手術後疼痛與脊髓背角內微小膠細胞衍生之 p38 絲裂原蛋白激活酶活化的關係之探討

A Study on The Correlation of Spinal Microglia-derived p38 Mitogen-activated Protein Kinase (MAPK) Activation And Post-surgical Pain

中文摘要

越來越多新的研究，顛覆了傳統上認爲疼痛只發生在神經元的觀念。它們發現，疼痛不僅侷限於神經元通路，還包括了背根神經節中的許旺細胞、衛星細胞，周邊神經的免疫系統，及脊髓中的神經膠細胞如微小膠細胞、星狀膠細胞等。其中，微小膠細胞最受到注意，且認爲此細胞與神經元的交互反應，會強化疼痛的演化及維持。

絲裂原蛋白激活酶 (Mitogen-activated protein kinase, MAPK) 代表了細胞外刺激對細胞內反應的一個媒介，其活化過程需藉由磷酸化來完成。此家族的一個重要成員 p38，逐漸被發現參與了許多神經病變性疼痛的敏感化。當神經損傷，活化了微小膠細胞中的 p38 後，會誘發一系列訊息傳遞，生成前發炎性細胞素 (proinflammatory cytokines) 及趨化激素 (chemokines)。但在手術後疼痛的機轉中，這一系列的變化並未被報告過。

手術後疼痛是一種可以預期並計畫性處理的疼痛。習慣上將此疼痛歸類於急性發炎性疼痛，但最新研究顯示，10-50% 的術後病患會發展成慢性疼痛，症狀類似神經病變性疼痛。雖然大部分的疼痛可經由嗎啡或消炎藥控制，但是藥物本身副作用卻增加病患的不適或危險性，所以有必要加入一些效果佳及低風險的止痛治療。

因此，本論文針對了手術後疼痛造成 p38 及與微小膠細胞的活化，探討這種機轉對術後止痛的治療；同時提出了非藥物的「電針止痛」，觀察是否可抑制 p38 活化，來減輕手術疼痛。

本論文分爲三大部分，首先，將研究手術後疼痛是否誘發 p38 活化。利用大鼠後蹠部切口的手術模型，證明 p38 磷酸化會出現在脊髓背角的微小膠細胞中，而其活化(磷酸化)與術後疼痛反應有極密切關係。接著，第二部份則在手術前注射 p38 抑制劑，不但可抑制 p38 磷酸化，也明顯減輕手術後的疼痛，顯示 p38 參與了手術疼痛的敏感化。第三部份討論針刺止痛研究。因爲針灸的機轉不明確，爲此我們發展出一套可靠的動物針灸止痛模型，發現電針不只可提高疼痛閾值，減輕福馬林疼痛，也減少了脊髓背角 Fos 的表現。利用此針刺模型，進一步驗證電針刺激在手術疼痛的作用。結果發現，電針刺激不僅可明顯減輕手術後疼痛，且可降低脊髓背角 Fos 表現神經元的數量，及抑制微小膠細胞中的 p38 磷酸化；暗示針灸對於抑制手術後疼痛，具有多層面的機轉。

綜而言之，本論文的目的在探討微小膠細胞及訊息傳遞分子 p38 在手術後的反應，並藉由抑制此因子，調控疼痛的發展。由於 p38 活化後衍生出的發炎免疫反應，可能對疼痛記憶有更重要的影響，未來的發展將朝這一大方向，作更深入及廣泛的研究。

英文摘要

A growing body of evidence indicates, in contrast to traditional concept, the development of persistent pain involves not only neuronal pathways, but also Schwann cells, satellite cells in the dorsal root ganglia, components of the peripheral immune system, and microglia and astrocytes in the spinal cord. Among these cells, microglia play a pivot role in that release of immunological mediators from these cells affect the neuron-glial interactions and critically enhance the establishment and maintenance of pain sensitization.

Mitogen-activated protein kinase (MAPK), when activated via phosphorylation, can mediates many extracellular stimuli to trigger intracellular responses. p38, one member of this MAPK family, has been found to be important in pain sensitization, especially in various neuropathic pain types. After nerve injury, the activated (phosphorylated) p38 triggers a series of signal cascades and induces release of proinflammatory cytokines and chemokines. Nevertheless, the similar mechanistic changes have not been studied in postsurgical pain.

Postoperative pain is an expectable pain and can be managed through the whole perioperative period. Though this type of pain is customarily categorized into acute inflammatory pain, the new studies revealed that there are 10-50% of postoperative patients suffering form chronic postoperative pain, with symptoms analog to neuropathic pain. Morphine or NSAID can control most of postoperative pain, however, their side effects result in patients' discomfort and sometimes complications. Therefore, it is necessary to provide new low-risk and high efficacious therapeutic methods.

Accordingly, the current thesis is focusing on the surgery-induced p38 and microglial activation, and to explore the analgesic effect on postoperative pain by inhibiting the p-p38. I also present a novel electroacupuncture model in rats, and use this non-pharmacological treatment to reduce postoperative pain and to alter the p38 activation.

There are three major parts in this thesis. In the first part I will examine the p38 activation by surgical pain. Using a plantar incision pain model, we found the phospho-p38 expression showed a close correlation with postoperative pain behaviors. Immunohistochemistry showed a rapid p38 activation following surgery and a colocalization within microglia. Next, a preoperative intrathecal treatment with p38

inhibitor was shown not only to prevent p38 activation, but also significantly attenuate postoperative pain. These findings indicate p38 contributes to the development of postoperative pain and can be viewed as a new avenue for clinic use.

In the third part, a study of electroacupuncture analgesia on surgical pain is conducted. Because acupuncture mechanism is still controversial, I developed a stable electroacupuncture (EA) model. Under low-concentration of gas anesthesia, EA stimulation showed an intensity-dependent analgesia, elevated pain threshold, attenuated formalin-induced hyperalgesia, and reduced Fos expression in the spinal dorsal horns. Then, we testify the EA effect on surgical pain. We found EA was effective in decreasing postoperative pain, reducing Fos expression and more importantly, inhibiting the p38 activation in microglia. This finding implicates that EA can regulate the sensitization of postoperative pain and provide a wide-range analgesic function.

In conclusion, the major issue in this thesis is to emphasize the importance of microglial and p38 activation in postoperative pain, and to regulate pain development through inhibition of this factor. Nevertheless, more lines of new evidence disclose that the neruoinflammatory and/or neuroimmunal reactions downstream to MAPK activation may have greater impact on pain memory, aiming at this horizon will be my future research efforts.