p⁷⁵ nerve growth factor receptor in the vesical urothelium of patients with neuropathic bladder: an immunohistochemical study

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Introduction: Nerve growth factor (NGF), apart from its role as a growth factor, appears to be involved in neuroimmune interactions and in tissue inflammation. Low-affinity nerve growth factor receptor (p⁷⁵ NGFR), if demonstrated in the urothelium, could provide the means for (1) NGF-mediated modulation of the urothelial response to inflammation; (2) NGF-mediated autocrine/paracrine regulation of urothelial proliferation; and (3) p⁷⁵ NGFR-mediated induction of apoptosis.

Objectives: To investigate the presence of p⁷⁵ NGFR in the vesical urothelium of patients with neuropathic bladder by immunohistochemical methods.

Setting: A hospital-based study of consecutive, unselected, adult patients of either sex with neuropathic bladder, undergoing procedure on the urinary tract in a Regional Spinal Injuries Centre located in the north-west of England.

Intervention: Cold cup biopsies were taken from the trigone of the neuropathic urinary bladder of 26 patients with neuropathic bladder. Immunohistochemical studies were performed using antiNGF-receptor human monoclonal antibody which reacts with the low affinity receptor (p⁷⁵ NGFR).

Results: Both neural and epithelial structures showed positive immunostaining for p⁷⁵ NGFR. The basal layer of the transitional epithelium showed strongly positive immunostaining for p⁷⁵ NGFR in all the 26 cases. The luminal layer of transitional epithelium showed varying degree of positive immunostaining in 12 patients. The nerve fibres showed positive immunostaining for p⁷⁵ NGFR. In many cases, the positively-stained nerve fibres were coursing very close to the basal layer of the urothelium almost entering the urothelium; however, no NGFR-positive intra-epithelial terminals could be seen. The positively-stained single nerve fibres and positively-stained thicker nerve bundles were seen in abundance in the submucosa but they were present in a sparse manner in the muscularis layer.

Conclusion: The presence of p⁷⁵ NGFR was demonstrated in the urothelium of neuropathic bladder of all the 26 patients with neuropathic bladder. This observation may have potential therapeutic implications.

Keywords: p⁷⁵ receptor; nerve growth factor receptor; neuropathic bladder

Introduction

Nerve growth factor

Nerve growth factor (NGF), initially considered to be important for maintaining survival and providing guidance for target projection of peripheral sensory and sympathetic neurons, is now known to display a greatly extended spectrum of biological functions. NGF appears to be involved in neuroimmune interactions and tissue inflammation, with NGF acting as a general 'alert' molecule capable of recruiting and priming both local tissue and systemic defense

processes following stressful events. NGF can thus be viewed as a multifactorial mediator modulating neuroimmune-endocrine functions of vital importance to the regulation of homeostatic interactions, with potential involvement in pathological processes deriving from dysregulation of either local or systemic homeostatic balances.¹

p75 Low-affinity nerve growth factor receptor (p75 NGFR)

The neurotrophin family is distinguished by a unique signal transduction system represented by two different

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Table 1 The clinical details of the patients with paraplegia/tetraplegia in whom biopsy had been taken from the trigone of the neuropathic bladder, and findings of the immunohistochemical studies for p⁷⁵ nerve growth factor receptor in the bladder biopsy

Serial number	Sex	Year of birth	Date of paralysis	Diagnosis	Findings of p ⁷⁵ nerve growth factor receptor immuno-histo-chemical staining of biopsy from the trigone of the urinary bladder
1	M	1931	01.03.1991	Traumatic tetraplegia	The basal layer of urothelium shows strongly positive staining. Numerous nerve fibres are present and they show strongly positive staining.
2	M	1954	19.07.1984	Traumatic tetraplegia C-5/6	Strongly positive staining of the basal layer of the urothelium. Positive staining of the nerve fibres in the submucosa.
3	F	1964	24.09.1983	Guillain Barre syndrome. Tetraplegia C-4	Positive staining of the basal layer of the urothelium. Positive staining of the thick nerve bundles which look almost like a neuroma.
4	F	1969	Since birth	Spina bifida paraplegia	Strongly positive staining of the basal layer of squamous metaplastic epithelium. Nerve fibres are approaching very close to the basal layer of the epithelium and are almost running into it. Large number of nerve fibres show positive staining and these nerve fibres run along the basal layer of the epithelium.
5	F	1947	Since birth	Spastic tetraplegia	Very strongly positive staining of the basal and luminal layers of the urothelium. Positively staining macrophages. Strong neural staining.
6	M	1964	08.07.1988	Traumatic tetraplegia C-5/6	Very intense basal staining; no staining of the intermediary layers of the urothelium; surface cells show positive staining. Positive staining nerve fibres run very close to the basal layer of the urothelium.
7	M	1930	17.12.1951	Traumatic paraplegia T-5	Very intense staining of the basal layer of the urothelium. Positively stained nerve fibres come close to the epithelium.
8	M	1948	June, 1967	Paraplegia T-6 Viral myelitis	Positive staining of all the layers of urothelium; the staining is definitely cytoplasmic. Strongly positive staining of the macrophages.
9	M	1935	20.12.1991	Traumatic tetraplegia C-5	Very definite staining of the basal layer of the transitional urothelium. No staining in the squamous metaplastic epithelium. Nerve fibres show positive staining. Lymphoid tissue shows no staining.
10	M	1947	24.12.1985	Traumatic tetraplegia C-5	Very intense staining of the basal layer of urothelium. Parabasal staining: Positive. The surface layer of the urothelium shows positive staining. Lymphoid tissue shows no staining. The nerve fibres, which are scanty, show positive staining.
11	M	1933	30.08.1976	Traumatic tetraplegia T-10	The basal layer as well as the luminal layer of the urothelium shows strongly positive staining. Nerve twiglets show strongly positive staining. Nerve twiglets run upto the basal layer of the urothelium.
12	M	1929	11.12.1982	Traumatic tetraplegia C-5	Low to moderate intensity staining of the basal layer of the urothelium. Nerve fibres show positive staining.
13	M	1938	12.06.1957	Traumatic tetraplegia C-5	The basal layer of the urothelium show strongly positive staining. The luminal layer of the urothelium show low intensity staining. Mast cells show positive staining. Nerve twiglets are staining positive.
14	M	1923	Since birth	Spina bifida paraplegia	The basal layer in squamous metaplastic epithelium shows positive staining.

Table 1 *Continued*

<i>Serial number</i>	<i>Sex</i>	<i>Year of birth</i>	<i>Date of paralysis</i>	<i>Diagnosis</i>	<i>Findings of p⁷⁵ nerve growth factor receptor immuno-histo-chemical staining of biopsy from the trigone of the urinary bladder</i>
15	F	1966	Since birth	Spina bifida paraplegia	Very strong staining of the basal layer of the transitional epithelium. The <i>surface</i> layer shows only weakly positive staining. Big plexuses of nerve fibres are seen which show positive staining.
16	M	1973	Since birth	Spina bifida paraplegia	The basal layer as well as the <i>luminal</i> layer of the urothelium show strongly positive staining. Nerve fibres show strongly positive staining. Positively staining nerve bundles are seen in the muscularis.
17	M	1957	31.05.1975	Traumatic tetraplegia C-6	The basal layer of the urothelium shows positive staining. Numerous nerve twiglets are seen which exhibit strongly positive staining.
18	M	1956	07.01.1973	Traumatic paraplegia T-5	Intense staining of the basal layer of the urothelium. Increased numbers of nerve bundles are seen in the submucosa.
19	M	1962	05.02.1982	Traumatic paraplegia T-6	Strongly positive staining of the basal layer of the urothelium. The nerve fibres show positive staining.
20	M	1934	29.04.1967	Traumatic paraplegia T-10	Strongly positive staining of the basal and <i>surface</i> layer of the urothelium. Very marked staining of the nerve fibres.
21	M	1921	15.12.1962	Traumatic paraplegia L-1	Strongly positive staining of the basal and <i>surface</i> layer of the urothelium. Numerous nerve twiglets show positive staining.
22	M	1944	1970	Multiple sclerosis	Strongly positive staining of the basal layer of the urothelium. Low to medium intensity staining of the <i>surface</i> layer of the urothelium. Network of nerve twiglets show positive staining.
23	M	1958	11.09.1994	Traumatic tetraplegia	Low to medium intensity staining of both basal and <i>surface</i> layers of the urothelium. Positively stained nerve fibres come upto the basal layer of the urothelium.
24	M	1971	September 1988	Traumatic paraplegia T-6	Basal layer of the transitional urothelium show positive staining. There is no staining in the squamous metaplastic epithelium. Positive staining of the nerve fibres is clearly noticable. Macrophages show positive staining. Lymphoid tissue is seen but there is no positive staining in the lymphoid tissue
25	F	1923	03.01.1944	Traumatic tetraplegia C-5, incomplete	Strongly positive staining of the basal layer of the urothelium. Nerve fibres show positive staining.
26	F	1961	10.08.1993	Tetraplegia C-5, Incomplete?, Viral myelitis	Strongly positive staining of the basal layer of the urothelium. Nerve fibres show strongly positive staining. Lymphoid follicle shows no staining. Macrophages show positive staining.

transmembrane receptors. Nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) bind to two types of cell surface proteins – the *trk* family of receptor tyrosine kinases, and the *p75*, or low-affinity neurotrophin receptor. The *Trk* receptors are distinguished by an extracellular domain with multiple repeats of leucine-rich domains, two cysteine-rich domains and IgG-related domains, and a cytoplasmic

region that is distinguished by a catalytic tyrosine-kinase domain, a small insert and a short cytoplasmic tail. The *p75* receptor has a distinctive extracellular-domain sequence that differs from that of *TrkA*, with four distinct cysteine-rich domains that are responsible for ligand binding. As all the neurotrophins are capable of binding to *p75* receptor, this molecule may be redefined as a neurotrophin receptor, rather than only a nerve growth factor (NGF) receptor.

Neurotrophins initiate biological actions by binding to cell surface receptors, followed by receptor-mediated internalization, increased cellular tyrosine phosphorylation, retrograde transport, and the activation of numerous effectors molecules, such as protein kinases, phospholipases, and transcriptional activators.² The neurotrophin receptor p75 has emerged as a receptor with a multiplicity of roles. As a neurotrophin receptor subunit, p75 profoundly affects tyrosine kinase function on many levels, providing an additional mechanism for cells to modulate neurotrophin actions. In addition, p75 also can function alone to regulate gene expression, cell migration, and apoptotic events. The different aspects of p75 activity may ultimately depend upon the cell context and environment, the ratio of tyrosine kinase to p75 expression, and the presence of neurotrophins.³

Bladder distension and NGF production by smooth muscle

Neuropathic bladder dysfunction may be associated with functional, (commonly) chronic, obstruction at the bladder neck and/or at the external sphincter. The urinary bladder undergoes anatomical, biochemical, pharmacological, and physiological changes in response to the functional demands of chronic obstruction.⁴ Acute bladder outlet obstruction, (as occurs when an indwelling urinary catheter is blocked), elicits a relative denervation of the bladder with axonal degeneration with subsequent reinnervation of smooth muscle as shown by ultrastructural studies.⁵ Mechanical stretch of cultured smooth muscle from the urinary tract elicits rapid accumulation of nerve growth factor messenger RNA followed by secretion of nerve growth factor protein.⁶

Low-affinity NGFR immunoreactive fibres in the human urinary bladder

Wakabayashi *et al*⁷ studied low-affinity nerve growth factor receptor (LNGFR) immunoreactive fibres in the specimens obtained during urological surgery from the anterior wall of the bladder body of nine neurologically normal patients. LNGFR immunoreactive nerve fibres were observed in the lamina propria, submucosal layer and muscle layer, but not in the epithelium. The distribution of LNGFR-positive fibres were more abundant in the lamina propria than in the muscle layer. Most of the LNGFR immunoreactive nerve fibres in the lamina propria ran freely, but some were accompanied by blood vessels. Double immunostaining for low-affinity nerve growth factor receptor and tyrosine hydroxylase (used as a marker of adrenergic nerves in the urinary bladder) demonstrated that some low-affinity nerve growth factor-positive fibre bundles contained tyrosine hydroxylase immunoreactivity and others did not; thus it is likely that LNGFR-positive fibre bundles enclose various nerves that are not exclusive to adrenergic nerves. As the density of

LNGFR-positive fibres was more abundant in the mucosal layer than in the muscle layer, Wakabayashi *et al*⁷ suggested that epithelial cells in the human urinary bladder could produce nerve growth factor in normal conditions, and the LNGFR-positive fibres in the mucosa might be related to the trophic action on the innervating neurons. However, these authors did not refer to the presence of LNGFR immunoreactivity in the non-neural tissues such as the vesical epithelium, macrophages, or the lymphoid tissue in the bladder biopsies they studied.

Is p75 neurotrophin receptor present in vesical urothelium?

In view of the presence of NGFR in epithelial structures such as the oral mucosa⁸ and in prostatic epithelium,⁹ it is likely that p75 NGFR may be located in the vesical urothelium. The presence of p75 NGFR in the urothelium could subserve the following physiological roles: (1) NGF-mediated autocrine/paracrine regulation of urothelial proliferation; (2) NGF-mediated modulation of urothelial response to inflammation; (3) The constitutive activity p75 NGFR in the induction of apoptosis (a process known as programmed cell death, which is an active form of cellular suicide, that typically requires ATP, involves new RNA and protein synthesis, and that culminates in the activation of endogenous endonucleases that degrade the DNA of the cell, thereby destroying the genetic template required for cellular homeostasis).

We undertook a study to investigate the presence of p75 NGFR in the vesical urothelium of patients with neuropathic bladder. If p75 NGFR is shown to be present in the urothelium, this finding may open the avenue for innovative treatment strategies targeted at the p75 NGFR, as therapeutic applications of neurotrophic growth factors are no longer a distant goal but rather a practical reality.¹⁰

Patients and methods

Cold cup biopsies of the trigone of the urinary bladder were taken with Storz rigid cystoscope 22 Fr. from adult patients of either sex with neuropathic bladder dysfunction undergoing a therapeutic procedure such as electrohydraulic lithotripsy of vesical stone(s), JJ stenting of the ureter prior to extracorporeal shock wave lithotripsy of renal stone(s), transurethral resection of bladder neck or division of external urethral sphincter. All the participants gave written informed consent for taking bladder biopsy. Of the two biopsy specimens taken from the trigone of the urinary bladder, one biopsy specimen was preserved in buffered formaldehyde for routine histopathological examination. The other biopsy specimen was covered with OTC compound to prevent dehydration, then it was snap frozen in liquid nitrogen. Thereafter, the biopsy specimen was stored at -80°C until the immunohistochemical studies were performed for p75 NGFR in a

single batch on all the specimens. Anti NGF-receptor human monoclonal antibody (Catalogue no. 1198–637) was purchased from Boehringer Mannheim UK (Diagnostics & Biochemicals) Limited, Bell Lane, Lewes, East Sussex BN7 1LG. This monoclonal antibody reacts with the low affinity receptor (p⁷⁵ NGFR). It does not react with the high affinity receptor (p¹⁴⁰ TrkA) or any other members of the Trk family (TrkB, TrkC).¹¹

Results

The clinical details of the 26 patients with a neuropathic bladder, and the results of the immunohistochemical findings of the individual patients are listed in Table 1. Seventeen patients had sustained a spinal cord injury; four had spina bifida with paraplegia; three patients had tetraplegia of non-traumatic origin; one patient had paraplegia due to viral myelitis; and one patient had multiple sclerosis. Immunohistochemical staining of bladder biopsies for p⁷⁵ Nerve Growth Factor Receptor in patients with neuropathic bladder revealed the following features: (1) Both *neural* and *epithelial* structures showed positive immunostaining; (2) The basal layer of the transitional epithelium showed strongly positive immunostaining for p⁷⁵ NGFR in all the cases (Figure 1); (3) The luminal layer of transitional epithelium showed varying degree of positive immunostaining (low to medium to strong intensity) for p⁷⁵ NGFR in 12 patients (Figure 2); in one of them (BL), the surface layer of the transitional epithelium showed positive staining and gave an impression as though it was secreting the material into the lumen; (4) In contrast to the above, in those bladder biopsy specimens in which the transitional epithelium had undergone squamous metaplasia, the basal layer of the squamous metaplastic epithelium showed varying degree of positivity (low to medium to strong intensity) for p⁷⁵ NGFR immunostaining, or no positive immunostaining at all (Figure 3); (5) The pattern of immunostaining in the epithelium was 'peripheral', suggesting that the p⁷⁵ NGFR immunoreactivity was located at the plasma membrane; (6) The nerve fibres coursing below the transitional epithelium showed positive immunostaining for p⁷⁵ NGFR. In many cases, the nerve fibres were running in a random manner in the submucosa (Figure 4). In some biopsy specimens, the positively-stained nerve fibres were coursing very close to the basal layer of the urothelium almost entering the urothelium (Figure 5); however, no NGFR-positive intra-epithelial terminals could be seen; (7) The positively-stained single nerve fibres and positively-stained thicker nerve bundles were seen in abundance in the submucosa (Figure 6); however, the positively-stained nerve fibres were present in a sparse manner in the muscularis layer; (8) The positive immunostaining for p⁷⁵ NGFR appeared continuous along the basal layer of the urothelium, and in squamous metaplastic epithelium

when present, without variations in intensity, independently of the presence and abundance of superficial nerve fibres; (9) In many cases, the macrophages in the submucosa showed positive staining for p⁷⁵ NGFR; (10) Lymphoid tissue (including lymphoid follicles) was seen in some of the bladder biopsies; but there was no unequivocal positive immunostaining of the cells within the lymphoid tissue.

Discussion

Positive immunostaining for p⁷⁵ NGFR was demonstrated in *neural* as well as *epithelial* structures in the

Figure 1 Photomicrograph of urinary bladder biopsy stained with anti-nerve growth factor receptor human monoclonal antibody. The basal layer of the transitional epithelium shows strongly positive immunostaining

Figure 2 Photomicrograph of urinary bladder biopsy stained with anti-nerve growth factor receptor human monoclonal antibody. The basal layer as well as the luminal layer of transitional epithelium shows positive staining

Figure 3 Photomicrograph of urinary bladder biopsy stained with anti-nerve growth factor receptor human monoclonal antibody. The squamous metaplastic epithelium shows no staining

Figure 4 Photomicrograph of urinary bladder biopsy stained with anti-nerve growth factor receptor human monoclonal antibody. The nerve fibres running in a random manner in the submucosa show positive immunostaining

biopsies taken from the trigone of patients with neuropathic urinary bladder dysfunction. Wakabayashi *et al*⁷ studied bladder tissue taken from the anterior walls of bladder body of nine neurologically normal subjects, and they did not observe low-affinity nerve growth factor receptor (LNGFR) immunoreactive fibres in the vesical epithelium. These authors did not allude to LNGFR immunoreactive staining by non-neural tissue such as the epithelium, the macrophages and the lymphoid tissue in the bladder biopsies studied by them. In the present study also, no NGFR-positive intra-epithelial terminals could be seen. However, p⁷⁵ NGFR immunoreactivity was demonstrated in the *urothelium* of all the 26 patients with a neuropathic bladder.

It is now apparant that p⁷⁵, a low-affinity receptor for nerve growth factor, is yet another cellular death

Figure 5 Photomicrograph of urinary bladder biopsy stained with anti-nerve growth factor receptor human monoclonal antibody. The positively-stained nerve fibres are seen coursing immediately subjacent to the basal layer of the urothelium

Figure 6 Photomicrograph of urinary bladder biopsy stained with anti-nerve growth factor receptor human monoclonal antibody. Positively-stained single nerve fibres and thick nerve bundles are seen in abundance in the submucosa

signalling protein. Lowering of NGF concentration, over-expression of p⁷⁵, or expression of less TrkA (a member of the tyrosine kinase growth factor receptor family) caused p⁷⁵ to signal death. Recent experiments have shown that inhibiting p⁷⁵ expression with antisense oligonucleotides can rescue neurons in severed sensory nerves from death.¹² It is probable that p⁷⁵ plays roles other than that of a 'death gene' product; p⁷⁵ might signal both cell survival and cell

death. In the neuropathic bladder, p⁷⁵ NGFR might play a role in: (1) cell proliferation and apoptosis; (2) regulating the inflammatory changes (cystitis) in the bladder mucosa; (3) development and maintenance of mucosal innervation; (4) modulation of the urinary bladder stretch response in which a physical stimulus of distension of the bladder is transduced into a cascade of biochemical responses culminating in the production of nerve growth factor by the smooth muscle cells.

The presence of p⁷⁵ NGFR in the luminal layer of the urothelium in 12 of the 26 biopsy specimens taken from patients with neuropathic bladder, and the presence of p⁷⁵ NGFR in the basal layer of the urothelium in all the biopsy specimens, may offer an opportunity for designing possible innovative treatment strategies targeted at the p⁷⁵ receptor. A molecular biological approach aimed at modulating the function of neuropathic bladder may become possible,¹³ and it might involve the administration of either the ligands for the p⁷⁵ NGFR which would activate the receptor, eg nerve growth factor, or the administration of substances that would block the action(s) of p⁷⁵ NGFR, as p⁷⁵ NGFR has multifarious functions, apart from its classical role as a growth factor receptors.

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