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Hyperuricemia and the small intestine: Transport mechanisms and co-morbidities☆

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A B S T R A C T

There is a global increase in cases of hyperuricemia over the last 10 years. A critical component of serum uric acid control is the transport of uric acid to the intestinal lumen, which accounts for 30% of the uric acid eliminated from the serum. This mini review looks at two important aspects of elevated uric acid: the dynamics of intestinal uric acid transport and hyperuricemia co-morbidities. Elevated serum uric acid can lead to gout and it can also impact other diseases such as diabetes, cardiovascular diseases and nervous system diseases. The level of uric acid in the intestine could be related to the potential for uric acid to impact other morbidities. We review the evidence for this and what it would mean for persons with elevated serum uric acid.

1. Introduction

Normal human urine is mostly comprised of urea, with a small amount of uric acid. The pKa of uric acid is about 5.75, so it exists in the form of urate in the urine and other bodily fluids under the pH value of 7.4[1]. Uric acid, also known as trioxypurine, is the end-product of purine metabolism and it is weakly acidic in the alcoholic form (Fig. 1). Uric acid produced after the oxidation of purines is excreted in the urine. The rate of uric acid production and excretion largely remains constant in humans. However, temporary fluctuations in uric acid concentrations occur and are mediated through metabolism, immunity, and other bodily functions. High and low serum uric acid concentrations depend on a state of balance between purine synthesis, food intake, and uric acid excretion. After metabolism and circulation, two-thirds of the uric acid is excreted by the kidneys, while the remainder enters the intestines (from the blood or the stomach) where it is further broken down by colonic bacteria and then eliminated.[1,2]

Thus, there are two main sources for the accumulation of uric acid: difficulty with purine metabolism; and impaired excretion of uric acid through the kidney and intestine. Normally, there is approximately 1,200 mg of uric acid in the body, about 600 mg of newly generated uric acid per day, and 600 mg of uric acid excreted at the same time, in approximate equilibrium.[3] Changes to this equilibrium (too much uric acid is produced in the body to be excreted or the uric acid excretion mechanism degenerates) can result in the accumulation of uric acid. When the blood uric acid concentration is greater than 7 mg/dL, the body fluids will become acidic, which triggers gout: a kind of crystallization associated with arthropathy resulting from monosodium urate deposition.[4] Gout is directly linked to hyperuricemia: resulting from a failure in purine metabolism possibly coupled to reduced uric acid excretion. Tophi (masses of uric acid crystals) are frequent in patients with gout. They can be localized subcutaneously in the auricle, around the joints, tendons, soft tissues and other surrounding tissues of patients.[5]

2. Uric acid transporters involved in intestinal elimination of uric acid

To prevent the conditions associated with gout, approximately 600 mg of uric acid has to be eliminated each day. A significant part of the body's uric acid homeostasis is mediated through the intestine. Control of intestinal uric acid flux is mediated through both passive and active transporters. There are three primary uric acid transporters in the intestine: GLUT9, ABCG2 and MRP4.

3. GLUT9

The gene encoding GLUT9 is a member of the glucose transporter family which is located at 4p15.3-p16 and contains 1 noncoding region exon and 13 coding region exons with two splice variants: the long isoform (GLUT9L) and the short isoform (GLUT9S). GLUT9 is mainly distributed in the liver, kidney, intestine, and placenta, and in the renal

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proximal convoluted tubules. GLUT9L is found on the basement membrane side (Fig. 2[6]), whereas GLUT9S is found on the luminal side.[7–9]

GLUT9S functions similarly to another transporter URAT1, which is encoded by SLC22A12, and these two genes (SLC2A9 and SLC22A12) are not only important susceptibility genes for hyperuricemia but also causative genes for low uric acid levels. Single nucleotide polymorphisms (SNPs) in this gene can significantly affect serum uric acid levels, resulting[10] (Fig. 2). On the basement membrane side of renal tubular epithelial cells, GLUT9L is closely associated with urate reabsorption, and cytological studies have shown that loss of GLUT9L in the kidney blocks urate reabsorption, suggesting that GLUT9 transporters are crucial in the process of urate reabsorption. A study of GLUT9 mutant mice revealed increased serum uric acid levels with a significant decrease in urinary uric acid excretion.[11] GLUT9 is a highly efficient urate transporter and can facilitate urate complexes with other urate transporters to mediate urate reabsorption and maintain a serum urate balance in vivo.[12]

Debosch et al. studied the function of GLUT9 transport of uric acid in the intestine in a knockout ($GLUT9^{-/-}$) murine model. In this study, the renal ability to clear uric acid remained normal.[13] Compared with the control mice, the uric acid levels in the blood and urine of the vehicle mice, which were mutated for intestinal GLUT9 only, increased.

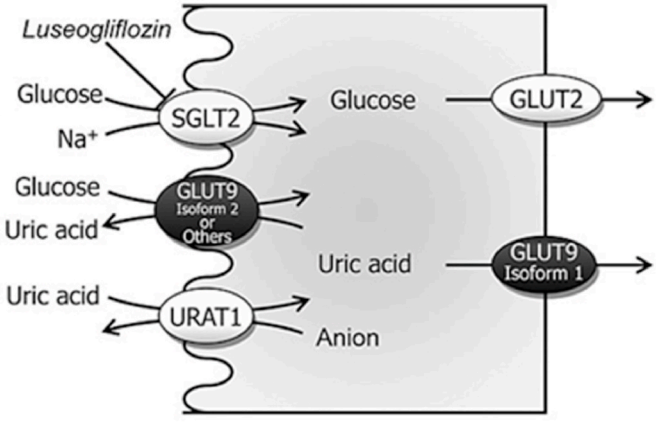

Fig. 2. Schematic diagram of compound transport by GLUT9.[6]

4. ATP binding cassette subfamily G member 2 (ABCG2)

The ABCG2 protein, encoded by a gene of the same name, is found on the apical surface of proximal tubular cells and intestinal epithelial cells. ABCG2 plays an important role in the excretion of urate by helping the small intestine and kidneys to perform urate excretion using energy from ATP (Fig. 3[14]). Its genetic single nucleotide polymorphisms (SNPs) can affect protein expression levels, transport efficiency of substrates, protein activity, etc.[15,16]

Functional deficiency of the ABCG2 gene in vivo results in increased serum uric acid levels. It has been found that 90% of young patients with gout (age of onset less than 30 years) have severe deficiency of expression of this gene and that patients with gout with mutations in ABCG2 have a significantly earlier age of onset than patients with functional ABCG2, indicating that loss of function of ABCG2 is associated with gout.[17,18] Knockout *ABCG2* mice had significantly lower intestinal excretion of uric acid, suggesting an important role for ABCG2 in controlling uric acid transport to the intestine.[19]

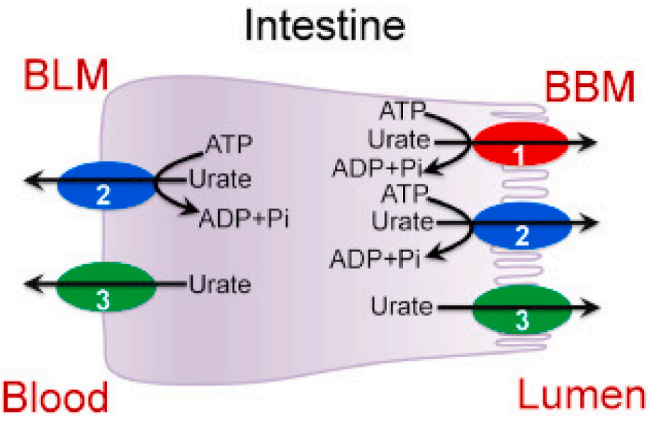

Fig. 3. Urate is transported out of the cell with the assistance of ABCG2.[14]

5. Multidrug resistance-associated protein 4 (MRP4)

MRP4 is a multi-drug resistance protein, meaning that cells can tolerate the lethal dose of a variety of drugs with different structures, functions, and pharmacological mechanisms by expressing MRP4. Tolerance can be seen for several drugs, simultaneously. MRP can participate in the transport of a variety of intracellular and extracellular complexes, adjust the distribution of intracellular substances and also participate in the transport of substances as a transport pump. These proteins are responsible for drug resistance in some tumors. MRP4 is widely distributed in human normal tissues, especially in the kidney, liver, small intestine and other organs.[22] Studies have shown that MRP4 can significantly promote the metastasis of human dendritic cells to lymph nodes, which has been suggested as an immunotherapy.[23]

MRP4 is located on the apical membrane of the renal proximal convoluted tubule to mediate urate excretion. It is involved in the transport of urate from epithelial cells into renal tubules. In addition, it transports other substrates in the kidney. A study in 2011 showed that the uric acid excretion in MRP4 knockout birds was 35% less than that of the control group.[24] MRP4 is also expressed in the basolateral membrane of hepatocytes. Uric acid is transported into the blood through MRP4 on the hepatic basolateral surface. However, the exact transport mechanism is still unclear. In 2019, a study by Xuedong et al. demonstrated that MRP4 is the main transporter of uric acid excretion when kidney and liver function are normal. Importantly, MRP4 in the intestine will exhibit compensatory increases in its expression when renal function and liver function are impaired, providing an intestinal mechanism for uric acid elimination.[25]

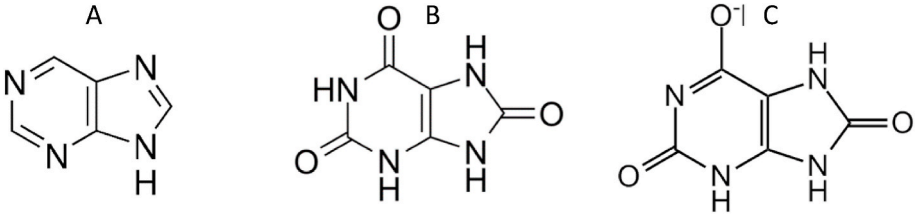

Fig. 1. Chemical structures of (A) purine, (B) uric acid, and (C) urate (from left to right).

6. Morbidities associated with hyperuricemia

6.1. Gout

Gout is a crystalline arthritis caused by the imbalance of purine metabolism and/or the abnormal excretion of uric acid. The clinical manifestations mainly include acute gouty arthritis, which can lead to renal dysfunction and major joint disability. Gout is not restricted to any particular geographic region, occurring throughout the world.[26] However, the pathogenesis and self-alleviation mechanisms of gout have not been fully elucidated and there are some difficulties in developing accurate clinical diagnoses.[27] Traditionally, hyperuricemia is associated with gout if the concentration of uric acid in the blood persists at more than 540 μmol/L. At this level the probability of gout will be greatly increased, and about 70%–90% of people will be affected.[28]

Although the clinical treatment of gout is extremely difficult, it can still be cured. The standard for a diagnosis of gout is to find birefringent acicular urate crystals in the patient's joint fluid or gout stones under a polarized light microscope. However, this kind of examination is invasive and at present, there are few hospitals that perform it. The majority of hospitals still use colchicine for diagnostic treatment of suspected gout patients, which is prone to missed diagnosis and misdiagnosis.

Acute gouty arthritis is caused by the accumulation of sodium urate crystals. Most of the forms of uric acid in the human body are sodium salts, which deposit and form crystals in cartilage, articular cavity, kidney, and soft tissue after circulation. Since abnormal sodium urate crystals were found in patients with gout, it is considered that sodium urate crystals are related to the clinical development of gout. The synovial fluid in the joint cavity of patients with gout can regulate uric acid, and the reduction of uric acid will increase the possibility of phagocytosis of remaining gout stones, which are highly inflammatory.[29] Some studies have confirmed that the synovial fluid in the articular cavity of gout patients is more likely to crystallize uric acid than that of patients with other joint diseases, so the accumulation of sodium urate crystals is closely related to the clinical development of gout.[30] There is more than one way that gout inflammation via sodium urate crystallization can occur. Examples include the Toll receptor, activating inflammatory substances and phagocytes. (1) The Toll receptor is a model receptor, which plays a key role in the generation of immune factors. Toll receptors transmit an inflammatory signal after the activation of urate crystallization and start the transcription of pro-inflammatory cytokines, resulting in gout inflammation. (2) Gout inflammation caused by the activation of inflammatory substances: inflammatory substances refer to macromolecular substances that can activate inflammatory enzymes and cytokines. The reaction mechanism of inflammatory substances-mediated gout inflammation is still unclear. (3) Inflammation caused by the activation of phagocytes: sodium urate crystallization will stimulate neutrophils in joint synovial cells. Neutrophils can form an extracellular network to phagocytize urate crystals, and when the formation of the extracellular network is inhibited, the phagocytosis of sodium urate crystals will be reduced.

One study showed that serum uric acid values increased and fluctuated greatly from birth to 3 Days; From 3 Days to youth, the value of blood uric acid was low (<5 mg/dL).[31] Hyperuricemia and gout are more common in middle-aged and elderly people over 40 years old, and their prevalence usually increases with age. The high incidence age of men is 40–49 years old, and women mostly suffer from gout after menopause. The prevalence age in developed regions and countries is earlier than that in developing countries. In western developed countries such as Europe and America, on average, about 2% of men are affected by gout. The prevalence of hyperuricemia and gout in American adults reach 21% and 3.9%, respectively.[32–34]

In recent years, with societal and economic progress, people's dietary structure has changed greatly, people's lifestyle and quality of life have been improved; however, the aging of the population is becoming more and more serious, and hyperuricemia has become a very common high-risk disease in older people. Early intervention and treatment of the disease are particularly important in the clinic. The incidence rate of hyperuricemia and gout in male patients is higher than that in females. The cumulative incidence rate of males is about 8.6%, but primary gout is 5.9%. The ratio of male to female gout prevalence is about 20:1, and hyperuricemia is about 2:1. The main reason for this difference is that androgen can promote the reabsorption of uric acid and inhibit the excretion of uric acid, while estrogen can promote the excretion of uric acid.[34] Compared with the age of male patients, female patients generally developed gout late, with an average difference of about 8.5 years. The incidence rate of gout in male adult patients is about 6.2% while that in women is only about 0.9%, according to one study.[35]

The fundamental approach for gout prevention and treatment is to quickly control the acute attack of gouty arthritis, prevent the recurrence of acute arthritis, reduce the high level of uric acid, prevent joint damage and kidney damage caused by urate deposition, dissolve the formed urate crystals and prevent the formation of new crystals. In addition, with the continuous development of molecular diagnostic methods and genomics, establishing a relationship between genotype and hyperuricemia will play an important role in the early screening and prevention of gout, prediction of disease development, treatment and prognosis evaluation, as well as the development of new drugs for gout and its associated diseases, such as diabetes.

6.2. Diabetes

There is increasing evidence that hyperuricemia is a predictive factor in the development of diabetes. Studies have demonstrated the positive correlation between elevated serum uric acid and risk of diabetes.[36–38] In recent years, retrospective studies and prospective studies have documented that changes in diabetes prevalence were positively correlated with uric acid concentration in the population.

A retrospective study of male veterans in the United States showed that the incidence of diabetes with UA < 7 mg/dL was 19%, 7 mg/dL < UA < 9 mg/dL was 23%, UA>9 mg/dL was 27%, and the incidence of hyperuricemia was significantly associated with the risk of diabetes in 1,923 diabetic patients who were followed up for 80 months.[39]

The relationship between the duration of diabetes and the uric acid level was analyzed by linear regression analysis. The study found that uric acid concentration increased before the diagnosis of diabetes, and then decreased with the duration of diabetes.[40] The uric acid level was associated with an increased risk of diabetes. In a prospective study by Framingham et al., serum uric acid was found to be a strong and independent risk factor for diabetes.[41] The incidence of type 2 diabetes also increased with increased serum uric acid levels. Data showed that the risk of type II diabetes increased by 15% when the serum uric acid concentration increased by 1 mg/dL, but this finding does not support a correlation between high uric acid levels and diabetes.[42]

A study of the association between serum uric acid level and prediabetes in China, with 500 China adults as the subjects, found that serum uric acid was associated with the control of blood glucose in type 2 diabetic patients. If the blood glucose was controlled within the appropriate range, the blood uric acid concentration would also be reduced as well. These studies indicate that blood uric acid is a potential biomarker for diabetes risk, although not all studies have found that there is a necessary link between them.[43]

In addition, some studies have found that the relationship between serum uric acid and the risk of diabetes is not consistent in different genders. Hyperuricemia is positively correlated with hyperinsulinemia, and the increase of uric acid may lead to insulin resistance, and women have stronger insulin resistance than men.[44,45]

6.3. Cardiovascular diseases

Hypertension is one of the most common clinical cardiovascular diseases and an important risk factor for cardiovascular disease

progression. Cannon et al.[46] established that 47% of patients with untreated hypertension had high uric acid, while 75% of patients with malignant hypertension had high uric acid. Every 59.5 μmol/L increase in serum uric acid level increases the risk of hypertension in children and adolescents by 50%.[47] A 20-year study on healthy people showed that the higher the baseline or end-point uric acid level, the greater the change of uric acid level before and after the experiment, the higher the risk of sudden hypertension.[48] Compared with the population without high uric acid, patients with high uric acid also had a significantly higher risk of developing hypertension from prehypertension within 5 years.[49] Compared with the low uric acid group, the risk of hypertension increased by 66% after 10 years in the group with elevated normal uric acid levels.[50]

Coronary artery calcification (CAC), as one of the markers of atherosclerosis (AS), can predict the occurrence of cardiovascular disease as well. Studies showed that the risk of CAC increased by 31% for every 59.5 μmol/L increase in serum uric acid level.[51] A study of 16,063 patients showed that the risk of coronary heart disease (CHD) increased by 14% for every 100 μmol/L increase in serum uric acid level.[52] Endothelial dysfunction is an important manifestation of coronary artery disease. Reactive hyperemia index (RHI) is often used to evaluate endothelial function. In patients with the acute coronary syndrome, serum uric acid level was positively correlated with RHI ($P < 0.001$), suggesting that serum uric acid level is related to the severity of coronary artery disease.[53] Other studies showed that the in-hospital mortality of ACS patients with serum uric acid levels higher than 356.9 μmol/L was significantly higher than that of ACS patients with serum uric acid levels lower than 356.9 μmol/L.[54] The analysis confirmed that the mortality of CHD increased by 12% with the increase of serum uric acid level of 59.5 μmol/L, suggesting that uric acid level can predict the prognosis of CHD patients.[55]

Heart failure (HF) is the terminal stage of many cardiovascular diseases. In one study the incidence of heart failure increased by 19% for every 59.5 μmol/L increase of serum uric acid level.[56] A 29-year follow-up period prospective study of 4,912 young patients in the United States divided subjects into Q1 group (71.3–257.8 μmol/L), Q2 group (257.9–308.9 μmol/L), Q3 group (309.0–368.3 μmol/L) and Q4 group (368.4–813.8 μmol/L) according to uric acid level. Compared with those in the Q1 group with low uric acid levels, the risk of heart failure in the Q4 group was increased by 6 times.[57] In elderly men receiving antihypertensive therapy, the incidence of heart failure increased by more than 2 times in the high uric acid group compared with those in the low uric acid group. The experimenters considered that serum uric acid level was more predictive of the risk of heart failure than other common risk factors such as body mass index, hypertension, angina pectoris, and diabetes.

6.4. Nervous system diseases

Parkinson's disease is a dyskinesia disease caused by the progressive degeneration and reduction of dopamine neurons in substantia nigra. Oxidative stress and nitriding stress are the main pathogeneses of Parkinson's disease. These stresses lead to the loss of dopaminergic neurons in substantia nigra in Parkinson's disease.[58,59] Uric acid can clear $ONOO^-$, chelate iron and stabilize ascorbic acid to prevent dopaminergic neuron apoptosis. Uric acid levels in substantia nigra, blood, and cerebrospinal fluid decreased in patients with Parkinson's disease. A series of large-scale epidemiological studies found that the incidence of Parkinson's disease in patients with hyperuricemia was low. The incidence of Parkinson's disease was lowest in patients with a high uric acid diet and gout history. The risk of Parkinson's disease in healthy people also decreased with the increase of uric acid. Clinical and imaging studies have found that the progress of early Parkinson's disease with hyperuricemia is slower than hypouricemia.[60] These results suggest that hyperuricemia may prevent the occurrence of and delay the progression through Parkinson's disease. Recently, the Parkinson's disease research group in the United States reported that oral inosine increased uric acid and can delay the development of dyskinesia in patients with early Parkinson's disease. The authors suggest that inosine can be used as a modified treatment for Parkinson's disease.[61]

Alzheimer's disease is dementia caused by chronic progressive degeneration of the central nervous system. The characteristic pathology of Alzheimer's disease is a β-Amyloid deposition and neurofibrillary tangles. Recent studies have found that oxidative damage is involved in the pathophysiological process of Alzheimer's disease. An autopsy showed that nitrated tyrosine in the hippocampus, cortex, and cerebrospinal fluid increased significantly in patients with Alzheimer's disease. Animal experiments showed that injection of $ONOO^-$ donor into hippocampus can cause Tau nitration and hyperphosphorylation, loss of the ability to bind and stabilize microtubules, and abnormal aggregation in neurons. Injection of uric acid can prevent Tau nitration and hyperphosphorylation. Through this mechanism hyperuricemia may decelerate the development of Alzheimer's disease.[62,63]

The researchers analyzed the data of the UK health promotion website from January 1, 1995, to December 31, 2013. 59,224 gout patients and 238,805 non-gout patients were included. There were 309 AD patients in 59,224 gout patients and 1,942 AD patients in 238,805 non-gout patients. Cox proportional regression model showed that the relative risk ratio of gout patients to non-gout patients was 0.71. This study found that gout has a negative correlation with AD in a large population, which supports the previous hypothesis that uric acid has a neuroprotective function and can also provide some benefit in the treatment of Parkinson's disease.[62]

Like other types of dementia, Alzheimer's disease first appears as mild cognitive impairment (MCI), which aggravates and develops into Alzheimer's disease. MCI represents the early stage of Alzheimer's disease. The level of 8-oxy-7,8-dihydro-2 ′- deoxyguanosine (8-oxodG) in the brain DNA of patients with Alzheimer's disease increased at autopsy, and 8-oxodG in leukocyte DNA of patients with Alzheimer's disease, Parkinson's disease, and MCI also increased. It can be seen that oxidative damage is a common pathological process of neurodegenerative diseases. The study found that the levels of uric acid in serum, brain tissue, and cerebrospinal fluid of patients with Alzheimer's disease and MCI decreased significantly. Hyperuricemia can delay the process from MCI to Alzheimer's disease. It is known that people with the apoE4 allele are more likely to develop Alzheimer's disease than those with apoE3. In the ApoE4 group with a high incidence rate of Alzheimer's disease, the plasma antioxidant capacity decreased and serum uric acid levels decreased. It is speculated that the decrease of serum uric acid level in patients with Alzheimer's disease may be involved in the occurrence and development of Alzheimer's disease. However, other studies have reported that uric acid levels in the blood and cerebrospinal fluid of patients with Alzheimer's disease are not related to cognitive function score and its decline. See Fig. 4 for detailed uric acid oxidation.[64]

6.5. Reducing hyperuricemia via intestinal transport of uric acid

There have been studies aimed at understanding the role that the intestine plays in uric acid homeostasis for the purpose of reducing serum uric acid levels. The idea behind these efforts is that reducing the amount of uric acid in the intestinal track has the potential to shift the overall equilibrium towards lower uric acid throughout the body.

There are three main mechanisms of action for currently used hyperuricemia treatments: (1) competing with xanthine oxidase enzymes to reduce the production of this compound in liver (e.g., allopurinol, febuxostat), (2) preventing kidney reabsorption by blocking uric acid transporters (e.g., URAT1, GLUT9) (e.g., probenecid, benzbromarone), or (3) degrading uric acid using recombinant urate oxidase (e.g., pegloticase,[12] rasburicase[13]). As with any therapeutic intervention, these approaches can have unintended side-effects including hypersensitivity, intolerance, diminished liver function, and compromised immune responses.[5,14] Allopurinol is an example of a drug that has

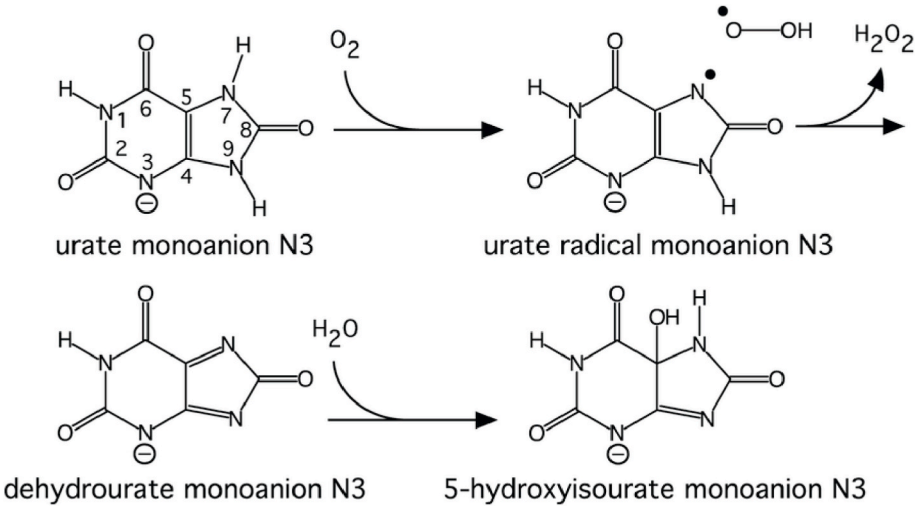

Fig. 4. The whole progress of UA oxidation.

negative outcomes, especially among patients with renal insufficiency.[15,16] For those patients intolerant of allopurinol there are options (febuxostat and benzbromarone), but these drugs can lead to compromised liver function and liver toxicity.[17] For these patients, recombinant urate oxidase therapy provides an alternative. However, these drugs appear to be suitable for short-term treatment only.[12,14]

As discussed above, there are two major pathways for uric acid removal in the intestine: polarized efflux from the basolateral to the apical (luminal) side of intestinal cells via transporters like BCRP (ABCG2)[18] and increased diffusion into intestinal lumen.[19] Sevelamer, a uric acid absorber as well as oral recombinant urate oxidase therapy[65,66] both showed that uric acid secretion into the intestinal tract can be enhanced, and the mechanism may well be due to the concentration gradient facilitated by luminal uric acid depletion. These studies demonstrated significantly lowered serum uric acid levels following its removal from the intestinal tract. Yet, only 23% of the subjects treated with sevelamer experienced a significant reduction.[67] As for the oral recombinant urate oxidase therapy, the presence of proteases in the gastrointestinal tract coupled with immune responses potentially triggered by recombinant proteins pose the main obstacles for extended use of this method.

7. Conclusion

There are three essential intestinal transporters of uric acid: ABCG2, GLUT9 and MRP4. Transporter activity regulates the disposition of up to 30% of bodily uric acid into the intestinal lumen. Uric acid homeostasis depends on intestinal transport. Elevated uric acid has been associated with several co-morbidities including diabetes, gout, cardiovascular diseases and nervous system diseases. The pharmaceutical space for treating elevated uric acid is limited and the drugs available have significant side-affects. It is therefore worth considering alternative strategies that potentially take advantage of the intestinal transport of uric acid to reduce hyperuricemia. The approach of reducing luminal uric acid could provide some relief for hyperuricemia, but that option is not currently available and has some hurdles to overcome before it is effective.

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