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The present study evaluated the effect of anagliptin on intimal hyperplasia following carotid artery injury in Sprague-Dawley rats. Sprague-Dawley rats weighing 280-300 g were injured using a 2F Fogarty balloon embolectomy catheter. The rats were divided into injury-(saline) and anagliptin-(10 mg/kg/day) treated groups. vascular injuries were induced in the left carotid artery, followed by evaluation of neointima formation at 28 days. The right and left carotid arteries were harvested and evaluated with histological evaluation, and the plasma activity of glucagon-like peptide 1 receptor (GLP-1), stromal cell-derived factor (SDF)-1α, interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α were detected by ELISA analysis. Treatment with anagliptin decreased balloon injury-induced neointima formation, compared with the injury group (P<0.01). Body weight and food consumption did not alter following treatment with anagliptin. Anagliptin caused an increase in the serum active GLP-1 concentration, compared with the injury group. In addition, serum SDF-1α was significantly decreased by treatment with anagliptin (P<0.001). Anagliptin altered the serum activity of IL-6, IL-1β and TNF-α (P<0.01). The results of the present study demonstrated that anagliptin appeared to attenuate neointimal formation by inhibiting in[redacted] cytokines and chemokines following balloon injury, and that treatment with a dipeptidyl peptidase 4 inhibitor may be useful for future preclinical studies and potentially for the inhibition of thrombosis formation following percutaneous coronary intervention.

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Balloon angioplasty, stenting and coronary arterial bypass grafting (CABG) are common interventions for the treatment of coronary artery disease (CAD), which is the leading cause of death globally and is expected to account for 14.2% of all deaths by 2030 (1). However, these interventir 2(i)11[redacted]njured site is

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ucts and the regulation of vascular homeostasis in the vessel wall is further disturbed. The damaged area is coated with platelets and macrophages which release thrombotic factors (fibrinogen and von Willebrand factor) and growth factors (platelet-derived growth factor and transforming growth factor) (4). According to the response-to-injury theory, the mechanism that initiates intimal thickening is that the growth factors which are released from platelets and ECs adhering to the damaged vessel wall stimulate the proliferation of SMCs within the first 24 h (5), and subsequently induce the proliferated SMCs in the medial layer to migrate to the intima during the 3rd and 4th days, ultimately leading to intimal hyperplasia. A previous study demonstrated that acute injury to the intima and media may produce hyperplasia and SMC proliferation, which occur at a rate proportional to the degree of injury (6). It has been additionally observed that intimal hyperplasia forms around the injury sites following balloon angioplasty (7).

Dipeptidyl peptidase 4 (DPP-4), also termed CD26, is a widely-expressed serine peptidase that exists on the surface of various cell types, although its expression level differs among cells (8). However, in different organs and tissues, including the lung, muscle and heart, approximately all tissue DPP-4 activity is due to its presence in the microvasculature (9). In the immune system, DPP-4 is associated with T-cell signal transduction as a co-stimulatory molecule.

Anagliptin, a specific DPP-4 inhibitor, is a novel oral anti-hyperglycemic agent which has been used to treat type

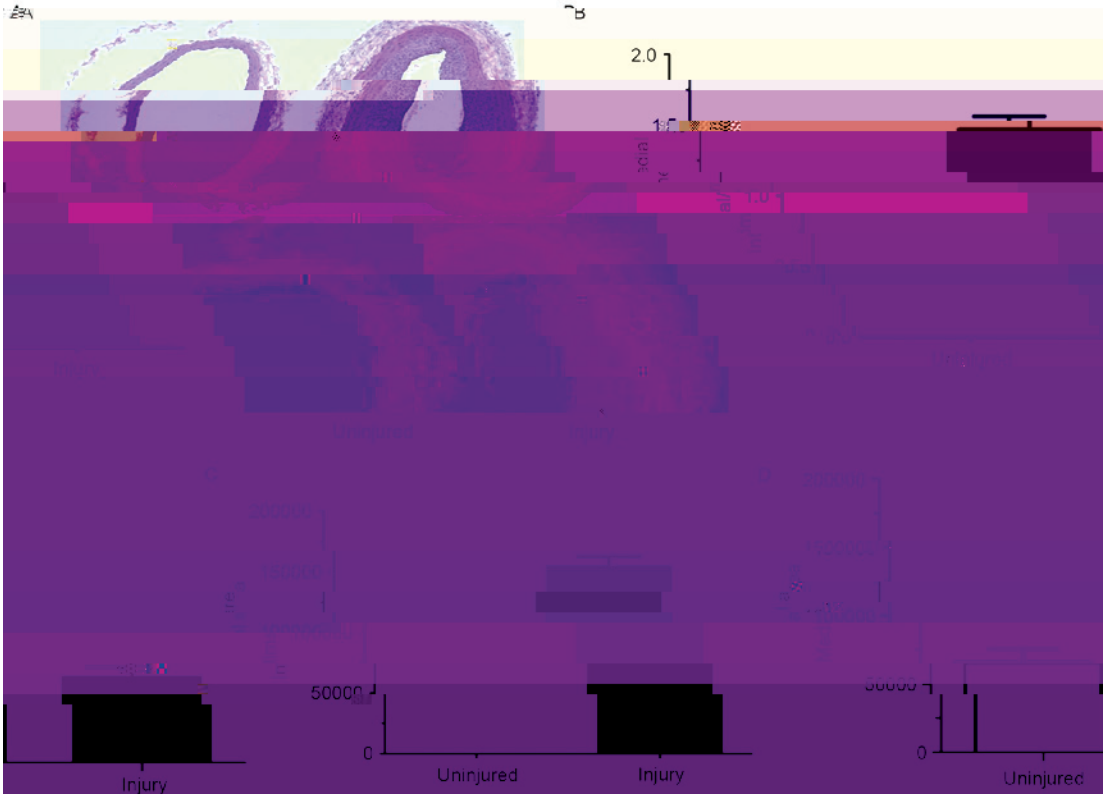


Figure 1. Neointimal hyperplasia formation following balloon injury. (A) Tissues were evaluated by hematoxylin and eosin staining (magnification, x200) at 28 days subsequent to balloon injury. The (B) ratio of intima/media (normalized to the uninjured tissue), (C) area of the intima and (D) area of the media were calculated and between the right and left carotid artery in the balloon injury group. Data are presented as the mean ± standard error of the mean.

ratio and the intimal area were equal to 0. Thus, the right common carotid artery served as the loading control Rat left carotid artery injuries were performed and neointima formation was evaluated at 28 days post-surgery. The left carotid artery was injured and exhibited a decreased lumen area (Fig. 1A and B) which corresponded to increased intimal thickening. The intima/media ratio was also increased in the left arteries compared with right arteries (Fig. 1C). However, there was no difference in the medial area between left and right carotid arteries (Fig. 1D).

In the histological sections obtained from the rats which were given anagliptin (10 mg/kg) twice daily, compared with the injury group, no significant differences between the right carotid arteries were observed. It was observed that the lumen area, intimal area and intima/media ratio was decreased at 28 days compared with the injury group (Fig. 2A). Notably, the medial area in the left carotid artery of both groups was same (Fig. 2B).

It has been hypothesized that the structural integrity of the IEL may be essential in the internal elastic lamina (IEL) rupture mechanism, in order to minimize intimal hyperplasia following vascular injury. The right carotid artery did not undergo vascular injury, and hence no intimal hyperplasia or IEL rupture was detected in the right carotid arteries of the injury and anagliptin groups. IEL rupture was subsequently analyzed in rat left carotid arteries, which were subjected to balloon injury in each group. Notably, balloon injury using the 2F Fogarty catheter did not result in any fractures in the whole IEL circumference, although intimal hyperplasia was still observed in the two groups (Fig. 2C). Intimal hyperplasia was decreased in the anagliptin group (Fig. 2C). The results of the present study indicated that there was no association between IEL rupture and intimal hyperplasia following balloon injury.

In order to examine the systemic in[redacted]n on metabolism, the food consumption and body weight of all the animals were determined at different time points. Following surgery, the body weight of both groups decreased at 4 days, and gradually recovered from 12 days until normal body weight was attained 28 days in the two groups (Fig. 3A). Daily analyses of food consumption illustrated decreased food intake at 4 days subsequent to balloon injury and recovery at 12 days, and this phenomenon was accordance with the loss of body weight observed in the groups (Fig. 3B). The results of the present study indicated that anagliptin attenuated neointima formation, independent of the glucose-lowering effect and body weight reduction among the groups.

GLP-1 is one of the substrates of DPP-4 which is rapidly degraded by the enzyme DPP-4 under normal conditions. Therefore, the concentration of GLP-1 may re[redacted] activity of DPP-4. ELISA analysis was employed for the detection of serum GLP-1 content which was collected from injury and anagliptin rats at the chosen time points (pre-operatively, and at 1, 7, 14, 21 and 28 days). The results demonstrated that

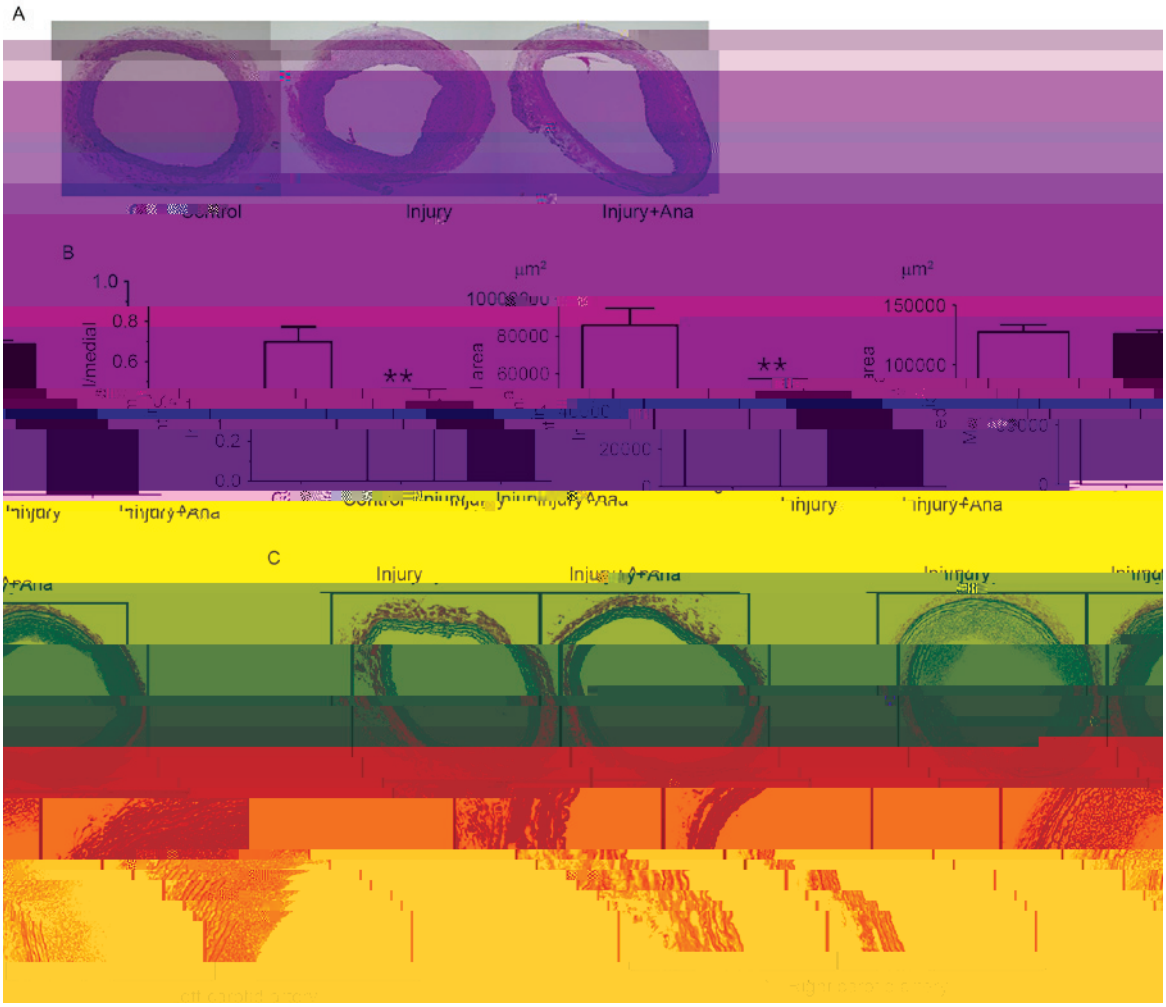


Figure 2. Anagliptin attenuates neointimal hyperplasia following balloon injury. (A) Tissues were evaluated by hematoxylin and eosin staining (magnification, x200) at 28 days following the commencement of treatment with anagliptin. (B) The intima/media ratio (normalized to injury), and the area of the intima and media were calculated in the injury and anagliptin groups. (C) Histological sections of injured and anagliptin-treated arteries were stained with elastic van Gieson (magnification, x200). **P<0.01 vs. injury. Data are presented as the mean ± standard error of the mean. Ana, anagliptin.

serum GLP-1 content in anagliptin group was significantly increased compared with the control group and peaked at 28 days (Fig. 4A) which suggested the activity of DPP-4 was decreased.

SDF-1α, one of the DPP-4 substrates that is degraded by DPP-4 through its cleavage, is a chemokine which induces EPCs to differentiate into ECs in order to protect the injured artery. Therefore, the serum SDF-1α concentration was measured. Anagliptin decreased the serum SDF-1α level (Fig. 4B). These data suggested that anagliptin increased the serum active GLP-1 concentration, and altered serum factors associated with EPC migration.

In [redacted] kines are among the most important accelerators of intimal hyperplasia. Therefore, the present study assessed the in[redacted] matory response following balloon injury. The serum levels of IL-1β, IL-6 and TNF-α were measured using ELISA analysis. The serum levels of IL-6, IL-1β, and TNF-α in the injury group were significantly increased compared with the group treated with anagliptin at 7 days (Fig. 5).

CAD is the leading cause of mortality and morbidity in the developed world (13). Numerous improvements have been made in coronary angioplasty, including balloon angioplasty, stenting and CABG, although a metallic scaffold device, alone or in combination with drug eluting stents (DESs) is considered to be the most effective method of treating CAD (14). However, studies have suggested that DESs delay re-endothelialization, which is important for the prevention of restenosis and stent thrombosis. Intimal hyperplasia develops as a result of SMC migration and proliferation, and the accumulation of the extracellular matrix, which is a normal adaptive response in arteries against hemodynamic stress and is a characteristic feature of arterial injury healing (15,16).

ECs regulate vascular physiology by producing a variety [redacted] molecules which have been identified, and regulate vascular homeostasis, including inhibiting the formation of thrombosis, coagulation, vasomotor tone and blood [redacted] (17). It has been demonstrated that defects in endothelial integrity and dysfunction may be the initial steps leading to the adhesion of phagocytic cells (18) and proliferation of SMCs (19)

in an injured artery, and may further induce atherosclerotic lesion and neointima formation (20). Therefore, EC loss is a factor that is a primary contributor to remodeling (21), and strategies to protect the endothelium and/or stimulate its repair following injury have been sought to reduce intimal hyperplasia formation.

Excessive intimal hyperplasia causes a decrease in blood [redacted] of the lumen and thrombosis formation by impairing the release of anticoagulants from ECs. A number of studies have been conducted using drugs to prevent intimal hyperplasia following balloon injury or stenting (22,23). Various mechanisms, including growth factors, in[redacted] metabolic disorders and blood [redacted] may cause intimal hyperplasia (24). Studies about the use of anagliptin for preventing intimal hyperplasia are novel and have not been published previously, however, positive results were obtained in the present study by using anagliptin in the prevention of intimal hyperplasia.

The intimal/medial ratio of the left carotid artery and the intimal area where the artery was injured were thicker compared with the right carotid artery in the injury group,

to elucidate whether the effect of anagliptin on neointimal hyperplasia was mediated by a decrease in the activity of DPP-4. Therefore, the plasma concentration of GLP-1, which may reflect the plasma activity of DPP-4, was measured. Serum GLP-1 concentrations rose with anagliptin treatment at the different time points indicating that anagliptin may inhibit the plasma activity of DPP-4 , with no difference being observed in the model group. Although the plasma levels of DPP-4 and GLP-1 were altered, body weight and food intake were not markedly different in the two groups. Notably, the active GLP-1 levels tended to increase in the anagliptin group compared with the injury group, although there was no significant difference preoperatively. A previous report indicated that the daily glucose levels of rats that were fasted for 4 h were similar in a group treated with vildagliptin compared with the control group, although they significantly decreased between week 2 and week 5

inhibiting the activity of DPP-4 may be a potential therapy to inhibit the process of intimal hyperplasia, and thus, this may also be a novel therapy for inhibiting restenosis following PTCA. A previous study reported that DPP-4 is released as an adipokine from visceral fat, and stimulates vascular (V) SMC proliferation through extracellular signal-regulated kinase/mitogen-activated protein kinase phosphorylation in VSMCs (40). Other DPP-4 substrates, including BNP (42), were not measured in the present study due to sample limitation. It was not possible to investigate all the mechanisms through which anagliptin may attenuate neointima formation following vascular injury. Further studies are required to clarify the vasculoprotective effect of anagliptin and to detect other DPP-4 substrates.

In conclusion, the results of the present study demonstrated that anagliptin was able to inhibit intimal hyperplasia by reducing the plasma level of SDF-1α, rather than by altering the process of metabolism. It was hypothesized that the potential mechanism involves decreasing the plasma level of SDF-1α to inhibit leukocyte adhesion and the migration of SMCs, and hence stimulate the migration and proliferation of ECs from the adjacent non-injured endothelium via additional factors released from the site of balloon injury. Future studies in the field of vascular biology following injury may positively affect the quality of life of patients who have undergone cardiovascular surgery by providing an improved understanding of endothelial functioning and recovery from injury, which is primarily responsible for intimal hyperplasia. The prevention of the intimal hyperplasia response may be effective in increasing lifespan following vascular reconstructive interventions, including surgical graft bypass or balloon angioplasty.

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