UNIVERSITI PUTRA MALAYSIA

A CORRELATION BETWEEN PROTEINURIA, ENZYMURIA AND KIDNEY HISTOPATHOLOGICAL CHANGES DURING EARLY RENAL DAMAGE INDUCED BY GENTAMICIN

SAIRAH BINTI ABDUL KARIM

FPV 2004 6

A CORRELATION BETWEEN PROTEINURIA, ENZYMURIA AND KIDNEY HISTOPATHOLOGICAL CHANGES DURING EARLY RENAL DAMAGE INDUCED BY GENTAMICIN

By

SAIRAH BINTI ABDUL KARIM

Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the Requirements for the
Degree of Master of Science

May 2004

DEDICATION

To my parents, Mr. Abdul Karim Jabar and Mrs. Noli Othman, my family members (especially Kak Awi and Abang Shaharul) who encouraged me to pursue a profession I would enjoy for a lifetime.

To my husband, Mohd. Fadhil and son, Faiz Isqandar, who bring me a great happiness.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

A CORRELATION BETWEEN PROTEINURIA AND ENZYMURIA AND KIDNEY HISTOPATHOLOGICAL CHANGES DURING EARLY RENAL DAMAGE INDUCED BY GENTAMICIN

By

SAIRAH BINTI ABDUL KARIM

May 2004

Chairman : Professor Rasedee Abdullah, Ph.D.

Faculty : Veterinary Medicine

This study was carried out to determine the correlation between urine proteins and urinary enzyme markers, aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT) and alkaline phosphatase (ALP) and tissue histopathological changes during early renal damage induced by gentamicin. Fifty five Sprague-Dawley rats, aged 7-8 weeks old were divided into two groups. One group of ten rats served as controls and forty-five rats were treated with gentamicin intraperitoneally to induce renal damage. Twenty four hour urine samples were collected daily for 3 days. On the third day, blood was withdrawn through cardiac puncture and kidney tissues from sacrificed rats were taken for histopathological analysis. Urine proteins were separated by sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE). Concentration of urine proteins, enzymes, serum urea nitrogen (BUN) and creatinine were analysed using a chemistry analyser. The

SDS-PAGE of urine proteins showed that proteinuria in gentamicin-treated rats consisted primarily of protein with low molecular weight. The separated urine proteins were mainly located in the molecular weight range of 10 to > 70 kDa. An extra protein band with molecular weight estimated to be approximately 11 kDa was also detected in the gentamicin-treated group and suggested to be an indicator of early renal disease. The electrophoretic patterns of urine proteins also demonstrated that the type of proteins leaking into urine had also provided some insight on the location and degree of renal injury. High urine enzymes concentrations specifically ALP had also reflected that the onset of injury was in the renal proximal tubular cells. This was confirmed by renal tissue histopathological evidences. Each parameter showed a high grading in gentamicin-treated group. Grade 3 cytoplasmic vacuolization (31%), grade 3 tubular dilatation (33.3%) and grade 3 cell detachment (33.3%) were the most prominent morphological changes in that group. BUN and serum creatinine did not show any obvious difference between the two groups. In conclusion, the elevated concentrations of low molecular weight urine proteins and urine enzymes appeared to be useful indicators for early renal damage as their elevation occurred before the BUN and serum creatinine produced any change.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PERKAITAN DI ANTARA PROTEINURIA DAN ENZIMURIA DENGAN PERUBAHAN HISTOPATOLOGI GINJAL SEMASA KEROSAKAN GINJAL AWAL YANG DIARUH OLEH GENTAMISIN

Oleh

SAIRAH BINTI ABDUL KARIM

Mei 2004

Pengerusi : Professor Rasedee Abdullah, Ph.D.

Fakulti : Perubatan Veterinar

Kajian ini dijalankan untuk menentukan perkaitan di antara protein urin dan penanda enzim urin, aspartat aminotransferase (AST), -glutamil transferase (-GT), dan alkalin fosfatase (ALP) dengan perubahan histopatologi dalam kerosakan renal awal yang diaruh oleh gentamisin. Lima puluh lima ekor tikus Sprague-Dawley, berumur 7-8 minggu, telah dibahagikan kepada dua kumpulan. Kumpulan pertama dengan sepuluh ekor tikus bertindak sebagai kawalan, dan empat puluh lima ekor lagi diperlakukan dengan gentamisin secara suntikan intraperitoneum untuk mengaruhkan kerosakan ginjal. Sampel dua puluh empat jam urin telah diambil setiap hari selama tiga hari. Pada hari ketiga darah diperolehi melalui cucukan kardium dan tisu ginjal daripada tikus yang telah dimatikan diperolehi untuk analisis histopatologi. Protein urin diasingkan melalui elektroforesis agar-agar natrium dodesil sulfat -poliakrilamida (SDS-PAGE). Kepekatan protein and enzim urin, nitrogen urea darah (BUN) dan kreatinina serum telah dianalisis

mengguna penganalisis kimia. SDS-PAGE untuk protein urin menunjukkan proteinuria dalam tikus terperlaku gentamisin terdiri terutama sekali daripada protein berat molekul rendah. Protein yang terasing itu terletak khususnya pada julat berat molekul 10 hingga >70 kDa. Satu lagi jalur protein yang berat molekulnya dianggar pada lebih kurang 11 kDa telah dikesan dalam kumpulan terperlaku gentamisin dan ini disarankan sebagai petunjuk kepada penyakit gingal awal. Pola elektroforesis protein urin telah menunjukkan yang jenis protein yang terkeluar kepada urin telah memberi gambaran kepada tahap dan tempat berlakunya kecederaan renal. Kepekatan enzim urin tinggi, khususnya ALP telah mencerminkan yang kecederaan mula tercetus pada sel tubul proksimal renal. Ini telah terbukti pada histopatologi tisu renal. Setiap parameter menunjukkan gred yang tinggi bagi kumpulan yang disuntik gentamisin. Gred 3 vakulisasi sitoplama (31%), gred 3 dilatasi tubul (33.3%) dan gred 3 penanggalan sel (33.3%) merupakan perubahan-perubahan morfologi yang ketara ditunjukkan oleh kumpulan tersebut. BUN dan kreatinina serum tidak menunjukkan sebarang kelainan yang jelas di antara kumpulan. Adalah disimpulkan yang peningkatan kepekatan protein berat molekul rendah dan enzim urin merupakan petunjuk berguna dalam kerosakan renal awal kerana peningkatannya berlaku sebelum ada sebarang perubahan dalam BUN dan serum kreatinina.

ACKNOWLEDGEMENTS

I would sincerely like to extend my warmest thanks and deepest appreciation to my main supervisor, Prof. Dr. Rasedee Abdullah for his guidance, advice and encouragement, which he patiently and unselfishly gave throughout the course of this study.

I also would like to thank to my co-supervisors, Assoc. Prof. Dr. Abdul Rahim Mutalib and Assoc. Prof. Dr. Mohd. Hair Bejo for their suggestions and support.

Sincere gratitude and appreciation are forwarded to Mr. Mohd. Halmi Othman and Mr. Abdullah Misron, the Hematology Laboratory in Microbiology and Pathology Department staff for their invaluable technical assistance during this study.

Last but not least, I would like to extend my gratitude to my loving husband, Mr. Mohd. Fadhil Ahmad, my son, Faiz Isqandar, my parents and family members for the constant support, love and encouragement.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

AAP	alanine aminopeptidase
ACE	angiotensin converting enzyme
ALP	alkaline phosphatase
ARF	acute renal failure
AST	aspartate transferase
BUN	blood urea nitrogen
CRF	chronic renal failure
ESRD	end stage renal disease
GFR	glomerular filtration rate
GGT	gamma glutamyl transferase
LDH	lactate dehydrogenase
NAG	N-acetyl –ß-D-glucosaminidase
NEP	neutral endopeptidase
PFD	Pirfenidone
RAS	rennin angiotensin system
RBP	retinal-binding protein
RRT	renal replacement therapy
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis

CHAPTER I

INTRODUCTION

Among human diseases, chronic renal failure (CRF) remains a serious health problem and the prevalence is increasing worldwide. Chronic renal failure is the most significant result of chronic kidney disease, characterized by a progressive loss of renal function. Damage to the kidneys over a period of ten years or more, may result in terminal kidney failure or end-stage renal disease (ESRD), which is the most feared consequence of kidney disease. According to Pierro *et al.*, (2001) the prevalence of ESRD is a good indicator of the burden of renal disease in a country. Therefore, ideally, effective programs should be directed at preventing the development of chronic renal insufficiency which could subsequent progress to ESRD. The early detection of kidney disease is very important.

Prevention through early detection of kidney diseases may serve as a solution to reduce the prevalence of renal disease. Treatment at the earlier stages of kidney disease will arrest the progression of the established kidney disease or even lead to the regression of the renal parenchymal damage. One epidemiological study suggested patients with kidney disease detected earlier had almost twice greater survival rate than those evaluated at a late stage (Mat Edelson, 2003). Unfortunately, many kidney diseases tended to be latent at onset and progress slowly making detection difficult. This is because kidneys have very high compensatory abilities that can compensate

up to 70% loss of functional mass and this unique feature had contributed to the difficulty of detection of renal insufficiency at very early stages.

At present, identification of patients with early renal disease relies heavily on blood and urine analysis in patients at increased risk for renal disease before the presence of significant symptoms. Analysis of urinary proteins and enzymes are accepted as reliable biomarkers and is very useful for the early diagnosis of tubule damage.

Even though there are numerous studies on enzymuria and proteinuria, it seems there is lack of information on their correlation with renal disease especially at the early stages of renal damage. Hypothesis of this study is that there are correlation between proteinuria, enzymuria and renal histopathological changes during early renal damage. Thus, this study was undertaken to determine their correlation and consequently ascertain the earliest indicator of renal damage. Gentamicin, an aminoglycoside antibiotic was used as the inducer for renal damage in this study and its effects were assessed to determined:

a. the relevant biochemical parameters that may be used as indicators of renal insufficiency during very early stage.
b. the rate of excretion of selected serum and urine biochemical parameters (blood urea nitrogen, serum creatinine, electrolytes,

protein, alkaline phosphatase, aspartate aminotransferase, gamma glutamyl transferase) during early renal damage.

c. the correlation between the extent of renal damage and urinary enzyme and protein excretions.

CHAPTER II

LITERATURE REVIEW

Progressive Renal Disease

Introduction

The progressive loss of renal function to end-stage renal disease (ESRD) is a common phenomenon in renal patients irrespective of the initial disease (Mai *et al.*, 2000). The most important causes of ESRD, at least in the United States, were hypertension and diabetic nephropathy (Raine, 1994), which are reliable predictors of risk for kidney disease. Patients who recover from an episode of acute renal failure may be at risk of developing chronic renal disease (Salem, 1999).

Nowadays, patients with various diabetic and non-diabetic glomerulopathies form the main contingent for renal replacement therapy (RRT) (Mai *et al.*, 2000). The number of patients receiving dialysis and transplantation continues to increase (Carl, 1995). These therapies are expensive, so that prevention is of prime importance. Arresting the rate of the deterioration of kidney failure will have a great impact and this may be achieved through accurate detection of early abnormal renal changes (Mai *et al.*, 2000).

Mechanisms

The mechanism underlying progression of renal disease is likely to be multifactorial. The nature of the progressive renal damage with various

etiologies is governed by such factors as hemodynamics, participation of the renin-angiotensin system (RAS) and progressive proteinuria (Mai *et al.*, 2000). All histological manifestations from patients with impaired kidney function show a rather similar histological picture of glomerulosclerosis, interstitial fibrosis, tubular atrophy and an interstitial infiltration (Vleming *et al.*, 1999). These seem to suggest that the progressive loss of kidney function results from a pathologic process that serves as a final cascade of events before renal failure. These events may be independent of the original etiology of the disease (Vleming *et al.*, 1999).

Disturbances in lipid metabolism, participation of cellular and molecular factors and subsequent accumulation of extra-cellular matrix component with the development of kidney fibrosis are closely related to the renal disease progression (Mai *et al.*, 2000). Recently much attention has been paid to the importance of persistence proteinuria, as an independent risk factor of renal disease progression (Giuseppe, 2000). There are now evidences which suggest a strong correlation between the degree of proteinuria and the rate of progression of renal failure in both diabetic (Breyer *et al.*, 1996) and non-diabetic (Ruggenenti *et al.*, 1998) renal disease patients. For this reason also, the National Kidney Foundation (NFK) has recommended that high-risk individuals undergo routine screening for proteinuria (Verna, 1999).

Treatment for Kidney Diseases

Treatment of kidney diseases is a complex issue and depends on the type of disease, the underlying cause, and the duration of the diseases. Clinically it is important to differentiate acute renal failure (ARF) from chronic renal failure (CRF) because generally acute disease is potentially reversible while the chronic form is not. Unfortunately, ARF may eventually go on to develop into CRF or end-stage-renal-failure if no treatment is instituted. Up to 10% of patients who experience ARF will suffer irreversible renal failure (Paula, 2000).

Treatment usually starts with addressing the original causes. In the case of acute kidney failure, treating the underlying cause may return the kidneys to normal function. Normally, initial treatments are focused on correcting fluid and electrolyte balances and uremia (Malay and Richard, 2000). Sometimes dietary restrictions (less salt and protein) are required until the kidneys are able to handle these substances. Diuretic drugs may help the body to excrete more water and salts. However, in CRF, drugs are used to stop the progression of the disease to ESRD.

When kidney disease does not respond to treatment with dietary restrictions and drugs, dialysis or kidney transplantation are the next treatments to consider. At least 500 000 people throughout the world are being routinely maintained by renal dialysis (Pierro *et al.*, 2001). Dialysis is a technique used

to remove waste products from the blood and excess fluids from the body of renal failure patients. Kidney transplantation on the other hand is a surgical procedure in which the diseased kidney is removed and replaced with a healthy kidney from a donor. This is the most effective form of renal disease therapy, with more than 12 000 procedures performed per year in the United State (David and Christopher, 1999). However, renal replacement therapy is expensive.

New Therapeutic Strategies

Renoprotection is a strategy, which has been developed to interrupt or reverse the progression of renal disease. Various modalities are now being used includes low-protein diet, anti-hypertensive therapy, and anti-fibrotic therapy (Mai *et al.*, 2000). However, it seems that the benefit of a low-protein diet in slowing the progression of renal failure is negligible (Pierro *et al.*, 2001). Instead an adequate early anti-proteinuric therapy may be a better approach. Anti-proteinuric therapy may arrest the progression of the renal disease or even lead to regression of the disease (Mai *et al.*, 2002).

Hypertension is recognized to be a strong independent risk factor for ESRD (Klag *et al.*, 1996). Renoprotection through lowering the blood pressure uniformly has shown to slow the disease progression and reduces renal damage (Anderson *et al.*, 1986). Among the anti-hypertensive drugs, the angiotensin-converting-enzyme (ACE) inhibitors are most effective in