JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
COMMENTARY

Cardioprotective effects of GLP-1(28-36a): A degraded metabolite or GLP-1's better half?

Cardiovascular disease (CVD) is one of the most serious health-related problems in patients with type 2 diabetes, especially in Western countries. Basic and clinical research on how to prevent CVD onset and progression in patients with type 2 diabetes has been an urgent task for decades. Glucagon-like peptide-1 (GLP-1) is one of two incretins that are responsible for ≥50% of postprandial insulin secretion and subsequent glucose excursion, and has been intensively investigated from the perspective of CVD due to its cardiovascular benefits in preclinical studies[1,2]. Dipeptidyl-peptidase (DPP)-4 inhibitors and GLP-1 receptor (GLP-1R) agonists are two major GLP-1-based therapies that are widely used clinically and have been gaining much attention for their possible cardiovascular benefits in patients with type 2 diabetes. Indeed, recent cardiovascular outcome trials (CVOTs) showed that some GLP-1R agonists, including liraglutide, semaglutide and dulaglutide, reduced the risk of major adverse cardiovascular events in patients with type 2 diabetes who have a history of CVD or multiple CVD risk factors[3–6]. Interestingly, CVOTs failed to show that the DPP-4 inhibitors, alogliptin, sitagliptin, saxagliptin and linagliptin, have benefits on major adverse cardiovascular events[3,7]. Although DPP-4 inhibitors and GLP-1R agonists both exert various biological effects through activation of GLP-1R signaling, the differing results of CVOTs for DPP-4 inhibitors and GLP-1R agonists calls for further investigation.

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Received 8 May 2020; revised 11 May 2020; accepted 14 May 2020

In response to meal ingestion, GLP-1 is released from the gut as an amidated 30-amino-acid hormone (i.e., GLP-1[7-36a])[8]. Degradation of GLP-1(7-36a) is rapidly catalyzed by DPP-4, producing the non-insulinotropic metabolite GLP-1 (9-36a) (Figure 1)[8]. GLP-1(7-36a) is also cleaved by neutral endopeptidase 24.11 (NEP24.11) to the non-insulinotropic GLP-1(28-36a) (Figure 1)[8]. The GLP-1R agonist liraglutide, which is resistant to degradation by DPP-4, but not by NEP24.11, reduced cardiac rupture and infarct size, and improved cardiac output in mice with acute myocardial infarction[9]. The DPP-4 inhibitor, sitagliptin, and genetic deletion of *Dpp-4* reduced mortality after myocardial infarction, and improved functional recovery after ischemia-reperfusion injury (IRI) *ex vivo*[10]. These results initially suggested that enhanced GLP-1R activation by GLP-1 (7-36a) leads to cardioprotective effects. However, a series of evidence now suggests that GLP-1 exerts cardioprotective actions through both GLP-1R-dependent and -independent pathways. Ban *et al.*[11] found that GLP-1(7-36a) increased left ventricular pressure after IRI in wild-type mice, but not in GLP-1R-deficient mice, whereas GLP-1(9-36a) significantly enhanced left ventricular pressure after IRI in both wild-type and GLP-1R-deficient mice. Importantly, the cardioprotective actions of GLP-1(9-36a) were blocked by exendin(9-39), yet were preserved in GLP-1R-deficient cardiomyocytes[12], suggesting that GLP-1(9-36a) or its metabolite interacts with a yet-to-be-identified receptor different from GLP-1R to exert cardioprotective effects. Although a dual receptor hypothesis for the cardioprotective action of GLP-1 and its metabolites has been discussed[13], the molecular mechanisms have been unclear until recently.

Recently, Siraj *et al.*[14] provided intriguing results that explain how GLP-1 might exert its cardioprotective effects in a GLP-1R-independent manner (Figure 2). The authors found that a NEP24.11-generated metabolite of GLP-1, GLP-1(28-36a), reduced myocardial infarct size, prevented cardiac dysfunction and protected coronary vascular cells from oxidative stress injury in both *in vivo* and *ex vivo* models of IRI[14]. Their rigorous work showed that GLP-1(28-36a) enters coronary artery endothelial cells through macropinocytosis and binds to mitochondrial trifunctional protein-α (MTPα), shifting substrate utilization from oxygen-consuming fatty acid metabolism toward oxygen-sparing glycolysis and glucose oxidation to increase adenosine triphosphate (ATP) production[14]. The increased intracellular ATP then modulates the ATP-sensor ,soluble adenylyl cyclase, thereby producing cyclic adenosine monophosphate and activating protein kinase A to exert cytoprotection from oxidative injury[14]. Importantly, the cardioprotective effects of GLP-1(28-36a) were lost in soluble adenylyl cyclase-deficient mice, but were comparable between wild-type and GLP-1R-deficient mice, confirming the GLP-1R independency.

Questions include the relevance of the findings by Siraj *et al.*[14] on the prevention of CVD onset and progression by GLP-1R agonists in patients with type 2 diabetes. Although human GLP-1-based GLP-1R agonists (e.g., liraglutide, semaglutide and dulaglutide) that show cardioprotective effects in CVOTs[3-6] are susceptible to cleavage by NEP24.11 (Figure 1), it remains to be seen if these cleaved products can bind to MTPα and

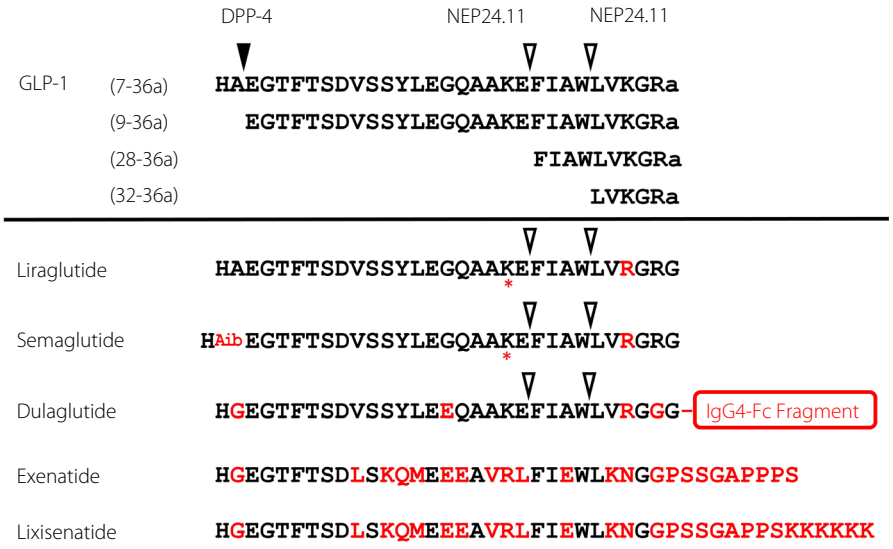

Figure 1 | Glucagon-like peptide-1 (GLP-1) and GLP-1 receptor (GLP-1R) agonists. The amino acid sequence of GLP-1, its metabolites and GLP-1R agonists are shown. Amino acids are shown by one letter code except for 2-aminoisobutyric acid as Aib; and amide is shown as "a". Amino acids different from those of human GLP-1 are shown in red. Lysine with an asterisk in liraglutide and semaglutide is conjugated with fatty acid derivatives; carboxyl-terminal glycine in dulaglutide is conjugated with immunoglobulin G4-Fc fragment. Closed and open triangles indicate cleavage sites by dipeptidyl-peptidase-4 (DPP-4) and neutral endopeptidase 24.11 (NEP24.11). Note that liraglutide and semaglutide can be degraded by NEP24.11, whereas exenatide is resistant to NEP24.11. No information on NEP24.11 sensitivity was reported for dulaglutide and lixisenatide.

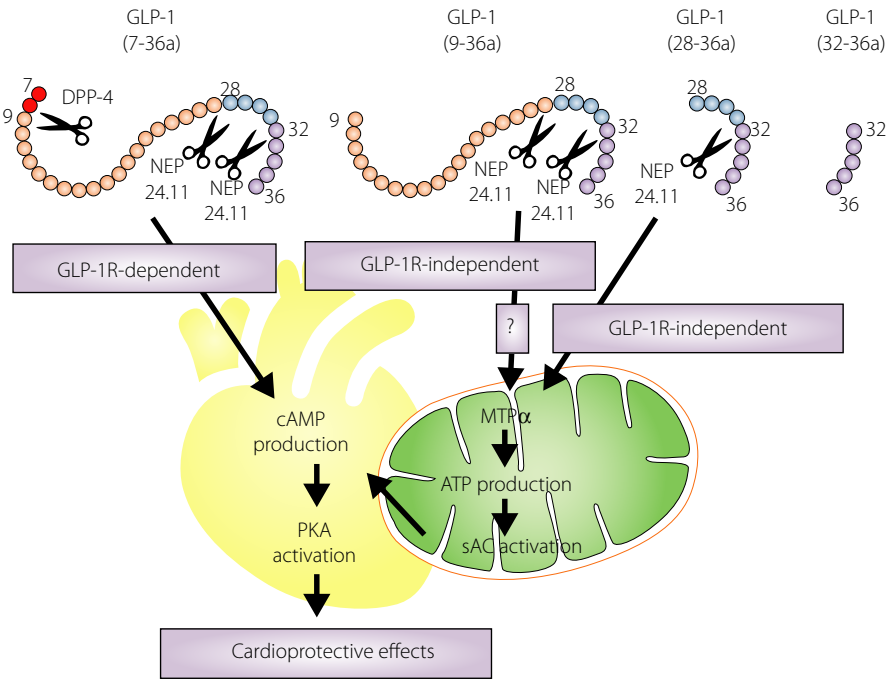

Figure 2 | Molecular mechanism underlying glucagon-like peptide-1 (GLP-1) exertion of cardioprotective effects in GLP-1 receptor (GLP-1R)-dependent and -independent manners. GLP-1 is released from the gut as an amidated 30-amino-acid hormone (i.e., GLP-1[7-36a]) after meal ingestion. GLP-1(7-36a) is rapidly degraded by dipeptidyl-peptidase-4 (DPP-4), producing the non-insulinotropic metabolite GLP-1(9-36a). GLP-1 (7-36a) is also cleaved by neutral endopeptidase 24.11 (NEP24.11) to the non-insulinotropic metabolite GLP-1(28-36a). GLP-1(28-36a) enters coronary artery endothelial cells through macropinocytosis and binds to mitochondrial trifunctional protein-α (MTPα), shifting substrate utilization to increase adenosine triphosphate (ATP) production and modulating an ATP-sensor, soluble adenylyl cyclase (sAC), thereby producing cyclic adenosine monophosphate (cAMP) and activating protein kinase A (PKA) to exert cytoprotection from oxidative injury.

shift substrate utilization. Exendin-4-based GLP-1R agonists (e.g., exenatide and lixisenatide) that failed to show cardioprotective effects in CVOTs[3,15] are resistant to cleavage by NEP21.11. Although exendin (9-39) can block the cardioprotective effects of GLP-1(9-36a)[12], it is unknown if exendin (9-39) can block the cardioprotective effects of GLP-1(28-36a), and if exendin-4-based GLP-1R agonists can bind to MTPα and shift substrate utilization. As the plasma levels of GLP-1(28-36a) have not been intensively investigated, it will be of interest to determine whether GLP-1(28-36a) levels that can be achieved by administration of DPP-4 inhibitors are sufficient to exert cardioprotective effects in diabetes. Further studies are required to determine whether the findings by Siraj *et al.*[14] can explain the differing cardioprotective effects of DPP-4 inhibitors and GLP-1R agonists in diabetes, and whether GLP-1(28-36a) can be utilized as a new anti-CVD therapy with lesser adverse effects than those associated with GLP-1R agonists (e.g., elevated heart rate), which the authors mention.

The findings by Siraj *et al.*[14] shed light on the effects of GLP-1 in other tissues and cell types. Previous studies showed benefits of GLP-1 and some GLP-1R agonists on non-alcoholic fatty liver disease in mice and humans, although the liver lacks expression of conventional GLP-1R[2]. It is possible that metabolites of GLP-1 and certain GLP-1R agonists interact with MTPα, which is a critical regulator of fatty acid β-oxidation and is tightly linked to the pathogenesis of non-alcoholic fatty liver disease[16]. Such GLP-1R-independent effects of GLP-1 might also be important in cells and tissues that express GLP-1R, including pancreatic β-cells. It is possible that binding MTPα with metabolites of GLP-1 and potentially GLP-1R agonists might contribute to amelioration of mitochondrial function, which is critical for glucose-induced insulin secretion. Based on the current findings by Siraj *et al.*[14], the various biological effects of GLP-1 and GLP-1-based drugs in diabetes need to be reconsidered by taking into account both the contributions of GLP-1R-independent and GLP-1R-dependent action. Although it has been over 100 years since the discovery of the incretin concept by Moore[17], and over 30 years since the discovery of GLP-1 as an incretin, the journey to understand the incretin system, and its role in health and disease continues.

ACKNOWLEDGMENT

This work was supported by grants from the Japan Society for the Promotion of Sciences (JSPS) (KAKENHI Grant 20K19673 [to Y Liu], and 20K11645 [to K Iizuka]) and Suzuken Memorial Foundation (to D Yabe).

DISCLOSURE

D Yabe received consulting or speaker fees from MSD K.K., Novo Nordisk Pharma Ltd. and Nippon Boehringer Ingelheim Co., Ltd. D Yabe also received clinically commissioned/joint research grants from Nippon Boehringer Ingelheim Co., Ltd., Eli Lilly and Company, Taisho Toyama Pharmaceutical Co. Ltd., MSD K.K., Ono Pharmaceutical Co. Ltd., Novo Nordisk Pharma Ltd., and Arklay Co. Ltd. K Iizuka received consulting or speaker fees from Novo Nordisk Pharma Ltd. and Kowa Company, Ltd. Y Liu, S Kubota and K Iizuka declare no conflict of interest.

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Doi: 10.1111/jdi.13302