桂枝茯苓丸對於子宮內膜異位症的細胞自噬之影響
The Effect of GFW on Autophagy in Endometriosis

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ABSTRACT

Endometriosis is defined as the growth of endometrial glands and stromal cells in a heterotopic location under the cyclic influence of ovarian hormones. Endometriosis is a common gynecological problem manifested primarily by chronic pelvic pain and infertility. It affects 7 - 10% of reproductive age women, 60% of women with pelvic pain, and up to 50% of women with infertility. An increasing body of evidence demonstrates the immunological etiology of endometriosis. Current treatments for endometriosis include surgery and meditation, however, the recurrence rate of both treatments remain high. Thus, an alternative strategy is required in improving the efficacy of the treatment of endometriosis. Autophagy is a highly regulated process in eukaryotic cells to disassemble and recycle unnecessary or dysfunctional organelles. Reduced autophagy is observed in the eutopic endometrium and ectopic endometriotic foci. Autophagy contributes to the pathogenesis of endometriosis by promoting the hyperplasia of endometriotic tissues, restricting apoptosis, and inducing abnormal immune response. In traditional Chinese medicine, endometriosis is manifested by stagnation of vital energy (qi) and blood stasis. Guizhi Fuling Wan (GFW) was first described in Chinese canonical medicine to treat stagnation of qi and blood stasis. Previous studies have shown that GFW can effectively inhibit the development of endometriosis. Therefore, the specific aims will test the hypothesis that GFW can induce autophagy during the development of endometriosis by examining the effects of GFW on autophagy during the development of endometriosis.

BACKGROUND

研究動機:

正常的子宮內膜僅出現在子宮腔內，如果子宮內膜出現在其他部位，就稱為子宮內膜異位症。這些異位的子宮內膜會受到雌激素調控(1)，會隨月經週期出血，造成各種臨床症狀。子宮內膜異位症會有相當廣泛的後遺症，像是續發性經痛、性交疼痛、不孕及腸胃道和尿道症狀等 (1)。子宮內膜異位症的成因眾說紛紜，其中「經血逆流」是被大家廣為接受的一個理論。經血如果沒有正常排出體外，而是經由輸卵管逆流到骨盆腔裡。大多數逆流的經血可以被身體自行吸收，但是有部分經血無法被吸收，最後附著在卵巢、輸卵管、或是骨盆腔表面，就會造成子宮內膜異位症。其他推測的致病機轉還有子宮內膜組織由血液淋巴系統傳送、自體免疫缺損。

細胞自噬是一種非細胞凋亡的計畫性細胞死亡(2)，生物個體能透過細胞自噬，在惡劣環境及營養不足的情況存活，細胞自噬亦可幫助移除堆疊錯誤的蛋白和清除受損之胞器，例如核糖體、過氧化體和粒線體等重要胞器；細胞自噬可透過抗原表現和細胞衰老，避免個體基因缺陷及細胞壞死等情況的發生(3)。細胞自噬的途徑可分為四個步驟，分別為啟動（Initiation）、擴張（Expansion）、關閉 （ Closure)、融合（Fusion）。在正常情況下，細胞自噬會發生在月經週期的腺性上皮組織及間質細胞上(4)。異位的子宮內膜及異位子宮內膜灶處的細胞自噬會減少，透過促進子宮內膜細胞及間質細胞的增生、限制細胞凋亡、誘導異常的免疫系統來促進子宮內膜異位症的發生(4-6)。

目前的治療方法大致上分為手術治療及藥物治療，手術治療有腹腔鏡手術及骨盆腔手術兩種。藥物治療在子宮內膜異位症治療佔重要角色，使用賀爾蒙製劑來達到抑制月經，進而使病灶萎縮不再生長，常用藥物有 GnRH analogues、danazol、dimetriose、progesterone、oral contraceptive pills、leuprin 與 gestrinone 等。但因為以上療法的復發率都很高，所以有找其他療法的需求。桂枝茯苓丸（GFW）已經被用來治療發炎一千多年了，它是由桂枝、茯苓、牡丹皮、桃仁、芍藥等組成(1)桂枝茯苓丸可以有效降低monocyte chemoattractant protein (MCP)-1跟cell adhesion molecules (ICAM)-1的表現，同時提高$CD4^+$跟自然殺手細胞的程度(1)，因此可以抑制子宮內膜的增生及入侵。

研究目的:

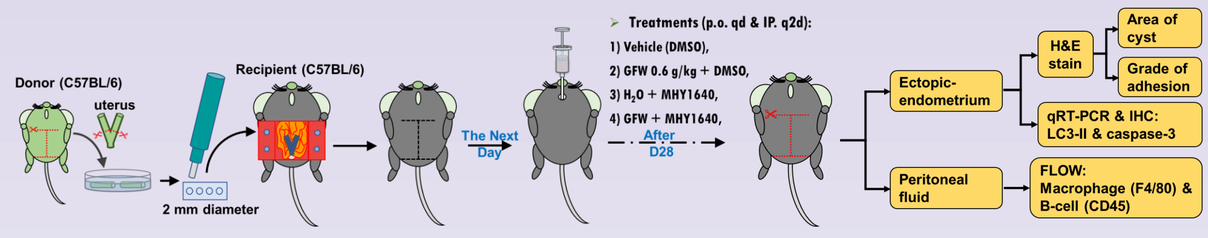
透過細胞自噬訊息途徑中的指標蛋白 LC3II，了解 GFW 在治療子宮內膜異位症中的促進細胞自噬的角色。小鼠若處理 mTOR 抑制劑（MHY1485）其子宮內膜異位症會更嚴重。而餵食桂枝茯苓丸的小鼠組別之子宮內膜異位症會得到改善，但若再處理 MHY1485 藥物所造成的效果則會和 GFW 抵消。 暗示細胞自噬在 GFW 抑制異位子宮內膜生成過程中扮演重要角色。

研究假說:

桂知茯苓丸促進細胞自噬的功效，對於抑制異位子宮內膜形成扮演著重要的角色。

METHODS

Experimental Flow Chart



Statistical Analysis

The area of endometriotic cyst and the quantification of immunohistochemistry were measured using NIH Image-J v1.53 DIA software. The statistical significance of results with equal variance was then examined by Student's t-test. $P < 0.05$ was considered statistically significant. All statistical differences were analyzed using SigmaPlot software 11.0 (Systat Software, San Jose, CA, USA).

RESULTS

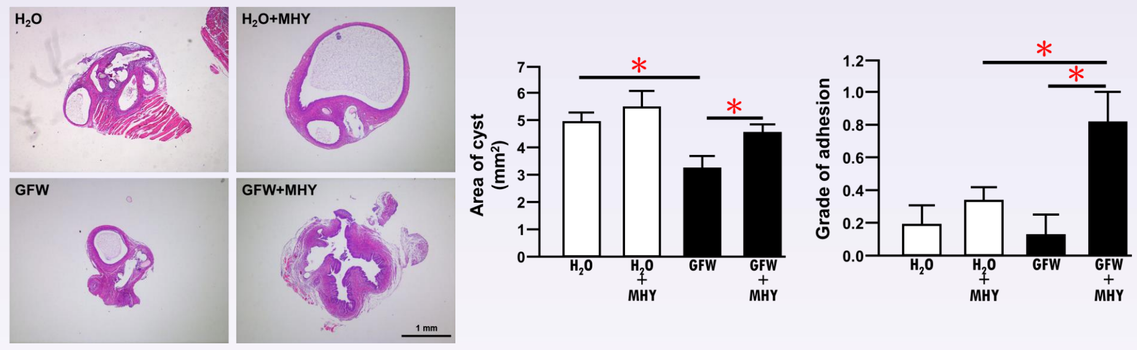


Figure 1. 處理細胞自噬抑制劑後，皆會增加異位的子宮內膜面積與和腹膜沾黏的程度。

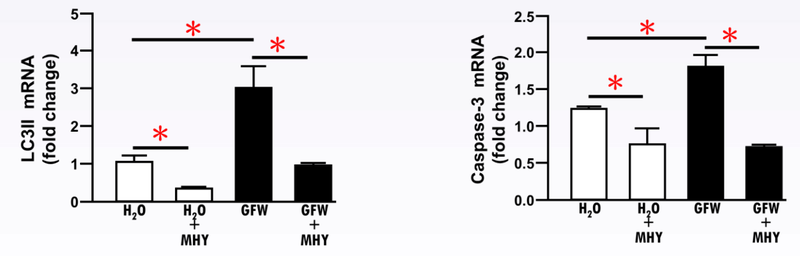


Figure 2. 處理細胞自噬抑制劑後，皆會降低 LC3II 與 caspase-3 在基因層次的表現。

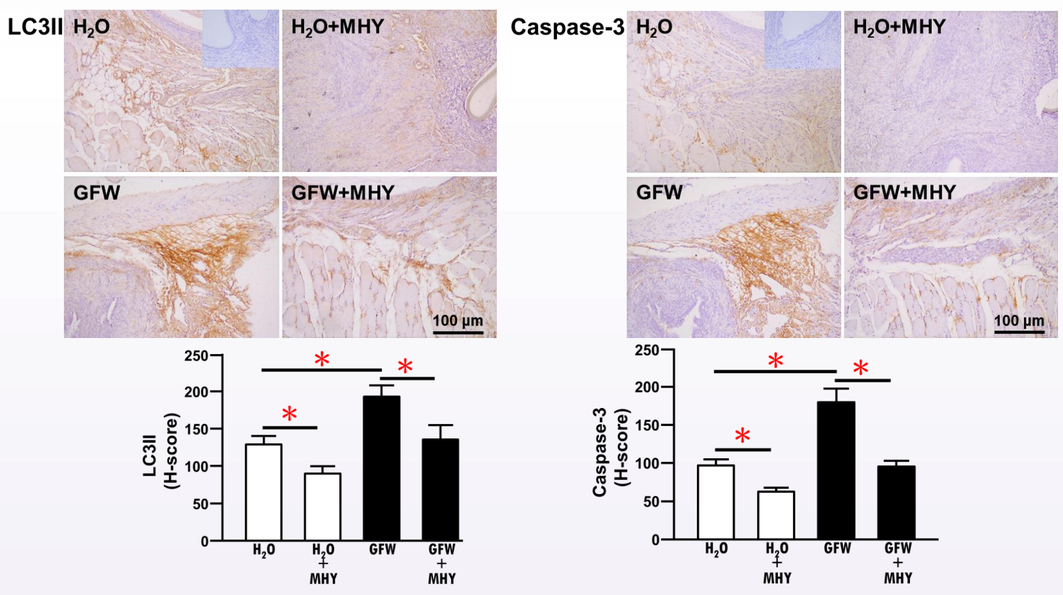


Figure 3. 處理細胞自噬抑制劑後，皆會降低 LC3II 與 caspase-3 在組織上的表現。

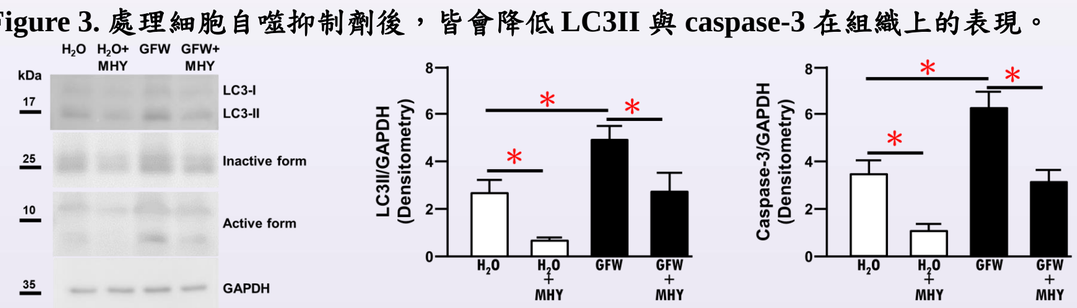


Figure 4.處理細胞自噬抑制劑後，皆會降低 LC3II 與 caspase-3 蛋白質量的表現。

CONCLUSIONS

桂枝茯苓丸已知用於治療血液停滯的傳統中藥，本研究藉由實驗小鼠處理細胞自噬抑制劑後檢視其相關基因表現，已了解到桂枝茯苓丸在抑制異位子宮內膜發展機制中所扮演的細胞自噬作用。未來可對此發展出新的預防方法及治療策略。

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