

行政院國家科學委員會專題研究計畫成果報告

中文計畫名稱: 胎盤粹取液對小腸機能之影響

英文計畫名稱: Influence of Placenta Extract on Intestinal Function

計畫編號: NSC 88-2314-B-002-282

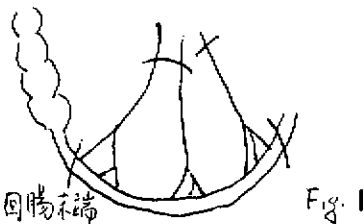
執行期限: 87年8月1日至88年7月31日

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一、中文摘要

Bovine 之 Placenta 抽出液, FBS(fetal bovine serum), 因有成熟牛血清沒有的各種 growth factor, 是做細胞培養時不可欠缺的 medium. 本計劃之主要目的是研究 Bovine 之 placenta 抽出液和 FBS 對 Lewis rat 做一段小腸虛血後小腸再生之影響。

小腸虛血之 experimental model 是如下



在回腸末端, 約 15cm 長度之回腸, 按圖一, 所有之血管用三號線結紮遮斷一切動靜脈血流, 虛血時間為 180 分

- (1) Placenta 溶液做 test solution, 讓 Lewis Rat 喝三天 test solution, 三天後在 Lewis Rat 之回腸做約 15cm 三小時的小腸虛血實驗, 實驗直後, 實驗後第一, 三, 五, 六天 kill rat, 取得小腸組織。
- (2) 裝有 test solution (FBS) 的 Alzet osmotic minipump, 裝入 rat 之腹壁皮下內, 用 polyethylene tubing 連結胃慶, 使 test solution (FBS) 能通過胃慶送到胃內做 luminal perfusion. 三天後在 Lewis male rat 之回腸做約 15cm 三小時的小腸虛血實驗, 1天, 2天, 3天, 5天, 6天後 kill rat, 取得小腸組織。

實驗得到的小腸組織, 用 formalin 固定後用 hematoxylin and eosin stain, 用 Montreal University 之 grading system 觀察小腸黏膜之 morphology 之變化, 並利用

Laser Scan Microscope 測定 villus height, width, density of villus, surface area per villus, 可以了解 mucosa surface area 之變化。mucosa surface area 之大小和小腸之 absorption capacity 有密切的觀係, 測量 crypt depth 能了解 crypt cell productin 之狀況, crypt cell 之 production 和小腸之 viability 有密切之觀係。更要測量小腸之 protein 來了解 mucosal mass 之行消長。

得到以下之結果 (1) placenta solution 不但沒有效果, 有反效果 (2) FBS 能保護小腸 Ischaemic damage, Reperfusion Injury.

關鍵詞: fetal bovine serum, 小腸虛血

二、英文摘要

Abstract

Bovine placenta extract and Fetal bovine serum (FBS) are known to have several growth factors and have been used as an important medium for cell culture. The objective of this study is to evaluate the effect of Bovine placenta extract and FBS for protect the rat small intestine from the ischaemic and reperfusion injury.

Young adult Lewis rats were used for the study. The experimental model of induction of intestinal ischaemia was as follows (Fig 1). The mesenteric arteries and veins that perfused 15cm long segment of ileum and both ends of ileal segment with the maginal vessels were ligated with NO.3 silk. The duration of ischaemia was 180 min.

The experimental methods were divided into two groups.

Group 1, Using placenta extract solution as the test solution. Experimental animals were given placenta extract solution by mouth for three days, then about 15 cm of terminal ileum was ligated for 3 hours. Immediately after 3 hours of total interruption of blood supply, and at days 1,3, and 6, rats were sacrificed and biopsy specimens of small bowel mucosa was obtained.

Group 2, Using FBS as the test solution. FBS was given by a subcutaneously implanted Alzet osmotic minipump, which was connected to the gastrostomy for luminal perfusion. Immediately after 3 hours of total interruption of blood supply, and at days 1,3, and 6, rats were sacrificed and biopsy specimens of small bowel mucosa was obtained.

The bowel morphology will be assessed using the grading system of Montreal University. Villus height, villus width, and crypt depth will be measured using Laser Scan Microscope, three or two dimensional measurement.

Keywords:

Fetal Bovine Serum, Ischaemic and Reperfusion Injury of Small Intestine

三、計畫緣由與目的

從過去在國外所發表的論文及本人在以前所發表的論文得知,用 Newborn 或 Fetal Lewis rat 小腸種在同種成鼠的腹壁上, intestinal graft 會存活且成長。從1994年7月本人等研究其機轉,對其沒有血管吻合,使植入腹壁上的腸片會存活的原因,做了一個假設,其不是植入的腸管片能很快的從宿主得到血管新生,而可能是母鼠生產前後的血液具有一些保護因子或成長因素,藉此保護腸管不致於壞死。我們一直探討這個機轉,結果得到以下的假設:

在母鼠懷孕之後,母鼠的生理機能會有變化而產生某種物質,這種物質是存在母鼠的血清內、Newborn 或 Fetal rat 的血清內、或胎盤裏面,使得Newborn 或 Fetal rat's intestine 種在同種成鼠的腹壁上時,雖然沒有血液的供給,也能存活3~4日,當新生血管產生後,便能很快的恢復其機能。

我們做的另外一個實驗是:經過aseptic technique 取下空腸的一小段,剝離腸間膜,用冷食鹽水 (4°C) 洗後,放在各種 solution 中,經過24小時、48小時、72小時、96小時取出小腸小段,用 hematoxylin and eosin stain 後,觀察其 morphology 之變化。結果知道 TAITA NO.4 溶液+ Bovine Placenta 溶液和 Fetal Bovine Serum 是對保持小腸黏膜機能最適當且最好的。這個研究計劃是要了解這種含 Bovine Placenta 或 Fetal bovine serum 之溶液,用continuous luminal perfusion對遮斷血流的小腸黏膜之 gross morphology,microscopic morphology 之影響。

四、結果與討論

(1) Test solution 使用 placenta 溶液之結果不能得到預期之結果,回腸一小段做 180 分之虛血並 reperfusion 後使用 placenta solution 沒有改善小腸黏膜之形態的變化,所以不進一步利用 Laser Scan Microscope 測定 villus 之變化,測量小腸 protein 。

(2) Test solution 使用 FBS 之結果如下:

(a)Morphology 之變化,用 Montreal University 之 grading system 觀察 Tabel 1, Fig 2 標示 Test solution 用 FBS 經過種在皮下的 Alzet osmotic pump, 以及胃慶做 luminal perfusion 的 experimental group 很明顯的比沒有使用 FBS 做 luminal perfusion,3 小時之回腸虛血之 control group, 對 reperfusion 後小腸黏膜 damage 之保護作用來得有效。

Table 1: 小腸形態之變化

Experimental Group			Control Group	
	各 n=6	平均	各 n=6	平均
Day 0	4,4,4,4,5,5	26/6=4.3	5,6,7,6,6,6	36/6=6.0
Day 1	4,5,4,5,5,5	28/6=4.7	4,5,4,2,7,5	27/6=4.5
Day 2	1,3,1,5,5,3	18/6=3.0	5,4,3,4,5,4	25/6=4.2
Day 3	0,0,0,3,3,1	7/6=1.1	4,1,1,4,2,3	15/6=2.5
Day 6	0,0,3,1,1,1	6/6=1.0	1,1,4,2,2,2	12/6=2.0

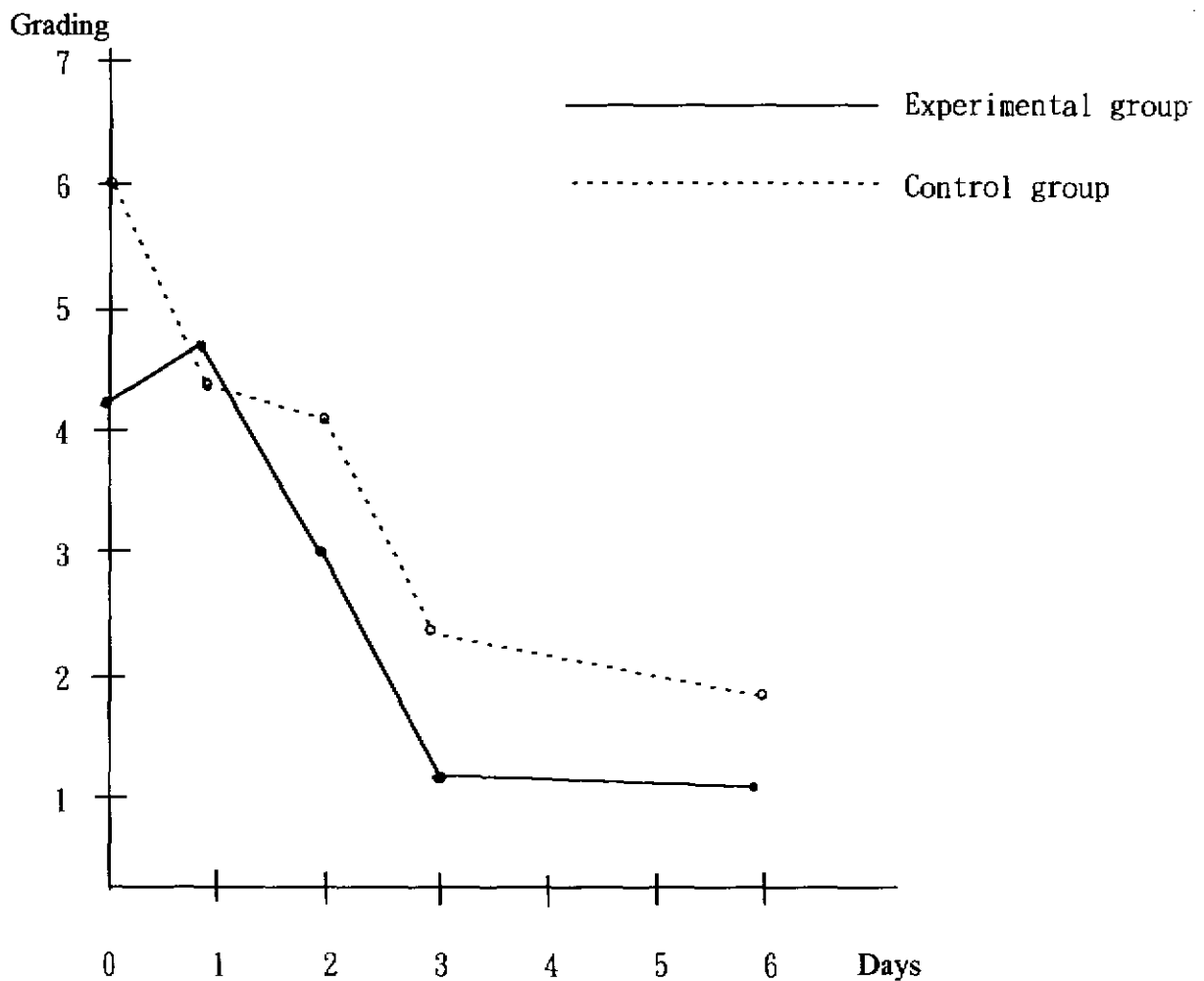


Fig. 2 Morphologic change (Montreal University, grading system)

Table 2: 使用 Laser Scan Microscope 測定纖毛之結果

Control group

	纖毛 height (μm)	纖毛 width (μm)	crypt depth (μm)	壁厚 (μm)	AREA (mm^2)
第三天	267.46	85.24	282.72	218.2	0.02080
	269.80	89.72	231.86	132.28	0.01772
	303.72	92.76	286.42	217.46	0.02392
平均	280.32	89.24	267.00	189.31	0.02081
第六天	295.20	87.38	286.90	206.84	0.02220
	278.94	86.62	253.86	145.06	0.02041
	287.07	87.00	270.35	175.99	0.02131

Experimental group

	纖毛 height (μm)	纖毛 width (μm)	crypt depth (μm)	壁厚 (μm)	AREA (mm^2)
第三天	335.14	85.50	252.40	220.62	0.02359
	309.34	121.70	269.88	176.54	0.03063
	260.76	94.00	236.16	165.38	0.02078
	303.12	105.86	223.5	217.72	0.02898
平均	302.09	101.75	245.45	195.07	0.02600
第六天	276.80	72.5	227.52	173.32	0.01839
	311.34	92.7	205.12	199.40	0.02385
	294.07	82.6	216.32	186.36	0.02112

由 Table 1 可知,

(1) 於第三天時 control group 仍有 1/3 在 grading system 4 以上,而 experimental group 只有 1/3 個 grade3, experimental group 形態較好

(2) 於第六天時 control group 則有 1/6 在 grading system 4 以上,而 experimental group 只有 1/6 個 grade3, experimental group 形態較好

但若比較 LSM 測定纖毛的結果 (Table 2), 則發現並沒有很大的差異。

五、計畫成果自評

本計畫在進行之際，歷經助理的轉換，其中浪費了不少的時間，也因為助理本身的背景不同，而使得實驗中需要高技術的部份亦有差異，如此一來便影響到了結果的判斷，就助理的部份而言，不安定的工作條件，最後影響到計畫的成果，是急需在制度上改善的。

在計畫部份，原本預定要做的 DNA 量測部份也因機器的老舊卻無經費更替而暫時擱置。

即便如此，我們依然照著計畫目標進行，由於這標題相關的研究在國際上尚無發現有研究者在進行，而本實驗結果也觀察到 FBS 對小腸黏模 **damage** 的保護確實有其效用在。我們將在繼續確定實驗相關細節後，提出論文發表於國際學術期刊上。

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