行政院國家科學委員會專題研究計畫　期中進度報告

人工關節溶解性鈦微粒對肺部及心血管系統的作用(1/2)

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人工關節溶解性鈦微粒對肺部及心血管系統的作用(1/2)
Effects of soluble titanium particles from total joint prosthesis on the pulmonary system and cardiovascular system

計畫編號：NSC 91-2314-B-002-248

執行期限： 91年8月1日 至 92年7月31日

主持人： 楊榮森 台大醫學院骨科

一、中文摘要

目前人工關節的置換已是骨科臨床上治療關節炎的常見手術，由於鈦具有高惰性及耐腐蝕性，因此鈦合金(Ti_6Al_4V)是其中一種廣被採用於手術醫材及人工關節的材質，但是使鈦植入物後，會釋出溶解態腐蝕產物及磨損微粒。本研究小組也曾證實，發生人工關節鬆脫病患的血液內及尿液的金屬離子含量(包括鈦)遠比正常人及未發生鬆脫者為高，顯見金屬元素可經循環系統分佈全身組織。這些金屬可在體內各處散佈，這些又可能再引起該部位的局部巨噬細胞進行各種反應，包括釋出一氧化氮，細胞動力素、分解酵素等，因此可能會對全身各處引起各種可能的生理或病理反應，例如肺臟、心血管組織等。近年來有關此方面的研究很少。第一年研究：為利用已建立的體外細胞培養模式，以探討人工關節鈦金屬釋放之溶解態鈦所引起的細胞生化學酵素變化及其互動關係。結果發現植入人工關節同材質的金屬(Ti_6Al_4V)塊動物2和4周後，其血液及肺部鈦元素含量明顯增高，且分離出之肺泡巨噬細胞對於細菌內毒素引起之一氧化氮生成及對於誘

導型一氧化氮生成表現上有減弱大鼠肺泡巨噬細胞亦可看到LPS刺激NO釋放及iNOS蛋白和mRNA量增加的情形皆會受到抑制。這些結果似乎暗示著肺部巨噬細胞在鈦作用下對於細菌內毒素引發之免疫反應會改變。另外，我們也發現當以溶解態二氧化鈦處理人類臍靜脈內皮細胞時eNOS蛋白表現會減少，且合併細菌內毒素處理時eNOS減少情形更明顯，但cyclooxygenase-2蛋白表現卻增加，此也似乎暗示著心血管系統功能可能會受到影響。

關鍵字：鈦植入物；一氧化氮；肺泡巨噬細胞；人類臍靜脈內皮細胞

Abstract

Total joint prosthesis replacement has become one of the most common procedures to treat arthritic patients in the clinical orthopedic practice. Titanium alloy (Ti_6Al_4V) is one of the materials widely used in common orthopaedic practice. The use of Ti implant results in the dissemination of soluble metallic corrosion products and particulate metallic wear debris in the long run. Previous reports focused on the release of metal debris that has been reported to be an important underlying mechanism for prosthesis-related late complications, such as loosening of prosthesis, local osteolysis, release of inflammatory factors. The first year will use in vitro cell culture model (easier to control the experimental conditions). We have found that the concentration of titanium (Ti) in the blood of patients with a loosened Ti-alloy prosthesis was elevated. An increase in levels of elemental Ti in blood and lung tissues of rats with an alloyed-Ti implant has also been found. The cellular reaction to an elevated elemental Ti level in the circulation remains unclear. We further performed experiments to examine the changes of inducible NO synthase (iNOS) expression in alveolar macrophages from alloyed-Ti implant rats. The elevation of nitrite and iNOS expression induced by lipopolysaccharide (LPS) was suppressed. Furthermore, the *in vitro* effect of a soluble form of Ti was further investigated. Ti inhibited the LPS-induced nitrite production and iNOS expression in alveolar macrophages from normal rats without any cytotoxic effects. These events were inhibited by co-incubation with Ti. These results indicate that elemental Ti may impair iNOS expression in alveolar macrophages, the inhibitory action of Ti on cellular responses of alveolar macrophages may be anti-inflammatory and depress local defense mechanisms related to microbial killing. Moreover, the expression endothelial nitric oxide synthase (eNOS) was decreased, while COX-2 expression was increased, in the presence of Ti. These results imply that Ti released from Ti-alloy may affect the function of cardiovascular system.

Keywords: titanium alloy, alveolar macrophage, human endothelial cells, nitric oxide synthase.

二、緣由與目的

隨著骨科醫學的進展，使人工關節的研發廣泛應用於嚴重關節炎病患的治療，多年來改善眾多病患的關節功能及生活品質，造福無數關節炎病患，臨床績效十分彰顯。人工關節置換是目前治療嚴重關節疾病的重要手術之一；鈦合金

(Ti_6Al_4V)是人工關節其中一種材質且是目前被廣泛採用者。鈦(Ti)曾被建議對於無骨水泥式人工髖關節是選擇性材質。但是有些研究也發現合金材質人工關節磨損導致可溶性金屬(包括鈦)釋出且會隨著循環到達全身；本研究小組也曾證實，發生人工關節鬆脫病患的血液內及尿液的金屬離子含量(包括鈦)遠比正常人及未發生鬆脫者為高，顯見金屬元素可經循環系統分佈全身組織。這些可溶性金屬可能進而在體內各處散佈，這些又可能再引起該部位的局部巨噬細胞進行各種反應，包括釋出一氧化氮，細胞動力素、分解酵素等，因此可能會對全身各處引起各種可能的生理或病理反應，例如肺臟、心血管組織等。近年來有關此方面的研究很少，因此，進一步探討人工關節磨損微粒的全身生理反應，可有助於我們明白這些長期置入體內的人工關節，所可能會造成的影響。本研究的主要研究重點在於利用細胞和組織培養模式及動物模式進行研究，以探討人工關節鈦金屬釋放之可溶性鈦，對肺臟及心血管系統的影響，以進一步探討人工關節金屬微粒所引起的全身性反應，以供推論人工關節置換手術後可能引發全身之反應。經由此研究我們可推論臨床上關節炎病患在接受人工關節置換手術後的可能反應，對於人工關節置換病患的臨床意義而言，本研究可提供非常珍貴的資料供參考，意義重大。本研究計畫第一年乃是利用已建立的體外細胞培養模式(肺泡巨噬細胞及血管內皮細胞)，以探討人工關節鈦金屬釋放之可溶性鈦所引起的細胞生化學酵素變化及其互動關係。

三、結果

試驗結果發現植入人工關節同材質的金屬(Ti_6Al_4V)塊動物(Wistar rats) 2和4周後，其血液及肺部鈦元素含量明顯增高，且分離出之肺泡巨噬細胞(alveolar macrophages)對於細菌內毒素(lipopolysaccharide, LPS)引起之一氧化氮(nitric oxide, NO)生成及對於誘導型一氧化氮(iNOS)表現上有減弱現象。外加溶生解態二氧化鈦(TiO_2) (0.01-0.06 mM)至大鼠肺泡巨噬細胞亦可看到LPS刺激NO釋放及iNOS蛋白和mRNA量增加的情形皆會受到抑制。這些結果似乎暗示著肺部巨噬細胞在鈦作用下對於細菌內毒素引發之免疫反應會改變。另外，我們也發現當以溶解態二氧化鈦(TiO_2)處理人類臍靜脈內皮細胞(HUVECs)時eNOS (endothelial nitric oxide synthase)蛋白表現會減少，且合併細菌內毒素處理時eNOS減少情形更明顯，但cyclooxygenase-2 (COX-2)蛋白表現卻增加，此也似乎暗示著心血管系統功能可能會受到影響。

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