Nesiritide protects endothelial function after balloon-induced trauma in the iliac artery in rabbits

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To assess the effect of nesiritide on the endothelial function of iliac arteries following endothelia trauma. Right iliac artery trauma was created with a balloon catheter. Ten rabbits were treated with a 4-week subcutaneous injection of nesiritide at a fixed daily dose of 0.1mg/kg. Ten rabbits received daily normal saline injection. Plasma endothelin 1 (ET-1), nitric oxide (NO), and Von Willebrand Factor (vWF) were measured before and after the therapies. Tissue proliferating cell nuclear antigen (PCNA) was measured after the treatment. After the treatment, in the therapeutic group, the area under internal elastic membrane and the residual lumen area were higher than in the normal saline group (P <0.05). The plasma levels of ET-1 (91.6±6.8 vs 114.9±6.3 ng/L, P =0.001), vWF (134.6±10.8% vs 188.8±10.4%, P =0.001) and the ratio of PCNA positive expression (11.7±4.2% vs 36.2±11.4%, P =0.005) in the therapeutic group was lower than in the normal saline group, while the plasma levels of NO was higher (89.7±9.3 vs 43.5±5.3 μmol/L, P =0.001). Nesiritide inhibited remodeling of rabbit iliac artery following endothelial trauma. The inhibition of vascular remodeling may be related to the alleviated endothelial dysfunction and reduced expression of tissue proliferating cell nuclear antigen.

Keywords: Plasma endothelin (ET-1). Nitric oxide (NO). von Willebrand Factor (vWF). Tissue proliferating cell nuclear antigen. Iliac artery.

INTRODUCTION

The B-type natriuretic peptide (BNP) is a vasoactive peptide. After more than two decades of active investigations, BNP has become a useful biomarker for the diagnosis of congestive heart failure and risk stratification of this prevalent and chronic condition (Egom, 2015; We *et al*. 2005a, 2005b, 2005c; Sun *et al*., 2007). BNP exerts protective effects against the post-myocardial infarction remodeling processes. In rats, BNP treatment inhibits extracellular matrix remodeling following acute myocardial infarction, and preserves ventricular function (Pan *et al*., 2011; Plante *et al*. 2014). BNP plays an important role in modulating vascular endothelial function, and predicts the endothelial function in human subjects (Menon *et al*., 2009; Pauriah *et al*., 2012). Nesiritide, a synthetic BNP, has been used to treat acute decompensated heart failure for more than a decade (Chen *et al*., 2013). Currently, there is a lack of understanding about the associations between nesiritide and vascular endothelial function. In human subjects, nesiritide treatment has been shown to suppress fibrosis and hypertrophy in the myocardium, and reduce end-diastolic volume a month after the treatment (Chen *et al*., 2009). In a porcine model of acute myocardial infarction, nesiritide infusion reduced the size of infarct and preserved coronary endothelial function (Lazar *et al*., 2009).

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Our previous studies in a rabbit model have found that nesiritide treatment inhibited vascular remodeling following arterial injuries, and the improved remodeling profile was due to reduced plasma aldosterone levels (Liu *et al*., 2010, 2012). However, it is unclear if nesiritide can protect injury-induced endothelial dysfunction. Endothelin-1 (ET-1) is an endogenous vasoconstrictor that is involved in systemic and pulmonary hypertension. It also impacts on cardiac function and is associated with a higher mortality rate in patients with heart failure (Perez *et al*., 2016). Nitric oxide (NO) is an endogenous vasodilator that regulates systemic and pulmonary circulation, as well as cardiovascular remodeling (Chen *et al*., 2014). von Willebrand Factor (vWF) is a molecule that is released from the vascular endothelium, leading to hypercoagulability and thrombosis. Balloon dilation of peripheral arteries were associated with an elevation in blood vWF (Yao, Wang, Kang, 2016).

Although BNP has been shown to improve endothelial function in a variety of chronic cardiovascular conditions, the impact of nesiritide treatment on plasma ET-1, NO, and vWF following vascular injuries has not been reported. In this study, we investigated the changes in plasma ET-1, NO, vWF and neointimal proliferation following nesiritide therapy in a rabbit model that we have previously reported (Liu *et al*., 2010, 2012).

MATERIAL AND METHODS

Induction of arterial trauma

The Animal Ethics Committee of our hospital approved the present study, and the study protocols have followed the national guidelines. All steps have been taken to minimize the animals' pain and suffering during and after the operations. A total of 20 male rabbits were used for this study. The weight of the animals was 2.3 ± 0.2 kg (2.1-2.6 kg), with a median age of 5 months. Arterial trauma was induced with a balloon-catheter (Liu *et al*., 2010, 2012). First, under room temperature, ketamine (20mg/kg) was intravenously injected. Under ultrasound guidance, we inserted a Medtronic balloon catheter (Medtronic, MN, USA) to the common iliac artery where the catheter was inflated to a 5-atmosphere pressure (Liu *et al*., 2010, 2012) to create trauma to the arterial wall. Subsequently, we divided the animals into two groups, with 10 animals in each group. In one group (the

therapeutic group), nesiritide (Shanghai GL Biochen, Shanghai, China) was injected subcutaneously at a daily dose of 0.1 mg/kg for a total of 4 weeks. This dosage was based on our previous studies in the same animal model, and proved to be the most effective (Liu *et al*., 2010, 2012). In the control group, normal saline was injected subcutaneously for the same period as the therapeutic group.

Histological studies

Haematoxylin and Eosin (HE) staining was used to study the vascular tissues which were collected at the end of the experiments (Liu *et al*., 2010, 2012). Tissue sections were studied by an imaging system (model ES2050, WD Technologies, Beijing, China). In each sections of the iliac artery, the area under internal elastic membrane (AUIEM), intimal area and residual lumen area were measured by two independent pathologists. The ratio of stenosis of the iliac artery was calculated by (AUIEM-residual lumen area)/AUIEM.

Plasma endothelin-1 (ET-1), nitric oxide (NO), and von Willebran factor (vWF)

Plasma ET-1 and vWF were measured by enzyme-linked immunosorbent assay (ELISA). Plasma NO was measured by a nitrate reduction method. A commercial ELISA kit (Bogoo Biological Engineering Co, Shanghai, China) was used for ET-1 and vWF measurement. The NO kit was provided by Jiancheng Institute of Biological Engineering (Nanjing, China).

Immunohistochemistry for proliferating cell nuclear antigen (PCNA)

A commercially available immunohistochemistry kit (Boster Biological Engineering, Wuhan, China) was used to determine PCNA in the vascular tissues. Vascular tissue sections were stained with mouse anti-rabbit PCNA antibodies, and the slides were examined by optical microscope ($\times$200). All cells in each of the 10 visual fields, including the cells with positive PCNA expression (brown stains) were counted and recorded manually. The ratio between PCNA positive cells and total cells was defined as the positive PCNA expression rate. Two readers performed the cell counts and the means of the two sets of readings were used as the results.

Statistical analysis

SPSS software (Version 13, IBM SPSS Statistics, NY, USA) was used in thestatistical analysis of data. Data expression was in the form of means ± standard deviation. Numerical data and categorical data were analyzed by an independent student's t and Fisher's exact test, respectively. Statistical significance was determined at p≤0.05.

RESULTS

Effect of nesiritide on the structure of the iliac arteries

The two remodeling measures (AUIEM and residual lumen areas) in the control animals were lower than the therapeutic group animals (P =0.025 and P =0.001, respectively, Table I), whereas the stenosis ratio in the study group was higher (P =0.001, Table I).

TABLE I - Comparison of remodeling indices between nesiritide and control group

	Control (n=10)	Nesiritide (n=10)	P
AUIEM (µm²)	493.7±139.3	643.2±134.1	0.025
Intimal area (µm²)	185.1±75.05	124.1±61.5	0.062
Residual lumen area (µm²)	308.6±86.5	519.1±93.5	0.001
Stenosis ratio (%)	37.1±9.2	18.54±7.8	0.001

AUIEM: area under internal elastic membrane.

PCNA expression in the iliac arteries

No positive PCNA expression was observed in the tissues of the normal iliac arterial tissues (Figure 1A). Traces of PCNA expression were found in the vascular structures of the animals from the therapeutic group (Figure 1B). However, strong PCNA expression were present in the endothelium, smooth muscles and interstitial cells of the iliac arteries of the control group rabbits (Figure 1C).
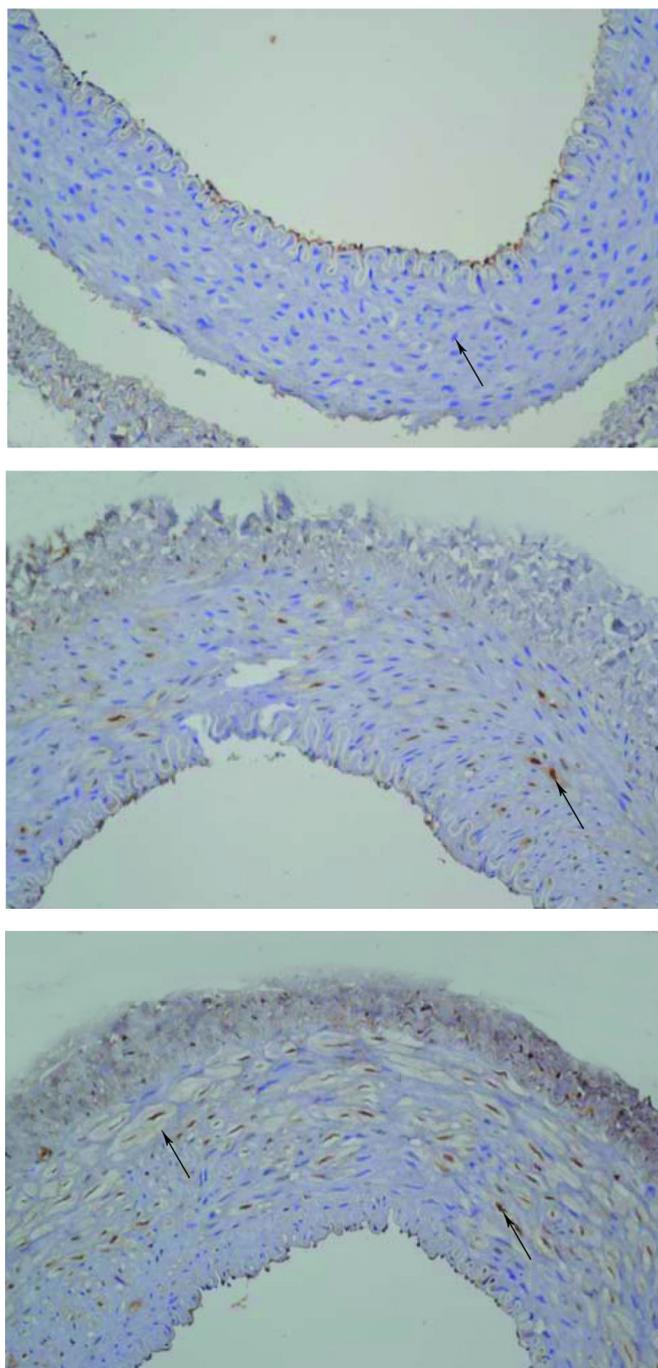
FIGURE 1 - Immunohistochemical PCNA staining (×200). Cells stained with brownish color were considered positive. (a) shows tissues from the non-injured left iliac arteries (n=10) from nesiritide group. No PCNA expression was found (arrow). (b) tissues from the injured right iliac artery tissue from the nesiritide group (n=10). Scattered PCNA expression displayed as yellow brown granules (arrow). The positive staining rate was 11.7±4.2%. (c) tissues from the injured right iliac artery from the control group (n=10) with dense PCNA expression (arrows). The positive staining rate was 36.0±11.2% (P <0.01 compared with nesiritide group).

Plasma levels of ET-1, NO and vWF

Baseline levels of ET-1, NO and vWF were obtained before the treatment, with no difference observed between the therapeutic and control group rabbits (Table II, P >0.05). In all rabbits, the levels of plasma ET-1 and vWF were increased, while NO levels were decreased at the end of the 28-day treatment. However, the post-treatment levels of plasma ET-1 and vWF levels in the therapeutic group rabbits were lower than the control group rabbits, while the NO levels were significantly higher (Table II, P =0.001).

DISCUSSION

In this experimental work, we found that treatment with nesiritide for four weeks reduced the degree of vascular remodeling in the iliac arteries of the rabbits. We also found that there was a subdued increase in ET-1 and vWF in the systemic circulation after nesiritide therapies, and a greater elevation in the circulating NO levels. These results suggest that the alleviation in endothelial dysfunction and the improved biosynthesis of NO may be the contributing factors for the therapeutic effect of nesiritide against vascular trauma.

Vascular remodeling is characterized by disordered proliferation and migration of vascular smooth muscle cells, leading to vascular stenosis and tissue ischemia. Vascular remodeling is implicated in several cardiovascular diseases, such as pulmonary hypertension, ventricular hypertrophy, heart failure and myocardial infarction. Endothelium plays a critical role in regulating circulatory and vital organ functions, as well as vascular remodeling after vascular injuries or insults. Endothelial injuries and subsequent dysfunction form the pathological basis of several cardiovascular disorders, such as coronary artery and cerebrovascular disease, which may result in organ failure (Lerman *et al.*, 1991, 1995; Manoury, Montiel, Balligand, 2012; Meyer *et al.*, 2006). Under certain stimulatory conditions, ET-1, NO and vWF are released from the vascular endothelium or vascular walls to exert their regulatory effects. Therefore, the plasma levels of ET-1, NO and vWF can be used as the circulating biomarkers for endothelial function. Elevation of ET-1 and vWF, and decline in NO may reflect endothelial dysfunction (Lerman *et al.*, 1991, 1995; Manoury, Montiel, Balligand, 2012; Meyer *et al.*, 2006).

The main physiological function of ET-1 is to stimulate the growth of vascular smooth muscle cells and cause vasoconstriction (Lerman *et al.*, 1991, 1995). The effects of NO are much more complex. They include vasodilation, inhibition of platelet aggregation, proliferation and migration of smooth muscle cells (Manoury B). NO also prevents cardiovascular trauma or insult, and alleviates vascular remodeling. NO also helps to protect endothelium against physical or chemical injuries (Manoury, Montiel, Balligand, 2012). The physiological or pharmacological roles of vWF are not well understood. It seems vWF helps to clearing out circulating coagulation factor VIII, and is therefore involved in the coagulation processes, particularly in the presence of trauma to the vascular endothelium (Lenting, Christophe, Denis, 2015; Mannucci, 1998).

A number of experimental and clinical studies have demonstrated that BNP can suppress vascular remodeling and improve hemodynamics by improving endothelial function. In cultured pulmonary arterial smooth muscle cells, BNP inhibited proliferation and migration of the

TABLE II - Comparison of plasma ET-1, NO and vWF levels between nesiritide and control group

	Control (n=10)		Nesiritide (n=10)		P (Post-treatment)
	Baseline	Post-treatment	Baseline	Post-treatment	
NO (µmol/L)	95.56±5.85	43.45±5.33[1]	96.51±6.48	89.66±9.25[1]	0.001
ET-1 (ng/L)	75.71±5.63	114.93±6.26[1]	74.98±5.96	91.59±6.76[1]	0.001
vWF (%)	102.94±11.42	188.84±10.36[1]	104.70±10.56	134.59±10.81[1]	0.001

[1] $P <0.01$ compared with baseline value of the same group. NO: nitric oxide; ET-1: endothelium 1; vWF: Von Willebrand factor

cells induced by angiotensin II (Hsu *et al*., 2014). BNP also inhibits angiotensin II or ET-1 induced ventricular hypertrophy (Volpe, 2014). Treatment with BNP for four weeks was associated with a reduced endothelial expression of vascular endothelial growth factor, and reduced stenosis after vascular injuries (Liu *et al*., 2010). Furthermore, intravenous infusion of a BNP, such as nesiritide, decreased pulmonary capillary wedge pressure, which is beneficial to the treatment of acute decompensated heart failure (Volpe, 2014).

In the present study, we found that nesiritide therapies diminished the degree of the remodeling of the iliac artery following physical trauma to the blood vessels. Furthermore, nesiritide therapies was associated with a greater reduction in circulatory ET-1 and vWF levels, and a greater elevation in the NO levels. These results indicate that nesiritide improved the endothelial function after the vascular trauma, which may have contributed to the attenuated vascular remodeling in the injured iliac arteries.

Tissue PCNA expression is a result of cell proliferation. Therefore, the levels of tissue PCNA have been used as a marker for cell proliferation (Suzuki *et al*., 2000). Other investigators have found that, in a rat model of vascular trauma, overexpression of PCNA was present in thickened neointima of the damaged blood vessels (Wei *et al*., 1997). In the present study on a rabbit model, no PCNA expression was found in the normal iliac arteries. However, PCNA expression was identified in the traumatized iliac arteries, with a higher PCNA expression identified in the control group rabbits. These results suggest that nesiritide may have inhibited neointimal proliferation and vascular remodeling by suppressing vascular smooth muscle proliferation.

It remains unclear as to how nesiritide treatment reduced the plasma levels of ET-1 and vWF, and increased NO levels in our rabbit model of traumatic iliac artery injuries. Several animal and human studies have confirmed that natriuretic peptides stimulate NO production through the natriuretic peptide clearance receptor (NPR-C) (Houben *et al*., 2005). Stimulation of this NPR-C receptor decreased cAMP levels by adenylyl cyclase inhibition through an inhibitory guanine nucleotide-regulating protein (Houben *et al*., 2005). However, there is limited information about the cellular or molecular mechanisms by which brain natriuretic peptides or nesiritide influenced the endothelial expression of ET-1 or vWF. Therefore, further studies in this area are warranted.

CONCLUSIONS

In this rabbit model, nesiritide improved endothelial function and inhibited neointima proliferation. The improved vascular remodeling following nesiritide treatment are associated with an elevated plasma levels of NO, and reduced levels of ET-1 and vWF, and reduced expression of PCNA in the vascular tissues. These novel results may have several potential clinical implications. Prophylactic treatment with nesiritide may reduce neointima proliferation or endothelial dysfunction following percutaneous coronary intervention (PCI), and diminish post-PCI coronary restenosis. Nesiritide may also be used to prevent vascular trauma from chronic insults, such as diabetes mellitus or hypertension, preventing ischemic injuries to vital organs such as kidneys or the heart. However, these potential clinical benefits require further investigations.

There was no conflict of interest to declare.

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