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The gastric wall in systemic sclerosis patients: a morphological study

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Summary

Organ failure secondary to fibrosis is the main cause of morbidity and death in patients with systemic sclerosis. Gastrointestinal tract dysmotility is a major visceral manifestation, clinically ranging from an asymptomatic form to severe paresis. Although the oesophagus is the most frequently affected part of the gastrointestinal tract, all other segments can be involved. The present study was undertaken to evaluate the histopathological changes of the gastric wall in a series of full-thickness biopsies from systemic sclerosis patients who underwent gastric surgery due to severe gastroesophageal involvement. Gastric biopsies were processed for light microscopy and transmission electron microscopy. The histological and ultrastructural observations revealed a generalized fibrosis affecting all the gastric wall layers. The most severe changes were observed in the muscularis mucosae and muscle layers. Wide areas of marked focal fibrosis with dense collagen bundles and elastic fibre deposition surrounding smooth muscle cells were found. Myofilaments and thickened dense bodies were severely disarranged or absent in most smooth muscle cells. Nerve fibres showed ultrastructural alterations, such as oedematous axoplasm and scarce cytoskeletal elements. Abundant elastic and collagen fibres enveloped nerve fibres, nerve endings and interstitial cells of Cajal, thereby separating them from smooth muscle cells and blood microvessels. This study provides evidence for a prominent fibrosis and severe ultrastructural alterations of smooth muscle cells and nerve fibres as the main histopathological hallmarks in the gastric wall of systemic sclerosis patients.

Key words

Systemic sclerosis, scleroderma, gastric wall, fibrosis, enteric nervous system, transmission electron microscopy

Introduction

Systemic sclerosis (SSc, scleroderma) is a multisystemic connective tissue disease characterized by prominent widespread small vessel vasculopathy, autoimmunity and progressive fibrosis of the skin and various internal organs including the lung, heart, kidney, and gastrointestinal tract (Varga and Abraham, 2007).

Gastrointestinal involvement is one of the earliest clinical manifestations appearing almost as early as Raynaud's phenomenon, and is commonly observed in up to 90% of patients with the limited and the diffuse cutaneous forms of SSc (Abu-

Shakra *et al*., 1994; Sjögren, 1996). Although the oesophagus is most frequently implicated, any part of the gastrointestinal tract may be affected and may significantly impact patient quality of life, morbidity and survival (Young *et al*., 1996; Steen and Medsger, 2000; Forbes and Marie, 2008). The gastric manifestations of SSc are a severe bleeding caused by antral vascular ectasia, and, more commonly, an electrophysiological dysfunction that leads mainly to delayed gastric emptying, contributing in turn to gastroesophageal reflux (Sallam *et al*., 2006; Forbes and Marie, 2008). Most of the the gastrointestinal complications originate from decreased gastrointestinal motility and include oesophageal dysmotility and reflux oesophagitis, gastroparesis, intestinal pseudoobstruction, diarrhoea/constipation and fecal incontinence (Young *et al*., 1996).

Gastrointestinal dysmotility in SSc is hypothesized to result from a primary vascular injury or neurogenic/myogenic dysfunction evolving into smooth muscle cell atrophy and replacement by collagenous fibrosis (Sjögren, 1996; Sallam *et al*., 2006). However, the small number of histopathological studies performed up to now could not clarify the pathogenetic mechanisms leading to the complex spectrum of SSc gastrointestinal symptoms (Roberts *et al*., 2006; Manetti *et al*., 2007; Manetti *et al*., 2008). In particular, a few ultrastructural examinations of the oesophageal and rectal wall have been made (Russell *et al*., 1982; Malandrini *et al*., 2000), and only one ultrastructural study of the muscle coat of the stomach in a case of SSc has been performed by our research group (Ibba-Manneschi *et al*., 2002).

In the present study, we reexamined the histopathological changes of the gastric wall in a larger series of SSc cases with significant gastroesophageal involvement using histochemistry and transmission electron microscopy (TEM).

Materials and Methods

SSc gastric biopsy samples, histochemistry and TEM

Full-thickness biopsy samples of the gastric anterior wall, near the greater curvature, were obtained from the fundus, corpus and/or antrum of three SSc patients (all female) with severe gastroesophageal involvement who underwent surgery (total gastrectomy or antrectomy with Roux-en-Y anastomosis). Two patients had the diffuse cutaneous form and one patient had the limited cutaneous form of SSc according to the classification of LeRoy *et al*. (LeRoy *et al*., 1988). Biopsies were taken with full informed consent and the study was approved by the Institutional Review Board. Each gastric biopsy was divided into two specimens and processed for light microscopy and TEM, respectively.

For histochemical analysis, the specimens were fixed in 10% buffered formalin, dehydrated in a graded alcohol series, and embedded in paraffin. Additional paraffin embedded full-thickness gastric blocks from 4 SSc patients (1 male, 3 female) were obtained by reviewing the pathological records at the Careggi Hospital, Florence, using the keywords ''scleroderma'' and ''systemic sclerosis''. Cases were included in the analysis if the review of the medical record identified clinical features that satisfied the criteria of LeRoy *et al*. (1988) for a diagnosis of SSc, and were all diffuse cutaneous SSc. Sections (5 μm thick) were stained either with haematoxylin and eosin or

with Masson's trichrome to evaluate histopathologic changes and tissue fibrosis. The sections were observed under a light microscope (Eclipse E400, Nikon, Tokyo, Japan) and photographed by digital camera (Coolpix 2500, Nikon).

For TEM, biopsies were immediately fixed in cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) at room temperature. The specimens were routinely processed and embedded in epoxy resin (Epon 812). Semithin sections (2 μm) were cut and stained with toluidine blue-sodium tetraborate and observed under a light microscope. Ultrathin sections (≈70 nm) were obtained from the pathological areas chosen after observation by light microscopy and stained with uranyl acetate and alkaline bismuth subnitrate for examination under a transmission electron microscope (Jeol 1010, Jeol, Tokyo, Japan).

Results

Light microscopy

In the stomach of SSc patients, Masson's trichrome staining revealed an accumulation of collagen in the lamina propria that became more severe in the lower layers of the gastric wall (figure 1A). The muscularis mucosae was thickened and showed dense and irregularly distibuted collagen bundles (figure 1A). In the submucosa, a prominent fibrosis around small blood vessels and ganglia was observed. In the circular and longitudinal muscle layers, wide areas of marked focal fibrosis surrounded smooth muscle cells (SMCs) (figure 1B). The involvement of the muscle layers showed a patchy distribution, with areas of either atrophic or normal smooth muscle fibres in the same tissue section. In the affected areas, SMCs were either hypercontracted or stretched and showed signs of degeneration (figure 1C). The ganglia of myenteric plexus were enveloped by dense collagen bundles and showed oedematous neurons and nerve fibres (figure 1D).

Transmission electron microscopy

At TEM, in the muscle layers of SSc gastric wall wide areas of marked focal fibrosis, characterized by collagen and elastic fibre depositions, were seen surrounding SMCs and widening the intercellular spaces (figure 1E). Considerable amounts of elastin were often found in the invaginations of SMC membrane (figure 1E). In the majority of SMCs, myofilaments and thickened dense bodies were severely disarranged or absent (figure 1E,F). Moreover, cytoplasmic vacuolization and swollen mitochondria were often observed in SMCs (figure 1E).

In the myenteric plexus, the axoplasm of nerve fibres was pale, oedematous, and scarce in neurotubules and neurofilaments, with occasional swollen mitochondria and lipofuscin bodies (figure 1G). Abundant elastic and collagen fibres enveloped nerve fibres, nerve endings and interstitial cells of Cajal, thereby separating them from SMCs and blood microvessels (figure 1G,H).

In the gastric muscle layers, blood capillary vessels showed preserved endothelial cells, while the perivascular basement membrane was occasionally thickened and delaminated (figure 1H). The lumen of several microvessels was partially or com-

pletely occluded by erythrocytes and neutrophils (figure 1H). Neutrophils extravasation was also observed. Mast cells, rich in granules or partially degranulated, were present around vessels, nerve fibres and SMCs (figure 1H).

Discussion

Organ failure secondary to fibrosis is the main cause of morbidity and death in patients with SSc (LeRoy *et al*., 1988; Steen and Medsger, 2000). Gastrointestinal tract dysmotility is a major visceral manifestation, clinically ranging from an asymptomatic form to severe paresis (Sallam *et al*., 2006). To our knowledge, many clinical studies have evaluated the SSc-related gastrointestinal manifestations (Forbes and Marie, 2008). However, only a few morphological analyses have been performed on the digestive system in SSc patients, and these were mainly on the oesophageal and rectal walls (Russell *et al*., 1982; Malandrini *et al*., 2000). In fact, the main limitation to perform such histopathological studies is the poor availability of gastrointestinal samples from SSc patients.

In the present study, we analyzed the morphology of the gastric wall in a series of full-thickness biopsies from SSc patients who underwent gastric surgery because of severe and refractory gastroesophageal reflux and gastroparesis. Our histological and ultrastructural observations revealed the presence of a generalized fibrosis affecting all the gastric wall layers. These findings are consistent with those of previous morphological studies on the gastrointestinal tract of SSc patients (Sjögren, 1996; Jaovisidha *et al*., 2005; Manetti *et al*., 2007), as well as with those of a recent study documenting a significant thickening of the walls of the upper gastrointestinal tract detected by endoscopic ultrasound (Zuber-Jerger *et al*., 2010). In our samples the most severe changes were observed in the muscularis mucosae and muscle layers. Indeed, wide areas of marked focal fibrosis with dense collagen bundles surrounding SMCs were found in the stomach of all SSc cases examined. Our ultrastructural analysis clearly showed relevant amounts of elastin in the invaginations of SMC membrane, as well as abundant collagen fibres not assembled in bundles near SMCs. These findings, together with the small number of fibroblasts found in the gastric muscle layers, suggest that elastin and collagen fibres might be actively produced by SMCs that could acquire a myofibroblast-like synthetic phenotype. The prominent fibrosis enveloping SMCs and widening the intercellular spaces, as well as the severe disarrangement or absence of myofilaments and thickened dense bodies may account for the impaired SMC contraction and its propagation from cell to cell.

Moreover, the ultrastructural alterations found in the nerve fibres, such as oedematous axoplasm and scarce cytoskeletal elements, suggest that the axonal transport and the electric transmembrane transmission may be impaired in SSc gastric muscle layers. Interestingly, these peripheral nervous system lesions are similar to those previously described in the skin of SSc patients (Ibba-Manneschi *et al*., 2005). In addition, the relevant deposition of collagen bundles separating nerve endings and interstitial cells of Cajal from SMCs may contribute to the significant gastric electrophysiological dysfunction and dysmotility observed in the clinics (Forbes and Marie, 2008).

Different theories have proposed for the gastrointestinal involvement: vascular damage, with concomitant or subsequent neurogenic abnormalities, or primary auto-

nomic dysfunction as initiating event in the pathogenesis of SSc gastrointestinal disease (Sallam *et al*., 2006). Our findings provide evidence for a prominent fibrosis and severe ultrastructural alterations of SMCs and nerve fibres as the main histopathological hallmarks in SSc gastric wall, while signs of vascular damage were only occasionally observed. However, further morphological studies on the stomach of SSc patients in early disease stage will be necessary to understand the sequence of pathological events, and, in particular, whether SMC alterations are primary or secondary to nerve fibre disarrangement and the fibrotic process.

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Figures

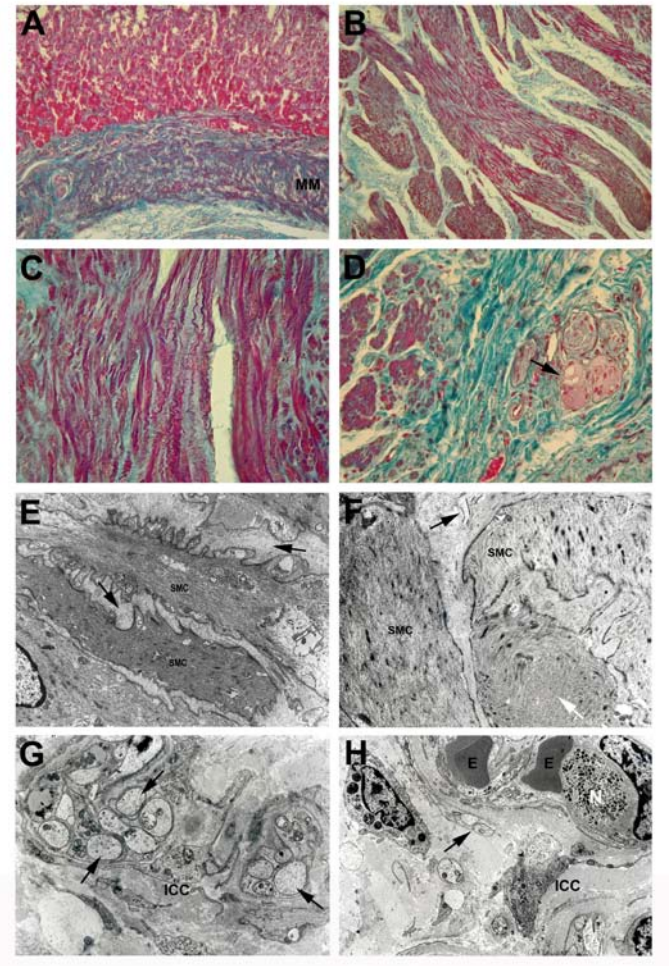

Figure 1 – Histochemical and ultrastructural analyses of the gastric wall in SSc patients. **A-D:** Masson's trichrome staining. **A:** Relevant fibrosis is evident in the lamina propria and muscularis mucosae (MM). **B-C:** In the circular and longitudinal muscle layers, wide areas of marked focal fibrosis surround smooth muscle cells. Note the disarranged and contracted muscle fibres. **D:** Ganglia of myenteric plexus are enveloped by dense collagen bundles and show oedematous neurons and nerve fibres (arrow). **E-H:** Ultrastructure of the muscle layers. **E:** Abundant collagen and elastic fibre deposition surrounds smooth muscle cells (SMCs). SMCs show cytoplasmic vacuolization, swollen mitochondria, and considerable amounts of elastin in the membrane invaginations (arrows). **F:** Myofilaments and dense bodies are severely disarranged (white arrow). Note the abundant collagen fibres not assembled in bundles near SMCs (black arrow). **G:** Myenteric plexus. The axoplasm of several nerve fibres appears oedematous and poor in neurotubules and neurofilaments (arrows). **H:** Abundant elastic and collagen fibres envelop nerve fibres (arrow), interstitial cells of Cajal (ICC) and blood microvessels. A blood vessel shows the lumen completely occluded by erythrocytes (E) and a neutrophil (N). A mast cell with granules is evident near a blood vessel. Original magnification: (A,B) x20; (C,D) x40; (E) x3000; (F,H) x5000; (G) x8000.