● REVIEW

Repositioning of dipeptidyl peptidase-4 inhibitors and glucagon like peptide-1 agonists as potential neuroprotective agents

Shaker A. Mousa[1, 2, *], Bassam M. Ayoub[1, 2]

1 Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, USA
2 The Center for Drug Research and Development (CDRD), Pharmaceutical Chemistry Department, Faculty of Pharmacy, The British University in Egypt, El-Sherouk city, Cairo, Egypt

Abstract

Repositioning of dipeptidyl peptidase-4 inhibitors and glucagon like peptide-1 receptor agonists is a breakthrough in the field of neural regeneration research increasing glucagon like peptide-1 bioavailability, hence its neuroprotective activities. In this article, the authors suggest not only crossing blood-brain barrier and neurodegenerative disease as off target for dipeptidyl peptidase-4 inhibitors and glucagon like peptide-1 receptor agonists, but also for ophthalmic preparations for diabetic retinopathy, which may be the latest breakthrough in the field if prepared and used in an appropriate nano-formulation to target the retinal nerves. The relation of neurodegenerative diseases' different mechanisms to the dipeptidyl peptidase-4 inhibitors and glucagon like peptide-1 receptor agonists should be further examined in preclinical and clinical settings. The repositioning of already marketed antidiabetic drugs for neurodegenerative diseases should save the high cost of the time-consuming normal drug development process. Drug repositioning is a hot topic as an alternative to molecular target based drug discovery or therapeutic switching. It is a relatively inexpensive pathway due to availability of previous pharmacological and safety data. The glucagon like peptide-1 produced in brain has been linked to enhanced learning and memory functions as a physiologic regulator in central nervous system by restoring insulin signaling. Intranasal administration of all marketed gliptins (or glucagon like peptide-1 receptor agonists) may show enhanced blood-brain barrier crossing and increased glucagon like peptide-1 levels in the brain after direct crossing of the drug for the olfactory region, targeting the cerebrospinal fluid. Further blood-brain barrier crossing tests may extend dipeptidyl peptidase-4 inhibitors' effects beyond the anti-hyperglycemic control to intranasal spray, intranasal powder, or drops targeting the blood-brain barrier and neurodegenerative diseases with the most suitable formula. Moreover, novel nano-formulation is encouraged either to obtain favorable pharmacokinetic parameters or to achieve promising blood-brain barrier penetration directly through the olfactory region. Many surfactants should be investigated either as a solubilizing agent for hydrophobic drugs or as penetration enhancers. Different formulae based on *in vitro* and *in vivo* characterizations, working on sister gliptins (or glucagon like peptide-1 receptor agonists), different routes of administration, pharmacokinetic studies, dose response relationship studies, monitoring of plasma/brain concentration ratio after single and multiple dose, and neurodegenerative disease animal models are required to prove the new method of use (utility) for dipeptidyl peptidase-4 inhibitors as potential neuroprotective agents. Furthermore, investigations of glucagon like peptide-1 receptor agonists' neuroprotective effects on animal models will be considered carefully because they crossed the blood-brain barrier in previous studies, enabling their direct action on the central nervous system. Combination therapy of dipeptidyl peptidase-4 inhibitors or glucagon like peptide-1 receptor agonists with already marketed drugs for neurodegenerative disease should be considered, especially regarding the novel intranasal route of administration.

Key Words: *repositioning; DPP-4 inhibitors; GLP-1RA; neural regeneration; blood-brain barrier; Parkinson's disease; Alzheimer's disease; diabetic retinopathy*

Insulin Actions in the Brain and Role of Brain Insulin Resistance in Neurodegenerative Diseases

Insulin has multiple functions in various organs beyond glucose hemostasis in the body. Insulin's action on energy homeostasis and various brain neuronal functions has been discussed in several reports as highlighted by Lee et al. (2016). Insulin resistance plays a key role in dementia and in type 2 diabetes mellitus wherein dementia is accelerated. Insulin and insulin-like growth factors including insulin-like growth factor-1 and insulin-like growth factor-2 are important in newborn development, cell differentiation, plasticity, and survival of the nervous system, with a greater role for insulin-like growth factor-1. In the adult brain, insulin and insulin-like growth factor-1 act as paracrine signals released from all neural cells. Preclinical evidences supported the impact of insulin/insulin-like growth factor-1 and glucagon like peptide-1 (GLP-1) signaling pathways including downstream targets and receptors distribution within the brain for treating Alzheimer's disease and Parkinson's disease patients (Bassil et al., 2014). We have performed a Scopus database literature review of articles published between 2012 and 2018 on re-purposing of gliptins and GLP-1 receptor agonists (GLP-1RA).

Neuroprotective Effects of Dipeptidyl Peptidase-4 (DPP-4) Inhibitors and Glucagon Like Peptide-1 (GLP-1) Receptor Agonists (GLP-1RA)

DPP-4 inhibitors represent novel antidiabetic treatment strategies by reducing glycaemia, sustaining insulin levels, and reducing glucagon levels in type 2 diabetes mellitus (Ahrén et al., 2004). DPP-4 inhibitors' actions are mediated indirectly through preservation of GLP-1 incretins that are mainly metabolized by the key enzyme DPP-4 (Andersen et al., 2018). Another recent addition is the GLP-1RA group of drugs that demonstrated high glycemic control efficacy with minimal risk of hypoglycemia, with the potential in restoring beta cell function (Aroda, 2018). Synthetic GLP-1RA has a major advantage in that it is less susceptible to degradation by DPP-4 enzyme than the natural GLP-1 incretins. Both DPP-4 inhibitors and GLP-1RA act by increasing GLP-1. GLP-1 is secreted in the distal small intestine and is also produced in the central nervous system, predominantly in the brainstem, to stimulate insulin secretion, enhance insulin signaling pathway, inhibit glucagon secretion, and protect β cells from apoptosis with reported neuroprotective functions beyond the anti-hyperglycemic effects (Yildirim Simsir et al., 2018). Evidence suggests that increasing plasma and brain GLP-1 levels have a potential neural regeneration effect across a range of experimental models (Athauda and Foltynie, 2016). Furthermore, a proof-of-concept human trial using GLP-1RA showed persistent improvements in motor and cognitive function (Athauda and Foltynie, 2016). Also, GLP-1RA showed inhibition of retinal nerve damage in diabetic retinopathy by decreasing apoptosis and activation of glial cells playing a protective role for blood retinal barrier cells (Pang et al., 2018).

DPP-4 Inhibitors, GLP-1RA and the Blood-Brain Barrier Crossing Challenge

Blood-brain barrier crossing studies are an interesting part of gliptins' repurposing and are still a controversy (Chen et al., 2015), while GLP-1 crossed the blood-brain barrier in all reported investigations (Hunter and Holscher, 2012; Athauda and Foltynie, 2016). In contrast, DPP-4 inhibitor (namely, linagliptin) showed neuroprotective effects that were attributed to peripheral functions rather than directly in the central nervous system since it does not cross the blood-brain barrier (Darsalia et al., 2013; Lin and Huang, 2016). Linagliptin-mediated neuroprotection occurs directly at the neuronal level because the GLP-1 receptor expression in the brain is exclusively in neurons (Darsalia et al., 2013). On the other hand, in reported studies GLP-1RA crossed the blood-brain barrier successfully, suggesting a direct effect on the brain GLP-1 after expression in the hippocampal area, which is an important site of adult neurogenesis, in addition to their effect on plasma GLP-1 levels that cross the blood-brain barrier after its direct enhancement (Hunter and Holscher, 2012; Athauda and Foltynie, 2016). A growing number of studies have demonstrated neuroprotective effects of GLP-1 receptor stimulation in models of Parkinson's disease and Alzheimer's disease, resulting in improvements in cognition, memory formation, motor, and non-motor deficits (Hunter and Holscher, 2012; Athauda and Foltynie, 2016). A rat tissue distribution study published by Srinivas (2015) suggested that the DPP-4 inhibitor, linagliptin, is a good substrate for P-glycoprotein, which not only limits the oral absorption but may also have a significant role to play in effluxing linagliptin from the brain and therefore rendering negligible entry of linagliptin into the rats' brain, although it was extensively distributed to the other organs. Moreover, the author suggested that the physiological consequence of Alzheimer's disease that leads to reduced blood-brain barrier resistance provides an opportunity for linagliptin to penetrate the blood-brain barrier, modulating the incretin levels *via* blocking DPP-4 enzyme localized in the brain and activating adenosine monophosphate activated protein kinase in the neuronal cells rendering neuroprotective properties. On the other hand, restoration of gut incretin levels by linagliptin may also indirectly help in the regulation of brain incretin hormones due to the overall establishment of glucose homeostasis and enhancement of insulin signaling pathway (Srinivas, 2015). Although many studies mention that previously developed DPP-4 inhibitors did not cross the blood-brain barrier, a recent study designed by Ayoub et al. (2018b) showed blood-brain barrier crossing of a novel once-weekly DPP-4 inhibitor, omarigliptin, based on its lipophilic properties and Log *P* value. In this study, the authors compared the blood-brain barrier crossing ability of trelagliptin and omarigliptin, but trelagliptin did not cross the blood-brain barrier through the oral route. In addition, a novel intranasal formulation for omarigliptin (a recently marketed once-weekly DPP-4 inhibitor) was developed and documented significant increase in brain/plasma ratio compared to oral omarigliptin (Ayoub et al., 2018b). Enhancing the blood-brain barrier crossing ability of omarigliptin based on its intranasal administration (Ayoub et al., 2018b) starts a new era for neuro-repurposing of gliptins. The potential of DPP-4 inhibitors and GLP-1RA might represent potential opportunity for treatment of patients with Alzheimer's disease given the current lack of any available effective Alzheimer's disease strategies.

Repurposing of DPP-4 Inhibitors and GLP-1RA for Neurodegenerative Diseases

The repositioning of already marketed antidiabetic drugs for neurodegenerative disease should save the high cost of the time-consuming normal drug development process. Drug repositioning is a hot topic as an alternative to molecular target based drug discovery or therapeutic switching. It is a relatively inexpensive pathway due to availability of previous pharmacological and safety data (Ayoub et al., 2018b). Both omarigliptin and trelagliptin were tested against MCF-7 breast cancer cell lines and showed half maximal inhibitory concentration (IC_{50}) values of 125 and 250 μg/mL, respec-

tively (Vacsera, Giza, Egypt). However, the relatively high value of IC_{50} and the absence of potent anticancer activity at lower concentrations, after National Cancer Institute screening (Rockville, MD, USA), excluded their repositioning as potent anticancer agents by Ayoub et al. (2018b) in contrast to the successful anticancer repurposing results obtained for linagliptin by Ayoub et al. (2018a) after studying its modulating effect towards Adenosine A3 receptor, showing an inhibitory profile against hepatocellular carcinoma cell lines with induction of apoptosis at G_2/M phase with increase in caspase-3 levels, accompanied by a down-regulation in gene and protein expression levels of adenosine A3 receptor with a subsequent increase in cyclic adenosine monophosphate.

The GLP-1 produced in brain has been linked to enhanced learning and memory functions as a physiologic regulator in central nervous system by restoring insulin signaling. Intranasal administration of all marketed gliptins (or GLP-1RA) may show enhanced blood-brain barrier crossing and increased GLP-1 levels in the brain after direct crossing of the drug for the olfactory region, targeting the cerebrospinal fluid. Further blood-brain barrier crossing tests may extend DPP-4 inhibitors' effects beyond the anti-hyperglycemic control to intranasal spray, intranasal powder, or drops targeting the blood-brain barrier and neurodegenerative disease with the most suitable formula. Moreover, novel Nanoformulation is encouraged either to obtain favorable pharmacokinetic parameters or to achieve promising blood-brain barrier penetration directly through the olfactory region. Surfactants other than the reported sodium lauryl sulphate by Ayoub et al. (2018b) will be investigated either as a solubilizing agent for hydrophobic

drugs or as penetration enhancers. Different formulae in *in vitro* and *in vivo* characterizations, working on sister gliptins (or GLP-1RA), different routes of administration, pharmacokinetic studies, dose response relationship studies, monitoring of plasma/brain concentration ratio after single and multiple dose, and neurodegenerative disease animal models are required to prove the new method of use (utility) for DPP-4 inhibitors as potential neuroprotective agents. Furthermore, investigations of GLP-1RA neuroprotective effects on animal models will be considered carefully because they crossed the blood-brain barrier in previous studies, enabling their direct action on the central nervous system. combination therapy of DPP-4 inhibitors or GLP-1RA with already marketed drugs for neurodegenerative disease will be considered, especially regarding the novel intranasal route of administration. For example, one of the marketed DPP-4 inhibitors or GLP-1RA (**Figure 1**) could be used in combination with one of the most common drugs for neurodegenerative disease: levodopa, carbidopa, deprenyl, tyrosine hydroxylase, apomorphine, anticholinergic drugs, metabotropic glutamate receptor 4 agonists, acetylcholinesterase inhibitors, N-methyl-D-aspartate receptor antagonists). Although Parkinson's disease and Alzheimer's disease are the most common forms of neurodegenerative disease, repurposing of DPP-4 inhibitors and GLP-1RA could be effective with the other neurodegenerative disease forms that induce neurons' death, like amyotrophic lateral sclerosis and Huntington's disease. Recent literature describing the therapeutic value of DPP-4 inhibitors for the treatment of neurological conditions are highlighted by Al-Badri et al. (2018).

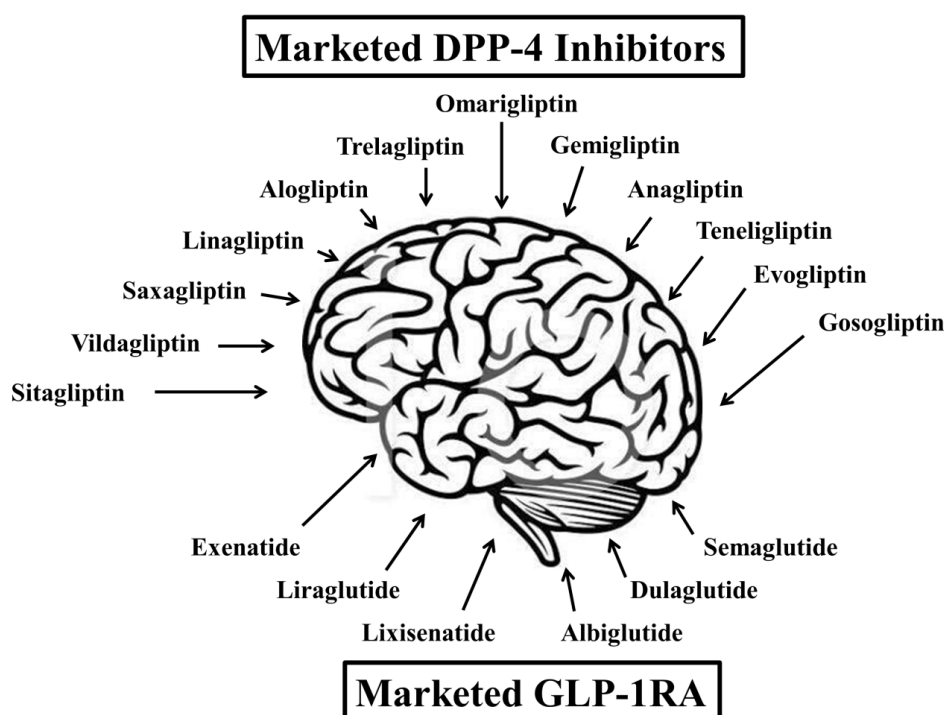If the suggested drugs, either alone or in combination,

Figure 1 Marketed dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon like peptide-1 receptor agonists (GLP-1RA).

show promising results in neurodegenerative disease animal models, they potentially may be used in combination formulas for patients who suffer from both diabetes mellitus and neurodegenerative disease rather than a complete repurposing for neurodegenerative disease. Because insulin resistance affects central nervous system in neurons and glial cells, defective brain insulin signaling directly plays a role in neurodegenerative disease pathogenesis by reducing brain metabolism so that diabetes mellitus is a major risk factor for neuropathy, affecting the control of cognition and neuronal function in brain. Neurodegenerative disease shares with diabetes mellitus impaired glucose metabolism. Many well-established studies linked diabetes mellitus as a risk factor for neurodegenerative disease, especially Parkinson's disease and Alzheimer's disease. So, working on the neuroprotective effects of the marketed antidiabetic drugs will add value as a secondary beneficial effect in addition to its insulin-related mechanism for high-risk patients with diabetes mellitus.

Conclusion

Repurposing DPP-4 inhibitors and GLP-1RA for neurodegenerative disease is a breakthrough in the field of neural regeneration research for two main reasons: (1) The reported neuroprotective effects of the first developed gliptins in animal models; (2) their mechanisms of action involve increasing GLP-1 bioavailability, hence its neuroprotective activities. The author suggests not only blood-brain barrier and neurodegenerative disease as off target for DPP-4 inhibitors and GLP-1RA, but also for ophthalmic preparations for diabetic retinopathy, which may be the latest breakthrough in the field if prepared and used in an appropriate Nanoformulation to target the retinal nerves.

Given the preclinical and limited clinical promise of DPP-4 inhibitors and GLP-1RA targets for a wide spectrum of neurodegenerative diseases, they are of great interest though indicating a potentially symptomatic or generic mechanism of action. In fact, neurodegenerative diseases are known to have rather different pathophysiological and genetic mechanisms, and the relation of these different mechanisms to the DPP-4 inhibitors and GLP-1RA should be further examined in preclinical and clinical settings.

Both DPP4 inhibitors and GLP-1RA cause similar adverse effects when given to humans, including pancreatitis, gastrointestinal symptoms, flu-like symptoms, skin rashes, dyspnea, and severe joint pain. In that regard, the intranasal administration route might attenuate such unwanted collateral effects and represents a unique advantage in minimizing such risk by increasing the delivery to the brain *versus* peripheral tissues.

References

Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A (2004) Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 89:2078-2084.

Al-Badri G, Leggio GM, Musumeci G, Marzagalli R, Drago F, Castorina A (2018) Tackling dipeptidyl peptidase IV in neurological disorders. Neural Regen Res 13:26-34.

Andersen ES, Deacon CF, Holst JJ (2018) Do we know the true mechanism of action of the DPP-4 inhibitors? Diabetes Obes Metab 20:34-41.

Aroda VR (2018) A review of GLP-1 receptor agonists: Evolution and advancement, through the lens of randomised controlled trials. Diabetes Obes Metab 20 Suppl 1:22-33.

Athauda D, Foltynie T (2016) The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in Parkinson's disease: mechanisms of action. Drug Discov Today 21:802-818.

Ayoub BM, Attia YM, Ahmed MS (2018a) Structural re-positioning, in silico molecular modelling, oxidative degradation, and biological screening of linagliptin as adenosine 3 receptor (ADORA3) modulators targeting hepatocellular carcinoma. J Enzyme Inhib Med Chem 33:858-866.

Ayoub BM, Mowaka S, Safar MM, Ashoush N, Arafa MG, Michel HE, Tadros MM, Elmazar MM, Mousa SA (2018b) Repositioning of Omarigliptin as a once-weekly intranasal anti-Parkinsonian agent. Sci Rep 8:8959.

Bassil F, Fernagut PO, Bezard E, Meissner WG (2014) Insulin, IGF-1 and GLP-1 signaling in neurodegenerative disorders: targets for disease modification? Prog Neurobiol 118:1-18.

Chen DY, Wang SH, Mao CT, Tsai ML, Lin YS, Su FC, Chou CC, Wen MS, Wang CC, Hsieh IC, Hung KC, Cherng WJ, Chen TH (2015) Sitagliptin after ischemic stroke in type 2 diabetic patients: a nationwide cohort study. Medicine (Baltimore) 94:e1128.

Darsalia V, Ortsäter H, Olverling A, Darlöf E, Wolbert P, Nyström T, Klein T, Sjöholm Å, Patrone C (2013) The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain: a comparison with glimepiride. Diabetes 62:1289-1296.

Hunter K, Holscher C (2012) Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. BMC Neurosci 13:33.

Lee SH, Zabolotny JM, Huang H, Lee H, Kim YB (2016) Insulin in the nervous system and the mind: functions in metabolism, memory, and mood. Mol Metab 5:589-601.

Lin CL, Huang CN (2016) The neuroprotective effects of the anti-diabetic drug linagliptin against Aβ-induced neurotoxicity. Neural Regen Res 11:236-237.

Pang B, Zhou H, Kuang H (2018) The potential benefits of glucagon-like peptide-1 receptor agonists for diabetic retinopathy. Peptides 100:123-126.

Srinivas NR (2015) Linagliptin-role in the reversal of Aβ-mediated impairment of insulin signaling and reduced neurotoxicity in AD pathogenesis: some considerations. CNS Neurosci Ther 21:962-963.

Yildirim Simsir I, Soyaltin UE, Cetinkalp S (2018) Glucagon like peptide-1 (GLP-1) likes Alzheimer's disease. Diabetes Metab Syndr 12:469-475.