Pancreatology 16 (2016) 225–230

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Original article

Somatostatin expression in the pancreatic cells of smoking and non-smoking chronic pancreatitis patients with or without diabetes

Mariola Śliwińska-Mossoń [a, *], Michał Jeleń [b], Halina Milnerowicz [a]

[a] Department of Biomedical and Environmental Analysis, Wrocław Medical University, Poland
[b] Division of Pathomorphology and Oncological Cytology, Wroclaw Medical University, Poland

ARTICLE INFO

Article history:
Available online 10 November 2015

Keywords:
Somatostatin
Diabetes
Chronic pancreatitis
Smoking
Immunohistochemistry
Hormone

ABSTRACT

Objectives: The aim of the analysis is to determine the location and degree of the hormone immunoreactivity in tissues of patients with chronic pancreatitis and diabetes.
Methods: The study was performed on 11 non-smoking and 12 smoking patients with chronic pancreatitis (CP) with/without diabetes. The hormone was located in the pancreatic tissues by means of the immunohistochemical method using somatostatin antibodies. The histopathological evaluation of the hormone expression intensity in tissue sections was carried out using the semi-quantitative method and was calculated by means of a digital image analysis.
Results: The hormone's strong immunohistochemical reaction and the modified D-cell location may be a result of the pancreatic tissue fibrosis process prevention in patients with CP. Changes in the intensity of SS immunoreactivity and the D-cell distribution in the pancreas of patients with CP and diabetes may possibly result from the additional hormone compensatory effect in the excessive glucagon secretion inhibition. Smoking patients with diabetes showed significantly higher hormone immunostaining in the pancreas compared to non-smoking patients without diabetes and healthy persons.
Conclusions: The severity of histopathological changes in smoking CP patients indicates that the cigarette smoke components may further exacerbate the inflammatory reactions. Patients with CP were found to have a strong immunohistochemical reaction to SS and changes in the distribution of D cells when compared to healthy patients. The strongest immunohistochemical SS reaction has been identified in the pancreatic tissue from smoking patients with diabetes.

Introduction

Somatostatin (SS) is a peptide hormone first described in 1973. Endocrine cells responsible for the biosynthesis and secretion of the hormone are present in many organs and tissues. Somatostatin has an extensive spectrum of activity, mainly of an inhibitory nature [1,2].

First of all, it is the exo- and endocrine secretion inhibitor [3,4]. Additionally, it has been demonstrated that the hormone exerts immunomodulatory effects as it suppresses the secretion of pro-inflammatory cytokines [2]. Somatostatin also participates in the processes inhibiting the growth and metastasis of tumor cells since it has an antiproliferative and antiangiogenic activity [3,4]. The physiological role of the peptide has led to its potential use in the treatment of pancreatic diseases [1,2,4]. There have been numerous studies on the effect of somatostatin in the prevention of pancreatitis after the endoscopic retrograde cholangiopancreatography, but these studies are quite controversial [4–6]. In chronic pancreatitis, the hormone suppresses the concentration of pro-inflammatory cytokines, which play an important role in the stimulation of stellate cells responsible for the gland fibrosis development. Therefore, somatostatin can contribute to a reduction of the connective tissue growth during chronic pancreatitis [7]. Furthermore, a frequent complication of the disease is diabetes type 3c [8]. The somatostatin secretion from the pancreatic D cells is stimulated by high glucose concentrations [9–11] and, therefore, the hormone may be involved in hyperglycemia compensation by inhibiting the glucagon secretion [12].

* Corresponding author. Department of Biomedical and Environmental Analyses, Wroclaw University of Medicine, Borowska 211, 50-556 Wroclaw, Poland. Tel.: +48 71 7840174; fax: +48 71 7840172.
E-mail addresses: mariola.sliwinska-mosson@umed.wroc.pl (M. Śliwińska-Mossoń), halina.milnerowicz@umed.wroc.pl (H. Milnerowicz).

http://dx.doi.org/10.1016/j.pan.2015.10.007

In view of the insufficient amount of research and information about the somatostatin expression in pancreatic diseases, the aim of the analysis is to determine the location and degree of the hormone immunoreactivity in pancreatic tissues from patients with chronic pancreatitis and diabetes.

Material and methods

Material and characteristics of the patients

The study was conducted on 23 patients with chronic pancreatitis (CP), admitted to the Department of Gastrointestinal and General Surgery, Wrocław Medical University, Poland, for the evaluation of their disease (Table 1). This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The study was approved by the Commission of Bioethics at Wroclaw Medical University in terms of ethics (No. KB: 4353/2011). All healthy persons and patients provided their informed consent in writing.

The patient classification was based on the following: routine clinical examination (medical history: pain appearance background, disease duration; physical examinations), ultrasonography (US) and computed tomography (CT) of the abdomen, in special cases: ERCP (endoscopic retrograde cholangiopancreatography) and MRCP (magnetic resonance cholangiopancreatography) of the pancreas and bile ducts as well as laboratory tests used in the assessment of pancreatitis. The physical examination, supplemented by the aforementioned diagnostic procedures, allowed in most cases a definition of the pancreatitis etiology. The diagnosis of chronic pancreatitis was combined with a history of alcohol abuse, where the patient consumed more than 40 g of alcohol per day for more than five years. The intake of alcohol was the main cause of chronic pancreatitis in the present study, accounting for about 70% of cases. The obstructive pancreatitis etiology was defined on the basis of ERCP and US tests. The family type of pancreatitis was not diagnosed. Cases, which could not be classified according to the above criteria, were defined as cases of idiopathic pancreatitis.

Data concerning smoking and alcohol consumption were obtained based on a direct personal interview conducted with each patient. Clinicopathological results such as: the disease duration from the moment of its occurrence, the endoscopic treatment, the type of surgery, the pharmacological treatment, the age of patients, the area from which the tissue collected, etc. were collected on the basis of archival materials. Based on the interview concerning smoking and on the elevated levels of cotinine (a nicotine metabolite in the plasma), the patients with pancreatitis were divided into: non-smokers and smokers. The obtained results helped to identify smoking patients as heavy smokers. Among the study group of patients: there were four non-smoking and six smoking patients with CP and diabetes. Diabetes was diagnosed based on the results of glucose tolerance, glycosuria and hypoglycemia tests (Table 1).

The blood plasma and tissues served as the study material. The venous blood was collected from fasting patients during the first 24 h of hospitalization. The plasma was prepared from blood clots collected to disposable S-Monovette EDTA tubes (Cat. No.: 03.1068.001, Sarstedt, Germany) and promptly mixed. The blood was centrifuged at 2500 g for 15 min, and the resulting plasma was aliquoted and stored in sealed Eppendorf tubes (Cat. No.: 25.1500.0, Eppendorf, Germany) at −80 °C until determination.

Twenty-three patients with CP (non-smoking, n = 11; smoking, n = 12) underwent surgery during which the tissue material was obtained. The control sections were derived from the pancreas of four healthy subjects who had died in traffic accidents. Patients were healthy; the presence of chronic pancreatitis and diabetes was excluded. All tissue sections were routinely fixed in a phosphate-buffered 10% formaldehyde solution and embedded in paraffin. Serial 4-μm thick sections were placed on histological slides (Super Frost Plus, Cat No.: 041300, Menzel-Gloser, Germany). The hematoxylin and eosin stained preparations were verified histologically in the Division of Pathomorphology and Oncological Cytology, Wrocław Medical University.

Methods

The tissue sections were deparaffinized and rehydrated in an alcohol series. The sections were then incubated with 3% hydrogen peroxide to block the intracellular activity of peroxidase. The non-specific binding was blocked with 0.05 M Tris–HCl buffer, pH 7.4 with 1% BSA (DAKO Antibody Diluent, Cat. No.: S0809, DakoCytomation, UK). The sections were then coated with a solution of antibodies against somatostatin (Cat. No.: A0566 DakoCytomation, UK). After washing the sections with 0.05 M Tris–HCl buffered saline (TBS, Cat. No.: 170-6435, Bio-Rad, USA) with 0.1% Tween 20 (Cat. No.: P1379, Sigma–Aldrich, Germany), visualization of the Ab–Ag complex (antibody–antigen) was performed by means of test LSAB2-HRP (Cat. No.: K0637, DakoCytomation, UK), using biotinylated secondary IgGantibodies (Cat. No.: OS03B, Calbiochem, UK). The peroxidase activity was localized with regard to the 3,3-diaminobenzidine in the imidazole-HCl buffer, pH = 7.5 (DAB, Cat. No.: K0637, DakoCytomation, UK). At the final stage, the sections were washed and counterstained with hematoxylin (Chem Mate™, Cat. No.: S2020, DakoCytomation, UK). The preparations were closed in the glycerin gel and allowed to dry.

A negative control was performed for each tissue section, replacing the primary antibody with the anti-rabbit immunoglobulin control IgG antibody (Cat. No.: X0931, DakoCytomation, Carpinteria, California, USA) – an antibody characterized by the

Table 1
Characteristics of patients with pancreatitis.

	Non-smoking patients		Smoking patients	
	Without diabetes	With diabetes	Without diabetes	With diabetes
The number of patients	7	4	6	6
F/M	2/5	1/3	1/5	2/4
The age range [years]	45–63	42–53	41–52	35–50
The average age [years]	50.7	47.3	46.5	43.0
The duration of the disease from the onset [years]	6.4 ± 0.8[1]	9.0 ± 3.5[2]	11.4 ± 3.1[1]	12.8 ± 3.1[2]
Alcohol/no alcohol	3/4	3/1	4/2	6/0
The number of cigarettes [n/24 h]	–	–	21.7 ± 5.2	25.8 ± 5.8
Cotinine concentration [ng/ml]	–	–	380.7 ± 78.6	365.0 ± 110.3

K – female, M – man.
Statistically significant differences: [1]$p = 0.008$; [2]$p = 0.04$.