Experimental Gerontology 75 (2016) 37–41

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Short report

Utilizing the age-related widening of the gingival crevice as a potential non-invasive vaccination route: Prospects for elderly vaccination

Marni E. Cueno *, Muneaki Tamura, Keisuke Seki, Manabu Ohya, Kuniyasu Ochiai *

Department of Microbiology, Nihon University School of Dentistry, Tokyo 101-8310, Japan

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 19 November 2015
Accepted 27 November 2015
Available online 17 December 2015

Keywords:
Gel-encapsulation
Gingival crevice
Gingival-vaccination
Xanthan gel

ABSTRACT

Gingival crevice (GC) increases with age allowing periodontopathic bacteria and its products to enter. We hypothesize that by mimicking this event we can utilize the GC as a potential vaccination route. Here, we used 20 wk-old (young) and 77 wk-old (old) Sprague–Dawley rats. Initially, we elucidated the difference between oral-administration and oral-supplementation in both young and old rats and, subsequently, we determined the optimal component concentration for xanthan gel-encapsulation. Next, through molecular docking, we simulated xanthan gel-encapsulation of a representative antigen (for this study we used influenza H5N1 hemagglutinin) in order to verify that target epitopes were not blocked. Lastly, we compared the antibody titer among gingival-vaccinated rats (old and young) and, likewise, we evaluated the antibody titer produced via the gingival route as compared to other vaccination routes (intradermal, oral, sublingual). Rat blood serum was collected for further downstream analyses. Throughout the study, we were able to establish the following conditions: higher target components enter old rats via oral-supplementation; 100 μg mL^{-1} is the optimal component concentration for xanthan gel-encapsulation; and xanthan gel-encapsulation leaves antibody epitopes exposed. More importantly, we observed that gingival-vaccinated old rats have higher antibody titer as compared to young rats and, likewise, we found that antibody titer elicited via gingival vaccination is comparable to other mucosal vaccination routes. Thus, we propose that the GC has the potential to serve as a non-invasive vaccination route.

1. Introduction

The elderly population is growing worldwide and several among the elderly suffer from frequent and often more severe infections as compared to the younger population (Aspinall et al., 2007; Gavazzi and Krause, 2002). A major concern affecting the elderly is immunosenescence or the age-related decline in response to infection by both the innate and adaptive immune systems (Dorrington and Bowdish, 2013; Montecino-Rodriguez et al., 2013), thereby, increasing the susceptibility of an aging individual to an infectious disease (Weinberger et al., 2008). This emphasizes the importance of preventing infection among the elderly.

Vaccination has long been used to protect people from infections, however, in the elderly population vaccine efficacy (particularly for influenza) is low resulting to fewer antibodies being produced and a reduction in secondary immune response is observed compared to healthy adults (Cadeddu et al., 2012). Age-related decrease in vaccine efficacy was previously proposed to be attributed to decreased naïve B and T cells ascribable to skewing of the hematopoietic stem cells and reduced output of lymphoid precursors, respectively (Dorrington and Bowdish, 2013; Ongradi and Kovesdi, 2011). Moreover, current vaccination strategies developed for the general population (catering to the younger and healthier individuals) would either be insufficient or ineffective in stimulating a significant immune response in the elderly population (Aspinall et al., 2007; Dorrington and Bowdish, 2013). Considering the lack of approved vaccines for elderly use and limited vaccine efficacy of current vaccines, there is a growing interest to develop vaccine strategies that caters to the elderly population.

Part of the oral-pharyngeal cavity is the gingival mucosa which diminishes with age (Suda et al., 2000) resulting to a wider gingival crevice (GC) serving as an entry point for periodontopathic bacteria and its products (Kim et al., 2009; Wu et al., 2014). In this regard, we propose that this same flaw can be utilized as a vaccination route, however, this was never investigated. A better understanding and appreciation of the age-related widening of the GC may lead to an alternative non-invasive vaccination route which could be ideal for elderly vaccination.

2. Materials and methods

2.1. Animal handling

Throughout this study, we used 20 wk-old (young) and 77 wk-old (old) Sprague–Dawley rats (Nippon CLRA, Shizuoka, Japan). All rats

* Corresponding authors at: Department of Microbiology, Nihon University School of Dentistry, 1-8-13 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan.
E-mail addresses: marni.cueno@nihon-u.ac.jp (M.E. Cueno), ochiai.kuniyasu@nihon-u.ac.jp (K. Ochiai).

http://dx.doi.org/10.1016/j.exger.2015.11.018
0531-5565/

were handled as previously described (Cueno et al., 2014; Cueno et al., 2013a). Briefly, rats were housed in individual stainless steel cages throughout the experiment. The cages were placed in a room under controlled temperature (23–25 °C), relative humidity (40–60%), and lighting (12 h). Rats had free access to water and hard briquettes with 20–50 mL water and 2–5 briquettes being consumed daily. They were handled in accordance with the guidelines for animal studies of Nihon University (AP10D023).

2.2. Comparison and optimization

Xanthan gel (Meiji Corporation, Tokyo, Japan) was used to encapsulate all samples (1 g xanthan powder:20 mL liquid sample) as previously described (Cueno et al., 2013a; Cueno et al., 2015). Catechin component (Taiyo Chemical Company, Mie, Japan) was initially used for preliminary comparison and optimization since it is stable under room-temperature and oral conditions as we have previously demonstrated (Cueno et al., 2013a; Cueno et al., 2015), therefore, any possible measurement variations between samples or concentrations will not be ascribable to catechin degradation. One set of rats is composed of 3 rats per age group (young, and old). Catechin solution was orally-administered in one set while gel-encapsulated catechin (GEC) was orally-supplemented along the GC of the upper and lower molars in the other set for oral treatment comparison. In both sets, 200 μL of an 800 μg mL^{-1} catechin concentration was used (Cueno et al., 2013a). Similarly, 200 μL of varying GEC concentrations (50, 100, 200, 400 μg mL^{-1}) were used to treat old rats (n = 4) via oral supplementation in order to determine the optimum concentration. Heart blood was collected 24 h post-treatment using a 25 G × 1" needle while rats were under intraperitoneal anesthesia (pentobarbital sodium). Subsequently, blood samples were centrifuged at 1000 × *g* for 20 min in 4 °C to isolate the blood serum which were then used to verify the presence of *O*-methyl derivative (by-product of catechin degradation inside the body) amounts through high-performance liquid chromatography.

2.3. Computational and structural analyses

There are several well documented infectious diseases commonly found among the elderly (Dorrington and Bowdish, 2013; Weinberger et al., 2008) and one example of a common elderly infection that occurs annually is influenza which consequentially has been associated with increased morbidity (Gavazzi and Krause, 2002). Influenza H5N1 hemagglutinin (HA) (GeneTex) was used as a representative antigen throughout this study. We verified the exposure of target antibody epitopes after gel-encapsulating hemagglutinin (GEH). Molecular docking of multiple xanthan molecules (CID: 7107) onto the representative H5N1 hemagglutinin (HA) crystal structure (PDB ID: 3S11) was performed using HexServer (Macindoe et al., 2010).

2.4. Antibody titer measurement

Gingival-vaccination (oral-supplementation of GEH along the upper and lower molar GC) was performed in two sets of experiments. One set compared the antibody titer measurements between gingival-vaccinated young and old rats, whereas, the other set compared antibody titer measurements among old rats vaccinated through our proposed vaccination route (gingival) and other previously established vaccination routes (intradermal, oral, sublingual). Each vaccinated rat (n = 4) was given the same optimized antigen concentration. Rats orally-supplemented with just the xanthan gel were used as control. Vaccination through the GC and sublingual route were performed using GEH while vaccination through the intradermal and oral route utilized HA in liquid form. Heart blood was obtained 14 days post-vaccination and blood sera were isolated as earlier described. Blood serum obtained in each rat treatment was serially diluted (until ×512) with blood serum obtained from untreated old rats. We emphasize that since the main objective of this study is to determine the prospect of utilizing the gingival crevice as a possible vaccination route, we believe that blood collection 14 days post-vaccination would suffice to check for antibody response induction.

Antibody titer was determined through ELISA (OD 450 nm). Briefly, antigen coating (at 1 μg mL^{-1} concentration) on polystyrene plates were done using sodium bicarbonate–sodium carbonate buffer (Polysciences, Inc). Blocking (24 h) was performed using PBS with 1% BSA blocking buffer (GeneTex). Influenza-A H5N1 antibody (Aviva Systems Biology) and anti-rat IgG-HRP (Santa Cruz Biotechnology) were used as primary and secondary antibodies, respectively. SIGMAFAST™ OPD tablets were used as substrate for peroxidase detection. Washing in-between steps was done using the PBS/Tween® Solution (Applichem). Hydrochloric acid (1.0 M) was used as stop solution.

2.5. Statistical analyses

Statistical analyses were performed in all comparisons made throughout the study. Initially, Andersen–Darling normality test was performed for each comparison and, if passed ($p > 0.05$), the statistical significance of differences was determined by either Student's *t* test (two-tail) or Tukey's test. A significance level of 95% ($p < 0.05$) was considered statistically significant.

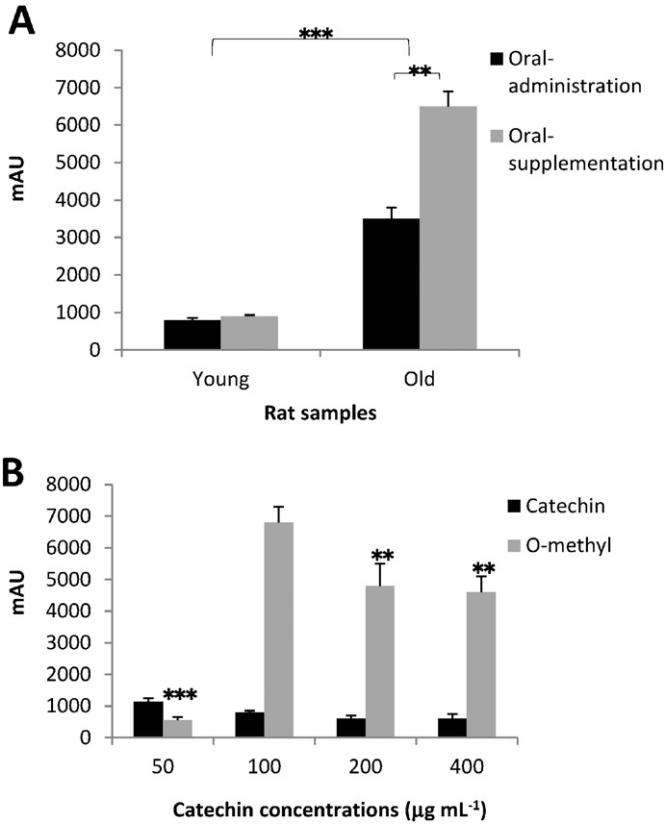

Fig. 1. Higher catechin components enter the blood serum of old rats via oral-supplementation. (A) Catechin oral-administration and oral-supplementation. *O*-methyl derivative levels after catechin oral-administration and oral-supplementation are indicated. Results correspond to 3 replicates per independent sample (n = 3) of young (20 wk-old) and old (77 wk-old) Sprague–Dawley rats. (B) Optimized catechin concentration for xanthan gel-encapsulation. Catechin and its by-product (*O*-methyl derivative) are indicated. Results correspond to 3 replicates per independent sample of old (77 wk-old) Sprague–Dawley rats (n = 4). Statistical analyses were performed using Anderson–Darling normality test and, if passed ($p > 0.05$), Student's *t* test ($^{**}p < 0.01$; $^{***}p < 0.001$).