出國報告（出國類別：參加國際會議）

赴瑞典參加第 45 屆歐洲腎臟醫學會暨歐洲透析移植醫學會大會並發表論文

服務機關：國防部軍醫局

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派赴國家：瑞典

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摘要

職參與此次醫學會之主要目的在發表由國軍左營總醫院與高雄榮民總醫院所合作關於「代謝症候群」與「高血壓」之間的病理機轉研究成果。由於代謝症候群及高血壓是現今的臨床醫療上的兩大課題，這類的疾病不僅使用了大量的醫療資源（費用），它們所造成的併發症（中風、心血管疾病、末期腎病變等）更是造成社會與家庭的重大負擔。因此本研究成果在未來的臨床運用上具有相當的潛力。本研究成果受到與會的各國研究高血壓與代謝症候群的學者所肯定。除發表論文外，職亦參與大會之各項研討會，特別是與軍陣醫學相關的「急性腎衰竭」相關的研討會，由各國專家學者的報告中，職個人無論在增進個人臨床的醫療診療技能或設計動物實驗架構上都有豐碩的收穫。

目次

一、目的：

職本次奉派出國之主要目的爲赴瑞典斯德哥爾摩參加「第 45 屆歐洲腎臟醫學會暨歐洲透析及移植醫學會(European Renal Association & European Dialysis and Transplant Association)年會」並發表論文。職本次發表的論文題目爲「腦幹孤立束核神經細胞胰島素阻抗性是造成餵食果糖大鼠高血壓的成因 (Neural Insuin Resistance in the Nucleus Tractus Solitarii Induces Hypertension in Fructose-fed Rats)」。本次大會粗估有超過 6000 位以上的腎臟醫學、移植醫學、高血壓等醫學領域的學者專家及臨床醫師參加，其中大部分爲歐洲地區的學者，但世界各地包括美加、巴西、北非、中東、印度、日本、韓國均有學者參加。因此，藉此大會發表論文不但可以將我們的研究成果發表，更可透過與各國專家學者的討論進一步的了解未來研究的方向。此外，藉由各領域學者的專題報告，讓職更加掌握腎臟醫學界的最新研究方向及新的臨床治療原則。

二、過程：

此 7 日之行程，包含 4 日之會議，並無特殊單位之參訪，爲純學術交流與醫療知識之更新與精進。職於 97 年 5 月 9 日遊高雄出發，經倫敦轉機於 5 月 10 日抵達斯德哥爾摩。

5 月 10 日

大會報到並參加大會開幕儀式。大會開幕儀式由大會主席 Peter Stenvinkel 主持。會中說明本次大會的重要討論課題並說明 2007 年歐洲腎臟醫學會各項工作綱要。

5 月 11 日

本日發表職與高雄榮民總醫院教學研究部所共同提出的論文：「腦幹孤立束核神經細胞胰島素阻抗性是造成餵食果糖大鼠高血壓的成因 (Neural Insuin Resistance in the Nucleus Tractus Solitarii Induces Hypertension in Fructose-fed Rats)」。論文大綱如下：

前言：我們先前的研究已證實再腦幹孤立束核胰島素扮演了一個心臟血管系統調控的重要角色。本研究的主要目的在探討是否孤立束核的神經細胞發生了胰島素阻抗會造成代謝症候群大鼠產生高血壓。

方法：WKY 大鼠餵予 10%之果糖溶液作爲其飲用水，使其成爲罹代謝症候群之大鼠。我們測量餵予果糖大鼠在孤立束核直接注射胰島素的血壓心跳的變化，以 ELISA 方式測量孤立束核內內生性的胰島素含量，以 SDS-PAGE 的方式研究再孤立束核內是哪一個信息傳導分子發生異常造成餵食果糖之大鼠孤立束核內胰島素的信息傳遞發生異常。

結果：餵食果糖二週後大鼠的血壓已顯著的上昇。在此時間點，孤立束核內的胰島素有顯著的上昇。而胰島素在餵食果糖大鼠孤立束核的血壓心跳調節作用顯著的下降。而免疫墨漬研究顯示在餵食果糖大鼠孤立束核的IRS1^{S307}的磷酸化程度顯著的上昇。而IRS1 的下游信息傳遞分子包括AktS473 和eNOSS1177 均顯著的下降。而rosiglitazone可以回復IRS1 與eNOS磷酸化的變化。

結論：在餵食果糖的大鼠的孤立束核中確實會發生胰島素抗，而孤立束核的胰島素阻抗會造成代謝症候群大鼠發生高血壓。而其致病機制在於 IRS1 位於 307 位置的絲胺酸過度磷酸化所造成。

因「代謝症候群」與「高血壓」間的關係目前人有許多的不同理論，而且它們對臨床治療有相當大的影響，因此本論文引起部分此方面之學者的共鳴。

此外，職本日亦參加大會所舉辦的多項討論會，而本日的大會的主會議是由美國德州大學西南醫學中心的 Makoto Kuro-o 教授主講，題目爲「Klotho as a regulator of fibroblast growth factor signaling and phosphate/calcium metabolism」。內容主要說明 Klotho 蛋白可以調節各種細胞生長因子（包括 insulin/IGF-1, Wnt, multiple iron channel）進而保護細胞免於氧化壓力的傷害，但其中的分子機轉目前仍尚待研究。

5 月 12 日

本日主要參與大會各項研討會，本日的大會的主要會議是由美國田納西范登堡大學醫學院 Eric Neilson 教授主講，題目爲「The fibroblast in progression of kidney disease」。內容主要說明在腎臟功能衰退的過程中，纖維細胞扮演重要角色，而纖維細胞的來源是由上皮-間葉轉化 (epithelial-mesenchymal transformation, EMT) 所形成，一旦纖維細胞形成，它可以就由局部的生長因子調控而增生 (proliferation)，當局部的發炎或傷害持續，纖維細胞就會不斷的增生並分泌大量的膠原蛋白；而這些作用使腎臟組織逐漸被破壞，使腎臟通能逐漸惡化。而目前在治療慢性腎衰竭的治療方向是研究如何降低細胞激素的產生，進而減少纖維細胞的產生。

本日另參與的討論會計有：「血管生理學－由基礎與臨床之方面討論 (Vascular Biology – Basic and clinical aspects)」、「加護中心腎衰竭病患的照護 (The care of the ICU patients with ARF)」。

5 月 13 日

本日主要參與大會各項研討會，本日的大會的主要會議是由德國海德堡

Eberhard Ritz 教授主講，題目爲「Is “essential hypertension” a kidney disease?」。內容主要說明腎臟疾病會誘發高血壓的發生，然而高血壓又促進了腎功能的惡化。而目前的機轉顯示高血壓是由腎臟所引起的。有幾個可能的機轉，包括：血壓－利鈉 (pressure-natriuresis)關係向右偏移，不正常的活化腎素－血管收縮素(renin-angiotensin)系統被活化，腎臟傷害造成交感活性上升，血管舒張因子產生下降，主動脈加速老化使得脈搏壓（pulse pressure）增加。

本日另參與的討論會計有：「高血壓 (Hypertension)」、「最新的臨床研究 (Last minute trials」等。

5 月 14 日

本日由斯德哥爾摩啓程經倫敦轉機，於 5 月 15 日返國。

三、心得及建議：

1. 本次奉派參加此次國際會議使職有機會在國際會議中發表論文，藉由與各國學者討論，增加職對目前基礎醫學研究主題的許多新的研究方向，將有助於職日後研究計畫的設計。建議相關主管單位可以增加臨床軍醫官參加國際會議的機會，透過參與各種國際會議可以提升臨床軍醫的研究與臨床工作的知識與技能，進而提升整體醫療品質。
2. 本次發表之論文係職透過院際合作，由國軍左營總醫院之民診基金支助研究計畫所需經費，同時使用高雄榮民總醫院的軟硬體設施，同時獲得高雄榮民總醫院教研部資深研究員曾清俊教授的指導進行研究所獲得的成果。職感謝各級長官對國軍醫院民診基金研究計畫的支持，以及各級長官對於各國軍醫院研究計畫可與當地之研究機構、學校合作的正確指示。希望這種合作計畫能加強實施，同時建議相關主管單位可擬定獎勵方案，吸引更多軍醫同仁的參與，藉以提升各軍醫院之研究水準。

附錄一 本次論文摘要 (刊載於 NDT plus, 2008:(1): suppl 2, ii77)

was studied in our experiments: ERK, MEK, JNK, p38. Cells were grown in normal and serum starvation media.
Results: Incubation of HK-2 cells in the high glucose medium with 25 mM concentration of glucose caused decrease of phosphorylation extent of MEK, ERK and JNK, that means downregulation of their activity lower than control level in all experimental time points: 1, 4, 7 days of incubation. p38 kinase phosphorylation increased after the 7 days of incubation of the cells in the high glucose medium. Inhibition of ERK 1/2 activity, was accompanied by the cells proliferation reduction.
Application of Angiotensin I and II (100 ng/ml during 10 min) against the background of cells incubation in the high glucose medium (during 1 day) decreased activity of MAP kinases more intensively. This indicates that in our experiments effect of Angiotensins was mediated through the AT II receptors (Tadashi Inagami, 1999), which signal pathways activate phosphatases of MAPK, i.e. lead to dephosphorylation of MAP kinases.
Thus, Angiotensin application intensified effect of incubation in high glucose medium on the MAPK activity. At the same time, influence of Angiotensine I and Angiotensine II had the same tendency. This indicates that in our experiments Angiotensine I was intracellularly transformed into Angiotensine II with the help of ACE (Angiotensine Converting Enzyme).
Conclusions: Thus, with the help of studied experimental model it was shown, that Angiotensine I and II treatment against the background of high glucose medium inhibits activity of MAPK signal pathways to the level less than control.
Disclosure: This abstract was possible thanks to a grant given by ERA-EDTA short term fellowship. Ref: ERASTF 9.00-06.

SP155 NEURONAL INSULIN RESISTANCE IN THE NUCLEUS TRACTUS SOLITARII INDUCES HYPERTENSION IN FRUCTOSE-FED RATS

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Introduction and Aims: It was thought that hypertension of metabolic syndrome is due to impaired NO production in the peripheral blood vessels. However, human and animal studies revealed sympathetic overactivity were present in the metabolic syndrome. Our previous study demonstrated that insulin plays a cardiovascular (CV) regulatory role in the nucleus tractus solitarii (NTS), one of the cardiovascular regulatory centers in the brain stem. We also demonstrated that the CV regulatory effects of insulin in the NTS were accomplished through activating PI3K-PKB/Akt-NO signaling pathways. It is interesting to know whether the sympathetic overactivity of the metabolic syndrome is due to insulin resistance in the NTS. The aims of this study were to investigate whether the neuronal cells in the NTS would develop insulin resistance in rats with metabolic syndrome, and whether development of insulin resistance in the NTS cause hypertension in the metabolic syndrome rats.
Methods: Six-week-old male Wistar-Kyoto (WKY) rats were fed with 10% fructose water with/without rosiglitazone (10 mg/kg) for 2-3 weeks. To test whether insulin resistance may develop in NTS, exogenous insulin (6 mIU) was microinjected into NTS stereotaxically and the CV parameters were recorded. We also measured the endogenous insulin content in the NTS by enzyme-link immunosorbent assay (ELISA) method. NO production in the NTS was measured by NO analyzer. To test which signaling molecule was defect that cause insulin resistance in the NTS, SDS-PAGE and immunoblotting were used to measure the singaling molecules in the NTS.
Results: Blood pressure (BP) of fructose-fed rats (FFR) was significantly elevated after 2-week fructose feeding. Insulin resistance index of pheriph-eral system (HOMA-IR) did not elevated yet, but endogenous insulin in the NTS was significantly elevated in FFR at the same time. The CV responses of exogenous insulin in the NTS were diminished in FFR. While in the rosiglitazone-treated FFR, BP and endogenous insulin in the NTS were de-creased to control level. The CV responses of exogenous insulin in the NTS were restored in the rosiglitazone-treated FFR. The immunoblotting results demonstrated the phosphorylation of $IRS1^{S307}$ was significantly elevated in FFR. While the phosphorylation of its downstream molecules, Akt^{S473} and $eNOS^{S1177}$, were significantly decreased as compared with the control group. In the NTS of rosiglitazone-treated FFR, the phosphorylation of $IRS1^{S307}$ was decreased, and the phosphorylation of Akt^{S473} and $eNOS^{S1177}$ were restored.
Conclusions: In conclusion, the neuronal cells in the NTS could develop insulin resistance in FFR, and the neuronal insulin resistance in the NTS contributes to the hypertension of metabolic syndrome. The mechanism of insulin resistance in the NTS is phosphorylation on the serine 307 residue of IRS1, which interfere with insulin signaling and subsequent NO production in the NTS.

SP156 REGULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN EARLY DIABETIC NEPHROPATHY

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Introduction and Aims: The pathogenesis of diabetic nephropathy (DN), the leading cause of end-stage renal failure in most parts of the world, remains unclear todate although several factors have been incriminated including alterations in renal nitric oxide (NO) generation. Recently we characterized a newer rat model for nephropathy in type II diabetes (Prabhakar et al, JASN 2007) and also reported that eNOS expression was increased along with increased urinary VEGF excretion in ZSF rats,. The factors that regulate enhanced renal NO production in early DN has been the focus of our recent invesigations. Expression of shortened alternatively spliced variants especially eNOS 13 gene result in heterodmerization and reduction of eNOS activity. We tested the hypothesis that dysregulation of eNOS translation and/or enhanced phosphorylation by Akt (protein kinase B) may account for enhanced renal NO levels in DN. In addition, we examined renal VEGF levels since VEGF in known to activate eNOS.
Methods: Male obese ZSF rats aged 8 weeks were fed on high calorie diet for 4 weeks to maintain hyperglycemia. Lean ZSF rats served as controls. At 12 weeks, the rats were euthanized, kidneys harvested and renal tissue homegenates were examined for mRNA expression of eNOS. Quantitative RT-PCR was performed to determine if there were changes in the expression of Akt or eNOS intron 13 using primers specific to the mRNA region of Akt gene and 5' end of intron 13 gene. Urine and blood samples were also collected at the time of sacrifice for examination of VEGF, NO metabolites in urine (NOx) and plasma creatinine and creatinine clearance. NOx was measured by chemilumenescence while VEGF was measured by ELISA. A set of obese ZSF rats were sacrificed at 8th week to serve as additional time controls, after obtaining urine and blood samples.
Results: Obese ZSF rats developed proteinuria and systemic hypertension by 12th week and were hyperlipidemic. As shown in the Table, hyperfiltration in ZSF rats at 12th week was associated with increased urinary VEGF and NOx levels compared to the levels in 8th week and to lean control rats.

Urinary NOx and VEGF levels in lean and obese ZSF rats

	Uprot (mg/kg BW/day)	Ccr (L/kg BW/day)	Urinary VEGF (ng/G creat)	Urinary NOx (μM/kg BW)
Lean ZSF12 wks	216±31	5.13±0.28	161±35	19.7±6.4
Obese ZSF 8 wks	239±39	5.32±0.19	121±22	17.8±5.2
Obese ZSF 12 wks	512±45*	6.88±0.44†	486±83*	32.1±7.9*

*P<0.01 vs. obese ZSF at 8 wks. †P<0.05 vs. obese ZSF at 8 wks.

In 12 week old obese ZSF rats, mRNA expression of eNOS was enhanced while eNOS intron-13 was decreased compared to obese 8 wk old obese ZSF rats. Furthermore the Akt expression increased three folds at 12th week compared to 8th week in obese ZSF rats.
Conclusions: These data suggest that eNOS mediated NO production in the kidney is enhanced in early phase of DN. While increased renal VEGF may be a major mechanism leading to increased eNOS protein expression, decreased alternate splicing (intron 13) and enhanced Akt expression facilitating increased eNOS phosphorylation, both resulting in increased eNOS activity may be significant additional factors contributing to increased eNOS in early DN.