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Morphological and immunohistochemical comparison of intrapancreatic nerves between chronic pancreatitis and type 1 autoimmune pancreatitis

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ABSTRACT

Objectives: The abdominal pain associated with chronic pancreatitis (CP) may be related to the increased number and size of intrapancreatic nerves. On the other hand, patients with type 1 autoimmune pancreatitis (AIP) rarely suffer from the pain syndrome, and there are no previous studies concerning the histopathological findings of intrapancreatic nerves in patients with type 1 AIP. The current study is aimed at investigating the differences in the histopathological and immunohistochemical findings of intrapancreatic nerves in patients with CP and type 1 AIP.

Methods: Neuroanatomical differences between CP and type 1 AIP were assessed by immunostaining with a pan-neuronal marker, protein gene product 9.5 (PGP9.5). The number (neural density) and area (neural hypertrophy) of PGP9.5-immunopositive nerves were quantitatively analyzed. Furthermore, the expression of nerve growth factor (NGF), and a high affinity receptor for NGF, tyrosine kinase receptor A (TrkA), was assessed by immunohistochemistry.

Results: Both neural density and hypertrophy were significantly greater in pancreatic tissue samples from patients with CP than those with normal pancreas or type 1 AIP. NGF expression was stronger in type 1 AIP than in CP, whereas TrkA expression in type 1 AIP was poorer than in CP.

Conclusions: Although CP and type 1 AIP are both characterized by the presence of sustained pancreatic inflammation, they are different in terms of the density and hypertrophy of intrapancreatic nerve fibers. It is possible that this may be related to the difference in the activity of the NGF/TrkA-pathway between the two types of pancreatitis.

1. Introduction

Chronic pancreatitis (CP), which is predominantly caused by chronic alcohol abuse, is radiologically characterized by atrophic pancreas with ductal dilation and parenchymal calcification [1]. On histopathology, the pancreas in these patients is typically characterized by inflammatory cell infiltration along with atrophy of the acinar cells and fibrosis. The clinical presentation of patients with CP frequently includes pain, impaired digestion, and diabetes mellitus. Pancreatic pain, described as upper abdominal pain radiating to the back, is the most frequent symptom, occurring in at least 75–85% of patients with CP [2,3]. The recurrent or persistent abdominal pain in CP is often difficult to manage, adversely affecting the quality of life of patients [4]. The pathogenesis of pain in CP is not completely understood, although the hypertrophy and increased branching of intrapancreatic nerves have been recently recognized as possible important causative factors [5–9]. These pathomorphological alterations of intrapancreatic nerves in CP appear to be induced by the upregulation of nerve growth factor

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(NGF) in damaged acinar cells and ductular complexes, and subsequent activation of the high-affinity tyrosine kinase receptor A (TrkA), a receptor for NGF, in the primary afferent nerve terminals [10].

Type 1 autoimmune pancreatitis (AIP), in contrast, is characterized by focal or diffuse pancreatic enlargement, irregular narrowing of the main pancreatic duct (MPD), and a dramatic response to steroid therapy [11–13]. Serologically, IgG4 levels are frequently elevated in these patients [14]. The histological features of type 1 AIP include periductal infiltration of lymphocytes, abundant IgG4-positive plasma cells, storiform fibrosis, and obliterative phlebitis, specifically lymphoplasmacytic sclerosing pancreatitis (LPSP). The clinical picture in these patients commonly includes obstructive jaundice, diabetes mellitus, and accompanying extrapancreatic lesions. However, abdominal pain is usually minimal or completely absent [15–17].

In view of the differences in the prevalence of pain between CP and type 1 AIP, we aimed to compare the pathomorphological alterations of intrapancreatic nerves in the two types of pancreatitis based on pan-neural marker, protein gene product 9.5 (PGP9.5), labeling and NGF and TrkA immunoreactivity of their pancreatic tissue samples.

2. Materials and methods

2.1. Patients and tissues

This study was approved by the Kansai Medical University's ethics committee. Pancreatic tissue samples were collected from 33 patients (16 with CP, 11 with type 1 AIP, and 6 with normal pancreas) that underwent surgical resection at Kansai Medical University Hospital in the time period between 1993 and 2014. The tissue samples of normal pancreas were collected from the pancreatic tissue adjacent to resected serous cyst neoplasms. The following clinical information on patients was collected: sex, age at surgical resection, alcohol and smoking habits, diabetic status, hyperamylasemia, pancreatic imaging, and period between first diagnosis to surgical resection. The intensity of pain prior to the surgical operation was graded by using a short pain scale: grade 0 = absent, grade 1 = mild or moderate (abdominal discomfort or pain that is non-disabling but requires analgesics), grade 2 = severe (pain that is disabling and controlled only by analgesics) [8].

For histological analysis, formalin-fixed and paraffin-embedded specimens were prepared, and consecutive 4-μm thick sections were cut. Pancreatic sections were deparaffinized and rehydrated by using xylene and graded descending series of alcohol before staining.

2.2. Histological analysis of intrapancreatic inflammation

The severity of intrapancreatic inflammation in hematoxylin-eosin (HE) stained sections was evaluated by a previously described scoring system [18,19]. Briefly, pancreatic tissue was scored for abnormal architecture, glandular atrophy, fibrosis, and pseudotubular complexes as follows: 0, absent; 1, minimal (<10% of the total pancreatic parenchyma); 2, moderate (10–50%); 3, severe (>50%). Inflammatory score was calculated by adding the scores for each histological parameter.

2.3. Immunohistochemistry

Endogenous peroxidase activity was blocked in sections for immunohistochemical staining by using 3% H_2O_2/methanol for 30 min. After washing in Tris buffered saline (TBS) solution for 15 min, each section was incubated for 10 min in protein blocking reagent without serum (Dako, Kyoto, Japan). Subsequently, the slides were incubated overnight at 4 °C with polyclonal antibodies against PGP9.5 at 1/400 dilution (Ultraclone Ltd., Isle of Wight, UK), NGF at 1/100 dilution (Serotec Ltd., Oxford, UK), and TrkA at 1/100 dilution (Santa Cruz Biotechnology, Santa Cruz, USA). Next, the slides were incubated with secondary antibodies, by using a commercial kit (Chem Envision kit/HRP, Dako, Kyoto, Japan), following the manufacturer's instructions. Finally, antibody binding was detected by using 3,3'-diaminobenzidine (DAB) (Dojindo, Kumamoto, Japan). Images were obtained with a microscope (Olympus, Tokyo, Japan).

2.4. Evaluation of immunostaining for PGP9.5, NGF, and TrkA1

The quantitative assessment of PGP9.5-immunoreactive nerves in the pancreas was made by a modification of the method described by Ceyhan GO et al. [20]. Entire sections of pancreatic tissue stained with PGP9.5 were scanned by using NanoZoomer Digital Pathology System (Nanozoomer 2.0-HT slide scanner; Hamamatsu, Hamamatsu City, Japan). The total number of PGP9.5-immunopositive nerve fibers within the pancreas was counted in the digital color images, and the neural density was expressed as the total number of nerves within the total area of the pancreas including acinar parenchyma and fibrosis in the interlobular spaces (per mm^2). In addition, the proportion of PGP9.5-immunopositive nerve bundles in the entire pancreatic area was calculated by using a software program (Win Roof 2013, Mitani Corporation, Tokyo, Japan) and presented as neural hypertrophy (%). As PGP9.5 immunoreactivity is known to be present not only both in neural tissue and in pancreatic endocrine cells [21], PGP9.5-immunopositive endocrine cells were morphologically differentiated and excluded from the quantitative analysis.

The assessment of NGF expression was performed based on staining intensity in ductal cells and acinar cells in the pancreas. The degree of intensity of immunoreactivity was determined by using the following criteria: negative (−), weak (±), moderate (+), and strong (++). TrkA expression in nerve fibers was also evaluated in a similar manner.

Histological scoring for inflammation and evaluation of immunostaining for PGP9.5, NGF, and TrkA1 were performed by two independent observers blinded to patient status.

2.5. Statistical analysis

Results were expressed as mean ± standard error (SE). T-test was performed to analyze clinical data pertaining to sex, history of alcohol intake, smoking habit, diabetic status, hyperamylasemia and pancreatic imaging. Age and grading of pain was analyzed by chi-square test. Fisher's exact test was used to compare neural density and neural hypertrophy of PGP9.5 positive nerves between samples from patients with CP and type 1 AIP. The correlation between neural density, neural hypertrophy and inflammatory score was investigated by using linear regression analysis, and the Pearson's correlation coefficient was calculated. Significance was defined as $P < 0.05$. All statistical analyses were performed by using STATA version 14 (Stata Corporation, College Station, TX, USA).

3. Results

3.1. Clinical data

The clinical characteristics of the CP and type 1 AIP groups are shown in Table 1.

The etiologies of CP were alcohol in 14 patients (88%) and idiopathic in 2 (12%). Nine of 16 patients with CP underwent

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