Animal Stone Disease

Subjects: Pathology

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Animals have stone disease too. There are several animal models for the research of human stone disease. Rodents are the most frequently used for stone research, although they are not prone to forming crystals in the kidneys. Ethylene glycol (EG), sodium oxalate and -hydroxyproline are common lithogenic agents. Dogs and pigs were also reported as a study animal for stone disease. However, the breeding costs and body size are too high. The most-used genetic study animal for stone disease was the mouse, but it was high-cost. Calcium oxalate (CaOx) crystals can also be light microscopically observed in the Malphigian tubules of *Drosophila melanogaster*, induced by adding EG to the food. Genetic studies of flies can be done by cross-breeding, and this has a lower cost than using mice. The fly model also has several advantages, including minimal breeding equipment, the fact that it is easier to reach larger numbers in a short time with flies, that crystals can be observed under microscopy, and that they allow genetic study. We suggest the fly will be an ideal animal model for stone research in the future.

Keywords: stone disease ; calcium oxalate ; Drosophila melanogaster ; animal models

1. Introduction

Humans are not the only animal with urolithiasis. Robbinson et al. reviewed PubMed for various animal species, and found a total of 919 citations regarding affected urolithiasis [1]. The reported cases of stone disease in two- or four-legged animals included non-mammals, and mammals such as dogs, cats, birds and turtles. In Taiwan, an early report of stone disease has been achieved not only in humans, but also in monkeys (*Macaca cyclopis*) [2]. Stone disease is a universal phenomenon throughout the animal kingdoms; therefore, the use of animal models to study the disease may have the advantage of similar pathogeneses, and may also have benefits both for humans and animals [3]. In this review, we searched the current types of animal models used in the stone disease research literature on PubMed, in order to share with the readers.

2. Animal Study for Stone Disease in Taiwan

In Taiwan, the first documented use of rats as a study animal for stone disease was reported by Lee et al. in 1991 [4]. They used rats as study animals, and ethylene glycol (EG) as the lithogenic agent to characterize the calcium oxalate (CaOx) crystals in the kidney. In 1996, adult Sprague-Dawley rats were used to study the effects of sex hormones on stone disease [5]. The lithogenic agent used was 0.5% EG in the feeding diet. Their results demonstrated that testosterone enhances urolithiasis, and estrogen inhibits CaOx formation [5]. Thereafter, many following urolithiasis researchers also used the rat as a study animal [6][7][8][9]. Huang et al. added 0.75% EG to the drinking water to study the rats' nephrolithiasis, and found that free radicals occurred mainly in the blood during stone formation [6]. They further studied the effect of EG in the rat model, and found that free radicals are responsible for oxalate toxicity [9].

The rat model was also used to investigate or screen potential agents for preventing urolithiasis. Tsai et al. investigated the effects of a stone-preventing traditional Chinese herbal formula, Wulingsan (WLS), using an EG-induced nephrocalcinosis rat model [7]. The results indicated that WLS effectively inhibited calcium oxalate crystals from being deposited in the rat's kidney. They also used the same model to study Zhulingtang for the effect of stone prophylaxis [10]. Lin et al. used this model to study extracts of *Flos carthami* for stone prevention [11]. *Flos carthami* is a traditional Chinese herb drug, which is active in enhancing blood flow and reducing blood stasis. The result from their study indicated that *Flos carthami* has the ability to inhibit the crystal deposition of CaOx in EG-fed rats.

The rat model was also used in a proteomic study of rat kidneys deposited in by CaOx crystals [12]. The renal cortex was harvested from EG-induced male Sprague-Dawley rat kidneys, and from controls, to compare the protein profile by means of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The results found a reduced amount of albumin in the EG-treated group. Chen et al. used the rat model to study the expression of alanine-glyoxylate aminotransferase 2 in EG-induced kidneys [13].

In an endemic era of melamine-contaminated milk causing stone disease, many studies have focused on the major complication, i.e., stone disease [14][15]. Chen et al. studied the effects of melamine and cyanuric acid on kidney stones in rat [16]. During acute intoxication, melamine and cyanuric acid injured the proximal tubular cells and subsequently blocked the distal tubules. The results from their study revealed that crystals were distributed in both the proximal and distal tubules in rats. Chen et al. used polarized microscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy microanalysis to study melamine-induced stone disease in a fly model [17]. They found stone compositions induced by melamine, not only by itself but also mixed with uric acid and CaOx.

Using the Malpighian tubules of *Drosophila melanogaster* is part of a powerful new animal model for studying stone disease. There is a world-leading team studying stone disease using *Drosophila melanogaster* in Taiwan. The composition of the crystal in the Malpighian tubules was identified by scanning electron microscopy, and was easily observed under polarized microscopy [18]. This novel animal model has been used to test the effect of potassium citrate, a clinical CaOx-preventive drug, and as the positive control in a further study. This model has been further used to screen some potential antilithic or lithogenic agents, such as commercial drinks, herbal medicines, cola, ractopamide and hydroxycitric acid [17][19][20][21][22][23].

3. Preventive Agent

Potassium citrate is a typical preventive agent for CaOx stones, and is also a good positive control for animal studies. There were several reports from animal studies regarding its antilithic effect. Tested herbal medicine, such as Wulingsan, Zhulingtang and *Salviae miltiorrhizae*, had been proven to have a potential antilithic effect, but this necessitates further clinical application to prove [7][10][11][24][25]. Wulingsan had been trialed clinically in a short period, and had a diuretic effect without interfering electrolytes [26]. However, a nation-wide population study of Wulingsan did not find its preventive effect clinically [25]. HCA has proven its antilithic effect both in vivo and in vitro [27][28][29]. However, further clinical trials for proving HCA's effect are needed.

4. Future Perspective

Table 1 depicts the advantages and disadvantages of several animal models. All lithogenic agents are not normally oral food for humans. The fly is an invertebrate animal lacking real kidneys, liver, lungs, etc. Rodents are popular experimental animals for a variety of research. However, rodents' nocturnal animal behavior is unlike that of humans. Dogs and pigs are relatively larger animal for study, for which the breeding costs are high. There is no ideal animal model for studying urolithiasis. We recommend fly as a future study animal, on account of its many advantageous characteristics. Genetic studies of flies help identify potential candidate genes responsible for stone disease [30]. Cohen et al. support using the *Drosophila* excretory system in order to study many human diseases [31]. They reviewed the anatomy and physiology of the fly's Malphigian tubule, and proposed that the fly is an excellent model for studying many renal functions, renal stone diseases and cancer-promoting processes. Therefore, the fly model has wide future applicability.

Table 1. Comparisons of advantages and disadvantages in variable animal models.

Animal	Fly	Rat	Mouse	Pig	Dog
Cost	Low	Intermediate	High	Low	High
Research organ	Malphigian tubules	Kidney	Kidney	Ureter	Kidney
Preparation of crystal observation	Direct observe under Polarizing microscopy	H&E stain before microscopy	H&E stain before microscopy	H&E stain before microscopy	H&E stain before microscopy
Biochemical measurement	Not available	Available	Available	Available	Available
Lithogenic agent	EG, LHP,	EG, NaOx	EG, NaOx	EG+VD, LHP	Not available
Requirement of animal ethic	-	Yes	Yes	-	Highly recommended

EG: ethylene glycol, NaOx: sodium oxalate, LHP: l-hydroxyproline, VD: vitamin D.

5. Conclusions

There were several animal models useful for the study of stone disease, including rat, mouse, fly, dog and porcine. EG currently seems to be an ideal lithogenic agent. The fly model seems to have future prospective use in studying human stone disease, due to its many advantages, such as low cost, high numbers, and its allowing of genetic studies.

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