Brief Communication

MOLECULAR METABOLISM

The incretin hormone GIP is upregulated in patients with atherosclerosis and stabilizes plaques in ApoE$^{-/-}$ mice by blocking monocyte/macrophage activation

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ABSTRACT

Objective: The incretin hormones GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic peptide) are secreted by the gut after food intake leading to pancreatic insulin secretion and glucose lowering. Beyond its role in glucose control, GLP-1 was found in mice and men to beneficially modulate the process of atherosclerosis, which has been linked to improved cardiovascular outcome of patients with diabetes at high cardiovascular risk treated with GLP-1 receptor agonists. However, little is known on the role of the other main incretin in the cardiovascular system. The aim of this study was to characterize GIP in atherosclerotic cardiovascular disease.

Methods and results: Serum concentrations of GIP were assessed in 731 patients who presented for elective coronary angiography at the University Hospital Aachen. While GIP concentrations were not associated with coronary artery disease (CAD), we found 97 patients with PAD (peripheral artery disease) vs. 634 without PAD to have higher circulating GIP levels (413.0 $\pm$ 315.3 vs. 332.7 $\pm$ 292.5 pg/mL, $p = 0.0165$). GIP levels were independently related to PAD after multivariable adjustment for CAD, age, sex, BMI, hypertension, diabetes, CRP, WBC, and smoking. To investigate the functional relevance of elevated GIP levels in human atherosclerotic disease, we overexpressed GIP (1–42) in ApoE$^{-/-}$ mice fed a Western diet for 12 weeks using an adeno-associated viral vector system. GIP overexpression led to reduced atherosclerotic plaque macrophage infiltration and increased collagen content compared to control (LacZ) with no change in overall lesion size, suggesting improved plaque stability. Mechanistically, we found GIP treatment to reduce MCP-1-induced monocyte migration under In vitro conditions. Additionally, GIP prevented proinflammatory macrophage activation leading to reduced LPS-induced IL-6 secretion and inhibition of MMP-9 activity, which was attributable to GIP dependent inhibition of NfκB, JNK-, ERK, and p38 in endotoxin activated macrophages.

Conclusion: Elevated concentrations of the incretin hormone GIP are found in patients with atherosclerotic cardiovascular disease, while GIP treatment attenuates atherosclerotic plaque inflammation in mice and abrogates inflammatory macrophage activation in vitro. These observations identified GIP as a counterregulatory vasoprotective peptide, which might open new therapeutic avenues for the treatment of patients with high cardiovascular risk.

Keywords Incretin; GIP; Atherosclerosis; Plaque stability; PAD; Macrophages

1. INTRODUCTION

The two major incretin hormones GLP-1 (glucagon-like peptide-1) and GIP (glucose-dependent insulinotropic peptide) are secreted by enteroendocrine cells following nutrient intake leading to insulin secretion and glucose control [1]. This mode of action is currently used for the treatment of patients with type 2 diabetes [2]. Beyond its glucoregulatory role GLP-1 has been found to mediate various protective pleiotropic effects in different organ systems [3]. For example, we and others found GLP-1 to reduce and stabilize atherosclerotic lesions in ApoE$^{-/-}$ mice by directly blocking monocyte migration and preventing inflammatory activation of monocytes/macrophages [4, 5]. Two recent clinical trials (LEADER and SUSTAIN-6) showed improved cardiovascular outcomes in diabetic patients at high cardiovascular risk after treatment with the GLP-1-receptor agonists liraglutide and semaglutide on top of standard antidiabetic

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therapy [6, 7]. Interestingly, in both studies, GLP-1-receptor agonists reduced cardiovascular endpoints most likely through a reduction in atherosclerosis-related events. However, the role of the other main incretin hormone, GIP, in the cardiovascular system, beyond its insulinotropic function, is still largely unknown. Experimental work by Nagashima et al. demonstrated that infusion of GIP into non-diabetic ApoE$^{-/-}$ mice on an atherogenic diet for 4 weeks was able to reduce lesion size [8]. A study by Nogi and colleagues could reproduce the anti-atherosclerotic effects of GIP also in diabetic ApoE$^{-/-}$ mice [9], which was mechanistically linked to a reduction of plaque macrophages and direct GIP-receptor-mediated inhibition of foam cell formation. However, evidence is lacking on the effects of GIP on composition and stability of atherosclerotic plaques. Experimental and clinical imaging studies identified atherosclerotic plaques of patients with diabetes compared to non-diabetic patients to be more instable due to a thin fibrous cap and less collagen content with high amounts of proinflammatory macrophages, thus leading to high susceptibility of early plaque rupture and life threatening cardiovascular complications like consecutive myocardial infarction [10, 11]. Therefore, identifying new approaches to target plaque morphology and stability is of particular importance to improve cardiovascular outcomes in patients with diabetes. Here we investigated the role of GIP in atherosclerotic cardiovascular disease with a focus on plaque morphology.

2. METHODS

2.1. Clinical study

We analyzed blood samples from 731 patients in our cardiovascular biobank (542 male and 189 female); these patients underwent elective coronary angiography at the University Hospital Aachen (Department of Cardiology). Stable coronary artery disease (CAD) was present in 474 patients, and PAD (peripheral artery disease) was present in 97 patients, as presented in Table 1. Blood was collected in a random nonfasting manner. After centrifugation at 2,000 g at 4 °C for 20 min, serum aliquots of 1 mL were frozen immediately at −80 °C. Total GIP serum levels were determined by using a commercial ELISA kit (Millipore) according to the manufacturers' instructions. Study protocols and biosampling were approved by the local ethics committee and conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki (ethics committee of the University Hospital Aachen, RWTH Aachen University).

2.2. Experimental study

Recombinant adeno-associated viral constructs: Vectors carried transgene cassettes encoding b-galactosidase (LacZ) as control or GIP (1−42) under control of a cytomegalovirus (CMV) promoter. The pseudotyping strategy was used to generate AAV vectors encapsidated in an AAV8 capsid (AAV2.8) as previously reported [4, 12]. Successful vector cloning was proved by sequencing and restriction digests. Vectors were purified by standard cesium sedimentation. Titers were determined via Taq-Man RealTime polymerase chain reaction (PCR).

Animals: C57BL/6 ApoE$^{-/-}$ mice were purchased from Taconic, USA. Six-week-old male mice were housed under specific pathogen−free conditions in either individually ventilated or filter top cages with a 12-h light/12-h dark cycle with free access to autoclaved water and regular chow diet ad libitum. Mice were i.v. injected with AAV vectors via tail vein (LacZ vs. GIP 1−42; 5×10^{12} particles/mouse). Mice were switched to a western diet (39 kJ% fat, 41 kJ% carbohydrates and 20 kJ% protein (ssniff EF R/M acc. TD88137AQ8 mod.; ssniff Spezialdiäten GmbH, Germany) 4 weeks after i.v. vector injection on which they remained for a total of 12 weeks. Body weight was monitored

Table 1 — Baseline characteristics and GIP measurements.

Parameter	All patients	PAD (no)	PAD (yes)	No CAD	PAD (no)	PAD (no)	Stable CAD	PAD (no)	PAD (yes)
n	731	634	97	257	242	15	474	392	82
Age	65.3 ± 13.2[a]	64.5 ± 13.4	70.3 ± 10.2	60.6 ± 15.8	60.3 ± 15.8	65.8 ± 15.7	67.9 ± 10.7	67.2 ± 11.0	71.2 ± 8.8
BMI	28.3 ± 5.6[b]	28.3 ± 5.7	28.2 ± 4.9	28.6 ± 7.1	28.6 ± 7.2	27.8 ± 4.6	28.2 ± 4.6	28.2 ± 4.6	28.3 ± 5.0
Sex (male)	542 (74.3%)[c]	466 (73.7%)	76 (78.4%)	154 (60.4%)	146 (60.8%)	8 (53.3%)	388 (81.9%)	320 (81.6%)	68 (82.9%)
Hypertension	527 (72.2%)[a]	447 (70.5%)	80 (83.3%)	145 (56.6%)	137 (56.6%)	8 (57.1%)	382 (80.6%)	382 (80.6%)	72 (87.8%)
DM	239 (32.7%)	199 (31.4%)	40 (41.2%)	63 (24.5%)	61 (25.2%)	2 (13.3%)	176 (37.1%)	138 (35.2%)	38 (46.3%)
CRP	10.5 ± 16.7[d]	10.5 ± 17.4	10.1 ± 11.9	11.0 ± 16.9	11.0 ± 17.2	10.2 ± 10.4	10.2 ± 16.6	10.2 ± 17.5	10.0 ± 12.3
WBC	7.4 ± 2.5[e]	7.4 ± 2.5	7.2 ± 2.4	6.9 ± 2.1	7.0 ± 2.1	6.1 ± 1.5	7.6 ± 2.6	7.6 ± 2.7	7.4 ± 2.4
Smoker	289 (39.6%)[a]	235 (37.1%)	54 (56.3%)	89 (34.6%)	78 (32.2%)	11 (73.3%)	200 (42.3%)	157 (40.1%)	43 (53.1%)
GIP level	343.5 ± 296.7 (272.0, 73.1-545.6)[f]	332.7 ± 292.5 (258.4, 71.2-533.9)[1]	413.0 ± 315.3 (376.2, 118.2-624.5)[1]	370.7 ± 314.9 (310.8, 73.5-567.4)[2]	360.5 ± 310.4 (292.1, 71.0-551.0)[3]	531.6 ± 352.1 (475.0, 230.5-1008.2)[3]	328.9 ± 285.8 (252.9, 72.6-533.3)[2]	315.7 ± 280.1 (241.1, 71.2-519.5)[4]	391.2 ± 305.5 (368.1, 95.4-615.5)[4]

Continuous variables are expressed as mean ± SD and (median, Q1 − Q3) in case of skewed data. Categorical variables are shown as absolute and relative frequencies. [a] 1 missing value, [b] 12 missing values, [c] 2 missing values, [d] 118 missing values, [e] 92 missing values, [f] 11 missing values. [1] PAD vs. No PAD p-value = 0.0165, [2] CAD vs. No CAD pvalue= 0.1869, [3] No CAD: PAD vs. No PAD p-value = 0.0632, [4] Stable CAD: PAD vs. No PAD p-value = 0.0389.

