Review

An update on the animal models in hyperuricaemia research

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Received on November 2, 2016; accepted in revised form on March 10, 2017.

Clin Exp Rheumatol 2017; 35: 860-864.

Key words: hyperuricaemia, animal model, nephropathy

Competing interests: none declared.

ABSTRACT

Hyperuricaemia is a metabolic disease caused by purine metabolic abnormalities, mainly due to the increased formation or reduced excretion of uric acid. In recent years, it has been proved that hyperuricaemia is an important risk factor for chronic kidney disease (CKD) and cardiovascular disease, and it also takes part in the pathophysiology of metabolic syndrome. In addition, more attention has been concentrated on the pathogenesis or treatment of hyperuricaemia. Since establishing an animal model on hyperuricaemia is the foundation for further researches, several methods to establish the hyperuricaemia model have been developed. In this article, remarkable progress on the modelling approach are summarised, and a comparison study on different methods of developing hyperuricaemia animal models was conducted.

Pathogenesis of hyperuricaemia

In general, renal stones and blocking tubules caused by hyperuricaemia could lead to kidney disease, while asymptomatic hyperuricaemia is considered harmless to the kidneys (1). However, the molecular mechanisms of hyperuricaemia demonstrated that urate-lowering therapy in CKD patients with asymptomatic hyperuricaemia could improve renal function (2). Urate lowering therapy (ULT) is the most common treatment for hyperuricaemia patients, but patients do not always adhere to this treatment and this has an adverse effect on the outcome (3).

It has been proved that uric acid (UA) is the end product of endogenous or exogenous purines (4). Endogenous purines count for about 80%, however, exogenous purines also play a crucial role in this metabolic process. In most mammals, uric acid may generate allantoin by uricase, and allantoin is more soluble than uric acid. During the evolution of primates, the urate oxidase gene was lost (5, 6), and an increase in filtered urate reabsorption was observed, resulting in a 10-fold rise in serum uric acid (4). The mechanism of this mutation is not clear. Recently, more evidence has demonstrated that the elevation of the level of serum uric acid could accelerate the progress of chronic kidney disease (CKD), cardiovascular disease (7, 8), and even metabolic syndrome (9). Therefore, the importance of hyperuricaemia has been understood, and an appropriate animal model is needed.

Determination of hyperuricaemia animal models

Primates, poultry and rodents have often been adopted as the animal model. Although primates are the closest relatives to humans, they have a lower serum uric acid level than human beings but this model is expensive and its technology complex, thus, few studies adopted primates as the animal model (10). Inbred chickens or other birds were used to create the animal model, and although poultry has the same metabolic end product as humans, the great variety in composition between the species make it an inappropriate choice (11). The rodent is the most common laboratory animal. The end product of purine in rodents is allantion, and urate oxidase can be controlled by gene knockout or by drugs. For this reason, it tends to be the choice made by the majority.

Methods to establish an animal model

Establishing a hyperuricaemia model using gene knockout

The uricase gene is lost in human beings. To investigate the influence of uricase gene deficiency, the direct way is to disrupt the urate oxidase gene, as in mutant mice. The absence of this

gene could lead to a significant hyperuricaemia (10 times higher serum uric acid than the normal one) and uric acid crystal deposition. The crystals deposit in the kidneys of mutant mice in 6 days, and most of the knockout mice died before 4 weeks of age (12). Gene knockout can eliminate the main difference between humans and mice in uric acid metabolism, but the complex technology and high mortality rates of animal models indicate that this is not a good choice for establishing a model.

Establishing a hyperuricaemia model using oxonate acid

• *Potassium oxonate*

Except for mutant animals, scientists have considered that the uricase-inhibitor, oxonate acid (11), could be an original conception to induce a hyperuricaemia model. Rats were treated with 2% potassium oxonate for 7 weeks in order to induce a mild hyperuricaemia (1.5–2-times increase in serum uric acid). There was no remarkable change in renal function (measured by BUN levels); renal tissue was normal under routine light microscopy; and no urate crystals deposited in the kidneys. However, immunohistochemical stains revealed an early interstitial fibrosis (13). It suggested that the animal model induced by 2% potassium oxonate is always used to investigate the relationship between hyperuricaemia and hypertension because of its minor effect on the kidney. In addition, it is noted that potassium oxonate is always added to rat foods, and the dosage of this drug fed to each rat is imbalanced and uncontrollable.

In addition, the hyperuricaemia model could also be induced by gavage with potassium oxonate (250 mg/kg) for seven consecutive days (14-16) or intraperitoneally injected (ip.) with potassium oxonate (200 mg/kg or 250 mg/kg) in one single dose (17, 18). These methods are used to induce acute hyperuricaemia. The levels of serum uric acid were elevated in both these methods, and H&E staining revealed the karyopyknosis of renal tubular epithelial cells in cortex and the hyaline casts in the medulla. The former has no evidence of pathological examination to prove the renal injury.

Table I. Methods to establish an animal model.

Gene knockout (12)	—
Oxonate acid	2% potassium oxonate for 7 weeks (13) Potassium oxonate (250 mg/kg) for seven days (14-16) Potassium oxonate (200 mg/kg or 250 mg/kg) for once (17, 18)
Potassium oxonate and yeast extract	Yeast extract (15 g/kg, bid) and potassium oxonate (250 mg/kg, qw) for 6 weeks (20) YEP (21 g/kg) and potassium oxonate (200 mg/kg) for 6 weeks (21)
Oxonate acid and uric acid	2% oxonic acid and 0.1 mmol/l uric acid for 5 weeks (22) 5% oxonic acid and 2.5% uric acid for 10 days (23) 2% oxonic acid and 6 mg/dl uric acid for 6 weeks (24) 2% oxonic acid and 1.5% uric acid for 35 days (25)
Potassium oxonate and ethambutol	Ethambutol (250 mg/kg/d) and potassium oxonate (200 mg/kg/d) for 6 weeks (29)
Adenine and potassium oxonate	Adenine (100 mg/kg/d) and potassium oxonate (1500 mg/kg/d) for 3 weeks (34) or 4 weeks (35)
Adenine and ethambutol	adenine (150 mg/kg) and ethambutol (250 mg/kg) for 14 days (36) adenine (100 mg/kg) and ethambutol (250 mg/kg) for 21 days (37)
Yeast and adenine	10% yeast and adenine (100, 150, 200, 250, 300 mg/kg, respectively) for 48 days (38)
Fructose	10% fructose for 58 days (40) or 4 weeks (41)
Others	potassium oxonate (200 mg/kg) and hypoxanthine (500 mg/kg) (42) potassium oxonate (250 mg/kg) and hypoxanthine (300 mg/kg) (43)

• *Potassium oxonate and yeast extract*

Yeast extract contains a body of nucleic acid and proteins. It can increase the activity of xanthine oxidase (XOD) and produce more uric acid to disturb normal purine metabolism (19). Rats were treated with yeast extract (15 g/kg, bid, oral administration) and potassium oxonate (250 mg/kg, qw, intraperitoneal injection) for 6 weeks. The kidneys in the hyperuricaemia model rats were characterised by brown urate crystal deposition, inflammatory cell accumulation, as well as fibrosis of the tubulointerstitium (20). Also, the hyperuricaemia model can be induced by intragastrically administered yeast extract powder (YEP) (21 g/kg/day) and intraperitoneally injected with potassium oxonate (200 mg/kg/day) for 6 weeks, resulting in a significant increase of the serum UA level and evident morphological changes in the kidney (21). The pathogenesis of the model induced by potassium oxonate and yeast extract is similar to the hyperuricaemia which is induced by a high-purine diet in humans. This model could be used to explore the influence of drugs (*i.e.* Febuxostat) on hyperuricaemia and the pathogenesis of human hyperuricaemia.

• *OA and uric acid*

Most researchers have reported that there were changes in tubules and tubulointerstitium in rats fed on an experimental diet (2% oxonic acid, 2.5 ml/100 g, three times a day and 0.1 mmol/l uric acid in drinking water) for 5 weeks (22). The same results were also obtained using 5% oxonic acid and 2.5% uric acid for 10 days (23), as well as 2% oxonic acid and 6 mg/dl uric acid in drinking water for 6 weeks (24). The formation of uric acid crystals was also discovered in rats fed on 2% oxonic acid and 1.5% uric acid for 35 days (25). Besides the increased level of serum uric acid, all of those experiments have induced tubule injury, including atrophy, dilatation, hyperplasia, as well as fibrosis in the tubulointerstitium. In addition, UA crystals can deposit in the tubules and interstitium, which aroused the intracellular lysosomes rupture and mitochondrial reactive oxygen species (ROS) production release (26), lead-

Table II. Comparisons between animal modelling methods.

Methods	Advantages	Disadvantages	Applications
Mutant mice	Eliminate main difference	Complex technology High mortality rate	—
Potassium oxonate	Develop hyperuricaemia and renal injury (treated with OA and other substances)	Develop mild hyperuricaemia with normal light microscopy presentation (treated with OA only)	Hypertension model Hyperuricaemia model
Adenine	Develop hyperuricaemia and renal damage Inhibit the excretion of uric acid	Toxicity of adenine	Chronic renal failure model Hyperuricaemia model
Ethambutol	Inhibit the excretion of uric acid	Nephrotoxicity of ethambutol	Hyperuricaemia model
Fructose	Develop hyperuricaemia and metabolic syndrome	Complications of the model	Hyperuricaemia and metabolic syndrome model

ing to renal inflammation and causing further damage. The deposition of UA crystals in experiment rats is similar to urate nephropathy in human beings. It may provide an adequate animal model to investigate the effects of UA crystal in medical, pharmacological and toxicological areas. Uric acid participates in the pathogenic mechanism directly, so some studies made to investigate the influence of drugs that prevent uric acid production are difficult to accomplish in this model.

• *Potassium oxonate and ethambutol*

It is estimated that approximately two-thirds of uric acid is excreted by the kidneys. In patients with primary hyperuricaemia, the increased reabsorption or decreased secretion of uric acid in the proximal tubule accounts for 90% of the pathogenesis of the disease (27). Increased serum uric acid caused by an anti-uricosuric agent (ethambutol) could reduce uric acid excretion in the proximal tubule (28). After treatment with ethambutol (250 mg/kg/d) and potassium oxonate (200 mg/kg/d) on rats for 6 weeks, the serum uric acid was increased significantly and the uric acid in urine was decreased (29). The pathogenesis of models induced by the agents mentioned above is consistent with hyperuricaemia in humans, but hepatorenal toxicity of ethambutol restricts the use of this model.

Establishing a hyperuricaemia model using adenine

Adenine can be converted into AMP by adenine phosphoribosyltransferase. With the increase of adenine, 2, 8-dihydroxyadenine (DHOA) may be produced by xanthine oxidase simultaneously. The slightly soluble feature of DHOA in urine results in precipitation and renal damage (30). Thus, adenine is always used to establish the model with chronic renal failure (31, 32). Recently, however, it has been confirmed that adenine can also be used to induce a hyperuricaemia animal model.

• *Adenine and potassium oxonate*

To establish an animal model with hyperuricaemia, three methods, including adenine (100 mg/kg) and potassium oxonate (1500 mg/kg/d) (Group I), adenine (150 mg/kg/d) and potassium oxonate (600 mg/kg/d) (Group II), adenine (20 mg /d) and ethambutol (50 mg/d) (Group III), have been compared by Jiang Qian *et al*. (33). The degree of renal damage in descending order is Group III, Group II, and Group I. All groups showed increasing trends in uric acid level, and the most obvious one is Group I. Compared with Group I, more serious renal damage in Group II revealed that 100 mg/kg of adenine is a safe and effective dosage to establish a hyperuricaemia model. As for Group III, the most serious pathological damage of renal tissue may suggest the nephrotoxicity of adenine and ethambutol. The hyperuricaemia nephropathy rat model was established by the treatment of adenine (100 mg/kg/d) and potassium oxonate (1500 mg/kg/d) for 3 weeks (34) or 4 weeks (35). In experimental rats, severe renal interstitial fibrosis and glomerular sclerosis have been observed, as well as increased serum creatinine and urea nitrogen levels. With the inhibition of uric acid excretion, adenine was able to convert into uric acid. Therefore, the serum uric acid level was increased, and endogenous purines were elevated by potassium oxonate because it inhibits the activity of uricase. Oral administration with adenine and potassium oxonate is a useful method to establish the animal model, but it should be used with caution because of the toxicity of adenine.

• *Adenine and ethambutol*

The method of establishing a hyperuricemia nephropathy (HN) model is oral administration with adenine (150 mg/kg) and ethambutol (250 mg/kg) for 14 days. The levels of serum UA, Cr and BUN were significantly increased in treatment group. Inflammatory cell infiltration, urate crystal deposition and tubular swelling in kidney tissues were also detected (36). The same biochemical parameters could be observed under other conditions, for example, administration with 100 mg/kg adenine and 250 mg/kg ethambutol for 21 days. However, the latter had no evidence of renal damage (37). Few studies have embraced adenine and ethambutol as the method to induce an animal model, because the result of the experiment may be influenced by the nephrotoxicity of these drugs.

• *Adenine and yeast*

As mentioned, yeast contains rich purine and protein. The rat model was established by 10% yeast, along with

the different doses of adenine (100, 150, 200, 250, 300 mg/kg) for 48 days. Infiltration of inflammatory cells, interstitial fibrosis and elevated serum uric acid or creatinine levels were observed in the experimental group. The high dose of adenine even induced renal failure and death (38). Adenine and yeast were converted to purines and increased the level of serum uric acid. High-dose adenine is associated with an elevated level of 2, 8-dihydroxyadenine which deposits in the kidney and causes renal damage. The models induced by adenine and yeast are usually used to investigate hyperuricaemia nephropathy complicated with chronic renal failure.

Establishing a hyperuricaemia model using fructose

In the process of fructose metabolism, besides the influence of high fructose ingestion on the urate excretion in kidney, accumulation of its phosphorylated products and continuous consumption of ATP in liver also contribute to increased uric acid levels. For example, consumption of beverages with high-fructose can elevate the level of serum uric acid (SUA) (39). In order to investigate the effect of high fructose consumption on SUA level, Li Liyu *et al*. (40) fed rats with 10% fructose for 58 days. In the model group, increased SUA level, reduced number of glomeruli, and thickened capillary wall in the kidney were revealed. In addition, Chun Huama *et al*. (41) also induced a hyperuricaemia model by 10% fructose for 4 weeks. Besides the increased serum uric acid level, proliferative glomeruli, infiltrated inflammatory cells and podocyte injury were also observed in the model group (41). The model induced by 10% fructose manifested with hyperuricaemia, hypertension, insulin resistance, and even metabolic syndrome. Thus, the model induced by 10% fructose is not suitable for studying simple hyperuricaemia but it is always used to investigate hyperuricaemia combined with metabolic syndrome.

Others

There are some other methods to induce a hyperuricaemia model, such as potassium oxonate (200 mg/kg ip.) combined with hypoxanthine (500 mg/kg gavage) (42) or potassium oxonate (250 mg/kg ip.) combined with hypoxanthine (300 mg/kg gavage) (43). Both of these methods could induce hyperuricaemia, and they are often used for pharmaceutical experiments.

Comparison between different hyperuricaemia animal modelling methods

In the past 40 years, the serum uric acid level has been found to be increased not only in American adults, but also in adolescents, with an increasing rate of 0.003 mg/dl per year (44). Hyperuricaemia can accelerate the process of renal disease, cardiovascular disease and metabolic syndrome (45). There is a very urgent need to establish an animal model in order to investigate the pathogenesis of hyperuricaemia. The modelling methods are shown in Table II.

However, hyperuricaemia of humans characterised by excretion dysfunction cannot be completely simulated by any of the existing animal models. There are advantages and disadvantages in different animal models. Therefore, the determination of rational models should take account experimental purposes and conditions. These modelling methods also require further improvements.

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