肉毒桿菌神經毒素對芥末油注射大白鼠後腳掌引發之發炎反應的抑制效果

The Inhibitory Effects of Botulinum Neurotoxin on Inflammation Induced by Mustard Oil Injection into Rat's Hind Paw

中文摘要

芥末油 (Mustard oil) 接觸正常人體及動物組織時，會選擇性興奮週邊感覺 C-纖維，並造成明顯的發炎反應；缺乏感覺神經 (特別是 C-纖維)的組織，所引起的發炎反應就會降低而不明顯。這種現象顯示週邊感覺神經 C-纖維是造成發炎反應的重要元素之一。貯藏在 C-纖維末梢小泡的 Substance P 具備強烈的血管擴張(vasodilation)效力，當 C-纖維興奮時 Substance P 會被釋放並擴散到周圍組織中，進而引發所謂的神經性發炎反應。肉毒桿菌神經毒素 (Botulinum neurotoxin, BOTOX) 可以阻止乙醯膽鹼 (Acetylcholine) 自運動神經纖維末梢的小泡釋放出來，造成肌肉麻痺。許多實驗證據顯示：幾乎所有的真核細胞生物，從低等單細胞生物的酵母菌類到高等靈長類動物的大腦神經細胞，其控制細胞小泡與細胞膜附著、黏合，並將含於小泡內之化學物質釋放出來之機制十分雷同，因此 Substance P 與乙醯膽鹼可能有相似的釋放機制。我們大膽假設 BOTOX 也可以阻止 Substance P 自 C-纖維末梢釋放，進而抑制神經性發炎反應的產生。本研究希望藉由動物實驗來探討： (1) BOTOX 預先注射對芥末油注射大白鼠後腳掌引起的發炎反應 (包括血漿外滲以及組織腫脹)的抑制效果；(2) 此一抑制效果的作用時間；(3) BOTOX、局部麻醉劑 Lidocaine 及 Substance P 受器拮抗劑 L-733060 對芥末油引發的發炎反應的抑制效果，並依不同藥劑之特殊作用部位推論 BOTOX 抑制發炎反應的作用機制。生理食鹽水、Lidocaine 或 BOTOX 被分別預先注射 (Pre-inject)到 Sprague-Dawley 大白鼠 (250-350 g)的後腳掌，而 L-733060 (Substance P 的 Neurokinin-1 受器的抑制劑)則以靜脈注射方式投予。生理食鹽水、Lidocaine、 L-733060 注射後 10 或 15 分鐘，或 BOTOX 注射後 1 至 10 天之後， 再將芥末油注射到之前注射過的後腳掌以引發發炎反應。芥末油注射前與注射後，我們以排水法測量後腳掌的體積，兩者的差值定義爲腫脹程度。在芥末油注射前十分鐘將伊氏藍 (Evans Blue)溶液經由頸靜脈注射進入大白鼠體內，俟大白鼠犧牲後取下其後腳掌，使用分光光度計測量腳掌組織的伊氏藍滲出量來定量發炎所導致血漿外滲 (Plasma extravasation)之程度。實驗結果如下：(1) 芥末油注射於預先注射生理食鹽水之後腳掌引起嚴重的腫脹及血漿外滲； (2) 預先注射 5 U 之 BOTOX、 Lidocaine 或 L-733060 可有效減低發炎反應；(3) 5U BOTOX 在注射 7 天後開始發揮效果，此一效果維持至少十天。此一結果顯示 BOTOX 可能具有抑制神經性發炎之效果！

英文摘要

In normal human body and animal tissues, the contact with mustard oil may selectively stimulate peripheral sensory C-fibers, leading to a significant inflammation reaction; such evoked inflammation is less significant for tissues lacking sensory nerves (especially C-fibers). This indicates that the C-fiber is a vital factor for inflammatory reaction. Substance P, stored at the distal vesicles of C-fibers, has a strong effect of vasodilatation. Upon the excitation of C-fibers, substance P is released and diffuses into surrounding tissues, resulting in a so-called neurogenic inflammation. Botulinum neurotoxin (BOTOX) could prevent the release of acetylcholine from the distal vesicles at the motor nerve fibers to lead to muscular paralysis. Many experiments have showed： that the control mechanism for the adherence and adhesion between cellular vesicles and cell membrane, as well as the release of chemical substances into surrounding environment are extreme similar among almost all eukaryotic organisms, from yeasts of a lower level unicellular organism to advanced primates. Therefore, substance P and acetylcholine may be released via a same mechanism. We confidently assumed that BOTOX also could prevent the release of substance P from the distal end of C-fiber and then to prevent the neurogenic inflammation. This study was aimed to probe into the following issues: (1) the inhibitory effect of a pre-injection of BOTOX on the inflammation, including plasma extravasation and tissue swelling at the hind paw of climacteric rats induced by a mustard oil injection, (2) the duration for such inhibitory effect, (3) the comparison between the inhibitory effect of BOTOX, lidocaine (a topical anesthetic), and L-733060 (an antagonist for substance P Neurokinin-1 receptor) on the mustard oil-evoked inflammation, and furthermore the action mechanism of BOTOX to inhibit the inflammation based on the specific sites of action of these various agents. Normal saline, lidocaine, or BOTOX was separately pre-injected to the hind paw of male Sprague-Dawley rat (250-350 g) while L-733060, an inhibitor of NK-1 receptor of substance P, was administrated intravenously. At 10 or 15 minutes after the injection of normal saline, lidocaine or L-733060, or 1 to 10 days after the pre-injection of BOTOX, mustard oil was injected to the same site to induce an inflammation. The volume of the hind paw was measured by displacement of water and the difference between the volume before and after the injection of mustard oil was calculated and referred to be the swelling level. At 10 minutes before the injection of mustard oil, an Evans Blue solution was administrated by a jugular vein injection to climacteric rat. After scarifying the rat, the hind paw was removed for measurement of the Evans Blue excretion was measured by a spectrophotometer to define the inflammation-induced plasma extravasation. Results as below： (1) serious swelling and plasma extravasation was evoked by an injection of mustard oil to a hind paw

with a pre-injection of normal saline, (2) a pre-injection of BOTOX of 5 U, Lidocaine, or L-733060, all could effectively reduce the inflammation, (3) a BOTOX pre-injection of 5 U commenced to act at 7 days after the injection with a duration for at least 10 days. This study has revealed that BOTOX has an inhibitory effect on neurogenic inflammation!