Int J Biol Med Res.2015;6(3):5211-5215

Contents lists available at BioMedSciDirect Publications

International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Review Article

Uricase and its clinical applications

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ARTICLE INFO

Keywords:
Uricase
hyperurecemia
Gout

ABSTRACT

Uricase the therapeutic enzyme used to regulate the concentration of uric acid in the serum. The uric acid is the metabolic product of purine degradation. Normally the concentration of uric acid in the serum is 6.8 mg/dL, beyond this the hyperurecemia condition occurs. These then results into the inflammatory pain in the joints and tissues, the disease is called Gout. Currently 8.3 million people in the US, around 3 % population are suffering from Gout. The concentration of uric acid varies according to age and sex. The enzyme uricase is not expressed in the human being, but it is produced by microorganisms, plants and other animals. The uricase not only used to detect uric acid level in serum and urine but also used to control its concentration. The uricase acts on uric acid and convert it into the more soluble compound allantoin. The uric acid is poorly soluble i.e. ~ 11 mg/dL compared to allantoin which is ~147 mg/dL. The uricase biosensors are developed to regulate the uric acid level.

INTRODUCTION

More than three decades ago allopurinol was considered as a first line drug which regulate uric acid synthesis by inhibiting xanthine oxidase (XO) enzyme. . In addition to allopurinol, febuxostat is also used to reduce the synthesis of uric acid by inhibiting the XO enzyme and is approved by the European Medical Agency in 2008 and US FDA in 2009. . In contrast to allopurinol it acts on both reduced as well as oxidised form of XO. . Partial relief from inflammation can be obtained by administration of hydroxychloroquine and non steroidal anti-inflammatory drugs. .

However these drugs are prone to have enormous side effects, so in order to regulate the uric acid level in serum or soft tissues, enzymatic treatment is considered to be the better way without any side effects. The enzyme uricase is well known for the treatment of gout disease. The gout disease is in response of accumulation of uric acid crystals which is the product of purine metabolism. Uricase (urate oxidase) EC 1.7.3.3 a tetramer therapeutic enzyme which oxidatively opens the purine ring of uric acid and forms allantoin, CO_2 and $H2O_2$ as shown in equation no. 1. . Allantoin is highly water soluble compound compared to the uric acid. Uricase is also acts as diagnostic enzyme used to determine the concentration of urate in serum and urine. . In addition to the uricase, peroxidase can also be alternatively used to estimate the uric acid concentration. . Uricase enzyme is highly conserved present in mammals, plants, fungi, bacteria and yeasts but is absent in humans due to evolutionary mutations in uricase gene. . Uricase catalyses the following reaction:

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$$\text{Uric acid} + H_2O + O_2 \longrightarrow \text{Allantoin} + CO_2 + H_2O_2 \quad \text{...Eqn No. 1}$$

Uric acid is poorly water soluble as ~11 mg/dL compared to the product allantoin which has solubility ~ 147 mg/dL. . Hence allantoin is easily excreted through the urine.

Table no.:1 Physico-chemical properties of Uricase:

Physico-chemical Properties	
Molecular Weight (Dalton)	145000-150000
Isoelectric point	4.3
Optimum pH	9.5
Optimum Temperature (°C)	30-35
Denaturation Temp. (°C)	40-60

Table no.:2 Uricase Sources

Sources	Opt. pH	Opt. Temp.	Enzyme activity	Reference
Bacillus subtilis	8.5	35	42.77 U/ml	(13)
Microbacterium spp.	8.5	37	1.0 U/ml	(18)
Pseudomonas aerogenosa	5.5	30	7.1 U/ml	(19)
Streptomyces exfoliates	8.0	45	0.5 U/ml	(56)
Bacillus cereus	8.5	30	15.43 U/ml	(57)
Bacillus thermocatenulatus	8.5	25-80	1.25 U/ml	(7)
Gliomastix gueg	8.5	25	275.98 U/ml	(58)
Gliocladium viride	7.5	30	63.14 U/ml	(59)

Candida utilis	**8.5**	**25**	**1.04 nkat**	**(60)**
Aspergillus niger	**9.0**	**30**	**47.40 U/ml (extracellular)**	**(61)**
Aspergillus niger	**9.0**	**30**	**12.90 U/ml (intracellular)**	**(61)**

Table no. 3: Uricase activity inhibition by Compound:(20)

Compound	Inhibitory Conc. (mM)	% Inhibition
Cu	0.02	75
Fe	1.0	35
Zn	2.0	80
Co	1.0	60
Ni	1.0	40
Potassium thiocyanate	10.0	93
Potassium ferrocyanide	15.0	10

Table no. 4: Effects of increase uric acid production

Increased Uric acid Production (62)	
Primary	**Secondary**
Purine metabolism enzyme defects	Specific enzyme defects
	Myelo or lyphoproliferative disorders
	Infectious mononucleosis
	Chronic haemolytic anaemia
	Gaucher's disease
	Obesity
	Exercise
	Ethanol abuse

Table no. 5: Effects of decreased uric acid excretion rate

Decreased uric acid excretion (62)	
Primary	**Secondary**
Idiopathic	Hypertension
Familial Juvenile gouty nephropathy	Renal insufficiency
	Hyperparathyroidism
	Diuretic drugs and low dose aspirin
	Sarcoidosis

Table No. 6: Concentration of uric acid with age and sex

Person	**Concentration of Uric acid (mg/dL) (63)**
Child	3.6
Mature male	7.3
Mature female	5.9

Table no.:1 Physico-chemical properties of Uricase:

The above parameters are considered for the optimum uricase activity like pH 9.0 to 9.5, temperature 30 to 35°C.

Table no.:2 Uricase Sources

From table no. 2, it can be understood that the uricase activity is highly dependent on temperature, pH and the source. And also can be observed that the fungus cultures are the overproducers of uricase. In addition to the microbial source, uricase is also reported from different leguminous plants as cowpea, soybeans, bean, and the leaf of chickpea, broad bean, wheat and tobacco leaves . Also uricase is found in leaves, stems, roots and seeds of flax plant (Linum usitatissimum). .

2. Uricase Activity:

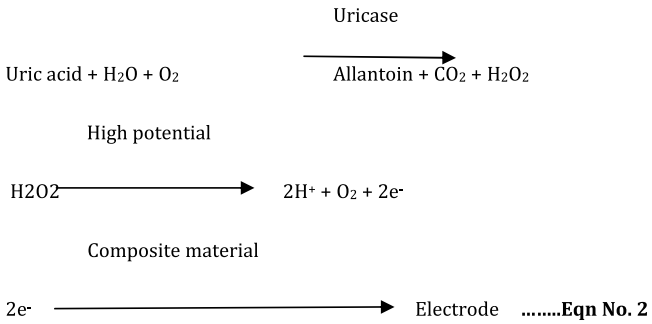
The uricase activity is assayed with respect to uric acid concentration either by disappearance of substrate uric acid detected by a decrease in the absorbance at 293 nm or increase in absorbance of product allantoin in presence of uricase. .

The activity of purified uricase is inhibited by the metallic complexes and some heavy metals shown in table no 3. The 93 % of inhibition of uricase activity is observed with Potassium thiocyanate at concentration of 10 Mm.

Table no. 3: Uricase activity inhibition by Compound:

3. Uricase Biosensor:

The biosensor can be developed to detect uric acid qualitatively as well as quantitatively by immobilisation of uricase either in matrix e.g. polypyrrole , or on gold/amino acid nanocomposites , or on chitosangraft polyaniline composite film or is covalently immobilized with the help of glutaraldehyde as a cross linker onto electrochemically synthesized polyaniline films. . Due to the covalent attachment the efficiency and half life of the uricase is increased. The affinity of the Uricase towards the substrate uric acid is increased from 3.4 x 10-1 mM/L to 5.1 x 10-3 mM/L. . The following electrochemical reaction takesplace at the time of response measurement: . The number of electrons generated is directly proportional to the substrate uric acid conversion by uricase.



.....Eqn No. 2

The sensor is capable of detecting uric acid from serum at the rate of 100 serum samples in 94 min.–.

Gout is one of the most common autoinflammatory arthritis disorder caused due to hyper accumulation of uric acid crystals (especially monosodium urate crystals (MSU)) in the joints and serum. . This results into the development of Lesch Nyan syndrome. . Recently it has been estimated that above 8.3 million people in United States at around 3% of the populations are suffering from gout. . Along with MSU crystals two other types of crystals are also contribute to gout as calcium pyrophosphate dehydrate leads to pseudogout and basic calcium phosphate (BCP/hydroxyapatite). . Bone erosion is commonly observed in the chronic gout disorder due to the accumulation of MSU which results into the joint damage and deformity. . Uric acid is the end product of purine metabolism and the hyperurecemia condition arises after the deposition of uric acid beyond its solubility point

6.8 mg/dL. . However the low concentration of uric acid acts as an antioxidant which scavenges serum free radicals and protects against cancer. . The concentration of uric acid is regulated by the endogenous metabolism, reabsorption and excretion rate by the kidney. . The most common cause of hyper uric acid accumulation is the reduced renal clearance. . This results into the hypertension and increase in the kidney related disorders. Along with these, alcohol consumption , obesity, hypertension, use of diuretic drugs and low dose of aspirin also contribute to the gout. . Hypertriglyceridemia is being observed in 80% gout populations. . The disease is most probably common in the middle aged men and is increasing in the elder populations along with postmenopausal women. . The current research on animals and humans conclude that the accumulation of monosodium urate crystals results into the stimulation of inflammatory response which indeed activates the nitric oxide, prostaglandins and proinflammatory cytokines as IL-1, IL-1β, which are produced by macrophages, dendritic cells, monocytes and inflammasome complex. . Anti-inflammatory therapies including colchicines, nonsteroidal anti-inflammatory drugs, glucocorticoids are commonly used decades ago for the treatment. .

4. Classification of Gout:

The gout has been classified as

4.1 Primary gout:

It is being classified as an abnormal uric acid metabolism without any known symptoms.

4.2 Secondary gout:

It is caused by an acquired disorder.

Table no. 4: Effects of increase uric acid production

Table no. 5: Effects of decreased uric acid excretion rate

5. Diagnosis of Gout:

The physical identification of gout can be done by observation to patient's big toe. The interphalangeal joint of the big toe of skeletal remains of patient possesses cavitations, erosions and osteophytic margins. . The abnormal metabolite concentrations in serum and urine are principle indicators of many pathogenic conditions including xanthinuria, hyperurecemia and gout. .

The various diagnosing techniques are available as,

5.1 Conventional Radiography (CR)

The CR involves the passing of X-rays through the body part onto the flat detector which then produces the projectional image. .

5.2 Ultrasonography (US)

Now a days, US is prominently used for the detection of gout which directly shows MSU crystal deposition in early gout. . US of bones and joints is non-invasive, nonirradiating, and cheap comparatively to the other techniques. .

5.3 Magnetic Resonance Imaging (MRI)

MRI have efficient sensitivity to detect bone erosion rather than US which detects MSU deposition. .

Besides these, several other methods are there to determine the concentration of uric acid such as high performance liquid chromatography (HPLC), chemiluminiscence, electrochemical method and UV-Visible spectroscopy.

5.4 Diagnosis and Quantification of Uric acid by Uricase

Based on the uricase enzymatic reaction, the concentration of uric acid in serum and urine is determined by measuring the absorption spectra at 293 nm by UV-Visible spectroscopy. . The absorption spectra measures the decrease in concentration of uric acid after reacting with uricase. .

6. Purine Metabolism Pathway: (55)

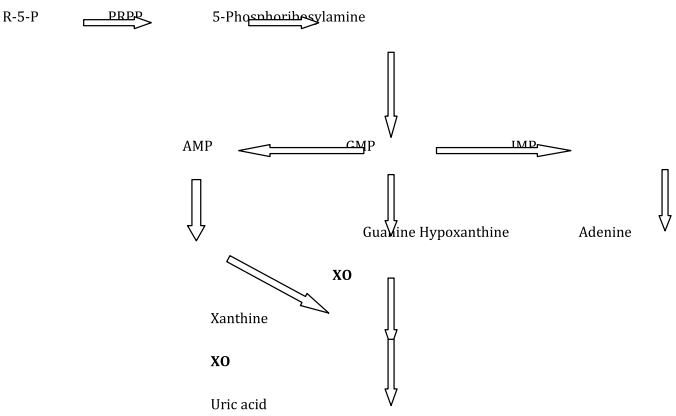


Table No. 6: Concentration of uric acid with age and sex

Table no. 6 shows the variability of concentration of uric acid with respect to age and sex. After attainment of menopause, the female uric acid concentration becomes nearly equal to the matured male. Hence the chances of MSU crystal formation are more in the males exceeding age 50.

7. Conclusion:

Hence it seems from this review article that the concentration of MSU crystals can be reduced into the soluble form i.e. allantoin with the help of uricase enzyme. The uricase can be produced on large scale by using above mentioned microbes. The enzyme can be used without any side effects in contrast to the drugs. The uricase biosensors are prominently used to measure uric acid level qualitatively as well as quantitatively. Hence finally it is concluded that the uric acid level in serum can be regulated by the use of uricase as a therapeutic drug.

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