一氧化氮在於蟹足腫致病機轉中的角色探討

The role of nitric oxide in pathogenesis of keloid formation

中文摘要

一氧化氮（NO）已經成為許多生理學上功能的一個重要的調控者。最近的報告提出一氧化氮參與傷口治癒的過程。蟹足腫疤痕是過度的傷口癒合過程，同時一氧化氮在於傷口癒合的發炎治癒階段中扮演很重要角色。起因於一氧化氮在於蟹足腫疤痕形成時所扮演的角色目前尚未完全研究清楚。本論文研究探討一氧化氮在於蟹足腫疤痕形成時的作用機轉。誘導型一氧化氮合成酵素的表現和一氧化氮的產生在蟹足腫疤痕組織中是上升的，但是在於配對的週邊皮膚組織中並沒有上升。此外，外源性一氧化氮可以造成蟹足腫疤痕纖維母細胞(KF)的膠原蛋白質第一型的表現具有劑量－依賴性的增加。一氧化氮也造成膠原蛋白第一型的表現具有時間－依賴性的增加，並且在於蟹足腫疤痕纖維母細胞暴露於一氧化氮 24 小時後達到最高鋒。

為了要更明確定義 NO 卅 cGMP 信號路徑在於蟹足腫疤痕致病病因中的潛在效果，在本研究中進行探討外源性的一氧化氮（從一氧化氮提供者釋放出）在蟹足腫疤痕纖維母細胞的膠原蛋白質表現的提高效果可藉由膠原蛋白第一型蛋白質和 TGF－β1 的表現增加。DETA NONOate，一種一氧化氮的提供者，被添加入蟹足腫疤痕纖維母細胞的培養中，做為外源性的一氧化氮，釋放一氧化氮到培養基中。膠原蛋白的表現被測試然後決定在蟹足腫疤痕纖維母細胞中總可溶性膠原蛋白與膠原蛋白第一型的增加量。蟹足腫疤痕纖維母細胞中的 cGMP 濃度以 EIA 方法測量。外源性的一氧化氮被發現提高膠原蛋白的表現而且提升細胞內 cGMP 的濃度。而且，評估提升的細胞內 cGMP 濃度對於膠原蛋白和 TGF－beta1 表現的效果，同時 cGMP 和 TGF－beta1 是以 ELISA 的方式來測量。PDE 的抑制劑像是 IBMX（3-isobutyl－1-methylxanthine），Vinpocetine，EHNA，Milrinone 和 Zapriast，已經被報告降低 PDE 水解細胞內 cGMP 的作用，並且造成細胞內的 cGMP 濃度的增加，誘發 TGF－beta1 的製造和蟹足腫疤痕纖維母細胞的膠原蛋白合成。在這研究中，我們發現 PDE 活性的抑制作用加強外源性一氧化氮增加膠原蛋白合成的效果。提升的細胞內 cGMP 濃度是由外源性的一氧化氮所誘發或是由 PDE 抑制劑來抑制 PDE 的水解活性所造成的。提高細胞內 cGMP 濃度可增加蟹足腫疤痕纖維母細胞的 TIMP-1 和 HSP47 的表現。外源性的一氧化氮明顯地加強蟹足腫疤痕纖維母細胞中 TGF－beta1 對應的增加的 TIMP-1 和 HSP47 的製造。

總結這些的結果可以推導一個結論：蟹足腫疤痕病變的過度膠原蛋白形成可能始於蟹足腫疤痕纖維母細胞的一氧化氮卅 cGMP 信號路徑來快速的增加 TGF－beta1，TIMP-1 和 HSP47 的表現所導致的。

英文摘要

Nitric oxide (NO) has emerged as an important mediator of many physiological functions. Recent reports have shown that nitric oxide participates in the wound healing process. Keloids arise from the aberrant wound healing process and nitric oxide plays an important role in the inflammation stage of wound healing. However, its role in keloid formation remains unclear. This study aimed to investigate the effects of nitric oxide on keloid formation. The inducible nitric oxide synthase expression and nitric oxide production were elevated in keloid scar tissues but not in matched perilesion skin tissues. Furthermore, exposure of keloid fibroblasts (KF) to exogenous nitric oxide resulted in increased expression of collagen type I in a dose-dependent manner. Nitric oxide exposure also induced time-course dependent collagen I expression that peaked at 24 hours in keloid fibroblast.
In order to better define the potential effect of NO/cGMP signal pathway in the keloid pathogenesis, the enhancing effect of exogenous NO (released from NO donor) on collagen expression in the keloid fibroblast as well as on the induction of collagen type I protein and TGF-beta1 expression in the KF was studied in this investigation. The DETA NONOate, an NO donor, was added to the KF, as the exogenous NO, to release NO in the culture medium. The expression of collagens was then determined by assaying the total soluble collagens and collagen type I in the KF. The cellular concentration of cGMP was measured by EIA in the KF. Exogenous NO was found to enhance the expression of collagens and elevate the cellular levels of cGMP. Moreover, to evaluate the effect of the elevated cellular cGMP levels on the expression of collagen and TGF-beta1, both cGMP and TGF-beta1 were measured by ELISA. The inhibitors for phosphodiesterase (PDE), such as IBMX (3-isobutyl-1-methylxanthine), Vinpocetine, EHNA, Milrinone and Zapriast, which have been reported to reduce the ability of PDE and subsequently produce an increase of cellular cGMP, induce the production of autocrine TGF-beta1 as well as the synthesis of collagen in the KF. In this investigation, the inhibition of the PDE enzyme activity was observed to enhance the effect on the collagen synthesis, and was induced by exogenous NO. Elevation of cellular cGMP levels was observed to be induced by NO or blocked by the hydrolysis activity of phosphodiesterase (PDE) by the PDE inhibitor. The elevated levels of cellular cGMP were noted to enhance the expression of TIMP-1 and HSP47 in KF. Exogenous NO was found to significantly accelerate the production of TIMP-1 and HSP47 in the primary KF with a corresponding increase in the production of TGF-beta1.
The results have led to a conclusion, that is: The excess collagen formations in the keloid lesion may be attributed to the NO/cGMP signal pathway by initiating a rapid increase in the expression of TGF-beta1, TIMP-1 and HSP47 in the KF cells.