Enhanced Permeation of Methotrexate *In Vitro* by Ion Pair Formation With L-Arginine

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Received 3 September 2008; revised 29 October 2008; accepted 17 November 2008

Published online 30 December 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21663

ABSTRACT: Ion paired solutions of methotrexate in L-arginine/water/propylene glycol systems were evaluated for their potential to enhance the permeation of methotrexate across rabbit nasal mucosa *in vitro*. The partition coefficient of methotrexate in the methotrexate: L-arginine ion paired systems was observed to be 24 times greater than that of the methotrexate system without L-arginine. The ion pair formation between methotrexate and L-arginine was confirmed by a decrease in the conductivity of the systems in the presence of propylene glycol, a dielectric constant reducing agent. The permeation of methotrexate across the rabbit nasal mucosa from the ion paired systems was observed to be significantly greater ($p < 0.05$) as compared to control systems of methotrexate solution in water and a sodium salt. Furthermore, a threefold increase in the flux of methotrexate was observed when propylene glycol was added to the ion paired systems. These results suggest that methotrexate: L-arginine ion paired systems have potential in improving the permeation of methotrexate across rabbit nasal mucosa and may form the basis for further development of an intranasal therapeutic system of methotrexate. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:3633–3639, 2009

Keywords: methotrexate; ion pair; L-arginine; nasal mucosa; permeation; propylene glycol

INTRODUCTION

Methotrexate, an antineoplastic agent, is widely used in the treatment of many malignant and nonmalignant disorders. Presently, malignancies of the central nervous system are usually treated by lumbar puncture or the intrathecal administration of a high dose of methotrexate. These routes of administration are invasive, require special surgical procedures, and are difficult to use in routine clinical settings. On the other hand, intranasal administration of methotrexate can provide for a more convenient and noninvasive alternative. Wang et al.[1] have previously investigated the possibility of a direct drug transport of methotrexate from the nasal cavity to the cerebro-spinal fluid after intranasal administration in rats.

In order to achieve effective systemic absorption of a drug through the nasal mucosa, the drug moiety must have good solubility and lipophilicty for it to be administered in acceptable volumes of less than 0.3 mL in the nostrils. Unfortunately, methotrexate is a poorly soluble and hydrophilic drug, and thus possesses low permeability across mucosal membranes. Ion pairing concepts for a hydrophilic drug with an appropriate counter ion, in order to improve its permeability and hence bioavailability have been well established by the research activity of Neubert et al.[2,3] Also, the permeation of ion paired cationic as well as anionic hydrophilic drugs has been researched *in vitro* and *in vivo* by numerous investigators.[4–15]

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Journal of Pharmaceutical Sciences, Vol. 98, 3633–3639 (2009)

The objective of this study was to enhance the permeation of methotrexate across rabbit's nasal mucosa by using L-arginine as an ion pairing agent, in order to assess the feasibility for the development of an intranasal delivery system for methotrexate. Furthermore, the effect of addition of propylene glycol on the stability, partition coefficient and permeation of methotrexate was also investigated.

MATERIALS AND METHODS

Materials

Methotrexate, ethanol, *n*-octanol, propylene glycol, L-arginine, and triethylamine were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium pentobarbital injection was purchased from Henry Schein, Inc. (Port Washington, NY) and all other chemicals were HPLC or analytical grade and used as received. Water was deionized and distilled in the laboratory.

Methods

Analytical Methodology

Methotrexate (MTX) was quantitatively analyzed by a Hewlett Packard 1050 series HPLC system with a symmetrical C_{18} column (5 μm, 150 mm × 3.9 mm ID) (Waters Corporation, Milford, MA). The mobile phase consisted of a mixture of methanol:triethylamine/phosphate buffer (25:75) pH 7.3 and the flow rate was set at 0.8 mL/min. MTX was detected at 303 nm with a retention time of 2.71 min. The limit of detection in the HPLC assay was 0.1 μg/mL, and the limit of quantitation was 0.5 μg/mL.

Partition Coefficient Studies

The apparent partition coefficient of MTX between *n*-octanol and solutions of L-arginine (ARG) at pH 7.0 was determined using the shake-flask method.[16] Briefly, ARG solutions of different concentrations were prepared and 2 mL of each solution were taken in screw capped test tubes to which an equal volume of *n*-octanol was added. Each phase had been pre-saturated with the other by equilibration overnight before the experiments. A known quantity of MTX was then added to the *n*-octanol/ARG solution mix and MTX was allowed to partition between the polar and nonpolar solvents at room temperature (25°C) for 24 h till equilibrium was achieved.

A solution depletion technique was employed for the determination of the partition coefficient based on the following equation:

$$P_{\text{app}} = \frac{C_0 - C_\infty}{C_\infty}$$

where C_0 is the initial concentration of drug in the aqueous phase and C_∞ is the final concentration of the drug in the aqueous phase after equilibrium was established.

Conductivity Measurements

The electric conductance of MTX solutions incorporating ARG, and in the presence of different solvents of varying dielectric constants was determined using a traceable VWR digital conductivity meter.

Preparation of the Nasal Mucous Membranes

New Zealand white rabbits (2.0–2.5 kg), purchased from Marland Breeding Farms Inc. (Hewitt, NJ), were euthanized by sodium pentobarbital injection and two pieces of the nasal mucous membranes (1.2 cm × 2.8 cm) were carefully stripped from the rabbit's nasal septum. After excision of the nasal mucous membrane, any adhering cartilage was removed and the nasal membrane was rinsed in oxygenated Kreb's Ringer solution. All the permeation experiments were conducted using freshly excised nasal mucosal membranes and as per the protocols approved by the Animal Care Committee, St. John's University (Queens, NY).

In Vitro Permeation Studies

In vitro permeation studies were conducted using side-by-side permeation cells (Crown Glass, Somerville, NJ) maintained at 37 ± 0.5°C by a thermostatic circulating water bath (VWR). The freshly excised nasal mucosa was immediately mounted on the permeation cells with the nasal mucosal epithelia facing the donor cell and exposing a surface area of 0.16 cm^2. The receptor cell was then filled with Kreb's Ringer solution (3.5 mL, pH 7.4) and the donor cell was filled with the test solution (3.5 mL). Samples (200 μL) were withdrawn from the receptor cell at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h, and replaced with fresh Kreb's Ringer solution in order to keep the receptor