誘導性氧化氮合成酶;在 dihydropyridine 引起的牙齦組織增生現象中的角色

The Role of Inducible Nitric Oxide synthase in the Pathogenesis of dihydropyridine-induced Gingival Overgrowth

中文摘要

Dihydropyridine 是在臨床上常用於治療高血壓藥物中的一種，長期服用的患者特別是男性，有一定的比例會產生牙齦增生的現象。我們的團隊先前的研究中發現這類患者牙齦纖維母細胞中睪固酮受器的表現量是一般人的兩倍。而 IL-1β 和睪固酮受器之間的作用似乎和牙齦增生有相關性。此外在文獻回顧中發現氧化氮合成酶的其中一種,誘導性氧化氮合成酶(iNOS)則是會被 IL-1β 活化，在短時間內釋出大量的氧化氮。氧化氮在許多組織裡扮演多重的角色，例如調控結締組織生長因子、血管擴張、神經訊息傳遞、抑制血小板附著凝集以及宿主對細菌、真菌和寄生蟲的免疫反應等等。但是 dihydropyridine 類的藥物對和類巨噬細胞共同培養且以多脂多醣刺激的健康牙齦纖維母細胞反而抑制了誘導性氧化氮合成酶活性和氧化氮的產生。為了了解在藥物引起的牙齦增生中，誘導性氧化氮合成酶是否正是影響睪固酮受器表現的原因之ㄧ，我們將牙周手術期間取下牙齦標本，培養成的牙齦母細胞分類成,健康(4 個樣本)、服用 dihydropyridine 造成牙齦增生(DIGO cells)(6 個樣本)共兩個組別，分別以滿盤前 48 小時以不加刺激的對照組，加入 IL-1β (10 ng/ml)刺激模擬牙周發炎，加入 nifedipine (0.34 μM)模擬服藥病人血清中藥物濃度以及合併 IL-1β (10 ng/ml)和 nifedipine (0.34μM)模擬服藥且有牙周發炎產生牙齦增生的情形，在四種不同的刺激條件下，分析兩個組別 real time PCR 的結果去檢測 iNOS 和睪固酮受器 mRNA 的表現，western blot 的結果去找出睪固酮受器的蛋白質表現，以及細胞上清液中氧化氮代謝物-亞硝酸鹽的濃度。以 Mann-Whitney U-test、Student t test 和 Spearman correlation coefficient 去檢視其差異程度和相關性。結果發現，無論健康、服用 dihydropyridine 造成牙齦增生兩個組別的細胞，受到 IL-1β 刺激後，iNOS mRNA 表現和上清液亞硝酸鹽濃度均顯著增加，而睪固酮受器 mRNA、睪固酮受器蛋白質反而顯著減少。只受到 nifedipine 刺激時，兩組細胞各種表現和對照組沒有顯著差異。當 IL-1β 和 nifedipine 合併刺激時，兩個組別的 iNOS mRNA 表現和上清液亞硝酸鹽濃度反而都顯著減少，睪固酮受器 mRNA、睪固酮受器蛋白質卻顯著增加，特別是服用 dihydropyridine 造成牙齦增生組別睪固酮受器蛋白質表現比健康組別細胞增加的多。這意味著對於因為高血壓長期服用 dihydropyridine 造成牙齦增生的病人來說，牙周發炎因子扮演了重要的角色。

英文摘要
Object: Dihydropyridine is one of the common medicine for hypertension with gingival overgrowth in some patients. Our previous studies found that there was two fold of androgen receptor (AR) in these patients to normal people. Interaction between interleukin-1β (IL-1β) and AR were related to gingival overgrowth. In addition, one kind of isoforms of NO synthases, inducible nitric oxide synthase (iNOS) , which is typically induced by inflammatory and immune stimuli such as interleukin-1β (IL-1β) and produces large amount of nitric oxide (NO). NO plays multiple important roles in many tissues , for example, regulation for the connective tissue growth factor (CTGF), homeostatic functions, including vasodilatation; neurotransmission; inhibition of platelet adhesion and aggregation; host defense against infectious agents like bacteria, fungi and parasites, and tumor cell killing. Nifedipine is a special calcium channel blocker which dose-dependently inhibits iNOS expression and NO production in normal human gingival fibroblasts which were co-cultured with RAW264 cells (macrophage-like cells) and stimulated with lipopolysaccharide (LPS). In order to understand weather iNOS effects AR expression in gingival overgrowth. Material and method: Two types of periodontal tissue: healthy (n = 4) and dihydropyridine induced gingival overgrowth (n = 6) in periodontal surgery. Each of these groups was stimulated with the control, IL-1β (10ng/ml), nifedipine (0.34μM) and IL-1β (10 ng/ml) combined with nifedipine (0.34μM) at 48 hrs before confluent state to intimate the condition of patients who took dihydropyridines and had DIGO. The data of real time PCR analysis of iNOS, AR mRNA and western bolt expression of AR protein were compared in these two groups. Besides, Griess reagent was used to detect the nitrite concentration respectively in the supernatant of all samples. Mann-Whitney U test、Student t test and Spearman correlation coefficient were used to examine the difference and correlation. Result: Under the stimulation of IL-1 β, mRNA expression of iNOS and nitrite concentration increases significantly while mRNA appearance of AR、 AR protein content decrease conspicuously. When nifedipine was added, no significant difference was indicated. Significant decrease of mRNA expression of iNOS and nitrite concentration were shown when IL-1β combined with nifedipine were administered. However, the AR mRNA and AR protein expression conversely raised, especially in the DIGO cells. Conclusion: The activation of iNOS as stimulated by IL-1?? is reversely related to the expression of AR mRNA in DIGO cells. While DIGO cells stimulated with IL-1β in conjunction with nifedipine, AR expression is significantly activated. It implies that inflammatory cytokine plays an important role in the anabolic overgrowth of gingival tissue when patients with hypertension are undertaking long term dihydropyridine therapy.