Evaluation of β-Blocker Gel and Effect of Dosing Volume for Topical Delivery

QIAN ZHANG,[1] DOUNGDAW CHANTASART,[2] S. KEVIN LI[1]

[1]Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, Ohio 45267
[2]Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

Received 9 September 2014; revised 13 January 2015; accepted 20 January 2015

Published online 18 February 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24390

ABSTRACT: Although topical administration of β-blockers is desired because of the improved therapeutic efficacy and reduced systemic adverse effects compared with systemic administration in the treatment of infantile hemangioma, the permeation of β-blockers across skin under finite dose conditions has not been systematically studied and an effective topical β-blocker formulation for skin application is not available. The present study evaluated the permeation of β-blockers propranolol, betaxolol, and timolol across human epidermal membrane (HEM) from a topical gel in Franz diffusion cells *in vitro* under various dosing conditions. The effects of occlusion and dosing volume on percutaneous absorption of β-blockers from the gel were studied. The permeation data were compared with those of finite dose diffusion theory. The results showed that skin permeation of β-blockers generally could be enhanced two to three times by skin occlusion. The cumulative amounts of β-blockers permeated across HEM increased with increasing dosing volume. An adequate fit was obtained between the theoretical curve and experimental permeation data, indicating that the experimental results of the gel are consistent with finite dose diffusion theory. In conclusion, the findings suggest the feasibility of using topical gels of β-blockers for infantile hemangioma treatment and topical application with skin occlusion is preferred. J Pharm Sci 104:1721–1731, 2015

Keywords: β-blocker; topical; percutaneous; hemangioma; finite dose; occlusion; skin; diffusion; partition coefficient; stability

INTRODUCTION

Infantile hemangiomas are benign tumors in children occurring in 5%–10% infants. Most of the infantile hemangiomas resolve naturally without any need of treatment, but approximately 10% of the affected infants require therapeutic intervention because of complications.[1,2] Systemic propranolol, an adrenergic β-blocker, has recently become the main treatment for infantile hemangioma.[3–5] However, because of the concerns of potential side effects associated with systemic β-blockers, a safer and more convenient therapy is needed.[6,7] Topical β-blockers are a promising alternative in the treatment of skin hemangiomas that can improve therapeutic efficacy and reduce systemic adverse effects. Currently, topical skin formulations of β-blockers are not commercially available, and topical β-blocker treatments have been carried out using commercial topical eye drops of timolol, a β-blocker, that are not optimized for skin delivery.[8,9] Topical gels are appealing in the development of a topical β-blocker product because they are convenient to use and have a broad range of applications in cosmetics, pharmaceuticals, and medicine.[10,11] Because of their high water content, gels also allow easier migration of the drugs within the vehicle and hydrate the skin that can promote skin permeation compared with other semisolid formulations.[12]

Previously, skin permeation of four β-blockers, propranolol, betaxolol, timolol, and atenolol was investigated for their potential in topical delivery for the treatment of infantile hemangiomas using side-by-side diffusion cells *in vitro* under different pH conditions in the donor solution.[13] The experiments in this previous study were performed under the infinite dose condition to evaluate the steady-state permeability coefficients of the skin for the β-blockers. The results in this study showed that the apparent permeability coefficients of the β-blockers across human skin increased with their lipophilicity and the pH of the donor solution. This suggests that the lipoidal pathway in the stratum corneum (SC) was the major skin transport mechanism for the β-blockers. However, the increase in the permeability coefficients for the β-blockers was less than the 10-fold increase per pH unit expected from the theoretical permeability coefficient versus pH relationship, indicating that the development of an alkaline topical dosage form for effective local delivery of the β-blockers might not be necessary. Although the previous study has provided important information on the possibility of topical treatment of infantile hemangioma using β-blockers, the experiments in the study were designed for mechanistic understanding of skin permeation of β-blockers and did not mimic the *in vivo* situations in clinical setting. Skin permeation of the β-blockers under finite dose conditions was not studied and topical skin formulations were not developed for the β-blockers.

Infinite and finite doses are commonly used in skin permeation studies to evaluate percutaneous absorption of drugs *in vitro*.[14] In infinite dose experiments, the permeant is often applied in a comparatively large dose in which steady-state permeation is achieved and the steady-state flux of the permeant can be determined using the linear region of a cumulative amount delivered versus time plot. Although the infinite dose experimental design is capable of providing scientific information such as steady-state permeability coefficients for mechanistic interpretation, the finite dose method can better resemble the *in vivo* situation in practice. In the finite dose experiments, the applied dose is usually limited and the depletion of the permeant in the donor chamber over time generally results in

Correspondence to: S. Kevin Li (Telephone: +513-558-0977; Fax: +513-558-4372; E-mail: kevin.li@uc.edu)

Journal of Pharmaceutical Sciences, Vol. 104, 1721–1731 (2015)

Table 1. Physicochemical Properties of the β-Adrenergic Blocking Agents

β-Blocker	Molecular Weight (g/mol)	p*K*a	log $K_{o/w}$
Propranolol	259.3	9.5 ± 1.2^a	3.3^a
Betaxolol	307.4	9.4^b	2.8^b
Timolol	316.4	9.2^c	2.1^c

[a]Values from the literature.[16]
[b]Values from the literature.[17]
[c]Values from the literature.[18]

a nonlinear cumulative amount delivered versus time profile. Because of such differences, finite dose permeation data are different from those of the steady state obtained under the infinite dose condition.[15] In general, the cumulative amount of a drug delivered across skin (or the flux) is related to the concentration of the drug in the dosage form (the driving force), and the same drug delivery profile is expected in permeation experiments at the same drug concentration in the donor chamber under the infinite dose condition. When different total amounts of the drug are applied on the skin in the donor chamber (i.e., at same concentration but different dosing volumes) under the finite dose condition, this will result in different extent of drug depletion and drug permeation profiles (i.e., cumulative amount delivered versus time or flux versus time profiles) leading to a dosing volume effect.

The objectives of the present study were to (a) compare the permeation profiles of β-blockers in skin delivery from topical gels under occlusive and nonocclusive conditions at finite doses, (b) investigate the effect of dosing volumes of the gels on the permeation of the β-blockers, (c) evaluate skin permeation of the β-blockers under the finite dose condition based on a finite dose diffusion model, and (d) examine the stability of the β-blocker gels during storage. Three β-blockers propranolol, betaxolol, and timolol were selected as the model drugs in the present study. Table 1 summarizes the physicochemical properties of the β-blockers. Permeation experiments with human epidermal membrane (HEM) were performed in Franz diffusion cells *in vitro* with the β-blocker gels at different dosing volumes to mimic the *in vivo* setting in practice. The following questions for the topical gel formulations were to be addressed. (a) What are the amounts of β-blockers that can be delivered with the topical gel formulations under the finite dose conditions? (b) What are the effects of dosing volume and drug lipophilicity upon skin permeation of the β-blockers from the gels? (c) Does skin occlusion affect skin permeation of the β-blockers and what is the extent of this effect? The present study would address these questions and provide information on the feasibility of topical skin delivery of the β-blockers for potential use in the treatment of infantile hemangiomas. These findings could also assist in future development of a β-blocker dermatological gel formulation as well as the identification of an effective dosing protocol for topical β-blocker delivery.

EXPERIMENTAL

Materials

D,L-Propranolol hydrochloride and betaxolol hydrochloride at purity ≥98% and 1-octanol at purity ≥99% were purchased from Sigma–Aldrich (St. Louis, Missouri). Timolol maleate and hydroxyethyl cellulose (5000 cps) NF (HEC) were purchased from Letco Medical (Decatur, Alabama). Betaxolol hydrochloride ophthalmic solution (0.5%) was purchased from Falcon Pharmaceuticals (Fort Worth, Texas). Sodium azide (NaN_3) was obtained from Acros Organics (Morris Plains, New Jersey). Methyl paraben and propyl paraben were obtained from Professional Compounding Centers of America (PCCA) (Houston, Texas). HPLC grade methanol and ethyl alcohol were purchased from Pharmaco-AAPER (Shelbyville, Kentucky). HPLC grade glacial acetic acid was obtained from EMD Chemicals (Gibbstown, New Jersey). Triethylamine, sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Fisher Scientific (Pittsburgh, Pennsylvania). Phosphate-buffered saline (PBS), pH 7.4, consisting of 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride, was prepared by PBS tablets (MP Biomedicals, LLC, Solon, Ohio) and deionized water. PBS was preserved with 0.02% (w/v) NaN_3 except when PBS was used in the β-blocker gel preparation. The pH of all the solutions was checked with a pH meter (Oakton Instruments, Vernon Hills, Illinois) and adjusted to pH 7.4 with 10% NaOH or 10% HCl when necessary.

Preparation of HEM

Excised split-thickness human cadaver skin from posterior torso of male aged between 19 and 69 years was obtained from the New York Firefighters Skin Bank (New York, New York). The thickness of split-thickness skin before heat separation was measured by a vernier caliper and found to be ~0.05–0.06 cm. HEM, composed of SC and viable epidermis, was separated from the dermis by heat separation.[19] Briefly, the cadaver skin was immersed in PBS at 60°C for 1 min. After heat treatment, the dermis was gently peeled off from HEM by a pair of forceps under immersion in PBS. The HEM was then patted dry with Kimwipe, wrapped in aluminum foil, and stored in a freezer at –20°C for later use.

Preparation of β-Blocker Gel

Topical gels of 4 mg/mL propranolol hydrochloride, 5 mg/mL betaxolol hydrochloride, and 5 mg/mL timolol maleate (equivalent propranolol, betaxolol, and timolol concentrations of 3.5 mg/mL, 4.5 mg/mL, and 3.7 mg/mL, respectively) were prepared with HEC and PBS. Briefly, the drug solution was prepared by dissolving the required amount of the drug in PBS (without NaN_3) in a vial. Then, an appropriate amount of HEC (2% w/v) was dispersed gradually in the drug solution. The dispersion was mixed using a magnetic stirrer until a clear transparent gel was obtained. The gel was then stored in a refrigerator (4°C) to provide maximum hydration and clarity and for later use.

HEM Permeation Study

The β-blocker gels were evaluated in skin permeation experiments using Franz diffusion cells. Prior to the permeation studies, the HEM samples were cut into the desired sizes (~1.5 × 1.5 cm^2) and allowed to hydrate in PBS at 4°C in a refrigerator overnight. Each fully hydrated HEM sample was supported by a Millipore filter (0.45 μm nitrocellulose) and mounted on the Franz diffusion cells with a rubber gasket placed between the SC and donor chamber to provide better sealing of the edges between the diffusion half cells. The diffusion cell setup provided