PATHOPHYSIOLOGICAL SIGNIFICANCE AND THE ROLE OF EXOGENOUS HYDROGEN SULFIDE (H_2S) IN A COMBINED STATE OF HYPERTENSION AND DIABETES AND ITS EFFECT ON RENAL EXCRETORY AND HAEMODYNAMIC FUNCTIONS

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By

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To

My beloved daughter late Fatima Fiaz

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LIST OF ABBREVIATIONS

HTN	Hypertension
EDRFs	Endothelium-derived relaxing factors
NO	Nitric oxide
EDCFs	Endothelial-derived constrictors factors
TXA2	Thromboxane A2
ANG II	Angiotensin II
PKC	Protein kinase C
ROS	Reactive oxygen species
RAAS	Renin angiotensin aldosterone system
STZ	Streptozotocin
DOCA	Deoxycorticosterone acetate
H_2S	Hydrogen sulfide
CBS	Cystathionine beta synthase
CSE	Cystathionine gama lyase
L-NAME	L-nitro-L-arginine-methyl ester
PAG	DLpropargyl glycine
NaHS	Sodium hydrosulfide
MAPK	Mitogen active protein kinase
WKY	Wistar Kyoto
SHR	Spontaneously Hypertensive rats
DOCA-salt HTN	Deoxycorticosterone acetate hypertension
g	Grams

NIBP	Non invasive blood pressure
mmHg	Millimeter mercury
mg /dl	Milligram per desiliter
mg/kg	Milligram per kilogram
i.p.	Intraperitoneal
ml	Milliliter
PWV	Pulse Wave velocity
µM	Micro moles
Ucr.	Urinary creatinine
Pcr.	Plasma creatinine
Bw	Body weight
MAP	Mean arterial blood pressure
SBP	Systolic blood pressure
HR	Heart rate
$U_{Na}V$	Absolute sodium excretion
FE_{Na}	Fractional sodium excretion
UFR	Urine flow rate
BPU	Blood perfusion unit

SIGNIFIKAN PATOFISIOLOGI DAN PERANAN EKSOGENUS HIDROGEN SULFIDA DALAM KEADAAN HIPERTENSI BERSAMA DIABETIS DAN KESANNYA KE ATAS FUNGSI EKSKRETORI DAN HEMODINAMIK GINJAL

ABSTRAK

Hipertensi dan diabetes merupakan morbiditi yang sering wujud bersama. Kedua-duanya saling mengaruh dan eksaserbat di antara satu sama lain. Pradispos hipertensi dan diabetes terhadap perkembangan penyakit kardiovaskular (cardiovascular disease, CVD) dan penyakit ginjal adalah komplikasi utama mereka. Kewujudan bersama hipertensi dan diabetes akan mengakibatkan perkembangan nefropati. Endogenus H_2S dikenali sebagai pemindah gas baru. Tisu vaskular mampu menjana jumlah H_2S yang boleh dikira dengan kepekatan ~46μM dalam serum tikus. H_2S terlibat dalam mengawal atur tekanan darah dan juga mengawal fungsi glomerular (vaskular) dan tubular ginjal. Kajian ini dijalankan untuk menguji hipotesis bahawa eksogenus H_2S merendahkan tekanan darah serta mengurangkan perkembangan nefropati dalam tikus diabetes hipentensi. Model tikus hipertensi SHR dan DOCA-salt digunakan. Hipertensi DOCA-salt dihasilkan daripada tikus WKY. Diabetes diaruh dengan satu injeksi tunggal streptozotocin (STZ) pada dos 40 mg/kg dalam suatu sediaan sejuk penampan natrium sitrat (0.1 mol/L, pH 4.5) secara intraperitoneum. Satu kumpulan diabetes diberikan NaHS, satu penderma H_2S, pada dos 56μmol/kg secara intraperitoneum dalam salin pada masa yang sama setiap hari selama 5 minggu. Tekanan darah diukur pada tikus yang sedar dan pada akhir tempoh rawatan, ia diukur pada tikus yang dibius Di samping itu, PWV (pulse wave

velocity) dan perfusi darah kortikal ginjal juga diperhatikan. Pengumpulan data metabolik bagi kajian fungsi renal dilakukan pada hari 0, 21 dan 33 daripada 35-hari kajian. Tahap H_2S, dalam plasma and urin, kepekatan kreatinin dan elektrolit diukur pada tiga keadaan yang berbeza sepanjang tempoh 35-hari. Tahap H_2S dalam plasma dan urin serta kepekatan kreatinin diukur secara spektrofotometri. Data, min±SEM dianalisis menggunakan ANOVA dan kesignifikanan secara statistik ditetapkan pada $p< 0.05$. Kumpulan tikus hipertensi diabetes mempunyai tekanan darah yang lebih tinggi, tahap H_2S dalam plasma dan urin yang rendah serta ketidakfungsian ginjal sebagimana yang terbukti berdasarkan kreatinin plasma yang meningkat, klearans kreatinin dan pengurangan nisbah natrium, kalium dalam urin dan perfusi darah kortikal ginjal. Di samping itu kencing tikus hipertensi diabetes mempunyai lebih tinggi nadi gelombang halaju. Tambahan pula, tikus diabetes mempamerkan peningkatan klearans kreatinin, natrium plasma, kumuhan ekskresi natrium mutlak dan pecahan kumuhan natrium. NaHS, penderma eksogenus H_2S mengurangkan tekanan darah, meningkatkan tahap H_2S dalam tikus diabetes hipertensi serta memulihkan ketidakfungsian ginjal aruhan -STZ. Di samping itu, eksogenus H_2S mengurangkan natrium plasma dan meningkatkan klearans kreatinin dan kumuhan natrium mutlak dalam tikus yang dirawat. Dapatan kajian ini mencadangkan bahawa pengambilan atau pemberian eksogenus H_2S merendahkan tekanan darah dan secara signifikannya merendahkan aruhan – STZ dalam kreatinin plasma, mengurangkan nisbah natrium : kalium dalam urin dan mengurangkan perfusi darah kortikal ginjal, yang mampu memberi perlindungan terhadap perkembangan nefropati aruhan-STZ dalam tikus hipertensi.

PATHOPHYSIOLOGICAL SIGNIFICANCE AND THE ROLE OF EXOGENOUS HYDROGEN SULFIDE (H_2S) IN A COMBINED STATE OF HYPERTENSION AND DIABETES AND ITS EFFECT ON RENAL EXCRETORY AND HAEMODYNAMIC FUNCTIONS

ABSTRACT

Hypertension and diabetes is a common co-morbidity that often coexists. Both diseases serve to induce and exacerbate each other. Hypertension and diabetes predispose the development of cardiovascular disease (CVD) and renal disease as their major complications. Coexistence of hypertension and diabetes results in rapid development of nephropathy. Endogenous H_2S is recognized as a novel gaseous transmitter. Vascular tissues are capable of generating the measurable amounts of H_2S with a concentration of ~46µM in the rat serum. H_2S is involved in the regulation of blood pressure and also implicated in controlling the renal glomerular (vascular) and tubular functions. Present study was undertaken to test the hypothesis that exogenous H_2S lowers the blood pressure and decreases the progression of nephropathy in hypertensive diabetic rats. SHR and DOCA-salt hypertension rat models were used in this study. DOCA-salt hypertension was produced from WKY rats. Diabetes was induced with a single injection of streptozotocin (STZ) at a dose of 40 mg/kg in freshly prepared ice cold sodium citrate buffer (0.1 mol/L, pH 4.5), intraperitoneally. One set of diabetic groups received NaHS, a H_2S donor, at a dose of 56µmol/kg intraperitoneally in saline at the same time daily for 5 weeks. Blood pressure was measured in conscious rats and at the end of the treatment period in surgically prepared anesthetized rats. In addition, pulse wave velocity (PWV) and renal cortical blood perfusion were also observed. Metabolic data collection for renal

function study was performed on days 0, 21 and 33 of a 35-day study. Plasma and urinary H_2S levels, creatinine concentrations and electrolytes were measured on three different occasions throughout the 35-day period. Plasma and urinary H_2S and creatinine concentrations were measured spectrophotometrically. Data, mean±SEM were analyzed using ANOVA and statistical significance was set at $p < 0.05$. Diabetic hypertensive groups of rats had higher blood pressure, low plasma and urinary H_2S levels and renal dysfunction as evidenced by increased plasma creatinine, creatinine clearance and decreased urinary sodium to potassium ratio and renal cortical blood perfusion. In addition diabetic hypertensive rats had higher pulse wave velocity. Moreover diabetic rats exhibited increased creatinine clearance, plasma sodium, absolute sodium excretion and fractional sodium excretion. NaHS, a donor of exogenous H_2S reduced the blood pressure and pulse wave velocity, increased the H_2S levels in hypertensive diabetic rats and reversed the STZ-induced renal dysfunction. In addition, exogenously administered H_2S decreased the plasma sodium and increased the creatinine clearance and absolute sodium excretion in treated rats. The findings of the present study suggest that exogenously administered hydrogen sulfide lowers the blood pressure and significantly reverses the STZ-induced increase in plasma creatinine, decrease in urinary sodium potassium ratio and reduced renal cortical blood perfusion, thereby conferring the protection against the progression of STZ-induced nephropathy in hypertensive rats.

CHAPTER 1
INTRODUCTION

1.1 Heart

1.1.1 Basic anatomy

The heart is a muscular organ found in all animals with circulatory system (including all vertebrates), that is responsible for pumping blood throughout the blood vessels by repeated, rhythmic contractions. The term "cardiac'' related to the heart comes from the Greek word *kardia*, for "heart" (Cohen, 2004).

The human heart is composed of cardiac muscle, which is an involuntary striated muscle tissue found only in this organ, and connective tissue. On the average human heart, beats at 72 beats per minute, roughly beats 2.5 billion times during an average sixty five years of lifespan, and weighs approximately 250 to 300 grams (9 to 11 oz) in females and 300 to 350 grams (11 to 12 oz) in males (Kumar *et al.*, 2005).

Heart is placed obliquely anterior to the vertebral column and behind the body of sternum so that 1/3 of it lies to the right and 2/3 lies to the left of median plane. It is enclosed in a double-walled sac called the pericardium. The superficial part of this sac is called the fibrous pericardium (Gavaghan, 1998). The outer wall of the human heart is composed of three layers. The outer layer is called the epicardium, or visceral pericardium since it is also the inner wall of the pericardium. The middle layer is called the myocardium and is composed of muscle which contracts. The inner layer is called the endocardium and is in contact with the blood that the heart pumps and it

also merges with the inner lining (endothelium) of blood vessels and covers heart valves (MedicaLook, 2007).

The human heart has four chambers, two superior atria and two inferior ventricles. The atria are the receiving chambers and the ventricles are the discharging chambers. The right ventricle discharges into the lungs to oxygenate the blood. The left ventricle discharges its blood towards the rest of the body *via* the aorta. The pathway of blood through the human heart consists of a pulmonary circuit and a systemic circuit. Blood flows through the heart in one direction, from the atria to the ventricles, and out of the great arteries. This is made possible by the presence of four valves which are the tricuspid valve, the mitral valve, the aortic valve, and the pulmonary valve (Marieb & Nicpon, 1990).

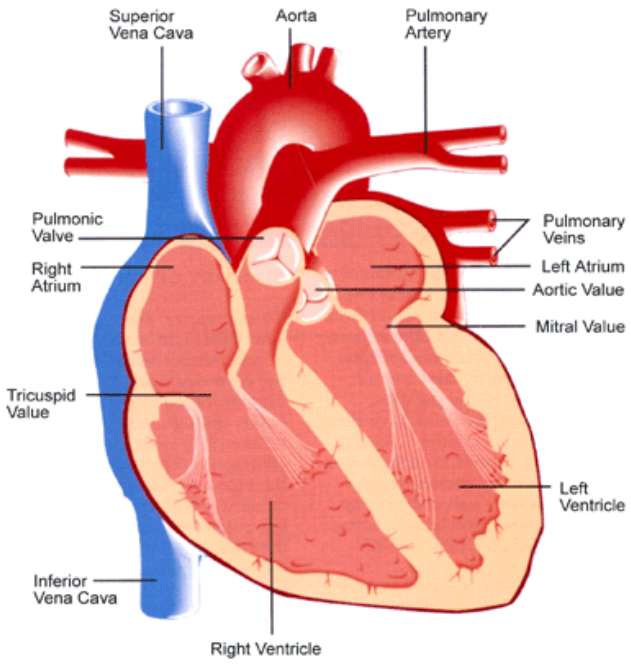

Figure 1.1 Chambers and four valves of the heart along with the main blood vessels entering and leaving the heart, adapted from (Chung, 1990).

1.1.2 Physiology of heart

Heart acts as a functional syncytium and is divided into right side and left side heart. Function of the right side of heart is to collect de-oxygenated blood, in the right atrium, from the body via superior and inferior vena cavae and pump it, through the tricuspid valve into the lungs termed as pulmonary circulation. In the lungs there is oxygenation of blood through the passive process of diffusion. The left sided heart collects oxygenated blood from the lungs into the left atrium. From the left atrium the blood moves to the left ventricle, through the bicuspid valve, which pumps it out to the body *via* the aorta. On both sides, the lower ventricles are thicker and stronger than the upper atria. The muscle wall surrounding the left ventricle is thicker than the wall surrounding the right ventricle due to the higher force needed to pump the blood through the systemic circulation (Scott, 1986).

The one complete beat of the heart, that is one systole followed by one diastole, is referred as one cardiac cycle. The duration of one cardiac cycle in human beings is 0.8 seconds. Systole lasts for 0.3 seconds and diastole lasts for 0.5 seconds (Guyton, 2006).

1.2 Blood vessels

Blood leaves the heart through the arteries, which conduct the oxygenated blood (except in the case of the pulmonary artery) to the various tissues and organs. Deoxygenated blood returns from the tissues and organs to the heart *via* a set of vessels, called veins (except the pulmonary vein) (Kahle *et al.*, 1993).

The blood vessels are composed of arteries, arterioles, capillaries, venules, and veins. The actual exchange of oxygen, carbon dioxide, foodstuffs and waste matter between the blood and the tissue fluid occurs in microscopically small vessels, called capillaries (Ivy Rose Holistic, 2003).

Structurally, vessel walls are composed of three "tunics" (layers).

1. Tunica intima (innermost): Consists of endothelium (simple squamous epithelium) which forms a smooth, flat, low friction surface. All vessels have this layer, and all but the teeny-tiniest have a basement membrane associated with the endothelium.

2. Tunica media (middle): Consists of smooth muscle cells and elastic connective tissue surrounding the interna. This layer is responsible for vasoconstriction and vasodilation.

3. Tunica adventitia (outer): Consists of connective tissue, surrounds entire vessel loaded with lots of collagen, and some elastin (Derrickson & Tortora, 2006).

1.3 Kidney

1.3.1 Basic anatomy

In human beings, there are two kidneys one on each side of spine, located in the abdominal cavity called the retroperitoneal space. They are approximately at the vertebral level T12 to L3 (Walter F. & Boron, 2004).

Each adult kidney weighs between 125 and 170 grams in males and between 115 and 155 grams in females. The left kidney is typically slightly larger than the right (Glodny B *et al.*, 2009).

The kidney has a bean-shaped structure. On medial side of each kidney there is a depression called hilum, at which the renal artery and nerve enters the organ, and the renal vein and ureter leaves the kidney (Marieb & Hoehn, 2007).

Kidney is divided into two major structures, outer is the renal cortex and inner is called renal medulla. Nephrons, the urine-producing functional unit of the kidney, span the cortex and medulla (Shier, 2003). The initial filtering portion of a nephron is the renal corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids and ultimately drain into a single collecting duct. The tip, or papilla, of each pyramid empties urine into a minor calyx, minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter (Applegate, 2000).

The kidneys receive blood from the renal arteries, left and right, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys

receive approximately 20% of the cardiac output. Each renal artery branches into segmental arteries, dividing further into interlobar arteries which penetrate the renal capsule and extend through the renal columns between the renal pyramids. The interlobar arteries then supply blood to the arcuate arteries that run through the boundary of the cortex and the medulla. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli (Walter F. & Boron, 2004).

After filtration, the blood moves through a small network of venules that converge into interlobular veins. As with the arteriole distribution, the veins follow the same pattern, the interlobular veins provide blood to the arcuate veins then back to the interlobar veins which unite to form the renal vein exiting at the hilus of the kidney. The renal veins return the blood to inferior vena cava (Vander, 1995; Applegate, 2000).

The kidney and nervous system communicate via the renal plexus, whose fibers course along the renal arteries to reach the kidney. Input from the sympathetic nervous system triggers vasoconstriction in the kidney, thereby reducing renal blood flow. The kidney is devoid of input from the parasympathetic nervous system (Bard, 2003).

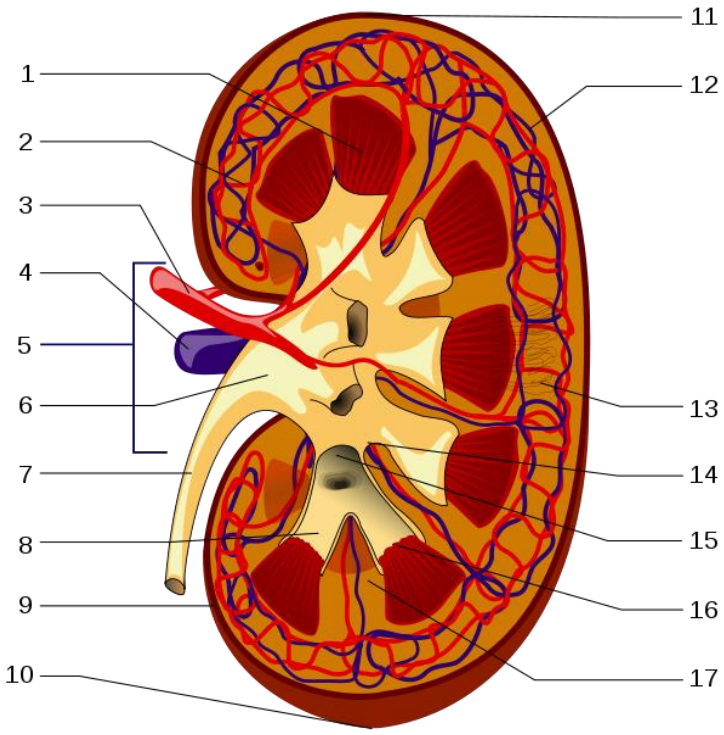

Fig 1.2 Anatomy of kidney
1. Renal pyramid • 2. Interlobular artery • 3. Renal artery • 4. Renal vein 5. Renal hilum • 6. Renal pelvis • 7. Ureter • 8. Minor calyx • 9. Renal capsule • 10. Inferior renal capsule • 11. Superior renal capsule • 12. Interlobular vein • 13. Nephron • 14. Minor calyx • 15. Major calyx • 16. Renal papilla • 17. Renal column.

Adapted from (GNU Free Documentation License 3 November 2008 Version 1.3)

1.3.2 Physiology of kidney

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in combination with other organs, particularly those of the endocrine system (Edemir *et al.*, 2011).

Most of the kidney's functions are completed by the simple mechanisms of filtration, reabsorption, and secretion that take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make the glomerular filtrate that will eventually become urine. Reabsorption is the transport of molecules from this ultrafiltrate and into the blood. Secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine (Guyton, 1991b).

1.4 Blood pressure

Blood pressure is defined as the pressure exerted by the blood against any unit area of vessel wall (Guyton, 1991a). Blood pressure is the resultant of the activity of heart and blood vessels. Simply, blood pressure is equal to cardiac output multiplied by total peripheral resistance. The normal blood pressure ranges are systolic blood pressure 90-119 mmHg and diastolic blood pressure 60-79 mmHg (Chobanian *et al.*, 2003).

Many interrelated physiological mechanisms are involved in maintaining the blood pressure including sympathetic and parasympathetic nerves, baroreceptors, circulatory hormones and local autoregulatory mechanisms. Derangement in these factors contributes to the elevation of blood pressure (Beevers *et al.*, 2001).

1.5 Hypertension

Hypertension is a common worldwide health problem and about 972 million individuals are suffering from this chronic illness (Hajjar *et al.*, 2006). It is an important health challenge in developed as well as in developing countries. The yearly estimated cost on hypertension treatment in USA exceeds USD 66 billion (Turek *et al.*, 2010).

Hypertension is a chronic medical illness, defined as a sustained elevation of blood pressure above normal. It is also referred to as high blood pressure and abbreviated as HT, HTN or HPN. The word "hypertension", generally refers to systemic, arterial hypertension (Maton, 1993). It is associated with increase in the peripheral vascular resistance and structural changes in the wall of blood vessels (Tanoue *et al.*, 2003). Persistent hypertension is one of the risk factors for stroke, ischemic heart diseases such as myocardial infarction and angina pectoris, congestive heart failure, aneurysm and chronic renal failure (Pierdomenico *et al.*, 2009). Hypertension is also linked with retinopathy and encephalopathy (Porta *et al.*, 2005). A mean arterial pressure of 50% or more above average is linked with high mortality rate until and unless treated efficiently (Guyton & Hall, 2005).

Hypertension is classified as essential (primary) hypertension and secondary hypertension. In essential or primary hypertension there is no detectable medical cause to explain the rise in blood pressure. It is common; about 90-95% of the patients have essential hypertension. Secondary hypertension indicates that the high blood pressure is a result of another condition (i.e., secondary to), such as kidney disease (Guyton, 2006).

1.6 Experimental models of hypertension

A number of rat models of hypertension are available for research purpose, including inbred strains, congenic lines, transgenic animals and recombinant inbred strains. All these models have been extremely helpful in exploring the physiological mechanisms that underlie the hypertension (Yagil & Yagil, 2000).

All these models are broadly divided into three classes based on etiology i.e. genetic, renal and pharmacologically induced. The most common genetic models of hypertensions are spontaneously hypertensive rats (SHR), severe hypertension stroke prone (SHR-SP) and Dahl salt sensitive rats (DSS). Depending on renal etiology unilateral nephrectomized DOCA-salt rat (UniNx DOCA salt) and renovascular hypertension (Goldblatt 2K1C rat) are commonly used. As far as the pharmacologically induced models are concerned L-NAME and angiotensin II treated rats are widely accepted.

1.6.1 Essential hypertension/SHR

Spontaneously hypertensive rats (SHR) are the model of essential hypertension. Since 1960s they are widely used to study the cardiovascular diseases (Pinto *et al.*, 1998). The development of hypertension starts at the age of 5 to 6 weeks and typical vascular and cardiac structural changes are evident at approximately 40-50 weeks of age (Conrad *et al.*, 1995). The development of hypertension is linked to the altered structural and functional changes in kidney (Rettig, 1993). In addition the over activity of sympathetic nervous system and increase in the renal nerve activity contribute to the hypertension (Oparil, 1986).

1.6.2 Deoxycorticosterone acetate (DOCA)-salt hypertension

DOCA-salt induced hypertension is a model of secondary hypertension, in which one kidney of the rat is removed and is administered with deoxycorticosterone acetate (DOCA) to produce the hypertension. It is a model of volume expansion. The pathophysiology is different as administered DOCA-salt leads to the salt and water retention resulting in hypertension. The increased level of sodium and water induced by DOCA is maintained, as there is only one kidney which is unable to handle this sodium and water overload. The level of renin remains low and hypertension is maintained by sodium and water retention, so extracellular volume expansion is the key pathophysiological mechanism in this model (Soszynski *et al.*, 1997). The other possible mechanisms include the decrease in arterial baroreceptor's reflex activity in the development of salt sensitive hypertension (Grisk & Rettig, 2004). There is also the contribution of renal efferent nerve activity in the development of hypertension through increasing the renal sodium retention as in this model the renal denervation postpones and decreases the severity of hypertension (Oparil, 1986). So DOCA-salt induced model is sodium loaded, volume overloaded and low renin hypertension.

1.7 Diabetes mellitus

There has been an increase in the prevalence of diabetes mellitus over the past 40 years, both in the US and worldwide. The worldwide prevalence of diabetes in year 2000 was approximately 2.8% and is estimated to grow to 4.4% by the year 2030, that will lead to a rise in the number of diabetic patients from 171 million in 2000 to 350 million in 2030 (Wild *et al.*, 2004).

Diabetes is defined as a chronic metabolic disorder that affects the metabolism of carbohydrates and other nutrients as a result of impaired insulin release and/or insulin resistance resulting in hyperglycemia (Tierney, 2002). Diabetes mellitus is classified into Type I and Type II diabetes mellitus. Type 1 diabetes mellitusis characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to a deficiency of insulin. It is either immune-mediated or idiopathic (Boon & Davidson, 2006). The majority of Type 1 diabetes is of the immune-mediated in nature, where the loss of beta cells is due to autoimmune attack mediated by T-cell (Johns Hopkins Autoimmune Disease Research Center, 2007). On the other hand Type II diabetes mellitus is characterized by the presence of insulin resistance or reduced insulin sensitivity, and with relatively reduced insulin secretion. This defective response of body tissues to circulating insulin involves the insulin receptor in cell membranes (Rother, 2007). The exact cause and mechanism is not fully understood in type II diabetes mellitus. Certain risk factors are associated with increased incidence of diabetes such as obesity, as 55% of patient diagnosed with type 2 diabetes mellitus have central obesity (Eberhart *et al.*, 2004). Other factors include ageing, increasing body mass and decreased demands of physical activity and family history (Kopelman, 2000). Environmental factors also have been implicated in type II diabetes mellitus (Lang *et al.*, 2008). Genetics are strongly linked with both types of diabetes mellitus (Walley *et al.*, 2006).

1.8 Hypertension and diabetes mellitus

Hypertension and diabetes often coexist. The diabetic persons have increased prevalence of developing the hypertension, one prospective study (that included 12,550 adults), indicates that the development of diabetes in hypertensive patients is 2.5 times greater as compared to normotensive subjects (National High Blood Pressure Education Program Working Group, 1994). Similarly, previous data suggests that there is increased prevalence of diabetes in hypertension and approximately 20% of hypertensive individuals have coexisting diabetes (Contreras *et al.*, 2000). Moreover, both diseases serve to induce, as well as exacerbate each other (Sowers & Epstein, 1995). Both hypertension and diabetes predispose to the development of cardiovascular disease (CVD) and renal disease as their major complications (Sowers, 2004b). The combined presence of hypertension and diabetes in patients increases the risks of cardiovascular diseases by 75% (Adler *et al.*, 2000). Diabetes mellitus and systemic hypertension promote the process of atherosclerosis, and their combination further increases this risk (Fuller, 1985).

1.8.1 Complications of diabetes and hypertension

Hypertension and diabetes are associated with marked abnormalities of cardiovascular structure and functions. Hypertension and diabetes both can induce coronary heart disease, infraction, cerebrovascular accidents, nephropathy and retinopathy and peripheral vascular diseases (George *et al.*, 2000).

Complications are generally divided into microvascular and macrovascular diseases. Microangiopathy is associated with retinopathy, neuropathy and nephropathy. Whereas the macroangiopathy is mostly evident with accelerated

atherosclerosis affecting the vital organs i.e. heart and brain (Calles-Escandon & Cipolla, 2001).

Endothelial dysfunction is considered as the first step in the pathogenesis of micro and macro vascular complications of both diseases (Wong *et al.*, 2010). Endothelium, the inner lining of blood vessels, releases certain chemical substances in response to acetylcholine. These substances are divided into two types according to the functions they perform (Wong *et al.*, 2010).

Endothelium-derived relaxing factors (EDRFs) including nitric oxide (NO), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factors (EDHFs). All of them reduce the vascular tone. Opposite to the beneficial EDRFs endothelium also produce vasoconstrictors substance called as endothelial-derived constrictors factors (EDCFs). Prostaglandin H2, thromboxane A2 (TXA2), leukotrienes, endothelin, and superoxide anions are included in this category (Vanhoutte, 2009).

A critical balance is required between EDRFs and EDCFs in order to maintain the vascular health and function. Hypertension and diabetes tend to disturb this balance by either increasing or decreasing the production of one or both (Vanhoutte *et al.*, 2009).

Hyperglycemia associated with diabetes modifies the endothelial function through a number of complex mechanisms including oxidative stress (Laight *et al.*, 2000), glycation of protein and lipids (Vlassara *et al.*, 1992) and activation of protein kinase C (Hink *et al.*, 2001). Similarly the endothelial dependent vasodilatation is

impaired in different animal models of hypertension including spontaneous hypertensive rats (Lüscher *et al.*, 1990), salt induced hypertension (Jiménez *et al.*, 2007) and renovascular hypertension (Rühlc *et al.*, 2006).

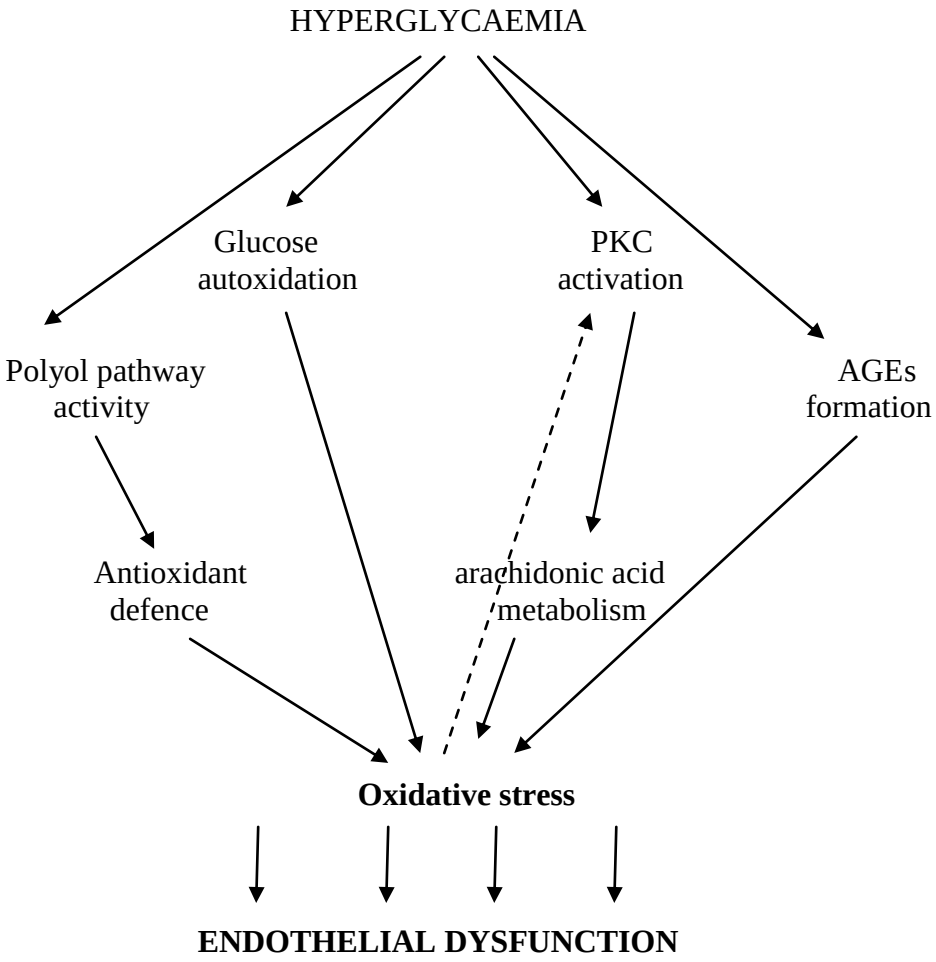

Fig 1.3 Outline and interactions of hyperglycaemia-induced metabolic pathways potentially involved in the pathophysiology of endothelial dysfunction (Adapted from (De Vriese *et al.*, 2000).

*AGEs= advanced glycation products, PKC= protien kinase C

1.8.2 Diabetic nephropathy

Nephropathy is the hallmark of microvascular complications of diabetes. Enough supporting evidences are present that the basic pathophysiological mechanisms eventually leading to nephropathy are similar in type 1 and 2 diabetes (Parving, 2001). Hypertension either related to the diabetes or not, additionally damages the kidney resulting in complex patterns of nephropathy. The risk for the development of nephropathy is strongly linked to genetic factors and only approximately 40–50% of patients with either type 1 or type 2 diabetes will ultimately develop nephropathy (Lindner, 2003).

Haemodynamic and structural changes are important in the development of diabetic nephropathy. Glomerular haemodynamic changes including hyperfiltration and hyperperfusion have been sighted as a key factor in the development of diabetic nephropathy and are found very early in the disease process (Ruggenenti *et al.*, 2001). Elevated intraglomerular pressure has been linked to an increase in mesangial cell matrix production and thickening of the glomerular basement membrane, eventually leading to glomerulosclerosis (Wolf *et al.*, 2000). Local haemodynamic stress also contributes to the structural changes of diabetic nephropathy by the local activation of cytokines and growth factors (Wolf, 2004).

Abnormalities in sodium reabsorption have been linked to glomerular hyperfiltration in diabetic nephropathy (Thomson *et al.*, 2004), as the diabetes induced hypertrophy of tubules causes the increased reabsorption of sodium chloride (Chen *et al.*, 2001).

As far as the structural abnormalities are concerned diabetes can affect all the compartments of the kidney. The structural changes range from the thickening of basement membrane to mesangial expansion, glomerulosclerosis (diffuse, nodular) fibrin cap lesion, capsular drop lesion, endothelial foam, podocyte abnormalities, tubular atrophy, interstitial inflammation and interstitial fibrosis (Wolf, 2004).

On the molecular level, five major pathways have been implicated in glucose-mediated vascular and renal damage including increased polyol pathway flux, increased hexosamine pathway flux, activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), increased advanced glycation end-product (AGE) formation, stimulation of angiotensin II (ANG II) synthesis and activation of the protein kinase C (PKC) pathway. All of these pathways reflect a single hyperglycaemia-induced process of overproduction of reactive oxygen species (ROS) and oxidative stress (Wolf, 2004).

Another factor which is linked to nephropathy is the activation of renin angiotensin aldosterone system (RAAS) found in tubular cells (Wolf & Ziyadeh, 1997). Hyperglycemia stimulates expression of renin and angiotensinogen in mesangial and tubular cells (Vidotti *et al.*, 2004). This stimulation results in an increase in local ANG II concentrations which in turn through autocrine and paracrine pathways induce the production of different cytokines and growth factors (Wolf & Ziyadeh, 1997). Previous experimental studies indicate that a high glucose-mediated generation of ROS is responsible for the upregulation of angiotensinogen in proximal tubular cells (Hsieh *et al.*, 2002). In fact, it has been shown that angiotensin

II is involved in almost every pathophysiological process implicated in the development of diabetic nephropathy (Wolf, 2004).

Generally, as far as complications of both diseases are concerned hypertension and diabetes attack the common organs for end stage damage and potentiate the adverse prognostic effects of each other.

1.9 Animal models of diabetic nephropathy

Several rodent models with spontaneous diabetes such as fatty Zucker rat, Kuo kondo mouse, New Zeland obese mouse and Cohen diabetic rat (Janssen *et al.*, 1999) are known but not completely assisting us to reveal the multifaceted pathophysiology of diabetes and its complications. Apart from these models, diabetes can be induced with streptozotocin (STZ) and other chemical agents and thus providing another set of animal model of diabetes. STZ induced diabetic rats develop typical diabetic haemodynamic and structural abnormities that correspond to the early stage of diabetes. These lesions include hyperfiltration, progressive thickening of basement membrane, albuminurea and renal hypertrophy (O'Donnell, 1993; Nobrega *et al.*, 2004).

1.9.1 SHR diabetic and DOCA (Deoxycorticosterone acetate)-salt HTN diabetic

A large number of studies used STZ induced diabetes either with salt loaded hypertension (Korner *et al.*, 1993) or with genetic hypertension (use of SHR) (Cojocel *et al.*, 2005) in order to get the severe and rapid renal injury. It was reported earlier that the rise in creatinine clearance after the induction of diabetes was greater in the SHR-STZ animals suggesting that this may be a better model for studying 'hyperfiltration' in diabetes. Similarly, SHR-STZ developed albuminuria more rapidly than WKY-STZ despite similar GFR in the two strains (Cooper *et al.*, 1986). This proposes that hypertension may have a specific action in the acceleration of diabetic renal disease, independent of GFR.

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The severity of diabetic lesions in these animals ranges from early diabetic nephropathy to full blown diabetic renal disease depending upon the duration of diabetic stage. SHR diabetic and DOCA-salt hypertension diabetic have demonstrated accelerated tubulointerstitial injury in form of vacuolization of tubular cells, tubular atrophy, infiltration of inflammatory cells and subsequent fibrosis of renal tissue (Cooper *et al.*, 1986; Usui *et al.*, 2003; Cojocel *et al.*, 2005).

1.10 Pathophysiology of hypertension with diabetes

Several epidemiologic studies provide evidence for co-existence of hypertension and diabetes and indicate towards a common genetic and environmental factor promoting both diabetes and hypertension. Similarly, a combined state of hypertension, insulin resistance, hyperlipidaemia and central obesity have been documented (Reaven, 1988). It is documented that about 25–47% of persons with

hypertension have insulin resistance or impaired glucose tolerance (Lind *et al.*, 1995). The relationship of insulin resistance, diabetes and hypertension is very complex but interrelated. Increased activity of renin angiotensin aldosterone system (RAAS) in hypertension and diabetes has been proposed (Ogihara *et al.*, 2002). This up regulation of RAAS results in the formation of reactive oxygen species (ROS) which in turn affects glucose utilization and blood pressure in a setting of diabetes and hypertension (Sowers, 2004a). At the same time RAAS mediates the increase in oxidative stress which further contributes to insulin resistance (Blendea *et al.*, 2005). Furthermore the increased formation of angiotensin II results in increased aldosterone secretion from the adrenal gland leading to increased water and sodium absorption and hence aggravation of hypertension.

Moreover increased secretion of aldosterone increases sympathetic nervous system activity and reduces baroreceptor's activity (McFarlane & Sowers, 2003). As such, renin angiotesin aldosterone system (RAAS) is mainly linked with hypertension combined with diabetes. In addition, quite a few other pathophysiological events are also suggested such the elevation of intracellular calcium concentration, vascular smooth muscle cell proliferation and atherosclerosis, and reduction in nitric oxide concentration or its bioavailability (Modan & Halkin, 1991). It has also been shown that increased level of insulin also affects the reactivity of large vessels to vasodilator agents (Johnstone *et al.*, 1993; Westerbacka *et al.*, 1999).

1.11 Hydrogen sulfide (H_2S)

Hydrogen sulphide (H_2S), along with nitric oxide (NO) and carbon monoxide (CO), forms a group of biologically active gases that are termed as gasotransmitters or gasomediators. H_2S, NO and CO once solely considered as metabolic poisons. Many other gases, including O_2 and CO_2, play equally, if not more, important and fundamental roles in human biology, yet H_2S, NO, and CO have attracted attention because they exert fine, modulator control over cellular functions by influencing a range of intracellular signaling processes (Li *et al.*, 2011).

H_2S has the bad public image of a deadly "gas of rotten eggs", recognized having a toxic potential equal to cyanide for last 300 years. As a toxicant, it mainly affects the brain, kidney and lungs (Beauchamp *et al.*, 1984). H_2S is the most recently discovered member in the family of gasotransmitter and, perhaps not surprisingly, has attracted a great deal of interest among the researchers over the past few years. H_2S can be generated endogenously in many types of mammalian cells (Stipanuk & Beck, 1982). Endogenous H_2S is recognized as a novel gaseous transmitter (Wang, 2002). The H_2S concentration of rat serum is ~46μM (Zhao *et al.*, 2001). Besides the circulating H_2S, a significant amount of H_2S is produced in various tissues such as liver, kidney, pancreas, brain and blood vessels. For instance, the physiological concentration of H_2S in brain tissue has been reported to be 50–160 μM (Hosoki *et al.*, 1997). Recent studies have shown that vascular tissues are capable of generating the measurable amounts of H_2S (Zhao *et al.*, 2001).

1.11.1 Production of H_2S

To date, quite a few scientists have comprehended the formation of H_2S molecule naturally, at levels enough to modify cell function without causing cell death. H_2S is synthesized in mammalian tissues via endogenous enzymes and by nonenzymatic pathways. As far as the enzymatic formation of H_2S is concerned, two pyridoxal phosphate-dependent enzymes, cystathionine beta synthase (CBS) and cystathionine gama lyase (CSE) are responsible for the majority of the endogenous production of H_2S in mammalian tissues that use l-cysteine as the main substrate (Stipanuk & Beck, 1982). The expression of CBS and CSE has been identified in many human and other mammalian cells, including those from the liver, heart, blood vessels, kidney, brain, skin fibroblasts and blood lymphocytes (Levonen *et al.*, 2000).

The expression of CBS and/or CSE is tissue specific. CBS is considered as a major endogenous enzyme for H_2S production in the brain (Abe & Kimura, 1996). On the other hand CSE enzyme is concerned with the endogenous production in cardiovascular system. As in rat mesenteric artery and other vascular tissues CSE is the only H_2S-generating enzyme that has been identified, cloned, and sequenced. The mRNA of this enzyme was expressed solely in vascular SMCs as detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and *in situ* hybridization. No transcript of CSE was found in the endothelial layers of intact vascular tissues or cultured endothelial cells. Expression levels of CSE mRNA varied in different types of vascular tissues (Zhao *et al.*, 2001).

Previously it was considered that the vascular smooth muscle which expresses the CSE enzyme is the major source of H_2S production. Recently it was reported that

the CSE enzyme is also expressed in the endothelium of mice aorta (Yang *et al.*, 2008a).

Similarly it was reported that endothelium is another source of H_2S but the enzymes responsible for the production of H_2S are different as compared to smooth muscle H_2S production. It has been demonstrated that 3-mercaptopyruvate sulfurtransferase (3MST) and cysteine aminotransferase (CAT) are localized to vascular endothelium in the thoracic aorta and produce H_2S in rats. Both 3MST and CAT were expressed in the endothelium. Lysates of vascular endothelial cells produced H_2S from cysteine and α-ketoglutarate (Shibuya *et al.*, 2009).

Whereas most emphasis has been placed on the enzymatic formation of H_2S, the generation of this gas from bound sulfur (intracellular sulfur stores) may be important in certain cells and under certain conditions. Bound sulfide most likely occurs when H_2S interacts with cysteine thiols to form stable persulfides, which, under reducing conditions, can release stored H_2S confirming a nonenzymatic source of H_2S (Searcy & Lee, 1998). H_2S *in vivo* is metabolized by oxidation in mitochondria or by methylation in cytosol or scavenged by methemoglobin and is excreted mainly by the kidney as free or conjugated sulfate (Beauchamp *et al.*, 1984). Notwithstanding these biochemical means for H_2S catabolism, H_2S is a powerful reducing agent and is likely to be consumed by endogenous oxidant species in the vasculature, such as peroxynitrite, superoxide, and hydrogen peroxide (Li *et al.*, 2011)

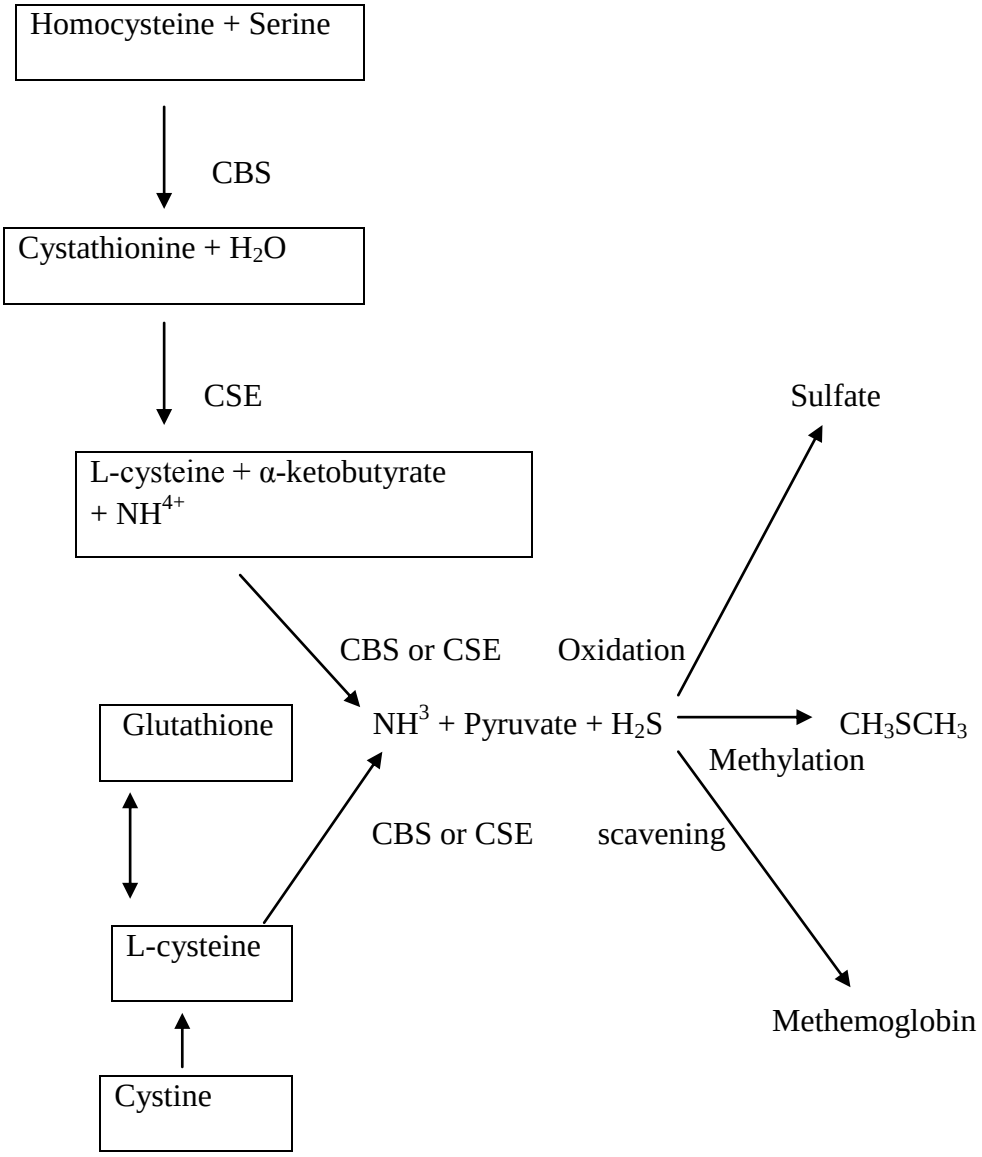

Fig.1.4 Endogenous enzymatic production and metabolism of H_2S, Adapted from (Wang, 2002)

1.11.2 Physiological actions of H_2S

To date, much research into H_2S has been centered upon its effects on individual body systems. Even though many systems have come under the spotlight, the effect of this gas on the cardiovascular system and in inflammation has attracted the most attention. Many other possible roles for endogenous H_2S have been hypothesized in, for example, the peripheral and central nervous systems, pain appreciation and neurodegeneration, control of gastrointestinal and urogenital function, and endocrinology.

Some of the physiological actions of H_2S which have been characterized since its discovery in relation to cardiovascular system are as follows:- it has been shown to relax vascular smooth muscle cells, induce vasodilatation of isolated blood vessels, reduce blood pressure (Elrod *et al.*, 2007), inhibit leukocyte–endothelial cell interactions *in vivo* (Zanardo *et al.*, 2006), is a potent anti-inflammatory molecule, a potent antioxidant (under chronic conditions such as diabetes and hypertension), effectively inhibit apoptosis of a number of cell types (Kimura & Kimura, 2004), exert potent effects on mitochondrial function and respiration like NO (Nicholls, 1975), protect the myocardium in ischemia reperfusion (I/R) injury (Bian *et al.*, 2006) and has profound effects on vascular growth formation and neointimal hyperplasia (Meng *et al.*, 2007). Moreover H_2S known to be cardioprotective in isolated rat hearts following coronary artery ligation, ischemia, or lipopolysaccharide (LPS) injection (Li *et al.*, 2011).