不同壓力與氣體對於裸鼠皮膚的穿透影響

Effect of different pressure and gas on nude mice skin transport

中文摘要

藥物穿皮輸送系統主要被皮膚最外層的低穿透性之角質層所限制，目前被廣泛地研究如何克服這層屏障，包括了使 chemical enhancers，iontophoresis，electroporation 以及 sonophoresis 等方法．而本實驗的主要目的是利用不同的壓力，氣體及處理時間探討裸鼠皮膚的穿透機制及影響．使用的壓力分別是 5，7.5，10 或 12.5 lb/in2，並以氮氣與氧氣 (95% O2+5%CO2)，分別給與 30 秒，60 秒或 90 秒，處理後，檢測小分子螢光性物質 (Fluorescein Isothiocyanate (FITC) Isomer I (Mw 332)與 Pyrene (Mw 202))與大分子物質 (胰島素 (Mw 5500))，來進行體外穿透實驗．此外，利用粒徑 0.046, 0.11, 0.5 與 2.0 μm 具螢光的 Latex beads，，探討壓力對於皮膚孔徑大小的影響，觀察組織切片，皮膚電阻測試，掃描式與穿透式電子顯微鏡下結構的變化；另外使用傅立葉變換紅外光譜儀，差式掃瞄熱量分析儀，蛋白質定量分析法觀察氮氣與氧氣對於皮膚組織脂肪與蛋白質組成之影響．

實驗結果顯示，增加藥物穿透的主要機制是由於角質層上的孔洞形成．在 FITC 小分子穿透，控制組與實驗組以 12.5 lb/in2, 90 秒，氮氣處理後，最有顯著差異；二組的擬穿透係數分別為 4.65±0.45´ 10-7 cm/sec 與 7.24±0.77´ 10-7 cm/sec cm/sec；在 Pyrene 方面，則為 372.02±27.28´ 10-7 cm/sec 與 490.92±50.05 ´ 10-7 cm/sec．另外在經氧氣處理後，在 FITC 方面，控制組與實驗組的穿透係數分別為 4.47±0.22´10-7 cm/sec 與 5.55±0.87´ 10-7 cm/sec，而在 Pyrene 方面，則為 299.68±19.26´ 10-7 cm/sec 與 374.83±32.07´ 10-7 cm/sec．在皮膚電阻測試方面，經由氮氣與氧氣處理過後呈現明顯減少，其中以氮氣具有顯著差異．另外在 latex beads 的穿透實驗上，經由氮氣與氧氣處理過後的皮膚，四種不同大小顆粒皆能有效地增加穿透．藉由組織切片得知，氮氣對於皮膚組織之 Langerhans cell 有增加趨勢，氧氣則無；但是氧氣對於皮膚真皮層細胞則觀察到有增生的現象．從傅立葉變換紅外光譜儀數據得知，實驗組在 1550, 1454 cm-1 的吸收峰有明顯減少；由差式掃瞄熱量分析得知，控制組與實驗組的相轉移溫度分別為 61.74 與 59.61 ˚C；在蛋白質含量分析上，控制組的蛋白質含量有從 208.74 減少至 159.60 mg/ml；在皮膚水份含量上，控制組與實驗組水份含量分別為 40.03 與 38.23 %，皆具有統計上的差異 ($p<0.05$)．由以上結果得知，對於小分子的物質是可以有效地經由此方法來增加穿透，而大分子的物質是無法增加穿透的．

英文摘要

The major limit of transdermal drug delivery system is the low skin permeability of the stratum corneum (SC). Several approaches have been extensively studied to overcome these skin barrier properties, including the use of chemical enhancers, iontophoresis, electroporation and sonophoresis.

The main purpose of this study was applied different pressures, gas (N2, O2+CO2 mixture) and treated time on nude mice skin to evaluate ability of drugs. Using different pressures (5, 7.5, 10 or 12.5 lb/in2) and applied time (30, 60 or 90 seconds) with nitrogen or oxygen gas, in vitro of drugs transport by two small fluorescent molecules [fluorescein isothiocyanate (FITC)(Mw 332) and pyrene (Mw 202)], and large molecule [insulin (Mw 5500)] were performed. In addition, series of fluorescent latex beads (0.046, 0.11, 0.5 and 2.0μm diameter) was investigated the effect of pressures on skin transport.

Furthermore, we utilized scanning electron microscopy (SEM), transmission electron microscopy (TEM), fourier transform infrared spectrometer (FTIR), differential scanning calorimetry (DSC) and protein assay to evaluate the content of proteins and lipids loss influenced by pressure effect as well as observation of histological changes of skin with nitrogen and oxygen.

The results showed a main mechanism of increasing drug transport was due to pore formation (≈10μm) on stratum corneum by SEM and TEM observation. Apparent permeability coefficients (P) of FITC treated with 12.5 lb/in2 of nitrogen for 90 seconds was significantly increased from 4.65±0.45´10-7 cm/sec to 7.24±0.77´10-7 cm/sec ($p<0.05$). Also using pyrene marker, was also increased from 372.02±27.28´10-7 cm/sec to 490.92±50.05´10-7 cm/sec, respectively. Similarity with oxygen treated (12.5 lb/in2, 90 seconds) of FITC and pyrene was found from 4.47±0.22´10-7 cm/sec to 5.55±0.87´10-7 cm/sec ($p<0.05$), 299.68±19.26´10-7 cm/sec to 374.83± 32.07´10-7 cm/sec ($p<0.05$), respectively. In skin resistance test, nitrogen or oxygen treated skin resistance were decreased and four particle size of latex beads were able to pass across skin effectively. However, nitrogen treated skin was observed some Langerhans cell number increased and oxygen treated skin increased dermis layer by histology evaluation. FTIR data shows that two peaks of absorbance were decreased ($p<0.05$) at 1550 and 1454 cm-1. DSC data also shows phase transition temperature (Tm) shifted from 61.7 to 59.61 °C ($p<0.05$) as well as protein contents decreased from 208.74 to 159.60 mg/ml. Furthermore, water content test appeared decreased from 40.03 to 38.23 % in skin. In addition, small molecules can enhance skin transport, but large molecule will not.