行政院國家科學委員會專題研究計畫成果報告

計畫題目:長期間斷性缺氧減低腎臟對缺血再灌流傷害優勢機轉之分子生物與神經生理研究

計畫編號：NSC 88-2314-B-002-399
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一、中文摘要

腎臟移植或缺血灌流所誘發之細胞凋亡為術後腎功能減低主因之一。本實驗室曾首用間斷性缺氧來處理腎臟發現其對缺血灌流之傷害具忍受性。為探測間斷性缺氧是否能減低腎臟移植或缺血灌流所誘發之細胞凋亡，我們利用 TUNEL **免疫染色**, DNA **階梯生化證據及定量** PCR **與西方墨點法探討熱休克基因與抗致死基因** bcl-2 **的表現。結果發現間斷性缺氧前處理可加強** HSP70 **與** bcl-2 **之表現並而減低** TUNEL **染色細胞** (apoptotic cell)**之數目**,**與** DNA **階梯之表現並進而減低腎缺血灌流所造成之腎功能損傷。**

關鍵詞： 細胞凋亡; **缺血灌流傷害**; **移植**; **缺氧前處理**

Abstract

Apoptosis induced during the harvest and implantation of renal transplants or in ischemia/reperfusion (I/R) injury can reduce renal function. To prevent apoptosis formation, many strategies have been endeavored. Recently, a series of studies in our laboratory have found that the kidneys of rats exposed to intermittent hypoxia adjust to their situation, becoming more tolerant to ischemic insults. To explore the beneficial mechanism induction after hypoxia preconditioning, we examined the expressions of HSP and anti-death gene (Bcl-2) in the rat kidney during the hypoxic period and I/R. The aims of this study were to elucidate the role of hypoxic preconditioning in the I/R induced renal tubular apoptosis of the rat kidneys. Hypoxic preconditioning was induced by placing the female Wistar rats (weighing 210-240 g) in an altitude chamber set at 5,500 m for 4 weeks. The bilateral renal artery occlusion was performed 45 min by hemoclip and then we removed the clip to reperfuse the damage kidneys for 1, 2, 4, 10, 15 and 24 hours. We used TUNEL, DNA laddering, quantitative PCR and western blot to evaluate the expression of apoptosis. We found that the severe apoptotic expression in the damaged kidneys was 45-min ischemia followed by 4-hour reperfusion and was attenuated by hypoxic preconditioning. Hypoxic preconditioning up-regulated bcl-2 and HSP70 expression in the I/R kidney. It is suggested that hypoxic preconditioning can attenuate the I/R induced apoptosis in the rat kidney and the protective mechanism may be ascribed to the overexpression of HSP70 and bcl-2.

Keywords: APOPTOSIS; REPERFUSION INJURY; TRANSPLANTATION, HYPOXIC PRECONDITIONING

二、緣由與目的

APOPTOSIS, found in the tissue subjected to ischemia/reperfusion (I/R) damage, impairs the renal tubular cells and reduces renal function[1]. To preserve the organ function and

morphological integrity after I/R, many tactics, such as induction of heat shock protein (HSP) by thermal stimulation[2] or drugs administration[3], have been endeavored. Recently, a series of studies in our laboratory have found that the kidneys of rats exposed to intermittent hypoxia adjust to their situation, becoming more tolerant to ischemic insults[4,5,6]. To explore the beneficial mechanism induction after hypoxia preconditioning, we examined the expressions of HSP and anti-death gene (Bcl-2) in the rat kidney during the hypoxic period and I/R.

三、方法

Treatments of Animals.

Hypoxic preconditioning was induced by placing the female Wistar rats (weighing 210-240 g) in an altitude chamber set at 5,500 m for 4 weeks as described previously[4,5,6]. The age-matched sea level (SL) control rats were similarly treated and studied at the same time. After 4 weeks in the altitude chamber, chronically hypoxic (high altitude; HA) and SL rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Renal ischemia was induced by completely occlusion of the renal artery for 45 min. Following ischemia, the rats were allowed to recover for different time frames of reperfusion in an individual metabolic cage. After urine collection, the rats were sacrificed with overdose anesthetics and the kidneys were removed.

In Situ Apoptosis Assay (ISAA).

The 6-μm thick sections of the kidney were prepared, deparaffinized, and stained by the terminal deoxynucleotidyl transferase-mediated nick-end labeling method (TUNEL) according to the method of Gavrieli et al.[7]

DNA Extraction and Electrophoresis.

DNA extraction and electrophoresis were performed as described previously by Facchinetti et al.[8].

Immunoblot Analysis for HSP70 and Bcl-2.

Protein samples were isolated from the kidneys and the expression of HSP70 and Bcl-2 was determined by western blot as described previously[2,9].

Statistical analysis.

All values are expressed as mean ± SE. For comparisons of group data, one-way analysis of variance (ANOVA) and then the Student's unpaired *t*-test were conducted.

四、結果與討論

With the TUNEL reaction and DNA laddering (Table 1), apoptosis formation was most evident in the kidney subjected 45-min ischemia and 4-h reperfusion in both groups. However, a less degree of renal tubular apoptosis was found in HA groups (7%) when compared to SL group (16%). Additionally, 24 h after I/R, a less expression of tubular necrosis in the corticomedullary junction and normal renal excretion ability were found in the

HA group. These data indicated hypoxic preconditioning reduced renal damage during I/R.

After 4 weeks of hypoxic preconditioning, HSP70 and Bcl-2 were overexpressed in the kidney (Table 1). After 4 weeks of hypoxic preconditioning, HSP70 and Bcl-2 were overexpressed in the kidney (Table 1). HSP70 is a member of the family of proteins known as chaperones. These proteins can function to promote correct protein folding and prevent inappropriate protein interactions, because during I/R, tertiary structure of proteins may change sufficiently to alter function by free radicals and marked increase in intracellular calcium[2]. In contrast to the pattern of expression for HSP70 during hypoxic preconditioning, Bcl-2 is also up-regulated in the hypoxia-treated kidney. Bcl-2 has a role in suppressing the production of reactive oxygen species and lipid peroxidation[9,10], and a regulatory effect on intracellular calcium levels. The anti-apoptotic function of Bcl-2 in response to various stimuli is well known[9,10]. Over-expression of Bcl-2 has been demonstrated to prevent neuronal death and induce tolerance against ischemic neuronal death[11]. Taken together, the ability to overexpress HSP70 and Bcl-2 within the kidney and protect the tubular cells without any apparent detrimental effects introduces the possibility of future therapeutic opportunity.

In conclusion, hypoxic preconditioning up-regulates Bcl-2 and HSP70 expression in the rat kidneys and attenuates renal ischemia/reperfusion inducing apoptosis formation.

Table 1. Bcl-2 and HSP70 Gene Expression and Renal Function between the kidneys of SL and HA rats

	Time after Reperfusion (hr)					
Group	C	0	1	4	10	24
SL (n=4)						
Apoptotic cells	0±0	0±0	2.3±0.9	16±3	0±0	0±0
DNA ladder	-	-	+	++	-	-
Bcl-2	+	+	+	+	ND	ND
HSP70	-	+	+	+	ND	ND
Urine flow (μl/min)	18±4	0±0	16±4	28±6	11±3	2±1
HA (n=4)						
Apoptotic cells	0±0	0±0	1.9±0.5	7±3*	0±0	0±0
DNA ladder	-	-	+	++	-	-
Bcl-2	++*	++*	++*	++*	ND	ND

HSP70	++*	++*	++*	++*	ND	ND
Urine flow (μl/min)	21±4	0±0	17±5	23±5*	21±5*	17±4*

The apoptotic cells were calculated for each sample by counting the number of TUNEL positive stained cells divided by the total numbers of cells counted ×100. The results of Bcl-2 and HSP70 were expressed as: –: < 100 units; +: 100 to 200 units; ++: >200 units, with units calculated from the signal intensity from a densitometer. * P<0.05 when compared to SL.

五、成果自評

本計畫之成果已發表在 Transplantation Proceedings, 31, 2012-2013, 1999. **本研究使用之間斷性缺氧技術及數據與實驗前之推論及初步結果極為相近。間斷性缺氧所誘發之保護蛋白**(HSP70 **與** Bcl-2)**是抗氧化能力甚強之基因。若能以基因治療法應用於器官之強化與保存與減低器官缺血灌流傷害是極具醫學應用價值。另一方面之神經生理研究發現四種受器會受某一** cytokine **影響此一結果已在** 2000 **年** Kidney International 57 volume **中發表。這一研究將是世界首例以神經生理來觀測細胞傷害之文獻。**

六、參考文獻

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