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Chronic rhinitis and chronic tonsillitis of staphylococcal genesis in rabbits as laboratory animal model for experimental research

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ABSTRACT

Current treatment and prevention of chronic rhinitis (CR) and chronic tonsillitis (CT) highlights the need for clinical trials in the respective laboratory model, of better options, including new medicaments. Therefore, to understand CR and CT via the laboratory model, the chronic pro-inflammatory process was simulated in laboratory animals ("Chinchilla" rabbits) using the reference *Staphylococcus aureus* strain 209P (ATCC 6538-P). Initial sensitizing of the laboratory animals with the inactivated causative agent and subsequently infecting the required area with the reference-strain made it possible to work out a reproducible model of both chronic rhinitis and chronic tonsillitis. Adequacy of CR and CT development was confirmed by the results of microbiological and pathomorphological investigations. The proposed laboratory model can be used to solve theoretical and practical medical current issues, as well as pharmacological topical problems.

INTRODUCTION

Chronic rhinitis (CR) and chronic tonsillitis (CT) form a large part of the oto-rhino-laryngology workload. According to recent literature, chronic rhinitis and chronic tonsillitis make up between 2 and 15% of the total population suffering from ORL pathology [1,2,3]. Moreover, according to statistical data for the last 8 to 10 years, the number of patients with CR and CT has increased significantly [1,2,3]. Difficulties in the treatment of CR and CT are due to the diversity of the etiopathogenetic mechanism of its development, and, therefore, one of the topical issues of practicing medicine is the optimization of the treatment of CR and CT in the acute stage. This requires scientists to develop an adequate and reproducible model for testing various methods, including new medicaments, under experimental conditions. The dominant bacterial factor of chronic rhinitis is mostly *Staphylococcus aureus*. In different countries of the world, for the modelling of purulent-inflammatory processes, in particular, endophthalmitis, osteomyelitis, endocarditis, etc.,

the reference strain *Staphylococcus aureus* 209P (ATCC 6538-P) is widely used as it has permanent biological properties which is available for obtainment [4].

The aim of the work was to develop a laboratory model of both chronic rhinitis and chronic tonsillitis of staphylococcal genesis.

MATERIALS AND METHODS

In order to establish viable laboratory models, reference strain *S. aureus* 209P (ATCC 6538-P) was obtained from the museum of microorganisms of the State Institution "I.I. Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine". A suspension of *Staphylococcus* was prepared according to the McFarland turbidity standard, using a Densi-La-Meter® device (Lachema, Czech Republic). This was then adjusted to an optical density of 1 and 3 units, respectively, on the McFarland scale in accordance with the instrument instructions and information letter on innovations in the system of Public Health Services No.163-2006 "Standardization of preparation of microbial suspensions", Kyiv, Ukraine.

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Synchronization of cultures was carried out with the help of low-temperature action.

To simulate acute catarrhal rhinitis in the laboratory animals for experimental research, 20 µl of 0.1% dextran solution (C₆H₁₀O₅) per kilo of body weight with molecular mass (Mr) of 70,000 was administered [5]. Since it was necessary to obtain a chronic inflammatory process, and, therefore, to establish a laboratory model, it was decided to infect the mucous membrane of the nasal cavity with 0,1% dextran solution once, but with 30 µl in each nostril. After 24 hours, standardized suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of either 1 or 3 units, respectively, on the McFarland scale, was used to infect the mucous membrane of the nostrils.

Cytostatics, for instance, cyclophosphamide, is most often used to suppress the immune response of animals. The immune response of the carrier, on one hand, should be suppressed, to create conditions for active integration of the pathogen, and, on the other hand, it should be sufficient enough, to inhibit the active development of the inflammatory process. Therefore, in certain research groups, we used laboratory animals which were preliminary sensitized by the microorganism. Initially, the standardized sensitizing agent-suspension of the heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* was subcutaneously administered a day before infecting the mucous membranes of the nose of the laboratory animals, either once or twice, respectively, with an interval of 1 day.

The object of the study was to derive a laboratory animal (rabbits of the breed “Chinchilla”) model for experimental research and for this purpose, to assess the anti-infective resistance of their mucous membranes. In the clinical trials, rabbits (n=31) of the breed “Chinchilla” weighing between 2.0 to 2.5 kg were used. However, they had the two weeks of quarantine. Clinical trials with the laboratory animals were guided by the following regulatory documents: DVST 421-88 “Animals laboratory. Technological process to comply with the main provisions of the Council of Europe Convention on the Protection of Vertebrate Animals used for experimental scientific purposes” (18.03.1986), ETS Directive of 24.11.1986, No. 609, Order of the Ministry of Health of Ukraine of 01.11.2001, No. 281

To be able to produce chronic inflammatory process in the laboratory animal model with long-term persistence of the pathogen, they were consequently divided into the following groups:

For chronic rhinitis:

- I group (n=10) of rabbits (laboratory animal model) were infected thrice with 0.1 ml of a suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale and with an interval of 7 days after dextran was initially administered in the nasal mucosa;
- II group (n=10) of rabbits (laboratory animal model) were sensitized once with subcutaneous injection of 0.1 ml of a suspension of heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale. Thereafter, dextran was administered in the nasal mucosa and after 24 hours, the rabbits were infected once

intranasally with 0.1 ml of a suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale.

- III group (n=10) of rabbits (laboratory animal model) were initially sensitized once with subcutaneous injection of 0.1 ml of a suspension of heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale. Thereafter, dextran was administered in the nasal mucosa and the rabbits were infected twice: after 24 hours, with 0.1 ml of a suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale, and after 7 days, with a density of 3.0 units on the McFarland scale.
- IV group (n = 10) of rabbits (laboratory animal model) were sensitized once with subcutaneous injection of 0.1 ml by suspension of heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale. Thereafter, dextran was administered in the nasal mucosa and the rabbits were infected thrice: after 24 hours, firstly with 0.1 ml of a suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale and subsequently, secondly and thirdly, after intervals of 7 days, in an amount of 0.1 ml with a density of 3.0 units on the McFarland scale;
- V group (n=10) of rabbits (laboratory animal model) were sensitized twice with subcutaneous injection of 0.1 ml by suspension of heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale. Thereafter, dextran was administered in the nasal mucosa and the rabbits were infected thrice: after 24 hours, with 0.1 ml of a suspension cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale, and, subsequently, secondly and thirdly after intervals of 7 days in an amount of 0.1 ml with a density of 3.0 units on the McFarland scale;
- VI group (n=7) were control/intact rabbits (laboratory animal model).

For chronic tonsillitis:

- I group (n=10) of rabbits (laboratory animal model) were infected once in the upper pole of palatine tonsils with 0.1 ml of the suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale;
- II group (n=10) of rabbits (laboratory animal model) were sensitized once with subcutaneous injection of 0.1 ml by suspension of heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale. Thereafter, the palatine tonsils were infected once with 0.1 ml of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale;
- III group (n=10) of rabbits (laboratory animal model) were sensitized once with subcutaneous injection of 0.1 ml by suspension of heat-killed (at a temperature of 80°C for 60 minutes) cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale. Thereafter, the palatine tonsils were infected once

with 0.1 ml of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 1.0 unit on the McFarland scale. After 10 days, the palatine tonsils were infected again with 0.1 ml of the suspension of live cells of *S. aureus strain 209P (ATCC 6538-P)* with a density of 3.0 units on the McFarland scale;

- IV group (n=5) were control/intact rabbits (laboratory animal model).

The observation period was 28-30 days. On the 7th, 14th, 21st and 28th days, throat swabs were taken, respectively, from the surface of the tonsils of the experimented laboratory animals in order to assess the degree of colonization of *Staphylococcus aureus*.

The concentration of microorganisms was determined according to the following formula by counting the colony-forming units (CFU) in the amount of inoculum with dilution loss,

$$X = \frac{10NM}{m}, \text{ where:}$$

- X – CFU,
- 10 – constant coefficient with plating 0.1 ml suspension,
- N – number of colonies
- M – dilution (10, 100, 1000 times, etc.),
- m – volume of culture plate.

The obtained indicators were expressed in decimal logarithms (lg CFU/ml).

RESULTS AND DISCUSSION

All laboratory animals were previously screened for *Staphylococcus aureus*. The dose administered for infection process and the multiplicity were selected experimentally in such a way so as to ensure that the rabbits had zero-mortality rate, a minimal number of septic states, chronic inflammatory process development due to the localization of the process and the formation of an acute purulent-inflammatory process.

The laboratory animals were observed for 90 days with a periodic determination of the degree of colonization of the microorganism on the surface of the mucous membranes of the nasal cavity (Table 1).

Table 1. Results of microbiological studies of the degree of colonization of golden staphylococcus on the surface of the mucous membranes of the nasal cavity of the experimental animal models

Animal grouping	The degree of colonization of golden staphylococcus (lg CFU/g) on the nasal mucosa membranes of the experimental animal models on the observation day (M ± m)			
	25~30 days	40~45 days	60~65 days	90 days
I, (n=10)	development of sepsis in 80% of all animals	-	-	-
II, (n=10)	4.3±0.7	not extracted	not extracted	not extracted
III, (n=10)	5.1±0.5	not extracted	not extracted	not extracted
IV, (n=10)	5.3±0.4	3.0±0.5	not extracted	not extracted
V, (n=10)	6.1±0.5	4.1±0.3	3.8±0.4	3.1±0.2
VI, (n=7)	not extracted	not extracted	not extracted	not extracted

Studies have shown that in the first group, in 80% of all animals, acute purulent nasal inflammatory process was developed with the transition to a septic state which

subsequently led to their lethal end, and, therefore, which did not provide the desired technical result.

In rabbits of the II-IV groups, after 25-30 days, the chronic inflammatory process was chronicized, with a gradual elimination of the pathogen from the surface of the mucous membranes of the nose. Thus, it was determined that the carrier of the pathogen did not last for a maximum of 40-45 days, which, therefore, did not provide the desired result. The results of pathomorphological studies after 100 days of observation of animals of groups II-IV showed no signs of damage or inflammation of the tissues.

However, in the V group, in 100% of all laboratory animals which were sensitized twice and intranasally infected thrice after administration of dextran in the nasal mucosa, 25-30 days thereafter, a microbiological picture of chronic nasal infectious of staphylococcal aetiology that prolonged for 90-100 days showed that the development of the pathogen persistence corresponds to the desired microbiological criteria of the bacterial carrier [6].

After 100 days, pathomorphological studies in rabbits of group V showed a spectrum of histopathological changes: the density of cells forming the respiratory epithelium was raised, while, in certain areas, dystrophic changes in the ciliated epithelium with ciliary oedema and gluing were observed and the number of goblet cells were decreased. Moreover, sub-epithelial cells were characterised by expanded blood vessels with extravasation and loosening of adjacent tissues with moderately expressed cellular infiltration with lymphoid elements (17-22 in the field of vision). The above signs took place in 100% of the animals, which indicates the reproducibility and adequacy of the development of chronic inflammation of the bacterial genesis of the nasal mucosa.

In contrast, laboratory animals with chronic tonsillitis of groups I-III showed a deterioration of the general state (lack of appetite, decreased mobility, etc.) a day after the introduction of the live staphylococcus strain, whereas the laboratory animals of group IV were active throughout the study.

Therefore, it was demonstrated experimentally that the clinical signs of acute tonsillitis (a marked increase in reddening and oedema of palatine arches and tonsillar fossa) were observed on the 7th day of animals in group I animals. This was confirmed by a Microbiological Index: the degree of colonization of the tonsils surface of experimental animals with golden staphylococcus was 8.2 ± 0.1 lg CFU/ml (Table 2).

Table 2. Results of microbiological studies of golden staphylococcus on the surface of the tonsils of experimental animals

Animal grouping	The degree of settling of golden staphylococci (lg CFU/ml) on the surface of the tonsils of experimental animals at the observation day, (M ± m)			
	7 days	14 days	21days	28 days
I, (n=10)	8.2±0.1	3.0±0.5	not extracted	not extracted
II, (n=10)	6.1±0.7	5.0±0.4	3.0±0.2	not plated
III, (n=10)	7.4±0.3	6.0±0.8	6.1±0.5	5.0±0.3
IV, (n=7)	not extracted	not extracted	not extracted	not extracted

On the 14th day, with regard to the animals of group I, the clinical signs of acute tonsillitis disappeared and the amount of *S. aureus* decreased by 2.3-3.3 times (p<0.001),

as compared to the corresponding parameters on the 7th day of observation. Excretion of *S. aureus strain 209P (ATCC 6538-P)* was noted on the 21st day of observation. The decrease of immunoreactivity was carried out by preliminary sensitization of laboratory animals to the infectious agent.

Our study showed that in the animals of group II, on the 7th day of observation, clinical signs of acute tonsillitis were also determined. However, the degree of colonization of the surface of the tonsils of *S. aureus strain 209P (ATCC 6538-P)* was 1.2-1.5 times lower ($p < 0.01$) compared to the corresponding parameters of group I. On the 28th day of observation, *Staphylococcus aureus* was not excreted from the surface of the tonsils of experimental animals.

To simulate the chronic purulent-inflammatory process in the animals of group III, in addition to preliminary sensitization with an infectious agent, repeated infection was carried out. We found that the clinical signs of acute tonsillitis, which gradually faded, were present in the rabbits of group III on the 7th day of observation. After repeated infection with a higher dose of the pathogen, the microbiological indices obtained indicated the etiologic significance of *Staphylococcus aureus* in the development of the inflammatory process. Herein, the degree of colonization of the tonsil surface with golden *Staphylococcus* averaged 14 and 21 days, respectively 6.0 ± 0.8 and 6.1 ± 0.5 lg CFU/ml. After 28 days, the population density of *S. aureus 209P (ATCC 6538-P)* on the tonsil surface dropped, but remained high though. The above signs occurred in 100% of the laboratory animals of group III – which indicates the reproducibility of the established model of chronic tonsillitis.

CONCLUSION

A reproducible laboratory model of the inflammation of the nose and tonsils of staphylococcal genesis were developed which can be used in the Microbiological/ Bacteriological Laboratories of Research Institutes. Furthermore, Biotechnology Industries can use these models to study the pattern of formation of chronic staphylococcal pathogenesis, and, hence, to assess the potential effectiveness of the methods for remedial therapy.

Patented inventions of Ukrainian Utility models were established based on clinical trials [7,8]. The authenticity of the inventions could be verified at the Ukrainian Patents Database: «www.uapatents.com» and at State Enterprise «Ukrainian Intellectual Property Institute (Ukrpatent): www.uipv.org». This confirms the granting of exclusive rights for the invention patent of Ukraine. Moreover, in Ukraine, an invention is deemed the result of the intellectual activity of a person in any technological field.

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