

Analysis of Animal Model Based on Clinical Features of Frostbite

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Abstract. To analyze the modeling methods and characteristics of frostbite animal model, and to compare with the anastomosis of traditional Chinese medicine and Western Medicine. Methods: Summarize the characteristics and modeling methods of frostbite in animal models, according to the clinical characteristics and diagnostic criteria of traditional Chinese medicine and Western medicine, analyze anastomosis between corresponding clinical symptoms and frostbite animal model, discusses the advantages and disadvantages of the existing frostbite animal model and promotion suggestion. Results: At present, there are few modeling methods of frostbite, which reflect the characteristics of the clinical symptoms of frostbite in a certain extent, but it lacks the animal model which can reflect the characteristics of Chinese medicine and Western Medicine, also lacks methods for evaluating frostbite models. Conclusion: Establish frostbite evaluation method is feasible, The establishment of combination disease with frostbite model is the focus of research in the future.

Introduction

Frostbite is the body exposed to low temperature environment, excessive heat loss caused by lasting cold, so that the body heat balance is disrupted, leading to systemic or local acute freezing injury [1]. Frostbite parts are the face, ears, nose, hands, feet and other poor peripheral blood circulation, The main manifestations of patients with pale skin, cold, pain and numbness, itching when the temperature is high, skin erosion or ulcers may occur and other phenomena [2]. According to the nature of the damage, Frostbite can be divided into frozen injury and non freezing injury. Non freezing damage caused by low temperature and humidity below 10°C above freezing point, such as frostbite, trench foot, immersion foot, etc.[3]. Freeze injury is freezing tissue cells caused by the freezing of the low temperature, According to the degree of frostbite, it can be divided into I degree, II degree, III degree, IV degree frostbite, the injured part is epidermis layer and dermis layer, then into the whole skin or subcutaneous tissue, even to the muscle, bone and other tissues[4]. Frostbite is not severe, but seriously affect people's quality of life, so the simulation of these clinical symptoms, making higher degree of animal model, It's of great significance on the prevention of frostbite, pathogenesis, treatment and nursing.

Frostbite Etiology and Pathogenesis

Frostbite Modern Medicine Etiology and Pathogenesis

The pathogenesis of frostbite is not very clear at present. The research shows that frostbite is closely related to vascular endothelial cell injury and its the changes of function, and the changes

of blood coagulation and hemorheology[5]. The body exposed to 0 degrees Celsius environment for a long time, easy to accelerate the human body heat radiation, vascular contraction or expansion, local or systemic blood and oxygen supply to reduce, tissue metabolism decrease, heart rate slower, skin and blood circulation disorders caused by limb tissue freezing [6]. When the temperature continues to decrease, the tissue freezes, Rapid freezing form intracellular ice crystals, Changing the cell inside and outside environment, increase osmotic pressure in intracellular, cell dehydration, resulting in material lost and deplete of energy metabolism , then the cell dead [7]. Free from the frozen, blood vessels expanded during rewarming, sedimentation quickly when the blood into the capillaries, exudate increased, formatting edema. Plasma extravasation, blood concentration, leading to thrombosis and microcirculation, make the organization more ischemia, and even lead to tissue necrosis [8].

Etiology and Pathogenesis of Frostbite in Traditional Chinese Medicine

Chinese medicine theory think that frostbite is due to lack of yang, skin frost, Qi and blood running sluggish, Meridian obstruction, Stagnation of blood stasis, skin lost nutrition. Qi and blood weakness or fatigue, heat and cold, freezing and heating will lead to frostbite, owing to cold evil, meridian blocking, Qi and blood congestion corbel, light injury shallow, only skin collaterals and Qi and blood stagnation, patient lost warmth, moisten and damaged. Serious injury is deep, injury to muscle, vein and blood coagulation acerbity barrier, the affected part lose nutrition, then fester, what’s worse, it can damage their bones. Chinese medicine clinical treatment take promoting blood circulation and relieving pain, improve microcirculation, improve the body warm ability as the basic treatment principle.

Diagnostic Criteria and Clinical Characteristics of Frostbite

Diagnostic Criteria and Clinical Manifestations of Frostbite in Western Medicine

Frostbite often occurs in the peripheral blood circulation of poor parts and exposed parts, such as hands and feet, nose, auricle, cheek, etc., Cold skin pale, cold, pain and numbness, Local representation is similar to burn after rewarming, but the local swelling is usually not obvious, there is burning, itching or tingling, Frostbite diagnosis can be divided into four degrees according to the depth and severity of the injury, The following reference to <Atlas of diagnosis and treatment of frostbite>, protocol frostbite diagnostic criteria [9], See Table 1.

Table 1. Diagnostic criteria and clinical manifestations of frostbite in Western Medicine.

Frostbite Type	clinical manifestations
I degree	① hyperemia or hydroncus, pale skin and turned red. ② No blister. ③ The skin dry, heating, high temperature. ④ Skin redness, burning, pain and itching.
II degree	① hyperemia and hydroncus, ② blister, blister fluid is serous or pale. ③ substrate is bright red and humid, The skin high temperature. ④ Full thickness skin frostbite, local skin redness, itching, tender feeling sensitive, obvious pain.
III degree	① Vesicular edema and hemorrhage, blister fluid is bloody, dark red, after injury 2 weeks to black eschar exfoliation, scab, exposed granulation tissue. ② Full thickness skin necrosis ③ substrate is purple or dark red, The skin is blue, low temperature. ④ The formation of black hard crust after the blisters and necrosis of the skin dry, thick and hard, feel dull pain.
IV degree	① Blood blister fluid, dark red, brown or dark purple, small size, serious without blisters. ② Total thickness of frozen limb skin necrosis ③ The skin pale, bluish gray or purplish blue; ④ lose skin tenderness or dullness. ⑤ Severe pain in the limbs.

Diagnostic Criteria and Clinical Manifestations of Frostbite in Traditional Chinese Medicine

Frostbite is a common skin disease caused by cold and wet irritation, Chinese medicine called it as the frozen sores, frozen wind, it should be nourishing blood and warming kidney and meridians, dispelling cold in Chinese medicine treatment, diagnostic standard reference to <Diagnosis and treatment of skin and surgical diseases in traditional Chinese Medicine[10], See Table 2.

Table 2. Diagnostic criteria of frostbite in traditional Chinese Medicine.

differentiation of symptoms and signs for classification of syndrome	Symptom	Tongue and pulse
Deficiency of Kidney Yang and Qi-blood type	grey, dark red or purple, hydroncus, burning and itch, mostly older and weaker.	pale tongue, thin pulse
Cold evil invading the peripheral blood stasis type	The skin is dark purple, swelling, nodules, ulcer,water blister, local pain or numbness, cold limbs.	dark tongue,white fur, deep tense pulse
Evil toxin invagination type	skin redness, severe pain, large ulcer area, accompanied by chills, fever, serious nonsense.	red tongue, yellow fur, slip, pulse

Research Status of Frostbite Animal Model

Selection of Model Animals

Commonly animal used in frostbite model, General selecting animals similar to human body structure, function, metabolism and disease characteristics, There are rats, mice, rabbits, cats, dogs, pigs, monkeys, squirrel monkeys, sheep [11], The rats, mice, rabbits, cats and monkeys were used most, at present, most of the rodents are selected as the research object, mouse, rabbit, monkey skin structure are close to human, high survival rate, good repeatability, easy operation, more economical, It is beneficial to the experimental study on the treatment of local frostbite.

Modeling Methods and Clinical Goodness of Fit

The pathogenesis of frostbite is not very clear, At present, there are some related theories: cell injury and vascular injury. , the tissue cells freeze at low temperature, which can cause cell damage and death in the process of melting. Frostbite causes tissue necrosis, disturbance of blood circulation is one of the most important causes of severe cold injury due to tissue necrosis. At present, the study of animal models of frostbite has been shown in Table 3.

Table 3. Analysis of frostbite animal model.

principle	animal model	modeling method	animal	model characteristics	Clinical application of TCM and Western Medicine
Liquid nitrogen freezing method	Different degrees of frostbite model[12]	bare skin, silver leaching -196 C in liquid nitrogen, immediately attach test site of skin, paste the skin 3S, 5S, 10s	Rabbit / rat, mouse / pig	advantage:simple and reliable, easy to control temperature, more in line with the experimental study of local frostbite, symptoms approach the clinical. disadvantage: time is not well controlled, not accurately controlled the degree of frostbite,	accord with table 1 I-IV all, goodness of fit≥90%,

		sore surface, cause local frostbite		different time of frostbite is difficult.	
	Deep frostbite model of tibialis anterior [13]	free bilateral tibialis anterior muscle, metal strip is placed on the surface of the tibialis 90s anterior muscle after liquid nitrogen freezing.	rat	advantage: It is suitable as an animal model for myoblast transplantation disadvantage: instability model, incomplete injury, difficulty operation, intraoperative inflammatory infection, troublesome operation, narrow scope research	accord with table 1 IV degree ②③④⑤ goodness of fit≥70%
	Foot frostbite model [11]	Foot immersion - 25°C, 90% ethanol 10/5 min, 1 times measuring temperature per 15s	Rabbit / rat, mouse	Advantage: simple and easy, stable model, good repeatability, the surface of the wound is uniform, which is convenient for observation disadvantage: individual with large differences, Lacking objective indicators,	accord with table 1 I-III degree①②③④ goodness of fit≥90%, IV degree①③④⑤, goodness of fit≥70%,
Ethanol immersion freezing method	Qi deficiency frostbite model [14]	Overfatigue+nte mperance of taking food, low temperature ethanol and water immersion method.	rat, mouse	advantage: high success rate, no anesthesia, low freezing temperature, short time simple and reliable operation, it is beneficial to the experimental study of frostbite disadvantage: individual with large differences, inconsistent degree of injury, unstable model.	accord with table 1 I-II degree①②③④ goodness of fit≥90%, accord with table 2 with Deficiency of Kidney Yang and Qi-blood type, goodness of fit,≥80%
Simulated high altitude hypoxia Complex freezing method	Plateau freezing model [15]	Nitrogen dilution method is used to maintain the oxygen content to simulate the experiment height in the cabin, maintain cabin temperature in refrigerator.	Rat / pig / dog / sheep	advantage: simple structure, convenient use, can be simulated to study the plateau conditions of refrigeration in the laboratory. disadvantage: simulating plateau conditions is not easy to achieve, model instability, lacking objective indicators, narrow scope of application.	accord with table 1 II-III degree①②③④ goodness of fit≥90%, accord with table 2 with Cold evil invading the peripheral blood stasis type, goodness of fit,≥80%

Discussion

Chinese medicine theory considered that frostbite caused by Yang deficiency, Qi-stagnancy and blood stasis, the body suffered pathogenic cold caused muscle meridian blocking, Qi and blood congestion astringent, makes the skin lose nutrition, then damaged, This is consistent with the modern understanding of the microcirculation disorder of frostbite. topical drug in clinical treatment of frostbite with wine, cream, lotion, liniment or decoction, The main composition of

the efficacy of TCM is nourishing qi and blood, warm channel and expelling cold, promoting blood circulation to remove meridian obstruction, it is to improve the body surface ending blood circulation in modern pharmacology. TCM treatment of the disease focus on the combination of local and systemic treatment.

A variety of animals have been used in the frostbite model as the experimental object, but according to the research of medical science, it is more convincing to use mice, monkeys and pigs as experimental objects in the frostbite model. This is because the skin of these animals have a high degree of similarity when compared with people, including the thickness of the epidermis and the density of hair on the surface of the skin, the fat layer of the skin, the body fluid and metabolic mechanism in the skin of frostbite. The rat is cheap and convenient for a large number of quantitative studies. The rabbit is gentle, easy to fix, injuring and medicating won't have a side effect not because of struggle, at the same time, the rabbit medium sized, both sides can contrast itself. In the study human body of severe frostbite, it is suitable for monkeys, monkey skin thick, large individual, and it takes a long time to repair severe frostbite wound, it can fully observe the repair process and mechanism, more suitable animal model for the experimental study on the treatment of severe cold medicine

Summary

It had little relevant information about the establishment of frostbite animal models at home and abroad. According to the analysis of the animal model above, we can see that there are few types of frostbite, most animal models can reproduce the clinical symptoms of different degrees of frostbite. Liquid nitrogen freezing method and ethanol immersion freezing method were the main modeling method, but they were difficult to control the actual degree and area of frostbite. To study the effects of different drugs on animal experiments with different degrees of frostbite, to minimize the individual differences, and control the temperature, time, rewarming rate and medication time, to ensure the frostbite wound cleaning, Preventing infection is an important condition to ensure wound repair, It is important to have good frostbite animal model to study the mechanism and repair of frostbite.

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