行政院國家科學委員會專題研究計畫成果報告

##

公豬精漿中精子活動力抑制蛋白的出現與分佈之探討

The emergence and distribution of the sperm motility inhibitor (SMI) in boar

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題目是 The secretory origin and temporal appearance of the porcine β-microseminoprotein (sperm motility inhibitor) in the boar reproductive system.)

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摘 要

關鍵詞：豬精漿, 豬精子活動力抑制蛋白, 西方墨點法, 組織化學免疫反應

使用高效能液相電泳儀(HPLC), 從豬精漿中純化豬精子活動力抑制蛋白(porcine sperm motility inhibitor, SMI) ,再利用此蛋白質製造出 polyclonal antibody。

取不同年齡 (初步取用初生、一個月、二個月、三個月、四個月等)公豬的生殖器官、消化器官、呼吸器官等,製成組織切片,或研磨後,以 SDS-PAGE 分離,再以 Western blotting 檢視其免疫反應, 並使用接上 FITC 的二級抗體 (FITC-conjugated secondary antibody), 使切片呈現螢光, 再以影像分析軟體統計含有 SMI 的細胞數目與其分佈, 並且得知有否因年齡不同而有不同, 由此檢視並推測其可能的生理功能。

ABSTRACT

Keywords : Porcine seminal plasma, porcine sperm motility inhibitor, Western blotting, histochemical immunity

Porcine sperm motility inhibitor is extracted and purified through HPLC (high performance liquid chromatography). After this purification, the protein (SMI) is used to induce polyclonal antibody.

Boars of different age are used (neotal, one month, two months, three months, four months, etc.). The organs of reproductive, digestive, respiratory system are dissected. Paraffin sections and tissue homogenates for Western blotting are prepared to reveal the immunohistochemical reaction. FITC-conjugated secondary antibody is also used for image process to count the cells which contain SMI.

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簡介背景

以往對於精漿 (精液中非細胞的部分)的研究較少，但目前逐漸有不少針對精漿內物資,特別是蛋白質方面 (Rufo *et al.*, 1982; Kemme *et al.*, 1987; Kwok *et al.*, 1993; Jeng, *et al.*, 1993; Iwamoto *et al.*, 1992, 1993, 1995; etc.)，開始有不少報導，精漿與生殖生理的關係遂日益被重視。

其中的豬精子活動力抑制蛋白(porcine sperm motility inhibitor, SMI)是本人從豬精漿中，以高效能液相電泳(HPLC) 純化得到的蛋白質，此精子活動力抑制蛋白對於豬精子的活動力，具有依劑量增加而增加之抑制作用，且在遇到豬卵巢濾泡液後可恢復精子的活動力(Jeng, *et al.*, 1993)。Iwamoto 等人從豬精漿分出的SPMI (sperm motility inhibitor) 是一個dynein ATPase inhibitor (Iwamoto et al., 1992, 1993, 1995), 有137 amino acid residues, 乃由精囊分泌, 與SMI, 雖然二者均可抑制精子的活動力，卻不是同一個蛋白質。

對於 SMI 之研究方面，由已知的 Amino acid sequence 得知，此蛋白質與 β- microsemino-protein 極相似 (Chao, *et al.*, 1996), 但 β-microseminoprotein 的功能目前亦仍未明，僅猜測與 mucus 之分泌有關。另外 SMI 可降低豬精子自發性的頭帽反應 (spontaneous acrosome reaction) (Jeng and Chang, 1997), SMI 可進入豬精子內部 (Jeng *et al.*, 1998, 已投稿未發表資料), 使之對 SMI 的生殖生理功能愈發有研究意義。

有關 SMI 的分泌來源，分佈與有否因年齡不同而有不同分泌量之確定，因爲與其生理功能大有關聯，故需要一全面性之研究。

Materials and Methods

研究進行步驟及方法

1. 豬精子活動力抑制蛋白 (porcine sperm motility inhibitor, SMI) 之純化
   本計畫將取用豬精漿, 以高效能液相電泳(HPLC) 純化其中的 SMI
2. 抗體的備製, 使用紐西蘭大白兔製造出 polyclonal antibody。
3. 豬器官的取得
4. 不同年齡豬器官的組織切片
5. Western blotting 免疫反應的檢視
6. 螢光影像的處理與分析

Results and Discussions

Immunohistochemical studies and western blotting indicated that porcine β-microseminoprotein did not appear in the prostate tissue before day 30. At day 60 and older, it appeared only in the prostate gland.

The western blotting analysis of tissue extracts showed that the prostate gland is the only origin for the synthesis of porcine β-microseminoprotein. All the other tissues such as seminal vesicle, bulbourethral glands, prostate, epididymis, testis, stomach, duodenum, jejunum, ileum, trachea, spleen and liver did not show any immunoreaction with the SMI antiserum.

The western blotting analysis of prostatic extracts showed that the prostate glands older than 60 days have immunoreaction with the SMI antiserum. The upper band is very likely the SMI dimer based on its immunoreaction and the molecular weight.

The secretory origin of porcine β-microseminoprotein is also confirmed by light microscopy using the immunohistochemical localization method. The staining was located in the cytoplasm of the epithelial cell and within the secretory duct of prostate gland as brown pigments. The control sections incubated with normal rabbit serum show no staining except some light background in the epithelium and the seromucous gland in the submucosa of the trachea.

The HPLC profile for the purification of porcine β-microseminoprotein. It reveals that porcine β-microseminoprotein is the major protein component of prostate gland. The molecular weight was found to be 10,066, as determined by mass spectrometry.

DISCUSSION

In our previous study (Chao et al., 1996) we found that porcine SMI is identical to β-microseminoprotein. Its human analogue has been characterized in man and the ape and is one of the predominant proteins in the secretion of the human prostate gland (Lilja and Abrahamsson, 1988). The human β-microseminoprotein has been found to be present in several non-prostatic tissues by immunohistochemical staining (Weiber et al., 1990) and northern blot analysis (Ulvsbäck et al., 1989). Its distribution profile seems to suggest some association with mucus secretion (Fernlund et al., 1996).

In the present study we found that porcine SMI was clearly detected by immunohistochemical staining of the prostate gland. Other non-prostatic tissues including the seminal vesicle, bulbourethral gland, testis and epididymis of the reproductive system; the stomach, duodenum, jejunum, and ileum of the digestive system and the trachea of the respiratory system showed very little immunoreaction. The western blotting analysis revealed an immunoreactive band of about 52 kDa in all tissues examined which obviously is not related to SMI. This immunoreactive band was also observed in the control group stained with normal serum. It is likely that some unknown proteins that could recognize the serum components (probably the immunoglobulins) were responsible for this nonspecific immunobinding. This positive reaction is in agreement with a very weak staining in the control group treated with normal rabbit serum. The presence of β-microseminoprotein in the trachea of the pig as reported by Fernlund et al. (1994) could be due to this nonspecific immunoreaction.

The present report reveals that the human and porcine β-microseminoproteins, although homologous in sequence, are so different in its tissue distribution profile. There is no doubt that β-microseminoprotein is a prostatic protein and must have some functions in the reproductive system. Our previous report suggested one possible function of β-microseminoprotein; that is, to reversibly and mildly reduce the sperm motility which can be restored by the female follicular fluid (Jeng et al., 1993). It makes sense that the sperm motility is temporarily inhibited before entering the female reproductive tract. The fact that the mature spermatids appear in the boar of MeiShan strain at 60 days of age (Cheng, 1983) is in agreement with our present finding that SMI is not synthesized until this age. These all suggest that the expression of SMI may be coordinated to the course of sexual maturation. Of course, further investigations are needed to clarify this point.

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