行政院國家科學委員會專題研究計畫成果報告

用於減肥治療之 norepinephrine 經皮輸移系統的研究

Investigation of norepinephrine transdermal delivery systems for anti-obesity therapy

計畫編號：NSC 90-2320-B-041-004
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一、中文摘要

無數奈米微粒組成之軟膏用以作為新副腎素之經皮輸移系統；奈米微粒包覆新副腎素是以固體凍膠粉碎技術來製備，新副腎素-親水膠體奈米微粒的粒子大小是以鐳射粒徑分析儀來檢測，至於該微粒之藥物負載與體外藥物釋離則是以高效液相層析儀來檢測，軟膏的流變性質是以 Cone and Plate 黏度計來描述，另外，藥物與聚合物間的交互作用是採用熱卡式分析儀來評估。結果顯示，以固體凍膠粉碎技術製備之新副腎素-親水膠體奈米微粒的粒子大小介於 40 奈米至 2000 奈米之間，奈米微粒的粒徑與藥物包埋率受膠體種類與含量的影響，基於藥物釋離結果與 Higuchi 作圖得知，新副腎素是包埋於親水膠體基質中，隨著最初 30% 至 40%新副腎素快速突釋後，可維持 24 小時的持續釋放效果。軟膏含高濃度細小親水膠體奈米微粒所展現與時間有關的性質，可歸納為擴張性與搖變稠的特性，為了長期的安定性，本研究結果建議，含新副腎素-親水膠體奈米微粒之製劑應以凍晶粉末於低溫下貯存，使用前加二次蒸餾水還原。熱分析的結果指出，各組成分之間不論是在製造過程亦或凍晶粉末加水還原，均未有化學交互作用產生。上述結果對爾後發展其他減重藥物經皮輸移系統將有所助益，

關鍵詞：新副腎素，軟膏，奈米微粒，釋離，體外實驗

Abstract

Ointments composed of numberless nanoparticles were used for norepinephrine (NE) transdermal delivery. Nanoparticles encapsulating NE were prepared using the solid jelly pulverization technique. The size of the NE- hydrogel nanoparticles was assessed using a LS Particle Size Analyzer. For these nanoparticles, drug loading and in vitro drug release was quantified using a HPLC assay. The rheological properties of ointments are characterized by Cone and Plate Viscometer. In addition, any drug-polymer interactions were assessed using a differential scanning calorimeter (DSC). The results show that solid jelly pulverization technique yielded NE hydrogel nanoparticles with a mean diameter of 40 nm to 2000 nm. The particle size and encapsulation efficiency of nanoparticles were affected by the hydrogels and their amount. Based on our release profiles and Higuchi plots, it appears that NE is trapped in the hydrogel matrix. Following an initial burst release of 30% to 40% NE, the release was sustained over the 24-hours study period. The time-dependent behaviour of ointments including a high

concentration of small hydrogel nanoparticles is referred to as dilatant and exhibit shear thickening. For the long term stability, the results suggested that the product containing NE nanoparticles should be stored in the state of lyophilized powder at lower temperature and reconstituted by adding double distilled water before used. The DSC measurements indicated that the chemical interaction does not occur among the components during manufacturing processes and reconstituting lyophilized powder. The above results will be helpful to possible development of the other anti-obesity drug transdermal delivery systems.

Keywords: Norepinephrine, Ointment, Nanoparticles, Release, in vitro studies

二、Introduction

Obesity is rapidly becoming a worldwide epidemic, with significant consequences in terms of clinical burden and economic costs in treating its complications, [1-3] however, the long term safety data of drugs which were used to treat obesity are not yet available and the use of these drugs may be limited by seriously adverse effects. [4-9] So effective new approaches are urgently needed. Norepinephrine induced lipolysis that has been proved in a cell-free system consisting of the lipid droplets and hormone-sensitive lipase (HSL), but it rapidly metabolized before reaching the systemic circulation and therefore ineffective after oral administration. [10-16] In order to achieve and maintain an adequate concentration of drug at the side of action for a prolonged period of time so as to improve the therapeutic efficacy, in the work, we attempt to develop a transdermal delivery system by the use of ointment dosage forms. The major part of the ointment is a gel-like structure composed of hydrogel with nanoparticles encapsulating norepinephrine. Compared to the free drug, nanoparticles have advantage of minimal irritation. The study will last for two years. In the first year, we investigate the effect of variation in the composition and preparation condition of nanoparticles on the viscosity, adhesive of products and drug release. The physicochemical properties of ointments will be determined by Cone and Plate viscometer, texture analyser and differential calorimetry. The effect of ointment physicochemical properties on the release of norepinephrine from ointment base will be discussed by *in vitro* studies. The above results will be to design further *in vivo* studies and helpful to possible development of anti-obesity drug transdermal delivery systems.

三、Results and discussion

The formulations used in the experiments are shown in Table 1. When preparing, add a definite amount of water, heat up to melt, then cool to coagulate into jelly. The jelly strength is 25 g/cm. The nanoparticles were obtained by using a solid phase pulverization technique. The distribution of particle size was measured by LS Particle Size Analyzer, and was controlled within a range of 40 nm to 2000 nm. Assay of entrapped norepinephrine was determined by filtering the nanoparticles dispersion under

vacuum through a 0.025 μ m filter. The filtrate containing free drug was estimated by HPLC. The % encapsulation efficiency of norepinephrine in nanoparticles of various formulations was calculated using the following expression and shown in Fig 1 and 2.

% Encapsulation efficiency = (Total drug – drug in aqueous phase) X 100 / Total drug

Agar and gelatin gels are termed thermal gels. Gels often contract spontaneously and exude some of the fluid medium. The increased amount of agar or gelatin resulted in a significant in the effect that is known as syneresis. This might be the reason for the decreased efficiency of encapsulation of higher agar or gelatin content in nanoparticles. On the other hand lyophilic sols form gels in a different manner. The macromolecules may form a network simply by entanglement. e.g. Acacia and Tragacanth. There were no significant difference within encapsulation efficiency of nanoparticles by adding Acacia or Tragacanth.

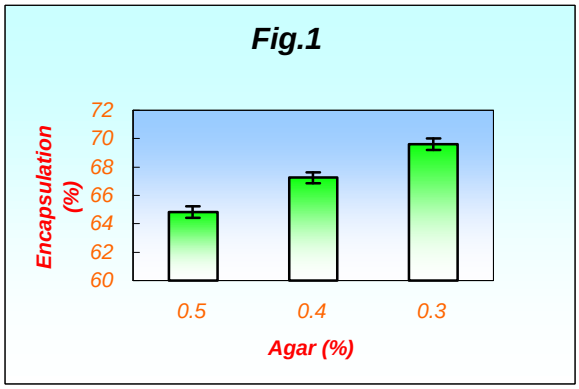
The rheological properties of ointments including a high concentration of small hydrogel nanoparticles are characterized by Cone and Plate Viscometer. As shown in Fig 3, the viscosity increases with increase in shear rate and time. The time-dependent behaviour of ointments is referred to as dilatant and exhibit shear thickening. The results are different from the similar materials are most usually typified by aqueous dispersions of hydrocolloids. Moreover, the viscosity of ointments will increase as agar content of nanoparticles increase.

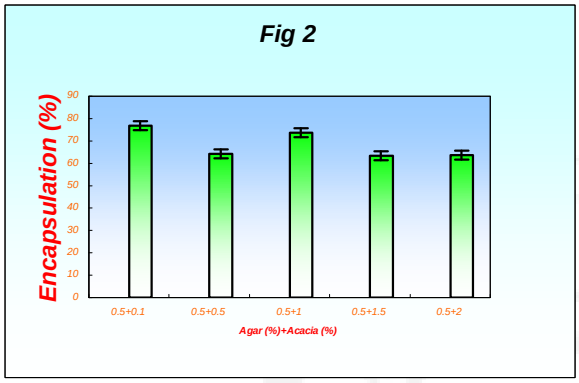
The in vitro release of norepinephrine from the hydrogel nanoparticles was carried out in a way similar to apparatus of the 15-ml vertical Franz diffusion assembly. The apparatus was maintained at 37 ±0.5 ° C with a water jacket. An aliquot of the sample was taken at appropriated times, and the concentration was determined by HPLC method. The results show that following an initial burst release of 30% to 40% norepinephrine, the release was sustained over the 24-hours study period. A Higuchi plot was approximately linear for all the formulations, indicating that norepinephrine is entrapped within the hydrogel matrix.

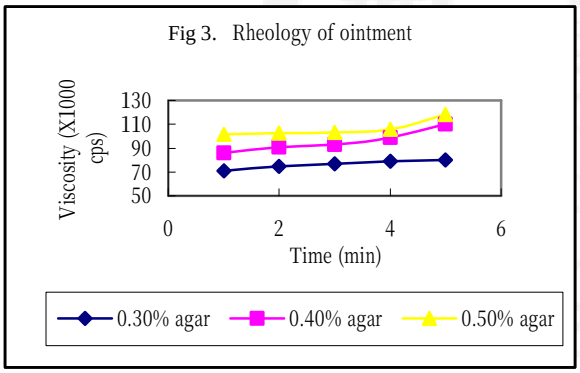
Differential scanning calorimeter was used to establish physical-chemical properties of hydrogel nanoparticles. The DSC measurements indicated that the chemical interaction does not occur among the components during manufacturing processes and reconstituting lyophilized powder.

Table 1 Formulation of jelly-like hydrogel nanoparticles

Component	Content (w/w %)
Agar	0.1 0.5
Acacia	0 0.05
Tragacanth	0 0.05
Gelatin	5 25
Water	Add to 100







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計畫參與人員：王俊傑

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中　華　民　國　91 年　10 月　25 日