幾丁聚醣對痤瘡丙酸桿菌之生長及其脂酶;活性抑制之探討 The Inhibitory Effect of Chitosan and SACCHACHITIN on the Growth of Propionibacterium acnes and the Activity of Lipase

中文摘要

痤瘡丙酸桿菌 (Propionibacterium acnes, P. acnes) 是痤瘡致病原因之一，其所分泌的胞外酵素 Lipase 在分解皮脂成為甘油 (Glycerol) 及游離脂肪酸 (Free Fatty Acids, F.F.A.) 之路徑上扮演 key enzyme 之角色。本研究目的主要探討幾丁聚醣 (Chitosan) 及 SACCHACHITIN 是否具有抑制 P. acnes 生長及抑制 Lipase 活性之能力。經由 Lipase 活性之定性實驗，藉由觀察 F.F.A.是否與 Victoria Blue B (V.B.B) 吸附產生沈澱，及油酸是否釋出，我們發現幾丁聚醣與 SACCHACHITIN 能有效抑制沈澱物之形成及油酸釋出，同時溶解於培養液中之殘餘 Victoria Blue B 可做為 Lipase 活性及 P. acnes 生長之負相關指標。在最小抑制濃度 (Minimum inhibitory concentration, MIC) 實驗，我們發現幾丁聚醣具有劑量-效應依隨(Dose-dependent)之抑制 Lipase 活性效果，並測得最小抑制濃度為 0.025% 。經由油酸之釋出定量 Lipase 活性之實驗，我們同樣證實，不論高分子量、中分子量、低分子量之幾丁聚醣及 SACCHACHITIN 皆具有顯著降低油酸產生之效果，而高分子量之幾丁聚醣有較高之抑制能力，其機轉尚待進一步研究，不排除此抑制 Lipase 活性係源自於抑菌之連帶效應。此外，在 MTT 分析試驗，我們證實 Chitosan 從 0.5~0.02% 濃度範圍亦具有 Dose-dependent 之抑菌功效，SACCHACHITIN 受其難溶性質影響，不易依吸光值推估其抑菌能力，但從 0.01% 濃度樣品及定性實驗觀察，仍可測得其抑菌能力。此外，我們以 oleic acid 取代 Triolein，重複 Lipase 活性定性實驗系統，證實 V.B.B 確實與 oleic acid 結合，產生沈澱，由此結果足以確立 V.B.B 殘留量與 Lipase 活性之負相關指標意義。為了解本實驗系統之抑菌途徑及相關機轉，我們設計了 Glycerol compensation assay，嘗試以 Glycerol 加入含 Chitosan 之菌液培養，發現 Glycerol 非 P. acnes 之主要碳/能源。綜合以上所有實驗結果，說明 Chitosan 及 SACCHACHITIN 明顯具有抑菌之能力，Lipase 活性隨之受其影響，機轉尚待研究，但此結果說明此天然物具研發成為治療痤瘡安全、有效而成本低藥物之潛力。

關鍵字：痤瘡丙酸桿菌、Lipase、抑制劑、Victoria Blue B、幾丁聚醣、SACCHACHITIN

英文摘要

Propionibacterium acnes (P. acnes) play an important role in the pathogenesis of acne vulgaris . Extracellular lipases produced by P. acnes in vivo act as key enzyme in

the hydrolyzation pathway of native sebum triacylglycerols to glycerols and free fatty acids (FFA) The purpose of the study reported here was to investigate whether or not chitosan and SACCHACHITIN possessed the inhibitory potency on the growth of P. acnes and the activity of lipase. Putative lipase activity was detected by observation of the interactive precipitate between FFA and Victoria Blue B (VBB). Result of the investigation demonstrated the inhibitory effect of chitosan and SACCHACHITIN on the precipitate formation and the release of FFA. Meanwhile, the unreacted VBB in the culture broth could be a negative correlation index of lipase activity and P. acnes viability. The evaluation of minimum inhibitory concentration (MIC) revealed the dose-dependently inhibitory effect of chitosan on putative lipase activity, and the MIC was measured around 0.025% for chitosans of different molecular size.. Further investigation of lipase activity was detected by measuring the amount of oleic acid produced from triolein. The result suggested that all of high molecular weight chitosan, medium molecular weight chitosan, and low molecular weight chitosan possessed the potencies of significantly reducing the release of oleic acid. Among the chitosan samples tested, high molecular weight chitosan showed the most effective. Although the results showed significant inhibitory effect , there still lacked sufficient data to prove the inhibitory mechanism of chitosan on lipase activity. In addition, there was a probability that the inhibitory effect on lipase activity was based on the inhibitory effect on P. acnes viability. In order to determine whether chitosan possessed the inhibitory effect on P. acnes viability, MTT assay was performed. Results demonstrated that chitosan dose-dependently inhibited P. acnes viability. SACCHACHITIN was not suitable to evaluate its antimicrobial function by spectrophotography because of its poor solubility. However observation from the samples of 0.01% concentration and other qualitative analysis, also showed its antimicrobial potency in MTT assay. To repeat the confirmation assay system of lipase activity, we used oleic acid as a substitute for triolein and demonstrated the binding of Victoria Blue B to oleic acid, thus resulting in precipitate formation. Therefore, we proved that the unprecipitated VBB was a negative correlation index to lipase activity. For the detection of antimicrobial mechanism and pathway, the glycerol compensation assay was carried out by introducing glycerol into the culture broth containing chitosan. The result showed that glycerol was non-essential carbon/energy source in P. acnes nutrition. Altogether, these studies suggests that chitosan and SACCHACHITIN could inhibit the P.acnes viability and consequently inhibit the putative lipase activity in vitro,. therefore,chitosan or SACCHACHITIN may be a potential medicine in acne therapy