APD371: A Potent, Highly Selective, Full Agonist of the Human CB2 Receptor
With Sustained Analgesic Effects in Rodents

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INTRODUCTION
Cannabinoid receptor antagonists have been extensively explored to modulate a wide variety of pain modalities. The cannabinoid 2 (CB2) receptor has received significant attention as a target that may provide pain relief without the central nervous system (CNS) liabilities associated cannabinoid 1 (CB1) receptor modulators.1 Although selective CB2 receptor agonists have shown great promise in preclinical models, the efficacy observed in these models has not translated into the clinic. In detailed pharmacologic studies of selective CB2 agonists, we encountered abnormal pharmacokinetic (PK)/pharmacodynamic relationships for numerous compounds in an osteoarthritis (OA) pain animal model, where efficacy was not consistent with PK and were not reproducible across studies. We believe the observed PK/PD relationships are not driven by off-target effects, but rather result from complex PK/PD relationships driven by a critical step in receptor recycling required for maintenance of efficacy. In rodent pain models, CB2 receptor agonists were generally efficacious in the first administration but then rapidly lost efficacy despite sustained plasma exposures. In contrast, it was seen 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 rat CB2 receptors with >1000-fold selectivity versus the CB1 receptor, as a critical step in receptor recycling required for maintenance of efficacy. In rodent pain models, CB2 receptor agonists with >1000-fold selectivity versus the CB1 receptor pharmacology of APD371, a highly selective, full efficacy CB2 receptor agonist, for multiple pain-related behaviors was evident. We found that most reported CB2 agonists for 1 hour at 37°C, then washed extensively.

RESULTS

Figure 1. Acute tachyphylaxis of antinociceptive effect of a partial cannabinoid 2 (CB2) agonist in an osteoarthritis mouse model.

Figure 2. Receptor desensitization: proposed mechanism for the acute tachyphylaxis of cannabinoid 2 (CB2)-mediated activity observed in vivo.

Figure 3. Antinociceptive efficacy of APD371 in animal models of pain.

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

METHODS

Methods: Performed in a clinical trial and human CB1 and CB2

HEK293 cell lines using the PathHunter technology (DiscoverX, Fremont, California).

Performed in a clonal rat and human CB1 and CB2/ovary (CHO) cells were prelabeled with Alexa488 conjugated antihemagglutinin (Sigma-Aldrich) and then stimulated with forskolin (Sigma-Aldrich) to induce internalization of the hemagglutinin (HA)-epitope-tagged CB2-Chinese hamster ovary (CHO) cell line. Receptor internalization was quantified by high content analysis using the Cellomics ArrayScan (ThermoFisher, Waltham, Massachusetts).

Cyclic Adenosine Monophosphate (cAMP) Modulation: G-protein-mediated changes in intracellular cAMP were measured, using standard protocols, in in vitro cell lines using the HTRF (Cisbio Bioassays, Bedford, Massachusetts) platform.

Receptor Internalization: Hemagglutinin-epitope tagged (HA)-Chinese hamster ovary (CHO) cells were prelabeled with Alexa488 conjugated antihemagglutinin, then incubated in 10 mM forskolin (Sigma-Aldrich) for 1 hour at 37°C, washed, and then imaged. Generalized locally on an IncuCyte 100 (Sartorius, Philadelphia, Pennsylvania). In vitro efficacy of APD371 in cAMP, β-arrestin, and receptor internalization assays for the rat and human CB2 receptor.

Receptor Desensitization Assays: CB2/CHO cells were treated either with 100 nM CP55,940 and 100 nM CP55,940 for 1 hour at 37°C, then washed extensively and then imaged. APD371 or a vehicle control was added to the washed cell lines for 2 hours, and the cells were then imaged.

Receptor Internalization: Hemagglutinin-epitope tagged (HA)-Chinese hamster ovary (CHO) cells were prelabeled with Alexa488 conjugated antihemagglutinin, then incubated in 10 mM forskolin (Sigma-Aldrich) for 1 hour at 37°C, washed, and then imaged. Generalized locally on an IncuCyte 100 (Sartorius, Philadelphia, Pennsylvania).

Mouse Model of Neuropathic Pain: Partially hepatectomized Sprague Dawley rats were administered APD371 osmotic pump delivery or vehicle treatment for 8.3 days.

SzT Model of Partially Perforated Duodenal Neuropathy: Sprague Dawley rats were injected ip with a 0.5% concentration of WGA/HRP solution and killed at 3 hours after injection. Animals were sacrificed and tissue was fixed in Bouin’s solution followed by standard histology. A blinded observer counted the number of nerves in the duodenal nerves. Effects of APD371 on myelinated nerves were measured using a laser Doppler flowimeter at 3 and 4 hours after compound administration.

SUMMARY AND CONCLUSIONS

• Selective CB2 partial agonists show transient efficacy in rodent model of OA pain, despite sustained plasma exposure
• Partial agonists induce CB2 receptor desensitization in vitro, presumably because of insufficient receptor internalization and recycling, following activation
• APD371 is a highly selective, full agonist at the CB2 receptor in recombinant cells and exerts robust efficacy at endogenous human CB2 receptors in primary cells
• APD371 does not induce desensitization of CB2 receptor signaling in vitro
• Following single administration, APD371 demonstrates sustained efficacy in OA pain model, commensurate with its PK profile
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REFERENCES

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