利用雙紡錘孢子蟲草之細胞壁組成做爲傷口癒合機轉之研究

Mechanisms Involved in the Wound Healing of the Cell Wall from Cordyceps bifusispora

中文摘要

幾丁質促進傷口癒合之機轉，目前並不清楚，本研究室先前的研究發現，真菌來源之幾丁質可以促進角質細胞及纖維細胞增生，並能抑制 MMP-9 (Matrix metalloproteinase) 的活性，但並無法全盤說明促進傷口癒合的機轉。此外，文獻中亦顯示，在慢性傷口中發現其生長因子 (Growth factor) 有異常偏低而 MMPs 有不正常偏高的情形。因此，假設幾丁質可以抑制 MMPs 或是其他的 proteinase 而使生長因子不被過度分解，甚至幾丁質可促使傷口組織分泌生長因子，使生長跟破壞得到一個調節平衡，進而促使傷口癒合。

雙紡錘孢子蟲草 (Cordyceps bifusispora, Eriksson) (Phytocordyceps ninchukispora, Su & Wang) 屬麥角菌科之真菌，係 1984 年於台灣發現的新種。本實驗之目的是利用不同於?子菌之雙紡錘孢子蟲草液態培養之細胞壁做爲一項新的幾丁質來源，並開發傷口新材質並研究其傷口癒合之機轉。

經液態培養靜置 21 天之雙紡錘孢子蟲草細胞壁分析，結果顯示出其含有 N-acetyl-D-glucosamine 的含量爲 33%，將雙紡錘孢子蟲草細胞壁經鹼處理，冷凍乾燥後，與甲基纖維素混合成爲 ASCOCHITIN 軟膏。利用大白鼠 (Wistar rats) 做動物實驗，在分別於脊髓兩側利用打孔開六個 6mm 圓形鏡相之傷口，再將 ASCOCHITIN 軟膏塗抹於傷口上，發現可顯著的促進傷口癒合，並且取下的傷口樣本以 immunoassay 的方法分析其 transforming growth factor-beta 1 (TGF-β1) 、platelet derived growth factor (PDGF) 和 vascular endothelial growth factor (VEGF)，其中對於 TGF-β1 在第一及第二天出現顯著差異，對於 PDGF 則是在第二及第四天出現顯著差異。結果顯示 ASCOCHITIN 軟膏可維持 TGF-β1、PDGF 和 VEGF 在傷口中的濃度。Zymography 的結果也顯示 ASCOCHITIN 軟膏可能抑制了傷口中微生物或嗜中性球所分泌的蛋白水解?而促進了生長因子的生成。

英文摘要

Chitin is reported as an accelerator of wound healing. Previous reports indicate that chitin accelerates the proliferation of keratinocytes、fibroblasts , and inhibit the activity of MMP-9 (Matrix metalloproteinase-9). However the details of the mechanism are not clear. In this study, culture of Cordyceps bifusispora was used for a new source of chitin. In the cell wall of liquid culture at 28℃ for 21 days, approximate 33% of N-acetyl-D-glucosamine was obtained. Full thickness skin punch (0.6cm ϕ) was performed on the back of Wistar rats. Application of the cell wall of C. bifusispora (ASCOCHITIN) significantly induced wound healing and then transforming growth factor-beta 1 (TGF-β1) 、platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) were assayed by immunoassay

method in wound samples. As a result, ASCOCHITIN significantly increased the production of TGF-β1、PDGF and VEGF in the early stage of the healing process. The results also indicated that the cell wall components might inhibited proteases from microorganisms or neutrophils in the wound area that resulted in a higher level of the growth factors. MMP-9 content in the wound area was also analyzed by zymography and the result was in good agreement to that of the growth factors.