探討超氧化物歧化?與鄒族退化性關節炎的關係及重金屬污染對分子之影響

Study on the Cu,Zn-SOD gene of the Tsou's osteoarthritis and the possibile effect from environmental toxic metals to the Cu,Zn-SOD molecule

中文摘要

退化性關節炎 (osteoarthritis, OA) 的發生原因爲由於細胞激素 (cytokines) 的誘發，使得活性氧分子 (ROS) 參與細胞外的分解路徑，造成軟骨部分損傷，而導致退化性關節炎的發生。而銅鋅超氧化物歧化? (Cu,Zn-SOD) 的作用爲清除 ROS 中的超氧陰離子，由 Cu,Zn-SOD 基因所表現可以推測與退化性關節炎有關。Cu,Zn-SOD 在歐洲多國被使用在臨床治療肌肉骨骼發炎 (musculoskeletal inflammation)，特別是退化性關節炎的治療，具有長效的效果，因此超氧化物在退化性關節炎的生化病理方面扮演重要角色。我們觀察台灣鄒族 退化性關節炎發生率偏高，可能與關節液的 Cu,Zn-SOD 活性有關，推測因爲基因的異常，或是蛋白活性異常所造成。利用自動化核酸定序儀來進行定序鄒族 58 人，對照台灣 48 人及泰雅族 20 人之 exon 序列，結果沒有發現有任何序列的改變，因此推測 SOD1 基因序列是非常保守的，可能與退化性關節炎無關。鄒族退化性關節炎比例偏高，可能是因爲環境污染或是土壤重金屬污染，造成 Cu,Zn-SOD 蛋白質活性異常或有其他原因，還尚待研究。Cu,Zn-SOD 屬同源雙體分子 (homodimer) 之抗氧化酵素，酵素活性下降時，除可能與退化性關節炎有關外，亦可能造成肌肉萎縮性側索硬化症 (Amyotrophic Lateral Sclerosis, ALS)。過去在 E.coli SOD overexpression system 中加入重金屬以干擾 Cu,Zn-SOD 金屬結合位置的實驗發現，環境中重金屬的污染，可能因上述機制而改變生物體內 Cu,Zn-SOD 的蛋白質結構，進而影響其酵素活性。因此，本研究中選擇以砷、鎘、鉻、汞和鉛這五種廣泛環境污染之重金屬元素，來觀察重金屬污染對細胞內 Cu,Zn-SOD 分子結構的影響。

利用大腸桿菌在相同濃度的銅和鋅來過量表現 Cu,Zn-SOD 蛋白，酵素活性測試則顯示，在較高濃度的砷或汞下表現的 Cu,Zn-SOD 蛋白，其酵素活性因重金屬濃度之增加相對減少。以感應偶合電漿原子放射光譜儀 (ICP-AES) 偵測 Cu,Zn-SOD 蛋白內各金屬元素含量發現，當砷或汞濃度提高時，Cu,Zn-SOD 蛋白中的含銅量沒有改變；而其含鋅量則有顯著地減少。顯示重金屬影響 Cu,Zn-SOD 蛋白質之活性降低，是經由影響鋅在 Cu,Zn-SOD 的結合能力。利用圓振二向色性分析二級結構的變化，發現重金屬砷或汞會造成 α-helix 增加和 β-sheet 的減少。結合砷、鎘、鉻、汞和鉛五種金屬，在低濃度下亦會影

響 Cu,Zn-SOD 的活性及改變鋅離子的結合。
本研究結果推測環境污染物多重重金屬，影響 Cu,Zn-SOD 蛋白質之活性降低，可能是因爲少數重金屬的取代造成鋅的大量流失，進而改變 Cu,Zn-SOD 構型。希望本實驗能對於金屬介入分子內之可能機制，以及退化性關節炎的致病機轉提供一些新的研究方向 。

英文摘要

Osteoarthritis is characterized by focal loss of cartilage due to an up-regulation of catabolic pathways, induced mainly by pro-inflammatory cytokines. Reactive oxygen species (ROS) have been proposed to involved in this extracellular-matrix-degrading activity, chondrocyte oxidative status responsible for cartilage damage occurring in primarily degenerative joint disease. We presumed that osteoarthritis is associated with sod1 gene. SOD for parenteral administration is in clinical used in several European countries, where it is prescribed principally for treatment of musculoskeletal inflammation, especially osteoarthritis. We hypothesized that the prevalence of osteoarthritis was high in Tsou was thought associated to the SOD activity of articular fluid. It may be accounted by the abnormality of gene or protein activity. In order to explore the relationship of osteoarthritis and sod1 gene, we sequenced 56 Tsou, 48 Taiwanese and 20 Atayal. We hadn't found any polymorphism or point mutation of sod1 gene. We demonstrated that sod1 gene is unusually stable, so it was hard to find any polymorphism. It is possible that environment population or toxic metals of soil induced abnormality of protein activity.
Copper/Zinc-superoxide dismutase (Cu,Zn-SOD) is a homodimer antioxidant enzyme. The reduce in enzyme activity of Cu,Zn-SOD (SOD1) is one of the reasons that caused the familial amyotrophic lateral sclerosis (FALS). The pollution of heavy metal in the general environment may interfere metal binding site of the SOD protein, change its conformation, and lead to a reduced activity of Cu,Zn-SOD in the living being. Among the heavy metals, arsenate, cadmium, chromium, mercury and lead are the most toxic and widely polluted. Therefore, they have been selected to interact with Cu,Zn-SOD in this study.
The activities of purified Cu,Zn-SOD from E.coli overexpression system in LB broths containing various concentrations of toxic metals were compared. In addition, the activities of purified Cu,Zn-SOD proteins, from E.coli cultured in higher concentrations of toxic metals, decreased dose-dependently. By ICP-AES, we demonstrated that adding of toxic metals significantly increased the content of toxic metals, but reduced its zinc content of the Cu,Zn-SOD protein.
In conclusion, the presence toxic metals have affected the expression of Cu,Zn-SOD in E.coli, but they decreased the enzyme activity probably by replacing the binding of

zinc to the metal binding sites. Therefore, the presence of heavy metals in the culture broth might cause incorrect binding of metal ions to the SOD protein, and result in decreased the enzyme activity of SOD, which is a pathogenic reason of osteoarthritis. The obtained information may offer some directions to the studies on pathogenic mechanism of sporadic osteoarthritis.