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Changes in neurons, neuroendocrine cells and nerve fibres in the lamina propria of irradiated bowel

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Summary. Damage to bowel often complicates radiotherapy for abdominal and pelvic malignancy. The symptoms of chronic irradiation enteropathy, which often include intractable diarrhoea, are generally attributed to vascular injury. We have examined specimens of bowel resected from patients who had been therapeutically irradiated to assess the extent of injury to the enteric nerve plexuses. To facilitate visualisation of nerve fibres and cells of neural or neuroendocrine origin, sections were immunostained with antibodies to neuron-specific enolase or PGP 9.5, widely used markers of nerves and neurons. Electron microscopy was performed in selected cases. In 27 out of 33 specimens the number of nerve fibres in the lamina propria was obviously increased compared to that in control material. Scattered cells with the histological, immunohistochemical and ultrastructural features of ganglion cells were noted within the lamina propria in 23 of the specimens, and in 18 cases so-called neuroendocrine cells, not normally seen in this location, were also present. These radiation-induced changes in the innervation of the bowel may contribute to the symptoms of chronic radiation enteropathy.

Key words: Radiation – Bowel – Nerves – Ganglion cells – Neuroendocrine cells

Introduction

Therapeutic irradiation of the abdomen and pelvis for malignant tumours often results in permanent injury to the large or small bowel (Schofield et al. 1983; Weisbrot et al. 1975; Yeoh and Horowitz 1988; Fischer et al. 1989). The most important factor in determining the effect of abdominal irradiation is the degree of fixation of the bowel (Berthrong and Fajardo 1981). The entire duodenum and the upper jejunum are fixed in place at the ligament of Treitz. The terminal ileum is also relatively fixed through its attachments to the immobile caecum. Similarly, the rectosigmoid area of the large bowel is relatively immobile and, because of its close anatomical association with many of the organs commonly irradiated in the pelvis, it is extremely susceptible to damage during the course of therapeutic irradiation.

The early effects of irradiation are rarely seen by the surgical pathologist but the severe chronic reaction often produces intractable symptoms which may be life threatening and require surgical resection (Edington et al. 1988). Most patients develop symptoms 6 months to 5 years after irradiation, but some may present after periods of up to 29 years with diarrhoea (often bloody), crampy abdominal pain with episodes of subacute intestinal obstruction, rectal bleeding and pain, nausea, vomiting and, less commonly, malabsorption.

Histological examination of the irradiated bowel shows extensive fibrosis and obliterative vascular changes (Fajardo and Berthrong 1988) which have led to the conclusion that post-irradiation enteropathy results solely from vascular insufficiency of affected bowel. It has also been noted, though, that the submucosal and myenteric ganglia and nerve fibres are often distorted by post-irradiation fibrosis and ganglion cells, in particular, may appear bizzare (Berthrong and Fajardo 1981). Our preliminary observations of quantitative changes in nerve fibres, ganglion cells and neuroendocrine cells in irradiated bowel have prompted us to carry out a detailed examination of the enteric nerve plexus in sections of resected large and small bowel from 30 patients with symptoms of chronic radiation enteritis.

Materials and methods

Thirty patients who had previously been irradiated for a variety of neoplastic conditions underwent surgical resection of damaged bowel (Table 1). The resections were performed at the Middlesex Hospital between 1976 and 1985. The 30 patients consisted of 8 males and 22 females who ranged in age from 27 to 85 years (mean 55 years). The patients were irradiated for the following

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Table 1. Clinical data of irradiated and control patients

Case	Age (years)	Sex	Clinical diagnosis	Radiation dose (rad)	Interval after last radiation (months)
1	30	F	Carcinoma of cervix	6090	4
2	77	F	Carcinoma of vagina	3865	1
3	69	F	Carcinoma of cervix	7560[a]	83
4	41	F	Carcinoma of cervix	8650	20
5	46	F	Carcinoma of cervix	5570	9
6	71	M	Carcinoma of bladder	6720	19
7	79	M	Oesophageal carcinoma with spinal metastases	5400	Not known
8	66	F	Carcinoma of cervix	4710[a]	7
9	85	M	Carcinoma of bladder	8130	9
10	38	F	Carcinoma of cervix	5760	8
11	46	F	Carcinoma of uterus	5670	21
12	67	F	Carcinoma of cervix	6150	13
13	64	F	Carcinoma of ovary	5280	18
14	37	M	Non-Hodgkin's lymphoma	3050	3
15	64	F	Carcinoma of cervix	5970	19
16	51	F	Carcinoma of cervix	8750	69
17	47	F	Carcinoma of uterus	5500	164
18	27	F	Carcinoma of cervix	Not known[a]	15
19	39	F	Carcinoma of cervix	Not known	41
20	76	M	Carcinoma of prostate	4750	5
21	48	F	Carcinoma of cervix	7400	1
22	61	F	Carcinoma of cervix and ovary	10750	192
23	42	F	Carcinoma of cervix	5590	69
24	63	F	Chondrosarcoma	7000	25
25	51	M	Hodgkin's disease	3900	11
26	65	F	Carcinoma of bladder	5840	55
27	64	M	Carcinoma of prostate	6840	34
28	27	F	Carcinoma of cervix	>5200	Not known
29	54	M	Carcinoma of bladder	6000	112
30	60	F	Carcinoma of vulva	12740	42
31	87	F	Carcinoma of colon	0	
32	79	M	Carcinoma of colon	0	
33	73	M	Carcinoma of colon	0	
34	75	M	Carcinoma of colon	0	
35	76	M	Carcinoma of colon	0	
36	68	F	Colonic polyp	0	
37	12	M	Investigation of stunted growth	0	
38	68	F	Investigation of anorexia	0	
39	70	M	Investigation of malabsorption	0	

[a] Received intracavitary irradiation

conditions: carcinoma of the cervix (15 patients), carcinoma of the vulva (1 patient), carcinoma of the vagina (1 patient), carcinoma of the bladder (4 patients), uterine carcinoma (2 patients), ovarian carcinoma (2 patients), lymphoma (2 patients), prostatic carcinoma (2 patients), chondrosarcoma (1 patient) and oesophageal carcinoma with spinal metastases (1 patient). It should be noted that 1 patient had both ovarian and cervical carcinoma. The patients presented with symptoms of radiation enteropathy 1 month to 16 years after irradiation. Twenty-six of the patients had received only external irradiation, 2 patients with carcinoma of the cervix had been treated with both intracavity and external irradiation, 1 patient with cervical carcinoma had been given intracavitary irradiation alone and in 1 case the route of administration was not documented in the available hospital records. For 26 of the patients, the dose of irradiation ranged from 3050 to 8750 rad. One patient, with carcinoma of the cervix, who subsequently developed a carcinoma of the ovary, received fractionated doses amounting to 10750 rad, and another, with vulval carcinoma received 12740 rad in total. In 2 patients (1 of whom was treated with intracavitary irradiation alone) the dose of irradiation was not documented. The resections were performed between 1 month and 22 years after the initial irradiation. Thirty-three sections of large and small bowel were examined since in some cases both large and small bowel were received from the same patient.

Nine control cases, yielding a total of 12 blocks of small and large bowel, were examined (Table 1). These blocks were from histologically uninvolved resection margins of bowel removed for carcinoma, and from small bowel resected or biopsied for other benign, non-inflammatory conditions. Of the 9 control cases, 6 were males and 3 were females. The mean age was 67 years with a range of 12–87 years.

All of the sections were routinely processed to paraffin wax. Five-micrometre-thick sections were stained with haematoxylin and eosin (H&E) or immunostained with antisera to two neural

antigens, PGP 9.5 and neuron-specific enolase (NSE). NSE is a widely used marker of neurons, nerve fibres and neuroendocrine cells. PGP 9.5 (Rode et al. 1985) is a marker with similar specificity to that of NSE. The secondary antibody was conjugated with alkaline phosphatase, which produced a red reaction product. In 1 or 2 cases sections were stained with lead-haematoxylin to identify the cytoplasmic granules of neuroendocrine cells. Electron microscopy was performed in selected cases.

Results

All of the sections of bowel examined showed the typical features of subacute or chronic radiation changes. These included hyaline thickening of the walls of medium-sized and large blood vessels, atrophy and fibrosis of the muscularis propria, bizarre fibroblasts and ganglion cells in the submucosa, telangiectasis of blood vessels in the lamina propria and occasional mucosal ulcers. In H&E-stained sections, large cells having the morphological appearance of ganglion cells were identified in the lamina propria; this was still clearly demarcated from the submucosa by the muscularis mucosae. The most common change, in 27 of the 33 sections, was a moderate to severe degree of nerve fibre hyperplasia in the lamina propria of both large and small bowel (Table 2). These changes were noted in the H&E sections but were much more easily seen in the sections immunostained for PGP 9.5 and NSE (Figs. 1A, B, 2A).

Table 2. Summary of histological findings

Case	Specimen	Lamina propria nerve fibres	Lamina propria ganglion cells	Lamina propria neuroendocrine cells
1	Large bowel	++[a]	+	+
2	Large bowel	+++	++	+
3	Large bowel	+++	+	0
4	Large bowel	+++	+	0
	Small bowel	+++	++	+
5	Small bowel	+	0	0
6	Large bowel	++	0	0
7	Large bowel	++	++	+
	Small bowel	++	++	+
8	Large bowel	+	+	0
	Small bowel	+++	+	+
9	Large bowel	++	+	+
10	Large bowel	+++	0	0
11	Small bowel	+++	++	+
12	Large bowel	+	0	0
13	Large bowel	+++	+	+
14	Large bowel	+	0	0
15	Large bowel	+++	++	+
16	Large bowel	+++	+	+
17	Large bowel	+++	++	+
18	Small bowel	+++	++	+
19	Large bowel	++	0	0
20	Large bowel	+	0	0
21	Large bowel	+++	+	+
22	Small bowel	++	0	0
23	Large bowel	+	0	0
24	Small bowel	+++	++	+
25	Small bowel	++	+	0
26	Small bowel	+++	+	+
27	Small bowel	+++	+	+
28	Large bowel	++	+	+
29	Large bowel	++	+	0
30	Large bowel	++	0	0
31	Large bowel	++	+	0
32	Large bowel	+	0	0
	Small bowel	+	+	0
33	Large bowel	+	0	0
	Small bowel	+	+	0
34	Large bowel	+	0	0
	Small bowel	+	0	0
35	Large bowel	++	0	0
36	Large bowel	++	+	0
37	Small bowel	+	0	0
38	Small bowel	+	0	0
39	Small bowel	+	0	0

[a] 0 none; + occasional; ++ moderate numbers; +++ numerous

Most of the hyperplastic nerve fibres aggregated near the muscularis mucosae, but in the more severe cases the fibres extended into the lamina propria towards the mucosal surface. In these cases the density of the nerve fibres in the lamina propria was very similar to that adjacent to the muscularis mucosae.

Immunohistochemical staining confirmed that the large ganglion-like cells noted in the H&E-stained sections were labelled by the two neural antibodies (Fig. 2B). In addition, several clusters of smaller neuroendocrine cells (NEC) were noted in the lamina propria in some of the sections of irradiated bowel (Fig. 2C).

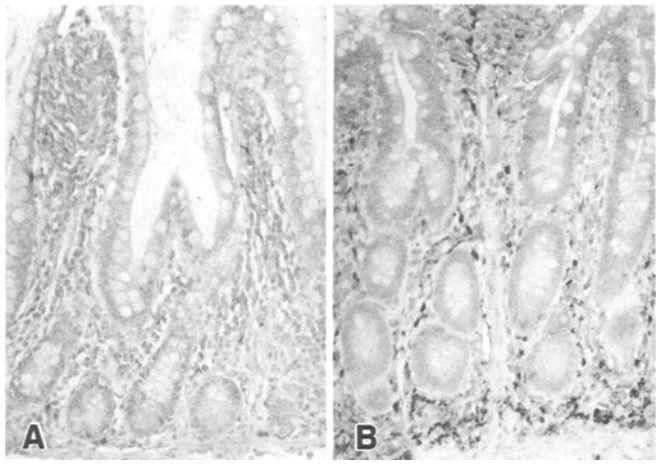

Fig. 1. Sections of control (**A**) and irradiated (**B**) small intestine immunostained with anti-PGP 9.5. There is marked hyperplasia of nerve fibres in the lamina propria of the irradiated bowel, ×110

In only 4 of the 12 large and small bowel control cases was an occasional ganglion cell identified in the lamina propria (Table 2). Neither typical nor atypical NEC were identified and where ganglion cells were noted they were very sparsely distributed and found with difficulty. In the irradiated bowel sections, however, ganglion cells, and NEC were more numerous and identified more easily. Ganglion cells were seen in the lamina propria in 23 of 33, and NEC in 18 of 33 irradiated specimens (Table 2). In general, cases with more severe degrees of nerve fibre hyperplasia had more ganglion cells and NEC in the lamina propria.

Electron microscopy also showed large cells in the lamina propria with vesicular, eccentric nuclei and prominent nucleoli having the typical ultrastructural features of ganglion cells (Fig. 2D). The cytoplasm contained abundant rough endoplasmic reticulin, and in some cases lipofuscin. Occasional swollen, synaptic terminals were also identified. Smaller neurosecretory cells with prominent dense cored vesicles were also identified.

An attempt was made to correlate several clinical parameters with the histological and immunohistochem-

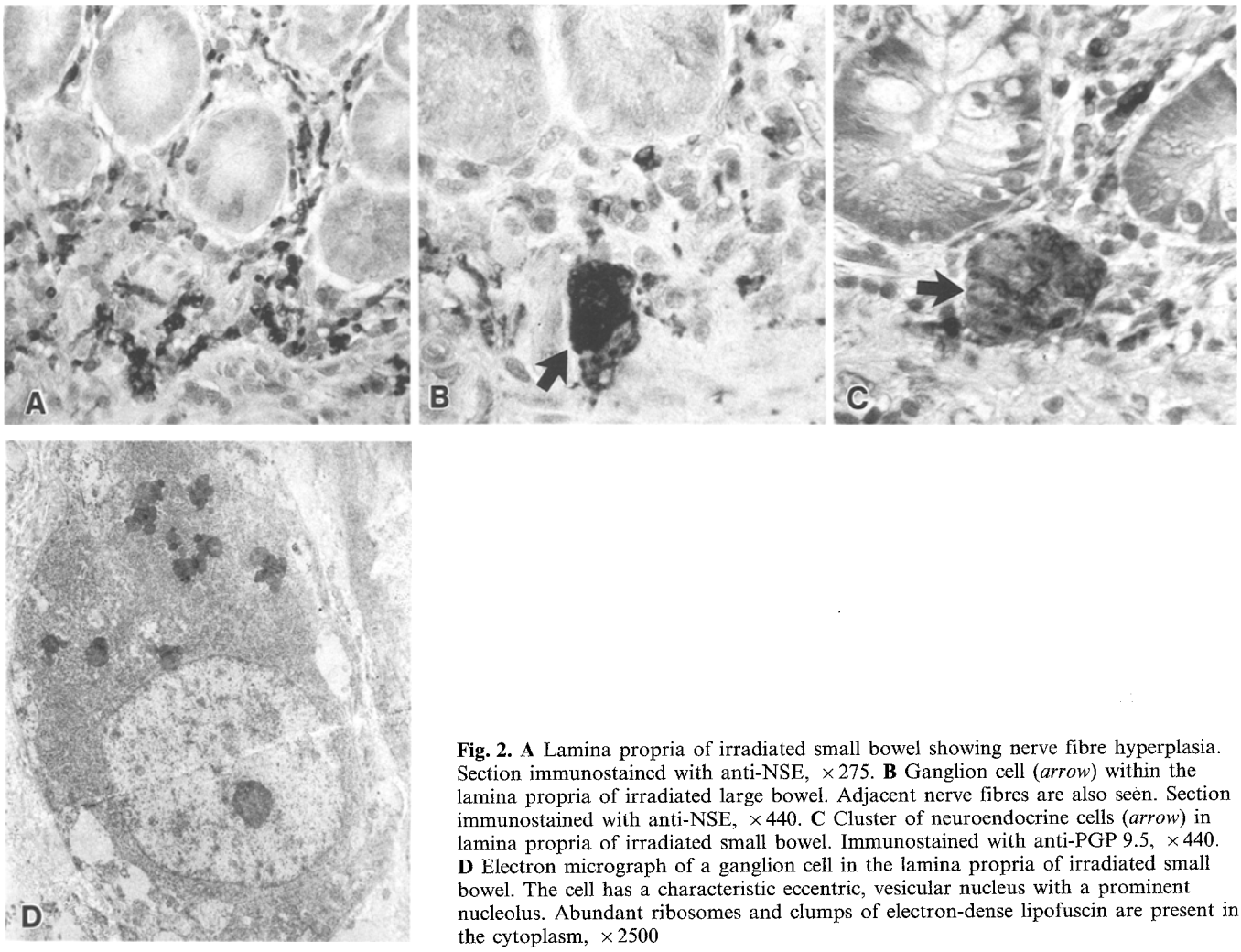

Fig. 2. A Lamina propria of irradiated small bowel showing nerve fibre hyperplasia. Section immunostained with anti-NSE, ×275. **B** Ganglion cell (*arrow*) within the lamina propria of irradiated large bowel. Adjacent nerve fibres are also seen. Section immunostained with anti-NSE, ×440. **C** Cluster of neuroendocrine cells (*arrow*) in lamina propria of irradiated small bowel. Immunostained with anti-PGP 9.5, ×440. **D** Electron micrograph of a ganglion cell in the lamina propria of irradiated small bowel. The cell has a characteristic eccentric, vesicular nucleus with a prominent nucleolus. Abundant ribosomes and clumps of electron-dense lipofuscin are present in the cytoplasm, ×2500

ical changes. There was no discernible correlation between the clinical symptoms, the specific morphologial features, the dose of radiation, and the time interval between irradiation and clinical presentation.

Discussion

Small and large bowel differ slightly with regard to radiosensitivity (Berthrong and Fajardo 1981). Mucosal cell turnover cycles are slightly longer in the large than in the small bowel (4–8 days vs 3–6 days). Individual susceptibility and fractionation schemes also contribute to the degree of radiosensitivity. Enteric injury is uncommon with doses below 4500 rad. In patients who receive in excess of 6000 rad, up to 37% can be expected to present with colonic injury, 10% with rectal injury and 25–50% with small intestinal injury. In many of the patients studied, the total dose of irradiation ranged from 4500 to 5500 rad, but in some cases the dose exceeded 6000 rad and in others the dose was as low as 3050 rad. In spite of this wide range of irradiation exposure all of the patients showed histological features of post-irradiation changes in samples of resected bowel and all presented with signs and symptoms of chronic radiation enteropathy. No correlation was found between the clinical symptoms and either the dose of irradiation received or the time interval between irradiation and clinical presentation.

Both small and large bowel are richly supplied with sympathetic and parasympathetic nerve fibres which innervate the three major plexuses in the bowel wall – the myenteric plexus of Auerbach, located between the two layers of the muscularis propria, and the submucosal plexuses of Meissner (just outside the muscularis mucosae) and Henle (just inside the circular muscle coat) (Lake 1989). The neural plexuses consist of neurons, nerve fibres and supporting Schwann cells. Cholinergic (parasympathetic) neurons play an important role in the co-ordination of peristalsis, whereas noradrenergic (sympathetic) activity causes relaxation of the bowel wall. According to Lake (1989), the finding of neurons in the lamina propria of bowel has been regarded as being abnormal and is said to reflect a disturbance in the orderly migration of neurons. However, Lake observed that occasional neurons may be found, albeit rarely, in bowel biopsies which appear otherwise normal, as in the present study.

Recently several secretory products other than noradrenalin and acetylcholine have been identified within a third group of neurons in the bowel wall plexuses – so-called peptidergic neurons (Gershon and Erde 1981). A variety of biologically active peptides which include vasoactive intestinal polypeptide (VIP), substance P, somatostatin, bombesin and pancreatic polypeptide have been identified by immunocytochemical techniques. Excessive production of VIP, as occurs in the Werner-Morrison syndrome, leads to severe diarrhoea. Aggregates of ganglion cells, NEC and Schwann cells, closely associated with bundles of nerve fibres, were recently described in the lamina propria of the appendix by Papadaki et al. (1983). The function of these lamina propria complexes (LPCs) is not known. Serotonin was identified by immunohistochemical techniques in the cells of appendiceal LPCs (Rode et al. 1983) and it was suggested that release of serotonin by the LPCs may initiate inflammation and produce the pain associated with appendicitis (Rode and Dhillon 1983). Several pathological conditions are known to be associated with alterations in the neuronal populations of the bowel wall (Smith 1970). Hypoganglionosis or aganglionosis may occur as a congenital (e.g. in Hirschprung's disease) or an acquired defect (e.g. in Chaga's disease) which results in an impairment of peristalsis (Gershon and Erde 1981). In chronic ulcerative colitis there is a variable increase in the number of ganglion cells in the myenteric plexus (Smith 1972), which may be related to the increase in intestinal motility that occurs in these patients. A substantial increase in the number of ganglion cells was observed in both the myenteric and submucosal plexuses in patients with Crohn's disease (Bishop et al. 1980). In these patients there was also an increase in the number of nerves and ganglion cells and in the intensity of immunohistochemical staining of both VIP-containing nerve fibres in the mucosa and submucosa of the bowel. Scattered gastric neuroendocrine cells were observed in the lamina propria of the stomach in patients with chronic gastritis (Strachura et al. 1981), but since normal material was not examined it is not known whether NEC are normally present at this site.

In the present study, histological examination of irradiated bowel showed not only the fibrosis and obliterative vascular changes that are widely recognised in irradiated bowel but also increased numbers of nerve fibres, ganglion cells and NEC, not previously described in this condition. The cause of these abnormalities remains speculative. The hyperplasia of nerve fibres may be analagous to that occurring in traumatic neuromas. Bishop et al. (1980) suggested that severe transmural inflammation may stimulate the similar proliferation of ganglion cells noted in Crohn's disease, but it is difficult to reconcile apparent increases in the number of ganglion cells with the concept that neurons are terminally differentiated cells not capable of division. NEC and ganglion cells in the gastrointestinal tract are known to secrete substances which exert marked effects on intestinal motility and mucosal secretions. An alteration in the distribution of nerve fibres and in the ganglion cell or NEC population in the lamina propria of irradiated bowel may contribute to some of the symptoms of radiation injury, in which case the identification of the secretory products of these cells could have important therapeutic implications.

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