APD371: A Potent, Highly Selective, Full Agonist of the Human CB_2 Receptor With Sustained Analgesic Effects in Rodents

John W. Adams,[1] David Unett,[2] Todd Anthony,[2] Joel Gaitlin,[3] Ibragim Gaidarov[2]

[1]Arena Pharmaceuticals, Inc., San Diego, California, USA; [2]Beacon Discovery, San Diego, California, USA; [3]Human Longevity, Inc., San Diego, California, USA

INTRODUCTION

Cannabinoid receptors have been extensively explored as targets to modulate a wide variety of pain modalities. The cannabinoid 2 (CB_2) receptor has received significant attention as a target that may provide pain relief without the central nervous system (CNS) liabilities associated cannabinoid 1 (CB_1) receptor modulation.[1,2] Although selective CB_2 receptor agonists have shown great promise in preclinical models, the efficacy observed in these models has not translated into the clinical setting. In detailed pharmacologic studies of selective CB_2 agonists, we encountered abnormal pharmacokinetic (PK)/pharmacodynamic relationships for numerous compounds in an osteoarthritis (OA) pain animal model, whereby analgesic efficacy was rapidly lost, even while systemic drug exposures remained very high. We found that most reported CB_2 agonists are partial agonists, particularly in promoting receptor internalization following activation, a critical step in receptor recycling required for maintenance of efficacy. In rodent pain models, CB_2 partial agonists were highly efficacious in the first hour following administration but then rapidly lost efficacy despite sustained plasma exposures. In contrast, the in vivo efficacy of full agonists was maintained as long as plasma levels remained sufficient to achieve receptor activation. These studies allowed the identification of APD371, a full agonist at human and rodent CB_2 receptors with >1000-fold selectivity versus the CB_1 receptor. This unique pharmacologic profile provided efficacy in numerous animal pain models commensurate with the compound's PK profile. These data support the unique receptor pharmacology of APD371, a highly selective, full efficacy CB_2 agonist that is currently being evaluated in phase 2 clinical trials for visceral pain associated with Crohn's disease.

METHODS

β-Arrestin Recruitment: Performed in a clonal rat and human CB_1 and CB_2/HEK293 cell lines using the PathHunter technology (DiscoverX, Fremont, California).

Dynamic Mass Redistribution Assays: Compounds were evaluated on the indicated cell types in label-free dynamic mass redistribution assays (Corning Epic®, Tewksbury, Massachusetts).

Receptor Internalization: Hemagglutinin-epitope tagged CB_2-Chinese hamster ovary (CHO) cells were prelabeled with Alexa488 conjugated antihemagglutinin antibody, exposed to test compounds for 2 hours at 37°C and then fixed. Receptor internalization was quantified by high content analysis using the Granularity Module on an InCell Analyzer 1000 (GE Life Sciences, Pittsburgh, Pennsylvania).

Cyclic Adenosine Monophosphate (cAMP) Modulation: Gαi-mediated reductions of forskolin-stimulated intracellular cAMP were measured, using standard protocols, in a CB_2-CHO cell line using the HTRF (Cisbio Bioassays, Bedford, Massachusetts) platform.

Receptor Desensitization Assays: CB_2-CHO cells were treated with either vehicle or 10 mM test CB_2 agonists for 1 hour at 37°C, then washed extensively. Gαi-mediated reductions of forskolin-stimulated intracellular cAMP in response to CP55,940 or other reference CB_2 agonists were measured using the HTRF (Cisbio Bioassays) platform.

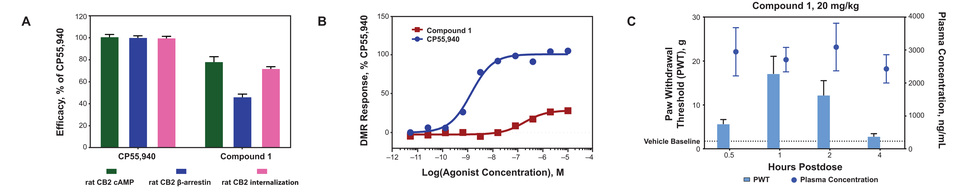
Monosodium Iodoacetate (MIA)–Induced Rat OA Model: MIA (2 mg) was injected intra-articularly into the left knee of each Sprague Dawley rat. Acute effects of CB_2 agonists were measured on day 14 or later following MIA injection. Indicated doses of compounds or vehicle (0.5% methylcellulose) were given orally. Pain was measured as paw withdrawal threshold using a Von Frey apparatus at baseline and at 1, 2, 4, and 6 hours after compound administration. For chronic studies, rats were implanted subcutaneously with osmotic pumps loaded with vehicle or APD371 designed to provide steady-state APD371 plasma levels at about 200 ng/mL. A satellite group of rats was used for PK analysis.

Paclitaxel Model of Neuropathic Pain: Paclitaxel was injected intraperitoneally (2 mg/kg) on days 0, 2, 4, and 6. Pain was measured as paw withdrawal threshold using a Von Frey apparatus 12 days after the first dose. After baseline measurements, indicated doses of APD371 or vehicle (0.5% methylcellulose) were administered orally. Gabapentin (100 mg/kg) was administered intraperitoneally. Pain was measured at 1, 2, 4, and 6 hours after compound administration.

STZ Model of Painful Peripheral Diabetic Neuropathy: Sprague Dawley rats were injected intraperitoneally with streptozotocin (STZ) at 50 mg/kg. Animals were hyperglycemic by 72 hours after injection administration. Animals were considered diabetic when nonfasting blood glucose values were >250 mg/dL. Development of allodynia was monitored on a weekly basis using Von Frey filaments. Effects of APD371 (10 mg/kg) given orally were measured using a Von Frey apparatus at baseline and at 1, 4, and 6 hours after compound administration.

RESULTS

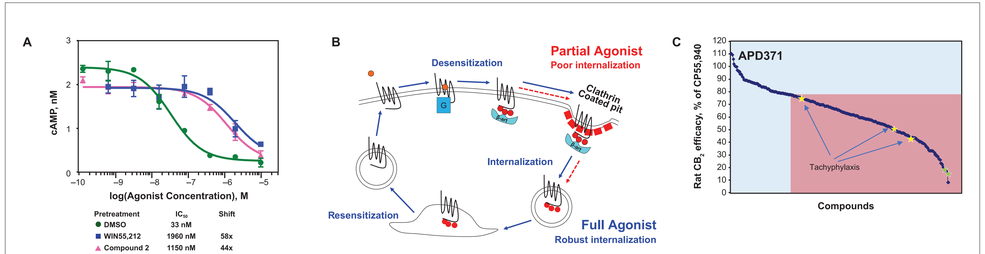
Figure 1. Acute tachyphylaxis of antinociceptive effect of a partial cannabinoid 2 (CB_2) agonist in rat osteoarthritis pain model.



(A) In vitro efficacy of CP55,940 (full CB_2 agonist) and compound 1 (partial CB_2 agonist) in cAMP, β-arrestin recruitment, and receptor internalization assays for the rat CB_2 receptor. **(B)** Compound 1 is a partial agonist in DMR assays in primary rat splenocytes. **(C)** Pharmacodynamics/pharmacokinetics relationship for compound 1 in rat osteoarthritis pain model. Antinociceptive efficacy is rapidly lost while plasma levels remain high (2430 ng/mL). Paw withdrawal threshold is plotted along with the plasma drug levels at the indicated time points after oral drug administration, 20 mg/kg.

cAMP, cyclic adenosine monophosphate; CB_2, cannabinoid 2; DMR, dynamic mass redistribution.

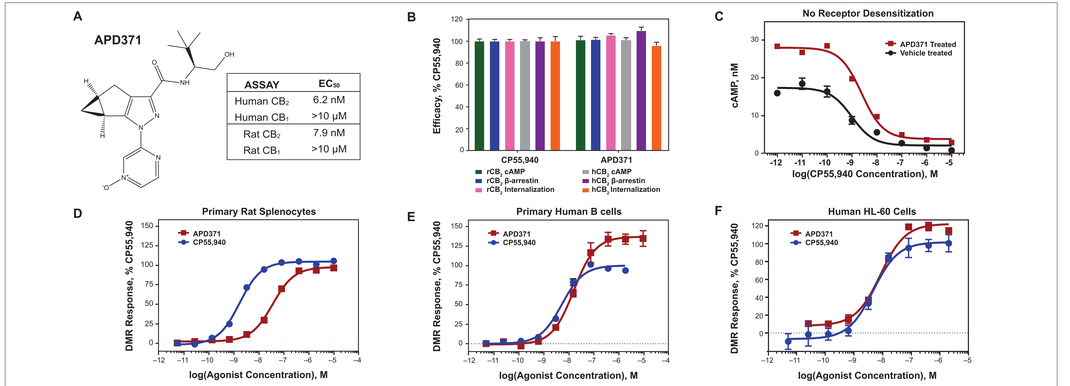
Figure 2. Receptor desensitization: proposed mechanism for the acute tachyphylaxis of cannabinoid 2 (CB_2)–mediated activity observed in vivo.



(A) Pretreatment with partial CB_2 agonists (WIN55,212 or compound 2) induces functional desensitization of rat CB_2 receptor and leads to a large shift in agonist potency upon agonist rechallenge. **(B)** Working hypothesis: CB_2 receptors activated by partial agonists produce an acute response which is rapidly desensitized because of receptor phosphorylation. Receptor dephosphorylation and resensitization is inefficient because of the absence of robust receptor internalization, leading to rapid loss of in vivo efficacy. CB_2 receptors activated by a full agonist efficiently undergo the full cycle of receptor activation/desensitization/internalization/dephosphorylation and recycling, leading to maintenance of in vivo efficacy as long as plasma drug levels remain sufficient for receptor activation. **(C)** CB_2 agonists exhibiting rapid tachyphylaxis are partial agonists in rat CB_2 receptor arrestin recruitment assays. Very few full agonists (relative to CP55,940) were found.

cAMP, cyclic adenosine monophosphate; DMSO, dimethyl sulfoxide; IC_{50}, half maximal inhibition concentration.
Stars indicate examples of CB_2 partial agonists that showed tachyphylaxis in vivo (yellow) or receptor desensitization in vitro (green).

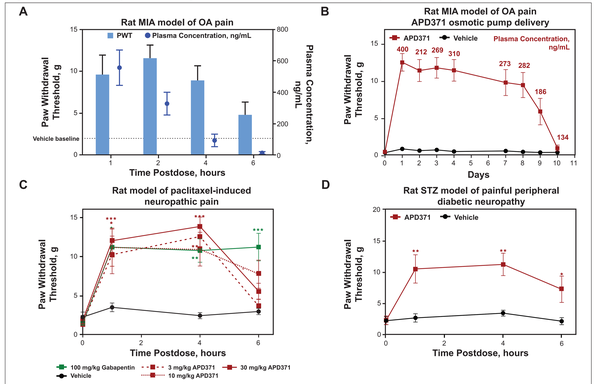
Figure 3. APD371, highly selective cannabinoid 2 (CB_2) agonist with full efficacy in numerous assay platforms in recombinant and primary cells.



(A) Structure and CB_2/cannabinoid 1 pharmacology of APD371. **(B)** In vitro efficacy of APD371 in cAMP, β-arrestin, and receptor internalization assays for the rat and human CB_2 receptor. **(C)** Pretreatment with APD371 does not induce functional desensitization of rat CB_2 receptor for agonist rechallenge. **(D)** APD371 induces fully efficacious response in DMR assays in primary rat splenocytes. **(E)** APD371 induces fully efficacious response in DMR assays in human B cells. **(F)** APD371 induces fully efficacious response in DMR assays in human HL-60 cells.

cAMP, cyclic adenosine monophosphate; DMR, dynamic mass redistribution; hCB2R, human CB_2 receptor; HL-60, human promyelocytic leukemia cell; rCB2, rat CB_2 receptor.

Figure 4. Antinociceptive efficacy of APD371 in animal models of pain.



(A) Pharmacokinetic/pharmacodynamic relationship for APD371 in rat MIA model for OA pain. Antinociceptive efficacy of APD371 is sustained for at least 4 hours, and only decreases at 6 hours, when plasma drug levels are <20 ng/mL. **(B)** Efficacy of APD371 in rat MIA model for OA pain is sustained for at least 9 days while plasma levels of APD371 are maintained above 200 ng/mL by osmotic pump administration for 8.3 days. **(C)** Activity of APD371 in rat paclitaxel-induced neuropathic pain model. Efficacy of APD371 is sustained for 4–6 hours. **(D)** Activity of APD371 in rat STZ model of painful peripheral diabetic neuropathy. Efficacy of APD371 is sustained for 4–6 hours. PWTs are plotted at the indicated time points after drug administration.

MIA, monosodium iodoacetate; OA, osteoarthritis; PWT, paw withdrawal threshold; STZ, streptozotocin.

SUMMARY AND CONCLUSIONS

- Selective CB_2 partial agonists show transient efficacy in rodent model of OA pain, despite sustained plasma exposure
- Partial agonists induce CB_2 receptor desensitization in vitro, presumably because of inefficient receptor internalization and recycling, following activation
- APD371 is a highly selective, full agonist at the CB_2 receptor in recombinant cells and retains full efficacy at endogenous CB_2 receptors in primary cells
- APD371 does not induce desensitization of CB_2 receptor signaling in vitro
- Following single dosing, APD371 demonstrates sustained efficacy in OA pain model, commensurate with its PK profile
- Upon chronic administration, APD371 maintains full efficacy in OA pain model as long as plasma drug levels are maintained
- These data support the unique profile of APD371 that is currently being evaluated in a phase 2 clinical trial for visceral pain associated with Crohn's disease

REFERENCES

1. Dhopeshwarkar A, Mackie K. *Mol Pharmacol*. 2014;86(4):430-437.
2. Anand P et al. *Brain Res Rev*. 2009;60(1):255-266.

ACKNOWLEDGMENTS

Medical editorial assistance was provided by ApotheCom, San Francisco, CA.

FUNDING

This study and associated medical editorial assistance was funded by Arena Pharmaceuticals, Inc.

Poster presented at the American Pain Society Scientific Summit; March 4-6, 2018; Anaheim, California