前列腺素膠原蛋白凝膠釋放系統

Collagen Gel Delivery System for The Percutaneous Application of Prostaglandins

中文摘要

膠原蛋白爲一天然聚合物，經過胃蛋白酵素修飾螺旋纖維蛋白末梢蛋白鏈之 telopeptide-poor 膠原蛋白，形成低抗原性、生體相容性、生體可降解性及無毒性之材質，具有優於合成聚合物之特性。但由於膠原蛋白分子間及分子內交叉鍵結的特性，及在純化過程重組膠原蛋白分子交叉鍵結的形成，使材質的再現恆降低。因此在本實驗中，綜合文獻中各種不同備製方法，探討在不同酸溶液，溫度，胃蛋白酵素對膠原蛋白比例，及不同冷凍乾燥條件，對所備製得到的膠原蛋白材質特性之影響。經由掃描式電子顯微鏡觀察，在不同前處理和冷凍乾燥條件，膠原蛋白可以顯現不同的纖維狀、孔隙性纖維膜狀及緻密的膜狀結構。利用比對 SDS-PAGE 電泳分析及 SizeExclusion HPLC，分析膠原蛋白不同螺旋蛋白結構的含量與單體及多聚合體分子量分佈的現象，以經由 pH2.5 鹽酸溶液 20℃ 消化反應，並於以 0.5M 醋酸下冷凍乾燥之膠原蛋白材質，具有較少的多聚合體含量，及良好的纖維特性及水合性，是不錯的材質選擇。

經由皮膚滲透釋放前列腺素 E1 而達到療效的研究是目前重要的課題之一，但由於前列腺素 E1 化學不安定性與穿皮滲透量低限制其臨床使用，因此應用化學修飾具親脂性藥物分子以促進穿皮滲透，爲一非常具有前瞻性的模式。本實驗以化學修飾之前列腺素 E1 甲基酯爲模式藥，並以不同比例酒精共溶媒系統，以裸鼠皮穿透的體外實驗，評估前列腺素 E1 甲基酯穿透皮膚的特性。於前列腺素 E1 甲基酯安定性實驗顯示，其會經由前列腺素 A1 甲基酯轉換形成前列腺素 B1 甲基酯，但鮮少甲基酯的水解反應；但於裸鼠皮穿透實驗顯示前列腺素 E1 甲基酯，可穿透皮膚並轉換形成前列腺素 E1 ，40%左右的酒精含量有最高的滲透速率。比對前列腺素 E1 三種酒精含量溶液的滲透也發現，僅有極少量前列腺素 E1 穿透，遠比前列腺素 E1 甲基酯少約 10 倍量。進一步應用膠原蛋白材質作爲劑型設計，以 1%膠原蛋白凝膠並用酒精共溶媒系統，實驗證明前列腺素 E1 甲基酯仍可經由膠原蛋白凝膠釋放，及形成前列腺素 E1，但滲透速率稍有下降。

英文摘要

Natural polymer oftelopeptide-poor collagen by the treatment of pepsin is low antigenic, biocompatible, biodegradable and is less toxic than synthetic polymers. With the possible formation of inter- and intra-molecular crosslinkmgs during purification and reconstituted process, the properties of collagen usually were found to be poorly reproducible. After a system survey of reported methods of purification

used by various labs, a detailed examination of how digestion medium and its temperature, the ratio of collagen to pepsin, and the freeze-drying conditions affecting the properties of collagen was initiated. The morphological characteristics examined by scanning electron microscopy reveals that fibril collagen, porous fibril membrane or dense membrane is all possible formed dependent on the pretreatment and freeze-drying conditions. The helical structure (α, β, γ) , the content of monomers and oligomers in each collagen sample obtained by various conditions were analyzed by the SDS-PAGE electrophoresis and size exclusion HPLC. Both methods show that the collagen sample obtained by the pretreatment with pepsin under pH 2.5 HCl solution and freeze-drying in 0.5 M acetic acid give less oligomers. Its fibril characters and easy hydration also expresses that the method to obtain this collagen sample is the best choice for the purification.

Transdermal delivery system for prostaglandin (PGE1) to achieve therapeutic effect has less been one of major research nowadays. But the chemical instability and low penetration rate limit the clinical application. Therefore, a methylester derivative of PGE1 with more lipophilic character to enhance the penetration rate would be valuable. In this study, in vitro transdermal delivery on hairless mouse skin of PGE1 and its methylester from buffer solution with various content of alcohol was investigated. In the initial stability studies, it was found that PGE1 methylester was mainly degraded to PGA1 methylester and then PGB1 methylester, but the hydrolysis of methylester to PGE1 was minor. During penetration, it was found that PGE1 methylester was converted to PGE1 in the skin. There showed a maximal penetration rate for PGE1 methylester from buffer solution with 40 % alcohol content. PGE1 methylester also penetrate faster than that PGE1 from all three alcohol content examined. When 1 % collagen was used as gelling agent, PGE1 methylester still shows a similar pattern of penetration but with a slower penetration rate.