(1) (1)嘗試以陽離子交換樹脂層析法純化豬精漿內已知的精子活動力抑制蛋 白 SMI 之研究;(2)維生素 K3 對人類子宮頸癌細胞之抗癌活性及作用機轉之 研究

(1) The Utilization of Cation Exchanger to Purify Sperm Motility Inhibitor (SMI) from Porcine Seminal Plasma;(2) The Anitumor Acitivy and Machanistic Study of Vitamin K3 against Human Cervical Carcino

中文摘要

（一）精漿 (sem inalplasm a) 中的物質主要由雄性副性腺的分泌物組成，如精囊 (sem inalvesicle)，前列腺 (prostate gland) 以及尿道球腺 (bulbourethalgland) 的分泌物等。SMI (sperm motility inhibitor) 乃由豬精漿中分離純化，含 91 個胺基酸，具有抑制公豬精子活動力及降低其頭帽反應頻率的蛋白質。今另嘗試以陽離子交換樹脂層析法純化之。利用陽離子交換層析柱 carboxym ethylcellulose chrom a-tography (CM52) 分離豬精漿液中蛋白質，收集 20 mM HEPES，pH 7.9，1 mM EDTA 的緩衝沖
洗液，再以陽離子交換層析柱 phosphocellulose chrom atography (P11) 吸附後，在含氯化鉀 0~0.1 M 之 20 mM HEPES，pH 7.9，1 mM EDTA 緩衝鹽
溶液中，可沖提出 SMI。純化的蛋白質經濃縮並去除鹽份後，即以 SDS-PAGE，HPLC 及質量質譜分析儀分析。以質量質譜分析儀分析此蛋白質質量爲 (10,067*1) dalton。證實豬精漿液在經過 2 次陽離子交換層析純化後，可獲得純化之 SMI蛋白質。吾更進一步根據其於體內分布，選用肺炎雙球菌測試對其生長能力的影響，結果顯示並無抑制作用。 （二）維生素 K3 (VK3，2-甲基-1,4- 奈昆) 是兩種天然維生素 K1 及 K2 的人工合成類似物。VK3 對不同嚙齒動物及人類腫瘤細胞具有廣泛之抑制生長作用。而且已知 VK3 是維生素 K 族中最強之抗癌藥。在美及臺已進行第一、二期之臨床試驗。以 sulforhodam ine B (SRB) 染色法得知 VK3 對人類子宮頸癌 SiHa 細
胞株的半致死劑量爲 37 微莫耳濃度。在作用機轉之研究中，發現 VK3 在 SiHa 細胞中可引起： (1) 細胞週期運轉延遲於 S 或 G2/M 期，(2) 改變具細胞週期特異性蛋白質之表現或磷酸化之程度，如 CDK1 激活갛，Cdc25 磷酸갛，Cyclins A 及 E。爲了闡明 VK3 直接標的物，以人工合成一段十一個胺基酸胜갺鏈（含遍存於蛋白質酪胺酸磷酸갛 (protein tyrosine

phosphatase）及雙重特異性蛋白質磷酸걍 (dualspecificity protein phosphatase）活化中心）與帶有放射性之VK3作用證明，VK3可和此鏈內之cysteine (Cys) 胺基酸結合，使酵素失去活性，但無法與由Cys突變爲serine (Ser) 的胜것鏈結合。VK3可能是目前臨床抗癌藥物中，第一個被發現爲蛋白質酪胺酸磷酸걍的抑制劑。上述結果顯示 VK3 可能因與Cdc25磷酸걍之Cys結合，使其活性散失進而使CDK1激活酵素處於高度磷酸化而失去活性，導致癌細胞無法進行細胞週期運轉而死亡。

英文摘要

(PART I): Seminal plasma has been shown to be composed of fluid from male accessory sex glands, such as seminal vesicle, prostate gland and bulbourethal gland etc. Sperm motility inhibitor (SMI) was previously purified from porcine seminal plasma as described and the utilization of cation exchanger to purify was also tried in this report. SMI was shown to contain 91 amino acids, and to decrease the sperm motility and its frequency of acrosomal reaction rate of spermatozoa. Carboxymethyl cellulose chromatography (CM52) was used to partial purify SMI protein from porcine seminal plasma. The fractions containing SMI protein were obtained in the flow through buffer (20 mM HEPES, pH 7.9 and 1mM EDTA; PC buffer) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and reversed-phase high performance liquid chromatography (RP-HPLC). After the SMI was checked and surely contained in the collected fractions, the phosphocellulose chromato-graphy was used for the further purification. And then the purified SMI protein was found in the gradient PC buffer containing 0 ~ 0.1 M KCl. The result was also checked by SDS-PAGE, RP-HPLC and mass spectrophotometer. The molecular weight of SMI determined by mass spectrophoto-meter is (10,067*1) dalton, which is the same as previously reported by others.SMI was identical to b-microseminoprotein and was also found in the mucosa of respiratory and digestive tract. The Streptococcus pneumonia was chosen to clarify the role of SMI (2 and 20 mg) in the mucous secretory tissue. No inhibitory effect on the growth of Streptococcus pneumonia was found after 24 h incubation in a humidified 5 % CO2 incubator. (PART II): Vitamin K3 (VK3, 2-methyl-1,4-naphthoquinone) is the synthetic

derivative of two naturally occuring vitamin K1 and K2. It has been shown that VK3 exhibits a broad spectrum of antitumor activity in rodent and human tumor cells. Among these three VK congeners, VK3 exerts the most toxic effect toward cancer cells. A phase I/II clinical trials of VK3 has been performed in both U.S. and Taiwan. The IC50 of VK3 against human cervical carcinoma SiHa cells is 37 mM as determined by sulforhodamine B (SRB) colorimetric assay. In the mechanistic study of VK3*s action on SiHa cells, we found that VK3 induces: (1) the cell cycle arrest or delayed at the S or G2/M phase, (2) the alterations of the expression or phosphorylation status of cell cycle specific proteins, such as CDK1, Cdc25 phosphatase, Cyclins A and E. For the elucidation of the direct target of VK3*s action on cells, the synthesized undecapeptide, containing the conserved cysteine (Cys) active domain of protein tyrosine phosphatases family and dual specificity protein phosphatases family, were incubated with 3H-VK3 in vitro. VK3 was found to bind directly to the peptide containing Cys residue but not to the mutant couenterpartner containing Cys mutated to serine (Ser). Among the clinically used anticancer drugs, VK3 might be the first drug that acts as an inhibitor of protein tyrosine phosphatases. Our results suggest that binding of VK3 to the Cys residue at the active site of Cdc25 phosphatase generates the hyperphosphorylated inactive form of CDK1 which in turn induces cell cycle arrest, leading to the cell death.