# 探討補益類中藥----龜鹿兩仙膠作用於人體體幹細胞增生與分化之效能評估研究

**To investigate the influence of Guilu Erxian Gao on the proliferation and differentiation of human tissue stem cells**


中文摘要

龜鹿兩仙膠( GEG)爲傳統醫學上具骨修復效能與治療骨質疏鬆症的中藥複方，但至今其藥理仍缺乏科學驗證。本論文的目的是利用人體造血及間質幹細胞的特性來系統性的探討龜鹿兩仙膠對成體組織細胞增生與分化的影響及功能性基因體的評估，藉此鑑定出龜鹿兩仙膠之藥理作用。實驗結果顯示：(1)造血系統的影響方面：由血球前趨細胞群落形成分析( Colony forming assay；C.F.A.)得知龜鹿兩仙膠( GEG)會增多血球群落總數，並促進顆粒與巨噬細胞系造血細胞之分化( granulocyte and macrophage lineage )；而其動物性成份—龜板( T.P.)與鹿角( C.C.)僅能增多總群落數但對造血性幹細胞的分化無影響。(2)非造血系統的影響方面：(a)神經分化方面，除具有分化爲成熟神經細胞的能力也保有其原具有的神經膠細胞分化潛能；(b)對血管分化生成具有促進效果；(c)軟骨分化方面，會加速並增多軟骨球的生成；(d)硬骨分化方面除會加速並增加骨礦化外( Bone mineralization)，也有加速骨母細胞增生與分化的效用。

除上述之藥效應證外，從成分藥材分析結果發現(1)動物性中藥— 龜板爲龜鹿兩仙膠促進硬骨與軟骨分化之主要效力成份； (2)人蔘爲促進血管分化生成效能之成份。但基於保育觀念興盛，本論文進而探索可能取代龜板之中藥材，我們利用傳統中藥的經驗醫學進行蒐尋，找出七個與骨生成與骨修復相關的中藥材— 丹蔘( S.M.)、骨碎補( D.F.)、何首烏( P.M.)、杜仲( E.U.)、續斷( D.A.)、山茱萸( C.O.)、牛膝( A.B.)…等。先藉由 Von-Kossa 染色篩選出最可能取代龜板之中藥材爲骨碎補、續斷、牛膝。接著進行與龜鹿兩仙膠相同的實驗，以評估取代效用。結果顯示(1)取代方對造血系統之影響方面：骨碎補與欲取代之龜板相似，能增多血球總群落數但對造血性幹細胞的分化無影響。另ㄧ中藥牛膝具有增多紅血球系母細胞並降低顆粒與巨噬細胞系母細胞群落形成。(2)取代方對非造血系統影響方面：杜仲、丹蔘能促進血管之分化生成；骨碎補、續斷會促進軟骨球形成，而骨碎補、續斷、牛膝則會促進硬骨分化。綜合上述實驗結果可知骨碎補與續斷可能爲最具取代龜板的中藥材。

在分子層次更進一步驗證龜鹿兩仙膠之分子藥理，在間質幹細胞分化爲硬骨細胞的早期，不論龜鹿兩仙膠、龜板或欲用來取代龜板之骨碎補與牛膝，皆會降低 osteoclast 相關基因 IL-11 的表現；在分化爲硬骨細胞的中期則不論龜鹿兩仙膠、龜板或欲用來取代龜板之骨碎補與牛膝，osteoblast 相關基因 BMP-2 皆會增強表現，且在分化爲硬骨細胞的晚期除牛膝外也都會增強細胞基質 Type I Collagen 的

表現。
雖說骨碎補之龜板替代方具有促進硬骨與軟骨分化的效用，但由於功能性基因體上的表現仍有些許異同且在鹼性磷酸酶染色與鹼性磷酸酶活性分析實驗中，骨碎補在 100μg/mL 具有加速骨母細胞分化的能力但在 200μg/mL 時卻出現類似細胞毒性反應使細胞停止生長與分化，藥物有效且安全的作用濃度狹窄，因此新配方的組成仍待討論，有待更進一步進行動物實驗驗證其效果並尋找適合的安全使用劑量。

英文摘要

Guilu Erxian Gao( GEG) is a well known traditional Chinese medicine that has been frequently used in the treatment of osteoporosis and other bone diseases, however, the pharmacological base function has not been elucidated.
In the present study, we utilize human tissue stem cell to evaluate the influences of GEG on the hematopoietic and non-hematopoietic mesenchymal system. In the hematopoietic response, GEG shown to elevate the total hematopoietic colony numbers, and to promote Granulocyte and Macrophage lineage differentiation. In non- hematopoietic stem/progenitor system, GEG shown to promote osteogenesis, chondrosphere and endothelial tube formations, and sustain the neuron-glial cell differentiations.
We found that the promoting osteogenic and chodrosphere formation function by GEG is mainly come from the Tortoise plastron (T.P.) ingredient and the vasculogenic effect by Radix Ginseng ( R.G.).
In order to prevent use of T.P. component from the endangered specie animal, several substitute herb candidates have evaluated. Among the candidates we found Dipsacus asper (D.A.), Achyranthes bidentata (A.B.), and Drynaria fortunei (D.F.) are useful substitutes for T.P.
Specifically, we found that D.F. as T.P. promotes the total hematopoietic colony forming activity, and Achyranthes bidentata (A.B.) shown promoting the Erythroid lineage differentiation and inhibits the Granulocyte and Macrophage lineage differentiation. In mesenchymal tissue responses, we found that D.F. and D.A. accelerate and increase the chondrosphere formation and S.M. and E.U. promote the vasculargenesis; We further confirmed that D.F. , A.B., and D.A have the ability to advance the bone mineralization. In the Functional genomic evaluation, we investigated that the IL-11 expression was down regulated and the BMP-2 and OPN gene expression enhanced by T.P., D.F. and A.B.. At the late stage of osteoblast differentiation, T.P. and D.F. shown enhance the gene expression of Type I collagen., but not the A.B.
Althought the substitute T.P. component of GEG needed to be further studied by in

vivo animal or clinical investigation, current study provides a cell based molecular pharmacological evidences for GEG prescription and three potential candidates for the T.P. substitute.