Transmucosal Delivery of Testosterone in Rabbits Using Novel Bi-Layer Mucoadhesive Wax-Film Composite Disks

STEVEN JAY, WILLIAM FOUNTAIN, ZHENGRONG CUI, RUSSELL J. MUMPER

Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 907 Rose Street, Lexington, Kentucky 40536-0082

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ABSTRACT: Testosterone exhibits very low oral bioavailability because of its low aqueous solubility and extensive first-pass metabolism. The purpose of this study was to develop a novel bi-layer mucoadhesive wax-film composite (WFC), and to test the relative bioavailability of testosterone via the buccal route in rabbits. The release rate of testosterone from optimal WFCs (3/8-in. diameter) per unit surface area was 5.6 μg cm^2 mL^{-1} min^{-1} and was zero-order. Bi-layer WFCs (average weight of 14 ± 2.6 mg and thickness of 186 ± 34 microns) containing 4 mg of testosterone were applied to the buccal pouch of anesthetized New Zealand white rabbits. Rabbits ($n = 3$) injected intravenously had C_{max} and area under the curve values of 1200 ± 46 ng/mL, and $48{,}227 \pm 12{,}995$ ng*min/mL, respectively. Rabbits ($n = 3$) dosed via the buccal pouch had C_{max}, T_{max}, and area under the curve values of 127 ± 13 ng/mL, 200 ± 35 min, and $24{,}221 \pm 1543$ ng*min/mL. The relative bioavailability for rabbits treated with the WFC was $50.2 \pm 3.2\%$ with a coefficient of variation of 6.4%. It was concluded that these bi-layer mucoadhesive WFCs disks could deliver physiologically relevant amounts of insoluble drugs such as testosterone across the buccal mucosa. J Pharm Sci 91:2016–2025, 2002

Keywords: buccal; Eudragit®; polycarbophil; sodium lauryl sulfate

INTRODUCTION

Buccal delivery for the transmucosal absorption of drugs into the systemic circulation offers a number of advantages over oral delivery, especially for those drugs that have poor oral bioavailability and/or those drugs that suffer from extensive first-pass metabolism in the liver.[1,2] Conceivably, buccal delivery systems provide easy administration, thereby increasing patient compliance. Many different types of buccal delivery systems have been developed including sprays, solutions, erodable or nonerodable multi-layer adhesive films, adhesive tablets, and lollipops.[1,3–8] Several peptides have been delivered via the buccal route including thyrotropin-releasing hormone, insulin, octreotide, leuprolide, and oxytocin, among others.[1,2,6,9,10] In most cases, the relative bioavailability of these peptides by the buccal route was quite low (i.e., 0.1–5.0%), but was significantly increased by the inclusion of chemical penetration enhancers. In contrast, recent studies in humans have shown that the relative bioavailability of small lipophilic drugs by the buccal route (such as buprenorphine, testosterone, atipamezole, fentanyl, butorphanol, melatonin, and nifedipine, among others) are relatively high (i.e., 20–80%) and routinely comparable to nasal administration.[1–5,7,8]

The barriers for effective buccal delivery are similar to those for oral delivery including substantial physical barriers (mucous layer that lines a multi-layered epithelium) and chemical barriers caused by the presence of a multitude of peptidases and proteolytic enzymes.[1,2] These barriers

Correspondence to: Russell J. Mumper (Telephone: 859-257-2300, extension 258; Fax: 859-323-5985; E-mail: rjmump2@uky.edu)

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contribute to the relatively low permeability of the buccal mucosa. However, the buccal mucosa is well vascularized. Rapid and extensive drug absorption is possible, especially for those drugs that are small, lipophilic, and remain stable to enzymatic degradation. Further, unlike formulations intended for injection or nasal administration, buccal delivery systems do not have to be sterile, which greatly reduces manufacturing costs. Chemical penetration (absorption) enhancers such as surfactants, bile salts, and fatty acids have been used to markedly increase the bioavailability of drugs via the buccal route. However, there are no approved products on the market with designated penetration enhancers, so the inclusion of these chemicals in buccal delivery systems may be undesirable.[1]

Cui and Mumper[11] have recently described a novel bi-layer mucoadhesive wax-film composite (WFC) composed of a pH-sensitive mucoadhesive layer and a pharmaceutical wax as the impermeable backing layer. The mucoadhesive layer consisted of Noveon® AA-1, a crosslinked polyacrylate polymer, and Eudragit® S-100, the pH-sensitive anionic polymer polymethacrylic acid-*co*-methyl methacrylate. The pharmaceutical wax was DENTSPLY® Utility Wax. Cui and Mumper reported that bi-layer mucoadhesive WFC, containing either plasmid DNA or subunit protein antigen, applied to the buccal pouch in rabbits produced enhanced antigen-specific antibody and proliferative immune responses to expressed antigen or subunit protein antigen over those responses observed after conventional subcutaneous injection of adjuvanted protein antigen.[11] It was also demonstrated that the adhesion time of these WFCs to both glass (simple *in vitro* model) and the rabbit buccal pouch could be controlled by modifying the relative ratio of these two excipients, the thickness of the disk, and the extent of the wax-backing layer. These novel WFCs potentially provide advantages over other reported buccal delivery systems mentioned above because of the following: 1. relative low cost, 2. ease of production, 3. controllable mucoadhesion time from minutes to hours, and 4. biocompatibility and bioerodability.

The overall objective of these studies was to further develop the bi-layer mucoadhesive WFCs for the transmucosal delivery of testosterone, and to determine the relative bioavailability after buccal administration to that of intravenous administration in rabbits. To this end, factors influencing the adhesion of the WFCs both *in vitro* and *in vivo* as well as the rate of release of testosterone under the conditions of temperature, media, and drug loading were also investigated.

MATERIALS AND METHODS

Materials

Polycarbophil (Noveon® AA-1, USP) was obtained from BF Goodrich (Cleveland, OH). Polymethacrylic acid-*co*-methyl methacrylate (Eudragit® S-100) was a generous gift of Röhm America Inc. (Piscataway, New Jersey). Testosterone and potassium phosphate dibasic were purchased from Aldrich Chemicals (Milwaukee, WI). DENTSPLY® Utility Wax was obtained from DENTSPLY International (York, PA). Sodium hydroxide NF, ethanol (95%) USP, and sodium lauryl sulfate (SLS) NF, were purchased from Spectrum Laboratory Products (Gardena, CA). Testosterone enzyme-linked immunosorbent assay (ELISA) kits were acquired from IBL Immuno-Biological Laboratories (Hamburg, Germany). Rabbit serum (cat. 16120-102) was purchased from Invitrogen Corporation (Carlsbad, CA)

Preparation of WFCs Containing Testosterone

Placebo WFCs were made as previously described by Cui and Mumper.[11] Briefly, ethanol-based gels were first prepared by mechanical mixing for up to 3 h using a Caframo Mixer. The final clear mucoadhesive gel consisted of Noveon® AA-1 (3% w/w) and Eudragit® S-100 (1% w/w) in ethanol. For testosterone WFCs, the appropriate amount of testosterone was first dissolved in the mucoadhesive gel. The mucoadhesive gel solutions were sonicated for 10–15 min to remove air bubbles. Next, 7 mL of each ethanol-based gel was cast in a plastic circular hollow ring (diameter = 6.2 cm; total area = 30.175 cm^2) fixed on Mylar film. Ethanol was removed by drying the ethanol-based gels overnight under various conditions (i.e., 0°, 5°, 25°, and 55°C) to produce optimal mucoadhesive mono-layer films. The films were dried to a constant weight (Mettler AC100 balance; sensitivity = ±0.1 mg). The mono-layer films (diameter = 6.2 cm) were coated with melted DENTSPLY® Utility Wax by carefully dipping one side of the mucoadhesive film into the melted wax at 88°C for 1–2 s. The thin wax coating on the films was allowed to harden at ambient condition.