行政國家科學委員會專題研究計畫成果報告

上皮生長因子對十二指腸潰瘍修復之調節

The regulation of epidermal growth factor on the healing of duodenal ulcer

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一、中文摘要

本研究主要是測定內生性 EGF（epidermal growth factor；EGF）與口服外生性 EGF，對醋酸誘發十二指腸潰瘍老鼠之腸道黏膜修復所扮演的角色。本實驗以雄性 Sprague-Dawley 老鼠(約 200-250 克重)，老鼠隨機分成四組（每組十隻，包括病理切片與拍照各一隻）：控制組（無潰瘍、無口服 EGF），無潰瘍口服 EGF 組，潰瘍無口服 EGF 組，及潰瘍口服 EGF 組，另外有十隻不接受任何手術之正常老鼠，作為 negative control。所有無潰瘍老鼠皆接受假手術（sham operation），潰瘍老鼠則以醋酸誘發。醋酸誘發潰瘍手術的進行：以巴比妥酸鹽麻醉下打開腹腔，並以一塑膠管（內徑 4.5 mm）裝盛醋酸 30%、70 μL，沾附十二指腸外層十秒（由 pilot study 決定其醋酸的使用劑量與時間），如此會造成醋酸接觸範圍的 mucosa 與 submucosa 層細胞壞死，但不會傷及肌肉層，或使周圍器官穿孔或壞死。在移開醋酸接觸後，縫合腹腔，並使老鼠從麻醉中恢復，手術當天（day 0）只給水，隔天（day 1）再給予正常食物。平日記錄老鼠體重、攝食量與飲水量。手術隔天後，每天於下午三點左右將 recombinant human EGF，劑量為 60 μg/kg，溶在無菌的去離子水 35 mL，置於潰瘍老鼠的飲水中餵食，並記錄精確的口服劑量。無口服 EGF 的老鼠則口服相同量的無菌去離子水。為測定潰瘍修復速率、EGF 分泌、與胃中酸鹼質的改變，在第 1、5、10、15 天時犧牲（約下午三點左右）。當天老鼠以巴比妥酸鹽麻醉，收集血液，測量胃壁酸鹼值，以刀片刮下胃及十二指腸黏膜，稱其濕重。以測定十二指腸黏膜中 DNA、RNA 及 protein 的含量，作為評估黏膜生長的指標。結果顯示，不論有無潰瘍、投予 EGF，對老鼠體重、胃中酸鹼值、攝食量均無影響，僅有在手術實施後兩天內，潰瘍組老鼠攝食量明顯低於無潰瘍組。在手術實施後第一天，潰瘍組老鼠黏膜的 DNA 含量明顯低於無潰瘍組；第五天時，各組之 DNA 含量並無差異、RNA 含量以添加 EGF 之潰瘍組最低、蛋白質含量以無添加 EGF 無潰瘍組最高；第十天時，無潰瘍有 EGF 組之 RNA 含量顯著高於無潰瘍無 EGF 組、而有 EGF 有潰瘍組的蛋白質含量高於無潰瘍有 EGF 組。第十五天時，各組之 RNA 含量並無差異、潰瘍並投予 EGF 組之

DNA 含量顯著高於其他各組、且其蛋白質含量高於無潰瘍投予 EGF 組。在各自組別的時間恢復效應中，有潰瘍有 EGF15 天組的 DNA 與 RNA 顯著高於同組的 5 天、10 天組，而第 10 天的蛋白質顯著高於同組的 5 天、15 天組。

關鍵詞：上皮生長因子、十二指腸潰瘍修復、黏膜生長、老鼠

Abstract

This study was to investigate the mucosal healing effects of endogenous and exogenous epidermal growth factor (EGF) on acetic acid-induced duodenal ulcer in rats. Sprague-Dawley rats (200-250 g) were randomly divided into four groups: control (no ulcer without oral EGF), oral no ulcer with oral EGF, ulcer without oral EGF, and ulcer with oral EGF groups (n=10, including one rat for pathological examination and photograph, respectively). In addition, 10 normal rats did not be operated as the negative control. The rats without ulcer were done by sham operation, and the rats with ulcer were induced by acetic acid. The rat was anesthetized by thiopentone sodium and the abdomen was opened. A plastic tube (4.5 mm inner diameter) with 70 μL of 30% acetic acid was applied to the surface of the duodenum for 10 sec (the dosage of acetic acid and the applied duration were determined by the pilot study), which causes immediate necrosis of the entire thickness of the mucosa and submucosa exactly within the area of acetic acid application without penetration or perforation to the muscular layer and the surrounding organs. After removal of acetic acid, the abdomen was closed, and the rat was allowed to recover from the anesthesia. The rat with ulcer received only water on the day of operation (day 0), and was fed a normal diet ad libitum next day (day 1). The weight, food intake, and water intake of the rats were routinely recorded. The next day after operation at approximately 3:00 PM, the rats were orally administration of recombinant human EGF (60 μg/kg) in 35 mL sterile deionized drinking water, and oral EGF intake was exactly recorded. To determine the healing rate, EGF secretion, and the changes of pH values in the stomach, the rats were sacrificed on day 1, 5, 10, and 15 at around 3:00 PM. After the rats were anesthetized by thiopentone sodium, blood was collected, pH values of the gastric wall were determined, and the wet weight of the mucosa was measured. The amounts of DNA, RNA, and protein in the mucosa were determined as the indicator of mucosal growth. The results showed that oral EGF had no effects on body weight, pH values of the stomach, and food intake in the rats with or without ulcer. Food intake decreased in the rats with ulcer compared to those without ulcer after 2 days of operation. The amount of mucoal DNA significantly decreased in the rats with ulcer compared with those without ulcer

on day 1. No significant differences were observed in DNA content on day 5. The ulcerous rats with oral EGF had the lowest RNA content, and the sham-operated rats without oral EGF had the highest protein content on day 5. The sham-operated rats with oral EGF had significantly higher RNA content than those without oral EGF, and the ulcerous rats with oral EGF had higher protein content than the sham-operated rats with oral EGF on day 10. On day 15, RNA content was not different, the ulcerous rats with oral EGF had significantly higher DNA than others, and higher protein content than the sham-operated rats with oral EGF. In the time-course study, the ulcerous rats with oral EGF had significantly higher DNA and RNA content on day 15 compared with those on day 5 and 10, and higher protein content on day 10 compared with those on day 5 and 15.

Key words: epidermal growth factor, healing of duodenal ulcer, mucosal growth, rats

二、緣由與目的

上皮生長因子是由53個胺基酸所組成的多　　鏈，分子量約6000 Da。EGF 可在哺乳動物的各種體液與組織中發現，控制上皮與間質細胞的增生與分化(1)。此外，EGF 與 EGF 家族相關之　　對維持與修復腸道黏膜扮演相當重要之角色(2)。EGF 可由十二指腸布隆納氏腺（Brunner's gland）(3)、小腸巴內特氏細胞（Paneth's cells）、唾液腺、胃黏膜頸細胞（mucous neck cells），和潰瘍相關細胞（ulcer associated cell lineage，UACL）（最近被證實為分布於受傷部位的一種腺體結構），連續分泌到腸腔中(4)，其在胃液與十二指腸中的濃度各別是0.42-2.15 和 10.8 ng/mL(5、6)。有報告指出，EGF 可促進老鼠胃黏膜之 DNA 合成與含量(7、8)。有證據顯示，醋酸誘發胃炎的老鼠中，其潰瘍周圍的 EGF receptor 和製造 EGF 的細胞會增加(9)。而人類位在潰瘍黏膜與相鄰潰瘍處的細胞，其 EGF 的分泌亦會增加(4)。這些都證明，EGF 在潰瘍修復上，扮演一個很重要的角色。目前對於十二指腸的內生性 EGF 分泌，並無完整的“ 時間效應研究數據“（time course study data）；對口服（外生性）上皮生長因子與十二指腸修復作用，亦仍有爭議。選擇口服 EGF 的理由，是因為此在臨床上是一個較容易、較可實行給予病患的方式。因此，我們設計三向因子變異數分析（有無潰瘍×有無給予 EGF×不同時間點），分別探討十二指腸潰瘍本身及口服 EGF 對由醋酸誘發的十二指腸潰瘍的影響。

本研究主要目的為：

1）測定內生性胃腸道 EGF 含量是否會因醋酸誘發的十二指腸潰瘍而改變。

2）探討口服 EGF 是否影響醋酸誘發的十二指腸潰瘍修復作用，包括黏膜 EGF 的含量、黏膜的生長、及胃酸鹼值的改變。

三、結果與討論

本實驗的結果如下：

1）DNA、RNA、Protein 含量：

a) 手術後第一天：潰瘍組老鼠黏膜的 DNA 含量明顯低於無潰瘍組，證實醋酸對腸黏膜細胞的確造成傷害。

b) 手術後第五天：各組之 DNA 含量並無差異、RNA 含量以添加 EGF 之潰瘍組最低、蛋白質含量以無添加 EGF 無潰瘍組最高，在此時間點中，EGF 對潰瘍修復並無明顯的影響。

c) 手術後第十五天：各組之 RNA 含量並無差異、潰瘍並投予 EGF 組之 DNA 含量顯著高於其他各組、且其蛋白質含量高於無潰瘍投予 EGF 組。在此階段中，EGF 對於潰瘍細胞有顯著的修復情形，對於無潰瘍細胞也有促進其生長的現象。

d) 時間恢復效應：有潰瘍有 EGF15 天組的 DNA，RNA 顯著高於同組的 5 天、10 天組。

2）攝食量、體重、胃部酸鹼值：整體而言，不論有無潰瘍、投予 EGF，對老鼠體重、胃中酸鹼值、攝食量均無影響，僅有在手術實施後兩天內，潰瘍組老鼠攝食量明顯低於無潰瘍組。因此，在本實驗中，EGF 並無法藉由調節胃酸來改善潰瘍的情形。

先前的研究顯示投與 EGF 可調節老鼠(10-14)與人類(15)潰瘍之修復。Olsen 等人亦提出報告：老鼠實驗中，分別由醋酸或 cysteamine-HCl 誘發的慢性胃潰瘍(11)和十二指腸潰瘍(12)，經添加 EGF 30 μg/kg/day 於飲水中，分別持續 25 和 50 天，皆可促進其修復，而修復效果與 cimetidine（一種 H_2 receptor 拮抗劑）相類似。在本實驗中，添加 EGF 60 μg/kg/day 於飲水中，與無潰瘍組比較，添加 EGF 在第 5 天時會降低潰瘍細胞的蛋白質含量，在第 10 天時會降低潰瘍細胞的 DNA 含量，在第 15 天時會增加潰瘍細胞的 DNA 含量。

四、計畫成果自評

本計畫原本需測量內生性十二指腸黏膜的 EGF 分泌量。因黏膜樣本已做 DNA、RNA 與蛋白質的分析，所剩下的黏膜量有限，目前正在克服測量 EGF 的問題，故並無顯示於本結果中。除此遺憾之外，本研究針對口服上皮生長因子對十二指腸潰瘍黏膜的修復，已做一不同時間點的詳盡探討，但對內生性十二指腸黏膜的 EGF 分泌量的影響，則需進一步研究。

五、參考文獻

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