Preliminary study on the construction of animal model for non-puerperal mastitis

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Research

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Abstract

Objection: This qualitative study explored the key factors affecting the occurrence of non-puerperal mast (NPM and the construction of an animal model which is closer to the characteristics of the disease.

Background: The etiological factors and pathogenesis of NPM are unknown, and the incidence, misdiagnosis rate and recurrence rate are high. Previously reported modeling methods vary, reproducibility is poor, and disease characteristics cannot be well characterized, so new animal models are urgently needed for research.

Methods: In this study, using female Wistar rats to construct a non-puerperal mastitis animal model by intraperitoneal injection of metoclopramide to simulate hyperprolactinemia + autologous milk injected into the mammary gland to simulate ductal secretions.

Results: Pathological results showed that 2 weeks after injection in group C (intraperitoneal injection of metoclopramide for 2 weeks + breast injection of maternal milk mixture twice) and group D (intraperitoneal injection of metoclopramide for 2 weeks + breast injection of maternal milk mixture three times), lipid stasis, periductal inflammation, and massive plasma cell and lymphocyte infiltration were observed,similar to the lesions in patients with human plasma cell mastitis.

Conclusions: We believe that we can can establish a non-puerperal mastitis model by altering prolactin levels and ductal secretion stasis in rats.

Background

Non-puerperal mastitis (NPM) is a group of non-puerperal women with unknown etiology, benign, non-specific inflammatory diseases, including mammary duct ectasia (MDE)/periductal breast Inflammation (periductal mastitis, PDM), granulomatous lobular mastitis (granulomatous lobular mastitis, GLM). Existing epidemiological data show that the disease can occur at all ages, with an incidence rate ranging from 1.41-5.5%[1, 2], and a misdiagnosis rate as high as 56.9%-73.1%[3]. The recurrence rate after fistula surgery is 23%[4]. It has the characteristics of long duration and easy recurrence. Affected patients suffer from the disease and are often mistaken for breast malignancies, which bring great psychological burden to patients and seriously affect the quality of life. However, our understanding of the disease is still insufficient, and there are few basic studies on this disease so far. The study of animal models of non-puerperal mastitis is still in its infancy, and there is no mature modeling method. We believe that the occurrence of non-puerperal mastitis is related to endocrine disorders and secretion stasis blocking the duct, and it is now planned to construct a rat model of non-puerperal mastitis by intraperitoneal injection of metoclopramide and autologous milk injection into the rat mammary gland.

Methods

Experimental materials

200±20g Wistar non-pregnant female mice, 5 days postpartum and lactation Wistar nest mice, purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. Shanghai Branch (SPF grade, license number: SCXK(Shanghai) 2017-0011).
Metoclopramide(specification:10mg:1ml,N.05190803,manufacturer: Shanghai Hefeng Pharmaceutical Co.,Ltd.).Oxytocin Injection(Specification:1ml:10IU,National Medicine Standard H31020862, Manufacturer: Shanghai Shanghai First Biochemical Pharmaceutical Co., Ltd.),Chloral Hydrate (250G, N.A600288-0250, Manufacturer: Shenggong Bioengineering(Shanghai)Co.,Ltd.,complete Freund's adjuvant (50ml,N.P2036,manufacturer:Shanghai Biyuntian Biotechnology Co.,Ltd.), incomplete Freund's adjuvant(50ml,N.P2031,manufacturer: Shanghai Biyuntian Biological Technology Co.,Ltd.). Rat PRL Elisa kit (manufacturer: Shanghai Hengyuan Biotechnology Co., Ltd., N.20H09L11)

Modeling methods

Milk Collection

Refer to the collection method described in the literature[5], the specific operation method is as follows: before the experiment, all the experimental items are placed in an ultra-clean table for ultraviolet disinfection for 20 minutes; during the experiment, the lactating female rat are pressed 2-3ml/kg with 10% chloral hydrate minal cavity for anesthesia. After the rat was unconscious, the rat abdomen was quickly depilated with depilatory cream. After the hair was cleaned, the exposed skin of the chest and abdomen was disinfected with an alcohol cotton ball. Inject 10IU/mL oxytocin into the rat's abdominal cavity at 2ml/kg according to the rat's body weight. Wear sterile gloves to take milk. Gently squeeze around the nipple to make a small amount of milk appear on the nipple. Use the right hand to transfer 200ul. The device sucks the milk away at any time, and discharges the milk into a 1.5ml cryotube that has been sterilized in advance. After the milk is collected, the mother mouse is disinfected again, and the milk is frozen at -20°C for later use.

Mixed Preparation

On the night before use, the mother's milk was mixed with complete Freund's adjuvant or incomplete Freund's adjuvant in a ratio of 1:1, and placed on a shaker at 4 ℃ overnight to prepare a milky white oil-in-bag milk mixture, and rat mammary gland injection was performed on the second day.

Animal grouping

Wistar female rats were randomly divided into 4 groups, group A: normal control group, 2 female rats; group B: single-use gastric compound group, 2 female rats, each female mouse at a dose of 30 mg/kg[6] at 9:00 on the day, 0.6ml metoclopramide was injected subcutaneously on the back for 2 weeks; group C: 6 female rats, on the basis of group B, at the same time each female mouse was injected with 0.2ml oil through the third and fourth pairs of mammary glands respectively Milk suspension (milk + complete Freund's adjuvant), 0.2ml of oil-in-oil suspension (milk + incomplete Freund's adjuvant) was injected again after 1 week; group D: 8 female rats, on the basis of group C Above, after 2 weeks, the mammary

glands were again injected with 0.2ml of emulsion-in-oil suspension (milk + incomplete Freund's adjuvant).

Injection Method and Route

Rats were admitted to the animal room SPF grade feeding environment to adapt to 5 days, at 9:00 every day, the Metoclopramidere admitted to the animal room SPF grade feeding environment to adapt to 5 days, at 9:00 every day, the stomach was subcutaneously injected on the back of rats for 2 weeks; the prepared mixed rats were injected into the subcutaneous fat pad through the 3rd and 4th pair of mammary glands of dams every other week.

Specimen Collection and Processing

Before the start of the experiment, blood was collected from the orbit of each rat in a quiet condition to retain serum for future use. After 2 weeks of metoclopramide administration, orbital blood samples were taken again, and rat serum was subjected to Elisa to detect changes in prolactin levels. Two rats were randomly selected at 1 and 2 weeks after injection in groups C and D. Blood samples were collected from the abdominal aorta after anesthesia, and the second, third, and fourth pairs of breast tissues were taken, washed with normal saline and fixed in paraformaldehyde for 24 hours before paraffin tissue embedding and HE staining for observation. Three weeks after the injection of D group, all the remaining rats were sacrificed and sampled, and the operation was the same as above.

Evaluation indicators

Observation of general conditions

After the first injection of the 3rd and 4th pair of mammary glands of rats, the size of mammary masses was measured once a week until sacrifice, and the skin changes, redness, swelling, and ulceration of the rats were recorded.

Pathological diagnosis

The diagnosis of non-puerperal mastitis mainly depends on the pathological diagnosis of the diseased tissue, ductal dilatation, secretion, granuloma formation, giant cell reaction, plasma cell infiltration, lymphocyte infiltration, eosinophil infiltration, histiocyte infiltration and other conditions in the breast lesion tissue. It is mainly based on Ackerman Pathological Diagnosis (the 10th edition) as the diagnostic criteria for non-puerperal mastitis.

Statistical methods

GraphPadPrism7.0 software was used for statistical analysis. The mean value of measurement data was expressed as `x±s t Test was performed for comparative analysis. $P<0.05$ was considered statistically significant.

Results

General observation

On the 12th day after model establishment, the model group successively showed ulceration and scab of the breast mass, with different size of the sore surface and degree of exudation, and by the end of the experiment, all of them converged and scabbed or desquamated. No rats died during the experiment. At the end of the experiment, the rats were in good general condition and had no abnormal stools.

Changes of PRL Levels in Rats

Elisa detection and analysis were performed on the sera obtained from rats before and after metoclopramide treatment, and compared with those before the experiment, the serum PRL level of rats was significantly increased after 2 weeks of metoclopramide treatment, as shown in Table 1.

Table 1 Effect of metoclopramide on prolactin levels in rats after 2 weeks of medication

group	number of animals	Prolactin（pg/ml）
before medication	8	93.09±3.144
after medication	8	127±5.104***

Note: Compared with that before administration, ***P＜0.001

Breast Mass Changes

There was no mass growth in the mammary gland of rats in the normal stomach recovery group. At 1 week after injection, compared with group C, group D had larger mass, but there was no statistical significance (P > 0.05); at 2 weeks after injection, there was no significant difference compared with group D, but there was no statistical significance (P > 0.05); at 3 weeks after injection, group D had larger mass than group C, with statistical significance (P < 0.01). See Table 2 for details.

Table 2 Size of rat mammary gland mass

group/mass（mm^3）	1 week after injection	2 weeks after injection	3 weeks after injection
group C	152.6±26.99	241.4±14.27	211.8±21.87
group D	177.9±22.33	235.7±26.78	348.6±21.18**

Note: 3 weeks after injection, compared with group C, **P＜0.01

Comparison of pathological results

In group B, the breast lobules were partially hyperplasia, with acinar and ducts slightly dilated, and a small amount of secretion was seen; there was no obvious infiltration of plasma cells and neutrophils in

the breast lobules and milk ducts, as shown in Figure 1B. 1 week and 3 weeks after the last injection in groups C and D, there were fat accumulation in the lumen, inflammation around the catheter, and a large amount of lymphocyte infiltration, but no obvious plasma cell infiltration, as shown in Figure 1 (C, E, F, H) . Two weeks after the last injection in groups C and D, lipid accumulation in the lumen, inflammation around the duct, infiltration of plasma cells and lymphocytes, and necrosis in some areas were seen, similar to those in patients with human plasma cell mastitis. Group D The performance is more typical, see Figure 1 (D, G).

Discussion

The etiological factors and pathogenesis of non-puerperal mastitis are not clear, and reports suggest that hyperprolactinemia caused by long-term oral antipsychotics[7] and pituitary adenomas[8] is directly related to this disease, which may be caused by chronic inflammation and fibrosis caused by dilated ducts and concentrated secretions leading to the occurrence of this disease.Pathological section analysis by Tedeschi[9] in patients with clinical plasma cell mastitis revealed that the material accumulated in the lactiferous ducts was similar to those chemical stimuli that escaped into the surrounding tissues, mainly some fatty acids, as well as a small mixture of phospholipids and cholesterol.It was also found that the composition of nipple discharge in the non-lactating state was similar to that in lactating milk, and the levels of free fatty acids, triglycerides, cholesterol, and phospholipids in nipple secretions during non-lactation were higher than those in colostrum[10].Rodman[11] used rabbits as the experimental subjects to inject pancreatized milk into the breasts of rabbits for modeling, but there was no specific experimental procedure in the literature, and the experiment could not be repeated; Li Daofang[12] found that patients with ductal ectasia syndrome mainly presented with abnormal secretion of estrogen, luteinizing hormone and prolactin, and non-pregnant New Zealand female rabbits were used for the experiment and the model was successfully established. However, the intervention method of exogenous estrogen and progesterone administration resulted in abnormal secretion of other hormones at the same time, and the design of the influencing factors was not rigorous enough.In this experiment, the model was induced by metoclopramide, which can directly act on hypothalamic dopamine receptors to induce an increase in prolactin levels, and we intervened the animals with metoclopramide to increase only prolactin levels.When modeling, the milk injected locally into the mammary gland of normal rats was obtained from Wistar lactating dams, so that the hormone influencing factors of modeling were single, while the injected milk was also obtained from homologous, with controllable variables, which better simulated the pathogenesis of the disease.

In this experiment, serum PRL was significantly increased 2 weeks after metoclopramide injection in rats, and the breast mass was still more obvious 3 weeks after injection in groups C and D, and in group D. A case of mastitis with similar pathological changes to human non-puerperal mastitis occurred 2 weeks after injection in groups C and D, and the infiltration of cooked plasma cells in group D was more obvious. Complete Freund's adjuvant can promote the formation of inflammation and slowly release the relevant antigenic components in milk, and Yu's experiment[13] also confirmed that it cannot induce the

production of a large number of plasma cells alone, so we believe that the abnormally increased prolactin level and ductal milk stasis are the intrinsic soil for the development of non-puerperal mastitis.

Conclusions

The construction of animal models of non-puerperal mastitis is a long process. The non-puerperal mastitis rat model established by the experimental method used in this experiment is the first model. Given the stability of the model, we need to expand the number of experimental animals, further refine the experimental conditions, standardize the pathological diagnosis criteria.When possible，the construction of animal models for larger mammals provides a more complete and reliable model for studying the etiology, pathogenesis and prevention of non-puerperal mastitis.

Declarations

Ethics approval and consent to participate

All experimental procedures were performed in accordance with the ethical requirements of experimental animals of Shanghai University of Traditional Chinese Medicine, in compliance with the relevant regulations on the welfare and ethics of experimental animals, and with humane care in accordance with the 3R principles for the use of experimental animals. Animal ethics number:PZSHUTCM200424003.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MLN carried out the scientific research and manuscript writing, performing statistical analysis. CHF was the initiator of the study and provided expert opinions for the study.

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Experiments from this study were performed at the animal Experiment Center of Shanghai University of Traditional Chinese Medicine.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

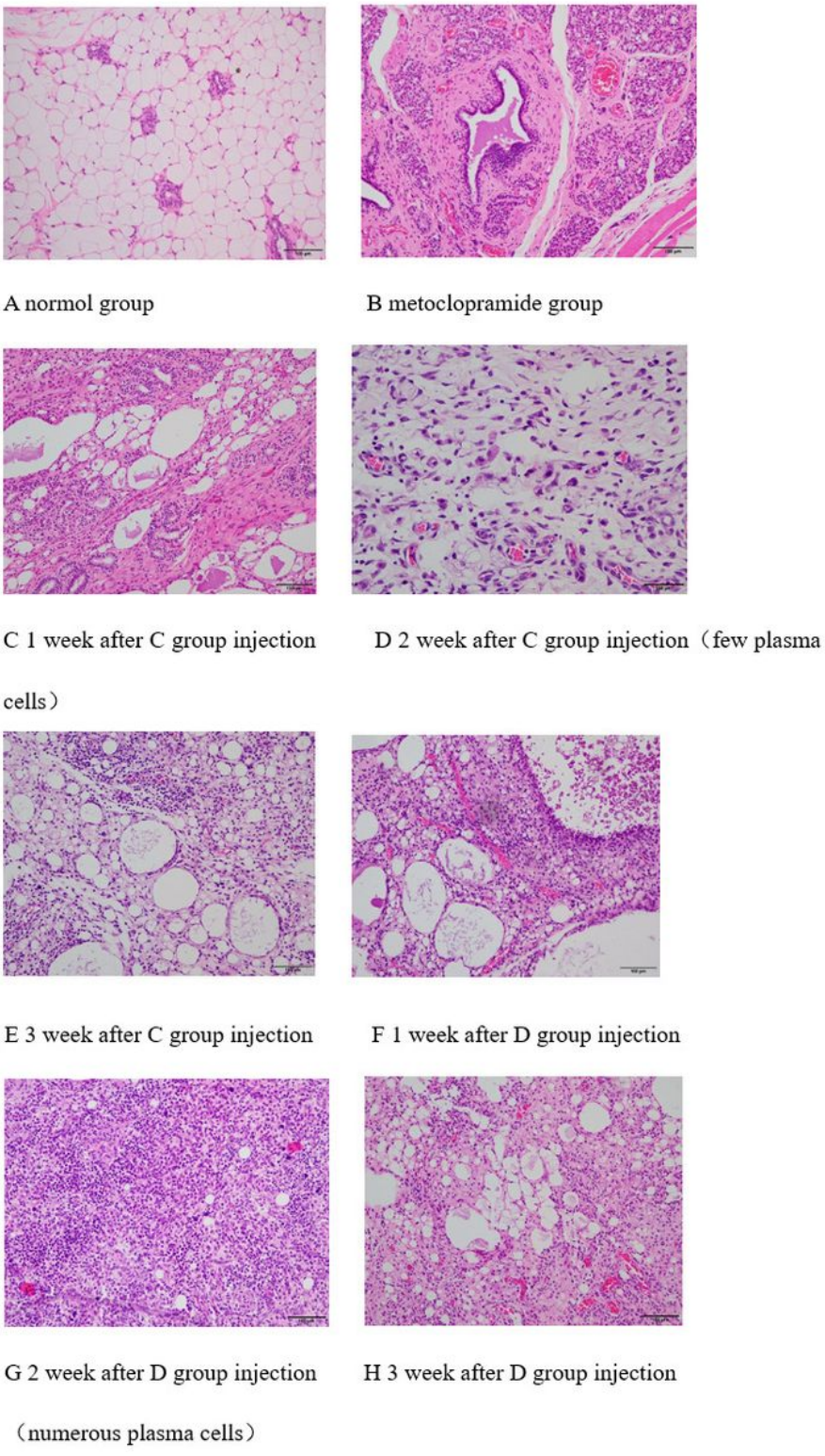

Figure 1

Histopathological morphology of mammary gland at different time points in rats of each group (HE × 200)