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In Vitro Dissolution Method for Evaluation of Buprenorphine In Situ Gel Formulation: A Technical Note

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INTRODUCTION

Buprenorphine is a highly lipophilic partial μ-opiate agonist. It has been used for several years for the treatment of drug addiction.[1,2] Buprenorphine is relatively well absorbed by most alternate routes, including the sublingual,[3-6] buccal,[6] and nasal routes.[7] Sublingual (8 mg/day) administration of buprenorphine is superior to oral administration (20 mg/day) of methadone.[8] A parenteral preparation containing 0.3 mg of buprenorphine in 1 mL solution, Buprenex, has been on the market for several years. Recently, the National Institute on Drug Abuse, in collaboration with a pharmaceutical company (Reckitt and Benckiser), has developed a sublingual buprenorphine tablet, Subutex, for the treatment of drug abuse. However, the current dosage forms are associated with a potential for abuse as well as a large variation in efficacy. For example, recently, several buprenorphine-related deaths among drug addicts in France have been reported.[9] Therefore, a new formulation of buprenorphine needs to be developed for opiate maintenance programs, because a means of administration that would deliver the drug slowly into the body, smoothing out blood levels and obviating serum highs, is desirable.[10] To provide long-term constant buprenorphine delivery, researchers need to develop a sustained-release formulation that could be administered subcutaneously and release the drug for approximately 1 month at relatively constant rates sufficient to treat addiction. The development of a subcutaneous buprenorphine sustained-release preparation would be beneficial to patients because buprenorphine is not known to cause any local irritation or tissue necrosis following subcutaneous injection.[11-13]

There are 2 primary approaches for fabricating a subcutaneous sustained-release formulation: (1) microencapsulation, in which the drug is encapsulated in a biocompatible polymer[14]; and (2) in situ gel, in which the drug is dissolved or suspended in a biocompatible polymer solution that solidifies in situ following injection.[15-19] In situ gel formulations are more likely to be accepted by patients because of the ease of administration. Several in situ gel formulations have been developed for the delivery of therapeutic agents. These injectable formulations are composed of a water-insoluble biodegradable polymer dissolved in a water-miscible biocompatible solvent. Following subcutaneous or intramuscular injection into an aqueous environment, the biocompatible solvent diffuses out of the polymer while water diffuses into the polymer matrix. In the presence of water, this polymer coagulates or precipitates, resulting in a solid polymeric implant. These novel in situ gel formulations have been used for the delivery of model proteins,[15] luteinizing hormone-releasing hormone antagonists,[16] growth factors,[17] anti-inflammatory agents,[18] and antitumor agents.[19] The drug release from these in situ gel systems is analogous to that reported for implant systems prepared ex vivo.

The long-term goal of our project is to develop a novel in situ gel formulation of buprenorphine. First, we needed to develop a reliable in vitro dissolution method for routine evaluation of in situ gel formulations.

MATERIALS AND METHODS

Materials

The copolymer poly(DL-lactic/glycolic acid) (PLGA) 50:50, RG 502 and 502H, inherent viscosity 0.2 dL/g, 13 500 molecular weight, was obtained from Boehringer Ingelheim (Ingelheim, Germany). A Spectra-Por dialysis bag (Sigma Aldrich, St. Louis, MO) (with cutoff 12 000-14 000 Da), buprenorphine HCl, n-methyl pyrrolidone, acetonitrile, glacial acetic acid, and tetrabutylammonium dihydrogen phosphate were obtained from Sigma Aldrich (St Louis, MO).

Methods

Preparation of In Situ Gel

Two different Resomer types of PLGA, RG 502 (an end-capped variety) and 502H (an uncapped variety), were used in these experiments to achieve different dissolution profiles. These 2 polymers were selected because they are similar except in polarity, and this difference was expected to result in different dissolution profiles of buprenorphine. To evaluate the in vitro dissolution method, it was necessary to evaluate whether the proposed method was capable of identifying

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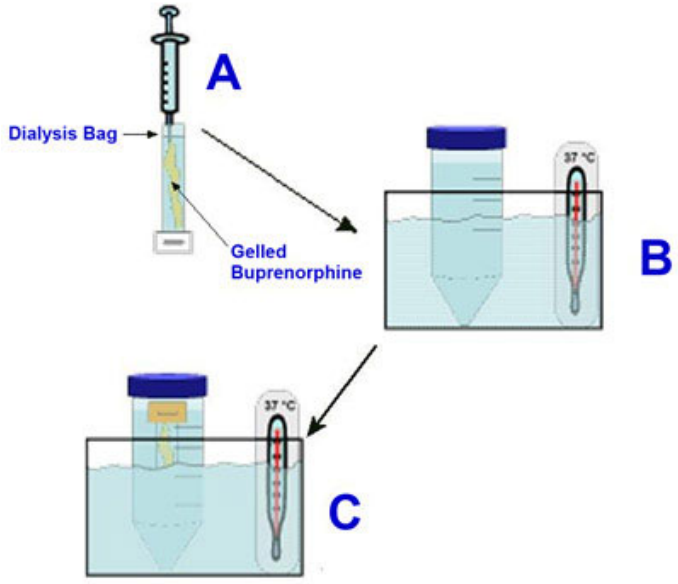

Figure 1. Schematic of the in vitro dissolution method for in situ gel formulation. (A) Copolymer solution containing buprenorphine was injected into a dialysis tube containing the dissolution medium. (B) Polypropylene tube containing the dissolution medium was immersed in a water bath. (C) Dialysis tube containing the in situ gel was immersed in the polypropylene tube.

the differences in dissolution profiles. A 50% (wt/vol) polymer solution was prepared by dissolving 250 mg of PLGA in 0.5 mL of n-methyl pyrrolidone. N-methyl pyrrolidone is a relatively nontoxic solvent commonly used for solubilization,[20] transdermal drug delivery,[21] and in situ gel formulation.[22] A specific amount (5 mg) of buprenorphine HCl was added to the polymer solution and mixed thoroughly for 60 seconds using a vibrating mixer (Vibra Model 231, Fisher Scientific, Pittsburgh, PA). The resultant drug/polymer solution was transformed into a gel by injecting it into a Spectra-Por dialysis bag containing 1.5 mL of the dissolution medium (Figure 1).

In Vitro Dissolution Study

The dissolution medium used in this study was either phosphate buffer (pH 7.4) or 0.15% Tween 80 solution. Two different dissolution media were used to identify the one that was capable of distinguishing the formulation differences. The dialysis bag containing the buprenorphine gel was immersed in a 50-mL polypropylene tube containing 40 mL of the dissolution medium. This volume was selected to maintain a sink condition throughout the dissolution study. The solubility of buprenorphine HCl in water is 17 mg/mL.[23] If drug release was as fast as possible (ie, 5 mg/day), the concentration of buprenorphine HCl would be less than 1% of the saturation solubility (the recommended concentration is 10% or less),[24] which is far less than the concentration usually recommended to maintain a sink condition during a dissolution study. All of the dissolution medium was removed at preset time intervals (0.5 hours, 1.5 hours, 4 hours, 7.5 hours, 1 day, 2 days, 4 days, 7 days, 11 days, 15 days, 18 days, 22 days, 26 days, 29 days, 36 days, 42 days, and 55 days) and analyzed for buprenorphine. The dissolution medium was replaced with fresh medium to maintain a sink condition. The amount of buprenorphine released during a sampling period was measured using high-performance liquid chromatography (HPLC). The experiments were conducted independently in triplicate.

HPLC Analysis

The analysis of buprenorphine was performed using a rapid and sensitive HPLC method.[25] Since our samples were relatively pure compared with plasma samples, the method was slightly modified to shorten the retention time for buprenorphine. In short, the chromatographic system consisted of a Waters Model 600 programmable solvent delivery module, a Waters Model 717plus auto sampler, and a Waters Model 996 photodiode array detector (Waters, Milford, MA). The chromatography was performed using a Supelcosil C-8 (5 μm, 4.6 × 250 mm; Supelco, Bellefonte, PA) column. The mobile phase consisted of 80% 0.05 M acetate buffer with 0.002 M tetrabutylammonium dihydrogen phosphate and 20% acetonitrile. A flow rate of 1.5 mL/min was used. The mobile phase was vigorously purged with helium gas for 15 minutes prior to use. The identity of the eluting peaks was verified using a diode array detector. Standard calibration curves ($r^2 > 0.99$) for buprenorphine HCl, ranging from 0.5 μg/mL to 16 μg/mL concentrations, were prepared. The concentration of buprenorphine in each sample was determined by intrapolating the peak height to the buprenorphine standard curve. Each experiment was performed in triplicate.

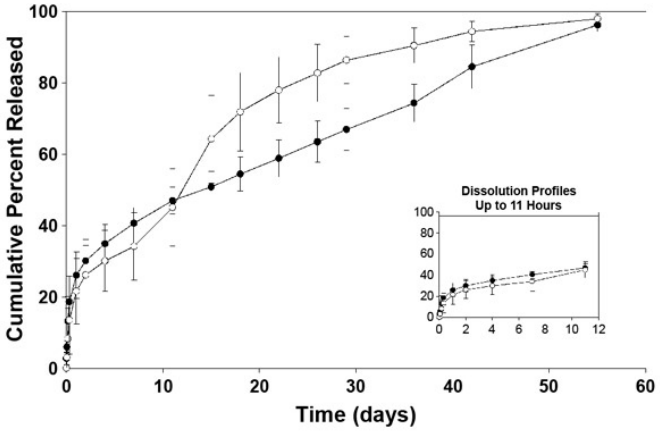

Figure 2. Dissolution profiles of buprenorphine formulation in phosphate buffer. RG 502 formulation (●); RG 502H formulation (○).

Statistical Analysis

The amount of buprenorphine released from the different formulations during the in vitro study was compared by Student *t* test using the SAS software package. A *P* value of < .05 was considered to be evidence of a significant difference.

Results and Discussion

Both formulations, RG 502 and RG 502H, formed a solid gel as soon as they came into contact with the dissolution medium. Figures 2 and 3 show the dissolution profiles of the formulations in phosphate buffer and Tween 80, respectively. Irrespective of the copolymer or the dissolution medium, the data obtained from this study were associated with very high standard error. This can be explained by evaluating the formation of the in situ gel. During the formation of the gel, buprenorphine was distributed throughout the matrix. Since the formation of the gel was a spontaneous process, there was little control over the distribution of buprenorphine throughout the matrix. Because of the differences in distribution between batches, the drug release also varied from batch to batch, which resulted in high SDs. Irrespective of the dissolution medium, both formulations showed less than 3% drug release within the first 30 minutes. The dissolution profiles in phosphate buffer showed significant differences ($P < .05$) in drug release from RG 502 and RG 502H after 11 days of dissolution. Despite the differences, drug release in this dissolution medium continued over 55 days. Drug release from RG 502 was linear from day 4 until the end, but RG 502H showed a significant "burst" between 11 and 15 days, with 45% to 64% released (Figure 2). Because of the uncapped nature of RG 502H, it is relatively more polar than RG 502. The dissolution profiles obtained in Tween 80 showed faster drug release than did those in phosphate buffer.

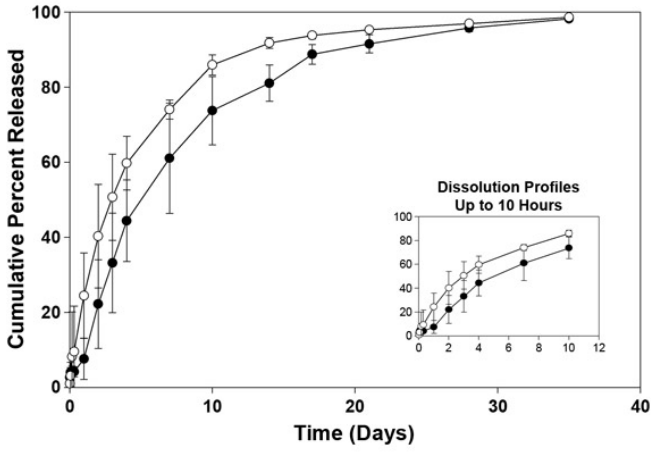

Figure 3. Dissolution profiles of buprenorphine formulation in Tween 80. RG 502 formulation (●); RG 502H formulation (○).

In phosphate buffer, RG 502H showed a slightly faster dissolution than RG 502, but the differences were not statistically significant ($P > .05$) (Figure 3). This faster dissolution in Tween 80 was due to the solubilizing characteristics of Tween. The dissolution in Tween was completed within 35 days, compared with 55 days for phosphate buffer. Tween may have enhanced the dissolution by increasing the solubility of buprenorphine. Unlike RG 502H in phosphate buffer, RG 502H in Tween 80 did not show any burst release during the period of dissolution, because both RG 502 and RG 502H experienced faster dissolution in Tween 80.

Conclusion

The in situ gel formulation of buprenorphine showed sustained drug release for a prolonged period of time. The drug release from RG 502 followed a linear pattern throughout the dissolution without any significant burst release. The amount of buprenorphine released during the first 30 minutes, irrespective of the type of Resomer or dissolution medium, was less than 3%. Drug release continued over 55 days in phosphate buffer and 35 days in Tween 80. The in vitro dissolution method developed during this study was capable of identifying formulation differences and thus will be useful for routine drug delivery research, particularly in situ gel formulation development research. In situ gel formulations are routinely compared using animal models,[21] so development of such an in vitro method will expedite formulation evaluation.

Acknowledgments

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