植生蟲草多糖體抗腫瘤代謝物 (PN-2) 之甲基化分析及利用流動細胞儀探 討免疫機能之研究

Methylation Analysis and Immuno-Flow-cytometric Studies on an Antitumor Polysaccharide PN-2 Puritied from Phytocordyceps ninchukispora Su et Wang

中文摘要

雙節棍孢子植生蟲草(Phytocordyceps ninchukispora Su & Wang)屬麥角菌科之真菌,係 1985 年於台灣發現的新屬植生蟲草(Phytocordyceps)之單一種。將此菌株進行液態培養,把培養液離心取其上清液經超微過濾器,分子篩及離子交換法可得到一純化的水溶性多糖 (PN-2) 。將此多糖進行成份分析並用新法做各種生理活性測試。利用 Chitinase 分析可確定此糖至少含三相鄰 N-Acetylgucosamine。將此糖經甲基化後水解再乙醯化，其衍生物經 GC 比對和 GC Mass 分析，可知其主鍵爲 $\beta 1 \rightarrow 4$ N-Acetylgucosamine，其第六 C 位置有些接 Galactose Mannose 和 Threitol，其鍵結爲 $1 \rightarrow 4$ 鍵結。本水溶性多糖(PN-2)可減少小白鼠皮下腫瘤惡化程度,延長壽命 21% , 並明顯減少皮下腫瘤面積 29% 。對 sarcoma-180 cell 腹水癌小鼠的體重可明顯降低 11%。在抗人工肺臟腫瘤轉移方面 i.v. PN-2 可降低肺中腫瘤結節數 88% 。爲解決人工計數肺中腫瘤結節數的不客觀性本次研究利用 CDDP,BrdUrd, PI 偵測 S-180 cell DNA 特性, 發現可利用 S-180 cell DNA 含量和 normal lung cell DNA 含量不同,而可用 Flow cytometry 正確計數腫瘤轉移肺臟中 S-180 腫瘤 cell 的百分比。爲了解 PN-2 抗腫瘤活性, 將 PN-2 和 S-180 cell in vitro 培養, 發現無細胞毒性。進行 Ames test 知其無抗突變能力,也無基因毒性。測試 PN-2 對末稍血液 T 、T4、T8、B. Macrophage 比率影響, 知其可使 T, T4, Macrophage 顯著上升 ($P<0.01$) 爲了解 PN-2 是否可降低 S-180 cell 分裂速度和能力, 以 PI, BrdUrd 雙染色法用 Flow cytometry 作 cell cycle 的測量,計算 PN-2 對 S-180 cell kinetic parameter 的影響, 結果顯示 PN-2 對 BrdUrd Labeling Index, DNA synthetic time (Ts), G/S/G2M 百分比皆無影響。但利用 BrdUrd 標幟 S-180 細胞追蹤測試則在 S-180 腹水癌中以 PN-2,i.p.注射, 可使 S-180 cell 因 macrophage 活性增加而被吞噬掉, 因而使 S-180 cell 在腹水中存活時間降低 45%。其間並發現 PN-2 有強烈 mitogen 作用, 可使週邊血液 WBC 分裂能力增加, PN-2 組 6.7±0.6%, PBS 組 4.2±0.5%,($P<0.01$)。以 i.v.投與 PN-2 發現副作用極小, 對週邊血液 WBC, RBC, PLT, HGB %L. %M. RDW, MPV, PDW, LYM, GRAN, HCT, MCH, PCT 値皆無影響,

僅對 MCV, MCHC 值有微小增加作用。

英文摘要

Phytocordyceps ninchukispora was a new species of the new genus Phytocordyceps in the family of clavicipitaceae found in Taiwan in 1985. In the liquid culture of P.ninchukispora, a water soluble polysaccharide (PN-2) was isolated. This polysaccharide was used for chemical structure and antitumer studies.Based on the data obtained from chitinase assay and GC,GC/mass spectrome- try, PN-2 appeared to be a polymer of β-1-4 N-acetylglucosamine as the main chain with a molecular weigut approximately 1*10 · The side chains of galactose, mannose and threitol meioties were linked by β 1-4-linkage to the 6 position of glucosamine residues of the polymer. PN-2 reduced subcutaneous tumor size by 29% and prolonged the life span of mice implanted with S-180 cells by 21%. PN-2 also reduced the nodule number in munine lung by 88% as demonstrated by artificial metastasis test of the 14th day after S-180 cell implantation. A flow-cytometric mesaurement was established to distinguish normal lung cell and S-180 cell in the present study. PN-2 had no direct cytotoxic effect on tumor cells and was neutral in the Ames test. The antitumor activities were evidenced by increasing total number of T cells, T4 cells and macrophages as demonstrated by flow cytometric analysis. PN-2 dramatically reduced the viability of the tumor cell as indicated by BrdVrd and PI staining,but had no effect on labeling index of Brdulrd, DNA synthesis time (Ts) and G1/S/G2M ratio. I.p. administration of PN-2 strongly activated macrophages and a prominent mitogenic effect which resulted in increasing WBC division ability. I.V.administration of PN-2 in mice did not change the peripheral blood profiler inclluding the percentage of WBC.RBC. PLT.HGB%L,%M,RDV,PDW,LYM,GRAN,HCT, MCH and PCT;but slightly increased the values of MCV and MCHC.