

# 行政院及所屬各機關出國報告

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## 小兒神經性膀胱之研究

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## 摘要

小兒泌尿道疾病患者中，至少有四分之一是因神經性膀胱引起的。原因可分為先天的，如脊柱裂，薦骨發育不良，腦性麻痺；後天性的，如中樞神經感染及脊椎損傷等。其中以先天性較常見，又以脊髓脊膜膨出為最多，佔出生率的千分之一。這些患者在原始原因治療完成之後，可能面臨神經性膀胱的後遺症。神經性膀胱除了引起尿失禁的問題，影響個人衛生及生活品質之外，最嚴重者甚至腎衰竭，而且不在少數。因此必須儘早診斷，迅速處理。診斷神經性膀胱最重要的工具，就是尿路動力學檢查。本研究的目的是，如何把尿路動力學檢查，應用在小孩子身上。及研究診斷出神經性膀胱之後，後續的處置。另研究神經性膀胱中，膀胱平滑肌細胞增生的機制。

關鍵詞： 小兒，神經性膀胱，尿路動力學檢查

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## I. 前言與目的

小兒泌尿道疾病患者中，至少有四分之一是因神經系統病變造成。引起小兒神經性膀胱的原因，可分為先天性及後天性，先天性的顯著的例子，如脊柱裂(spinal bifida)，薦骨發育不良(sacral agenesis)，腦性麻痺(cerebral palsy)，tether cord等。後天性的原因，如脊髓腫瘤，脊椎損傷及中樞神經感染等。以脊柱裂最常見，其中又以脊髓脊膜膨出(myelomeningocele)為最多，國外的統計，約千分之一的發生機會，即出生嬰兒一千位中有一位；依此比例推算台灣每年約有三千八百例新患者。在醫療不發達的時代，脊髓脊膜膨出引起神經性膀胱的病例，並不常見；因為這些脊髓脊膜膨出的患者，早期，將因脊髓脊膜細菌感染或腦室積水而死亡。但隨著醫療水準的進步，出生之後，隨即迅速手術閉合皮膚傷口，投予抗生素，及放置 ventriculoperitoneal shunt 管等，大部分的患者將存活下來；隨著年齡的成長，緊接著將有神經性膀胱引起尿失禁或尿滯留，便秘，及下肢畸形等後遺症的發生。

脊髓脊膜膨出的患者中，大部分的膀胱功能不正常，只有百分之五的膀胱功能正常；而且有將近三分之一的患者，將因膀胱功能不正常變成腎衰竭。發生腎衰竭的原因，膀胱和尿道括約肌之間的不協調，導致膀胱壓力上升，造成輸尿管尿液滯留或膀胱尿迴流的現象。另外膀胱和尿道括約肌之間的不協調，膀胱尿滯留，易造成細菌感染，重復性的腎臟腎盂炎，最後破壞腎臟的實質組織。這二者就是導致腎衰竭的原因。

不一定要背部有明顯的皮膚缺陷或神經學檢查異常，才表示有神經系統的問題。據統計，解尿功能異常但神經學檢查正常者中，以核磁共振攝影術檢查發現，百分之十六的人有脊髓異常現象。神經系統疾病引起的膀胱功能正常或異常，也不是持續不變，將可能隨著身體的成長，而發生改變。由此可之小兒尿動力檢

查的重要性.

小兒神經性膀胱研究的目的是有三: 1. 即在研究如何利用尿路動力學檢查, 診斷出小孩子不正常的膀胱功能. 2. 診斷出小孩子不正常的膀胱功能之後, 如何進一步去處置及追蹤. 3. 更進一步, 從細胞的層次研究神經性膀胱中, 阻塞引起膀胱平滑肌細胞增生的機制. 了解膀胱平滑肌細胞增生的機制, 除了能夠利用阻止細胞增生之外, 亦可利用於人工膀胱的培養.

## II. 過程

研究時間從 2000 年 9 月 28 日至 2001 年 12 月 30 日，於哈佛醫學校波士頓兒童醫院泌尿科進行。兒童醫院泌尿科對於小兒神經性膀胱的診斷與治療，擁有豐富的經驗及各類的患者，由著名的 Stuart B. Bauer 教授主持。項目包括 1. 小兒神經性疾病(膀胱)聯合門診，成員有小兒科，小兒泌尿科，骨科，神經外科專科醫師及社會工作者，針對神經系統疾病引起的各科問題，同時診治；特色是同一時間能夠非常有效率會商與解決問題，值得學習。2. 完整的尿路動力學檢查，包括膀胱功能測定及尿道外括約肌肌電圖，成員有小兒神經科醫師負責尿道外括約肌肌電圖的判讀，小兒泌尿科醫師負責膀胱功能的判讀與整個檢查過程的安排，技術員兩名實際操作尿路動力學檢查，尿路動力學檢查室的配置：隱密，溫暖及舒適，另備有吸引小朋友注意的玩具和照片；特色：小兒神經科醫師負責尿道外括約肌肌電圖的判讀，能夠偵測神經 denervation 之後外括約肌的變化。泌尿科實驗室，研究平滑肌細胞增生的機制，主持人 Michael R. Freeman 副教授是這一方面的專家。研究期間，承蒙 Stuart B. Bauer 教授，尿路動力學檢查室技術員 Mary Kelly 小姐，Michael R. Freeman 博士，及博士後研究員 Rosalyn Adam 之熱心指導，過程忙碌但順利，收穫豐碩。

### A. 尿路動力學檢查(Urodynamics)

為了讓檢查過程能夠順利進行，必須讓受檢者及家長充分了解整個檢查的過程。因此必須對受檢者及家長詳細解釋每一項檢查的步驟，及可能的遭遇；除了解釋之外，為了減輕患者之恐懼，可以給予印有檢查步驟的小冊子，在家閱讀。同時給家屬問卷填寫，關於懷孕過程，出生順不順利，生長發育情形，大小便習慣，大小便訓練及其它疾病病史等等，都可作為參考之用。

受檢者來到檢查室之前，先喝水脹膀胱，以便測定尿流速度(Uroflowmetry，

UFR)及同時透過單方向鏡面觀察解小便時的情形，是否有不當腹部用力的情況。緊接著進行膀胱壓力描繪圖檢查(cystometrography, CMG)，以 Xylocaine jelly 注入尿道潤滑之後，放置 7 Fr. 二叉(測膀胱內壓力) 或三叉尿管(同時測膀胱內及尿道括約肌內壓力)，完成之後，先測量殘餘尿量及送部分導尿液做細菌培養。殘餘尿量是膀胱壓力描繪圖檢查中重要因子。必要時，於直腸內放置導管，在貯存尿液階段及排尿過程，測量腹壓，以便區別膀胱不自主收縮或肚子用力。尿管放置妥當之後，讓病人咳嗽增加腹壓，看膀胱壓力曲線是否急遽上升，以便試著了解尿管壓力傳導是否通暢。小孩子，先以每分鐘 15 c.c.的速度，灌注 37°C 的生理食鹽水；至適當容量時，可以酌以增加灌注速度。在貯存尿液階段，觀察是否有膀胱不自主收縮(hyper-reflexia)或壓力大於 15 公分水柱，稱為 detrusor instability；膀胱壓力持續上升(hypertonicity)的現象，注意容量多少時壓力持續增加；評估膀胱測定容量，再與估計值比較(估計值：小於 2 歲，體重(kg) X 7；大於 2 歲，則是年齡 (年) +2 X30)，這項因子，可做為真正膀胱容量的參考。在檢查過程，儘量想辦法分散受檢者的注意力。諸如播放兒童喜愛的卡通影片或吹泡泡吸引受檢者的興趣，假如做完檢查，應該給予適當的獎勵，為下一次檢查做準備。灌注速度每分鐘宜慢於估計值的十分之一，否則易造成假陽性的壓力上升。一般先以每分鐘 15 c.c.的速度灌注，再酌以增加灌注速度。

是否給予鎮定劑，則是見仁見智；憂懼的受檢者，除了儘量安撫之外，可給予 meperidine (1 mg/kg body weight) 或 EMLA 藥膏(局部麻醉劑)。一般儘量不給其它藥物，或投予不影響受檢者合作及膀胱收縮功能的藥物。因膀胱功能異常，而服用其他它藥物者，例如 cholinergic drug : Bethanechol; anticholinergic drug: Oxybutynin (Ditropan), Detrol; sympathomimetic drug: Pseudoephedrine. 則於受檢查前 2 小時使用。

尿道外括約肌肌電圖(electromyography, EMG)係利用 24-gauge needle

electrode, 由會陰部(男生)或尿道口週圍(女生)穿刺至尿道外括約肌位置, 偵測肌電位, 以圖形變化或音效強弱稀疏, 表達肌電位的改變. 檢查目的有三: 1. 剛剛穿刺時, 注意尿道外括約肌肌電位圖形及音效變化, 這是靜止期的尿道外括約肌肌電位圖形及音效, 以便了解肌肉是否因 denervation 而 degeneration. 2. 在膀胱的灌注過程, 以 Crede 及 Valsalva 方式, bulbocavernosus 和肛門口 (anocutaneous )的刺激, 觀察外括約肌肌電位圖形及音效的變化; 正常狀況, 一刺激肌電圖電位或音效就會反射性增強; 否則, 表示 reflex circle 有問題, 下運動神經元的疾病( lower motor neuron disease). 3. 配合膀胱尿路動力學檢查, 在排空階段, 觀察肌電圖電位是否完全靜止. 假如沒有完全靜止, 稱為逼尿肌與尿道外括約肌間不協調(detrusor-sphincter dys-synergia, DSD), 表示上運動神經元 (upper motor neuron disease)有問題. 所以膀胱尿路動力學檢查時, 就必須想辦法讓受檢者排尿, 如哄小孩, 聽流水聲, 或以溫水澆大腿內側刺激排尿, 直到排尿才可以結束. 當然尿道外括約肌肌電圖的檢查, 不是一次即可得到結論, 必須與後續結果做比較, 才能下結論. 假如尿道外括約肌肌電圖的檢查結果, 就必須進一步實施核磁共振檢查脊髓(MRI), 看是否有 tethering cord 的現象.

除了尿道外括約肌肌電圖檢查之外, 詳細的病史, 相關病史, 如便秘, 尿失禁, 背痛, 尿路感染及下肢畸形. 身體理學檢查, 檢查背部是否有凹陷, 毛髮或正中血管瘤. 神經學檢查, 檢查肌腱反射, 神經感覺的範圍.

尿道壓力檢查(urethral pressure profile, UPP), 則是用來測定單點尿道阻力; 多項要素構成尿道阻力, 包括尿道周圍組織的彈性, 尿道括約肌產生的張力. 尿道壓力隨著膀胱儲存量的多寡而改變. 一般配合膀胱尿動力學檢查進行, 當膀胱內壓力上升時, 尿道阻力是否同時減少, 以此更加肯定膀胱不自主收縮, 非腹部使力所致; 另外當有尿道阻力減少的情況, 亦懷疑有膀胱不自主收縮的現象. 但該項檢查, 受限於尿管開口位置, 僅限於 6 歲以上者檢查. (附檢查結果).



建議，對於小兒神經性膀胱患者，5 足歲前，每年至少實施尿路動力學檢查一次，包括尿流速度檢查，殘餘尿量，尿道外括約肌肌電圖(EMG)，及膀胱壓力描繪圖檢查。如有必要再加上腎臟超音波檢查(Ultrasonography)，膀胱尿道排尿攝影(Voiding Cystourethrography, VCUG) 及核子醫學腎臟功能檢查。

## B. 小兒神經性膀胱之處置

當被檢出罹患神經性膀胱時，第一考慮避免腎臟功能的惡化，在初次或後續的檢查出逼尿肌與尿道外括約肌間不協調(DSD)的患者中，約有百分之七十一的機會，腎臟功能的惡化。即使是逼尿肌與尿道外括約肌間協調的患者，或尿道外括約肌無功能者，分別有百分之十七及百分之二十三的機會，腎功能惡化 (Bauer SB: Early evaluation and management of children with spina bifida. In King LR, ed: Urologic Surgery in Neonates and Young Infants. Philadelphia, W.B.Saunders Company, 1988, pp252-264). 所以除了密切追蹤，每年至少實施尿路動力學檢查一次，包括尿流速度檢查，殘餘尿量，尿道外括約肌肌電圖(EMG)，及膀胱壓力描繪圖檢查之外，對於膀胱不自主收縮(hyper-reflexia or hypertonicity)的患者，投予 anticholinergic 藥物，常用 Oxybutynin hydrochloride，使用劑量 1.mg/kg body weight Q12h 至 5 mg tid, 新生兒 1 mg，可以合併 Detrol 的使用；如有逼尿肌與尿道外括約肌間不協調(DSD) 及殘餘尿量過多的情形，則配合間歇性導尿 (Clean Intermittent Catheterization, CIC)，每 4 到 6 小時一次；從新生兒就開始給予間歇性導尿，讓小朋友自小就熟悉導尿，長大之後自然而然習慣導尿。尿道外括約肌功能減低者，給予 Pseudoephedrine，增加尿道括約肌部位的阻力；尿道外括約肌無功能纖維化者，予以間歇性導尿，以避免殘尿過多，易於膀胱尿迴流及細菌感染。間歇性導尿管，以小號的 nelaton tube 代替，使用過後清水沖洗，再放入微波爐中加熱殺菌。小兒神經性膀胱合併膀胱尿迴流(vesicoureteral reflux)的處理：合併膀胱尿迴流的患者，常有膀胱 hypertonicity，poor compliance 及逼尿

肌與尿道外括約肌之間不協調的現象，給予 oxybutynin 及間歇性導尿，避免膀胱壓力過大影響腎臟功能；同時給予預防性抗生素，避免尿路感染。以這種方式處理，百分之三十至五十五的患者，膀胱尿迴流的問題，得到解決。小兒神經性膀胱合併膀胱尿迴流手術治療的適應症，與原發性者相似；即重復性尿路感染，持續嚴重尿迴流，合併解剖構造異常，持續尿迴流至青春者。假如家屬無法配合導尿者，身體虛弱合不堪手術者，或間歇性導尿腎水腫沒有改善者，才建議做膀胱造口術。但是對膀胱發育造成影響。

尿失禁的問題，假如是尿道括約肌的阻力小引起的，如尿道外括約肌功能減低者，於訓練大小便階段前，或 5 歲前，不建議處理；尿道括約肌的阻力小者，自然的保護裝置，讓膀胱內壓力於 40 公分水柱高之前排空尿液。假如膀胱內壓力有機會超過 40 公分水柱高，百分之百的可能性造成上泌尿道水腫，腎功能異常，只是發生時間前後而已。年紀已夠大時，超過訓練大小便階段的年齡，希望脫離尿布，或五歲以上者，則採取不同的部驟來增加尿道括約肌的阻力。藥物，sympathomimetic drug: ephedrine 或 pseudoephedrine。手術治療，如膀胱頸重建手術，有 Young-Dees-Leadbetter procedure 或 Kropp urethral Lengthening procedure 及 Kropp modification (Salle, Ppork)，但術後必須自我間歇性導尿。另外 fascial sling procedure 或 bulking agent injection，亦足以達到增加尿道括約肌阻力的目的，但術後仍然需要自我間歇性導尿。植入人工尿道括約肌是另一種選擇，適用於膀胱能夠收縮者。尿道括約肌的阻力小合併膀胱容積過小者，除了採取增加尿道括約肌阻力的部驟之外，同時必須實施膀胱增大整型術，依身體的狀況，如利用輸尿管，小腸或胃修補膀胱，達到增加自身膀胱容量的目的。但因整形後的膀胱收縮功能有問題，仍須持續間歇性導尿，達到尿液排空的目的。目前，tissue engineering 技術發達，利用組織培養的人工膀胱是另一類的選擇，但仍在實驗階段。

## C. 膀胱平滑肌細胞增生的機制研究

PDGF-Stimulated DNA Synthesis in Human Bladder Smooth Muscle Cells Occurs Independently of Erk-MAPK Pathway Activation. (Hong-Lin Cheng, Rosalyn M. Adam, Stuart B. Bauer, and Michael R. Freeman. The Urologic Laboratory, Department of Urology, Children's Hospital and Harvard Medical School, Boston MA 02115)

### **Abstract**

**Introduction and objectives:** Bladder smooth muscle cells (SMC) exposed to mechanical forces increase expression of peptide growth factors. Excessive mechanical deformation can result in which expression and activity of growth factors are upregulated. In the current study we have examined the relative potencies of known SMC mitogens in promoting DNA synthesis in human bladder SMC and have characterized signal transduction pathway activation downstream of growth factor stimulation.

**Materials and Methods:** Confluent and quiescent primary cultured human bladder SMC were exposed to different doses of HB-EGF, PDGF or FGF-2 for 24 h. The extent of DNA synthesis was determined by uptake of radiolabeled thymidine into acid-precipitable material. To identify the signaling pathway(s) responsible for induction of DNA synthesis, cells were pretreated with pathway-selective pharmacological inhibitors prior to addition of growth factors and assay for DNA synthesis. Pathway activation was also assessed by immunoblot analysis, following growth factor and/or inhibitor treatment. Total cell lysates were fractionated by SDS-PAGE, blotted to nitrocellulose and immunoblotted with antibodies to the non-

phosphorylated and phosphorylated forms of p38 SAPK2, Akt and p44/p42 Erk.

**Results:** PDGF was found to be the most potent stimulator of DNA synthesis in human bladder SMC, promoting a ~5-fold increase in thymidine incorporation over baseline. HB-EGF and FGF-2 were relatively ineffective as mitogens in this assay. The PDGF-mediated DNA synthesis response was demonstrated to be specific since it was blocked in a dose-dependent manner with the PDGF receptor tyrosine kinase inhibitor, AG 1296. The inhibitors SB203580 and LY294002, which block signaling through p38SAPK2 and PI-3 kinase respectively, were also able to ablate the PDGF-stimulated DNA synthesis response whereas the MEK1 inhibitor PD98059, which blocks Erk/MAPK activation, did not affect PDGF-induced thymidine incorporation. Involvement of the p38SAPK2 and PI-3 kinase pathways was confirmed by immunoblot analysis using antibodies to the activated (phosphorylated) forms of p38 and Akt, which demonstrated increased phosphorylation of these signaling intermediates in response to PDGF-treatment. Furthermore, the PDGF-induced upregulation of phosphorylation of p38 and Akt was decreased in the presence of doses of SB203580 and LY294002, respectively, known to block the DNA synthesis response.

**Conclusions:** These data demonstrate for the first time that PDGF is a potent mitogen for human bladder SMC. The DNA synthesis response to PDGF in bladder SMC is mediated by signaling through the p38SAPK2 and PI-3 kinase pathways, but is independent of Erk-MAPK pathway activation.

## Introduction

The primary force impinging on cells of the bladder wall is that of mechanical deformation. Distension of the bladder wall during cycles of bladder filling and emptying under normal conditions enables storage of urine at low pressure. In cases of outlet obstruction, bladder wall distends beyond its normal physiologic limits leading to the initiation of molecular mechanisms that attempt to compensate for the pressure increase. One demonstrated outcome of obstruction is an increase in bladder mass which results from increased smooth muscle cell (SMC) mass (hypertrophy) and number (hyperplasia) [Levin et al., 1984; Levin et al., 1990; Levin et al., 1995; Monson et al., 1994, 1995; Buttyan et al., 1992, 1994; Chen et al., 1994].

Although the mechanisms underlying the growth response of the bladder wall to distension have not been fully defined, changes in peptide growth factor expression and activity at the local tissue level are known to occur. Altered expression of several growth factors and other molecules in response to mechanical stimuli has been demonstrated, including heparin-binding EGF-like growth factor (HB-EGF) [Park et al., 1998; Nguyen et al., 1999; Borer et al., 1999], basic fibroblast growth factor (bFGF), TGF  $\beta$  1 [Buttyan et al., 1992] and cyclooxygenase-2 (COX-2) [Park et al., 1997]. These data suggest that SMC exposed to mechanical forces may regulate their own proliferation through the elaboration of growth regulatory molecules, thereby contributing to the SMC hypertrophy and/or hyperplasia observed following outlet obstruction.

Platelet-derived growth factor (PDGF) is a major mitogen for connective tissue cells, smooth muscle cells and certain other cell types. The PDGF isoforms, binding to and activating two structurally related protein kinase receptors,  $\alpha$ -receptor and

-receptor, exert their cellular effects. Mechanical force increases PDGF-B and PDGF receptor expression in vascular smooth muscle (VSM) cells; And PDGF-B chain plays an important role in the proliferation of VSM cells.

We have previously demonstrated that neonatal rat bladder SMC exposed to cyclic mechanical deformation in culture undergo DNA synthesis which is mediated in part by selective upregulation of signaling through the p38SAPK2 mitogen-activated protein kinase (MAPK) pathway [Park et al., 1998; Nguyen et al., 2001]. Significantly, signaling through the Erk-MAPK pathway, which is known to mediate DNA synthesis in many cell types, was found to be dispensable for the stretch-induced DNA synthesis response in rat bladder SMC. In the present study, we have identified PDGF as a potent stimulator of DNA synthesis in primary culture human bladder SMC. Consistent with our previous findings with rat bladder SMC exposed to mechanical deformation, PDGF-induced DNA synthesis was found to be mediated through selective upregulation of p38SAPK2 activity, but occurs independently of Erk/MAPK activation.

## **Materials and Methods**

### Reagents

Dulbecco's modification of Eagle's medium (Cellgro); human PDGF-BB, HB-EGF, FGF-2 (R&D Systems); AG1296, PD98059, LY294002, SB203580 (Calbiochem); Phospho-Akt antibody, Akt antibody, Phospho-38 antibody, p38 antibody, Phospho-p44/42 MAPK antibody, p44/42 MAPK antibody (Cell signaling).

### Cell Culture

Human bladder smooth muscle cells (SMC) were isolated and propagated in culture as previously described [Atala et al. 1993; Borer et al., 1999]. Cells were maintained in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37 ° C in a humidified atmosphere of 95% air/5% CO<sub>2</sub>. Experiments were performed on cells between passages 2 and 5.

#### DNA Synthesis Assay

Cells were seeded at a density of  $1.5 \times 10^4$  cells/well in 24-well dishes and incubated for 72 hrs in DMEM/10% FBS. Cells were then treated for 24 hrs with appropriate growth factors in DMEM and inhibitors over a range of doses 2 hrs before; cells were labeled for the last 12 hrs of the treatment period with 1 µCi/well methyl-<sup>3</sup>H-thymidine. At the end of the incubation period, cells were fixed with 15% trichloroacetic acid (TCA), washed with methanol and allowed to air dry. Then cells were solubilized with 0.3N NaOH/1% SDS and, prior to analysis using a Rackbeta liquid scintillation counter (LKB Wallac, Gaithersburg, MD), the resulting solution mixed with Optifluor scintillant.

#### Immunoblot Analysis

Cells were seeded at a density of  $1 \times 10^5$  cells /well in 6-well plates and incubated for 3 days in DMEM/10% FBS till 80% confluence; then serum deprived for 48 hrs. Cells were pretreated without or with 20 µM SB203580, 10 µM LY294002 or DMSO (vehicle control) for 1 hr, followed by stimulation with 0.25 nM PDGF-BB for indicated times. Cell were harvested in lysis buffer (62.5mM Tris-Cl pH 6.8, 10% glycerol, 2% SDS, 1mM Na<sub>3</sub>VO<sub>4</sub>) and the protein concentration determined using the Micro BCA assay (Pierce Chemical Co.) Equivalent amounts of protein were

fractionated on 12% SDS-PAGE gels, the protein transferred to nitrocellulose membrane and the membranes probed with antibodies to the non-phosphorylated and phosphorylated forms of p38SAPK2 and Akt.

## Results

PDGF-B was found to potently stimulate DNA synthesis, as measured by methyl-<sup>3</sup>H-thymidine incorporation in human bladder SMC. In contrast basic FGF/FGF-2 and heparin-binding EGF-like growth factor (HB-EGF) were much less potent as mitogen for bladder SMC (fig.1).

To delineate which signal transduction pathways may be responsible for mediating the observed DNA synthesis response to PDGF-B treatment, cells were pretreated for 2 hours with pathway-specific inhibitors prior to stimulation with PDGF-B. The inhibitors used were as follows: AG1296, a specific inhibitor of the PDGF-B receptor tyrosine kinase; PD98059, an inhibitor of MEK1, specific for the Erk-MAPK pathway; SB203580, an inhibitor of p38-SAPK2 phosphorylation and pathway activation; and LY294002, an inhibitor of PI-3-kinase. Dose-dependent inhibition of PDGF-stimulated DNA synthesis in bladder SMC was observed by pretreatment of cells with AG1296, SB203580 and LY294002, but not with PD98059 (fig.2). These data implicate the p38SAPK2 and PI-3-kinase pathways in mediating the DNA synthesis response to PDGF and suggest that the Erk-MAPK pathway is not required for cell-cycle traverse in human bladder SMC.

To confirm the effects of the inhibitors observed in vitro, total cell lysates were prepared from cells pretreated with the effective dose of the inhibitors, and stimulated with 0.25 nM PDGF-B for different time point. Cells treated with PDGF-B but not



exposed to inhibitors served as controls. Lysates were fractionated by SDS-PAGE, transferred to nitrocellulose membrane and membranes were probed with antibodies to the phosphorylated and non-phosphorylated forms of Erk1/Erk2, p38SAPK2, and Akt which is downstream of PI-3-kinase. PDGF-B was found to stimulate phosphorylation of both p38 and Akt in a time-dependent manner, albeit with differing kinetics. Increased p38 phosphorylation was observed as early as 10 mins following PDGF-B treatment with peak phosphorylation observed at 60 mins. Akt displayed modest basal phosphorylation which was increased markedly within 5 mins of PDGF-B stimulation and peaked at 30 mins. Controls displayed little or no significant phosphorylation of either p38 or Akt (fig. 3A, fig. 4A).

In cells exposed to inhibitors of the p38SAPK2 or PI-3-kinase pathways, a decrease in PDGF-B stimulated phosphorylation of the appropriate effector was observed. SB203580 (20 $\mu$ M) was found to reduce p38 phosphorylation to baseline or close to baseline at all time points tested. The PI-3-kinase inhibitor, LY294002 (10 $\mu$ M), also reduced PDGF-stimulated Akt phosphorylation, although substantial Akt phosphorylation remained especially at the later time points (fig. 3B, fig. 4B).

To confirm that the Erk-MAPK pathway was functional in human bladder SMC and that the PD98059 was active in this cell type, SMC were pretreated without or with different doses of PD98059 for 30 mins and stimulated for 15 mins with 50 ng/ml FGF-2, previously demonstrated to be a bladder SMC mitogen. Lysates were prepared as described and blotted with antibodies to the phosphorylated and non-phosphorylated forms of Erk. FGF-2 was found to potently stimulate phosphorylation of Erk1/Erk2 and this phosphorylation was inhibited in a dose-dependent manner with increasing doses of PD98059 (fig.5). These data confirmed that the Erk-MAPK kinase

pathway was indeed functional in human bladder SMC, and was activatable by the appropriate ligand. Furthermore the data indicate that PD98059 was a potent inhibitor of Erk phosphorylation, and therefore the Erk-MAPK pathway at the doses employed in the DNA synthesis.

## **Discussion**

Previous studies in this laboratory and by others have demonstrated altered growth factor expression and DNA synthesis in response to application of mechanical force in bladder smooth muscle cells. Basic FGF, keratinocyte growth factor (KGF)/FGF-7 and HB-EGF are known to be upregulated in SMC exposed to mechanical deformation in in vitro and in vivo model systems. To determine whether increased growth factor expression was implicated in the DNA synthesis response in SMC, we performed an initial screen of several known SMC mitogens to compare their relative potency in stimulating uptake of thymidine in primary culture human bladder SMC. As anticipated, bFGF and HB-EGF stimulated DNA synthesis in bladder SMC, however they displayed modest potency relative to PDGF-BB which was the most potent factor tested. PDGF has long been known as an extremely potent mitogen for vascular SMC, however its activity on bladder SMC proliferation has not been investigated. In view of its ability to stimulate DNA synthesis in bladder SMC, PDGF-BB may be implicated in the increase in mass of the bladder wall following outlet obstruction. PDGF has not been directly implicated in the hypertrophic or hyperplastic response of the bladder wall to outlet obstruction with the proliferative response largely attributed to members of the FGF, EGF-like and IGF growth factor families. However PDGF-B gene expression is known to be regulated by shear-stress in certain cell types such as endothelial cells. PDGF-B released from such cells in response to mechanical stimuli may then act in a paracrine manner on underlying

SMC to achieve its effects. Although PDGF has not been identified as a mechanically-regulated molecule in bladder SMC in vivo or in vitro, it is possible that PDGF is elaborated by the epithelial component of the bladder wall, which is also exposed to mechanical deformation, where it can participate in paracrine stimulation of the associated SMC [Li et al., *Hypertens. Res.* 20 217-23; Wilson et al., *Hypertension* 31, 170-5; Wilson et al., *J Cell Biol* 123, 741-7; Sumpio *J Surg Res.* 44, 696-701; Sumpio *J Vasc Surg* 10, 570-1; Omer et al., 1992 *Am. J. Physiol/Renal* 263, R1284-90; Sadoshima et al., *JBC* 267, 10551-60].

Figure 1

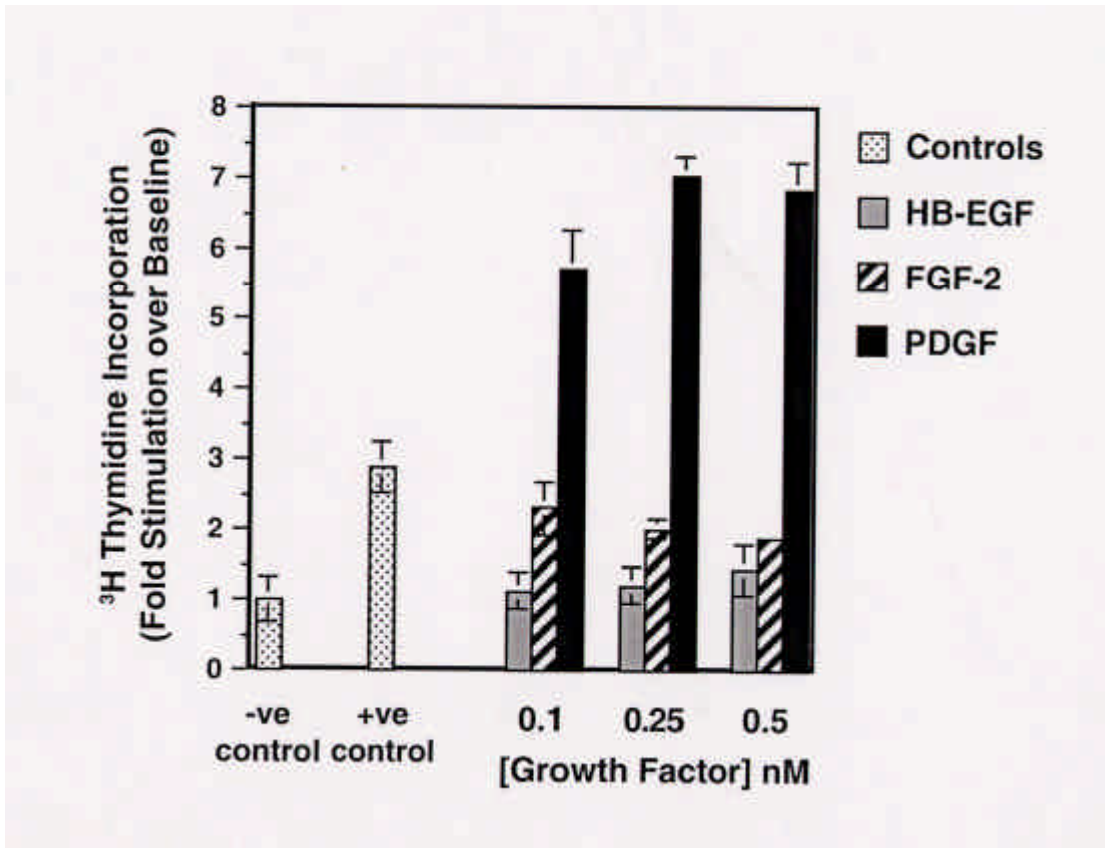
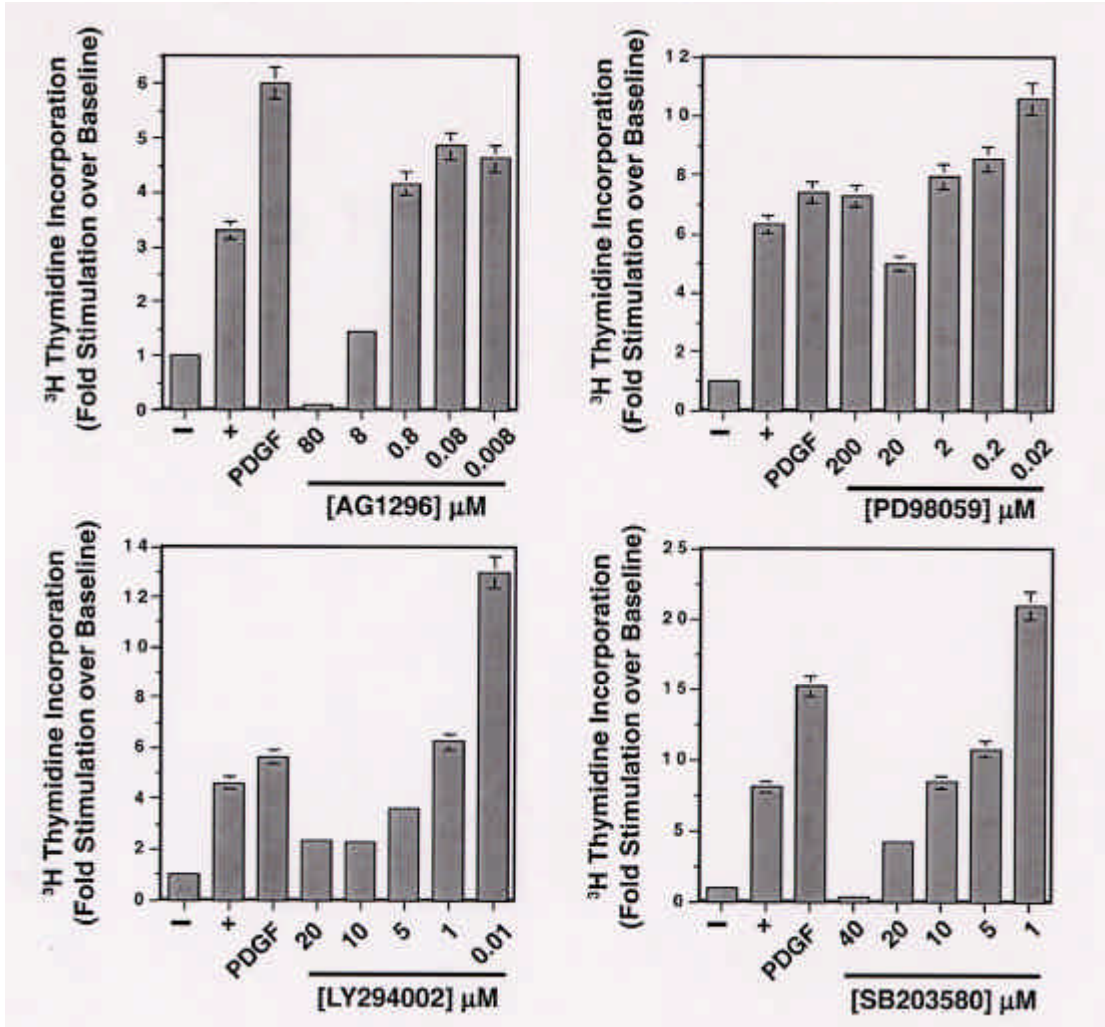
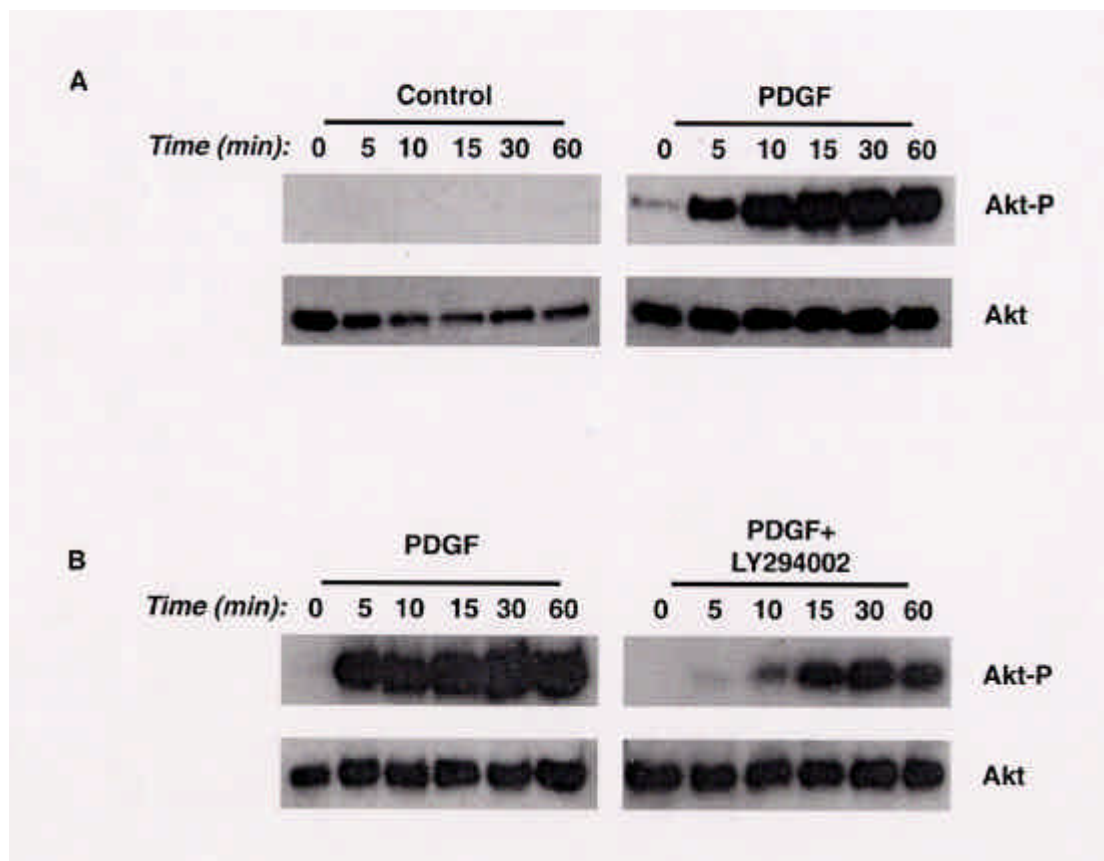


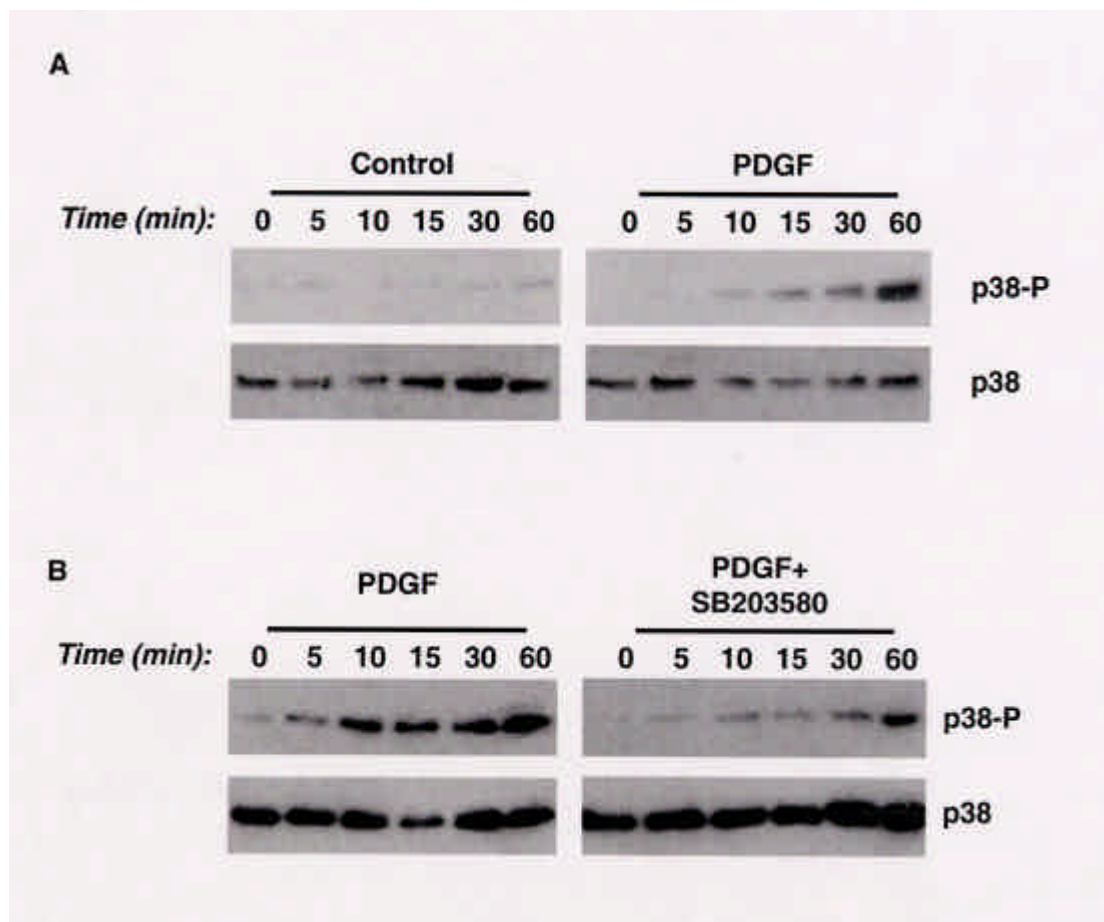
Figure 2



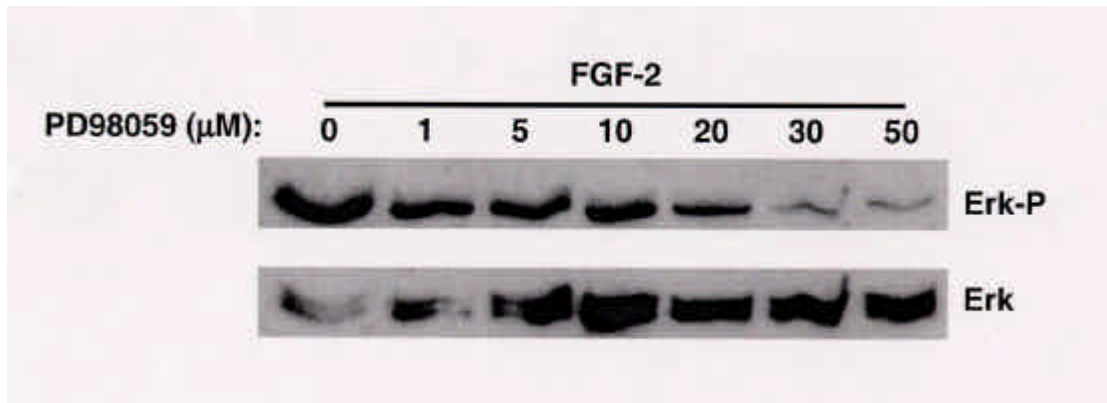
**Figure 3**



**Figure 4**



**Figure 5**









### III. 心得

小兒神經性膀胱是一種罕見的泌尿道疾病，直到 1960 年代引進 ventriculo-atrial shunt 來治療腦室積水(hydrocephalus)。從那時候起，神經外科醫師才積極的關閉縫合脊髓脊髓膜膨出(meningomyelocele)的皮膚，再配合抗生素的使用，得以克服嬰兒階段腦室積水及脊髓膜炎的問題，這些患者才能夠存活。漸漸地，包括神經性膀胱，越來越受到重視。其它後遺症如嚴重便秘，下肢變形，下肢行動困難等等問題，亦不等程度的同時存在。據估計脊柱裂患者中，百分之九十五的泌尿系統異常；百分之十至三十的新生兒，上泌尿道水腫；追蹤至五歲，上泌尿道水腫的情形，增加至百分之五十。Wu et al. 報告原始的影像學檢查結果，百分之二十八者腎水腫，百分之二十一者有膀胱輸尿管尿迴流。神經性膀胱發生的原因，分為先天性和後天性命，先天性的原因如，脊柱裂，薦骨發育不良，腦性麻痺，無肛症等；後天性者如，脊椎損傷及中樞神經感染等。其中以脊柱裂中的脊髓脊髓膜膨出最常見。脊髓脊髓膜膨出發生的原因，與胚胎發育階段葉酸(Folic acid)缺乏有關；因此目前媽媽懷孕過程普遍地補充葉酸，這類患者有減少的情形，但還是有新病例。神經性膀胱的影響，大類歸還為二項，1. 尿失禁，患者下部及會陰部，始終浸泡在尿液裡，容易引起濕疹，細菌霉菌感染，破皮及褥瘡，將影響個人的衛生及健康；長期包尿片，產生尿騷味，嚴重影響個人的生活品質與人格發展。2. 腎功能不全及腎衰竭，神經性膀胱患者中，逼尿肌與尿道外括約肌之間不協調，百分之七十二腎功能不全；逼尿肌與尿道外括約肌之間正常者，百分之二十二腎功能不全；尿道外括約肌沒有功能者，將有百分之十一腎功能不全；據統計在 1970 及 1980 年代，脊髓脊髓膜膨出的患者，到 7 歲時，將近三分之一的人腎功能惡化至腎衰竭的程度，因此社會國家將付出很大醫療成本，照顧這些患者。這項議題非常值得重視。

早期，為了避免腎衰竭的發生，針對逼尿肌與尿道括約肌之間不協調的病例，實施尿道括約肌切開術，造成低阻力的尿道括約肌，避免腎功能惡化；或採取輸尿管分流及輸尿管造瘻，不讓尿液進入到膀胱。神經性膀胱的處理，最重要的進步，始自 1972 年 Lapidès 提出間歇性導尿(clean intermittent catheterization, CIC)，這種被公認是一種既安全，可靠同時容易學習的方法。目前普遍地被接受來處理成人及小孩的神經性膀胱。小孩因神經性膀胱問題需要實施間歇性導尿，建議始自新生兒或一診斷之際，規則性的間歇性導尿，能夠完全排空膀胱尿液，有效地達到保護腎臟的效果；據報告，規則性的間歇性導尿，能夠遏止這類病人腎衰竭的發生。從新生兒就開始間歇性導尿的用意，讓小孩子習慣間歇性導尿，因為可能一輩子都需要導尿。青春期階段，叛逆性較大，必須密切追蹤，容易因不合作導致腎功能變化。Lapidès 的報告，218 位因為神經性膀胱造成感染或尿失禁患者，實施間歇性導尿後，百分之四十八的人沒有尿路感染的情況；雖然有百分之五十二的人，尿液內可以培養出細菌，但只有一次是腎盂腎炎。有兩位發生尿道炎或副睪丸炎。33 位原先有腎水腫，腎水腫的情況得到改善。以後的報告也顯示，雖然有菌尿症，但鮮少因此引起腎盂腎炎。間歇性導尿，對膀胱輸尿管尿迴流的問題也有幫助；Joseph 的研究顯示，33 位神經性膀胱的新生兒，16 位有膀胱輸尿管尿迴流，輕微尿迴流者中，百分之五十三消失，百分之四十七結果穩定。但 3 位嚴重尿迴流者，都沒有得到改善，都需要開刀治療。預期對於只有腎水腫的患者，實施間歇性導尿，也會改善上泌尿道的擴張。Lindehall et al 報告 87 位使用間歇性導尿的患者，11 位只有腎水腫沒有膀胱輸尿管尿迴流的患者，經過十年的追蹤，腎水腫的情形都得到改善。除了實施間歇性導尿之外，假如再加上膀胱鬆弛劑，對改善嚴重尿迴流的問題，也有助益。間歇性導尿亦是解決神經性膀胱引起的尿失禁的方法；Cass et al 報告 84 位小兒神經性膀胱患者，以間歇性導尿治療，經三年的追蹤，全乾 41 位 (49%)，偶而尿濕 14 位 (17%)，常尿濕 8 位 (9%)，半乾及半尿濕 7 位 (8%)，

全尿濕 14 位(17%); 百分之七十五有接受藥物控制膀胱的活動性. 因此間歇性導尿已變成泌尿科處理任何年紀膀胱功能的重要手段.

最常使用來控制膀胱 hyper-reflexia 及 hypertonicity 的藥物, 是 oxybutynin hydrochloride (Ditropan®), 這是一種膀胱逼尿肌的鬆弛劑, 能夠有效地降低膀胱內壓力及抑制膀胱不穩定的情況, 有效地增加膀胱容積. 配合間歇性導尿, 能夠解決輕微的膀胱尿迴流和非逆流性的上泌尿道擴張. 服用 oxybutynin hydrochloride 常見的副作用, 包括口乾及便秘.

約有百分之五十至六十的患者, 在併用膀胱逼尿肌的鬆弛劑與間歇性導尿之後, 仍然持續的尿失禁. 這種情形必須考慮是否膀胱容積減少, 尿道括約肌功能不足, 或兩項同時存在. 假如年齡夠大, 可以考慮手術治療. 膀胱容積減少, 以膀胱整形術來增加膀胱容量, 增加膀胱的組織來源可分為胃, 小腸, 大腸, 輸尿管或合併使用兩種以上組織. 使用部分胃組織的優點: 黏液少不容易妨礙導尿的進行; 不會吸收水分與電解質, 對腎功能不全者, 不會造成電解質不平衡. 缺點: 百分之五十的人, 發生小便疼痛與血尿的現象, 和胃組織分泌酸液有關. 使用腸組織的優點: 可以達到增大膀胱的效果; 缺點是黏液易阻塞導尿管, 大腸會吸收水分, 對腎功能不全者不利. 以一邊無功能擴張的輸尿管, 同樣是移行性上皮也是一種選擇. 目前以組織工程培養人工膀胱用來增加膀胱容積, 在未來又是另一種選擇. 尿道括約肌功能不足的手術治療, 目的增加尿道阻力. 方法包括, bulking agent 如 Teflon, Collagen, 注射在括約肌位置增加尿道阻力. 膀胱頸重建術, fascial sling 膀胱頸懸吊, 人工尿道括約肌植入, 都可以解決尿失禁的問題; 但術後仍須持續導尿, 同時密切追蹤膀胱功能, 一旦膀胱內壓力持續上升現象, 以藥物控制無效, 就必須進行膀胱整形手術. 假如膀胱容積減少與尿道括約肌功能不足, 同時存在, 就必須同時進行膀胱整形手術與增加尿道阻力手術.

間歇性導尿，是處理神經性膀胱的重要方法。但是常遭遇自我導尿困難的情形，原因可能是尿道狹窄，不正常的膀胱頸，或身體畸形不易導尿。因此必須想辦法解決。1980年，Mitrofanoff 描述利用闌尾連結膀胱與下腹部皮膚間，形成闌尾膀胱造口術(appendicovesicostomy)，膀胱端合併抗逆流構造，避免尿失禁；使用 10 至 12Fr. 導尿管，由皮膚開口導尿，Continence rates 約百分之九十至百分之一百。能夠很有效達到禁尿及導尿的目的。副作用，包括皮膚造口狹窄，造口突出，闌尾通道彎曲扭轉困難導尿。以上副作用均可以手術方式矯正。假如闌尾已被切除或另有用途，可以輸尿管，小腸或膀子管代替。Mitrofanoff 原則，是下泌尿道重建的重要貢獻；技術易學而且成功比率高。

小兒神經性膀胱患者，常合併嚴重的便秘。因此解決了尿失禁與腎水腫的問題之外，也必須解決大便不通的問題。傳統上，以鼓勵多喝水，多攝取纖維質，服用軟便藥，緩瀉劑或灌腸等方法處理；但效果非常有限，困擾患者和家屬的程度，比起尿失禁有過之而無不及。1990年 Malone et al. 採用 Mitrofanoff 的原則，發展出 antegrade continence enema (ACE procedure)。這種 enema 方式可以把整個大腸的糞便，完全清理乾淨，避免便秘的發生。方法是利用闌尾或小腸，連接盲腸與皮膚間，形成盲腸與皮膚間的造口術。同樣地，盲腸端形成抗迴流的構造，避免糞便失禁。成功率百分之七十至九十五，生活品質得到很大的改善。手術完成後，以 12 Fr. 導管灌注自來水充洗，量由少至多調整，間隔亦調整至最佳狀態。副作用，造口處狹窄。

尿路動力學檢查，是檢查神經性膀胱不可缺少的工具；以往普遍實施於成人患者。對小孩子而言，受限於配合度，或家屬的憂慮，國內這類檢查應用於小孩，並不多見。事實上，假如配合措施施行得當，要實施於小孩，並不困難。檢查結果，在早期診斷神經性膀胱的功能，與神經理學檢查，具有相輔相成的效果；尿路動力學檢查，有神經理學檢查無法完全取代的角色。另外，尿路動力學檢查，

對神經系統疾病的變化，也能夠早期發覺出來。

這類患者後續的追蹤，也重要的議題。因為原始神經系統疾病，不是保持靜止不變，卻可能發生變化。神經系統疾病會引起泌尿道，腸胃道及骨骼系統的后遺症；同樣地，後遺症的惡化，亦可能代表神經系統疾病仍在進行。如患者發生背痛，下肢畸形加劇，重復性的尿路感染，尿路動力學檢查結果發生改變，都要懷疑發生 tethering cord。因此需要在包括小兒神經科，小兒骨科，小兒泌尿科及神經外科等專科醫師的門診密切追蹤，以便即時矯正。

#### **IV. 建議**

1. 加強宣導神經性膀胱的影導及儘早診斷的必要.
2. 鼓勵實施尿路動力學檢查於小兒神經性膀胱患者.
3. 聯合與小兒神經性膀胱各專科醫師密切地追蹤.