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Characterization of pericardial and plasma ghrelin levels in patients with ischemic and non-ischemic heart disease

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ABSTRACT

Ghrelin is an endocrine regulatory peptide with multiple functions including cardioprotective effects. It is produced in various tissues among others in the myocardium. Pericardial fluid has been proven to be a biologically active compartment of the heart that communicates with the myocardial interstitium. Thus, pericardial level of certain agents may reflect their concentration in the myocardium well. In our study we measured acylated (active) and total (acylated and non-acylated) pericardial and plasma ghrelin levels of patients with ischemic and non-ischemic heart disease. Pericardial fluid and plasma samples were obtained from patients with coronary artery disease (ISCH, $n = 54$) or valvular heart disease (VHD, $n = 41$) undergoing cardiac surgery. Acylated pericardial ghrelin concentrations were found to be significantly higher in patients with ischemic heart disease (ISCH vs. VHD, 32 ± 3 vs. 16 ± 2 pg/ml, $p < 0.01$), whereas plasma levels of the peptide showed no difference between patient groups. Pericardial-to-plasma ratio, an index abolishing systemic effects on local ghrelin level was also significantly higher in ISCH group for both acylated and total ghrelin. Plasma total ghrelin showed negative correlation to BMI, plasma insulin and insulin resistance index HOMA-A. Pericardial acylated and total ghrelin concentrations were negatively correlated with posterior wall thickness ($R = -0.31$, $p < 0.05$ and $R = -0.35$, $p < 0.01$, respectively). Plasma insulin concentration and HOMA-A showed significant negative correlation with pericardial ghrelin levels. In conclusion, increased pericardial active ghrelin content and higher pericardial-to-plasma ghrelin ratio were found in ischemic heart disease as compared to non-ischemic patients suggesting an increased ghrelin production of the chronically ischemic myocardium. According to our results, pericardial ghrelin content is negatively influenced by left ventricular hypertrophy and insulin resistance.

1. Introduction

Ghrelin is an endocrine regulatory peptide with multiple functions. The hormone is mainly produced in the X/A-type cells of the gastrointestinal tract; however, its production has recently been described in several other tissues including the myocardium [1,2]. The active form of the peptide – uniquely among peptides – is n-octanoylated at Ser3 by ghrelin O-acyltransferase (GOAT) [3]. The acylated form is rapidly deacylated in the plasma by acyl-protein thioesterase 1 (APT-1) [4]. Thus, the vast majority of the circulating peptide is the non-octanoylated variant [5].

The ghrelin receptor has two known subtypes: growth hormone secretagogue (GHS) receptor type 1a, the classical ghrelin receptor mediating most of the biological effects of ghrelin, and type 1b with yet unknown physiological role. Recently, existence of other types of ghrelin receptors was also suggested [6]. Ghrelin receptors are expressed in almost all human organs including the myocardium and vascular smooth muscle [2,7,8]. In addition to ghrelin and ghrelin receptors, GOAT enzyme is also expressed in the myocardium suggesting the existence of a local paracrine ghrelin system in the heart [9].

Beside the regulation of the somatotropic cell function and growth hormone secretion, ghrelin also has a potent orexigenic effect by stimulating food intake and regulating energy homeostasis, gastrointestinal motility, gastric acid secretion, fat deposition and weight gain. The circulating level of the peptide is the highest in fasting state and is decreased after food intake. Ghrelin levels are negatively associated with body mass index and insulin level, whereas they positively correlate with insulin sensitivity [10]. The peptide possesses widespread cardiovascular effects. It increases cardiac index and stroke volume [11] and induces vasodilation and decrease of blood pressure. The latter are mediated through both direct vascular and central mechanisms [12]. Interestingly, ghrelin may induce vasoconstriction on the coronary vasculature [8,13,14]. The peptide was shown to exert multiple cardioprotective effects in various animal models of myocardial injury by reducing infarct size, by preventing early myocardial remodeling through anti-inflammatory effects, and by decreasing sympathetic activity [15–18]. Ghrelin may inhibit apoptotic cell death of myocardial and

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endothelial cells and may stimulate cardiomyocyte cell development and proliferation and also myocardial angiogenesis [19–23]. Recently, it was shown that chronic ghrelin treatment in rats with myocardial infarction decreases susceptibility of the heart for ventricular tachyarrhythmias [24]. Low myocardial ghrelin expression was found in explanted hearts of patients with terminal heart failure undergoing heart transplantation [25]. In turn, there are evidences that chronic application of exogenous ghrelin improves left ventricular dysfunction both in rodent models and in chronic heart failure patients [26–29]. Moreover, decreased circulating ghrelin levels were found to be associated with the presence and severity of coronary artery disease [30,31]. At the same time single nucleotide polymorphism of the GHSR-1a and the ghrelin genes were found to be associated with the risk for myocardial infarction and coronary artery disease in a German cohort [32].

The pericardial fluid was traditionally believed to serve only as a mechanical lubricant of the heart. However, a lot of data originated from both animal experiments and human investigations have shown that it can be considered as a biologically active compartment of the heart [33–37] and may participate in autoregulatory processes [38]. Concentrations of several endogenous products of the myocardium such as endothelin, atrial natriuretic peptide and adenin nucleosides were found to be higher in the pericardial fluid as compared to plasma levels reflecting an increased myocardial level of these agents [33,39,40]. Intrapericardial concentrations of certain agents may further elevate in pathological cardiac conditions, e.g. myocardial ischemia [41,42]. In turn, cardioactive agents administered pericardially may exert their characteristic effects to the heart with decreased elimination and prolonged action [37,43].

According to our best knowledge, no available data exist on pericardial fluid ghrelin concentration which may be more closely associated with the myocardial ghrelin production than the levels found in the systemic circulation. In order to investigate the role of ghrelin in ischemic heart disease, the aim of the present study was to evaluate the ghrelin content in the pericardial fluid, and to compare biologically active and total ghrelin levels in two different patient groups undergoing cardiac surgery due to ischemic or valvular heart disease. Correlations between hormone levels and functional and morphological cardiac variables, anthropometric and metabolic parameters of patients were also investigated.

2. Materials and methods

2.1. Patients

Central venous plasma and pericardial fluid samples were obtained intraoperatively from 54 patients with ischemic heart disease undergoing elective coronary artery bypass grafting (ISCH group) and 41 subjects with valvular heart disease undergoing open heart valvular surgery as a control group (VHD group: aortic valve replacement n = 25, and mitral valve replacement n = 16). Patients with acute myocardial infarction, infectious, malignant, renal and hepatic disorders were excluded. All subjects in the valvular disease group had normal coronary angiogram. All samples were collected after at least 12 h of fasting. Diabetic patients received human short acting insulin during the preoperative period to maintain euglycaemia. Samples in these patients were obtained at least six hours after the last insulin injection. All patients gave their written informed consent to the study. Regarding the chronic medication of the patients, higher percentage of ischemic heart disease patients took angiotensin converting enzyme inhibitors (ISCH vs. VHD: 71 vs. 41%, $p < 0.01$), statins (62 vs. 39%, $p < 0.05$), nitrates (60 vs. 10%, $p < 0.01$), and antidiabetic drugs or insulin (31 vs. 10%, $p < 0.05$), while higher portion of valvular heart disease patients took diuretics (16 vs. 37%, $p < 0.05$). No significant difference was observed in the percentage of patients taking beta receptor blockers, digoxin, angiotensin receptor and calcium channel blockers. Anesthesiological and surgical procedures were performed according to local standards. Pericardial fluid samples were taken by the heart surgeon immediately after opening the pericardial sac using a soft line connected to a syringe. Central venous blood samples were collected in EDTA-containing tubes (Vacuette No. 486502). All samples were treated with serine proteinase inhibitor aprotinin (500 KIU/ml) and were immediately centrifuged at 4 °C, 3000 rpm for 20 min. Samples were stored at −80 °C until the measurements.

2.2. Biochemical measurements

Acylated and total (acylated and non-acylated together) ghrelin levels were measured by radioimmunoassay according to the manufacturers' instructions using acylated ghrelin (GHRA-88HK) and total ghrelin (GHRT-89HK) RIA kits (Linco Research Inc., St. Charles, MO, USA). Plasma insulin levels were determined by enzyme-linked immunoassay (Biosource Europe, Nivelles, Belgium). Values of conventional laboratory variables were measured by laboratory automats. The insulin resistance parameter HOMA-A index was calculated with the following formula: blood glucose (mmol/l) × plasma insulin (μIU/ml) / 22.5.

2.3. Echocardiographic studies

All echocardiographic measurements were performed in our department as a part of the routine preoperative protocol using the same device (Toshiba Aplio SSA-770A, Tochigi, Japan). The following parameters were measured: left ventricular end diastolic (LVEDD) and end systolic (LVESD) diameter, septal (SWT) and posterior (PWT) wall diastolic thickness, right ventricular diameter (RV) and tricuspid annular plane systolic excursion (TAPSE). Left ventricular ejection fraction (EF) was calculated using the Simpson method. Left ventricular mass (LVM) was calculated according to the formula described by Devereux and Reichek: $LVM = 1.04 \times [(LVEDD + PWT + SWT)^3 - LVEDD^3] - 13.6$, where LVM is in grams, diameters and wall thickness parameters are in centimeters [44]. Upper limit of normal for both posterior wall and septal thickness is considered 10 mm in men and 9 mm in women, for left ventricular mass it is 200 g for men and 150 g for women [45]. The anthropometric, biochemical and echocardiographic data of patients are summarized in Table 1.

Table 1
Demographic and anthropometric data, metabolic and echocardiographic variables of the ischemic (ISCH) and valvular heart disease (VHD) patient group. * $p < 0.05$, ** $p < 0.01$ ISCH vs. VHD (Fisher's exact test for binary parameters, Mann–Whitney *U* test for continuous parameters).

Parameters (mean ± SEM)	ISCH (n = 54)	VHD (n = 41)
Age (years)	63 ± 1	66 ± 1
Sex (m/f)	**43/11***	**15/26**
Ratio of smokers (smokers/non-smokers)	14/40	5/36
Ratio of alcohol consumers (consumers/non-consumers)	6/48	3/38
Ratio of patients with hypertension (with/without)	**47/7***	**6/35**
Ratio of patients with DM (with/without)	**18/36***	**4/37**
BMI (kg/m^2)	28 ± 1	27 ± 1
Cholesterol (mmol/l)	**4.4 ± 0.2****	**5.1 ± 0.2**
Triglyceride (mmol/l)	1.6 ± 0.1	1.6 ± 0.1
Blood glucose (mmol/l)	5.5 ± 0.2	5.6 ± 1.2
Blood insulin (μIU/ml)	11.8 ± 1.9	9.5 ± 0.6
HOMA-A index	2.9 ± 0.4	2.5 ± 0.2
Ejection fraction (%)	**55 ± 1****	**62 ± 1**
Left ventricular end diastolic diameter (mm)	49 ± 1	47 ± 1
Left ventricular end systolic diameter (mm)	**36 ± 1****	**32 ± 1**
Septal wall thickness (mm)	14 ± 1	15 ± 1
Posterior wall thickness (mm)	12 ± 0	13 ± 1
Left ventricular mass (g)	309 ± 21	322 ± 24
Right ventricular diameter (mm)	29 ± 1	32 ± 1
Tricuspid annular plane systolic excursion (mm)	26 ± 1	28 ± 1

Bold highlighting represents sigificant differences.