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Smooth muscle contraction bands in intestinal infarction

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Summary. We studied microscopic sections of 24 cases of intestinal infarction looking for contraction bands (CB) in the muscularis propria of the bowel wall. Controls were 11 surgical and 11 autopsy cases from patients who did not suffer any form of ischaemic disease. The difference of CB frequency was significant ($P<0.001$) in infarction versus the surgical control group. Moreover, within the study group the CB frequency was also related to the severity of the ischaemic lesion ($P<0.01$). With immunostaining, CB were not reactive with antibodies against vimentin, desmin, actin or myosin. We propose that CB genesis in intestinal smooth muscle is related to hypoxia, possibly through altered homeostasis of calcium and catecholamine metabolism.

Key words: Contraction bands – Smooth muscle – Intestinal infarction – Ischaemic damage – Immunohistochemistry

Introduction

The pathology of intestinal ischaemia in man is commonly described in terms of changes affecting the mucosa and submucosa (Fenoglio-Preiser et al. 1989; Norris 1990; Whitehead 1976; Williams 1971). Although it is generally accepted that, with increasing severity of ischaemia, the full thickness of bowel wall may be involved by necrosis, structural changes in the muscular layer in the early stages of the tissue damage have received scant attention. In extensive reports on the subject, smooth muscle is often not described or is considered to be singularly resistant to ischaemic insult (Whitehead 1976). In an experimental study Ming and McNiff (1976) have examined the morphological alterations in the mouse intestinal muscularis in ischaemic conditions obtained by ligation of mesenteric arteries. They were able to observe an early change consisting of typical dense bands in smooth muscle cells. Similar lesions in human myocardium have been called contraction bands (CB) and have widely been studied in a variety of clinical and experimental conditions in many of which ischaemia may have played a significant role (Freifeld et al. 1983; Karch and Billingham 1986; Reichenbach and Benditt 1970; Sommers and Jennings 1964). More recently Salinas-Madrigal et al. (1987) reported the occurrence of CB in smooth muscles in various clinical conditions associated with visceral ischaemia and showed that these changes are neither artefacts nor are they induced by autolysis. We have undertaken this study in order to search for the presence of CB in surgical specimens of intestinal infarction, attempting to correlate its occurrence with the severity of ischaemic damage and with some pathogenetic models. Finally the composition of CB with regard to their content of intermediate filaments (vimentin and desmin) and in contractile proteins, (actin and myosin) has been investigated immunohistochemically.

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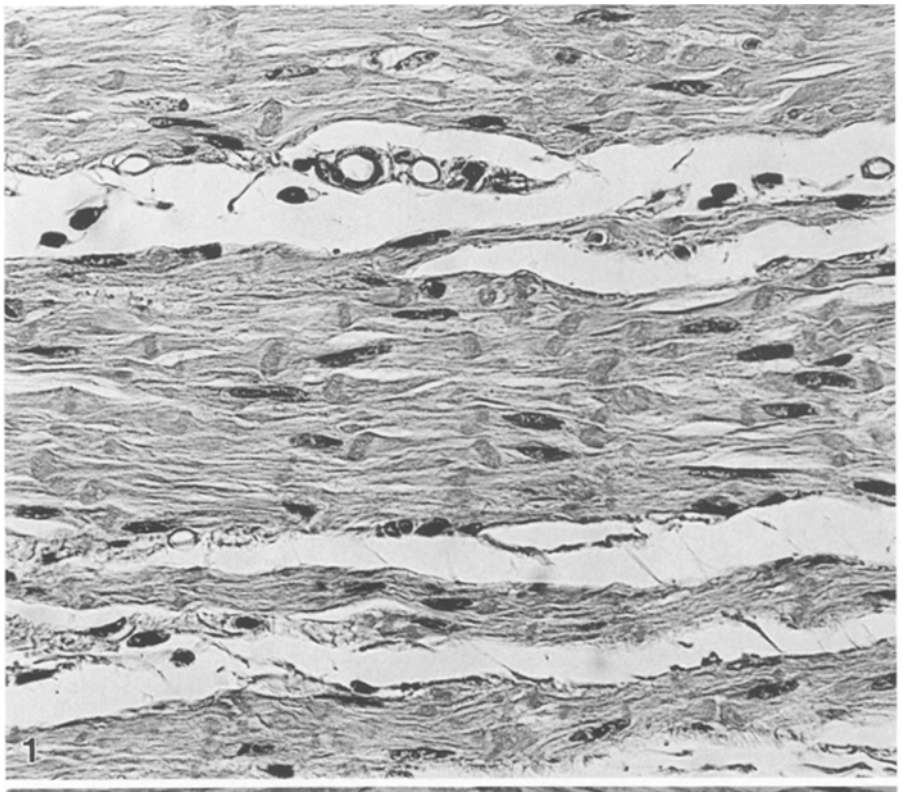
Materials and methods

We reviewed the major clinical and pathological features of 26 consecutive surgical cases of intestinal infarction. There were 16 men and 10 women with an average age of 68 years. Abdominal pain, vomiting and/or bloody diarrhoea were most frequent symptoms. All patients were treated by small bowel resection, ranging from 20 to 130 cm in length.

According to the classification proposed by Swerdlow et al. (1981) the lesions were labelled as mucosal (10 cases), mural (4 cases) and transmural infarctions (12 cases).

In order to evaluate the occurrence of CB in bowel wall in clinical conditions unrelated to direct evidence of intestinal ischaemia, we examined the small bowel of 11 right hemicolectomies for carcinoma and 11 autopsies performed on 2 still-births, 2 severely immature newborns, 2 ischaemic cardiopathies, 1 myocarditis in von Recklinghausen's disease, 1 traumatic shock and on 3 neoplastic diseases (1 meningioma of the base of the skull, 1 renal adenocarcinoma and 1 large bowel non-Hodgkin's malignant lymphoma). These surgical and autopsy control groups were selected excluding any case with clinical or pathological signs of mucosal or submucosal intestinal ischaemic damage.



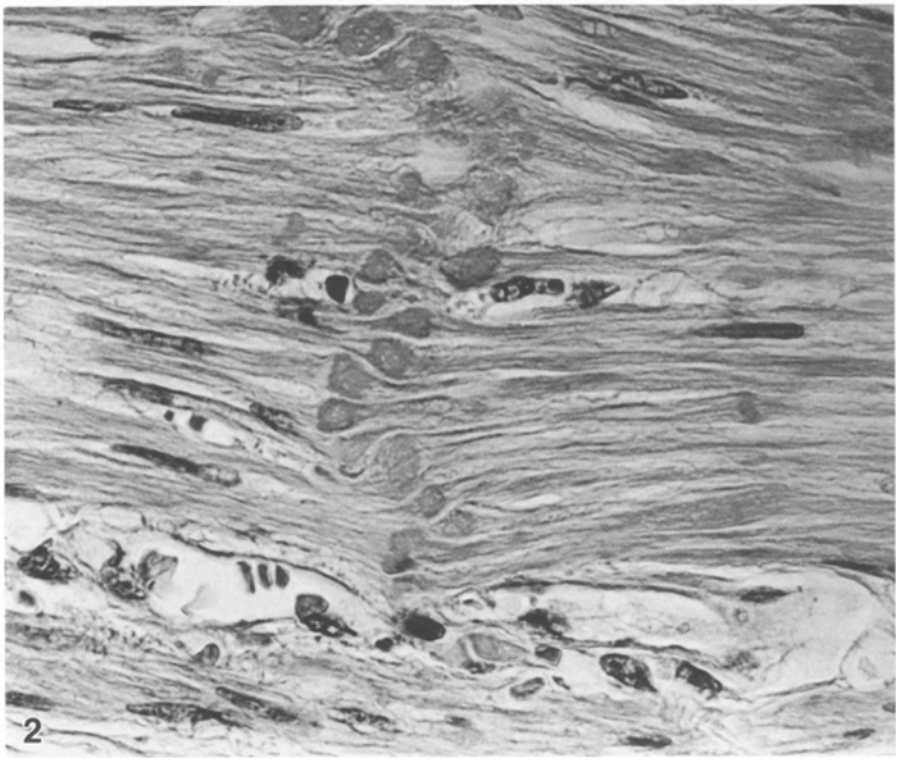


Fig. 1. Smooth muscle in intestinal infarction. Contraction bands appear as transverse, homogeneous structures in the cytoplasm of muscle fibres. H & E, ×250

Fig. 2. Contraction bands appear as nodules arranged to form a continuous row in contiguous cells. H & E, ×400

All study material was fixed in 10% buffered formalin and paraffin embedded. From each case of intestinal infarction, 2 to 10 representative samples have been processed.

Material from surgical and autopsy controls was taken so that from 2 to 4 and from 2 to 6 samples were examined, respectively. CB were sought in areas with preserved nuclei, adjacent to areas of fully developed necrosis in the muscularis propria. Two transmural infarctions were excluded because total bowel necrosis without any viable tissue was present. CB were counted with a 40× objective in ten consecutive fields in order to scrutinize about 1000 muscle cells in every slide, and in every infarction the frequency of CB was graded as: 0, absent; +, 1–9% of smooth muscle cells with CB; ++, 10–29%; +++, 30–100%.

Immunohistochemical study was performed with monoclonal antibodies to muscle specific actin (Ortho, Milan, Italy), vimentin (Dako, Milan, Italy) and desmin (Dako) at final dilution of 1:5000,

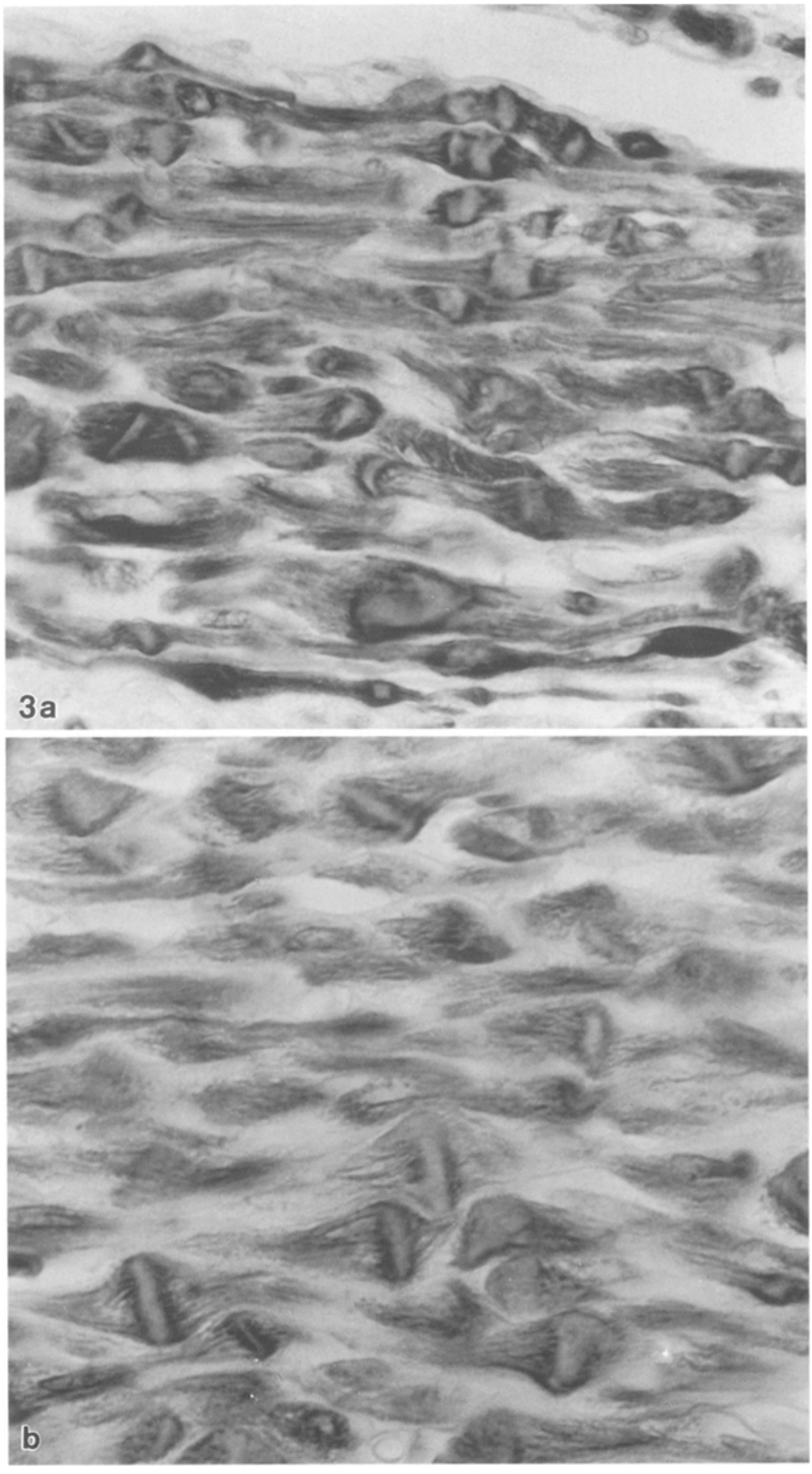


Fig. 3a, b. Immunoperoxidase staining. Contraction bands are unreactive to actin (**a**, ×250) and desmin (**b**, ×800)

1:10 and 1:50 respectively, using the peroxidase-antiperoxidase complex staining method (Sternberger et al. 1970) and with polyclonal antibodies for myosin (BioGenex, Milan, Italy) already prediluted, according to the avidin-biotin-peroxidase complex staining technique (Hsu et al. 1981).

Statistical analysis was performed using the chi-square method.

Results

The major lesion observed in intestinal infarction is coagulative necrosis, variably associated with inflammatory polymorphonuclear response, oedema, congestion and

Table 1. Distribution, frequency and grade of contraction bands

Intestinal infarctions				Controls	
Grade	Mucosal	Mural	Transmural	Surgical	Autopsy
+	3	3	3	4	2
++	1	1	4	0	3
+++	0	0	3	0	6
Total	4/10	4/4	10/10	4/11	11/11
%	40%	100%	100%	36.4%	100%

+, 1–9% of smooth muscle cells with contraction bands; ++, 10–29%; +++, 30–100%

haemorrhage in the wall. CB were evident in 40% of mucosal and in 100% of both mural and transmural infarctions, yielding 75% positivity (18/24) in the whole group (Table 1). They were identified as homogeneous, eosinophilic bands or nodules, transversely arranged along the longitudinal axis of the muscle fibres (Figs. 1, 2). The cellular diameter at the CB level was often increased. In transverse sections CB appeared as oval to round homogeneous hyaline disks, occupying the whole cytoplasmic area. Frequently CB were observed on the same circumferential plane, in cells close one to the other, giving a rhythmic image of parallelism (Fig. 2).

Immunohistochemically CB were non-reactive with all antisera employed (Fig. 3), whereas the adjacent cytoplasm stained with actin, myosin, vimentin and desmin.

In the surgical ileocolectomy control group CB were observed in 4 of 11 cases (36.3%). The lesions were minimal and their distribution was sparse (+) (Table 1). In the autopsy control group, in contrast, CB were present in all cases with a distribution pattern more evident and diffuse (+, 18%; ++, 27%; +++, 54%) (Table 1).

Analysis of CB frequency in study group versus the surgical control group showed a statistical significance of $P<0.001$. The same significance ($P<0.001$) has been observed between autopsy and surgical control groups.

Looking for differences between subgroups (i.e. mucosal, mural and transmural infarctions) in the study cases was unrewarding; we did not observe any statistical difference between CB frequency in mucosal versus mural infarctions. In contrast, the frequency of non-transmural (mucosal, plus mural) versus transmural infarctions revealed a minor but significant difference ($P<0.01$).

Discussion

Intestinal infarction is a severe pathological condition resulting from ischaemic damage. Many factors are responsible for the morphological alterations observed, of which the most important is the different sensitivity to anoxia of the various tissues of the intestinal wall. Although the bowel muscle has been regarded as one of the more resistant tissues to ischaemic insult (Whitehead 1976), in this study we have observed a large number of muscle cells with lesions that show CB in the majority of infarctions.

First described in myocardium, CB are regarded as a morphological marker of ischaemic damage (Freifeld et al. 1983; Reichenbach and Benditt 1970; Sommers and Jennings 1964). They are the result of hypercontraction and disruption of the orderly arrangement of myofilaments (Baroldi 1988; Karch and Billingham 1986). At the microscopic level this modification consists in a transverse cytoplasmic eosinophilic band. The presence of similar lesions in the smooth muscle of the great majority of our cases of intestinal infarction suggests the existence of a strong relationship between CB of smooth muscle and ischaemia. The frequency of CB was significantly greater in the infarction group than in the surgical control group ($P<0.001$). Moreover in the infarction group there was a significant difference in the frequency of CB in relation to the severity of the ischaemic damage, evaluated as non-transmural versus transmural infarction ($P<0.01$). Such a result, suggests a common pathogenetic pathway for both infarction and CB.

The tissue damage in ischaemia has been conventionally ascribed to ischaemia itself. Recent studies, however, have suggested a major role for the superoxide radical in ischaemic damage, generated during tissue reperfusion through the activity of the xanthine-oxidase enzyme (Bulkley 1987; Granger 1988; McCord 1985; Parks et al. 1982). The superoxide radical is an unstable and cytotoxic form of molecular oxygen able to alter endothelial permeability, with subsequent necrosis, by peroxidation of cellular membrane lipids (Granger 1988). Nevertheless the possibility that CB phenomenon is a reperfusion lesion induced by superoxide radical is not very likely, for different reasons. First, xanthine-oxidase activity is mainly located at mucosal layer (Granger 1988). It is therefore difficult to maintain the hypothesis that the by-products of the reaction are responsible for a lesion in the muscular layer. Second, CB have been observed, even if minimally and sparsely, in the surgical control group, where such a dramatic biochemical event, in the absence of any other lesion consistent with an ischaemic injury, appears very unlikely. Third, since CB can develop in the absence of circulation in incubated tissue (Ming and McNiff 1976), reperfusion injury do not seems mandatory in the pathogenesis of the lesion.

CB in myocardium have been found in different clinical and experimental settings, often but not always associated with ischaemia. Karch and Billingham (1986) suggest unifying pathogenetic concept: that an increased intracellular calcium level induced by catecholamines could produce CB through an exaggerated coupling of excitation contraction mechanisms (Milei et al. 1978). Actually a modification of intracellular calcium homeostasis seems to be involved in the pathogenesis of ischaemic damage (Cheung et al. 1986). Furthermore, an increased release of noradrenaline, responsible for long-lasting vasoconstriction, is even involved in the pathogenesis of intestinal ischaemic necrosis (Caplan and Hsueh 1990; Cueva and Hsueh 1988; Hsueh et al. 1988). This scheme is in keeping with the presence of CB in all autopsy group cases of the present study. In this group it is very likely that death has been preceded by an episode of splanchnic hypoperfusion with local release

of catecholamines and it is also possible that an increase of systemic circulating catecholamines has taken place after cardiac arrest (Worstman et al. 1984).

The occurrence of sparse CB in our non-infarction surgical group is possibly explained via a short hypoperfusion time due to anaesthetic drugs (Fenoglio-Preiser et al. 1989; Salinas-Madrigal et al. 1987) or to the surgical clamping of the mesenteric vessels, resulting in intestinal hypoxia. The finding of CB in this group, in which a complete return to normal appearance is expected, suggests that CB in many instances is a transitory or reversible lesion (Reichenbach and Benditt 1970).

In conclusion, our study shows a significant correlation between CB and intestinal infarction, with a major significance in the more severe ischaemic lesions. Our data are in keeping with the hypothesis that CB are lesions related to tissue hypoxia. If their pathogenesis is due to an ischaemic event, CB may be accounted an early and sensitive marker of tissue hypoxia in many clinical conditions, other than overt infarction. We cannot exclude that CB, in intestinal smooth muscle as in the myocardium (Karch and Billingham 1986), is a morphological expression of a generic physiopathological response associated with, but not exclusively induced by, hypoxia.

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