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# Dipeptidyl peptidase 4 as a therapeutic target in ischemia/reperfusion injury

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ABSTRACT

Dipeptidyl peptidase 4 (DPP4, DPPIV, CD26, EC 3.4.14.5) was discovered more than four decades ago as a serine protease that cleaves off N-terminal dipeptides from peptide substrates. The development of potent DPP4 inhibitors during the past two decades has led to the identification of DPP4 as a target in the treatment of type 2 diabetes. The favorable effect of DPP4 inhibitors is based on prevention of the in vivo inactivation of the incretin hormone, glucagon-like peptide-1 (GLP-1) by DPP4. Apart from GLP-1, a number of other biologically active peptides are truncated by DPP4. For these peptides, the physiological relevance of their truncation has yet to be fully elucidated.

Within the last 10 years, DPP4 inhibitors have been employed in several animal models of lung and heart disease, in which injury was induced by an ischemic insult followed by subsequent reperfusion. In this review, we present a state-of-the-art of the ischemia/reperfusion injury (IRI)-related pharmacological actions of DPP4 substrates, including GLP-1, stromal cell-derived factor-1 alpha and vasoactive intestinal peptide. Furthermore, we discuss the large body of experimental work that now provides compelling evidence for the advantageous impact of DPP4 targeting in IRI. However, possible risks as well as underlying mechanisms are yet to be elucidated before translating these promising treatment strategies into clinical practice.



**Contents**



## 1. Introduction

Dipeptidyl peptidase 4 (DPP4/DPPIV/CD26) cleaves off N-terminal dipeptides from peptides with preferably proline or alanine at the penultimate position (Lambeir et al., 2003). Several DPP4 inhibitors like sitagliptin, vildagliptin, saxagliptin and linagliptin are currently available for the treatment of type 2 diabetes and others are in advanced stages of clinical development. Their pharmacological action is based on the reduced cleavage of incretin hormone glucagon-like peptide-1 (GLP-1) by DPP4, preserving the insulinotropic action of this peptide (Deacon, 2011).

Nowadays, DPP4 inhibitors are being investigated for their applicability in other pathological conditions, both in animal studies and in clinical settings (Lambeir et al., 2008). There are several reasons to explore their application range. First, DPP4 inhibitors have a benign adverse effect profile and are generally well tolerated (Deacon, 2011; Monami et al., 2011; Karagiannis et al., 2012). Furthermore, GLP-1 has a role outside glucose regulation and in addition, DPP4 is able to cleave

*Abbreviations:* BM, bone marrow; BNP, brain natriuretic peptide; EC, endothelial cell; ERK, extracellular signal regulated kinase; GLP-1, glucagon-like peptide-1; GSK3β, glycogen synthase kinase-β; GLUT, glucose transporter; HIF-1, hypoxia-inducible factor-1; IRI, ischemia/reperfusion injury; MI, myocardial infarction; MMP, matrix metalloproteinase; NPY, neuropeptide Y; PI3K, phosphatidylinositol-3-OH kinase; SDF-1α, stromal cell-derived factor-1 alpha; Tx, transplantation; VIP, vasoactive intestinal peptide.

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other substrates. Clinical trials testing the effect of DPP4 inhibitors on the cardiovascular outcome in type 2 diabetes patients are ongoing. The anticipated completion date of these studies is 2014–5 and results are expected shortly thereafter (Frederich et al., 2010; Ussher & Drucker, 2012). In animal studies, the effects of DPP4 inhibitors are evaluated in osteoporosis (Kyle et al., 2011), heart failure (Gomez et al., 2012), atherosclerosis (Shah et al., 2011; Ta et al., 2011; Matsubara et al., 2012; Terasaki et al., 2012) and also in ischemia/reperfusion injury (IRI), mainly in heart and lung. Ischemic disorders in general are the main cause of death in humankind. Among those, ischemic cardiomyopathy following an acute myocardial infarction (MI) is the most important. In adults with type 2 diabetes, heart disease death rates are about 2 to 4 times higher than in adults without diabetes. Consequently, there is considerable interest in strategies that reduce cardiovascular morbidity and mortality in diabetic subjects (Buse et al., 2007). The microvascular complications can be altered by tight glycemic control, but the macrovascular complications appear not to respond to such regimens (Skyler et al., 2009). Next to the increased risk of MI in type 2 diabetes patients, nephropathy is another consequence of this disease associated with IRI. Nephropathy may reach the status that necessitates renal transplantation (Tx) and IRI is an inevitable consequence of this procedure (Wald et al., 1990). IRI in the setting of lung Tx potentially leads to primary graft dysfunction. This phenomenon affects 10–25% of all lung transplant recipients and is a major cause of early post-transplant morbidity and mortality (Christie et al., 2005). Moreover, survivors of primary graft dysfunction are at higher risk for the development of chronic allograft rejection, the main obstacle to long term survival.

IRI is characterized by an initial restriction of blood supply to an organ followed by the subsequent reperfusion with concomitant reoxygenation. During ischemia, the severe imbalance of metabolic supply and demand causes tissue hypoxia. Restoration of the blood flow and reoxygenation is frequently accompanied by an exacerbation of tissue injury and a profound inflammatory response (Eltzschig & Eckle, 2011). IRI is associated with modified local cytokine/chemokine secretion patterns, increased neutrophil recruitment, free-radical accumulation, lipid peroxidation and impairment of functional and structural integrity of the organ (Granger & Korthuis, 1995). Different DPP4 substrates, like GLP-1, vasoactive intestinal peptide (VIP) and stromal cell-derived factor-1 alpha (SDF-1α) are shown to have certain beneficial effects in different experimental settings following an ischemic insult. A prolongation of their biological activity might therefore explain certain protective effects of DPP4 inhibitors.

Possible negative effects of DPP4 inhibition were postulated recently by Krijnen et al., declaring that DPP4 activity on endothelium affects its thrombogenic nature. Within the damaged area of recently infarcted human hearts, DPP4 expression and activity on coronary microvascular endothelial cells (ECs) is decreased, coinciding with an increase in Tissue Factor expression and thus a switch to a prothrombogenic status of the endothelium. Moreover, treatment of human umbilical cord vein endothelial cells with diprotin A, a DPP4 inhibitor, increased the expression of endothelial Tissue Factor and consequently induced adherence of platelets to the ECs (Krijnen et al., 2011). However, it remains unknown whether the observed detrimental in vitro effects of DPP4 inhibition also occur in vivo.

The purpose of this article is firstly to critically review current literature concerning the effects of DPP4 inhibitors in experimental animal IRI studies. Secondly, we summarize the knowledge on some DPP4 peptide substrates in this setting. Lastly, the feasibility of translation into clinical practice is discussed.

## 2. Dipeptidyl peptidase 4 expression during hypoxia and ischemia/reperfusion injury

DPP4 expression seems to be influenced by the hypoxic state of cells and organs. In ECs in culture, the expression of DPP4, both at mRNA and protein level, is upregulated by hypoxia (Eltzschig et al., 2006). In 2008, DPP4 was detected as a novel target gene of hypoxia-inducible factor-1 (HIF-1) (Dang et al., 2008). HIF-1 is composed of HIF-1α and HIF-1β. While HIF-1β is constitutively expressed, HIF-1α protein stability is regulated by hypoxia. The HIF-1 complex activates more than 60 target genes involved in angiogenesis, glycolysis, vasodilation and erythropoiesis (Berra et al., 2006). Stabilization of HIF-1α within hypoxic tumors resulted in a 6-fold induction in DPP4 mRNA and a more than 10-fold induction of the DPP4 protein (Dang et al., 2008).

In the IRI setting, DPP4 expression varies between different experimental models. During ischemia in the rat muscle, DPP4 mRNA was upregulated (Lee et al., 2003) and DPP4 activity on blood mononuclear cells was increased in patients suffering from an acute coronary syndrome due to stenosis of the proximal left anterior descending artery (Moro et al., 2011). In the latter study, the DPP4 activity decreased early after the start of reperfusion during percutaneous transluminal coronary angioplasty (Moro et al., 2011). In contrast, Kanki et al. found no difference in DPP4 protein level between ischemic and non-ischemic myocardium (Kanki et al., 2011). Furthermore, in syngeneic lung Tx experiments applying cold ischemia, DPP4 activity in the graft was decreased (Zhai et al., 2007; Jungraithmayr et al., 2012). Differences among experimental models such as varying durations of ischemia and reperfusion, cold versus warm ischemia, different organs and species might explain these dissimilarities.

## 3. Dipeptidyl peptidase 4 targeting in ischemia/reperfusion injury

The relevance of DPP4 as a target in IRI has been demonstrated in several animal studies, mostly myocardial infarction (Zaruba et al., 2009; Post et al., 2010; Sauvé et al., 2010; Ye et al., 2010; Zhang et al., 2010; Huisamen et al., 2011; Ku et al., 2011; Theiss et al., 2011; Yin et al., 2011; Chinda et al., 2012; Hocher et al., 2012) and experimental lung Tx (Zhai et al., 2006, 2007, 2009; Jungraithmayr et al., 2010, 2012), either using DPP4 inhibitor treatment or DPP4 knock out animals. These studies are listed in Tables 1 and 2. Although direct comparison of studies is difficult due to different experimental settings and/or parameters analyzed, an overall beneficial effect is seen upon DPP4 pharmacological inhibition or genetic deletion. However, in one study, the long-term treatment with vildagliptin had no effect on cardiac remodeling post-MI (Yin et al., 2011). The reason for this dissimilarity is unclear. Apart from these animal studies, one pilot study in humans showed cardioprotection by sitagliptin in patients with coronary artery disease (Read et al., 2010).

Among the studies listed in Table 1, there are inconsistencies with regard to the involvement of DPP4 inhibitor-mediated effects on glucose homeostasis in the observed beneficial outcome after IRI. Sauvé et al. provided evidence for a cardioprotective role for DPP4 independent of the glucoregulation since an increased survival after MI was observed in normoglycemic *dpp4*$^{-/-}$ mice versus wild type mice. The increase in survival percentage was similar to the one observed after MI in diabetic mice treated with sitagliptin (Sauvé et al., 2010). In contrast, Huisamen et al. reported a reduction of the infarct size after myocardial IRI upon DPP4 inhibitor treatment in obese pre-diabetic rats but no effect in healthy animals (Huisamen et al., 2011). This dissimilarity can be related to different experimental models since Huisamen et al. performed cardiac IRI in an isolated perfused heart model whereas Sauvé et al. carried out an in vivo coronary artery ligation. The lack of efficacy of DPP4 inhibition in non-diabetic animals in the ex vivo model may be due to the absence of an ongoing supply of certain inhibitor-protected DPP4 substrates via the blood flow compared to the in vivo model. The renal IRI studies (Table 2) were either performed in diabetic (Vaghasiya et al., 2011) or non-diabetic animals (Glorie et al., 2012), both showing a reduction in serum creatinine levels upon DPP4 inhibition. Furthermore, since most of the studies in Tables 1 and 2 were performed in non-diabetic