REVIEW

Cardiovascular Effects of Incretin-Based Therapies

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■ Abstract

GLP-1-modulating therapies are a class of anti-diabetic drugs that improve glycemic control by stimulating glucose-dependent insulin secretion from pancreatic beta-cells. In addition, GLP-1-based therapies have a variety of extrapancreatic effects, including satiety induction and gastric mobility reduction, which extend to distinct cardiovascular actions. GLP-1 was found to reduce infarct size in the context of acute myocardial ischemia which depends on the activation of prosurvival pathways including PI3-kinase, Akt, and ERK1/2. Also, GLP-1 augments the left ventricular function in dilative and metabolic cardiomyopathy, possibly by increasing insulin independent cardiomyocyte glucose uptake. Furthermore, experimental and preliminary clinical evidence suggest vasoprotective efficacy of GLP-1 mediated by improved endothelial function and anti-inflammatory capacities leading to atheroprotection. Mechanistically, the GLP-1 receptor is relevant for glucose lowering efficacy of GLP-1. However, many of its vasoprotective actions have also been described for the GLP-1 metabolite (9-37), which does not activate the GLP-1 receptor, suggesting the presence of an additional, yet unknown, signaling pathway. Ongoing research investigates the relevance of these observations in human disease and underlying mechanisms, which are reviewed in the present article.

Keywords: type 2 diabetes · hypoglycemia · glycemic control · antidiabetic drug · incretin · GLP-1 · DPP-4 · exendin-4 · liraglutide · cardioprotective

Introduction

An epidemic growth of obesity parallels the world wide expansion of the western life style. This is associated with a dramatic increase of insulin resistance and type 2 diabetes as primary risk factors for cardiovascular disease and heart failure.

While an inverse association between glycemic control and mortality is well established, data demonstrating a prognostic benefit of glucose-lowering therapies in diabetic patients are limited. Although intensive blood glucose control reduces microvascular complications, effects on macrovascular disease, or mortality, remain disappointing [1]. Interestingly, improved prognostic outcome has been reported for some antidiabetic drugs, but not for others despite similar HbA1c-lowering capacities. Metformin was found to reduce mortality in a subpopulation of the United Kingdom Prospective Diabetes Study (UKPDS) and in other retrospective cohort studies [2-5]. This suggests strong relevance of drug-specific, HbA1c-independent effects for overall prognosis. In case of metformin, this might be due to its favorable side effects, namely body weight reduction and avoidance of hypoglycemia [6]. Therefore, the evaluation of new antidiabetic drugs should include a thorough metabolic and cardiovascular risk assessment.

GLP-1-modulating drugs have been introduced to the clinics in recent years. High expectations for favorable prognostic effects of these drugs have been raised by their potent HbA1c-lowering capac-

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Drug Development and Clinical Trials in T2D

ity, and their beneficial effect on body weight and the avoidance of hypoglycemia. These are potential key elements for cardioprotective therapy.

GLP-1 is an incretin hormone which is secreted by the intestinal L-cells in response to nutritional stimuli [7]. Circulatory GLP-1 binds to the GLP-1 receptor expressed on pancreatic beta-cell, leading to increased glucose-dependent insulin secretion. In addition, GLP-1 inhibits glucagon release from pancreatic alpha-cells and impairs gastric emptying, which in concert improves postprandial glucose metabolism [8]. Finally, GLP-1 has beta-cell protective, antiapoptotic effects, and induces satiety by stimulation central hypothalamic neurons [7, 8].

GLP-1 is produced in a protein convertase PC1/3-dependent manner from the preproglucagon gene which also harbors glucagon, GLP-2, and oxyntomodulin [9]. This leads to the secretion of GLP-1 (7-36-amide) and GLP-1 (7-37) which both feature similar activity on the GLP-1 receptor [10]. The half life of GLP-1 is limited to approximately 2 minutes. The ubiquitously present enzyme dipeptidyl peptidase-4 (DPP-4) cleaves off the first two amino acids of the peptide leading to rapid inactivation [7]. Thereby, the N-terminal-truncated GLP-1 metabolite (9-36-amide), or (9-37), is created which is unable to activate the GLP-1 receptor and rather act as a weak receptor antagonist [11-13]. Consistently, *in vivo* application of the GLP-1 metabolite (9-36) does not increase insulin secretion in pigs or humans [14, 15]. However, a number of reports suggest a cardiovascular effect of the GLP-1 metabolite [16].

Signal transduction of GLP-1 is mediated via the GLP-1 receptor which belongs to the glucagon superfamily of G-protein-coupled receptors and functions by increasing intracellular cyclic adenosine monophosphate (cAMP), calcium, and phospholipase C [7, 17]. Expression of the GLP-1 receptor is found in a variety of tissues including pancreatic islet cells, lung, heart, kidney, stomach, brain, endothelial cells, vascular smooth muscle cells, and cardiomyocytes [7].

The improvement of glucose metabolism by the multifactorial actions of GLP-1 has let to the development of a variety of new drugs which either mimic the GLP-1 peptide or increase the bioactivity of active, endogenous GLP-1 by inhibition of its degrading enzyme DPP-4 [17]. So far, two GLP-1 agonists have been approved for the clinic. These are exendin-4 and liraglutide [17]. Exendin-4 is a natural DPP-4-resistant peptide with only 53% homology to GLP-1 but full receptor activating capacity. Liraglutide, however, is a fatty acid-modified GLP-1 homolog which displays a prolonged half life by increased albumin binding capacity. Both agonists similarly improve glucose metabolism and cause HbA1c reduction by approximately 1%. Another positive effect is that they promote weight loss and thereby favorably impact on the vicious cycle of obesity and insulin resistance [17]. A major drawback of these agonists is their need for daily subcutaneous injections. However, long-acting GLP-1 analogues are currently in clinical development, allowing a once weekly application. In contrast, DPP-4 inhibitors can be taken orally resulting in an 80% reduction of DPP-4 activity and a 2-fold increase of circulating active GLP-1 peptide [18]. So far, sitagliptin, vildagliptin, and recently saxagliptin have been approved for clinical use. These drugs cause an HbA1c reduction of approximately 0.8%, but they have no impact on body weight [19].

While clinical outcome studies investigating the cardiovascular effects of GLP-1-based therapies have been initiated, results will not be available for a few years. However, a number of experimental and preliminary clinical data suggest a cardiovascular benefit of GLP-1-based therapies, which will be discussed in the following sections.

Abbreviations:

AGE - advanced glycation end product
AMPK - AMP-activated protein kinase
ApoE - apolipoprotein E
BAD - Bcl-2-associated death promoter
cAMP - cyclic adenosine monophosphate
CVD - cardiovascular disease
DPP-4 - dipeptidyl peptidase-4
eNOS - endothelial nitric oxide synthase
ERK1/2 - extracellular signal-regulated kinases 1/2
GLP-1 - glucagon-like peptide-1
GLUT4 - glucose transporter type 4
GSK - glycogen synthase kinase
HbA1c - glycated hemoglobin
HDL - high-density lipoprotein
LPS - lipopolysaccharide
MAP - mitogen-activated protein
MAPK - mitogen-activated protein kinase
mTOR - mammalian target of rapamycin
NF-κB - nuclear factor-kappa B
NOS2 - nitric oxide synthase 2
Nrf2 - nuclear factor (erythroid-derived 2)-like 2
NYHA - New York Heart Association
PC1/3 - prohormone convertase 1/3
PI3K - phosphoinositol 3-kinase
PKA - protein kinase A
PPAR - peroxisome proliferator-activated receptor
RAGE - receptor for advanced glycation end-products
RISK - reperfusion injury salvage kinase
UCP3 - uncoupling protein 3
UKPDS - United Kingdom Prospective Diabetes Study

GLP-1 and the heart

GLP-1 and acute myocardial infarction

Cardioprotective effects of GLP-1 were first reported in patients with acute myocardial infarction who received 72 h of continuous GLP-1 infusion post angioplasty, leading to a significantly improved left ventricular systolic function [20]. This prompted a variety of additional studies investigating the therapeutic potential of GLP-1 in the context of myocardial ischemia.

While rapid revascularization of occluded vessels remains the main treatment strategy for myocardial infarction, additional approaches try to preserve tissue integrity by increasing the cellular tolerability to ischemia. Thereby, expansion of myocardial infarction depends on ischemic exposure and reperfusion injury caused by radical oxygen formation during revascularization. Experimental interventions applying fractional ischemia before vessel occlusion or gradual reperfusion after myocardial infarction can reduce tissue necrosis by approximately 50% [21]. Conceptually, this so called ischemic pre- or post-conditioning is used to identify new targets for pharmacological treatment [22]. Among these targets, the reperfusion injury salvage kinase (RISK) pathway has been identified as a promising candidate, together with the prosurvival signals phosphoinositide-3 (PI3) kinase, Akt, and extracellular signal-regulated kinase 1/2 (ERK1/2) [23]. Activation of the RISK pathway during reperfusion reduces myocardial infarct size by 40-50% [23]. Ongoing research is looking for RISK-modulating drugs with GLP-1 being of potential interest.

Cardioprotective effects of GLP-1 in acute myocardial ischemia have been described in a series of species and models [20, 24-36] although not confirmed in all studies [37, 38]. A substantial proportion of these investigations were done under *ex vivo* conditions in isolated heart perfusion models [24, 27, 28, 31, 33-35], which successfully translated to short [20, 24-26, 29, 30, 32, 36] and longer lasting *in vivo* studies [27].

Administration of GLP-1 significantly reduced myocardial infarction size and led to a functional recovery of rodent heart perfusion models under *ex vivo* and *in vivo* conditions [24, 28, 31, 34]. Similarly, liraglutide prevented myocardial injury, improved survival in mice, and reduced post-infarction cardiac rupture [27]. This was also confirmed in DPP-4 knockout mice [39]. Furthermore, DPP-4 inhibition augmented myocardial function

following myocardial infarction in rodent models [40, 41]. In rabbits, pre- or post-ischemic application of GLP-1 reduced infarction size, with best results seen when administered during reperfusion [25, 26]. These studies were extended to dogs, in which GLP-1 augmented regional wall motion recovery following a brief period of ischemia [26]. In pigs, exendin-4 limited infarct size and improved functional left ventricular recovery when given for 72 h starting with reperfusion [32]. However, two additional studies in pigs failed to demonstrate cardioprotective effects of GLP-1 or liraglutide in the context of acute myocardial infarction [37, 38]. This discrepancy may result from the use of different GLP-1 agonists or the timing of application prior to or post myocardial infarction.

At present, investigations in humans are limited. Besides the initial study, reporting the beneficial effects of GLP-1 in the context of acute myocardial infarction [20], two additional small studies have been conducted. The latter investigated the effects of continuous GLP-1 infusion in patients undergoing coronary artery bypass graft surgery. No effect of GLP-1 on myocardial contractility and hemodynamics was found in either study. Whereas, GLP-1 reduced the need for circulatory inotropic, or vasoactive, drug support following surgery [30, 42]. In a dobutamin stress test in patients with ischemic coronary artery disease, administration of GLP-1, or sitagliptin, improved myocardial wall motion [29, 36]. In conclusion, GLP-1 convincingly proved cardio-protective in *ex vivo* and small animal models during acute ischemia. Results in large animal models remain conflicting, while studies in humans suggest cardioprotection, but they are not conclusive. Therefore, a prospective, double-blind clinical trial, investigating GLP-1 effectiveness in patients with acute myocardial infarction, remains very preferable. Also, it is not clear to what extent these direct GLP-1 effects can be found in patients treated with GLP-1 analogues or DPP-4 inhibitors.

Mechanisms of GLP-1-dependent cardioprotection during ischemia

Although GLP-1-dependent cardioprotection has been extensively studied, the relevant underlying mechanisms are still not fully understood. On cellular basis, GLP-1 was found to reduce cardiomyocyte apoptosis in response to ischemic/reperfusion injury [24, 25, 27, 32, 43]. This can be attributed to GLP-1-dependent activation of PI3K, AKT, and ERK1/2, which together are known as
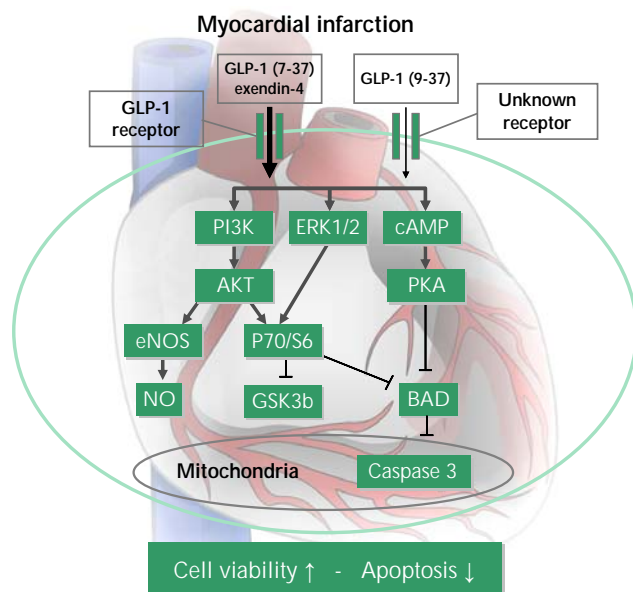
Figure 1. Effects of GLP-1 during acute myocardial infarction. PI3K: phosphoinositol 3-kinase. ERK1/2: extracellular signal-regulated kinases. PKA: protein kinase A. eNOS: endothelial nitric oxide synthase. BAD: Bcl-2-associated death promoter. GSK: glycogen synthase kinase 3.

the RISK pathway [24, 27, 32, 34, 43] (Figure 1). Furthermore, GLP-1 was found to activate the cell growth signaling cascade of mammalian target of rapamycin (mTOR) and p70s6K kinase, which are downstream of AKT and inhibit the proapoptotic protein BAD [24, 34, 43, 44]. However, this could not be confirmed in all models [27]. An additional antiapoptototic action of GLP-1 is attributable to the inhibition of GSK3β as another downstream target of the RISK pathway [27, 34, 43]. Finally, GLP-1 was found to increase the expression of redox sensitive transcription factors, namely nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and peroxisome proliferator-activated receptor (PPAR) delta [27].

Relevance of the GLP-1 receptor

Ongoing controversy debates the relevance of the known GLP-1 receptor as the target of GLP-1 actions in the cardiovascular system. This discussion is augmented by the persistent cardiovascular benefits associated with the GLP-1 metabolite (9-36), which does not activate the GLP-1 receptor [16].

Relevance of the GLP-1 receptor in the cardiovascular system has emerged through its broad expression in cardiomyocytes and endothelial cells, the decreased heart rate, elevated left ventricular (LV) end-diastolic pressure, and cardiac hypertrophy found in GLP-1 receptor knockout mice [33, 45]. It was also found that GLP-1 receptor knockout mice lose the liraglutide-dependent activation of prosurvival pathways in the heart [27]. Furthermore, GLP-1 receptor antagonist exendin-9 abolished the cardioprotective effects of GLP-1 in ischemia/reperfusion models. Similar effects were found with the inhibition of cAMP signaling as the downstream target of the GLP-1 receptor [24, 27, 28]. Also, in some experiments, simultaneous DPP-4 inhibition was required to reach GLP-1 effectiveness, suggesting that only the uncleaved GLP-1 (7-36) peptide was cardioprotective [24, 34]. This finding was further supported by observations of reduced myocardial infarction size, improved survival of DPP-4 knockout mice [39], and the augmented myocardial function seen with DPP-4 inhibition in rodent models [40, 41] and humans [29].

However, major relevance of the GLP-1 receptor has been challenged by other findings. Firstly, preserved GLP-1-mediated cardioprotection was found in hearts from GLP-1 receptor knockout mice [33]. Secondly, the GLP-1 metabolite (9-36)—which does not activate the GLP-1 receptor—was found to have infarct-sparing capacities similar to exendin-4 [35], although this was not confirmed in all studies [31, 33]. Interestingly, while exendin-4 lost its cell-protective effect in the absence of GLP-1 receptor, the effect was preserved for the GLP-1 metabolite [35]. This suggests the presence of an additional, yet unidentified, receptor only activated by the GLP-1 metabolite but not exendin-4 (Figure 1). However, this receptor seems to share significant structural and functional homology with the known GLP-1 receptor, as it was also found to increase cellular cAMP levels and was similarly antagonized by exendin-9 [35]. This suggests the presence of an additional G-protein-coupled receptor as target of the GLP-1 metabolite. Importantly, these observations question the use of exendin-9 as a tool to characterize specific effects of the known GLP-1 receptor.

GLP-1 and heart failure

GLP-1-dependent cardioprotection extends to the setting of chronic heart failure. This was firstly described in a dog model of pace maker-induced dilated cardiomyopathy. A continuous 48

h GLP-1 infusion improved cardiac function resulting in increased stroke volume and cardiac output, while simultaneously reducing heart rate, peripheral resistance, and plasma norepinephrine concentrations [46]. Similar effects were found in mouse models of primary dilated cardiomyopathy [47] and chronic ischemic [48], or metabolic, cardiomyopathy [49]. In humans, continuous GLP-1 infusion for 5 weeks augmented left ventricular ejection fraction and maximal oxygen uptake in a small study of patients with heart failures of classes NYHA III and IV [50]. In contrast, 48 h of GLP-1 infusion failed to improve cardiac function in a double-blind placebo-controlled crossover study in 20 patients with NYHA class II and III. This could have been due to the shorter application interval [51].

On molecular level, heart failure is characterized by a switch in primary substrate utilization from fatty acid oxidation in healthy subjects to glucose oxidation in heart failure patients [52] (Figure 2). The lower respiratory quotient of glucose thereby reduces myocardial oxygen demand [53]. This coping strategy is however defeated by a concomitant raise in local and systemic insulin resistance during heart failure, which limits glucose supply and causes a chronic state of energy deprivation [54]. Therefore, improvement of cardiomyocyte glucose delivery was identified as a target for heart failure therapy. GLP-1 directly increases cardiomyocyte glucose uptake, which happens independently of its insulinotropic actions and remains present under hyperinsulinemic clamp conditions [46]. Mechanistically, this might be attributable to increased GLP-1-dependent AKT activation and glucose transporter type 4 (GLUT4) translocation [47, 49]. It may also result from mitogen-activated protein (MAP) kinase and nitric oxide synthase 2 (NOS2)-dependent GLUT1 translocation [55]. Interestingly, in one study, the GLP-1 metabolite was also found to increase cardiomyocyte glucose uptake [11]. Additional cardioprotective effects of GLP-1 might result from a reduction of oxidative stress, reduced mitochondrial density, and increased UCP3 expression [55].

GLP-1 and the vessel wall

GLP-1 has vasoprotective effects. GLP-1-dependent improvement of vascular function has been reported in healthy subjects and type 2 diabetic patients with stable coronary artery disease [56, 57]. Continuous infusion of the peptide thereby rapidly increased acetylcholine, or flow-mediated, vasodilatation of the brachial artery

under hyperinsulinemic clamp conditions, demonstrating a direct and insulin-independent effect [56, 57]. Similar results were obtained from different experimental models in which GLP-1 increased vasorelaxation in coronary or pulmonary arteries in a cAMP- and endothelial nitric oxide synthase (eNOS)-dependent manner [33, 58]. In contrast, other findings suggested vascular smooth muscle to be of primary relevance for vasorelaxation in an eNOS-independent manner [59]. Importantly, eNOS inhibition abrogated 50% of the cardioprotective effect of GLP-1 following myocardial infarction, demonstrating a relevant contribution of the vascular system to GLP-1-dependent cardioprotection [33]. Interestingly, DPP-4 inhibition was able to abrogate GLP-1-dependent vasorelaxation, while this was preserved for the GLP-1 metabolite, even in the absence of the GLP-1 receptor [33]. This suggests that the GLP-1 metabolite was the primary cause of vasorelaxation. However, others reported similar improvement to endothelial function with exendin-4 as a non-cleavable peptide and classical activator of the known GLP-1 receptor [60].
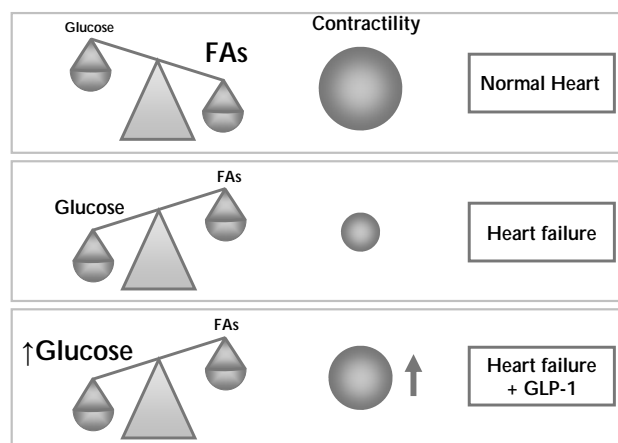

Figure 2. Modulation of myocardial substrate utilization by GLP-1 during heart failure. FA: fatty acids.

The vasorelaxative effects of GLP-1 might in part explain the antihypertensive potential demonstrated in long-term clinical trails using exenatide, liraglutide, or DPP-4 inhibitors [61-63], although most of the antihypertensive effects may be due to weight loss. Consistently, a 2-week GLP-1 treatment reduced blood pressure and proteinu-

ria in salt-sensitive hypertensive rats. This effect was paralleled by increased natriuresis and improved endothelial function [64]. However, these observations contrast to heart rate- and blood pressure-increasing effects of short-term GLP-1 application, as found by others [65, 66]. Potentially, this reflects a GLP-1-dependent bidirectional modulation of blood pressure in a time-dependent manner [67].
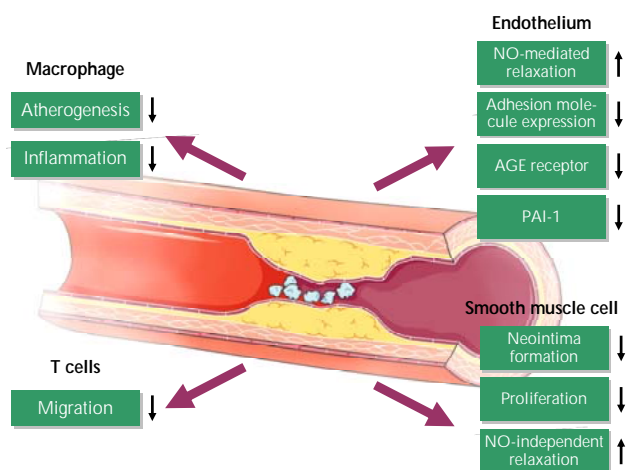

Figure 3. Vasoprotective effects of GLP-1.

Additional vasoprotective effects of GLP-1 have been described in acute vascular injury models in which exendin-4 reduced neointima formation and smooth muscle cell proliferation after wire injury [68]. Furthermore, chronic treatment with exendin-4 reduced atherosclerotic lesion formation in apolipoprotein E (ApoE)-deficient mice on a high fat diet [69]. Both studies reported reduced vascular inflammation under GLP-1 agonist treatment which was attributed to a variety of immune and vascular cells (Figure 3). GLP-1 was found to (*i*) reduce lipopolysaccharide (LPS)-dependent cytokine release from macrophages, (*ii*) inhibit the migration of T cells, (*iii*) repress endothelial cell adhesion molecule expression, and (*iv*) impair vascular smooth muscle cell proliferation [69-74]. These observations translated to a reduced mortality of exendin-4-treated mice challenged with LPS [75]. In macrophages, anti-inflammatory effects of GLP-1 have been attributed to inhibition of NF-κB in a cAMP- and protein kinase A (PKA)-dependent manner [69]. Similarly, GLP-1 was found to in-

hibit NF-κB in endothelial cells, although mediated by AMP-activated protein kinase (AMPK) [74]. However, neither cAMP, Akt, nor mitogen-activated protein kinase (MAPK) signaling was found to be responsible for antiproliferative effects by GLP-1 seen in vascular smooth muscle cells [68]. Anti-inflammatory effects of GLP-1 might also beneficially modulate the coagulation system, with decreased expression of plasminogen activator inhibitor being found in response to liraglutide in endothelial cells [72].

It was also found that GLP-1 can down-regulate the receptor for advanced glycation end-products (RAGE) in endothelial cells, which contributes to hyperglycemia-induced vascular inflammation in diabetes [71, 76]. Consequently, generation of reactive oxidant species in response to advanced glycation end product (AGE) exposure was reduced by GLP-1 agonist treatment [71]. Interestingly, similar anti-inflammatory and RAGE suppressive effects were reported for DPP-4 inhibition in diabetic rats resulting in reduced NF-κB activation [77]. These anti-inflammatory actions might also explain the improved survival of endotoxin-challenged rats when treated with DPP-4 inhibitors [75].

Furthermore, GLP-1 agonists have been found to improve lipid metabolism during longer term treatment intervals, leading to a reduction of triglyceride levels and increased HDL cholesterol [78, 79]. This effect seems to be due to a GLP-1-dependent improvement of postprandial lipemia [80], although it might also largely be secondary to improved glucose metabolism and body weight reduction.

The described effects on heart and vascular system, including the modulation of risk factors, might translate into long-term improvement in clinical prognosis. Indeed, a large retrospective database analysis recently compared cardiovascular events in diabetic patients treated since 2005 with exendin-4 or other antidiabetic drugs. Patients receiving exendin-4 were more likely to present with ischemic heart disease, obesity, hypertension, and/or other co-morbidities. However, the use of exendin-4 was associated with a 19% reduction in CV-events and a 12% reduction in CVD-related hospitalization, suggesting a cardiovascular benefits of GLP-1-based therapies [81].

Conclusions

Current experimental and early clinical data suggest that GLP-1-based therapies may modulate

vascular and cardiac function. As such, they exhibit protective effects in the cardiovascular system. Moreover, retrospective analyses of phase II and III studies with GLP-1 analogues and DPP-4 inhibitors showed a trend towards reduced cardiovascular events compared to placebo or comparator. This finding raised the hypothesis that these beneficial effects may translate into a reduction in cardiovascular morbidity and mortality in treated patients. Large randomized, prospective cardiovascular outcome trials are currently under way for various GLP-1-based drugs. The anxiously awaited results will clarify whether these therapies have the potential to reduce cardiovascular risk in patients with diabetes.

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Cardiovascular Effects of Incretin Therapy   The Review of DIABETIC STUDIES   Special Issue 389
Vol. 8 · No. 3 · 2011
Drug Development and Clinical Trials in T2D

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