Cytokine 90 (2017) 161–168

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Cross-talk between innate cytokines and the pancreatic polypeptide family in acute pancreatitis

Sayali A. Pendharkar, Ruma G. Singh, Maxim S. Petrov *

Department of Surgery, University of Auckland, Auckland, New Zealand

ARTICLE INFO

Article history:
Received 26 October 2016
Accepted 22 November 2016

Keywords:
Acute pancreatitis
Interleukin-6
Low-grade inflammation
MCP-1
Pancreatic polypeptide family
TNFα

ABSTRACT

Background and aim: Low-grade inflammation persists in patients with acute pancreatitis (AP) after hospital discharge, and is linked to metabolic disorders. Neuropeptide Y (NPY) is well recognized as an important mediator of inflammation in these patients but the role of the other two structurally similar peptides, pancreatic polypeptide (PP) and peptide YY (PYY), in inflammation has been sparsely investigated. The aim was to investigate the association between PYY, PP, NPY and circulating levels of innate cytokines in patients after AP.

Methods: Fasting blood samples were collected to measure PYY (ng/mL), PP (ng/mL), NPY (pg/mL), interleukin-6 (IL-6) (ng/mL), monocyte chemoattractant protein (MCP) 1 (ng/mL), and tumour necrosis factor (TNF) α (ng/mL). Modified Poisson regression analysis and linear regression analyses were conducted. Age, sex, ethnicity, obesity, diabetes, aetiology, time from 1st attack of AP, recurrence, severity, physical activity, and smoking were adjusted for in several statistical models. $P < 0.05$ was considered statistically significant.

Results: A total of 93 patients were recruited. Peptide YY was significantly associated ($p < 0.001$) with IL-6, MCP-1, and TNFα in the unadjusted and all adjusted models. Pancreatic polypeptide was significantly associated ($p < 0.001$) with IL-6, MCP-1, and TNFα in the unadjusted and at least one adjusted model. Peptide YY and PP together contributed 22.2%, 72.7%, and 34.6% to the variance of IL-6, MCP-1, and TNFα, respectively. Neuropeptide Y was not significantly associated with any of the three cytokines.

Conclusions: Peptide YY and PP are associated with circulating innate pro-inflammatory cytokines in patients after AP and cumulatively contribute to nearly half of the variance of IL-6, MCP-1, and TNFα. Future research is warranted to investigate the signaling pathways that underlie these associations.

1. Introduction

Acute pancreatitis (AP) is a classic inflammatory disorder associated with strong up-regulation of innate pro-inflammatory cytokines, in particular interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumour-necrosis factor (TNF) α [1,2]. While it is conventionally believed that AP self-resolves during the hospital admission, recent evidence shows that low-grade inflammation continues to persist after hospital discharge, and is linked to insulin resistance, diabetes mellitus (DM), and obesity [3]. Activation of the immune system is one of the most important pathogenetic factors that govern the aforementioned metabolic derangements; in particular DM, with which AP shares a bi-directional relationship [4,5]. While it has long been known that diabetes is an independent risk factor for development of AP [6,7], recent meta-analyses show that nearly 40% of patients develop new-onset pre-diabetes or diabetes after an attack of AP [8,9]. Although a similar bi-directional relationship between obesity and AP has not been established, a recent population-based study shows that obesity is a risk factor for developing AP with similar underlying inflammatory state and patients with high body mass index (BMI) are at a two times greater risk of developing AP. Evidence also shows that circulating pro-inflammatory cytokines levels are increased in individuals with metabolic disorders such as DM and obesity, long after their hospitalization due to AP [10,11].

The gut-brain axis has recently emerged as an important contributor to persistent low-grade inflammation in gastrointestinal (GI) diseases, DM, and obesity [12–14]. According to its most conventional definition, the gut-brain axis comprises the GI tract, the

* Corresponding author at: Department of Surgery, University of Auckland, Room 12.085A, Level 12, Auckland City Hospital, Private Bag 92019, Victoria Street West, Auckland 1142, New Zealand.

E-mail addresses: s.pendharkar@auckland.ac.nz (S.A. Pendharkar), ruma.singh@auckland.ac.nz (R.G. Singh), max.petrov@gmail.com (M.S. Petrov).

http://dx.doi.org/10.1016/j.cyto.2016.11.014
1043-4666/

nervous system, and the immune system. The interaction between these three constituents is mediated by a wide array of regulatory molecules, including gut peptides and neuropeptides [15,16]. In particular, neuropeptide Y (NPY), belonging to the pancreatic polypeptide (PP) family – one of the most phylogenetically conserved family of regulatory peptides, has been implicated in GI inflammation and in the pathogenesis of associated disorders, including but not limited to ulcerative colitis, Crohn's disease, and pancreatitis [17,18]. Neuropeptide Y is the most abundant peptide in the brain, which is expressed at all levels of the gut-brain axis and acts on the Y receptors, primarily Y1, Y2, Y4, and Y5 [17]. Neuropeptide Y activates and negatively regulates antigen-presenting cell function and T-cell function, respectively, and its involvement in GI inflammation is multifaceted [17]. Pancreatic polypeptide and peptide YY (PYY), the other two members of the PP family, share a remarkable structural similarity with NPY – all consisting of 36 amino acids and the same hairpin-shaped PP-fold tertiary structural motif [17,19]. Peptide YY is secreted by the L cells in the gut and preferentially binds to the Y2 receptor. Pancreatic polypeptide is secreted by the γ cells in the pancreas and preferentially binds to the Y4 receptor. [17]. Given the similar evolutionary origin and relative structural homogeneity of the three members of the PP family, we hypothesized that PYY and PP are functionally involved in GI inflammation, along with NPY.

Therefore, the aim of this study was to investigate the association between PYY, PP, and NPY and circulating levels of the innate pro-inflammatory cytokines (IL-6, MCP-1, and TNFα) in patients after AP.

2. Methods

2.1. Study design

The study was a follow-up study of AP patients who had been admitted to Auckland City Hospital (Auckland, New Zealand). The study protocol was approved by the Auckland District Health Board (ADHB) Institution (A+6139) and the Health and Disability Ethics Committee (13/STH/182) and all patients gave informed consent.

Individuals were recruited into the study if they had a primary diagnosis of AP based on the international guidelines [20]; were at least 18 years of age; and resided in Auckland at the time of the study. Individuals were excluded from the study if they had/were chronic pancreatitis; post-ERCP pancreatitis; malignancy; pregnant at time of AP or afterwards; intra-operative diagnosis of AP.

2.2. Sample acquisition and storage

All patients attended the clinic at 8 am and were required to fast for at least eight hours. A certified phlebotomist at the International Accreditation New Zealand accredited tertiary medical referral laboratory, LabPlus, Auckland City Hospital, collected venous blood into one plasma separation tube, one lithium heparin tube, one fluoride tube, and two ethylene-diamine-tetra-acetic acid tubes. The tubes were centrifuged at 4000*g* for 7.5 min at 4 °C. The plasma was then separated into aliquots of 400 µl, and the eppendorf tubes stored at −80 °C until further use.

2.3. Definitions

Body mass index (BMI): was determined using a digital medical scale with stadiometer. Patients were then categorized into three groups: healthy (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese ($\geqslant$30 kg/m^2) according to the most recent National Institutes of Health and World Health Organisation guidelines [21,22].

Normal glucose metabolism (NGM): fasting blood glucose (FBG) < 5.6 mmol/L and/or glycated haemoglobin A1c (HbA1c) $\leqslant$ 38 mmol/mol [23].

Pre-diabetes: FBG between 5.6 and 6.7 mmol/L and/or HbA1c between 39 and 48 mmol/mol [23] at the time of the study.

Diabetes: FBG $\geqslant$ 6.8 mmol/L and/or HbA1c $\geqslant$ 49 mmol/mol [23] at the time of the study.

Time from first attack of AP was defined as months elapsed from first hospital admission due to AP to the time of the study.

Severity of AP: was defined according to the 2012 Determinant-Based Classification [24,25].

Aetiology: was categorized as alcohol, biliary, or other (e.g. pancreas divisum, idiopathic pancreatitis, hypertriglyceridemia).

Recurrence of AP: individuals admitted with one or more episodes of confirmed AP since their first admission with AP to the time of their participation in the study were considered to have recurrent AP.

Physical activity: was recorded as patient's response, active or inactive, based on a questionnaire asking participants if they performed physical activity for at least two and half hours per week.

Smoking status: was recorded as patient's response, yes or no, based on a questionnaire asking participants if they smoked cigarettes or tobacco related products on a daily basis.

2.4. Laboratory assays

Blood tests for FBG, insulin, and HbA1c were conducted at LabPlus, an accredited medical laboratory at Auckland City Hospital. Insulin was measured using chemiluminescence sandwich immunoassay (Roche Diagnostics NZ Ltd) while an enzymatic colourimetric assay (F. Hoffmann-La Roche Ltd.) was used to measure FBG. Glycated haemoglobin A1c was measured using the boronate affinity chromatography assay (Trinity Biotech, Ireland).

Neuropeptide Y was measured using the Merck-Millipore (MA, USA) ELISA kits according to user's manual. The Rayto Microplate Reader (V-2100C, Santa Fe, Granada, Spain) with an absorbance range of 405–630 nm was used to read the results (pg/mL).

Pancreatic polypeptide, PYY, IL-6, MCP-1, and TNFα were measured using the MILLIPLEX® MAP Human metabolic hormone magnetic bead panel based on the Luminex xMAP® (Luminex Corporation, Austin, Texas, USA, 1995) technology. Results were quantified (ng/mL) based on fluorescent reporter signals recorded by the Luminex xPONENT® software (MILLIPLEX® Analyst 5.1). All assays were performed as indicated in the user's manual.

2.5. Statistical analyses

Data were presented as either frequency (%) or mean ± standard deviation. The statistical analyses were conducted in three steps.

First, a modified Poisson regression analysis, using the generalized linear model, was used to investigate the association between each peptide of the PP family and the three cytokines (IL-6, MCP-1, and TNFα). Each cytokine and peptide was categorized into quartiles based on pre-determined concentration ranges using the frequencies function. The *p*-trend across the quartiles was calculated by assigning each participant the median value in their quartile and assessing this as a continuous variable. Each peptide was investigated as an independent variable in one unadjusted and five adjusted models. The unadjusted model investigated the association between each peptide and prevalence of each cytokine. Model 1 was adjusted for demographics (age, sex, and ethnicity). Model 2, in addition, was adjusted for metabolic factors (BMI category and DM status). Model 3 was further adjusted for pancreatitis-related risk factors (aetiology, recurrence, time since first attack of AP,