UNIVERSITI PUTRA MALAYSIA

MERCURY-INDUCED NEPHROTIC SYNDROME: A CORRELATION BETWEEN PATHOLOGICAL CHANGES AND SERUM AND URINE BIOCHEMISTRY PARAMETERS IN RATS

SUHAIDAH IBRAHIM

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By

SUHAIDAH IBRAHIM

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometer
AT III	Antithrombin III
ATPase	Adenosine triphosphatase
BOD	Biological oxygen demand
BUN	Blood urea nitrogen
Ca^{+}	Calcium ion
Cd	Cadmium
CGN	Chronic glomerulonephritis
Cu	Cooper
EDTA-Na_2	Ethylenediamine tetraacetic acid disodium salt
EP	Extraction procedure
Factor V	Cofactor proaccelerin
Factor VII	Proconvertin (enzyme)
Factor VIII	Antihaemophilic factor
Factor IX	Christmas factor / Plasma thromboplastin component
Factor X	Stuart-Prower factor (enzyme)
Factor XII	Hageman factor
g	Gram
μg	Microgram

GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
HCl	Hydrochloride acid
H&E	Haematoxylin and Eosin
Hg	Mercury
$HgCl_2$	Mercury chloride
HNO_3	Nitric acid
H_2SO_4	Sulphuric acid
IgG	Immunoglobulin G
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IgE	Immunoglobulin E
K^+	Potassium ion
$K_2Cr_2O_7$	Potassium dichromate
kg	Kilogram
$KMnO_4$	Potassium permanganate
mg	Milligram
ml	Millilitre
MPGN	Mesangial proliferative glomerulonephritis
MT	Metallothionein
Na^+	Sodium ion
NaCl	Sodium chloride
NS	Nephrotic syndrome

pbb	Part per billion
Se	Selenium
SG	Specific gravity
SLE	Systemic lupus erythromatosus
$SnCl_2$	Stannous chloride
Zn	Zinc

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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March 1998

Chairman: **Associate Professor Rasedee Abdullah, Ph.D**

Faculty: Veterinary Medicine and Animal Science

Nephrotic syndrome (NS) is a renal disease featured mainly by proteinuria, hypoalbuminemia, oedema, and ascites. The etiologies could be diverse while the signs and symptoms are detected only at late stages of the disease. This study was conducted to assess the response of serum and urine biochemical indicators of renal failure and the compensatory mechanism/s that may be involved in maintaining optimum renal function following repeated exposure to mercury chloride ($HgCl_2$). A total of forty-five Sprague-Dawley rats aged between eight to ten weeks were injected intravenously through tail vein with 0.5 mg of $HgCl_2$/ kg body weight every alternate days for ten days. The same number of rats were injected with 1 ml of normal saline/ kg body weight and

served as controls. Five rats from each group were killed every four days commencing from the fourth day of the last injection.

There were significant changes observed in the concentration of blood urea nitrogen (BUN), serum creatinine, serum total protein, serum albumin, urine total protein, and urine albumin during the 42-day experimental period. The concentration of BUN begun to increase significantly ($P<0.05$) by day 22, but returned to normal values after the initial increase on day 30. While serum creatinine concentration fluctuated with two peak values on days 34 and 42. Loss of albumin from plasma was observed to be intermittent and urine total protein showed a late increase on day 34. Urine albumin showed a significant earlier increase ($p<0.05$) on day 18, but decreased toward control values for the next 8 days before increasing back to a peak value on day 42.

The deposition of mercury (Hg) following chronic exposure was high in the kidneys and the liver. The concentration of renal Hg was at peak values from day 14 to day 22 and gradually decreased thereafter. The renal tubular damage was observed to begin on day 18 and increased in intensity 26 days into the experiment reaching peak on day 42. There was also epithelisation of renal tubular epithelium. This response was greater on day 14 and quickly decreased thereon to disappear completely by day 28. The extensive damage of renal tubules which began on day 18 onwards could be due to an excessive loading of the metal beyond tissue elemental saturation and to the long retention of Hg in the tissues.

The study suggests Hg accumulated predominantly in the kidneys and produced a biphasic response of renal-associated biochemical parameters in which urine albumin is the possible early indicator to renal damage. Tubular epithelisation could be one of the mechanisms involved in maintaining the optimum renal function.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat-syarat untuk ijazah sarjana sains

SINDROM NEPROSIS TERARUH MERKURI: SUATU PERKAITAN DI ANTARA PERUBAHAN PATOLOGI DAN PARAMETER BIOKIMIA

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Sindrom nefrosis (NS) ialah satu penyakit renal yang dinyatakan oleh proteinuria, hipoalbuminemia, edema, dan asitis. Etiologinya mungkin pelbagai, sambil petanda dan simptom pula hanya dapat dikesan pada peringkat lewat penyakit ini. Kajian ini dikendalikan untuk menilai gerak balas petunjuk biokimia serum terhadap kegagalan renal dan mekanisme pampasan yang terlibat dalam penyenggaraan fungsi renal optimum berikutan pendedahan berulang kepada merkuri klorida ($HgCl_2$). Empat puluh lima ekor tikus Sprague-Dawley berumur lapan hingga sepuluh minggu disuntik secara intravena menerusi vena ekor dengan 0.5 mg $HgCl_2$/kg berat badan setiap selang satu hari selama sepuluh hari. Sejumlah sama tikus disuntik dengan 1 ml 0.85% natrium klorida

($NaCl_2$)/kg berat badan bertindak sebagai kawalan. Lima ekor tikus daripada setiap kumpulan dimatikan setiap empat hari bermula empat hari selepas suntikan terakhir.

Perubahan tererti ($P<0.05$) telah dicerapkan dalam kepekatan nitrogen urea darah (BUN), kreatinin serum, protein sepenuh dan albumin serum, protein sepenuh dan albumin urin sepanjang tempoh 42 hari ujikaji. Kepekatan BUN mula meningkat secara tererti ($P<0.05$) pada hari 22 dan kembali kepada normal selepas peningkatan awal, pada hari 30. Sambil itu kepekatan kreatinin serum beralun dengan dua nilai kemuncak pada hari 34 dan 42. Kehilangan albumin daripada plasma dicerapkan tidak selanjar dan protein sepenuh urin menunjukkan peningkatan lewat pada hari 34. Albumin urin menunjukkan peningkat tererti ($P<0.05$) lebih awal pada hari 18 tetapi menurun semula ke arah nilai kawalan selama 8 hari berikutannya sebelum ia meningkat semula kepada nilai kemuncak pada hari 42.

Pengenapan merkuri (Hg) berikutan pendedahan kronik adalah tinggi dalam ginjal dan hati. Kepekatan Hg renal berada pada nilai kemuncak daripada hari 14 hingga 22 dan beransur kurangan sejurus selepas itu. Kerosakan tubul renal dicerap bermula pada hari 18 dan meningkat keamatannya selepas 26 hari ke dalam tempoh ujikaji dengan mencapai kemuncak pada hari 42. Keepiteliuman tubul renal juga berlaku. Gerak balas ini lebih tinggi pada hari 14 dan cepat mengurang selepas itu untuk hilang terus pada hari 28. Kerosakan teruk tubul renal yang bermula pada hari 18 mungkin disebabkan oleh pembebanan berlebihan logam ini hingga melebihi ketepuan unsur tisu dan oleh penahanan Hg dalam tisu.

Kajian ini menyarankan Hg terkumpul secara keutamaan dalam ginjal dan menghasilkan gerak balas dwifasa parameter biokimia berkaitan renal, yang mana albumin urin mungkin merupakan petunjuk awal kepada kerosakan ginjal. Keepiteliuman tubul mungkin merupakan satu daripada mekanisme yang terlibat dalam menyengarakan fungsi renal optimum.

CHAPTER 1

INTRODUCTION

Mercury-Induced Nephrotic Syndrome: A Correlation Between Pathological Changes and Serum Biochemical Parameters

In recent years, there has been growing concern over the extent of environmental contamination with toxic metals due to industrial development. Among the fourteen environmental contaminants currently specified under the US Extraction Procedure (EP) Toxicity Test, Hg is rather high on the list.

The concentration of Hg in the environment is, in part, the result of waste products from manufacturing processes which utilise Hg or the disposal of products containing Hg. On a global basis, it is estimated about 10 metric tons mercuric waste per year is released into fresh water and about 480 metric tons per year into oceans (Von Burg and Greenwood, 1991). These mercuric wastes may sediment at the bottom of the lakes, rivers, and seas. There, the bacteria and fungi methylate the inorganic Hg to organic form and introduces the threat into the food chain (Hansen *et al.*, 1989).

Accumulated Hg in the food chain,

reservoir,

biotransformation and released into the atmosphere as elemental Hg.

The most common forms of Hg exposed to humans and animals can be categorised into three classes;

1972). Each class of Hg has different pharmacokinetic properties with regard to their uptake and absorption, distribution

Based on present evidence,

(Underwood,

is bioaccumulative. Mercury is known particularly to be a potential nephrotoxic agent (Bariety *et al.*, 1971). Methylmercury is by far recognised as the most toxic form of Hg and it represents great risks of irreversible functional damage to both humans and animals.

The two main routes of Hg entry into the body leading to toxicosis are ingestion (the food chain) and inhalation (atmostphere). Regardless of the chemical

route of entry, following

highest concentration of Hg (Greenwood *et al.*,1990). The association of Hg toxicosis and nephrotic syndrome has long been established (Mandema *et al.*,1963).

Studies so far have shown that chronic exposure to $HgCl_2$ could lead to an induction of biphasic membranous glomerulonephritis in Brown Norway rats (Sapin *et al.*, 1977;

human.

The kidneys, It could compensate up to 70% loss of functional mass.

contributed to the difficulty of detecting renal insufficiency at very early stages. Both biochemical and morphological techniques are still incapable of detecting early damage.

Thus,

NS with the following objectives:

a.

indicators of renal damage.

b.

serum and urine biochemical parameters.

c.

conjunction with the development of nephrosis.

CHAPTER 2

LITERATURE REVIEW

Nephrotic Syndrome

Introduction

Nephrotic syndrome (NS) is a renal disease of varied etiologies characterised by hypoalbuminemia,

hallmark of this condition is attributed to an increase in the glomerular permeability due to the loss of fixed negative charges on the glomerular membrane.

negatively charged polyanions,

excreted into urine (Coggins and Maffly,

Massive proteinuria is the most prominent feature of NS.

can excrete as much as 3.

1985;

concentration of albumin and forces the plasma fluid out of blood vessels into tissue interstitial spaces and causing ascites and oedema. In addition, the

elevated plasma renin and aldosterone activity due to hypovolemia,

protein loss,

retention of sodium,

extracellular fluid accumulation,

excess fluid which could further aggravate the edematous condition.

Classification of Renal Lesion in Nephrotic Syndrome

Glomerulonephritis,

disease of the kidney which begins in the glomerulus (Wilson,

50% of chronic glomerulonephritis (CGN) will eventually give rise to NS (Coggins and Maffly,

and Maffly,

membranous nephropathy;

and mesangial proliferative glomerulonephritis (MPGN) (Wilson,

Membranous Nephropathy

Almost one-third of NS cases originated from lesions of membranous nephropathy (Coggins and Maffly,

studies revealed thickened glomerular capillary wall due to deposits of immune complexes (Row *et al.*, 1975; The involvement of the immune mechanism can be demonstrated by immunoflourescence staining to show the presence of IgG and complement deposits (Wilson,

the total number of adult patients with idiopathy showed this form of NS at biopsy (Glassock *et al.*

The response of membranous nephropathy to corticosteroid and immunosuppressive therapy is poor with variable prognosis which either show complete remission,

progressing to chronic renal failure (Row *et al.*

Minimal Lesion Glomerulonephritis

Minimal lesion glomerulonephritis,

process disease (Wilson,

al., This is the only form of NS which does not involve immunopathology (Cameron *et al.*, 1974;

normal under light microscope but lipid accumulation is seen in the epithelial cells of the tubules whilst electron microscope studies revealed fusion of the foot processes (Wilson, 1986b). It

could be food allergy,

type of NS accounted for 80 - 90% of cases affecting children of primary glomerulonephritis aged between one to five years old (Coggins and Maffly,

prognosis is very good since the disease responds readily to corticosteroid treatment and rarely progress to renal failure (Kida *et al.*

Focal Glomerular Sclerosis

The third typical form of glomerulonephritis is focal glomerulosclerosis. A sclerotic process which takes place within the glomeruli with ensuing renal insufficiency. The pathogenetic mechanism initially involves deeper juxtamedullary nephrons and it may or may not be detected at early renal biopsy (Wilson, It is usually associated with tubular defects which lead to glycosuria,

acidosis,

et al., 1984).

Mesangial Proliferative Glomerulonephritis

Mesangial proliferative glomerulonephritis (MPGN) involves the proliferation of mesangial cells (Wilson,

IgA or IgM in the mesangium.

a lobular or "wire-loop" appearance under light microscope. This type of lesion is closely associated with lupus nephritis. A common manifestation of MPGN is hypertension and microscopic haematuria. Normally the prognosis is poor and may slowly progress to chronic renal failure (Wilson,

Etiologies of Nephrotic Syndrome

The causes of NS could be diverse,

urinary tract,