Introduction

Uric acid, and its monoanion urate, was traditionally considered to be a metabolically inert end-product of purine metabolism in man, without any physiological value. However, this ubiquitous compound has been proven to be a selective antioxidant *(Becker, 1993).* Yet, despite the apparent significant contribution of uric acid to serum antioxidant capacity, it was found to lead directly or indirectly to vascular injury *(Waring et al., 2000).*

Elevated serum uric acid, besides its documented link to gouty arthritis, has been reported to be closely associated with the metabolic syndrome and, as well, to be a correlate of the development and progression of cardiovascular and renal diseases *(Baker et al., 2005),* though the role of uric acid in this respect is still unclear. Also, *Waring and Esmail (2005)*, based on a large number of epidemiological studies, stressed the association between high serum uric acid concentrations and increased cardiovascular risk.

Further, *Berry and Hare, (2004)* stated that uric acid might negatively impact the health status,

perhaps by activating a complex viscious cycle involving inflammatory-related diseases. Further, its free radical-inducing effect, acting sometimes as pro-oxidant, could contribute to its injurious effect *(Berry and Hare, 2004; Kanellis and Kang, 2005).*

A deleterious effect of hyperuricemia on endothelial function, platelet adhesiveness and platelet function was suggested *(Alderman and Redfern, 2004).* This platelet dysfunction may reflect endothelial dysfunction in hypertensive patients with increased cardiovascular risk *(Waring et al., 2000; Johnson et al., 2003).* However, no enough or definite experimental data exist concerning the association of hyperuricemia with the different cellular elements of blood.

It was, thus, intriguing to investigate the effects of elevated serum uric acid levels on the physiology of the different cellular blood elements in order to throw more light on the role of uric acid as a cardiovascular risk factor.

Aim of work

This study was carried out to investigate the effect of hyperuricemia on the different cellular blood elements, which might help in explaining the hyperuricemia-associated cardiovascular ailments.

Review of Literature

Although the notion that uric acid is a risk factor for poor health outcome is not universally acknowledged *(Freedman et al., 1995; Culleton et al., 1999)*, several studies have demonstrated that uric acid is an independent risk factor for hypertension, diabetes, coronary artery disease, as well as cerebrovascular morbidity and mortality *(Awad,1996; Iribarren et al.,1999; Waring et al., 2000; Verdecchia et al., 2000; Awad et al., 2003b; Johnson et al., 2003; Carnethon et al., 2003; Nakanishi et al., 2003 ; Niskanen et al., 2004; Coutinho et al., 2007)*. Further, *Berry and Hare (2004)* stated that uric acid may negatively impact the health status, perhaps by activating a complex viscious cycle involving inflammatory-related disease.

Uric acid in physiologic fluids

Uric acid is a weak acid (pka 5.8), distributed throughout the extracellular fluid compartments *(Emmerson, 1996).*The normal blood uric acid level in humans is approximately 4mg/dL (0.24mmol/L*)* *(Ganong, 2005)*, and is somewhat higher in males than in females *(Becker, 1993).*

The predominant form of uric acid is determined by the pH of its milieu. Under physiologic conditions, i.e at the usual pH of physiologic fluids, only uric acid and its monosodium salt, sodium urate, are found, with sodium urate predominating *(Martin,1985).* A major portion of the body's urate exists in body fluids outside the vascular system, and the concentration of urate in serum is approximately twice its concentration in erythrocytes *(Henry, 1974).*

It is noteworthy that blood uric acid level was found by *Shinosaki et al. (1992) to* show a circadian rhythm, with the lowest level occurring early in the morning and the highest level at night.

Uric acid metabolism

In humans, uric acid is the end product of purine degradation. Purines are crucial for a wide range of normal physiological functions. They are essential building blocks for nucleic acids (deoxyribonucleic acid "DNA", and ribonucleic acid "RNA"), extra- and intracellular messengers (adenosine triphosphate "ATP" and G-protien coupled reactions), metabolic regulators (cyclic adenosine monophosphate "cAMP"), coenzymes and neurotransmitters. Purines are degraded ultimately to uric acid through the action of

the enzyme xanthine oxidase that converts xanthine to urate *(McLean, 2003)*.

In most mammals, the liver enzyme uricase (urate oxidase) is responsible for further metabolism of uric acid to allantoin, which is a more soluble waste product. However, humans lack the enzyme uricase, resulting in higher blood uric acid levels *(Hediger et al., 2005)*. This might provide humans a survival advantage over other primates because of the function of uric acid as antioxidant (*Wu et al., 1992; McLean, 2003). Watanabe et al. (2002*) have speculated that uric acid could contribute to the increased life span in humans, by providing protection against oxidative stress-provoked aging and cancer. Moreover, further studies suggested that urate may help arrest multiple sclerosis through scavenging the toxic compound peroxynitrite in the central nervous system *(Sotgiu et al., 2002; Toncev et al., 2002)*.

About one third of the daily urate load comes from dietary sources, with the remainder generated endogenously. Once urate has been formed, it can be eliminated by the gastrointestinal tract or kidneys, or deposited in tissues. Enteric excretion is responsible for handling one third of the daily urate load *(Sorensen and Levinson 1975)*. The remainder is

handeled primarly by the kidneys, being cleared from plasma by glomerular filteration *(Emmerson, 1996)*. Approximately 95% of urate is filtered by the glomeruli and subsequently undergoes bidirectional movement across the proximal convoluted tubules (PCT) *(Cannella and Mikuls, 2005).* About 98% of the filtered uric acid is reabsorbed, and the remaining 2% makes up approximately 20% of the amount excreted. The remainig 80% comes from the tubular secretion *(Ganong, 2005)*.

The movement of urate is accomplished via several recently described anion transmembrane channels *(Enomoto et al., 2002; McLean 2003; Rafey et al., 2003)*. The balance between the PCT's secretory and reabsorptive activites exerts a major influence on renal excretion of uric acid. Although the secretory capacity of the kidneys can increase with hyper-uricemia, the compensation is often not enough *(Cannella and Mikuls, 2005)*.

In humans, specialized transporters for urate excretion that are located in renal proximal tubular cells, intestinal epithelial cells as well as vascular smooth muscle cells (VSMCs), were identified; including the voltage-sensitive urate transporter

URAT1, the ATP-dependent urate export transporter MRP 4, and the organic anion transporters OAT1 and OAT3 *(Hediger et al., 2005)*. The urate transpoter URAT1 was found to be directly inhibited by uricosuric drugs *(Enomoto et al., 2002)*. The rate of URAT1 transcription was reported to be under hormonal regulation, being influenced by sex hormones, where URAT1 expression in male mice was found to be higher as compared to that in females *(Hosoyamada et al., 2004)*.

For an individual, urate concentration is determined by the balance between the rate of purine metabolism, both endogenous and exogenous, and the efficiency of renal clearance. Alteration in this balance may account for hyperuricemia . In the majority (90%) of patients with primary gout, hyperuricemia results from relative renal under secretion, whereas in 10% of patients, there is overproduction of endogenous uric acid *(Fam, 2002)*.

Antioxidant-prooxidant characteristics of uric acid

For years, soluble uric acid was considered to be biologically inert. Recently, there has been substantial interest in the role of uric acid as an antioxidant, being one of the major plasma non- enzymatic antioxidants together with ascorbate (vitamin C) *(Ames et al., 1981; Becker, 1993; Sandez-Lozada et al., 2006).* The concentration of uric acid is generally high in comparison to other non-enzymatic antioxidants. *Becker (1993)* reported that plasma concentration of uric acid is almost 10-folds higher than other antioxidants, such as vitamin C and vitamin E. Moreover, uric acid has access to all extracellular fluid compartments besides plasma; namely to lymphatics as well as cerebrospinal, interstitial, synovial, intraocular, and amniotic fluids, and is, thus, not compartmentalized as rigidly as other enzymatic scavengers *(Fleming et al., 1988; Stocker and Frei, 1991).*

Because of its high concentration and powerful antioxidant properties, as well as endogenous synthesis, uric acid has been proposed to play a pivotal role in the antioxidant defense system in humans, protecting against oxidative damage of lipids, enzymes and nucleotides *(Becker, 1993).*

With respect to its antioxidant actions, soluble uric acid has been shown to scavenge superoxide, singlet oxygen, hydroxyl radical and, as well, peroxynitrite *(Toncev et al., 2002).* It, also, chelates transitional metals, as oxo-heme oxidant, formed by peroxide reaction with hemoglobin that acts as an important initiator of lipid peroxidation in erythrocyte membrane *(Ames et al., 1981; Squadrito et al., 2000).* Moreover, uric acid stimulates the expression of extracellular superoxide dismutase *(Hink et al., 2002)*, and prevents its degeneration *(Hicks et al.,1993),*thus, increasing superoxide dismutation to hydrogen peroxide, and decreasing the availability of superoxide and its harmful interaction with nitric oxide. Moreover, the ability of uric acid to preferentially react with peroxynitrite has been shown to aid in the substantial antioxidant benefit *(Kuzkaya et al., 2005).*

Further, the enzyme cyclooxygenase was demonstrated to be prone to self-inactivation, which is believed to be due to the production of reactive oxygen species (ROS) in the course of the metabolism of arachidonic acid to prostaglandin H2. Urate, in physiological concentrations, has been found to prevent oxidative inactivation of cyclooxygenase, and

even to stimulate the production of prostacyclin and thromboxane from arachidonic acid *(Deby et al., 1981)*.

With regard to the prooxidant potential of uric acid, biochemical studies have demonstrated that urate can generate amino carbonyl radicals that are capable of amplifying the oxidation of liposomes and low density lipoprotein cholesterol *(Maples and Mason, 1988; Santos et al., 1999)*. This prooxidant effect of urate appears to be more pronounced in the setting of relative deficiency of the water soluble anti-oxidants, such as ascorbic acid *(Abuja, 1999; Bagnati et al., 1999; Patterson et al., 2003)*. *Cherubini et al. (2000)* reported that worse outcome in acute stroke was associated independently with low ascorbate and high uric acid levels.

Awad et al. (2003a) reported that the physiological antioxidant activity demonstrated in short term (2 hours) hyperuricemic rats was lost and became reversed into a pathological prooxidant one upon prolonging the duration of experimental hyperuricemia to 4 and 8 weeks.

The injurious effect of elevated uric acid concentration on the arterial-capillary vessel wall,

which might contribute to endothelial dysfunction, arterial-capillary vessel wall remodeling and accelerated atherosclerosis, was believed to occur through oxidative-redox stress *(Fang and Alderman, 2000; Hayden and Tyagi, 2002; Niskanen et al., 2004)*. In the atherosclerotic prooxidative environmental milieu, the original antioxidant properties of uric acid was reported to become paradoxically prooxidant. Such antioxidant-pro-oxidant urate redox shuttle seemed to rely heavily on its surrounding environment, including the pH, the surrounding oxidant milieu, and the depletion of other local antioxidants *(Hayden and Tyagi, 2004)*.

The antioxidant-prooxidant urate redox shuttle was suggested to be an important concept that was described and proposed to exist in the vascular milieu of atherosclerotic endothelium. Uric acid was reported to act as antioxidant in its physiological range, but becomes prooxidant when elevated, with loss of the supporting antioxidants, and in milieu of oxidative redox stress *(Hayden and Tyagi, 2004)*.

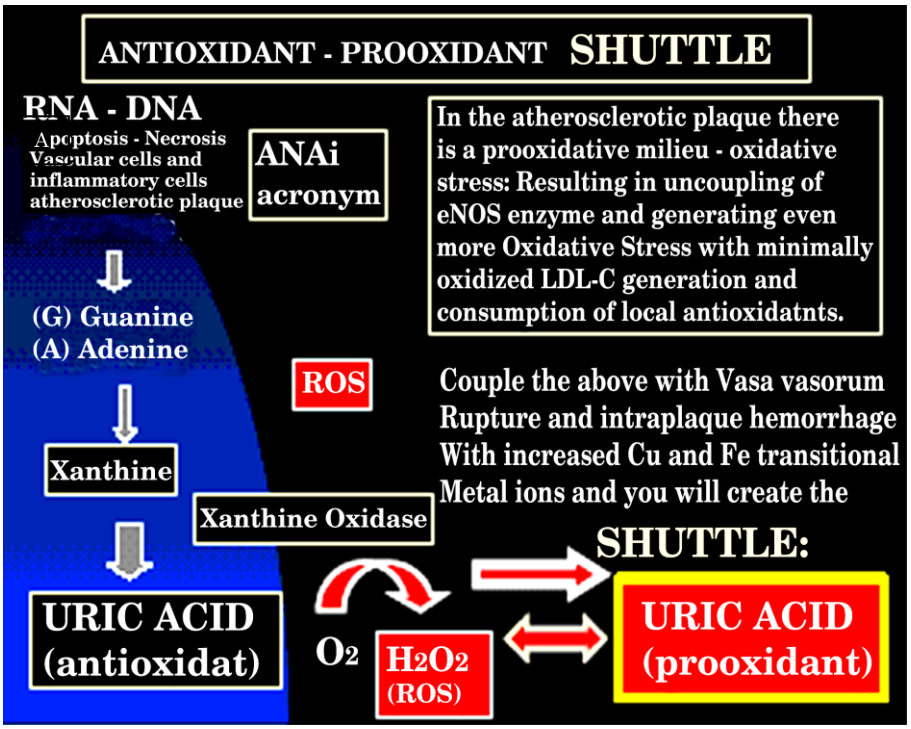

Fig. (1): Antioxidant-prooxidant urate redox shuttle *(Hayden and Tyagi, 2004).*

A pathophysiological role of xanthine oxidase has been related to its ability to generate reactive oxygen species that could cause both tissue structural damage and cell signaling interference *(Marsoni and Damia, 2004; Komaki et al., 2005)*. Moreover, cellular damage caused by chronic hypoxia was found to upregulate xanthine oxidase enzyme, leading to parallel increase of uric acid, and was associated with free radical production *(Berry and Hare, 2004).*

Hyperuricemia

Hyperuricemia in humans is defined by serum urate concentration >7.5mg/dl (0.45mmol/L) in men and >6.2mg/dl in women (0.37mmol/L*) (Ruggiero et al., 2006).*

When the uric acid/monosodium urate level exceeds their solubility in serum, the serum becomes supersaturated and crystals of sodium urate might precipitate and become deposited in soft tissues, particularly in or about joints *(Rodwell, 2003).*

Although gout is a well-known associate for hyperuricemia, yet high serum uric acid (SUA) concentrations were reported to be associated with a number of conditions that increase cardiovascular risk *(Waring et al., 2000)*. In fact, hyperuricemia has been often considered a part of the dysmetabolic syndrome or a marker of other coronary disease risk factors, such as hypertension, dyslipidemia, obesity, glucose intolerance as well as renal disease *(Facchini et al., 1991; Reaven, 1997; Johnson et al., 1999; Timar et al., 2000; Waring et al., 2000).*

Several possible pathological mechanisms linking hyperuricemia to cardiovascular disease were suggested; including the deleterious effects of elevated

uric acid on endothelial function, oxidative metabolism, platelet adhesiveness, hemorheology and aggregation *(Newland, 1975; Leyva et al., 1997; Butler et al., 2000; Doehner et al., 2002; Hoieggen et al., 2003; Alderman and Redfern, 2004)*.

Further, uric acid-mediated injury to cell membrane was believed to be the mechanism that contributes to the development of degenerative vascular disease in hyperuricemia *(Patetsios et al., 2001)*. One theory implicated urate to elicite cell membrane injury by linking to lysosomal membrane via hydrogen bonds, causing membrane lysis *(Mandel, 1979).* Another theory involved uric acid as a mediator of inflammation by directly activating complement factors *(Boogaerts et al., 1983)*. Moreover, uric acid might result in a free radical injury to the vessel wall *(Patetsios et al., 2001)*. In addition, possible adverse effects of elevated uric acid on the vasculature have been linked to increased chemokine and cytokine expression, induction of the renin-angiotensin system, increased vascular C-reactive protien (CRP) expression as well as to its free radical-inducing effect *(Kanellis and Kang, 2005).*

Recently, *Johnson et al. (2003*) stated that uric acid could, also, stimulate rat vascular smooth muscle