行政院國家科學委員會專題研究計畫執行進度報告

計畫題目:膀胱在過度澎脹解除後所產生的病理生理變化：自由基所扮演角色之探討

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一、 中文摘要

膀胱過度膨脹造成的氧化性傷害可能會造成膀胱收縮功能不良。缺氧預適應對於器官的缺血性傷害具有保護作用，然而，這樣的保護效果能否應用在膀胱過度膨脹/緩解所造成的氧化傷害目前尚未被提出報告。我們將大鼠分成海平面對照組（sea level group, SL）和長期缺氧組（high altitude group, HA，即將大鼠放置在模擬高度 5,500 m 的低壓低氧太空艙中，每天 15 小時，歷經四週)。我們輸入生理食鹽水將大鼠的膀胱分別過度膨脹一小時及兩小時（1 ml/100 克體重）而後緩解。使用多功能記錄器來測量此過程對兩組大鼠膀胱收縮壓的影響，並以超低感度的化學冷光儀來測定血液及活體膀胱表面自由基釋出量的變化。結果顯示 SL 鼠和 HA 鼠在遭受膀胱過度膨脹/緩解後，膀胱收縮壓皆顯著下降；然而，在過度膨脹緩解後 120 分鐘時，HA 鼠的膀胱收縮壓恢復情形較 SL 良好。在自由基的釋出量方面，膀胱過度膨脹/緩解會增加 SL 鼠血液以及膀胱表面的自由基釋放量，在過度膨脹緩解後的 5 分鐘，自由基的釋放量達到最高峰；在 HA 鼠膀胱過度膨脹/緩解過程，自由基的釋放量亦有增加的現象，然而，HA 鼠自由基的釋放量和 SL 鼠相比呈現明顯的減少。我們另測量 SL 鼠和 HA 鼠膀胱中抗氧化酵素 Cu/Zn SOD 及熱休克蛋白 70(Heat shock protein, HSP 70)的含量變化。結果發現 HA 鼠的 Cu/Zn SOD 和 HSP 70 的表現量比 SL 鼠者更為明顯。因此，我們推測 HA 鼠膀胱中 Cu/Zn SOD 及 HSP 70 的增加可能和膀胱過度膨脹/緩解之傷害過程的保護作用有關。

關鍵詞： 缺氧前處理；膀胱過度膨脹; 氧化傷害; 自由基;

Abstract

Oxidative stress resulting from bladder overdistension and relief (OD/RE) may contribute to micturition dysfunction. Hypoxia adaptation produced cardioprotection against ischemic insults whether this benefit extends to ameliorate OD/RE induced bladder damage is not yet defined. We compared rats kept at sea level (SL) and chronically hypoxia rats (high altitude; HA, induced by exposure to an altitude chamber at 5,500 m for 15 days/day). The bladders of SL rats and HA rats were subjected to 1-h or 2-h of saline overdistension (1 ml/100 gw body weight) and followed by relief. We analyzed bladder function by transcystometrogram and determined the amount of reactive oxygen species (ROS) from the whole blood *in vitro* and bladder surface *in vivo* by a chemiluminescence method. Results showed that OD/RE reduced the intravesical pressure in both SL and HA rats. However, the functional recovery of urinary bladder 2-hr after relief from bladder overdistention was significantly better in HA rats than in SL rats. In addition, ROS release from the blood samples and the bladder surface was increased in SL rats. The enhanced ROS release reached the peak 5 min after bladder relief. ROS release in HA rats was also increased, but less than that of the SL rats. The expression of Cu/Zn superoxide dismutase (Cu/Zn SOD) and heat shock protein 70 (HSP70) was upregulated in the hypoxia adapted bladder. Our results suggest that the increased Cu/Zn SOD and HSP 70 might be related to the acquisition of bladder protection against OD/RE.

Keywords: hypoxia preconditioning; bladder overdistension; oxidative stress; free radicals

二、 緣由與目的

Bladder overdistention is an unpleasant, painful experience that often takes place in patients with prolonged urinary retention secondary to bladder outlet obstruction [1]. Population studies reveal that acute urinary retention is a common event: a 60 year-old man who survives a further 20 years has a 23 per cent probability of experiencing an acute episode of urinary retention. Men with a clinical diagnosis of benign prostatic hyperplasia and a symptom score 8 or greater had the highest rates of acute urinary retention with age adjusted incidence 13.7/1,000 person-years [2]. Apart from patients with bladder outlet obstruction, bladder overdistention may also occurs in some females or young aged males, particularly in those who are enforced to over hold their urine due to professional inconveniences, such as sales lady in the department store, taxi driver and truck driver. Bladder overdistention may induce several detrimental effects on bladder functions. In animal studies, Lasanen et al have shown that bladder overdistention for 3 h result in transient damage to the cholinergic innervation, which may in turn explain the prolonged voiding difficulties often seen after catheterization of an overdistended bladder in a patient with urinary retention [3]. Overdistention may have long-lasting effects on the bladder. Prolonged micturition problems are often encountered after long-term bladder overdistention. By using histological and enzyme histochemical methods, Leppilahti et al [4] investigated the time course of bladder overdistention. He demonstrated that bladder overdistention cause transitional morphological changes in innervations which correlate with changes in micturition and bladder contractility. After bladder overdistention for 3 h, edema was seen beneath the mucosa of the rat urinary bladder at 12 h, with hyperemia and hemorrhages. The urothelium also showed some disruptions and degenerative vacuolization. The edema reached its maximum at 48 h, and large numbers of inflammatory cells were also seen. Damage to some muscle cells was also seen. These damages however are, almost completely healed within one week. [4]. If bladder distention progresses, the bladder may change from a state of compensation to decompensation, in which there are severe, irreversible alterations in bladder function [5]. In addition to the effects on urinary bladder itself, bladder distention may exert detrimental effects on systemic organs. Our recent experiment conducted on rat has demonstrated that bladder overdistention induces sympathetic over-activity, which in turn reduces renal blood flow and impairs renal function [6,7]. Similar findings have also been observed in clinical patients [1]. Another study conducted by our colleagues also showed that bladder distention in men produces coronary vasoconstriction. [8].

In our clinical practice, voiding dysfunction manifested as frequency, weak urinary stream and incomplete emptying of unknown etiology is not uncommon seen in apparent healthy females or young age males. Urodynamic studies in these patients commonly

demonstrated subnormal maximal flow rate and interrupted and prolonged flow pattern, without evidence of bladder outlet obstruction or overt neurological abnormalities. History tracking on these patients frequently disclosed experiences of frequent and/or prolonged bladder overdistention in the past. Given the facts that prolonged urinary retention may induce bladder dysfunction, it is tempting to speculate that the voiding dysfunction in these patients may be at least in part, a consequence of bladder overdistention. Interestingly, an epidemiological study conducted on adult Taiwanese females has shown that the prevalence rate of voiding problems in the adult females is much higher than encountered in clinical practice [9]. As women tend to over hold their urine due to sanitary considerations of the public toilets, it would be of great interest to investigate the relationship between voiding dysfunction and history of bladder overdistention in some apparently healthy subjects.

The detrimental effect of bladder overdistention on bladder function has also been studied in our laboratory recently. In rat subjected to bladder overdistention, bladder blood flow reduced by approximately 85% and regained after the evacuation of urine, mimicking an ischemia/reperfusion (I/R) situation. The reactive oxygen species (ROS) generated from bladder surface increased gradually during distention, reaching a peak level within 5 minutes after bladder emptying, then declined gradually to baseline level. Bladder contractility was affected significantly after bladder distention and took a longer time to recover when compared to the decline of ROS. The recovery of bladder function was significantly impaired in rat subjected to prolonged bladder distention [10].

There is growing evidence that ROS release may cause tissue injury through a variety of pathways. It may initiate a cascade of necrosis/apoptosis, and subsequent inflammatory infiltration in some organ subjected to I/R [11,12]. The mechanism responsible for post-reperfusion apoptosis is attributed to the release of ROS [11-13] or the increasing activity of endonuclease by elevation of calcium entry [14]. Buttke and Sandstrom [15] have proposed that ROS induce apoptosis by causing DNA damage, oxidation of lipid membranes or direct activation of the genes responsible for apoptosis.

三、方法

Surgery

Female Wistar rats weighing 200-250 g were housed at the Experimental Animal Center of National Taiwan University at a constant temperature and with a consistent light cycle (light from 0700 to 1800 hours). The animal care and experimental protocols were in accord with the guidelines of the National Science Council of the Republic of China (NSC 1997).

On the day of the experiment, rats were anesthetized with subcutaneous urethane (1.2 g/kg).

Urethane was chosen because it lacks ganglionic blocking properties, and the extrinsic neural input to the bladder is therefore maintained. After induction of anesthesia, the trachea was intubated to keep the airway patent. All animals were allowed to breathe spontaneously. PE-50 catheters were placed in both the left femoral vein, for administration of supplementary anesthetic, and the right femoral artery, for continuous arterial BP recording with a pressure transducer (P23 1D, Gould-Statham, Quincy, USA). The maintenance of deep anesthesia was determined by the persistence of miotic pupils as judged from frequent inspection and by the lack of heart rate and BP fluctuations in the absence of visceral stimuli. Body temperature was monitored continuously and maintained in a range of 36-38°C with a heat lamp. At the end of the experiments, the animals were sacrificed under deep anesthesia by intravenous injection of a potassium chloride saturated solution.

NM, CBOO

Eight rats were used for establishing the NM and CBOO models. The NM experiment was performed first. The urinary bladder was exposed through a midline incision in the abdomen. A PE-50 tube was inserted through the apex of the bladder dome, 3-4 mm into its lumen. The bladder catheter was connected via a T-tube to an infusion pump (Infors AG, CH-4103, Bottmingen, Switzerland) and a pressure transducer (P23 ID, Gould-Statham, Quincy, USA), for recording of IVP. The bladders were filled several times by continuous infusion of 0.9% saline (0.1 ml/min) at room temperature and were allowed to drain/micturate repeatedly. The infused volume at the onset of an efficient voiding contraction was defined as the micturition threshold volume of NM.

To induce CBOO in the same rat, we inserted another PE-50 tube into the bladder through the urethra and tied it in place by a ligature around the urethral orifice. The catheter was connected to a separate pressure transducer and an infusion pump via a T-tube connector. Transurethral filling (TUF, 0.1 ml/min) of 0.9% saline into the urinary bladder via the urethral catheter was done until rhythmic bladder contractions occurred, the infusion was then stopped, and the bladder was maintained under constant-volume conditions. The infused volume at the onset of isovolumetric contractions was defined as the threshold volume of CBOO (isovolumetric condition). Isovolumetric conditions of CBOO of the bladder were determined for 30 min.

Free Radical Determination

A technique for *in vivo* determination of free radicals in the insulted bladder by Chemiluminescence (CL) Analyzing System (CLD-110, Tohoku Electronic Industrial Co., Sendai, Japan) was introduced in our laboratory. The CL was measured in an absolutely dark chamber of the CL Analyzing System. Photon emission from the intact bladder was counted at 10 s intervals at 37°C and under atmospheric conditions. After surgery, the rat was placed into the sample chamber (Model TLU-17,) and the bladder was exposed for measuring CL. To record ROS production *in vivo* optically, MCLA (A5309, TCI-Ace) which is particularly

sensitive to superoxide, was injected directly into the control or bladder through bladder artery. MCLA-enhanced CL signal from the bladder surface was measured. For determination of CL in whole blood, 0.2 ml of renal venous blood was collected and analyzed the CL response by lucigenin as described previously. Blood CL was also determined to confirm the result from *in vivo* bladder.

Immunoblot Analysis for CuZnSOD and HSP70

Protein samples were isolated from the bladders, and the expression of CuZnSOD and HSP70 was determined by western blot as described previously.

三、 結果與討論

一、 膀胱過度膨脹/緩解對膀胱收縮壓的影響

膀胱灌注 1.5 ml 的生理食鹽水時，從結果可以看出膀胱收縮時，動脈血壓增加、心跳速率增加、膀胱血流下降、阻力（血壓/血流）增加以及膀胱收縮壓增加。從膀胱收縮壓原始圖中也可以看出，無論是 SL 鼠或 HA 鼠，膀胱過度膨脹兩小時/緩解後 30、60、90、120 分鐘時，膀胱收縮壓與基礎期相比較皆有下降的情形，而其下降數值可從表一中呈現出來。在 SL 鼠 OD1H 組（n=5）中，膀胱正常收縮壓數值約為 25.33 ±1.88 mmHg，然而，在過度膨脹緩解後 30、60、90 以及 120 分鐘時，膀胱收縮壓和基礎期相比，均有明顯下降的情形。而在緩解 120 分鐘時，膀胱收縮壓和緩解 30 分鐘時相比，其收縮壓有逐漸恢復的情形。同樣的，在 SL 鼠 OD2H 組（n=5）中，膀胱正常收縮壓數值約為 26.23 ±1.76 mmHg，而在過度膨脹緩解之後，膀胱收縮壓亦均明顯下降。而在緩解後 60、90、120 分鐘時，膀胱收縮壓和緩解後 30 分鐘時相比，其收縮壓亦有逐漸恢復的情形。在 HA 鼠方面，OD1H 組（n=5）和 OD2H 組（n=5）的膀胱正常收縮壓分別為 24.53 ±1.91 mmHg 和 27.43±1.81 mmHg，而在膀胱過度膨脹緩解後，其收縮壓數值和基礎期相比亦均顯著下降。而在 OD1H 組膀胱過度膨脹緩解後 120 分鐘時，其收縮壓數值和緩解後 30 分鐘相比，有逐漸恢復的情形。

另外，在 SL 鼠與 HA 鼠膀胱收縮壓下降量的比較中，在過度膨脹緩解後 30 分鐘時，OD1H 組中，SL 鼠膀胱收縮壓下降量和 HA 鼠相比，沒有顯著差異；而在 OD2H 組中，SL 鼠和 HA 鼠相比則有顯著差異，顯示 SL 鼠膀胱收縮壓下降較多。在緩解後 60 分鐘時，OD1H 組和 OD2H 組的膀胱收縮壓下降量，SL 鼠和 HA 鼠相比沒有顯著差異。而在緩解後 90 分鐘時，OD2H 組的膀胱收縮壓下降量兩組相比有顯著差異，顯示 HA 鼠的膀胱收縮壓有逐漸恢復的情形。直至緩解後 120 分鐘時，無論 OD1H 組或 OD2H 組，SL 鼠和 HA 鼠相比均有顯著差異，顯示 HA 鼠的膀胱收縮壓力雖有下降，但恢復情形較 SL 鼠良好。

二、 膀胱過度膨脹/緩解對膀胱內自由基釋出量的影響

（一）膀胱過度膨脹/緩解對動脈血中自由基釋出量之比較

1、SL 和 HA 鼠在膀胱分別過度膨脹一小時以及兩小時/緩解過程中，自由基釋出量的情形從原始圖中可以看出，SL 鼠在膀胱過度膨脹未緩解、以及緩解後 5 分鐘、60 分鐘以及 120 分鐘時，血液中自由基的釋出量均有增加的情形。在表二中，

SL 鼠 OD1H 組（n=8）在基礎期時血液中自由基的釋出量約為 101.78 ±6.06 counts/10sec，在過度膨脹時，自由基的釋出量顯著增加，而在過度膨脹緩解後 5 分鐘時，自由基的釋出量達到最大，直至緩解後 120 分鐘，自由基的釋出量和基礎期相比仍有增加的情形，然而在 120 分鐘時，自由基的釋出量和緩解後 60 分鐘時相比，已有逐漸下降的趨勢；在 OD2H 組（n=9）中，基礎期時自由基的釋出量約為 113.90 ±5.26 counts/10 sec，而在過度膨脹未緩解時自由基的釋出量明顯增加，而在過度膨脹緩解後 5 分鐘時，自由基的釋出量達到高峰，在 60 分鐘以及 120 分鐘時，自由基的釋出量逐漸減少，但和基礎期相比仍有增加的現象。在 HA 鼠方面, OD1H 組(n=7)在基礎期的自由基釋出量為 71.45 ±1.29 counts/10 sec，而在過度膨脹未緩解時自由基的釋出量未顯著增加，然而在緩解後 5 分鐘、60 分鐘以及 120 分鐘時，自由基的釋出量和基礎期相比有增加的情形，並且比過度膨脹未緩解時的釋出量多；在 OD2H 組（n=7）中，基礎期的自由基釋出量為 83.77 ±2.52 counts/10 sec，在過度膨脹兩小時未緩解時，自由基釋出量和基礎期相比明顯增加，直至緩解後 120 分鐘，自由基的釋出量仍有增加，不過在緩解後 60 分鐘和 120 分鐘時，其釋出量比緩解後 5 分鐘時減少。

2、SL 鼠與 HA 鼠在膀胱過度膨脹/緩解過程中，自由基釋出量的比較

在 SL 鼠和 HA 鼠血液中自由基釋出量百分比的比較上，在 OD1H 組中，過度膨脹未緩解時以及緩解後 5 分鐘，自由基的釋出量百分比在 HA 鼠均明顯比 SL 鼠來的少，而在緩解後 60 分鐘兩者無顯著差異，在緩解後 120 分鐘時，HA 鼠的自由基釋出百分比比 SL 鼠高，但無顯著差異；而在 OD2H 組中，自由基釋出量百分比在緩解後 60 分鐘時無明顯差異，而在過度膨脹未緩解、緩解後 5 分鐘、以及緩解後 120 分鐘時，HA 鼠均比 SL 鼠低。

（二）膀胱過度膨脹/緩解對活體膀胱表面自由基釋出量之比較

膀胱遭受過度膨脹/緩解整個過程中，自由基釋出量可以發現當 SL 鼠的膀胱遭受過度膨脹時，自由基釋出量增加，而在過度膨脹緩解瞬間，自由基釋出量急驟上升，而後並未有顯著下降的趨勢。在 HA 鼠也可以看到自由基的釋出量在過度膨脹時增加，而在過度膨脹緩解後，自由基的釋出量又比過度膨脹時增加。在 SL 鼠，不論是 OD1H 組（n=5）或 OD2H 組（n=5）的自由基釋出量，在任何時間點均明顯的高於其基礎期；而在 HA 鼠中，也有相類似的情形。至於 SL 鼠和 HA 鼠的比較上，在基礎期時自由基釋出量並無顯著的差異，然而，無論是 OD1H 組或 OD2H 組，在整個過度膨脹/緩解過程的任何時間點，自由基的釋出量在 HA 鼠均顯著地比 SL 鼠來得低。

三、 在 SL 鼠和 HA 鼠膀胱中抗氧化酵素及 HSP70 表現量的比較

（一）抗氧化酵素 Cu/Zn SOD 含量的比較

從各組織 Cu/Zn SOD 存在量的比較上可以看出, 膀胱組織所含 Cu/Zn SOD 比其他組織少。在量化後發現，將不同組織與肝臟相比，肺臟和膀胱中 Cu/Zn SOD 的含量顯著比肝臟低許多，顯示出膀胱中所含之 Cu/Zn SOD 的量較其他組織低。SL 鼠和 HA 鼠膀胱中 Cu/Zn SOD 含量的比較。將其結果量化後可以

發現，在正常情況下時，HA 鼠膀胱組織中的 Cu/Zn SOD 含量比 SL 鼠來的高。而在整個膀胱過度膨脹/緩解過程，HA 鼠膀胱 Cu/Zn SOD 的表現量均顯著比 SL 鼠增加。

（二）熱休克蛋白（HSP 70）含量的比較

SL 鼠和 HA 鼠膀胱中 HSP 70 含量的比較。將其結果量化後發現，在正常情況下時，SL 鼠膀胱所含 HSP 70 與 HA 鼠相比，未有顯著差異。而當膀胱遭受過度膨脹/緩解時，SL 鼠膀胱中 HSP 70 的表現量有比基礎期減少的趨勢。在 SL 鼠和 HA 鼠的比較上，HSP 70 的含量在每一時間點均明顯的高於 SL 鼠。

本實驗的結果可歸納成幾點：（一）當膀胱遭受急性過度膨脹/緩解後，海平面鼠和長期低氧鼠的膀胱收縮壓均顯著下降，但是，長期低氧鼠的膀胱收縮恢復情形較海平面鼠佳。（二）當膀胱遭受急性過度膨脹/緩解過程中，無論在活體膀胱表面或血液中，會增加超氧陰離子自由基的釋出量；然而，長期低氧鼠的自由基釋出量較海平面對照鼠顯著減少。（三）長期低氧鼠的膀胱組織 Cu/Zn SOD 的表現量高於海平面對照鼠。（四）長期低氧鼠的膀胱組織 HSP 70 的表現量高於海平面對照鼠。

膀胱急性過度膨脹/緩解之後，會降低膀胱的收縮壓力。而長期低氧鼠在膀胱急性過度膨脹/緩解過程所釋放的自由基量較少，同時，長期低氧鼠的膀胱中含有較高的 Cu/Zn SOD 酵素以及較高的 HSP 70。因此，Cu/Zn SOD 和 HSP 70 在長期低氧鼠對抗膀胱急性過度膨脹的傷害中，可能扮演了重要的角色。

五、成果自評

本計畫之成果已完成且已在期刊發表中。本研究發現膀胱急性過度膨脹/緩解之後會降低膀胱的收縮壓力且與自由基的產生相關。若能從增加抗氧能力的方式如缺氧預處理或高山運動等方式將可減低傷害而具醫學應用價值。This work was supported by the National Science Council of Republic of China (NSC89-2314-B002-350).

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