Lessons learned from conventional animals: Encouragement to use specific-pathogen-free animals

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A B S T R A C T

Experimental rabbits provide evidence for translational research regarding the pathogenies or treatment of human diseases. We developed a novel method for regenerating the middle ear mucosa using autologous cultured nasal mucosal epithelial cell sheets, and evaluated the wound healing process in the middle ear mucosa of experimental rabbits. Nonetheless, vigilant microbiological monitoring of experimental animals is essential to effectively prevent a decline in their health conditions, which may affect the research results. We experimented with contamination of Pasteurella multocida in non-specific-pathogen-free (SPF) rabbits (without microbiological monitoring). Most non-SPF rabbits had otitis media, whereas SPF rabbits did not, which affected their results during the mucosal regeneration study. The contamination was resolved by changing the experimental design from using non-SPF rabbits to that using SPF rabbits. It is crucial to use the SPF animals for any surgical intervention studies.

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In pre-clinical regenerative medicine and tissue engineering, mice may not be used for transplant surgery due to their small size. Rats are often used in preclinical medicine, and rabbits are used when dealing with smaller organs such as eyes [1], knee joints [2], or the trachea [3]. We have studied regenerative strategies for the middle ear mucosa as a subset of the respiratory epithelia, with an aim to improve the post-operative middle ear mucosa regeneration on the middle ear bone surfaces using autologous cultured nasal mucosal epithelial cell sheets applied after surgery. A successful rabbit preclinical study regarding wound healing of the middle ear [4] led to the publication of a human clinical study [5]. However, when conventional rabbits were used as experimental models, the preclinical study did not work at all.

We pre-screened rabbits for infections, such as sinusitis and otitis media, through nasal examination and endoscopic examination of the eardrum, and those with an evidence of infection were excluded (Fig. 1). However, an acute suppurative otitis media almost always developed in the middle ear mucosa wound-healing rabbit model (Fig. 2a and b). Some rabbits exhibited subcutaneous abscesses near the skin incision (Fig. 2c). Bacterial tests of nasal mucin, ear discharge, and subcutaneous abscesses detected P. multocida. By contrast, all SPF rabbits undergoing a routine microbiological monitoring did not exhibit any ear discharge (Fig. 2d and e). As is frequently the case, conventional animals screened before operation were not the same as the SPF animals.

P. multocida is a pathogen commonly infecting diverse animal hosts worldwide. In rabbits, infections due to P. multocida are commonly known as “snuffles”, that is, rabbit rhinitis. P. multocida can also infect humans bitten or licked by various carrier animals [6–8], and can cause sepsis in immuno-compromised individuals [9,10]. The prevalence of P. multocida infection in rabbits housed under laboratory conditions or on breeding farms has been reported to be between 15.8% and 94% [11]. P. multocida can infect rabbits asymptomatically and develop into an opportunistic infection when the host’s immune system is compromised [12]. The infection route can be through droplet infection, contact infection,
or birth canal infection from bacteria-positive rabbits. Infection with *P. multocida* causes acute otitis media, pneumonia, conjunctiva inflammation, septicemia, abscess formation, and rhinitis. Contents of the nasal discharge and abscesses are usually characterized by a creamy white pus. Although *P. multocida* is usually asymptomatic in rabbit carriers, opportunistic infections with virulent *P. multocida* can result from stress in animals or a declining health condition. In our case, *Pasteurella* infection seemed to be exacerbated by a direct surgical invasion or operative stresses after removal of the middle ear mucosa, as no ear infection was observed in the sham-operated animals without removal of the mucosa (Fig. 2b and e).

The contamination of *P. multocida* was resolved by changing the rabbit vendor. If we had not proceeded with the experiment with suitable modifications in the experimental protocol, this project would not have provided solutions to the encountered contamination. We hope that our experience will be of use to the reader who promotes or supports the application of a regenerative therapy.

**Declaration of Competing Interest**

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