Chitin oligosaccharides (oligomer of GlcNAc) protect mycelial growth of *Candida albicans in vitro* and *in vivo*.

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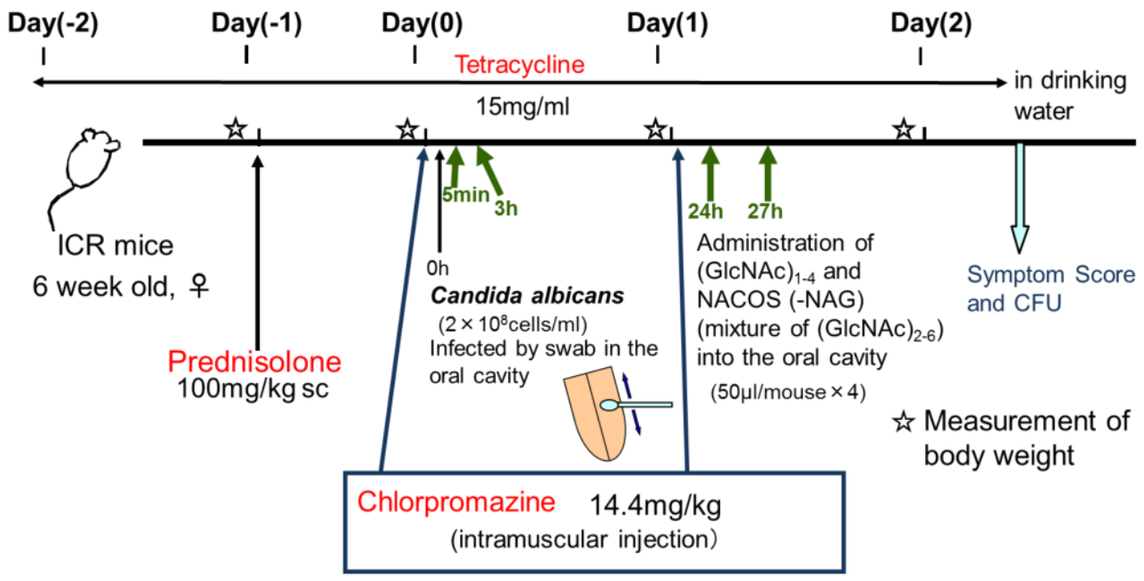
The amino sugar N-acetylglucosamine (GlcNAc) is well known to induce the mycelial growth of *Candida albicans*. We had reported that GlcNAc aggravated murine oral and gastrointestinal candidiasis. Here we investigated the effect of the oligomer of GlcNAc on mycelial growth of *C. albicans* and murine oral candidiasis. Surprisingly, chitin oligosaccharides inhibited mycelial growth of *C. albicans in vitro*. Inhibition of mycelial growth detected by the length of hyphae from 4h-culture of *C. albicans*; hyphae length elongation of *C. albicans* cells in the control culture, 25mM GlcNAc-, $(GlcNAc)_3$- and $(GlcNAc)_4$-supplemented culture were 89.77±20.38, 86.1±22.7, 67.18±27.96, and 42.25±22.4μm, respectively. Average length of the elongated hyphae in the case of $(GlcNAc)_3$ or $(GlcNAc)_4$ were significantly less than control or monomer ($P<0.01$). After 20h culture, each volumes of hyphae attached to the bottom of the culture plates were measured by crystal violet staining method. The hyphal volume of *C. albicans* after 20h-culture were decreased by the presence of the oligomer of GlcNAc (degree of polymerization = 2 to 6). The inhibitory effect of mycelial growth were observed in the case of three strains of *C. albicans*, TIMM1768, TIMM2640 and TIMM3163 (having fluconazole-resistant phenotype). To clarify the effect of chitin oligosaccharides *in vivo*, their effects on experimental murine oral candidiasis were examined. The mice infected with *C. albicans* were administrated 50μL of 12.5mM $(GlcNAc)_{1-4}$ four times (5min, 3h, 24h, 27h). Although mice given GlcNAc showed significant aggravation of oral symptoms of candidiasis after two days ($P<0.01$), mice given $(GlcNAc)_{2-4}$ showed significant improvement when compared with those of GlcNAc in oral symptoms ($P<0.01$). We discuss that application of chitin oligosaccharide as a nutritional supplement may have a valuable effect on oral health in people susceptible to oral or gastrointestinal candidiasis.

Objectives: The amino sugar N-acetylglucosamine (GlcNAc) is well known to induce the mycelial growth of *C. albicans*. We had reported that GlcNAc aggravated murine oral and gastrointestinal candidiasis[1, 2], and *C. albicans* adheres to chitin, polymer of GlcNAc[3]. Here we investigated the effect of chitin oligosaccharides, the oligomer of GlcNAc, on mycelial growth of *C. albicans* and murine oral candidiasis.

Methods: *C. albicans* TIMM1768, TIMM2640 and TIMM3136 from patients with candidiasis were used. Chitin oligosaccharides (GlcNAc) n (n = 1–6) were produced by partial hydrolysis of crab chitin by the method of Rupley[4]. One hundred microliters of *C. albicans* cell suspension were aliquoted into 96-well microtiter plates (1 x 10^4 cfu/well for germ-tube formation, 1 x 10^3 cfu/well for mycelial growth analysis), and 100-μl serial dilutions of chitin oligosaccharides (GlcNAc, $(GlcNAc)_3$ or $(GlcNAc)_4$) were added the wells which made final concentration of 50 mM/ml to 3.13 mM/ml and incubated at 37C in 5% CO_2 in air for 2h or 4h for germ-tube formation, for 20h for mycelial growth analysis. Animal experiments were performed according to the guidelines for the care and use of animals approved by Teikyo University (No.15-026). The experimental procedures for candidiasis model as shown below were according to Takakura [5].

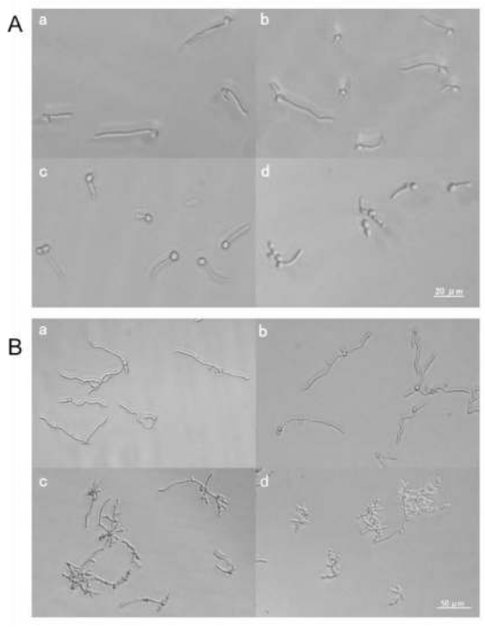


Group of immunosuppressed mice were inoculated *C. albicans* TIMM1768 cells and 50 μL of test samples were placed on the surface of tongues at 5min, 3h, 24h and 27h after inoculation (Control, n=6; 25 mM GlcNAc, n=6; 25 mM $(GlcNAc)_2$, n=6; 25 mM $(GlcNAc)_3$, n=6; 25 mM $(GlcNAc)_4$, n=6; 25 mM NACOS (-NAG) (mixture of $(GlcNAc)_{2-6}$), n=6).

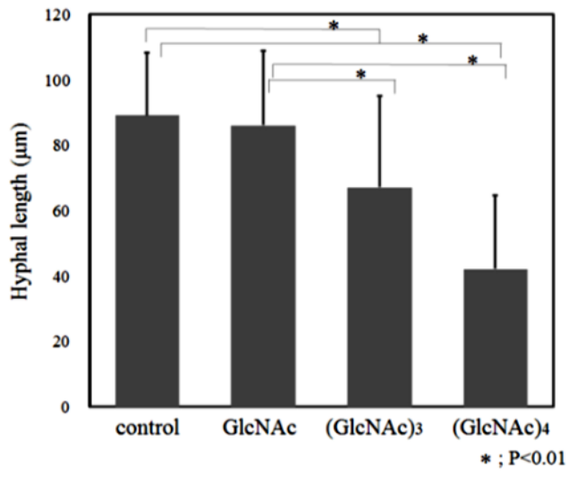
Results:

1. Chitin oligosaccharides inhibited mycelial growth of *C. albicans in vitro.*

Typical images of *C. albicans* TIMM1768 cultured with chitin oligo. A; 2h, B; 4h. a; control, b; 25mM GlcNAc, c; 25mM $(GlcNAc)_3$, and d; 25mM $(GlcNAc)_4$.



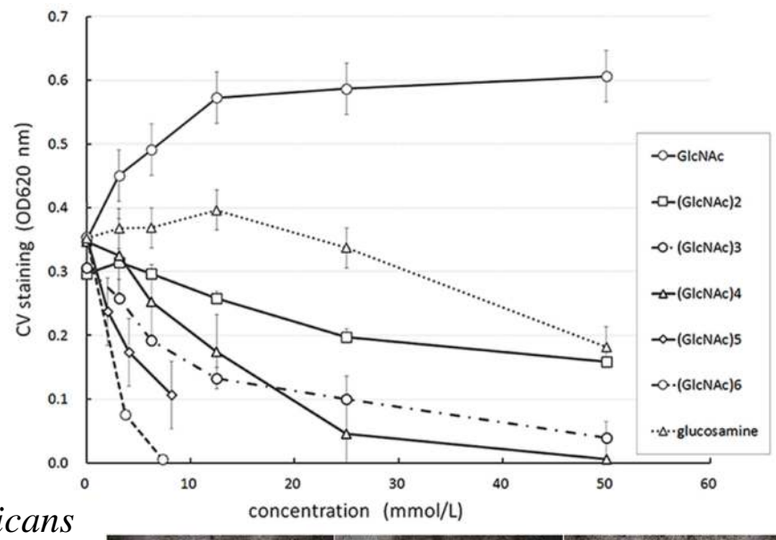
Length of hyphae were measured using Lenaraf 220b software (Vector Japan)



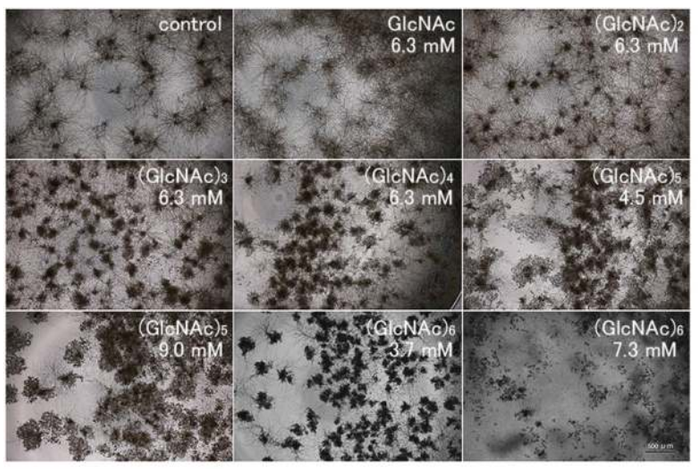
Inhibition of mycelial growth detected by the length of hyphae from 4h-culture of C. albicans; hyphae length elongation of C. albicans cells in the control culture, 25mM GlcNAc-, 25mM $(GlcNAc)_3$- and 25mM $(GlcNAc)_4$-supplemented culture were 89.77±20.38, 86.1±22.7, 67.18±27.96, and 42.25±22.4μm, respectively. Average length of the elongated hyphae in the case of $(GlcNAc)_3$ or $(GlcNAc)_4$ were significantly less than control or monomer ($P<0.01$).

2. The hyphal volume of *C. albicans* after 20h-culture were decreased by the presence of the oligomer of GlcNAc (degree of polymerization = 2 to 6).

After 20h culture, each volumes of hyphae attached to the bottom of the culture plates were measured by crystal violet method.



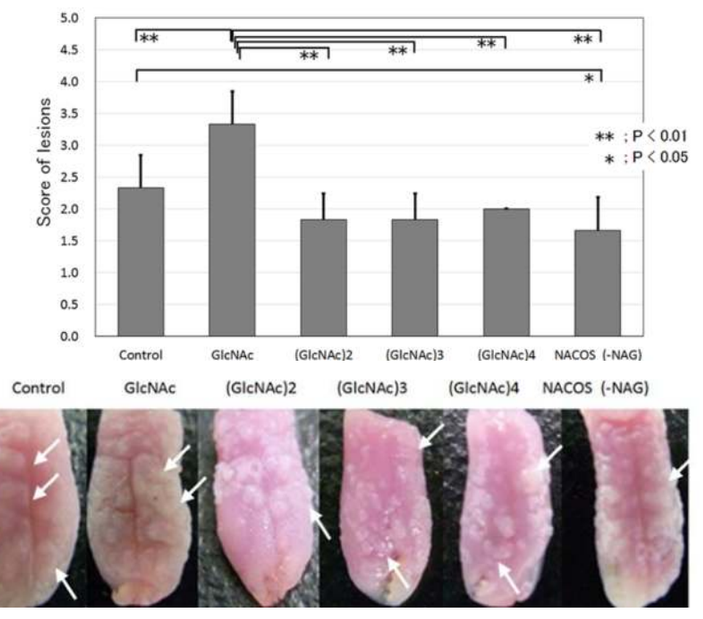
Typical images of *C. albicans* cultured with $(GlcNAc)_{1-6}$ were shown in the right figure.



The mycelial growth inhibition were observed in the case of 3 strains of *C. albicans*, TIMM1768, TIMM2640 and TIMM3163 (FCZ-resistant).

3. GlcNAc induced symptom severity of oral candidiasis, although oligomer of GlcNAc inhibited candidiasis.

The macroscopic evaluation of infection was expressed by scoring lesions from 0 to 4 on the basis of the extent and severity of whitish, curd- like patches (arrows) on the tongue surface according to Takakura [5] as follows: 0, normal; 1, white patches in less than 20%; 2, white patches in less than 90% but more than 91%; 4, thick white patches like pseudomembranes in more than 91%.



Conclusion: **Application of chitin oligosaccharide as a nutritional supplement may have a better effect on oral health in people susceptible to oral or gastrointestinal candidiasis.**

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Reference; 1. Ishijima SA et al. Med Mycol, 50; 252-258 (2012); 2. Ishijima SA et al. Med Mycol, 56E; E31-E39 (2015); 3. Ishijima SA et al. Med Mycol J, 58E;E15-E21 (2017); 4. Rupley JA. Biochem Biophys Acta, 83; 245-255 (1964); 5. Takakura N et al. Microbiol Immunol 47: 321-326 (2003)