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Nerve fibre proliferation in interstitial cystitis

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Summary. The aetiology of pain in interstitial cystitis is not understood, although it has been reported to be due to release of mediators from mast cell granules. Cystolysis and intravesical instillation of dimethyl sulphoxide have been shown to relieve pain in this condition. We have studied the nerve population within the bladder wall using immunohistochemical stains for protein gene product 9.5. A group of 18 cases of chronic interstitial cystitis and 12 controls; neuropathic bladder ($n=1$), chronic bacterial cystitis ($n=3$), systemic lupus erythematosus cystitis ($n=2$) and normals ($n=6$), were investigated. There were significantly more nerve fibres within the sub-urothelial and detrusor muscle layers in chronic interstitial cystitis than there were in normals. Patients with chronic cystitis of other aetiology did not have a significant increase in nerve fibre density within the bladder wall suggesting a specific association between nerve fibre proliferation and interstitial cystitis. Cystolysis is shown to deplete selectively the submucosal nerve plexuses without altering the nerve density within detrusor muscle. This finding explains the desensitisation of the bladder without impairment of detrusor function after this procedure.

Key words: Interstitial cystitis – Bladder nerves – Protein gene product 9.5 – cystolysis

Introduction

Interstitial cystitis (IC) is an idiopathic condition characteristically presenting with urinary frequency and suprapubic pain partially or completely relieved by voiding. It has been suggested that pain in IC results from release of agents such as histamine from granules within mast cells, which are abundant within the bladder wall in IC (Dixon and Hald 1986). Apart from a report in which it was postulated that pain in IC might result from sacral rhizopathy, which was demonstrated in eight cases, the nervous system has not otherwise been implicated as an aetiological factor in IC (Bohm and Franksson 1957). Extra-vesical denervation by cystolysis (Worth and Turner-Warwick 1973) and the intra-vesical instillation of dimethyl sulphoxide have been shown to relieve pain in IC (Perez-Marrero et al. 1988).

In spite of the above findings little attention has been paid to the role of nerves within the bladder wall in the transmission of sensation and pain in IC, although a single early study alluded to an increase in submucosal nerve density in IC (Hand 1949). The sensory nerve plexus of the bladder is thought to lie within the lamina propria and submucosa (Dixon and Gilpin 1987). The submucosal nerve plexus appears normal whilst nerve fibres within the detrusor are markedly depleted in patients with cholinergic dysautonomia in whom the detrusor is hypotonic but bladder sensation is not impaired (Kirby et al. 1985). This finding supports the theory that the submucosal nerve plexus is sensory in function. Vasoactive intestinal peptide (VIP) and substance-P have also been demonstrated in sub-urothelial nerves and have been implicated in the transmission of sensory impulses (Alm et al. 1977, 1978).

Protein gene product 9.5 (PGP 9.5) is a recently discovered cytoplasmic protein with a molecular weight of 27000 (Doran et al. 1983). It is present in neural and neuroendocrine tissue and has been shown to be an excellent general marker of nerve fibres (Rode et al. 1985; Gulbenkian et al. 1987; Wilson et al. 1988). We have investigated the innervation of the bladder in IC, chronic bacterial cystitis and normal controls after immunohistochemical staining for PGP 9.5.

Materials and methods

A series of 18 female patients (mean age 57.7 years) with IC of mean duration 9.3 years were studied prospectively with two cases

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of systemic lupus erythematosus (SLE) associated chronic cystitis and 10 age-matched controls (chronic bacterial cystitis $n=3$, neuropathic bladder $n=1$, normal bladder $n=6$). All cases of IC presented with suprapubic pain and urinary frequency, had submucosal petechial haemorrhage after cystodistension and an increase ($>20/mm^2$) in the mast cell count in the detrusor muscle on bladder biopsy (Kastrup et al. 1973). None of the control patients had a history of previous pelvic surgery. The IC cases could be subclassified according to previous treatment; cystolysis ($n=8$), intra-vesical DMSO ($n=3$) and no previous treatment ($n=8$). One case was studied before and after cystolysis and is therefore included in both groups.

Each case underwent cystoscopy, measurement of the bladder capacity (all >300 ml) and lateral wall "cold-cup" bladder biopsy under general anaesthesia. The biopsies were immediately fixed in formalin and imbedded in paraffin prior to cutting 4 μm sections. All sections were stained in one batch with polyclonal rabbit anti-PGP 9.5 using the avidin biotin method (Bains and Miller 1988; Doran et al. 1983; Wilson et al. 1984).

The sections were examined through a Leitz microscope at $\times 120$ magnification and there appeared to be an increase in nerve fibres within the IC cases compared to the control groups. In order to quantify the difference biopsies were analysed using a computerised microscopic video image analysis (CVIA) system (University of Adelaide, Zenith computers) (Jarvis 1986). The urothelium/submucosa and detrusor muscle areas were analysed separately and the number of nerve fibres, their areas in pixels and integrated optical densities (IOD) were evaluated. The results were analysed using the Mann-Whitney and Student's *t*-tests.

Results

Submucosa

Nerve counts within the submucosa in the IC and control groups were not significantly different (95.3% Confidence interval [CI], -14, 91, $p=0.102$). However, after separation of the IC cases into subgroups according to prior treatment differences in nerve counts between the groups were revealed (Table 1). The untreated IC group had significantly higher nerve counts than normal controls (95.2% CI, 49, 137, $p=0.0043$), IC cases treated by cystolysis (95.9% CI, 32, 129, $p=0.0039$) and IC cases treated with DMSO (96.8% CI, 30, 162, $p=0.019$). The latter group had only three cases and is therefore interpreted with caution. The differences between the IC group treated by cystolysis and normal controls, or those with chronic cystitis of other aetiology, were not statistically significant. In addition the chronic bacterial/SLE cystitis group did not have a significantly greater number of nerves than the normal controls. The single patient with IC that was investigated before and after cystolysis had a decrease in the submucosal nerve count from 163 to 77 nerve fibres per ten high-power fields.

The size and IOD of the nerves within the submucosa in the IC and control groups were not significantly different.

Muscle

Nerve counts within the detrusor muscle were significantly greater in IC cases than in normal controls (96% CI, 10, 165, $p=0.019$) and those with chronic bacterial/SLE cystitis (95.6% CI, 1, 160, $p=0.044$). Also, the untreated IC group as well as the IC cystolysis group had a significantly greater nerve count within muscle than normals (95.3% CI, 12, 175, $p=0.03$; 95.7% CI, 2, 187, $p=0.043$) (Table 1). The muscle nerve counts after cystolysis for IC were not significantly different from counts in untreated IC (96.2% CI, 106, -86, $p=1.0$) suggesting that this operation does not alter detrusor muscle innervation. Intravesical DMSO therapy reduced the mean intramuscular nerve prevalence in IC (Table 1) although this change was not statistically significant, possibly due to the small numbers studied (94.3% CI, -38, 192, $p=0.188$). This finding implies that DMSO might penetrate deep to muscle. The single patient studied before and after cystolysis did not have sufficient muscle in the second biopsy to permit a comparison.

Table 1. PGP 9.5 immunoreactive nerve counts (per 10 fields) in IC and control groups

	No. of Cases	Submucosa (Mean ±SE)	Detrusor (Mean ±SE)
All Interstitial Cystitis (IC)	18	101.8±12.6	133.7±16.4
Untreated IC	8	150.2±11.2	142.3±23.2
IC after Cystolysis	8	72.0±15.0	144.3±27.7
IC after Dimethyl Sulphoxide	3	52.0±16.3	71.0±27.0
Chronic Bacterial/SLE Cystitis	5	98.6±35.3	55.0±20.0
Normal Controls	6	61.2±14.0	51.2±16.3

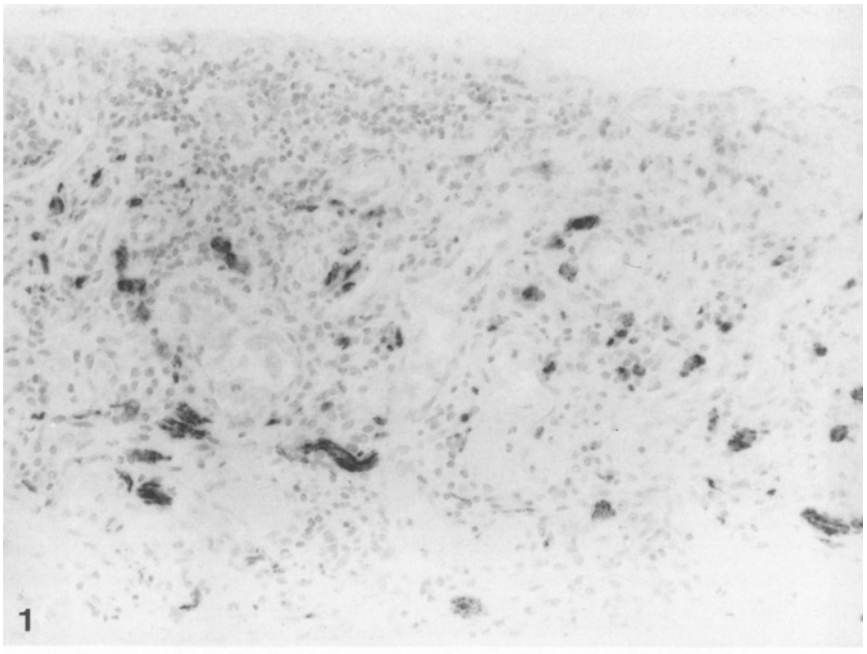
The size and IOD of the nerves within the muscle in the IC and control groups were not significantly different.

The bladder capacity at cystoscopy was not altered by cystolysis in the eight cases that had undergone this operation confirming the findings of the first report of this procedure (Worth and Turner-Warwick 1973). Alterations in the nerve population after this procedure did not therefore relate to contracture of the bladder.

Discussion

Protein gene product 9.5 is found within nervous tissue of all mammals, as well as reptiles and fish suggesting that it developed early in evolution (Jackson et al. 1985). PGP 9.5 was first isolated from human brain by high resolution two-dimensional electrophoresis and rabbit polyclonal anti-PGP 9.5 antibodies were developed later (Doran et al. 1983). Immunohistochemical staining for PGP 9.5 has shown it to be present within all nervous and neuroendocrine tissues including noradrenergic, substance-P and calcitonin gene-related peptide (CGRP) containing nerve fibres (Rode et al. 1985; Gulbenkian et al. 1987).

We have shown a significant increase in the number of PGP 9.5 immunoreactive nerve fibres within the submucosa and muscle layers of the bladder in interstitial



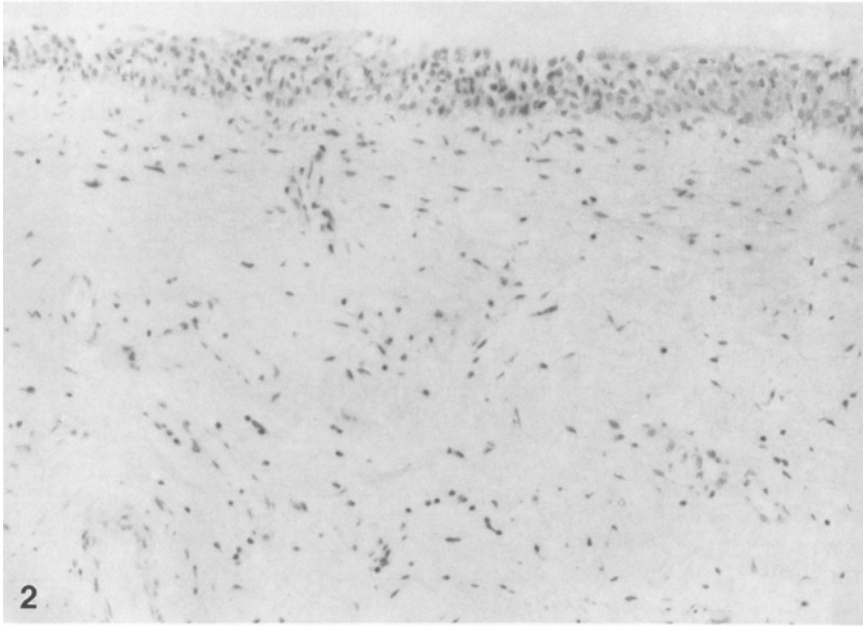


Fig. 1. PGP 9.5 positive neurones demonstrated within the bladder submucosa from a case of untreated interstitial cystitis (Avidin-Biotin-Peroxidase complex ×120)

Fig. 2. PGP 9.5 stained bladder biopsy from a normal control (Avidin-Biotin-Peroxidase complex ×120)

cystitis as compared to normal bladders. The aetiology of this nerve proliferation is not immediately obvious, however the increase of nerve fibres within the submucosa, where sensory nerve endings are thought to predominate, might explain the chronic suprapubic pain which is a characteristic feature of IC. Since PGP 9.5 is apparently present in all nerve tissues we are unable to define the transmitter substance within the sub-urothelial nerve fibres in IC. However, it is possible that these are nerves containing neuropeptides as putative transmitter substances, in particular substance-P and CGRP positive fibres, since these have been associated with transmission of pain in other organs and have been shown to stain positively for PGP 9.5 (Gulbenkian et al. 1987).

In other forms of chronic cystitis such as chronic bacterial and SLE-associated cystitis the mean nerve count was somewhat higher than the normal controls but there was no significant difference between the nerve counts in these patients and normal controls. In view of these findings it is therefore unlikely that the proliferation of nerve fibres within the bladder submucosa in IC results from chronic inflammation alone and it would appear to be a feature specific to IC.

The aetiology and significance of nerve proliferation within the detrusor muscle in IC is unclear, but could represent a continuation of the pathological process from the submucosa since IC is a transmural disease. The nerve fibres within the muscle are not likely to be

fibres passing from the submucosa since unlike nerve fibres within the submucosa they are not depleted after cystolysis.

Neurosurgical procedures, including differential sacral neurotomy and excision of the superior hypogastric plexus, have been shown to relieve pain in IC, therefore suggesting that painful stimuli may be transmitted via dorsal sacral roots or the sympathetic nervous system (Meirowsky 1969; Douglass 1934). More recently cystolysis, an extra-vesical supratrigonal denervation procedure, has been shown to relieve bladder pain in IC (Worth and Turner-Warwick 1973). Fortnightly intravesical instillation of dimethyl sulphoxide (DMSO) over two months has been shown to relieve pain and frequency in IC and the mechanism of this action is not completely understood since this chemical has a wide range of properties including anti-inflammatory activity and inhibition of nerve conduction (Perez-Marrero et al. 1988).

We have shown selective depletion of the submucosal nerve plexus after cystolysis and suggest that this accounts for the relief of pain after this procedure. Intravesical instillation of DMSO also appears to reduce the submucosal nerve population and also the detrusor muscle nerve population, although only three patients in this series had received this treatment and the change in muscle population was not statistically significant. It does however suggest that DMSO might exert its action through the full thickness of the bladder wall. In this study cystolysis has not been shown to alter the nerve density within the detrusor muscle which would explain the preservation of detrusor function after this procedure, and we presume that this is because this extravesical operation conserves intra-mural ganglia and hence also post-ganglionic intra-muscular nerve fibres.

Mast cells have been shown to be present in large numbers within the submucosa and detrusor muscle in IC and release of histamine and other agents from their cytoplasmic granules has been suggested as a possible aetiology of pain in IC (Kastrup et al. 1983). Mast cells have also been shown on electron microscopy to be intimately involved with autonomic nerve fibres and are thought to play a role in the development and proliferation of nerves (Heine and Förster 1975; Nyiri et al. 1977; Weisner-Menzel et al. 1981). In IC mast cells could release a nerve proliferation factor since the sites of increase in nerve fibre and mast cell numbers are the same. It is also possible that pain in IC might be related to stimulation of proliferating sensory nerve fibres within the submucosa with mast cells playing a role as mediators. Submucosal (but not intra-muscular) mast cell proliferation is a feature of chronic bacterial cystitis (Cornish et al. 1986) and this might explain the selective increase in submucosal fibres and supra-pubic pain which may be feature of the condition.

No previous studies have investigated the nerve population within the bladder wall in IC and this is surprising since bladder pain is the most disturbing symptom of the condition. Using polyclonal antibodies against PGP 9.5, which has been shown to be present in all nervous tissue including neuropeptide immunoreactive fibres, we have demonstrated an increase in the number of nerve fibres in the sub-urothelium and within the detrusor muscle in IC. Further studies are in progress to define the transmitter substances within these proliferating nerves. The results of this study are further evidence in support of the sensory role of the submucosal nerve plexus. Cystolysis leads to selective depletion of the sub-urothelial nerve fibre density and relief of bladder pain in IC suggesting that the increase in the nerve density within this area is an important factor in the mediation of pain.

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