



# Construction of animal models of uveitis with traditional Chinese medicine syndrome: a narrative review

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*Contributions:* (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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**Abstract:** At present, in the research of traditional Chinese medicine (TCM), it is necessary to use animal experiment method to verify. Although animal models of uveitis are mostly based on animal models with simple diseases, these models are not the most suitable choice for the research on TCM. TCM drug use follows the principle of treatment based on syndrome differentiation, which is the basis to achieve the expected effect of TCM. Therefore, the study of the mechanism of Chinese medicine on uveitis should choose animal models which are closer to TCM syndromes. Based on the modern medical animal model of uveitis and TCM syndrome modeling method, the author puts forward the methods of building model on animal model of TCM syndrome of uveitis according to the TCM syndrome classification of uveitis such as wind-heat, damp-heat and yin deficiency. Of course, more people are needed to verify its accuracy and science through experimental observation and research in the future, and constantly explore its standardized modeling method, so that it is more likely to be close to the characteristics of human disease and lay a foundation for the research of TCM treatment of uveitis.

**Keywords:** Uveitis; experiment autoimmune uveitis (EAU); endotoxin-induced uveitis (EIU); traditional Chinese medicine syndrome type (TCM syndrome type); animal model

Received: 10 October 2020; Accepted: 22 March 2021; Published: 30 March 2021.

doi: 10.21037/lcm-20-39

**View this article at:** <http://dx.doi.org/10.21037/lcm-20-39>

## Traditional Chinese medicine (TCM) syndrome of uveitis and status of experimental modeling

Uveitis is an inflammation of the iris, ciliary body and choroid. At present, in the world, the inflammation occurring in uveitis, retina, retinal vessels and vitreous body is called uveitis, and some people classify papillitis as uveitis. Uveitis has complex etiology, high incidence of disease and is prone to repeated attacks (1), which is a common

blindness eye disease, mostly occurring in young adults, with a blindness rate of 1.1–9.2% (2). Clinically, there are various classification methods according to etiology, inflammatory site, nature, pathological changes, and acute and slow onset.

In ophthalmology of TCM, uveitis belongs to the category of “iridocyclitis”, “chronic iridocyclitis” and “blurred vision”. The pupil is the water wheel of the five-wheel theory, which should be within the kidney and

bladder. The pathogenesis of TCM considers that uveitis mainly caused by exogenous wind-heat pathogen which attacks on eyes, or is caused by eye trauma, toxin swoop in and wind-heat attacks on eyes, or because of excessive heat in liver-gallbladder, flaring up of fire pathogen, attacking eyes, or exogenous wind-dampness heat, or is caused by improper diet and endoretention of damp heat, or because of body yin deficiency, old, long illness, infirmity, yin deficiency in the liver and kidney, flaring up of deficient fire. According to patients' eye symptoms and systemic symptoms, Peng divided uveitis into four syndromes: type of wind-heat of liver channel, type of liver-gallbladder excessive heat, dampness-heat amassment type, and yin deficiency and fire flourishing type (3). In recent years, according to the clinical experience of the combination of Chinese and western treatment of uveitis, various doctors have formed their own ideas of TCM syndrome differentiation. Bi put forward the pathogenesis theory of uveitis: "*heat is the standard, liver and gallbladder are the pivot, and the five organs are the basis*". His team created a four-dimensional syndrome differentiation system of "*meridians, pivot, manifestation and root cause*" (4). Zhao considers that wind-heat, wind-damp, dampness-heat and hepatic fire are the main pathogenic factors of uveitis, and she divided uveitis into seven syndromes, such as type of wind-heat in liver channel, type of dampness-heat of liver and gallbladder, type of wind-dampness with heat, type of spleen deficiency and dampness-heat, type of exuberant fire due to yin deficiency, type of binding of phlegm-damp and static blood, type of yang deficiency of spleen and kidney (5). Guided by the theory of wei-qi-ying blood combined with modern medical examination, Gao classified uveitis into three syndromes: type of heat-stagnation in qifen, type of heat affecting nutrient and blood and type of lingering pathogen due to vital qi deficiency (6). Ophthalmologists at home and abroad believe that the application of TCM has become a prominent feature in the treatment of uveitis in China, particularly the effect of oral Chinese medicine after treatment based on syndrome differentiation and the efficacy of comprehensive treatment scheme combing Chinese and western medicine deserve recognition (7). Based on better clinical effect, the animal experiment research of uveitis with treatment of Chinese and western medicine become more and more, but from the current research results, in the process of making models, researchers mainly choose recognized experiment autoimmune uveitis (EAU) and endotoxin-induced uveitis (EIU) in modern medical research. Nevertheless, the curative effect of TCM is based

on syndrome differentiation and treatment to achieve expected effect. Giving interventions of animal TCM syndrome in the animal experiment of uveitis of TCM is more likely to be close to the characteristics of human disease, and the research will be more valuable. But at present, there are few animal models of TCM syndrome of uveitis in China. Based on this, the author tries to describe the animal modeling method of TCM syndrome of uveitis.

We present the following article in accordance with the Narrative Review reporting checklist (available at <http://dx.doi.org/10.21037/lcm-20-39>).

## Common animal models of uveitis

### *EAU model*

The research shows that EAU is an autoimmune disease mediated by T cell immunity. The modeling process is realized by stimulating the systemic immunity of animal. Generally, the clinically visible uveitis occurs about 2 weeks after the active immunity. The reaction peaks after 1–4 days, and the inflammation gradually subsides after 6–9 days (8). The main antigens inducing EAU model include the retinal tissue proteins, including soluble antigen (s-Ag), interphotoreceptor retino in-binding protein (IRBP) and rhodopsin, etc. Domestic and international researches indicate that the model induced by s-Ag is often used to simulate the human uveitis response, and the lesion sites are mainly uvea and retina, because the antigenicity of s-Ag is basically the same in all animals and humans, while most human uveitis is the autoimmune response to s-Ag (9). Besides, there is non-retina antigen-induced EAU, for example, melanin-related antigen extracted and purified by Broekfyse induces experimental autoimmune anterior uveitis (EAAU) which is rapid onset, mainly involves anterior segment inflammation, mostly incidents bilateral. The pathological manifestations are similar to human acute preuveitis. EAAU is an ideal animal model for studying human preuveitis (10).

Common methods for animal treatment are as follows: preparing HS-AgP35 lyophilized powder into 4 mg/mL antigen solution and mixing HS-AgP35 with equal amount of CFA, fully emulsified to paste emulsion, intraperitoneally injecting chloral hydrate to anesthesia in rats, 0.1 mL HS-Ag emulsifier is injected into Lewis rats under the skin of double hind pads, double hind legs and back, and 0.1 mL of Diphtheria-Pertussis-Tetanus (DPT) triplet vaccine is intraperitoneally injected. After 1 week, the same method is

**Table 1** Common animal models of uveitis

Models	Modeling methods	Merits and demerits
EAU model	0.1 mL HS-Ag emulsifier is injected into Lewis rats under the skin of double hind pads, double hind legs and back, and 0.1 mL of DPT is intraperitoneally injected. After 1 week, the same method is used to secondary immunize	Rapid onset and the pathological manifestations are similar to human acute preuveitis. But the changes of anterior segment are not obvious
EIU model	1 g/L of 0.1 mL <i>S. typhi</i> normal saline solution is injected into rats' foot or vitreous cavity	Fast, stable and repeatable. But relatively short time, showing self-limited inflammation
Modified model	Injecting 450 µg/mL of 0.5 µL typhoid bacillus endotoxin into vitreous cavity through pars plana of rats the next day after the second immunization of the original EAU model	heavy reaction in the anterior segment, long duration of inflammation and good clinical observation effect

EAU, experiment autoimmune uveitis, EIU, endotoxin-induced uveitis, DPT, Diphtheria-Pertussis-Tetanus.

used to secondary immunize (11).

### *EIU model*

EIU is a relatively transient inflammatory response of the anterior segment induced by LPS. It belongs to the model of non-autoimmune uveitis, whose inflammatory manifestation and pathological process are very similar to human acute uveitis. Model induction generally use intestinal endotoxemia, mainly *Salmonella typhi* (*S. typhi*) or *Escherichia coli*. The pathological reaction appears within hours after endotoxin injection, and the inflammatory reaction peaks at 24 h and lasts for 48 h, and then gradually disappears, showing self-limited inflammation (12). This method is fast, stable and repeatable.

Common methods for animal treatment are as follows: 1 g/L of 0.1 mL *S. typhi* normal saline solution is injected into rats' foot (13) or vitreous cavity (14) to establish the EIU model.

### *Modified model*

The characteristics of EAU and EIU models are as follows: the EAU model has a long duration and is an ideal model for evaluating drug treatment strategies, but the changes of anterior segment are not obvious, which is not easy to observe. The EIU model shows early inflammation, with obvious anterior segment reaction but relatively short time, which is beneficial to clinical observation of ocular signs and classification of inflammation. Zhuo modifies the traditional method to establish an animal model of uveitis with long duration of ocular inflammation and convenient observation. The method is to inject 450 µg/mL of 0.5 µL typhoid bacillus endotoxin into vitreous cavity through pars plana of rats

the next day after the second immunization of the original EAU model (11). This method is designed to overcome the weak inflammatory response in the anterior segment of the EAU model, and to establish a new animal model with heavy reaction in the anterior segment, long duration of inflammation and good clinical observation effect (*Table 1*).

### **Modeling methods related to TCM syndromes of uveitis**

At present, animal experiments based on TCM syndrome are gradually increasing. Uveitis is related to wind, dampness, heat, yin deficiency of liver and kidney and deficiency of qi and yin in TCM pathogenesis.

#### *Wind-heat, liver-fire excess model*

The hypertensive animal model of hyperactivity of liver fire is equivalently transformed into animal macroscopic syndromes according to the standard of clinical syndrome diagnosis. The rotation tolerance test of rats and the score of the degree of irritability of rats were adopted to observe the number of ear and claw blood vessels and less fluid on tongue and so on (15,16). The migraine animal model of wind-heat syndrome is blow hot air by electric blower with 1 lever heat, 1 lever wind force, about 45 cm distance and temperature controlled at 39–41 °C. Continuous blowing for 7 days, the activity, fur color, eating and the times of scratching heads were recorded synchronously, and the image of tongue, auricle and conjunctiva were collected (17).

#### *Damp-heat model*

Damp-heat is mainly caused by the interaction of

“endogenous dampness” and “exogenous dampness”. Thus, impairing the spleen and stomach by greasy and surfeit flavor (or adding drinks) causes “endogenous dampness”, “artificial damp-heat environment” simulates “exogenous dampness”, and then pathogenic microorganisms (or immune induction) are used to induce specific diseases. The animal model established by this method conforms to the mechanism and clinical syndromes of damp-heat syndrome, and is an ideal syndrome model (18). The damp-heat modeling is also divided into equal predominance of dampness and heat, predominance of dampness, and predominance of heat (19). Xie *et al.* (20) adopted totally-enclosed automatic temperature and humidity control mold climate box to improve temperature and humidity, and simulated the unique damp-heat environment in Lingnan to make the air filled with dampness and create “endogenous dampness”. On the other hand, adopting improper diet and too much greasy flavor like “high protein + lard (high fat) + liquor + running for 30 min” simulated the “exogenous dampness”. Other studies have found that high-fat diet can make the model have the related performance of serum inflammatory factors in damp-heat syndrome, and the mechanism may be induced by intestinal inflammatory response by affecting intestinal flora, destroying gut mucosal barrier function and changing innate immunity (21).

### *Yin deficiency model*

Shi believes that the modeling thinking of yin deficiency syndrome can be divided into simulating syndrome characteristics of yin deficiency syndrome, simulating the etiology and pathogenesis of yin deficiency syndrome and simulating clinical high incidence of yin deficiency syndrome. Thyroxine, adrenocortical hormones, warm TCM, labor, diuresis, rage and wounding yin, or environmental diet can be used in this process (22).

### **Assumption of combining TCM syndrome in the animal modeling of uveitis**

#### *Modeling of acute or anterior uveitis and intermediate uveitis of uveitis combined with wind-heat and hyperactivity of liver-fire*

In Peng’s “*Eye fundus disease of integrated traditional Chinese and Western medicine*” (3), it is stated that the symptoms of wind-heat of liver channel of uveitis, such as eye pain, blurred vision, ciliary injection, turbid aqueous humor,

unclear iris texture, gradually small pupil, headache, fever, dry, red tongue, thin yellow fur and floating and frequent pulse, are all superficial manifestation of wind-heat; the symptoms of exuberance of liver fire of uveitis, such as eye pain rejection of press, photophobia and tears, ciliary injection, turbid aqueous humor, unclear iris texture, hypopyon, vaginal ulcers, mouth sores, bitter taste in mouth, yellow urine, dry stool, red tongue, yellow and dry fur, frequent and stringy pulse, are all the manifestation of exuberance of liver fire. The descriptions of these two symptoms are similar to those of acute or anterior uveitis and intermediate uveitis in the classification of Western medicine for uveitis. When selecting animal models of uveitis, EIU model shows rapid inflammatory response, obvious anterior segment and short duration. Its inflammatory manifestations and pathological process are very similar to human acute uveitis. Therefore, before the modeling of EIU, it can be used to treat animals with modeling factors of wind-heat and exuberance of liver fire. The model of the experimental animals can be designed as follows: from the first day of the experiment, the animals were blown hot wind by electric hair dryer at fixed time every morning, with the heat at 1 lever, wind at 1 lever, distance is about 45 cm, and the temperature is controlled at 39–41 °C. Continuous blowing for 7 days, and on 8 days 1 g/L of 0.1 mL *S. typhi* normal saline solution is injected into rats’ foot to establish the EIU model.

#### *Modeling of subacute, chronic or posterior uveitis combines with type of damp-heat and yin deficiency*

In Peng’s “*Eye fundus disease of integrated traditional Chinese and Western medicine*” (3), it is stated that the symptoms of wind-dampness complicated by heat of uveitis, such as eye pain rejection of press, photophobia and tears, visual deterioration, ciliary injection or mixed injection, unclear iris texture, iris swelling, deformed pupil, or membranous coverage, cloudy vitreous humor, joint swelling and muscle pain, yellow and greasy fur, frequent and soft pulse or stringy and frequent pulse, are the manifestation of damp-heat or wind-heat. The symptoms of hyperactivity of fire due to yin deficiency of uveitis, such as dry and blurred eyes, eye pain tends to come and go, ciliary injection, keratic precipitates, iris atrophy, lens opacity, dry, vexation, red tongue and less fur, thready and frequent pulse, are the manifestation of hyperactivity of fire due to yin deficiency. The descriptions of these two symptoms are similar to those of subacute, chronic or post-uveitis and panuveitis in the classification

of uveitis in Western medicine. EAU is accomplished by stimulating the systemic immunity of animals, and the occurrence of clinically visible uveoretinitis usually lasts 2 weeks. This model has no obvious changes in the anterior segment, but the model lasts for a long time, which is an ideal model for the evaluation of drug treatment strategies, and provides a good guarantee for the verification of the effect of traditional Chinese medicine decoction. The model of the experimental animals can be designed as follows: animals are fed in high temperature and humidity totally-enclosed automatic temperature and humidity control mold climate box, provided hot wind at the same time, and fed too much greasy flavor like “high protein + lard (high fat) + liquor+ running for 30 min”. After 7 days, 0.1 mL HS-Ag emulsifier is injected into Lewis rats under the skin of double hind pads, double hind legs and back, and 0.1 mL of DPT triplet vaccine is intraperitoneally injected. After 1 week, the same method is used to immunize twice; thyroid hormone suspension at a body mass of 300 mg/kg is gavaged for 30 consecutive days to establish the mice model of yin deficiency (23) or intraperitoneally injected with ACTH 16 u/kg once a day for 4 consecutive days to replicate the model of hyperactivity of fire due to yin deficiency in rats (24). After modeling, 0.1 mL HS-Ag emulsifier is injected into Lewis rats under the skin of double hind pads, double hind legs and back, and 0.1 mL of DPT triplet vaccine is intraperitoneally injected. After 1 week, the same method is used to secondary immunize.

### Outlook and shortages

Treatment based on syndrome differentiation is one of the major features of TCM. The efficacy of TCM is based on the discrimination of syndrome in TCM. Different from the only disease animal model of modern medicine, the animal model used in the research of TCM still needs to be guided by the theory of TCM, which should consistent with clinical syndromes of TCM (25), so that the research results can play a role in the clinical counterevidence and provide scientific support for the development of modern TCM. At present, we need to constantly explore and improve the animal model of TCM syndrome (26). Thus, the establishment of standardized animal models of TCM is particularly urgent, which is an important link to promote the modernization of TCM (27). In this article, the TCM syndrome animal model of uveitis design is the theoretical exploration based on the previous research achievements of other illnesses and diseases, and it is necessary to verify its

accuracy and scientificity through experimental observation and research, and constantly explore its standardized modeling methods. This lays the research foundation for the dialectical treatment of uveitis with TCM and integrated Chinese and western medicine.

### Acknowledgments

*Funding:* This work was supported by the Scientific Research Project of Hunan Education Department (18C0394); 2019 Hunan University Student Innovation and Entrepreneurship Training Program Project (1686); Hunan Graduate Scientific Research Innovation Project (CX2018B461); Hunan Provincial Engineering Technological Research Center for Prevention and Treatment of Ophthalmology and Otolaryngology Diseases with Chinese Medicine and Protecting Visual Function opened funding (2018YGC01); the Domestic First-Class Discipline Construction Project of Chinese Medicine of Hunan University of Chinese Medicine opened funding (2018JCYX13); the Domestic First-Class Discipline Construction Project of Chinese Medicine of Hunan University of Chinese Medicine; Hunan Graduate Scientific Research Innovation Project (2599); Central Government Finance the Construction of Local Colleges and Universities Project; Key Discipline Construction Project of Traditional Chinese Medicine Ophthalmology, State Administration of Traditional Chinese Medicine and Hunan Provincial Key Discipline Construction of Traditional Chinese Medicine Otorhinolaryngology.

### Footnote

*Reporting Checklist:* The authors have completed the Narrative Review reporting checklist. Available at <http://dx.doi.org/10.21037/lcm-20-39>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/lcm-20-39>). The authors have no conflicts of interest to declare.

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doi: 10.21037/lcm-20-39

**Cite this article as:** Lu J, Huang B, Wu YJ, Peng QH. Construction of animal models of uveitis with traditional Chinese medicine syndrome: a narrative review. *Longhua Chin Med* 2021;4:2.