Novel Core-Shell Zein Nanoparticles for Topical Delivery of Methotrexate

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ABSTRACT SUMMARY

Methotrexate is a folic acid antagonist that has potential anti-psoriatic activity. However topical delivery of MTX is limited by its hydrophilicity and charged nature. The objective of this study is to explore the feasibility of developing a topical formulation of MTX using core-shell zein nanoparticles. Zein is a biodegradable and water insoluble protein derived from corn. In this study core-shell MTX loaded zein nanoparticles were prepared using phase separation method. The MTX nanoparticles were characterized for the size, zeta potential, encapsulation efficiency and in-vitro release. The in-vitro skin penetration of MTX nanoparticles were studied using excised porcine skin.

INTRODUCTION

Psoriasis is a chronic inflammatory hyper proliferative skin disease which manifests as scaly, erythematous plaques. About 125 million people worldwide are affected with psoriasis. Majority of these patients receive inadequate treatment since the current therapies are ineffective or unsatisfactory. Topical therapy is the first line of treatment for psoriasis to slow down or normalize excessive cell proliferation and reduce the inflammation. Methotrexate is an effective anti-psoriatic drug that is currently administered by oral and injection routes. However the current MTX therapy is limited by the severe systemic side effects including bone marrow toxicity, decreased white blood cell and platelet counts, liver damage, diarrhea, gastric irritation, and ulcerative stomatitis. On the other hand, the topical skin delivery of the MTX is limited by its hydrophilicity and charged nature. To this end the aim of the present study is to develop a topical formulation of MTX using novel core-shell nanoparticles. The core-shell nanoparticles are made up of zein, a water insoluble plant protein as the core and lecithin/ pluronic as the shell which act as skin penetration enhancers. The zein due to its similarity to skin keratin combined with the skin penetration enhancing properties of lecithin/pluronic can be used to increase the skin penetration and skin retention of MTX.

EXPERIMENTAL METHODS

Methotrexate loaded zein nanoparticles was prepared using a phase separation method. Briefly zein and MTX were dissolved in 2 ml of 90% aqueous ethanol. This solution was added to an aqueous phase consisting of Pluronic F68 and lecithin in citrate buffer (pH 7.4) under probe sonication. Later the nanoparticle dispersion was stirred at 37°C for 3 hrs to evaporate the ethanol. MTX nanoparticles were separated by centrifugal filtration (MWCO 10kDa). The nanoparticles were lyophilized using trehalose as a stabilizer. Particle size, polydispersity index and zeta potential were determined by dynamic light scattering method (Nano-ZS, Malvern Instruments). The entrapment efficiency of methotrexate in zein nanoparticles was determined by UV spectrophotometry at 300nm. In vitro release studies were carried out using vial method in phosphate buffer (pH 7.4) and the MTX content was determined by UV spectrophotometer at 300nm. For comparison, MTX liposomes were prepared using thin film hydration method using lecithin and cholesterol. For skin penetration studies the MTX nanoparticles and liposomes were prepared by incorporating ³H labeled MTX along with the cold MTX. In vitro skin permeation studies were performed with full-thickness porcine ear skin (obtained from local slaughter house) and human skin (NDRI, Philadelphia, USA). The skin was mounted between the donor and the receptor compartments of the Franz diffusion cell. Free (100µg) and encapsulated MTX (MTX equivalent to 100µg) in phosphate buffer (pH 7.4) were placed in the donor compartment. The receptor compartment of the diffusion cell was filled with 0.01%w/v of methyl paraben in phosphate buffer (pH 7.4). Studies were performed at 37°C for 6 and 48hrs. Further in vitro skin disposition studies were carried by treating for 6 hrs and removing the formulation after 6 hrs and following the skin penetration for further 48 hrs. MTX in the receptor compartment and in the skin homogenate was determined using liquid scintillation counter.

RESULTS AND DISCUSSION

The particle size, polydispersity index and zeta potential of MTX nanoparticles was 239.5±13.1nm, 0.27±0.02, -18.56±0.07 respectively while, the particle size, polydispersity index and zeta potential of MTX liposomes was 266.3±17.2, 0.37±0.02, -15.29±0.09 respectively. The encapsulation efficiency of MTX in nanoparticles and was higher than in liposomes (41.52±3.76% vs 13.53±1.26). The release of MTX from zein nanoparticles followed by a zero order release profile with a small initial burst release (Fig. 1). In contrast all the MTX from liposomes was released within 48hrs.

Figure 1: Release of MTX from nanoparticle and liposome formulations in phosphate buffer (pH 7.4). MTX content was measured by UV Spectrophotometry at 300nm; Values are mean ± SD (n=3).
As shown in Fig. 2, the MTX skin penetration from nanoparticles and liposomes was 2 fold higher than a simple solution when treated for 6hrs.

MTX nanoparticles and liposomes showed significantly higher skin retention (2-3 folds) compared to free MTX after 48 hrs treatment (Fig. 4). At the same time, the amount of MTX in the receptor compartment was significantly reduced (3-4 folds) when encapsulated in nanoparticles and liposomes. Even though lower amount of MTX was retained in the human skin compared with the porcine skin, but no MTX was detected in the receptor compartment with nanoparticles and liposomes. The amount of MTX retained in the skin was found to be 4-8µg/ml. It was found to be in the therapeutic concentrations for the treatment of psoriasis.

CONCLUSION
Zein nanoparticles showed higher encapsulation and sustained release of MTX compared to liposomes. Further MTX loaded zein nanoparticles showed significantly higher skin retention of MTX in skin compared to free MTX and MTX liposomes. Overall the study demonstrates that zein nanoparticles are a promising topical delivery vehicle for MTX.

REFERENCES
1. National Psoriasis Foundation (www.psoriasis.org)

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