulin stimulatory effect at normoglycemic

concentrations. This means that GLP-1 has a **lower risk for hypoglycemia** than the sulfonylureas. GLP-1 receptors are present in the central nervous system and may play a role in the anorectic effect of the drugs. Type 2 diabetes patients undergoing GLP-1 infusion are **less hungry** (Masharani, 2017).

IV. NEW DRUG DEVELOPMENT

Lixisenatide, one of the new drugs that has been developed and adopted by the FDA in July 2016, is a non-insulin-dependent antiretroviral drug from the GLP-1 receptor agonist group (FDA, 2016).

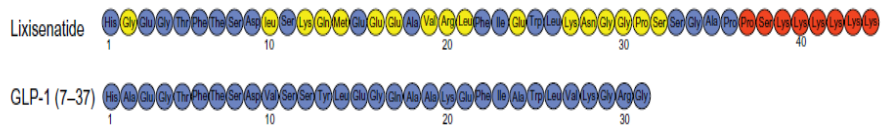

Figure 1 Structure of lixisenatide.
Notes: The yellow circles illustrate differences in amino acids compared with native GLP-1, and the red circles are differences compared with exendin-4.
Abbreviation: GLP-1, glucagon-like peptide-1.

Figure IV.1 Lixisenatide Structure (Petersen & Christensen, 2013)

The structure of lixisenatide consists of peptides as in the exendin-4 (exenatide) structure and consists of 44 amino acids. The difference with exendin-4 is found in the removal of the proline residue and the addition of six lysine residues C, where the above picture of the red circle shows the difference with exendin-4, and the yellow circle shows the difference with the structure of GLP-1 (Petersen & Christensen, 2013). Lixisenatide is able to withstand degradation by the DPP-4 enzyme due to the presence of six lysine residues C and the removal of the proline residue. The half-life of lixisenatide is 2-4 hours and is classified as short acting agonist GLP-1 receptor, in contrast to other long acting GLP-1s such as liraglutide and albiglutide. Despite having a short half-life, however, lixisenatide with a once-daily administration regimen can bind to the GLP-1 receptor strongly. This bond with this receptor is known from the concentration of median resistance (IC50) of lixisenatide is 1.4 nM, which is estimated to be four times larger than the GLP-1 bond itself. Meanwhile, liraglutide showed IC50 data of 0.11 nM, GLP-1 of 0.35 nM and an exenatide of 0.55 nM (Barnett, 2011).

B. SOME DIFFERENCE BETWEEN DRUG OF GLP-1 RESEPTOR AGONIST

GLP-1 receptor agonist agents have differences in delivery time, half-life, gastric emptying effect, max t, and dosage form.

Table IV.1 Pharmacological characteristics between GLP-1 and Lixisenatide receptor agonists (Horowitz, Rayner, & Jones, 2013)

Table 1 Pharmacologic characteristics of currently available GLP-1 receptor agonists and lixisenatide

Drug	Administration	$t_{1/2}$	t_{max}	Exposure	Sustained effect to slow gastric emptying
Exenatide IR [39]	Twice daily	2.4 h	2.1 h	Intermittent	Yes
Exenatide ER [45]	Once weekly	2.4 h	2 weeks[a]	Continuous	Minimal[b]
Liraglutide [41]	Once daily	11–15 h	9–12 h	Continuous	Minimal[c]
Lixisenatide[d] [46]	Once daily	2.8 h	1.25 h	Intermittent	Yes

ER extended release, *GLP-1* glucagon-like peptide-1, *IR* immediate release, $t_{1/2}$ terminal half-life, t_{max} time to maximum plasma concentration
[a] First peak, a second peak occurs at 6–7 weeks
[b] 75% less efficacy versus exenatide IR in head-to-head study [44]
[c] Flint et al. [42] and Degn et al. [43] analysis ongoing
[d] 20 μg dose

Several studies of lixisenatide have been performed and showed that lixisenatide was able to decrease HbA1C levels, showing significant differences in weight loss and also decreasing FPG levels more than those of other GLP-1 receptor agonist groups (Schmidt & et.al., 2014).

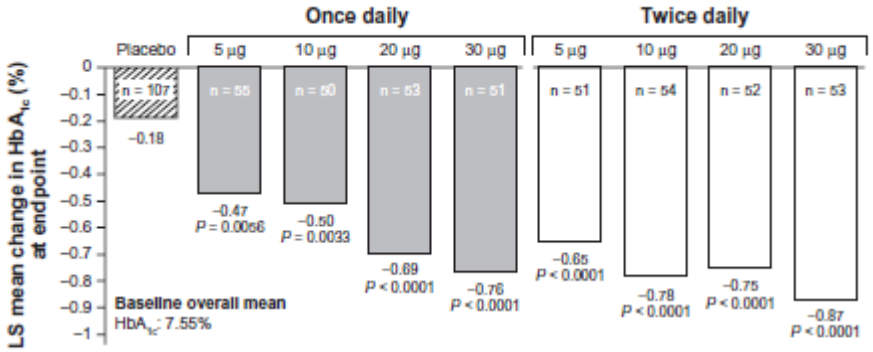

Figure IV.2 Changes in HbA1C after 13 weeks of therapy using lixisenatide with once daily or twice daily dosing regimens (Barnett, 2011)

In the graph above, a study of 542 patients with DMT2 (HbA1C ≥ 7.0% to <9.0%) who had metformin monotherapy was inadequate, and lixisenatide was administered with eight different dosing regimens (5, 10, 20, or 30 µg) with one-time administration times and twice daily, then compared with placebo for 13 weeks. The administration of lixisenatide has achieved a dose of 20 µg and 30 µg in the first 4 weeks, where the initial dose begins with 10 µg for a week, then raised 5 µg every week, to the expected dose. At week 13, significant statistical data were obtained on HbA1C decline from baseline to endpoint where the mean HbA1C decrease was 7.6%, each of which was 0.47%, 0.50%, 0.69%, and 0.76% for the once-daily administration regimen and 0.65%, 0.78%, 0.75%, and 0.87% in the twice-daily regimen (Barnett, 2011).

In terms of pharmacokinetics and safety in the use of lixisenatide in patients with renal impairment, there were also studies using a 5 µg subcutaneous dose of patients with normal kidney function (ClCr> 80 mL / min) of 8 people, then to 8 people with mild renal dysfunction (ClCr 50-80 mL / min), moderate (30-50 mL / min) and weight (<30 mL / min, but no dialysis required). From the research, it was found that patients with mild to moderate function disturbance showed no significant difference compared with healthy ones. However, in patients with severe renal impairment, there appears to be an increase in drug levels, so dose adjustment is required (Barnett, 2011).

In another study, 855 people, divided into 570 people in the lixisenatide group, and 285 were included in the group. Patients had previously received metformin and SU therapy, with an average BMI index of 30.2 kg / m2, and had diabetes averaged about 9.4 years 8.3%. Based on this study, significant decrease in HbA1C values was achieved at week 24 compared with placebo, where the mean HbA1C initial value of lixisenatide was 8.3% decreased to 7.4%, while the mean initial HbA1C level of placebo was 8 , 2% decreased to 8.1% (p <0.0001). Target values of HbA1C <7.0% and ≤ 6.5% were achieved significantly in patients from the lixisenatide group compared with the placebo group: 36.4% versus 13.5% for the HbA1C target <7.0%, and 19.3% versus 4.7% for target HbA1C ≤ 6.5% (p value <0.0001 for both) (Rosenstock *et al*, 2014).

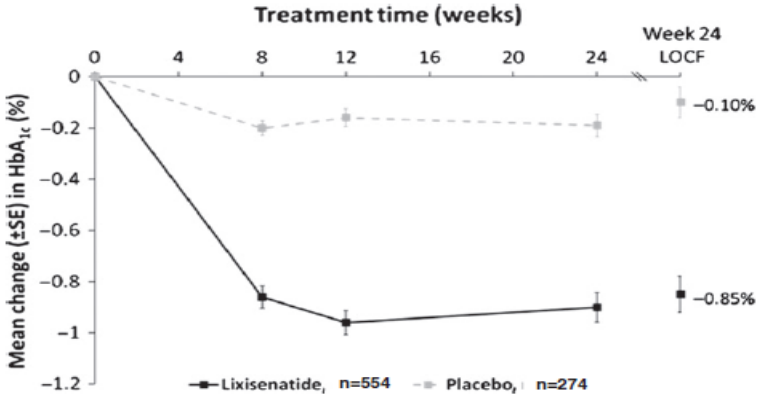

Fig. 1. Mean change in HbA_{1c} from baseline to Week 24 by visit. Week 24 LOCF data represent the LS mean change. HbA_{1c} = glycated hemoglobin; LOCF = last observation carried forward; SE = standard error.

Figure IV.3 Changes in mean HbA1C values from baseline to week 24 (Rosenstock *et al*, 2014)

Lixisenatide also significantly decreased the value of FPG (fasting plasma glucose) significantly from baseline to week 24 compared with placebo. The mean value of FPG lixisenatide decreased from 174.2 to 157.5 mg / dL, while placebo placebo value was 167.4 to 165.6 mg / dL (p <0.0001) (Rosenstock *et al*, 2014).

In terms of weight, lixisenatide also provides significant data in weight loss compared with placebo. On average there was a weight loss of 84.2 kg to 80.9 kg with the use of lixisenatide, and 84.5 kg to 83.6 kg with placebo. Overall, 14.4% of the lixisenatide group and 7.2% placebo obtained ≥5% weight loss from baseline to week 24 (Rosenstock *et al*, 2014).

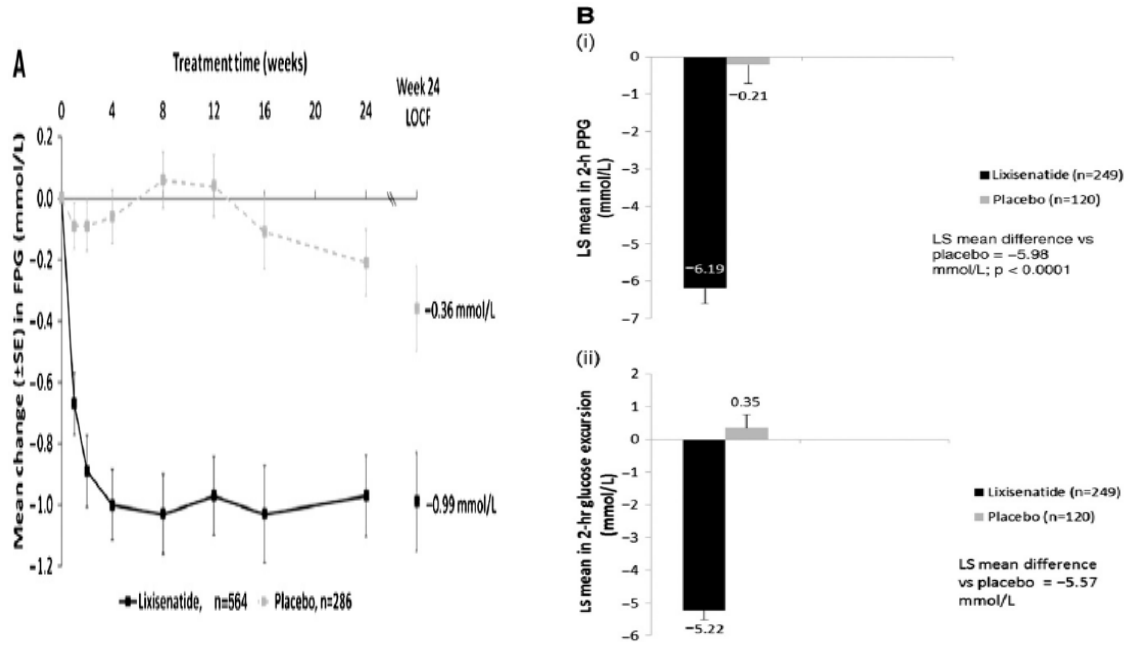

Figure IV.4 Changes in mean fasting plasma glucose (FPG) from baseline to week 24 (Rosenstock *et al*, 2014)

In the 2-hour PPG (postprandial glucose) test, the mean decreased from 299.3 to 191.2 mg / dL in the lixisenatide group increased from 298.2 to 300.3 mg / dL in the placebo group (p <0 , 0001). In the 2-hour test after eating glucagon, insulin, proinsulin and C-peptide levels decreased significantly with lixisenatide compared with placebo (Rosenstock, et al., 2014).

On the risk of hypoglycemia, the results did not differ significantly between the lixisenatide group and placebo, ie 15.3% versus 12.3% (not significant). It was mentioned that only one patient had severe hypoglycemia from the lixisenatide group (Rosenstock *et al*, 2014).

V. CONCLUSION

Type 2 Diabetes Mellitus is caused by deficiency of insulin secretion or combination of insulin resistance and β-cell dysfunction. Mechanism of antidiabetic agents is increasing insulin secretion, reducing hepatic glucose production, increasing insulin sensitivity, slowing carbohydrate absorption from intestine. GLP-1 receptor has a lower risk for hypoglycemia than the sulfonylureas. Lixisenatide as GLP-1 receptor agonists, are available and are increasingly used both as monotherapy, and "add-on" to other agents, particularly metformin, and more recently, basal insulin. Lixisenatide administrated once daily, resulted much better *t 1/2, t max, exposure, and sustained effect to slow gastric emptying* compared with another GLP-1 receptor agonists agents. Clinical responses to therapy from baseline to week 24, showed that Lixisenatide combined with basal insulin resulted decreases of mean HbA1c, mean FPG, mean change in body weight and mean insulin.

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