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Research paper

Alpha-synuclein immunoreactivity patterns in the enteric nervous system

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HIGHLIGHTS

- Enteric α-syn immunohistochemistry reveals specific and unspecific patterns.
- Various anti α-synuclein antibodies show different sensitivities and specificities.
- "Coarse aggregates" in neural structures are the most promising diagnostic marker.

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ABSTRACT

We aimed to compare immunoreactivity patterns of four different anti-α-syn antibodies in surgical specimens of the gastrointestinal tract of Parkinson disease and control cases.

Surgical specimens from stomach, small and large bowel of 6 PD cases and 12 controls were studied. Primary antibodies: anti-α-syn clone KM51, anti-phosphorylated α-syn Ser129, anti-α-syn clone 15G7 and anti-nitrated α-syn505. We found different immunoreactivity patterns: (a) coarse, Lewy-body-like aggregates labelled by the 4 antibodies and detected in 4/6 PD cases and in 1/12 controls; (b) distinct punctate cytoplasmic staining of ganglion cells labelled by anti-phosphorylated-α-syn and detected in 3/6 PD cases and 3/12 controls; (c) fine diffuse, synaptic-type staining of neural structures labelled by anti-α-syn-15G7 and anti-nitrated-α-syn505 and detected in all subjects.

We conclude that different specific and non-specific immunoreactivity patterns are detected in surgical specimens of gastrointestinal tract when using different anti-α-syn antibodies, as they recognize different epitopes and states of alpha-synuclein protein. Coarse aggregates in neural structures seem to be the most promising marker for the diagnosis of Lewy-body parkinsonism when evaluating abnormal α-syn in the gastrointestinal tract.

1. Introduction

Misfolded and aggregated alpha-synuclein (α-syn) is the major component of intraneuronal Lewy bodies (LBs), the neuropathological hallmark of Parkinson's disease (PD) [1]. Postmortem studies in subjects with disorders associated with LBs in the central nervous system (CNS) have shown that α-syn pathology also involves the peripheral autonomic nervous system in several organs such as the gastrointestinal (GI) tract, salivary glands, adrenal glands, heart

Abbreviations: Ab, antibody; α-syn, alpha-synuclein; CNS, central nervous system; ENS, enteric nervous system; GI, gastrointestinal; IBD, inflammatory bowel disease; IHC, immunohistochemistry; LB, Lewy body; LN, Lewy neurites; Mc, monoclonal; nα-syn, nitrated alpha-synuclein; pα-syn, phosphorylated alpha-synuclein; PD, Parkinson's disease.

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Table 1
Clinical characteristics of study subjects.

Case no.	Gender	Clinical diagnosis	Age of onset and/or at diagnosis (years)	Age at surgery (years)	Surgical specimen	Type of surgery	Fixation time
1	M	PD	53 y	55 y	Colon	Colonic endoscopic polypectomy	<24 h
2	F	PD	70 y	81 y	Jejunum	Jejunum resection, leiomyoma	48 h
3	M	PD	53 y	64 y	Stomach	Partial gastrectomy, adenoma with high grade dysplasia	5 days
4	M	PD	46 y	72 y	Colon	Sigmoidectomy, previous neoplasia.	48 h
5	F	PD	77 y	84 y	Colon	Right hemicolectomy, adenocarcinoma	72 h
6	M	PD	55 y	64 y	Jejunum	Jejunum resection, ulcerated ischaemia	72 h
7	F	Control	N.a.	35 y	Ileum	Transverse and right colectomy with distal ileum, IBD - CD	6 days
8	M	Control	N.a.	21 y	Colon	Right colectomy with distal ileum, IBD - CD	5 days
9	F	Control	N.a.	46 y	Colon	Transverse colectomy, IBD - CD	5 days
10	M	Control	N.a.	36 y	Ileum	Ileum resection, IBD–CD	72 h
11	M	Control	N.a.	54 y	Colon	Colectomy, IBD–UC	24 h
12	M	Control	N.a.	30 y	Ileum	Right colectomy with distal ileum, IBD–CD	5 days
13	M	Control	N.a.	59 y	Colon	Right colon/sigmoidectomy, adenocarcinoma	4 days
14	M	Control	N.a.	83 y	Colon	Rectum/sigmoidectomy, tubulo-villous adenoma	72 h
15	M	Control	N.a.	83 y	Colon	Right hemicolectomy, adenocarcinoma	24 h
16	M	Control	N.a.	71 y	Colon	Sigmoidectomy, previous adenocarcinoma	48 h
17	F	Control	N.a.	75 y	Colon	Transverse colectomy, adenocarcinoma	48 h
18	F	Control	N.a.	77 y	Colon	Sigmoid colon, adenocarcinoma	72 h

Abbreviations: CD Crohn's disease, IBD Inflammatory Bowel Disease, PD Parkinson's disease, UC ulcerative colitis.

and skin, among others [2–5]. Identification of α-syn deposits in the enteric nervous system (ENS) may be possible in PD patients as the ENS can be studied in routine biopsies [6–9]. However, variable results have been reported both in healthy subjects and in PD. These differences may be due to varying sites of the GI tract studied, to the use of different immunohistochemical techniques and antibodies, as well as to unequal interpretation of results (see Supplementary table for review). Some authors have highlighted the specificity and usefulness of phosphorylated alpha-synuclein (pα-syn) to discern PD patients from non-PD controls [6–13,15–17], whereas others have shown that ENS α-syn expression, even in phosphorylated forms, can be seen in subjects without PD [14,18].

The aim of our study was to define and compare the α-syn immunoreactivity patterns of four different commercial anti-α-syn antibodies in surgical specimens of gastrointestinal tract containing nervous structures in patients with PD and controls, and to discuss their possible biological relevance and diagnostic utility.

2. Material and methods

Samples were obtained from the archives of the Pathology Department of the Hospital Clinic in Barcelona, Spain. Gastrointestinal surgical specimens from 6 PD patients (age at surgery: range 55–84 years, median 68 years; disease duration: range 2–26 years) were available. Control material comprised gastrointestinal surgical specimens from 6 patients with inflammatory bowel disease and from 6 patients with colon cancer (samples were taken 2.5 cm away from the tumour) without known neurodegenerative disease (range age at surgery: 21–83 years, median 56.5 years). The male: female ratio was 2. Fixation time of specimens in 4% buffered formaldehyde ranged from less than 24 h to 6 days (median 72 h). Five micrometer-thick sections were cut from formalin-fixed and paraffin-embedded tissue blocks. Tissue specimens included stomach, colon and small bowel (jejunum and ileum) (Table 1). The most appropriate tissue blocks containing representative areas of submucosal and myenteric plexus were selected on haematoxylin and eosin stained sections. Immunohistochemistry was performed on an automated immunostainer (Dako Autostainer Plus and Autostainer Link 48) using four different primary antibodies directed against full-length, restricted epitope and posttranslationally modified (phosphorylated, nitrated/oxidized) alpha-synuclein, and different pretreatment conditions (Table 2). Detection of immunostaining was performed using the EnVision+ System-HRP labelled Polymer (DAKO, Code K4001) and diaminobenzidine was used as chromogen. Control of antibody specificity included omission of the primary antibody or preabsorption of the antibody (KM51) with full length recombinant alpha synuclein (rPeptide Cat.# S-1001-2). No immunoreaction was observed after these procedures.

Assessment of α-syn immunoreactivity (absence, presence and immunostaining pattern) was performed initially independently by three of the authors (EG, IA, and MC) who were blinded to clinical diagnosis and was followed by a joint discussion at a multiheaded microscope. The study was performed in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

3. Results

The following α-syn immunoreactivity patterns were identified in neuronal structures of the ENS of the specimens studied: a fine diffuse synaptic-type staining (Fig. 1A1, B1, C1 and A4), coarse aggregates in neuronal perikarya and neurites (Fig. 1D1–D3, C3), and a distinct punctate cytoplasmic staining of ganglion cells (Fig. 1B2 and C2).

The first, fine diffuse synaptic-type pattern was detected by 15G7 and anti-nα-syn antibodies, and was observed in all control and PD cases. This staining pattern was reminiscent of a physiological staining of neural structures, as virtually all neuronal perikarya and thick nerve fibres of myenteric and submucous plexus were diffusely stained.

The second pattern was in form of coarse aggregates in neuronal perikarya and cell processes (neurites), and was mainly observed in the myenteric plexus. These aggregates consisted of compact cytoplasmic inclusions and thickened, tortuous neurites. These aggregates were detected by the four antibodies and were found in 4/6 PD patients and in 1/12 controls at different biopsy sites.

The third pattern was a distinct punctate cytoplasmic staining of ganglion cells, which was only detected by anti-pα-syn immunohistochemistry. This pattern was observed in 3/6 PD patients (2 of them with coarse aggregates), and also in 3/12 control cases, none of them with coexistent coarse aggregates. This pattern was also mainly observed in the myenteric plexus at different GI levels, was not related with age and did not overlap with PAS+ lipopigment granules.

The site of the biopsy did not influence the pattern of staining. The detection of coarse α-syn aggregates was in general easier in the myenteric plexus. In the submucosa, they were inconspicuous and were encountered mainly in form of positive neurites,