ORIGINAL ARTICLE

In vitro and in vivo assessment of the abuse potential of PF614, a novel BIO-MD™ prodrug of oxycodone

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ARTICLE INFO

Keywords:
opioid abuse deterrence
Bio-MDTM oxycodone prodrug
PF614
extended-release opioid

DOI:10.5055/jom.2017.0366
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ABSTRACT

Objective: *The need for pain medication which will not lead to abuse is well recognized. Ensysce has designed prodrug analogs of the commonly used pain medications including hydromorphone, oxycodone (OC), hydrocodone, and morphine that limit their use to oral delivery, two of which are in clinical development. This study was undertaken to demonstrate that PF614, an extended-release prodrug of OC, allows the release of OC as designed when delivered orally, yet it resists ex vivo extraction with household chemicals and is pharmacologically inactive when administered by nonoral routes (nasal and parenteral), thereby substantially reducing its intravenous (IV) and intranasal abuse potential.*

Methods: *In vitro and in vivo methods were used to determine release of OC from PF614 and to show potential μ-opioid receptor activity. Plasma and cerebral spinal fluid levels of OC were evaluated following in vivo IV administration of PF614 in rats. In vitro extraction of OC from PF614 was explored using enzymes, common solvents, and household chemicals at room temperature and elevated temperature over time to determine release of OC from the prodrug.*

Results: *PF614 was stable with in vitro exposure to human plasma, saliva, and liver microsomes or culinary enzyme preparations. PF614 was stable (≥90 percent remaining as intact prodrug) under all room temperature conditions evaluated for 24 hours. At 80° C for 1 hour, no OC was released. Incubation at 80° C for 24 hours in vinegar or vodka produced a conversion to OC of 6 percent. Incubation with trypsin at 37° C converted PF614 approximately stoichiometric to OC with half-life of 4 hours. PF614's penetration of the central nervous system was 83-fold lower than OC and it had a 6.5-fold reduced potency as a μ-opioid agonist. Finally, oral PF614 delivers OC into plasma with an extended-release profile in dogs (reduced C_{max}; delayed T_{max}).*

Conclusions: *The Bio-Activated Molecular Delivery prodrug design limits the use of PF614 to the intended oral route of delivery with reduced potential for IV or nasal abuse, as it cannot be activated intravenously or nasally to provide an active opioid. Unlike existing opioid formulations, the extended-release profile of PF614 cannot be accelerated by chewing or ex vivo extraction to pharmacologically active substances.*

INTRODUCTION

Prescription opioid abuse and addiction are major burdens to society, resulting in significant costs, illnesses, and deaths.[1] The intertwined issues of (i) the widespread and increasing abuse of prescription opioids[2] and (ii) reluctance of prescribers to write prescriptions for opioid analgesics have resulted in the undertreatment of patients with moderate-to-severe pain.[3,4] It is imperative that patients have access to potent pain medications with products that reduce the possibility of abuse.

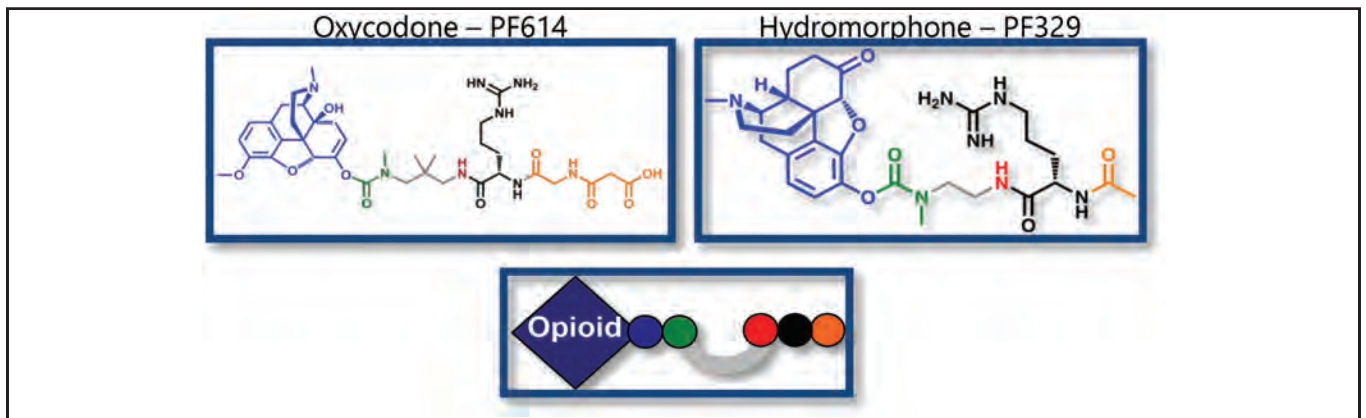

Figure 1. Chemical structure of PF614 and PF329 and general formula of the prodrug family showing the active opioid (blue) the cyclic urea linker (green/red) and the amino acid tail (black orange).

We have created a novel molecular delivery technology (BIO-MD™ [Bio-Activated Molecular Delivery]) designed to deter prescription opioid abuse by controlling bioavailability at the molecular level. Our goal was to design and develop a novel class of opioid prodrugs that effectively deter abuse and are not simply reformulated products. These prodrugs function as molecular delivery devices and are "activated" to release active opioid only when exposed to specific biological conditions (eg, in the presence of specific digestive enzymes in the intestinal tract). To this end, we have engineered tamper- and abuse-deterrent opioid prodrugs that demonstrate the key features listed in Table 1.

Our two lead BIO-MD™ products in clinical development PF329 and PF614 (Figure 1) are extended-release prodrugs of hydromorphone and oxycodone (OC) with a number of features aimed at resisting both oral and nonoral modes of abuse. This approach differs from current formulation-based strategies in a number of ways. Most important among these, the abuse-resistance provided by these products is unaffected by simple physical manipulations (eg, extraction and/or chewing of the dose form) and the bioavailability of active opioid following coingestion of multiple doses can be limited.

PF614 is a prodrug of OC. Following ingestion, the release of OC from PF614 proceeds via a 2-step process including (i) bioactivation in the small intestine via the digestive enzyme trypsin and (ii) a subsequent intramolecular cyclization-release reaction (Figure 2). The bioactivation step involves the hydrolysis of the amino acid residue by the proteolytic action of trypsin in the small intestine. This produces PFR06082, a nucleophilic amine intermediate, and L-arginine-glycine-N-malonate (PFR06112). The release of OC

Table 1. Key features of BIO-MD™ tamper- and abuse-deterrent opioid prodrugs

Key feature of prodrug	Rationale
Robust chemical stability	Deters tampering aimed at chemically converting prodrug to active opioid using readily available household chemicals
Not active at μ-opioid receptors and does not readily penetrate the CNS	Deters abuse via nonoral routes of administration
Does not readily convert to active opioid in the systemic circulation	
Conditional bioavailability: releases active opioid only when taken by prescribed route (ie, oral)	
Delivers therapeutic levels of well-established opioid agonist drug when ingested	Acceptable efficacy and safety profiles
Produces safe, well-tolerated metabolites	
Delivers opioid with desirable PK profile (ie, reduced C_{max} and delayed T_{max})	Deters abuse by reducing and delaying maximal effect (eg, euphoria)
Chewing or crushing dose form does not augment systemic exposure of active opioid	Prevents most prevalent mode of oral abuse (eg, chewing/crushing of extended-release formulations)
Multi-pill abuse deterrence	Deters oral abuse and misuse. Effectively limits exposure of active opioid following intentional (or unintentional) coingestion of multiple doses

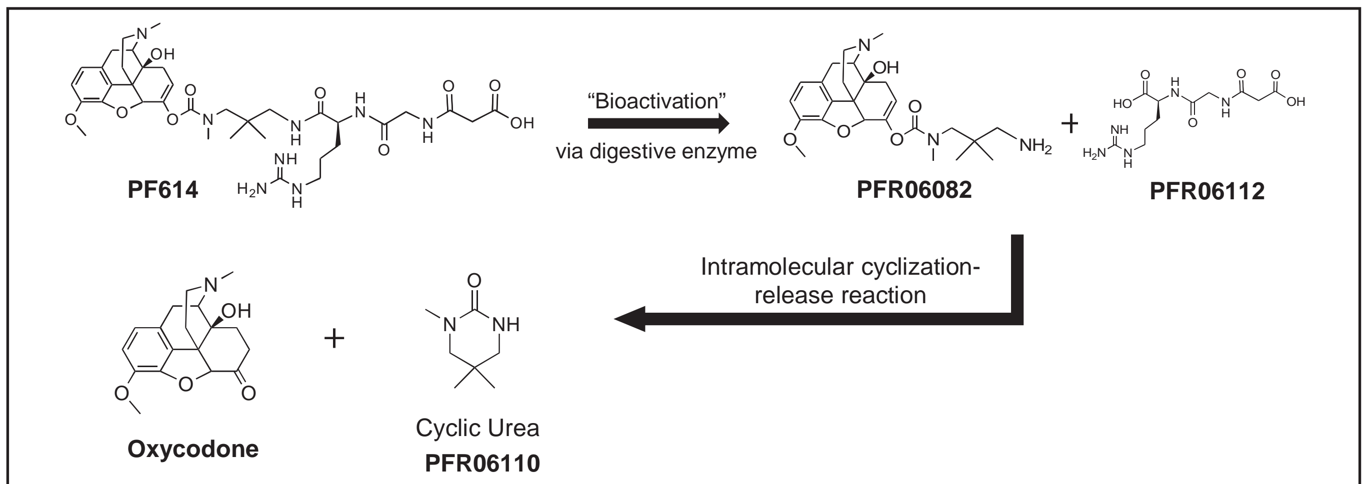

Figure 2. Mechanism of bioactivation and subsequent cyclization to OC from PF614.

occurs when PFR06082 undergoes a spontaneous intramolecular cyclization reaction. This reaction releases OC with concomitant formation of a cyclic urea metabolite (PFR06110). The time course of OC release from PF614 is a function of the kinetics of (i) the trypsin hydrolysis and (ii) the cyclization-release reaction. PF614 is expected to produce an analgesic effect similar to that of OC with reduced abuse potential as it was designed to provide release kinetics that deters abuse by the oral route and to substantially reduce the ability to abuse by injection or inhalation.

The objectives of the current studies were to determine the stability of an extended-release BIO-MD™ prodrug PF614 in common, commercially available chemical solutions, in enzyme preparations, and in human saliva and to measure the extent of their conversion to the active opioids. The studies were carried out exposing PF614 to distilled white vinegar, Windex®, vodka, and saturated aqueous sodium bicarbonate for up to 24 hours at room temperature (RT) and 80 °C. Similarly, it was explored whether activation and release of OC occurred in commercially available enzyme solutions of Adolf's® Tenderizer, AMO Ultrazyme® contact lens cleaner, or General Nutrition Corporation (GNC) Natural Brand™ Super Digestive Enzymes when PF614 was incubated at RT for up to 24 hours. Stability of PF614 was also assessed in human plasma, saliva, and liver microsomes. The concentrations of both the prodrug and active opioid, OC, were measured using an high-performance liquid chromatography-ultraviolet (HPLC-UV) method with the results of the study to provide a measure of the ease of extraction and potential abuse liability for these products.

Additionally, the activation of PF614 in the blood and penetration into the cerebral spinal fluid (CSF) was examined following intravenous (IV) administration to rats. These data were compared to an in vitro functional assay that compared PF614 to OC potential for agonist or antagonist activity at the μ-opioid receptor.

Finally, the pharmacokinetic (PK) profile of the PF614 release of OC in dogs was compared to that of immediate and extended-release OC to demonstrate that orally delivered PF614 provided lower C_{max} and delayed T_{max} than immediate release OC as has been suggested to reduce the abuse potential of opioid products.[5-8]

Taken together, our data support the development of this family of opioid prodrugs to overcome the abuse potential of this family of pain medication. PF329 has already demonstrated this expected extended-release profile in a phase 1a clinical trial; PF614 has moved into early clinical development. The BIO-MD™ technology platform could provide patients with a novel approach to treat pain with well-known opioid products in the near future.

MATERIALS AND METHODS

Test articles

PF614 (aka PFR06104 2HCl, lot 1; 98.9 percent purity) was supplied by PCAS-Nanosyn (Santa Rosa, CA). Extended-release OC hydrochloride: OxyContin (Purdue Pharma, Stamford, CT) and OC free base (Cerilliant Corp., Round Rock, TX; certified free base solution, catalog O-002, lot FE062707-01) were obtained from commercial sources.

Materials

Bovine type I pancreatic trypsin used was sigma #T8003, lot 010M7005, 12705 BAEE units/mg solid (Sigma-Aldrich, St. Louis, MO). Other commercial chemical products or products reported to contain active enzymes were purchased online or at a local grocery store—Adolf's® Tenderizer, original, unseasoned (Lawry's®; Unilever Englewood Cliffs, NJ; UPC 25100 01043): presumed active enzyme ingredient Papain; Ultrazyme® Enzymatic Cleaner for Soft Contact Lenses (Advanced Medical Optics, Inc. (AMO); Santa Ana, CA; product 90278; lot 27542): presumed active enzyme ingredient Subtilisin A; Natural Brand™ Super Digestive Enzymes (GNC; Pittsburgh, PA; product 180311; lot E11628): two capsules are reported to contain the active enzyme ingredients 7,000 DU amylase, 15,000 HUT protease 4.5, 250 FCCLU lipase AN, 11 AGU glucoamylase, 15 SAPU protease 3.0, 300,000 FCCPU bromelain, 2,000 HUT protease 6.0, 280,000 FCCPU papain, 100 CU cellulase, 125 DP malt diastase, and unspecified amounts of hemicellulase, β-gluconase, and phytase; Safeway Distilled White Vinegar (UPC 586322; RD 07043 711358; Pleasanton, CA); measured pH of 2.5; Windex® Original, glass cleaner with Ammonia-D® (SC Johnson; UPC 19800 40129; Racine, WI); measured pH of 10.9; Vitali Vodka, 80 proof (V.G. Corydon Distilling Products; UPC 21130 20825; Mira Loma, CA); measured pH of 3.2; saturated aqueous sodium bicarbonate; measured pH of 9.3.

Experimental

Experimental procedure for chemical solution stability. Incubations were conducted at both RT (19–23°C) and 80°C in individual microfuge tubes (80°C test solutions were preheated in a water bath for 10 minutes prior to the experiment). Incubations were initiated by adding 5 μL of a 120-mM PF614 stock solution in 0.1 percent aqueous formic acid to 95 μL of test solution and mixing briefly. At predetermined times (RT: 0, 1, and 24 hours; 80°C: 1 and 24 hours), 6.67 μL of 42.5 percent aqueous phosphoric acid (vodka, Windex®, and vinegar incubations) or 60 μL of 85 percent aqueous phosphoric acid (sodium bicarbonate incubations) were added to drive the pH to 4 or less to prevent further conversion to OC. OC was evaluated for degradation under similar conditions at 100°C. Samples were vortexed vigorously for 30 seconds before storing at −20°C between sampling times. After all incubations were completed, samples were diluted by adding 400 μL of water and vortexing vigorously for at least 10 seconds.

Experimental procedure for Adolf's® Tenderizer test solution. A test solution was prepared by combining Adolf's® Tenderizer with water at a concentration of 0.123 g/mL (the approximate concentration of a meat marinade recipe given on the product label). The resulting pH was measured to be ~6.5. Incubations were conducted at RT (19-23°C). Incubations were initiated as above. At predetermined times (0, 1, and 24 hours), 6.67 μL of 50 percent aqueous phosphoric acid was added to drive the pH to 4 or less to prevent further conversion to OC and samples were handled as in Experimental procedure for chemical solution stability section.

Experimental procedure for Ultrazyme® contact lens cleaner test solution. A test solution was prepared by dissolving one tablet of AMO Ultrazyme® Enzymatic Cleaner per teaspoon (5 mL) of water (pH ~ 9.5). Incubations were conducted at RT. Incubations were initiated as above. At predetermined times (0, 1, and 24 hour), 20 μL of 50 percent aqueous phosphoric acid was added to drive the pH to 4 or less to prevent further conversion to OC and samples were handled as in Experimental procedure for chemical solution stability section.

Experimental procedure for Natural Brand™ Super Digestive Enzymes test solution. A test solution was made by dissolving the contents of two capsules of GNC Natural Brand™ Super Digestive Enzymes in 5 mL of water to achieve a pipettable suspension with the highest concentration possible (pH ~ 5). Incubations were conducted at RT. Incubations were initiated as above. At predetermined times (0, 1, and 24 hours), 6.67 μL of 50 percent aqueous phosphoric acid was added to drive the pH to 4 or less to prevent further conversion to OC and samples were handled as in Experimental procedure for chemical solution stability section. Samples were vortexed vigorously for 30 seconds before storing at −20°C between sampling times. After all incubations were completed, samples were diluted by adding 400 μL of water and vortexing vigorously for at least 10 seconds.

Standard solutions for PF614 (0.188-10.0 mM) and OC (0.0313-6.00 mM) were made in 0.1 percent aqueous formic acid. Standards were diluted by adding 400 µL of water to a 100 µL aliquot of each standard and vortexing vigorously for at least 10 seconds. Samples and standards were prepared for analysis by centrifuging in a 96-well plate at 3,500 rpm (Beckman Coulter Allegra X-12 centrifuge, Fullerton, CA) for 10 minutes. An aliquot (200 µL) of the supernatants was transferred to a 96-well plate for analysis.

Trypsin activation of PF614. An in vitro incubation of PF614 in the presence of trypsin type 1 from bovine pancreas in 185 mM TRIS buffer, pH 8.0 with 2 mM $CaCl_2$ was conducted at 37°C. To quantify, PF614 disappearance with subsequent appearance of PFR06082, PFR06112, and OC, samples were analyzed using liquid chromatography/mass spectrometry (LC/MS) at multiple time points to obtain half-lives for PF614, the release of PFR06082, and the conversion to OC.

Ex vivo metabolism and activation in human saliva, plasma, and liver microsomes. A therapeutically relevant concentration of PF614 (5 µM) was incubated with human plasma and liver microsomes (S9 fraction) 37°C, with ß-nicotinamide adenine dinucleotide phosphate, D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and uridine 5' diphosphoglucuronic acid cofactors[9] for up to 6 hours (plasma) and 2 hours (microsomes). The percentage drug remaining was measured at 0, 60, 120, and 240 minutes after initiation of incubation. Samples were profiled to elucidate potential metabolite peaks by LC-MS and/or LC-MS/MS.

An in vitro study to determine the stability of PF614 (6 mM) in human saliva and the extent of its conversion to OC was carried out at RT for up to 24 hour. The percentage drug remaining was measured at 0 minute and various times up to 24 hours after initiation of incubation. Samples were profiled to elucidate potential metabolite peaks by LC-MS and/or LC-MS/MS.

Functional effects of PF614 and OC on the human µ-opioid receptor. An in vitro functional cellular assay was conducted to clarify the potential agonist or antagonist activity of PF614 compared to OC at the µ-opioid receptor using human recombinant CHO cells stably expressing the µ-opioid receptor (ChanTest CT6605). PF614 (4.57×10^{-09}–1.0×10^{-05} M) or OC (4.57×10^{-10}–1.0×10^{-06} M) were evaluated for agonist or antagonist activity following incubation for 10 minutes at 37°C. cAMP modulation (percent of control) was measured using homogeneous time-resolved fluorescence assay. Reference compounds evaluated under the same conditions confirmed the sensitivity of each assay.

In vivo analyses.

Animal welfare, care, and use statement

Animal studies were carried out by Covance Laboratories which is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All procedures in the protocol were in compliance with applicable animal welfare acts and were approved by the local Institutional Animal Care and Use Committee.

Plasma and CSF levels of PF614 and OC following IV administration to rats

PF614 (10 mg/kg) and OC (7.5 mg/kg) were administered intravenously to rats. At 2, 15, and 60 minutes after dosing, blood and CSF samples were obtained. Each sample was analyzed for PF614 and OC by LC/MS. Concentration values and CSF:plasma ratios were determined for each.

PK profile of OC release from PF614 following oral administration to dogs

PF614 (solution in 0.1 N hydrochloric acid; 9.1 mg/kg), OC (2 mg/kg), or OxyContin tablet (20 mg) were delivered orally to beagle dogs. Blood samples (approximately 1.0 mL) were collected predose and approximately 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours postdose after administration. Blood was collected in tubes containing potassium (K_2) ethylenediaminetetraacetic acid. Samples were maintained on chilled cryoracks and centrifuged (approximately 4°C for 10 minutes at 3,000 × g) within 20 minutes of collection. Plasma was harvested within 10 minutes of the end of centrifugation and placed in tubes containing formic acid (1 mL formic acid: 100 mL plasma). Harvested plasma was stored in a freezer, set to maintain −60 to −80°C, until analysis.

HPLC-UV analysis. Aliquots (40 µL) of the prepared chemical and enzyme solution samples were injected into an Agilent 1100 HPLC-UV system (Santa

Table 2. Standard curve results for HPLC analyses					
Analyte	LLOQ, mM	HLOQ, mM	Curve fit	Weighting*	Correlation coefficient, r
PF614	0.188	10.0	Linear	1/x	1.00
OC	0.0313	6.00	Linear	1/x	1.00

*Derived from the least-squares linear regression used to fit experimental data to the linear calibration curve. Weighting for Standard curve of 1/x selected where X = concentration.

Clara, CA). All analyte separations were performed on an Agilent Eclipse Plus C18 (4.6 id×100 mm, 5 µm particle size) reverse phase column (part 959996-902) maintained at 40°C. For analyses of the chemical samples, mobile phase A consisted of 0.2 percent ammonium formate buffer and mobile phase B consisted of 0.1 percent formic acid in 90:10 v/v acetonitrile:methanol. The chromatographic method used a linear gradient from 0 to 47 percent mobile phase B over the course of 5 minutes after holding 0 percent mobile phase B for 1 minute at a flow rate of 1.44 mL/min. The analytes were detected at 280 nm using a diode-array detector.

For analyses of the enzyme samples, mobile phase A consisted of 0.2 percent ammonium formate buffer and mobile phase B consisted of 0.1 percent formic acid in acetonitrile. The chromatographic method used a linear gradient from 12 to 45 percent mobile phase B over the course of 4 minutes after holding 12 percent mobile phase B for 1 minute at a flow rate of 1.44 mL/min. The analytes were detected at 254 nm using a diode-array detector. Table 2 provides standard curve results.

LC/MS analyses. Aliquots from saliva, plasma, or CSF samples of 10 µL were injected into Shimadzu Prominence, 20 series with a Synergi Polar-RP, 3.0×50 mm, 4 µm particle size column maintained at 40°C and a Sciex API 5000 MS detector. Mobile phase A consisted of 0.1 percent formic acid in water and mobile phase B consisted of 0.1 percent formic acid in acetonitrile; flow rate 1.1 mL/min. The chromatographic method used a linear gradient from 10 to 50 percent mobile phase B over the course of 1.2 minutes then to 95 percent over 0.3-minute holding at 95 percent mobile phase B for 2.3 minutes. The analytes were detected at retention times (RT): OC 1.05 minutes and PF614 at 1.39 minutes.

Data analysis. Quantitation was conducted by external standardization. Peak areas were best fit to linear calibration curves for PF614 and OC, using Applied Biosystems (Foster City, CA) Analyst 1.4.2 software. Test acceptance criteria required that the calculated concentration of the standards be within 25 percent of theoretical concentrations (accuracy). Three fourths of the standards should meet this criterion for the assay to be accepted. Sample concentrations below the LLOQ were assigned a value of zero. A dilution factor was applied to the samples to adjust for the phosphoric acid addition.

RESULTS

Stability in household chemicals, solvents, and enzymes

Stability of PF614 and OC was examined in common household chemicals and PF614 only in commonly available enzyme preparations. Amounts of PF614 remaining and OC generated in common, commercially available chemical solutions after 1 and 24 hours of incubation at RT and 80°C are shown in Figure 3. The data are expressed as a percentage of the initial molar concentration of PF614 (0 hour RT time point; n = 2). PF614 was stable (≥90 percent of prodrug remaining) under most of the conditions tested. OC was not detected as a degradation product under any condition tested at RT. PF614 degraded in vinegar (25 percent loss) or vodka (42 percent loss) at 80°C after 24 hours with ≤6 percent OC produced under any condition. The conversion to OC at 80°C was low with maximal conversion only 6 percent in vodka after 24 hours. The molar amount of OC produced did not account for the molar loss of PF614 under these conditions suggesting that either (a) PF614 was converted to alternative undetected products that contained OC or (b) OC was contemporaneously degraded under these conditions.

To investigate the fate of OC released from PF614 under these conditions, OC was incubated in the solutions and found to be stable for at least 24 hours under most of the chemical (vinegar, saturated aqueous sodium bicarbonate, vodka, and Windex®) and temperature conditions tested (RT, 80°C, and 100°C)

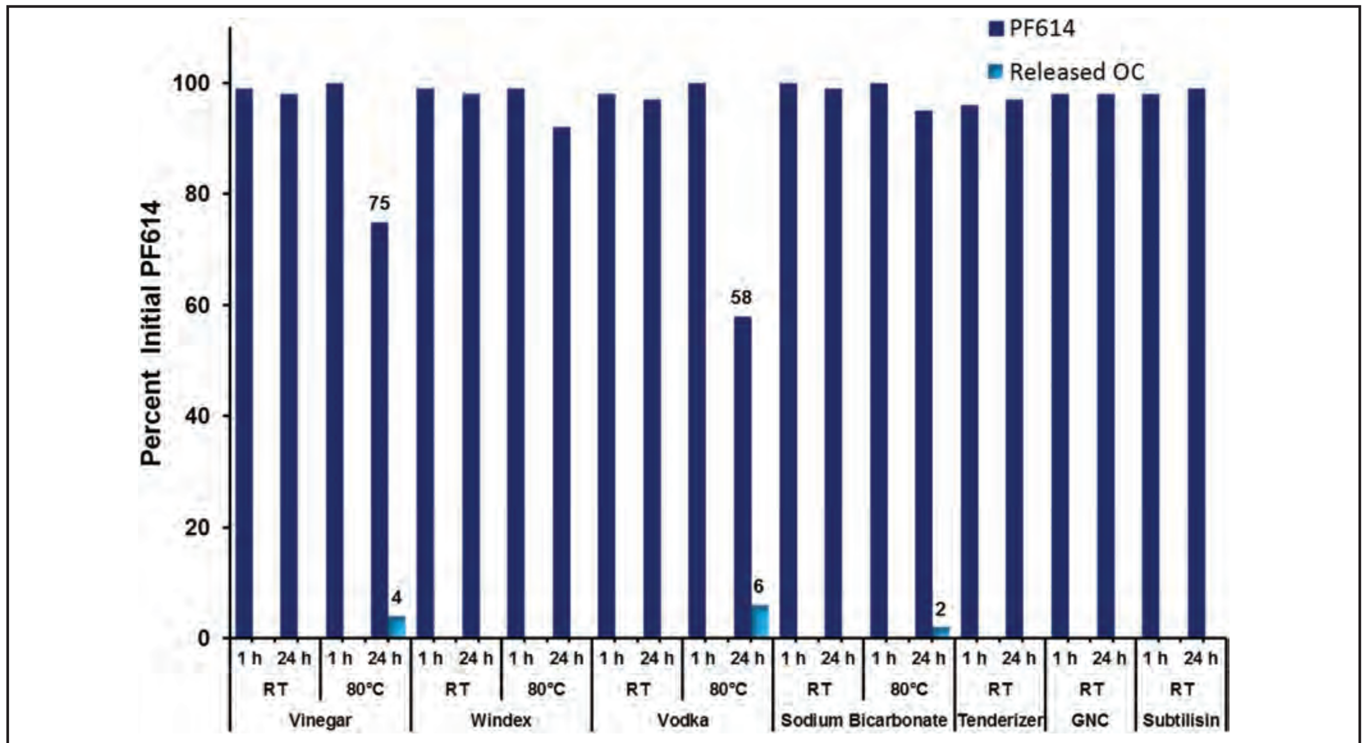

Figure 3. Mean concentration of PF614 remaining and OC appearance as a percentage of the initial molar concentration of PF614 in common, commercially available chemical solutions incubated for 1 and 24 hours at RT and 80°C (n = 2).

with the exception of saturated aqueous sodium bicarbonate and Windex® at 80°C and 100°C. OC was degraded 11 percent in sodium bicarbonate and 69 percent in Windex® over 24 hours at 80°C. Degradation of OC in the basic test solutions was more extensive when incubated at 100°C with 70 and 86 percent disappearing in sodium bicarbonate and Windex®, respectively, after 24 hours.

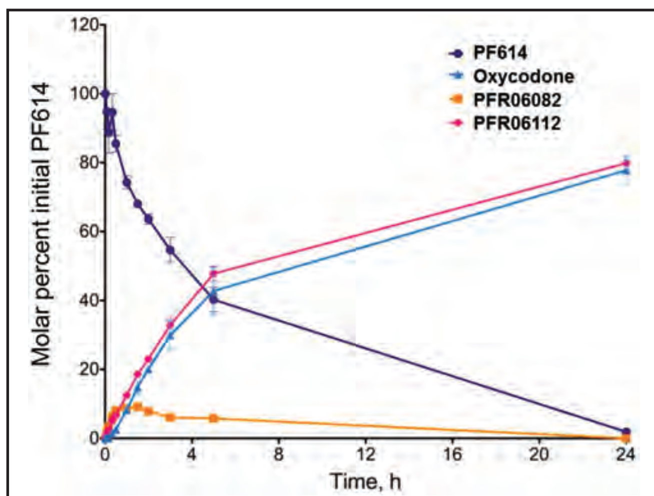

Figure 4. In vitro prodrug activation and delivery of OC from PF614 exposed to bovine pancreas trypsin type 1. Bar = SD.

Trypsin bioactivation

Exposure of PF614 in vitro to bovine trypsin established the half-life of bioactivation of the prodrug to PFR06082 and the release of OC (Figure 4). The half-life of PF614 in trypsin was determined to be 3.75 hours. No prodrug hydrolysis was observed with thermally denatured trypsin (data not shown).

Stability in human plasma, saliva, and liver microsomes

PF614 was stable in human plasma and liver microsomes. No ex vivo conversion to OC was detected at 2 hours when PF614 was exposed to microsomes or to plasma at 6 hours. To simulate mastication, PF614 was exposed to human saliva at RT up to 24 hours. The disappearance of PF614 and OC production in saliva is illustrated in Figure 5. PF614 was stable (>90 percent) out to 24 hours and no measurable OC was produced over the same period of time.

Determine functional effects of PF614 and OC on the human μ-opioid receptor

The human μ-opioid receptor functional assay evaluated PF614 and OC as a reference control. The

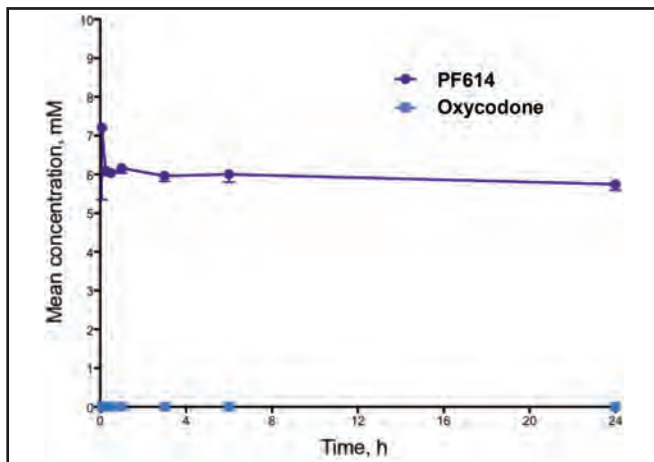

Figure 5 PF614 (6 mM) solution in human saliva over 24 hours at RT: mean PF614 concentration • and OC production ■ over time (n = 2).

mean EC_{50} was determined to be 4.2×10^{-07} M for PF614 and 6.5×10^{-08} M for OC over four independent measurements. The data showed that PF614 displayed reduced functional agonist activity at the human μ-opioid receptor binding site compared with OC of approximately 6.5-fold. PF614 displayed no measurable antagonist effect.

Plasma and CSF levels of PF614 and OC following IV administration in rat

Plasma and CSF concentrations of PF614 (10 mg/kg IV) and OC (7.5 mg/kg IV) were determined in rats at 2, 15, and 60 minutes after dosing. Concentration values and CSF:plasma ratios are summarized in Table 3. The CSF:plasma ratios at the 2 minutes postdose time point following IV administration of PF614 and OC to rats were determined to be 0.00247 and 0.210, respectively. These data indicate an 85-fold reduced ability of PF614 to penetrate the CSF relative to OC. The reduced ability of PF614 to access the central nervous system (CNS) together with its 6.5-fold reduced potency as a μ-opioid agonist is likely to result in an approximately 550-fold reduced potential for IV abuse (Abuse Profile = Relative Potency × Relative CNS Penetration).

PK profile of PF614 and its conversion to OC in dog

Following oral administration of PF614, OC was released as designed and absorbed in dogs with the AUC profile of 9.1 mg/kg PF614 similar to the PK profile of a 20-mg tablet of OxyContin® (Table 4). Importantly the mean absolute oral bioavailability of PF614 in its native form was low (4.0 ± 2.2 percent), therefore reducing possibly accumulation of the nonactivated prodrug in plasma. The OC plasma concentration-time profiles following oral administration of PF614 dosed as homogenous aqueous solutions was compared to the OC plasma concentration-time profiles following oral administration of OC at doses that provided similar AUC exposures of OC. The OC plasma concentration-time profile following oral administration of OC (2 mg/kg) demonstrated an immediate release profile, with a relatively high C_{max} (193 ± 69 ng/mL) occurring shortly after drug ingestion (0.50 ± 0.0 hour). The OC plasma concentration-time profile following oral administration of PF614 (9.1 mg/kg) demonstrated an extended-release profile, with a reduced C_{max} (36.2 ± 4.4 ng/mL) and delayed T_{max} (2.33 ± 1.2 hours; Table 5).

The extended-release profile of OC from a PF614 solution (9.1 mg/kg) was also compared to the OC plasma concentration-time profile following oral administration of OxyContin® tablets (20 mg tablet; ~1.8 mg/kg; Figure 6). Comparative PK parameters

Table 3. Mean (±standard deviation) plasma and CSF concentrations and relative CSF penetration of PF614 and OC at 2 min postdose following single dose administration to rats

Dose group/analyte	Plasma concentration, ng/mL	CSF concentration, ng/mL	Mean CSF:plasma ratio	Mean CSF:plasma fold reduced compared to OC
10-mg/kg PF614 IV				
PF614	56,600 ± 8,100	140 ± 160	0.00247	85
7.5-mg/kg OC IV				
OC	10,300 ± 2,600	2,160 ± 90	0.210	na
Abbreviation: na, not applicable.				

Table 4. Mean (±standard deviation) PK parameters of PF614 following single dose administration of PF614 to dogs*										
Species	**Dose route**	**Dose level, mg/kg**	**n**	**Fed/ fasted**	**C_{max}, ng/mL**	**T_{max}, h**	**AUC_{0-t}, ng h/mL**	**$AUC_{0-\infty}$, ng h/mL**	**$t_{1/2}$, h**	**F, percent**
Dog	PO	9.1	3	Fasted	338 ± 100	0.667 ± 0.29	656 ± 350	657 ± 350	3.17 ± 2.7	4.04 ± 2.2

*Data are for native (unmetabolized) PF614.

Table 5. Mean (±standard deviation) PK parameters of OC following single dose administration of PF614 to dogs*										
Species	**Dose route**	**Dose level, mg/kg**	**n**	**Fed/ fasted**	**C_{max}, ng/mL**	**T_{max}, h**	**AUC_{0-t}, ng h/mL**	**$AUC_{0-\infty}$, ng h/mL**	**$t_{1/2}$, h**	**$f_m \times F_a$, percent**
Dog	PO	9.1	3	Fasted	36.2 ± 4.4	2.33 ± 1.2	277 ± 20	310 ± 23	8.14 ± 0.69	35.7 ± 19

*f_m = fraction metabolized.

for released OC are presented in Tables 5 and 6. Based on these data, a five-fold dose ratio of PF614 to OxyContin® produced similar AUC exposures of OC. It is interesting to note that the plasma half-life of OC from PF614 (8.14 ± 0.69 hours) was greater than the half-life of OC from OxyContin® (4.89 ± 4.1 hours). The longer OC half-life from PF614 is expected to result in (i) a reduced peak to trough ratio relative to OxyContin® upon repeat dosing, and (ii) a steady state dose ratio of ~3.3 for PF614 compared to OC.

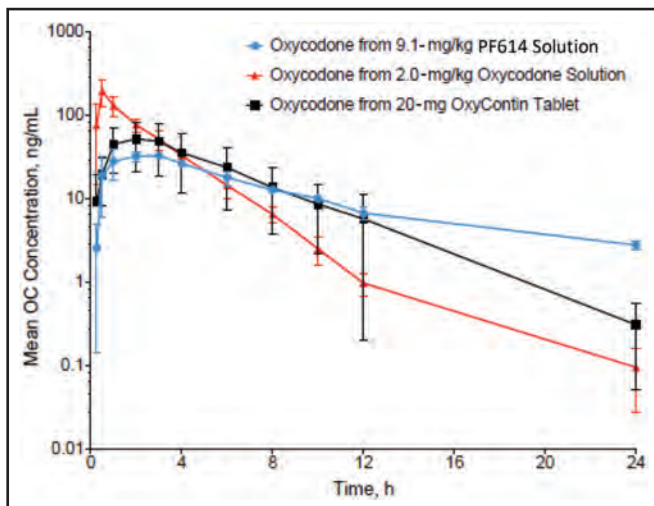

Figure 6 Log-linear mean ± SD plasma concentration versus time of OC following oral administration of PF614, OC, or OxyContin® to dogs.

CONCLUSIONS

These studies were undertaken to understand the potential for possible abuse by manipulation of an OC prodrug, PF614, and to demonstrate that when taken orally the prodrug did provide an extended-release PK profile. PF614 was found to be highly stable to all forms of manipulation and the conversion to OC was minimal under all the conditions. This is in contrast to Remoxy XRT™ (abuse-deterrent formulation of extended-release OC capsules developed by Pain Therapeutics, NDA 22-324) which showed that up to 15 percent extraction of OC was possible with shaking in beverages or household liquids by 60 minutes, and this increased to 35 percent extraction with the addition of crushing or grinding followed by exposure to the solutions.[10] The use of high temperature did not aid in the extraction of the active opioid from PF614 and this method of extraction is not considered to provide an avenue for abuse of PF614.

Abuse resistance—Nonoral routes

PF614 provides a number of important features expected to deter abuse via nonoral routes of administration. These include (i) 6.5-fold reduced activity versus OC at the μ-opioid receptor, and (ii) 85-fold reduced bioavailability to the central compartment compared to OC (as measured by CSF:plasma penetration in rats). The reduced ability of PF614 to

Table 6. Mean (±standard deviation) PK parameters of OC following single dose administration of OC solution or extended-release OxyContin® to dogs										
Species	Dose route	Dose level, mg/kg	n	Fed/ fasted	C_{max}, ng/mL	T_{max}, h	AUC_{0-t}, ng h/mL	$AUC_{0-\infty}$, ng h/mL	$t_{1/2}$, h	F, percent
Dog	PO	2.0*	4	Fasted	193 ± 69	0.500 ± 0.0	418 ± 54	419 ± 54	2.95 ± 1.2	43.9 ± 5.7
		1.8 mean†	4	Fasted	64.7 ± 8.8	2.75 ± 0.96	329 ± 160	330 ± 160	4.89 ± 4.1	37.0 ± 17

*Administered as an oral solution.
†Administered as OxyContin® 20-mg tablet; body weights ranged from 10.0 to 12.3 kg.

access the CNS together with its 6.5-fold reduced potency as a μ-opioid agonist is likely to result in a large reduction in nonoral abuse relative to OC. This along with the chemical stability of PF614 demonstrating inefficient conversion to OC when subjected to common household tampering methods provides support for the abuse-deterrent nature of the BIO-MD™ platform.

Abuse resistance—Oral routes

Resistance to abuse via the oral route has been designed for extended-release BIO-MD™ prodrugs which provide PK profiles that reduce "liking"[5-8] as compared to that of the parent products. The stability in human saliva study demonstrated that chewing PF614 would not release active OC. When ingested orally, PF614 itself is absorbed < 5 percent, but as designed it delivers OC with an extended-release profile that mirrors that produced by the extended-release OxyContin (ie, reduced C_{max} and delayed T_{max}) as does PF329, the extended-release hydromorphone prodrug that has been evaluated in a phase 1 trial.[11,12] Recent studies have suggested a correlation between the ratio of C_{max}/T_{max} and abuse potential. Unlike existing opioid formulations, the extended-release profile demonstrated by PF614 has a PK profile that is not affected by chewing/crushing/dissolving or coingestion with alcohol.[13] Furthermore, PF614 can be formulated as a combination product with a trypsin inhibitor to progressively decrease the bioavailability of OC, when excessive multiple doses are coingested (data not shown). This approach potentially offers a unique level of oral abuse resistance and overdose protection.[14]

Hence, the BIO-MD™ prodrug platform provides a highly novel approach to tackle the abuse problem of prescription drugs. It was designed to limit use of medication to oral delivery in an attempt to stem some of the abuse that has developed with a number of highly effect therapeutic products. PF614 has entered phase 1 clinical evaluation and should provide an alternative for patients who seek a pain modification product with a reduced potential for abuse.

ACKNOWLEDGMENTS

This study was supported by and undertaken by the authors who were full time employees of Signature Therapeutics, which has recently merged with Ensysce Biosciences Inc. Thank you to Jonathan W. Wray, formerly with Signature Therapeutics, who assisted in the preparation of the manuscript.

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