Study of Uric Acid and Ascorbic Acid as Strong Reducing Agents and Potent Antioxidants

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Hyperuricemia (HU) is associated with cardiovascular and renal disease. The aim of this study was to evaluate the role of uric acid as a risk factor for cardiovascular disease. Carotid Intima-media thickness (IMT) of the carotid arteries assessed noninvasively by ultrasonography is now validated as a sensitive marker for atherosclerosis and it is directly associated with increased risk of cardiovascular disease and is predictive of future cardiovascular events. Our study consisted of a group of 85 patients with CVD (cardiovascular disease) without HU (hyperuricemia) (male 58%, mean age ± S.D.: 49 ± 10 years), a group of 80 patients with CVD and HU (male 52%, mean age ± S.D.: 52 ± 10 years), and a control group of 80 healthy subjects (male 55%, mean age ± S.D.: 50 ± 11 years) hospitalized in the IVth Medical Clinic of University of Medicine and Pharmacy “Victor Babes” Timisoara in a one year period. The patients underwent complete clinically and paraclinically investigations (systolic and diastolic blood pressure, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and serum uric acid). All the patients in the study groups were also examined by high resolution B-mode ultrasound to measure the IMT of the common carotid artery. IMT values were significantly higher in the CVD patients groups with and without HU, compared to the control group (0.98 ± 0.28 mm, 1.41 ± 0.31 mm versus 0.36 ± 0.15 mm, respectively, p < 0.001). All patients with HU had significantly higher carotid IMT compared to the patients without HU. In this study we have shown that higher serum uric acid levels are associated with atherogenesis independently from CVD. Early screening for hyperuricemia and lowering serum uric acid levels might be beneficial in slowing progression of IMT in CVD patients

Keywords: uric acid, hyperuricemia, carotid intima-media thickness

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula C₅H₄N₄O₃. It forms ions and salts known as urates and acid urate such as ammonium acid urate. Uric acid is created when the body breaks down purine nucleotides. High concentrations of uric acid in blood serum can lead to a type of arthritis known as gout. The chemical is associated with other medical conditions like ammonium acid urate kidney stones [1-5] (fig 1).

Uric acid is a diprotic acid with pKₐ = 5.4 and pKₐ₂ = 10.3 [2]. Thus in strong alkali at high pH, it forms the dually charged full urate ion, but at biological pH or in the presence of carbonic acid or carbonate ions, it forms the singly charged hydrogen or acid urate ion as its pKa is greater than the pKa of carbonic acid. As its second ionization is so weak, the full urate salts tend to hydrolyze back to hydrogen urate salts and free base at pH values around neutral. It is aromatic because of the purine functional group.

As a bicyclic, heterocyclic purine derivative, uric acid does not protonate in the same manner as do carboxylic acids. X-Ray diffraction studies on the hydrogen urate ion in crystals of ammonium hydrogen urate, formed in vivo as gouty deposits, reveal the keto-oxygen in the 2 position of a tautomer of the purine structure exists as a hydroxyl group and the two flanking nitrogen atoms at the 1 and 3 positions share the ionic charge in the six membered pi-resonance-stabilized ring [2-7].

Thus, whereas most organic acids are deprotonated by the ionization of a polar hydrogen-to-oxygen bond, usually accompanied by some form of resonance stabilization (resulting in a carboxylate ion), uric acid is deprotonated at a nitrogen atom and uses a tautomeric keto-hydroxy group as an electron-withdrawing group to increase the pK value. The five membered ring also possesses a keto group (in the 8 position), flanked by two secondary amino groups (in the 7 and 9 positions), and deprotonation of one of these at high pH could explain the pK value and behavior as a diprotic acid. Similar tautomeric rearrangement and pi-resonance stabilization would then give the ion some degree of stability. (On the structure shown at the upper right, the NH at the upper right on the six membered ring is “1”, counting clockwise around the six membered ring to “6” for the keto carbon at the top of the six membered ring. The upper most NH on the five membered ring is “7”, counting counter clockwise around this ring to the lower NH, which is “9”.)

Uric acid is produced by xanthine oxidase from xanthine and hypoxanthine, which in turn are produced from purine. Uric acid is released in hypoxic conditions.

In humans and higher primates, uric acid is the final oxidation (breakdown) product of purine metabolism and is excreted in urine. In most other mammals, the enzyme uricase further oxidizes uric acid to allantoin. The loss of uricase in higher primates parallels the similar loss of the ability to synthesize ascorbic acid. Both uric acid and ascorbic acid are strong reducing agents (electron donors) and potent antioxidants. In humans, over half the antioxidant capacity of blood plasma comes from uric acid. The Dalmatian dog has a genetic defect in uric acid uptake by the liver and kidneys, resulting in decreased
conversion to allantoin, so this breed excretes uric acid, and not allantoin, in the urine [8-10].

In birds and reptiles, and in some desert dwelling mammals (e.g., the kangaroo rat), uric acid also is the end product of purine metabolism, but it is excreted in feces as a dry mass. This involves a complex metabolic pathway that is energetically costly in comparison to processing of other nitrogenous wastes such as urea (from urea cycle) or ammonia, but has the advantage of reducing water loss.

In humans, about 70% of daily uric acid disposal occurs via the kidneys, and in 5-25% of humans, impaired renal (kidney) excretion leads to hyperuricemia [11-13].

**Experimental part**

The study included 3 groups: the first group consisted of 85 patients (male 58%, mean age ± standard deviation: 49±10) with CVD without hyperuricemia (HU); the second group consisted of 80 patients with CVD and HU (male 52%, mean age ± standard deviation: 52±10); and the third group, was the control group represented by 80 healthy subjects (male 55%, mean age ± standard deviation: 50±11). The subjects from the control group had no cardiovascular or other systemic diseases and physical examination, electrocardiogram, chest radiography and two-dimensional Doppler echocardiography were normal.

Hyperuricemia was defined as the serum levels of >410 μmol/L in men, and >310 μmol/L in women.

**Carotid ultrasonography**

Subjects were investigated with a high-resolution B-mode operation system with linear transducers with 17 MHz frequency. To obtain a quality image, the optimal focal distance has to be between 30-40 mm, the optimal frame frequency 25 Hz and amplification setups must be done (for minimal intraluminal artifacts). The compensatory amplification has to be about 60 dB. Each subject rested in the supine position for several minutes in a temperature – controlled room. The brachial artery was identified at 5 cm proximal to the transiently bifurcation by using this High-resolution B-mode ultrasonography. After baseline imaging, a right arm cuff was inflated to >50 mm Hg above systolic blood pressure (SBP), for 5 min. After the cuff was deflated ischemia – induced distal hyperemia produced a transient increase of artery diameter. The relative change in mean arterial diameter was calculated as: % Dilation = [Maximum diameter – Baseline diameter] x 100 / Baseline diameter, where maximum diameter was the maximum mean diameter observed at 45 - 60 s after cuff release.

For carotid ultrasound study, the image was focused on the posterior (far) of the left carotid artery. A minimum of 4 measurements of the common carotid far wall were taken 10 mm proximal to the bifurcation to derive mean carotid IMT.

**Statistical analysis**

All the numeric variables were expressed as mean ± SD (standard deviation). Means were compared using analysis of variance of the Student t-test and Pearson’s correlation was used to test correlations and results. Statistical significance was defined as two – sided p < 0.05. The Anova One Way and Post Hoc Bonferroni tests were used to compare data. All statistical analyses were performed using Excel Microsoft Office 2003.

**Results and discussions**

Demographic data, distribution of traditional CV risk factors and the laboratory patient’s data are shown in table I. There is no significant statistical difference between groups concerning sex, age, cardiovascular profile risk and medical cardiovascular therapy, except of the serum total cholesterol (TC), triglycerides (TG) and low density lipoprotein – cholesterol (LDL – C).

The correlation between serum uric acid level and IMT in the control group was direct, strong and significant (α = 0.01) (fig 2-4).

In the group with CVD without HU we found a direct, medium and significant correlation between serum uric acid level and IMT (α = 0.05) (fig. 3) and in the group with CVD and HU, correlation between serum uric acid level and IMT was direct, strong and significant (α = 0.001) (fig. 4).

We also noticed that IMT values were significantly higher in patients with CVD, comparative with the control group. In the other two groups, with CVD, the patients with HU presented elevated IMT values comparatively with the patients without HU.
It was obtained the value of \( p < 0.001 \), meaning that between the IMT values for the three groups, the differences were significant (\( \alpha = 0.001 \)).

The values were compared for two by two groups, and in each case \( p \) was <0.001, meaning that there were significant differences (\( \alpha = 0.001 \)).

Conclusions

More than 50 years ago, Gertler noted an association between elevated levels of serum UA and coronary heart disease. Since then, several studies have attempted to establish whether UA is related to CHD events, independent of the known CVD risk factors.

Elevated levels of serum uric acid may be due to increased dietary intake of purines, increase in uric acid production, or decrease in its excretion. Differences in alcohol consumption, exercise, or dietary purine intake may cause transient hyperuricemia.

CVD are consistently associated with endothelial dysfunction and hyperuricemia is a strong predictor of hypertension and blood pressure progression. Therefore, individuals with essential hypertension constitute an interesting population in which to investigate the relationship between uric acid and endothelial dysfunction.

Even though hyperuricemia is often seen in CVD patients, the connection between them and the pathogenic mechanism is still unclear. Hyperuricemia has been linked to cardiovascular and renal diseases, possibly through the generation of reactive oxygen species (ROS) and subsequent endothelial dysfunction. Some other studies have reported that a high IMT value is strongly correlated with an increase of cardiovascular morbidity in patients with hypertension and hyperuricemia, but the role of hyperuricemia in the atherosclerosis process is not yet elucidated.

The thickness of the common carotid intima-media (IMT) measured by a noninvasive ultrasound technique is used as a marker of atherosclerotic disease and is directly associated with a high cardiovascular risk factor.

In our study we showed that IMT is higher in patients with CVD, with or without hyperuricemia, comparatively with the control group. We proved that this difference also exists between the two groups of CVD patients. We noticed that there were significant correlations between IMT, serum uric acid levels and other cardiovascular risk factors.

These results indicate that high levels of serum uric acid are associated with the atherogenic process, independently of hypertension.

Early screening for hyperuricemia and lowering serum uric acid levels might be beneficial in slowing progression of IMT in hypertensive patients. Thus, hyperuricemia induces endothelial dysfunction; this may provide insight into a pathogenic mechanism by which uric acid may induce hypertension and vascular disease.

References

Volumul Conservarea științifică a artefactelor din ceramică reprezintă o nouă și valoarosă valorificare a muncii de cercetare în domeniul investigațiilor chimico-fizice și al conservării științifice a bunurilor de patrimoniu a echipei ARHEOINVEST. Ea este înscripută în seria monografiei publicate de reputatul dascăl ieșean, cu scopul declarat de a prezenta studii asupra tuturor materiilor folosite în prelucrarea artefactelor de-a lungul timpului, precum și asupra materialelor suport ale operelor de restaurare.

În același timp, volumul constituie și un instrument de lucru pentru studenții de la restaurare, patrimoniu cultural, arheologie, pentru masteranzi și doctoranzi, pentru muzeografi și, în ultimul rând, pentru specialiști în conservare. Sunt prezentate pe larg aspectele legate de clasificarea mineralogică și fizico-chimică a argilelor, compoziția acestora, de temperatură, despre cauzele degradării și deteriorării ceramicii, prezentarea mai multor studii de caz etc. De asemenea, sunt descrise toate metodele și tehnici moderne aplicate în determinarea stării de conservare și în stabilirea autenticității bunurilor culturale din ceramică. 

Textul este însoțit de o ilustrație bogată, bine aleasă, care reflectă cele susținute în scris de către autori. Termenii folosiți, cele de specialitate și de conceptul creație sunt bine explicați, oferind cititorului posibilitatea de a cunoaște cât mai bine materialul folosit la nivel internațional și internațional. Bibliografia foarte bogată și de actualitate denotă cunoaștere și de specialitate și de actualitate denotă cunoaștere în detaliu a problematicii conservării, ridicând nivelul științific al demersului autorilor.

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