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In-vitro and in-vivo characterization of a buprenorphine delivery system

Sofie R. Kleppner, Raj Patel, Joseph McDonough and Lauren C. Costantini

Abstract

Buprenorphine is a mu-opioid receptor partial agonist with enhanced safety and comparable efficacy to methadone for treatment of opioid dependence. The sublingual formulation of buprenorphine, approved for treatment of opioid dependence, produces variable buprenorphine blood levels and requires frequent dosing that limits patient compliance. To achieve stable buprenorphine levels that may improve patient outcome, an implantable sustained buprenorphine delivery system was developed. Each implant consists of ethylene vinyl acetate copolymer and 90 mg buprenorphine HCl, and measures 26 mm in length and 2.4 mm in diameter. Steady-state release in-vitro was 0.5 mg/implant/day. In-vivo pharmacokinetics and safety were examined for up to 52 weeks in beagle dogs receiving 8, 16 or 24 subcutaneous implants. Plasma buprenorphine concentrations correlated with the number of implants administered. Peak buprenorphine concentrations were generally reached within 24 h after implantation. Steady-state plasma levels were attained between 3 and 8 weeks, and were maintained for study duration, with a calculated mean release rate of 0.14 ± 0.04 mg/implant/day. There were no test-article-related adverse effects. This delivery system can provide long-term stable systemic buprenorphine levels, and may increase patient compliance, thereby improving outcome for opioid-dependent patients.

Introduction

Opioid addiction is characterized by persistent drug use in spite of negative health, legal, social and personal ramifications. Current pharmacological treatments for opioid addiction include methadone and, more recently, buprenorphine. Buprenorphine is available in a sublingual formulation for the treatment of opioid dependence, and is gaining favour due to its comparable efficacy and superior safety profile to methadone and L-alpha-acetylmethadol (Strain et al 1994; Johnson et al 2000). Buprenorphine is a partial agonist at the mu-opioid receptor and an antagonist at the kappa-opioid receptor. Due to this mixed agonist–antagonist quality, a plateau or ceiling effect occurs in that high doses of buprenorphine do not cause significant complications (Lewis 1985; Walsh et al 1994).

Sublingual buprenorphine is easily administered; however, long-term maintenance treatment via this route is problematic for various reasons. First, plasma concentrations peak quickly and drop steeply with each sublingual dose, causing withdrawal symptoms (Lopatko et al 2003). Second, the sublingual route requires strict patient compliance. Third, sublingual buprenorphine can be diverted for illicit use or abused via crushing and intravenous injection (though the addition of naloxone to the formulation may reduce this form of abuse and diversion). Finally, buprenorphine requires frequent visits to the clinic for prescription refills, which include daily supervised dosing in some countries, a factor negatively impacting on compliance and overall treatment costs.

An implantable, long-term delivery formulation of buprenorphine could improve buprenorphine treatment by ensuring compliance, maintaining stable plasma levels of medication and reducing the likelihood of buprenorphine abuse and diversion. The delivery system described in this study is a non-erodible implant consisting of buprenorphine blended with ethylene vinyl acetate (EVA, a copolymer approved by the FDA for other implant applications). Each sterile implant contains 90 mg of buprenorphine and measures 26 mm in length and 2.4 mm in diameter. After subcutaneous implantation into patients, long-term

Titan Pharmaceuticals, Inc, 400 Oyster Point Blvd, Suite 505, South San Francisco, CA 94080, USA

Sofie R. Kleppner, Raj Patel, Lauren C. Costantini

Southwest Research Institute, 6220 Culebra Road, San Antonio, Texas 78238-5166, USA

Joseph McDonough

Correspondence: L. C. Costantini, Titan Pharmaceuticals Inc., 400 Oyster Point Blvd, Suite 505, South San Francisco, CA 94080, USA. E-mail: lcostantini@titanpharm.com

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plasma buprenorphine levels can be maintained, with one or multiple implants to achieve an individualized dose. Implants can be readily removed if dosing must be discontinued. In this study, we describe the development of these EVA-based implants, their release characteristics in-vitro and their release of buprenorphine for up to one year in-vivo with no adverse effects.

Materials and Methods

Formulation

Buprenorphine HCl (USP) was milled and screened (minus 120 mesh) and dry blended (75% by weight) with 25% EVA copolymer (UE 654-67EVA containing 33 wt% by weight vinyl acetate). The blended EVA/buprenorphine mixture was extruded via Microtruder screw extruder (Model No. RCP-025; Randcastle Extrusion Systems, Cedar Grove, NJ) to form a fibre 2.4 mm in diameter. The Randcastle Microtruder is a single screw extrusion system. The screw has a ¼-inch diameter (6.35 mm). The barrel temperature is controlled independently by three heating zones; one extra temperature controller is responsible for the die block. The diameter of the extrudate is controlled by the die orifice. Feeding zone and gear plate are water cooled. The extrudate is extruded horizontally and is collected for further processing. The extruded non-biodegradable fibre was cut into implants of 26 mm in length. After initial release testing of a subset of implants per batch (see below, In-vitro release), remaining implants per batch were washed in 95% ethanol (USP) at room temperature for 30 min to remove surface drug and thus minimize the initial release of buprenorphine. The washed implants were dried (air dried at room temperature for 30 min, then forced air at 40°C for 1 h followed by vacuum drying at 30°C for 24 h) to remove residual ethanol, and visually inspected. Surface and cross-sectional morphology were evaluated using scanning electron microscopy (SEM; Cambridge StereoScan Model 250). Implants were placed in moisture barrier foil pouches (one implant per pouch), heat-sealed and then sterilized using gamma irradiation (2.9–3.1 Mrads).

In-vitro release

Implants were weighed and then placed in a jar containing 50 mL 0.5% sodium dodecyl sulfate as the receptor phase. Since solubility of buprenorphine is low in water, to maintain sink conditions, sodium dodecyl sulfate was used as a surfactant to increase solubility. The jars were placed on an orbital table in an incubator at 37°C. Samples were taken at 24-h intervals by a complete change of the receptor phase. Sample solutions were analysed by high-performance liquid chromatography (HPLC) and the data plotted as mg of drug released per day against time in days. HPLC was performed using a Waters Symmetry C-18, 5 micron (4.6 mm × 250 mm; PN WAT054275) column at 40°C. The mobile phase was 1.0% sodium acetate, pH 5.0 (Solution A), and methanol (Solution B), at a ratio of 15% A and 85% B and a flow rate of 0.8 mL min^{-1}. The injection volume was 25 μL. Detection was accomplished by means of a UV/VIS (Waters Model 490) detector at a wavelength of 288 nm. Instrument control and data acquisition were facilitated using a Waters Millennium (V 2.15) software package. The external calibration was obtained using buprenorphine standard solutions prepared in methanol. Since solubility of buprenorphine is low in water, ethanol was used to obtain the required concentrations. After the 14-day release profile testing, the implants were placed in a 30°C vacuum oven to dry for approximately 24 h. The implants were then cut into approximately 2-mm pieces, weighed and placed in a 250-mL volumetric flask. Approximately 200 mL of methanol was added to the flask, and a stir bar was placed in the solution. The pieces were stirred for approximately 24 h at room temperature. Methanol was then added to a final volume of 250 mL, and samples were assayed for buprenorphine content via HPLC using the method described above.

In-vivo implantation

The in-vivo release of buprenorphine from implants was investigated in male and female beagle dogs. Young adult beagle dogs (Marshall Farms and Covance Research Products, Inc.), 7–12 kg, were housed in 12-h light–dark cycles and allowed free access to Certified 5007 Canine Diet or Purina Certified Canine Chow and tap water. General procedures for animal care and housing were in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and the Animal Welfare Standards incorporated in 9 CFR Part 3, 1991.

Before implantation, each dog was lightly anaesthetized with isoflurane. Dogs received 8 (n = 2), 16 (n = 2), or 24 (n = 18) buprenorphine-containing implants subcutaneously to the dorsal scapular region. An additional group of dogs received 24 EVA-only control implants (n = 16). A small incision was made through the skin and a trocar was inserted subcutaneously, then loaded with one implant. The stylet was inserted to hold the implant in place and the trocar was carefully removed, leaving the implant in the subcutaneous space. Each site was sutured closed and examined at each blood collection time point. One subset (n = 8) of 24-implant dogs was administered an additional 6 implants at 8.5 months to maintain at least 80% of steady-state plasma levels (C_{ss}) for 1 year (levels dropped below 80% C_{ss} at 8.3 months). All dogs were monitored for complications, including irritation, inflammation, infection or other site-specific adverse effects, as well as full toxicological profiles. The 16-implant dogs were explanted at 8 months to obtain off kinetics and clearance, and were followed for 4 subsequent weeks.

At the end of study, dogs were injected with thiopental sodium (30 mg kg^{-1}), blood was collected, and dogs were exsanguinated. One subset (n = 8) of 24-implant dogs was euthanized at 1 month, while the remaining dogs were on study for 9–12 months. Necropsy followed, and the skin adjacent to implant sites was reflected to expose implant sites. Tissue surrounding the sites was removed for histological analyses, and each implant was explanted and analysed for remaining content via HPLC. Tissue surrounding the implant sites were placed in 10% normal bovine serum and formalin fixed. All formalin-fixed samples were infiltrated and embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin

and eosin (H & E). Each of the sections was evaluated using an accepted grading scheme based upon ANSI/AAMI/ISO 10933-6 and ASTM F 981-99 guidelines.

Plasma analyses

Blood samples were collected (via vein puncture) into tubes containing ethylene-diamine-tetra-acetic acid (EDTA) at various times throughout study and analysed for buprenorphine and norbuprenorphine content via liquid chromatography/mass spectrometric analyses (LC/MS/MS; proprietary method on file at ABC Laboratories). The method was developed and validated for determination of buprenorphine and norbuprenorphine in beagle plasma in the linear range of 0.490–98.0 ng mL^{-1} with internal standard quantitation. Norbuprenorphine calibration range was 0.510–102 ng mL^{-1}. Standard curves were produced by linear regression using the peak area ratios of analyte to each respective deuterated analyte (used as internal standard).

Pharmacokinetics

The plasma concentration–time curve was used to determine C_{ss}, area under the curve (AUC) and release rate from implants during C_{ss}. AUC was calculated using the trapezoidal rule. The amount of drug remaining in implants was subtracted from the content of drug loaded into implant to determine the total amount of drug released. For release rate calculations, it was assumed that the concentration of drug in plasma is directly proportional to the amount of drug released during that time interval (i.e., all pharmacokinetic processes (release from implant, absorption into bloodstream, metabolism, elimination) were assumed to be dose-dependent and linear). The plasma concentration–time curve was divided into pre-C_{ss} phase and C_{ss} phase by visual inspection of data. The AUC was calculated for the entire curve, and for each of the designated phases. Drug released during C_{ss} = total drug released × AUC_{ss}/AUC_{total}. This amount was divided by the number of C_{ss} days and by the number of implants. The result is an estimate of amount of drug released per day per implant during C_{ss}.

Statistical analyses

Statistical analysis of the effects of time and washing on drug release in-vitro was performed using a repeated measures analysis of variance (after tests of normality showed that the values had a reasonable approximation to normal distributions). A post-hoc comparison of the individual groups was performed for each time point and successive time interval using analysis of variance test to discern between differences in drug release by washing. Statistical analysis of the effects of time and number of implants on drug release in-vivo was performed using a repeated measure analysis of variance on ranks (equivalent to Friedman's test; two of the groups had an N value of 2, thus a non-parametric approach was utilized). Two post-hoc comparisons were made: the first explored differences between groups at each time point to examine the absolute difference in plasma concentration between groups at each time point, and the second explored differences between groups at successive time intervals to examine the change in plasma concentration at each time interval (delta). $P < 0.05$ denoted significance in all cases. No adjustment for multiplicity was employed.

Results

In-vitro characterization

Buprenorphine implants were extruded with consistent dimensions of 2.4 mm ± 10% in diameter and 26.0 mm ± 10% in length (Figure 1A). Buprenorphine content averaged 90.0 mg/implant ± 10% (n = 16). An SEM photomicrograph of an implant in cross-section (Figure 1B) showed that EVA and buprenorphine are homogeneously distributed. Figure 2 illustrates the in-vitro release rate of buprenorphine from implants over time. Unwashed implants released 10 mg/day during the first day, and 5–6 mg/day over the following 4 days, resulting in an overall rate that would deplete the implants within 18 days in-vitro (by extrapolation). Release from the implants is predominantly dependent on surface area, but the high release rate also reflects a greater concentration of buprenorphine on

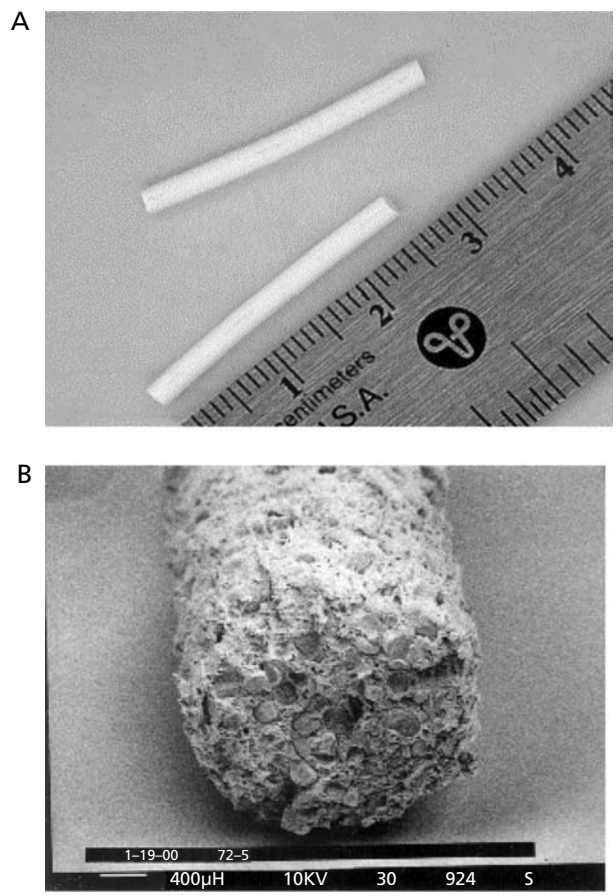

Figure 1 Images of buprenorphine implant. A. Scale image, dimensions are 26 mm in length × 2.4 mm diameter ±10%. B. SEM of cross-section of 75% loading buprenorphine in EVA showing a homogeneous mix of EVA and buprenorphine.

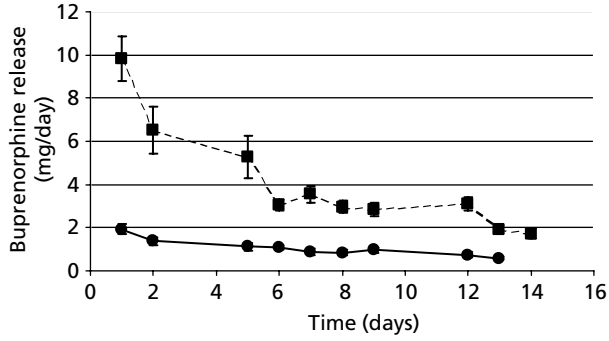

Figure 2 Buprenorphine is released in-vitro from washed and unwashed implants. In-vitro release of buprenorphine HCl from unwashed implants (squares) and implants washed for 30 min in EtOH (circles); n = 6 per group, ± s.d.

the surface and just beneath the implant surface. In an effort to minimize the initial release rate, the implants were washed with 95% ethanol (EtOH) for 30 min. The drug content of the implants that were washed for 30 min was also within the specifications of 90.0 mg/implant ±10%. The washed implants released 2 mg/day on the first day of release, and stabilized to approximately 1 mg/day over the subsequent 13 days.

The effect of time and washing on drug release was statistically examined, and washing was shown to have a significant effect on release rate over time ($P < 0.0001$, analysis of variance). Post-hoc analyses showed a significant difference in the change of release rate at each successive time point between unwashed and washed implants (delta) ($P < 0.0001$) until day 8, when the slopes of the lines of each group were no longer different ($P = 1.00$ between days 8 and 9, $P = 0.71$ between days 9 and 12). Release rates between days 12 and 13 were significantly different ($P < 0.0001$). The 30-min EtOH-washed implants were utilized for all subsequent studies.

In-vivo safety

All dogs tolerated the implant procedure well. Dogs receiving all doses of buprenorphine-containing implants were lethargic the first day after implantation, which correlated with maximum plasma concentrations (C_{max}), and was most likely due to the sedative nature of buprenorphine. No lethargy was observed in dogs receiving control (EVA only) implants.

No signs of toxicity or infection at the sites of implantation were observed in any dogs. Two dogs (24-implant) developed irritation considered secondary to skin preparation and implant procedure as opposed to a direct effect of the buprenorphine or control implants. Brief antibiotic treatment resolved the irritation.

Implants were easily retrieved from all dogs through a 3-mm incision using forceps at the end of treatment period. Minor adhesion to implants was observed, although this was not enough to hinder explantation. Upon explantation, no necrosis or visible vascularization was noted in the tissue adjacent to the implants in any dogs (Figure 3A). Microscopic examination of implant sites showed that tissues were within normal histomorphological limits. Histological analyses showed that the implant–tissue interfaces were characterized by a variable rim of fibrous tissue infiltrated by low to minimal numbers of macrophages, neutrophils, lymphocytes and plasma cells (Figures 3B and 3C). These aggregates of inflammatory cells were limited and had no biological significance. The implants were considered slight- to non-irritant when compared with the placebo implants (slight-irritant at 1 month, non-irritant at 10 months). The local response was biologically appropriate and was within the expected range for an article with its physical characteristics and difference in post-implantation duration.

One dog that received 24 implants was euthanized on day 321, after being found in a moribund state. Necropsy results suggested that this dog suffered from aspiration pneumonia. Plasma buprenorphine levels on day 321 were unusually high, but well below C_{max}, and resulted from impaired metabolism due to the moribund state caused by the pneumonia. A complete investigation of this dog was undertaken, and it was concluded that the sequence of events to explain the sudden death of this dog were as follows. Aspiration of foreign material (unknown event) leading to a chronic, unappreciated aspiration pneumonia with sudden death due to overwhelming sepsis secondary to acute release of bacteria or their toxic products from the infected, damaged lung. One dog that received control implants died on day 84; a definitive cause of death could not be established, although its occurrence was not considered related to the control implant.

Though 5.3% of study dogs showed a serious adverse event, it was concluded that neither of these events were related to buprenorphine implant. There were no test-article-related effects on clinical signs, body weights or food consumption. Based upon the results of this study, implants appear to be generally well tolerated for a period of up to 12 months following subcutaneous implantation in dogs.

Pharmacokinetics

Figure 4 illustrates plasma buprenorphine levels over time in dogs. Plasma levels achieved C_{max} within the first week in almost all dogs, and then decreased in a non-monotonic manner to C_{ss} at approximately 6 weeks post-implantation. Steady state was maintained through the duration of the study period, and was dose-proportional. Dogs receiving 16 and 24 implants maintained C_{ss} plasma levels 2.7- and 3.5-fold higher, respectively, than those receiving 8 implants. No buprenorphine was detected in dogs receiving control implants. Pharmacokinetic data are summarized in Table 1.

The effect of time and number of implants on plasma levels of buprenorphine was statistically examined between weeks 0 and week 36. Week 36 was utilized as the last time point for analyses since the sample size was significantly reduced after that time point due to sacrifice of subgroups of dogs and re-implantation of other subgroups of dogs. The overall analyses showed a significant effect of the number of implants on plasma levels over time via analysis of variance on ranked data ($P < 0.005$). There was a statistically significant interaction between time and treatment ($P < 0.0001$).

Post-hoc analyses at each time point showed significant differences among the 3 groups, except for the first time point (3; $P = 0.468$). Pair-wise comparisons at each time point

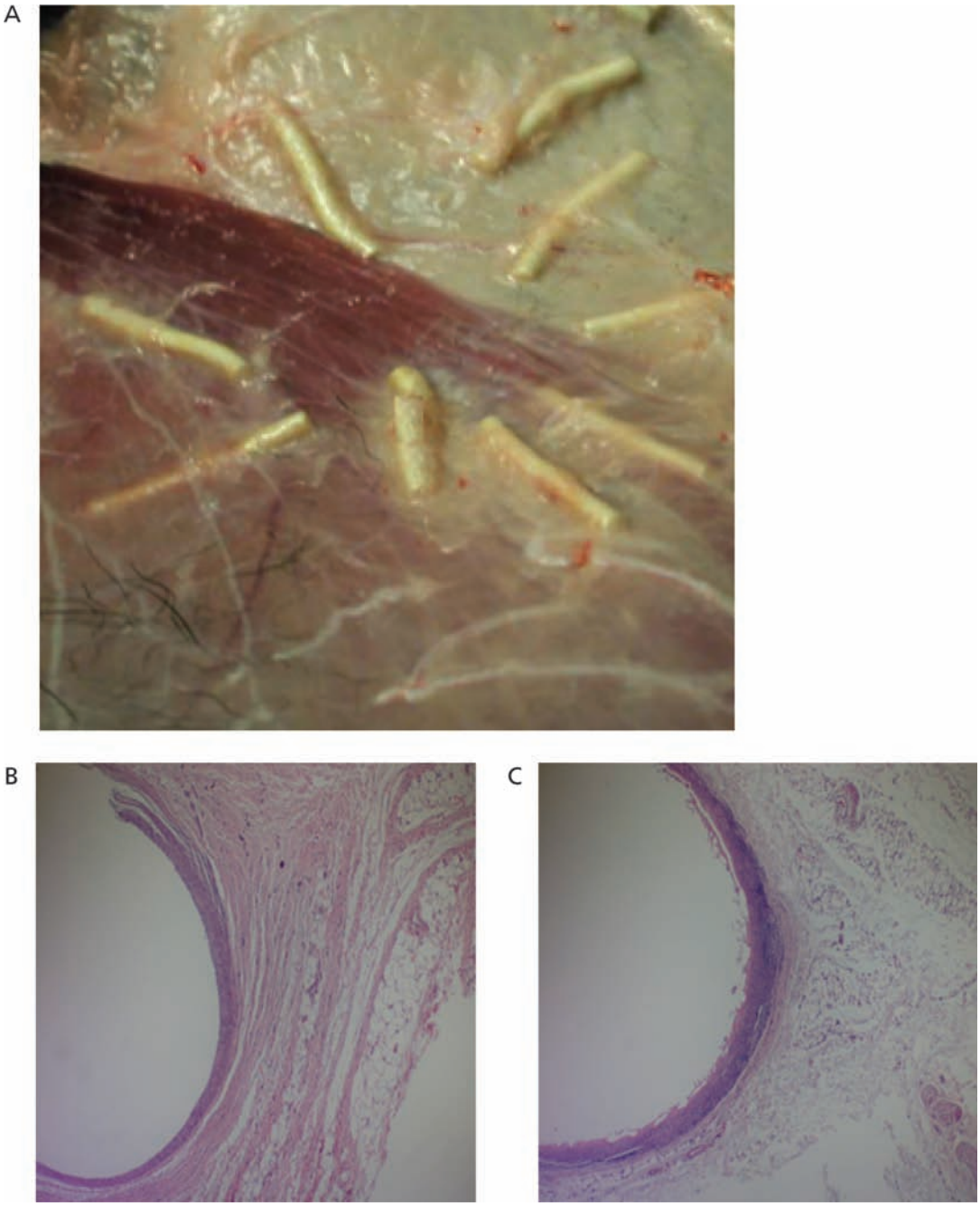

Figure 3 A. Example of subcutaneous implant site (skin adjacent to implant sites was reflected to expose implant sites) in beagle dog 9 months after implantation. B. Example of H & E stain of implant site in beagle dog 1 month after receiving placebo implant. C. Example of H & E stain of implant site in beagle dog 1 month after receiving buprenorphine implant.

between 8 and 16 implants showed few significant differences between groups, with $P < 0.05$ only at the later time points (weeks 28–34). Pair-wise comparisons at each time point between 16 and 24 implants also showed few significant differences between groups, except at early time points (9–24 h) and later time points (weeks 32–36). In contrast, pair-wise comparisons at each time point between 8 and 24 implants showed statistically significant difference ($P < 0.05$) at all time points, except at the 3-h time point ($P = 0.427$).

Post-hoc analyses on successive time intervals showed no significant difference in the slopes of the plasma concentrations between the 3 groups ($P > 0.05$), except at weeks 12 and 14, as well as the last time point analysed (week 36), when the slopes of the plasma concentrations diverged ($P < 0.05$). Pair-wise comparisons on successive intervals between 8 and 16 implants showed no significant differences between the slopes of the plasma levels except at week 18. Pair-wise comparisons on successive intervals between 16 and 24 implants

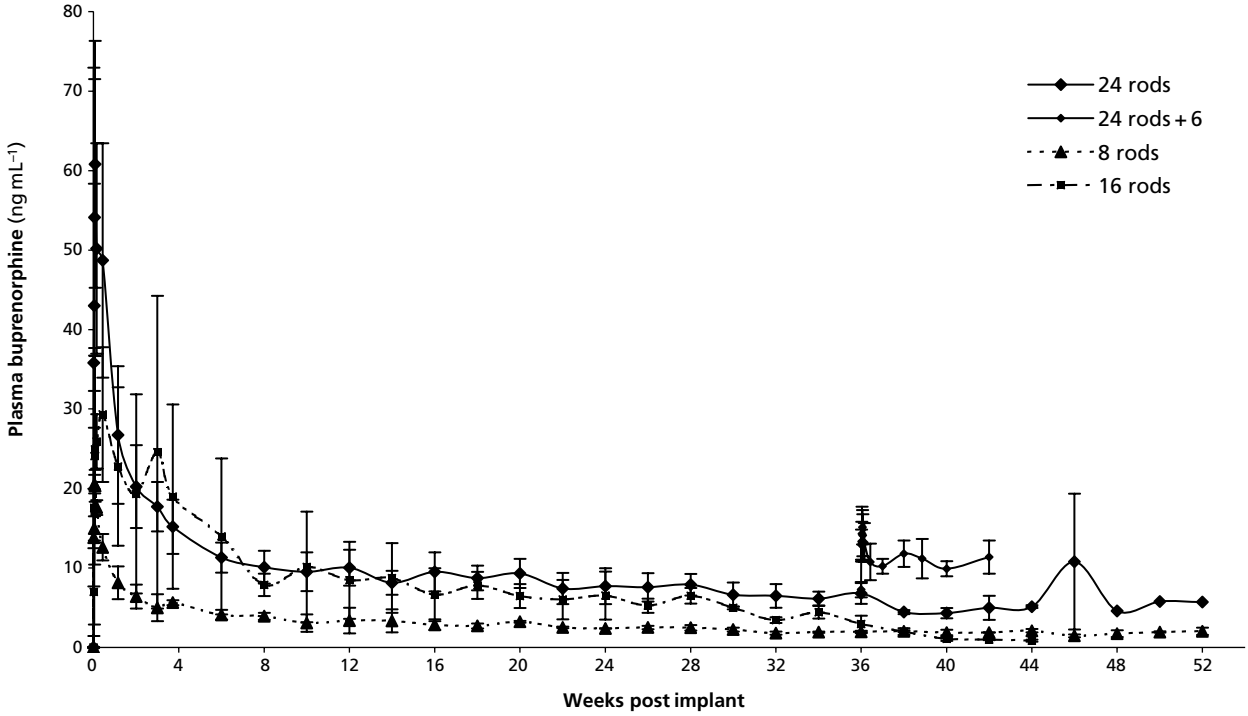

Figure 4 Buprenorphine plasma levels in dogs maintained for up to a year. Mean buprenorphine plasma concentration (± s.d.) from dogs receiving 8 (n = 2, triangles), 16 (n = 2, squares) or 24 (n = 18 week 0–4, n = 10 week 4–42, n = 2 week 42–46, n = 1 week 46–52, diamonds) buprenorphine implants. A subgroup of 24-implant dogs was implanted with 6 additional implants at week 36. Dogs receiving 16 implants were explanted at week 36 for off-kinetics (see text for details).

Table 1 Pharmacokinetics of buprenorphine subcutaneous implants in dogs

No. of implants implanted	C_{max} (ng mL^{-1})		T_{max} (study day)		C_{ss} (ng mL^{-1})
	Mean	Range	Mean	Range	Mean
8 (n = 2)	21.6	21.4–21.7	0.43	0.375–0.5	2.3 ± 1.0
16 (n = 2)	36.1	33.7–38.5	10.68	0.375–21	6.3 ± 3.7
24 (n = 18)*	70.4 ± 29.5	48.8–179	1.11 ± 1.06	0.125–3	8.1 ± 2.4

*n = 18 for days 0–30; n = 10 for days 30–294; n = 2 for days 294–322; n = 1 for days 322–364.

showed few significant differences between the slopes of the plasma levels, with $P < 0.05$ only at weeks 2, 12, 14 and 34. Pair-wise comparisons on successive intervals between 8 and 24 implants showed no significant differences between the slopes of the plasma levels at any time point.

Plasma norbuprenorphine levels were only quantifiable during the initial pulse of buprenorphine release (data not shown). Norbuprenorphine levels, when quantifiable, were approximately 3–5% those of buprenorphine. Given the lower limit of quantitation for norbuprenorphine (0.51 ng mL^{-1}), quantification was not expected when buprenorphine levels were below 10 ng mL^{-1}.

By week 36, plasma buprenorphine levels in eight of the 24-implant dogs reached approximately 80% of C_{ss}, the predetermined level at which supplemental implants were to be administered. Six additional buprenorphine-containing implants were inserted and plasma levels increased within 2 h. C_{ss} was attained within one week and was maintained through to the end of the study period in these dogs.

To determine how quickly buprenorphine is cleared from the blood upon removal of implants, dogs receiving 16 implants were explanted at week 36. Implants were surgically removed and plasma samples were taken over the following 12 h. Following removal of the implants in these dogs, plasma buprenorphine concentrations were quantifiable for 12 h, and below the limits of quantitation by 24 h. Dogs with 8 and 24 implants were surgically explanted at 10 or 12 months post-implant, and a subset of implants was assayed for remaining drug content to estimate the daily release rate per implant. At the time of removal, 70% of the original test implants and 8.3% of the week-36 test implants were broken into 2 or 3 pieces. No control implants were

broken. The mean buprenorphine release rate was calculated to be 0.14 ± 0.04 mg/implant/day during the C_{ss} phase.

Discussion

Results from this study show that buprenorphine implants maintain release in-vitro that can be manipulated by washing; maintain dose-proportional C_{ss} release in-vivo for 1 year with little variability between animals; and produce minimal adverse effects directly associated with the buprenorphine implant after 1 year of implantation. Serious adverse events (5.3%) were not directly related to implant.

The use of 8, 16, or 24 implants containing 90 mg of buprenorphine each is significantly higher than would be clinically utilized. However, these studies were carried out to measure the safety exposure of these implants, and therefore sought to show up to a 20-fold higher exposure than will be utilized in man.

The in-vivo pharmacokinetic study in dogs demonstrated an early, brief pulse of buprenorphine release followed by a decrease to C_{ss} levels, which were maintained up to 1 year following insertion. Steady state and C_{max} of buprenorphine in blood reflected the number of implants inserted. Peak buprenorphine levels occurred within 24 h of implantation in the majority of dogs, and by two days after implantation the mean plasma concentration had declined to 59% of C_{max}.

The lack of significant differences in plasma levels between the 3 groups at the early time point (3 h) indicates the high variability and thus high overlap in plasma levels 3 h after implantation. This can be expected due to the potential for less-accurate timing of sampling during this short duration after surgery, as well as potential variability in the subcutaneous physiology between dogs soon after implantation. The small sample size and the consequential use of nonparametric statistics also contribute to lack of adequate power to detect differences. Statistics also indicate differences in plasma levels between dogs receiving 8 and 24 implants, but not between 8 and 16, or 16 and 24 implants. The largest differences between groups is observed early in treatment (before week 6); this is also when the highest variability occurs. Even though the plasma levels attained in the three groups at steady state are dose dependent, they are not statistically different because the low plasma levels are masked by the variations observed among the dogs ($n = 2$ in 8-and 16-implant groups). Statistics also indicate that significant differences between the slopes of the plasma concentration curves occur only at weeks 12 and 14, indicating that the release characteristics are generally the same across groups, although the group implanted with the higher number of implants takes longer to attain steady state.

The release of buprenorphine from implants is dependent on the rate of dissolution and on passive diffusion through the polymer matrix. Therefore, the surface area of the implant determines the rate of release, as well as the washing procedure. Statistical analyses of in-vitro release indicates that the washed implants maintained a more-stable release rate from the beginning of study, whereas the release from unwashed implants showed a significantly higher burst at the earlier time points. The observed in-vitro buprenorphine release, however, was substantially higher than the in-vivo release. This can be attributed to the buprenorphine solubility and volume of dissolution media in the two environments. The subcutaneous space is constricted and has a low volume of tissue fluids compared with the constant stirring and large volume of dissolution media in the in-vitro chamber. The subcutaneous space can theoretically form a depot, but is unlikely to have done so in this study since buprenorphine cleared rapidly from the plasma after removal of the implants. The observed kinetics were similar to those reported for Implanon, an EVA-based contraceptive implant that releases greater amounts in-vitro than in-vivo, although the differences are not as great as those observed with the buprenorphine implants (likely because the drug content of Implanon is less than the drug content of the buprenorphine implants) (Geelen et al 1993).

Aside from lethargy and a reduction in food consumption on the day of implantation attributable to the transient pulse of buprenorphine, there were no test-article-related effects on clinical signs, body weights or food consumption. Two study dogs showed irritation considered secondary to skin preparation and implant procedure as opposed to a direct effect of the buprenorphine or control implants. No dog showed signs of serious infection or inflammation throughout the 1-year study. Though 2 study dogs showed a serious adverse event, it was concluded that neither of these 2 events were related to buprenorphine implant. Minor skin adhesions were noted in some dogs upon explantation but were noted as not clinically significant. Based upon the results of this study, implants appear to be generally well tolerated for a period of up to 12 months following subcutaneous implantation in dogs.

A number of the buprenorphine implants broke over the course of treatment. The breakage observed in some implants is likely due to the implant site (along the back), which is a vulnerable site given typical rolling behaviour in dogs. No significant differences in pK levels were seen in dogs with broken implants. Breakage would likely not have a significant effect on buprenorphine release, as buprenorphine implants are homogeneous and do not contain an inner core of drug that would dump upon breakage. Drug delivery from the buprenorphine implants is mainly governed by surface area. The surface area of the two ends is approximately 5% of the total surface area. Thus, delivery would increase by approximately 5% in an implant that breaks into 2 pieces, and by 10% in an implant that breaks into 3 pieces. Plasma levels were highest, and most variable, during the first several weeks after implantation. Given that the implants appear to break over time (8% of the implants that were inserted at week 36 were broken 4 weeks later, 30–40% of the original implants were broken in the 1-month dogs and 70% of the original implants were broken at 10 months), it is unlikely that this variability was due to broken implants.

The observed in-vitro and in-vivo kinetics are similar to those reported from an implant composed of nalmefene and EVA (Costantini et al 2004). The initial in-vitro release rate from nalmefene implants was approximately 10 times that of the release rate from buprenorphine implants, which was likely due to the high solubility of nalmefene ($130\,mg\,mL^{-1}$ vs buprenorphine $17\,mg\,mL^{-1}$, in water). These nalmefene implants were subsequently coated with additional EVA to attenuate drug

release. The coated implants attained a steady-state release of 0.36 mg/day in-vitro at 7 days, and maintained this release for 7 weeks. In-vivo steady-state release was maintained in rats from approximately week 3 through the end of a 24-week study, and was dose-proportional: C_{ss} was 3.3 ± 0.6 and 10.2 ± 2.3 ng mL^{-1} for 1 and 3 nalmefene implants, respectively. This illustrates the flexibility in release rate that can be obtained during the production of these EVA-based long-term delivery implants.

A phase I/II clinical study of these implants in opioid-dependent patients showed that two buprenorphine implants controlled withdrawal and cravings in 6 subjects previously maintained on sublingual buprenorphine at 8 mg daily, and that 4 implants controlled withdrawal and cravings in 6 subjects previously maintained on sublingual buprenorphine at 16 mg daily. No significant side effects were reported in either dose group (Saunders et al 2005). Additional larger trials are planned to illustrate the clinical utility of these implants.

Conclusions

These buprenorphine implants provide long-term stable blood concentrations in-vivo, eliminating the peaks and troughs observed with sublingual buprenorphine. Peak buprenorphine concentrations were generally reached within 24 h after implantation. Steady-state plasma levels were attained between 3 and 8 weeks and were maintained for the study duration. There were no test-article-related serious adverse effects.

This system is well suited for treating disorders that require strict compliance, such as opioid dependence, and may also prove useful for maintaining stable plasma levels of drugs for treating a variety of long-term disabilities. Drugs with high or low solubility can be incorporated into EVA implants, which can be washed or coated to manipulate the release properties. A long-term delivery system could be a significant improvement in treating chronic diseases by enhancing compliance, reducing adverse effects associated with peak/trough blood levels, providing constant therapeutic drug levels and improving long-term outcome.

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