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ORIGINAL RESEARCH

Tissue distribution and abuse potential of prucalopride: findings from non-clinical and clinical studies

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Abstract

Background: Prucalopride is a selective serotonin type 4 (5-HT_4) receptor agonist indicated for treatment of chronic idiopathic constipation (CIC) in adults (2 mg orally, daily). 5-HT_4 receptors are present in the central nervous system; therefore, non-clinical and clinical assessments were performed to evaluate the tissue distribution and abuse potential of prucalopride.

Methods: In vitro receptor-ligand binding studies were performed to assess the affinity of prucalopride (≤1 mM) for peptide receptors, ion channels, monoamine neurotransmitters and 5-HT receptors. The tissue distribution of ^{14}C-prucalopride (5 mg base-equivalent/kg) was investigated in rats. Behavioural assessments in mice, rats and dogs after treatment with single or repeated (up to 24 months) subcutaneous or oral doses of prucalopride (0.02–640 mg/kg across species) were performed. Treatment-emergent adverse events possibly indicative of abuse potential during prucalopride CIC clinical trials were evaluated.

Results: Prucalopride showed no appreciable affinity for the receptors and ion channels investigated; its affinity (at ≤100 µM) for other 5-HT receptors was 150–10,000 times lower than that for the 5-HT_4 receptor. In rats, <0.1% of the administered dose was found in the brain and concentrations were below the limit of detection within 24 hours. At supratherapeutic doses (≥20 mg/kg), mice and rats exhibited palpebral ptosis, and dogs exhibited salivation, eyelid tremors, decubitis, pedalling movements and sedation. All clinical treatment-emergent adverse events, possibly indicative of abuse potential, except dizziness, occurred in <1% of patients treated with prucalopride or placebo.

Conclusion: This series of non-clinical and clinical studies suggest low abuse potential for prucalopride.

Keywords: central nervous system, constipation, drug abuse, drug toxicity, pharmacokinetics, pharmacology, prucalopride, toxicology.

Citation

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Introduction

Prucalopride is a selective serotonin type 4 (5-HT_4) receptor agonist indicated for the treatment of chronic idiopathic constipation (CIC) in adults.[1] In humans, prucalopride is rapidly absorbed and extensively distributed, reaching the maximum plasma concentration (approximately 7.5 ng/mL or 20 nM [human therapeutic C_{max}])[2,3] 2–3 hours after a single 2 mg oral dose.[1] Prucalopride is primarily metabolized via demethylation and oxidation of the methoxypropyl moiety; 84% of the administered dose is recovered in urine and 13% is recovered in faeces.[1,4] R107504, the metabolite formed after O-demethylation and oxidation of the resulting alcohol to a carboxylic acid,[5] is the most abundant metabolite of prucalopride and accounts for 3.2% and 3.1% of the administered dose in urine and faeces in humans, respectively.[1]

The 5-HT_4 receptor is expressed throughout the human body. It is present in the gastrointestinal tract, where it is involved in the mediation of gastrointestinal functions, including peristalsis, secretion and vasodilation, which contribute to normal bowel function.[6] Given that gastrointestinal transit is likely to be impaired in CIC,[7,8] the 5-HT_4 receptor has become a therapeutic target in this dis-

ease. In addition to the gastrointestinal tract, the 5-HT_4 receptor is expressed in the urinary bladder, heart, adrenal glands and brain.[9] In the brain, expression levels are greatest in the basal ganglia, hippocampus and cortex.[9]

As well as bowel function, the 5-HT_4 receptor is implicated in a variety of other physiological functions and their pathophysiological variants, such as mood and depression or anxiety, food intake, obesity or anorexia, and memory and memory loss.[9] Changes in expression levels of 5-HT_4 receptors in the brain and central nervous system (CNS) have been shown to be associated with a number of brain disorders.[9] Studies of rodent models of depression and anxiety have demonstrated that 5-HT_4 receptor expression in the brain is upregulated after social defeat[10] or restraint stress,[11] and downregulated in response to unpredictable stress or maternal deprivation.[12] In humans, downregulation of 5-HT_4 receptor expression in the brain has been observed in patients with Alzheimer's disease and Huntington's disease,[13] and its expression has been shown to be correlated negatively with cognition and episodic memory.[14]

Drug products that are active in the CNS generally contain chemical ingredients that promote euphoria (or other changes in mood), hallucinations, or effects consistent with CNS depressants or stimulants. These drugs may be subject to abuse, defined as the intentional, non-therapeutic use of a drug product or substance to achieve a desired psychological or physiological effect.[15] Given the presence of 5-HT_4 receptors in the CNS, the abuse potential of prucalopride (i.e. the likelihood that abuse of this drug will occur given that it may be active in the CNS) was assessed using relevant data from a series of non-clinical in vitro and in vivo studies investigating the pharmacology, pharmacokinetics and toxicology of prucalopride and animal behaviour in response to treatment with prucalopride. Results from these studies are discussed in the context of findings from prucalopride clinical trials in patients with CIC during which any treatment-emergent adverse events (TEAEs) potentially related to drug abuse and misuse were recorded.[16–31]

Materials and methods

Overview

In vitro and in vivo non-clinical studies and phase II–IV randomized, double-blind, placebo-controlled clinical studies in patients with CIC were evaluated for data relevant to the assessment of whether prucalopride has activity in the CNS. Relevant assessments and data were selected based on guidance from the US Food and Drug Administration (FDA),[15] and included chemistry toxicology screening, receptor binding studies, in vivo tissue distribution, pharmacology, safety pharmacology and toxicology studies, and clinical trials to evaluate changes in behaviour that may be indicative of CNS activity. Data from these studies were evaluated collectively to assess the abuse potential of prucalopride.

All relevant studies in animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the institution's animal care and use committee. Animals were sacrificed by either CO_2/chloroform gas inhalation (mice and rats), decapitation (rats) or exsanguination via the carotid artery following anaesthesia (dogs, mice and rats). All clinical studies were performed in accordance with the ethical principles of the Declaration of Helsinki and were approved by institutional review boards or local independent ethics committees (further details are provided in the Supplementary Material; available at: https://www.drugsincontext.com/wp-content/uploads/2023/01/dic.2022-6-1-Suppl.docx). The in vivo work presented here is reported as per the Animal Research: Reporting of In Vivo Experiments (ARRIVE) Equator Network guidelines. Clinical data presented here are mostly from published clinical studies.[16–31]

In vitro receptor–ligand binding studies

A comprehensive set of receptor–ligand binding studies were conducted with prucalopride and its metabolites at a wide range of concentrations, from 0.1 nM to 1 mM. The effect of prucalopride on the uptake of monoamine neurotransmitters, peptide receptors, ion channel binding sites, monoamine neurotransmitter transporters and γ-aminobutyric acid was investigated. In addition, the functional effects of prucalopride (up to 100 μM) on intracellular concentrations of Ca^{2+} in intact mammalian cells expressing human [h] 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{4A} and 5-HT_{4B} receptors were assessed. The following receptors, ion channels or transporters were included: neurotransmitter receptor binding sites (serotonergic, adrenergic, dopaminergic, histamine and r-cholinergic muscarinic); drug receptor binding sites; ion channel ligand binding sites; peptide receptor binding sites (neurokinin, bradykinin, cholecystokinin and h-vasoactive intestinal peptide); lipid-derived factor binding sites; monoamine transporter sites; and [^{3}H] γ-aminobutyric acid (GABA) uptake in crude rat synaptosome preparations. Additional methodological details are provided in Tables S1 and S2.

Chemistry toxicology screening study

The identification of controlled substances, such as narcotics and psychotropic drugs, is the first step towards meeting compound compliance legal requirements. The ChemAxon Compliance Checker (Budapest, Hungary) is a combination of a software system and a

regularly updated content package, providing a flexible tool to efficiently screen chemical structures against the controlling legislation. Prucalopride was evaluated visually and computationally for structural relationships with controlled drug substances using the ChemAxon Compliance Checker.

In vivo studies

No specific inclusion or exclusion criteria were set for animals included in the studies detailed below; however, animals needed to pass a physical examination before the studies started. Confounders were not controlled for. Where applicable, control groups received solvent or similar and, where available, historical control data were used. Humane endpoints were defined as part of each study protocol and studies adhered to the 3Rs (replacement, reduction and refinement). The number of animals used for each experiment is detailed in the Methods and Results sections, and exclusions detailed where applicable.

Tissue distribution studies in rats

The tissue distribution of prucalopride in specific pathogen-free (SPF) Wistar rats (Hannover sub-strain; obtained from Charles River, Sulzfeld, Germany) was investigated in three separate studies: one using male rats (n=5; mean weight, 238±8 g; age not documented), one using non-pregnant female rats (seven groups of three; mean weight across all groups, 181±19 g; age not documented) and one using pregnant female rats (n=4; mean weight, 226±22 g; age not documented) dosed on day 18 of the gestation period. Rats were dosed orally with ^{14}C-labelled prucalopride succinate at 5 mg base-equivalent (eq.)/kg. The mean doses of radioactivity administered in male, non-pregnant female and pregnant female rats were 1.90, 0.27 and 1.08 MBq, respectively.

Measurements in male and pregnant female rats

Male rats were sacrificed at 0.5, 2, 4, 8 and 24 hours after dosing, and pregnant female rats were sacrificed at 0.5, 2, 8 and 24 hours after dosing (n=1 rat per time interval). Radioluminography of whole-body sections was performed using a Fuji BAS 2000 bioimaging analyser system (Fujifilm, Tokyo, Japan) to quantify total radioactivity. Detection was performed using Fuji Imaging Plates, BAS III (Fujifilm). Tissue levels of total radioactivity were calculated using Raytest-Tina packet software (Elysia-Raytest, Straubenhardt, Germany) and expressed as microgram equivalents of prucalopride-base per gram of tissue or millilitre of blood or plasma, based on the specific activity of the radiolabelled compound and the administered dose. Areas under the concentration *versus* time curves from 0 to 8 hours ($AUC_{0-8\,h}$) for total radioactivity were calculated using the trapezoidal method[32] and $AUC_{0-8\,h}$ tissue to blood and tissue to plasma ratios were calculated from $AUC_{0-8\,h}$ values.

Measurements in non-pregnant female rats

Non-pregnant female rats were sacrificed at 20 minutes and 1, 3, 8, 24, 48 and 96 hours after dosing (n=3 rats per time interval). Tissues were dissected and homogenized, and total radioactivity was measured using liquid scintillation spectrometry (either Packard model Tri-Carb 1900 TR or model Tri-Carb 2100 TR, Packard Bioscience, Meriden, CT, USA), with automatic external standardization and calculation from counts per minute into disintegration per minute. In addition, the concentration of unchanged drug was measured using a validated high-performance liquid chromatography assay with fluorescence detection (details are provided in the Supplementary Information file). $AUC_{0-8\,h}$ for total radioactivity and unchanged drug and $AUC_{0-8\,h}$ tissue to plasma ratios were calculated as described for male and pregnant female rats.

In vivo CNS and behavioural assessments performed during pharmacology, safety pharmacology and toxicology studies

A list of the in vivo studies and their related assessments are provided in Table S3.

Interaction of prucalopride with pentylenetetrazol

To further assess the possible CNS effects and abuse liability of prucalopride, its potential to increase or to antagonize the effects of the CNS convulsant pentylenetetrazol when coadministered, including tremors, tonic and clonic convulsions, and mortality, was evaluated in female SPF Wistar rats (weight, 110–120 g; age not documented; n=5 rats per dose of pentylenetetrazol) obtained from the breeding colonies of Johnson & Johnson, Beerse, Belgium. Rats were administered a single subcutaneous dose of prucalopride 40 mg/kg 1 hour before administration of intravenous doses of pentylenetetrazol (0, 5, 10, 20, 40, 80 or 160 mg/kg). An additional five female rats per dose group received the corresponding volume of solvent before administration of pentylenetetrazol. Tremors and convulsions were scored as 0 (absent), 1 (weak and delayed), 2 (weak but immediate), 3 (pronounced but delayed) or 4 (pronounced and immediate), and the 50% effective dose of pentylenetetrazol was calculated.

Behavioural assessments performed during pharmacology studies in mice, rats and dogs

All animals were obtained from Johnson & Johnson (Beerse, Belgium) breeding colonies. Male Swiss mice (n=5 per dose; body weight, 19–25 g), SPF Wistar rats (n=3–5 per dose; male and female; body weight of males, 225–275 g; body weight of females, 90–110 g)

and non-fasted beagle dogs (*n*=5 per dose; male and female; various body weights) were administered prucalopride up to 40 mg/kg (rodents) or 10 mg/kg (dogs) orally or subcutaneously. The age of the animals was not documented. In mice, behavioural changes or abnormalities were scored as present or absent at 15, 30, 45 and 60 minutes after prucalopride administration, and in rats at 1, 2 and 3 hours after prucalopride administration. The following behavioural changes and body functions were evaluated in mice and rats: sniffing, licking, rearing, preening, chewing, excitation, tremors, convulsions, lacrimation, salivation, diarrhoea, piloerection, passivity, sedation, prostration, catalepsy, ataxia, hypnosis, pinna and cornea reflexes, muscle tone, dyspnoea, hot plate reaction time, and acetic acid writhing. Dogs were observed over a period of 4 hours post dosing for overt behavioural phenomena such as salivation, lacrimation, defecation, diarrhoea, vomiting, hyperventilation, ataxia, loss of righting, sedation, catalepsy, vocalization, excitation, aggressiveness, tremors and convulsions. Additional methodological details relating to the assessments described are provided in the Supplementary Information file.

Behavioural assessments performed during cardiovascular safety pharmacology studies in dogs

Behavioural assessments were performed during three separate cardiovascular safety pharmacology studies in healthy, conscious, instrumented male beagle dogs of various ages (obtained from Johnson & Johnson breeding colonies, Beerse, Belgium). Details of the instrumentation procedure, which allowed monitoring of cardiovascular parameters, are provided in the Supplementary Information file. In one study, dogs (*n*=7; body weight, 11–13 kg) received a single oral dose of prucalopride 0.31 mg/kg and, in a second study, dogs (*n*=9; body weight, 9–13 kg) received a single oral dose of prucalopride 2.5 mg/kg. In a third study, dogs (*n*=7; body weight, 11–12 kg) were administered escalating doses of prucalopride (0.02, 0.04, 0.08, 0.16 and 0.31 mg/kg). Throughout the studies, changes in behaviour, including wakefulness, abnormal posture, respiration, sedation, agitation, excitation, aggression, convulsions, relaxation of the lower eyelids, vomiting, urination and defecation, were observed.

Behavioural and CNS-related assessments performed during single-dose and repeated-dose toxicity studies in mice, rats and dogs

Single-dose toxicity studies

The acute toxicity of a single oral dose of prucalopride 160–640 mg/kg was evaluated in adult Swiss mice (*n*=10 [five males and five females] per dose group; body weight of males, 27–32 g; body weight of females, 20–28 g). Two single-dose toxicity studies of prucalopride were also performed in adult SPF Wistar rats. In one study, four rats (two males and two females) were administered prucalopride 548 mg/kg and two rats (both female) were administered prucalopride 320 mg/kg (weight range of all rats, 212–260 g). In another study, rats (five males and five females; weight range, 185–289 g) were administered prucalopride 640 mg/kg. All mice and rats were obtained from Charles River (Sulzfeld, Germany).

Repeated-dose toxicity studies in rodents

In a 24-month oral carcinogenicity study, Swiss mice (body weight of males, 27–32 g; body weight of females, 21–27 g; approximately 5.5 weeks old) were administered prucalopride 10, 20 or 80 mg/kg per day (*n*=120 (60 males and 60 females) per dose group). In a 1-month, repeated-dose oral toxicity study, SPF Wistar rats (Hannover sub-strain; male and female; body weight range, 120–168 g; approximately 4 weeks old) were administered prucalopride 20, 40 or 80 mg/kg per day (*n*=20 [10 males and 10 females] per dose group). In a 24-month oral carcinogenicity study in SPF Wistar rats (Hannover sub-strain) of approximately 5 weeks of age, male rats (body weight, 87–131 g) were administered prucalopride 5, 20 or 80 mg/kg per day (*n*=60 per dose group) and female rats (body weight, 82–112 g) were administered prucalopride 5, 10 or 40 mg/kg per day (*n*=60 per dose group). All mice and rats were obtained from Charles River, Sulzfeld, Germany, or from Biological Research Laboratories, Füllinsdorf, Switzerland. In single-dose and repeated-dose toxicity studies of mice and rats, all animals were observed daily for signs of clinical or behavioural abnormalities, some of which were CNS related; these included ptosis, convulsions, tremors, salivation, sedation, ataxia and hypothermia.

Repeated-dose toxicity studies in dogs

In a 1-month, repeated-dose oral toxicity study, beagle dogs of approximately 6 months of age (*n*=4 (2 males and 2 females) per dose group; various body weights) were administered prucalopride 10, 20 or 40 mg/kg per day and in a 12-month, repeated-dose oral toxicity study, beagle dogs (*n*=8 (4 males and 4 females) per dose group; various body weights; 6–7 months old) were administered prucalopride 2.5, 10 or 30 mg/kg per day. All dogs were obtained from Johnson & Johnson (Beerse, Belgium) breeding colonies.

In repeated-dose toxicity studies of dogs, all animals were observed daily for signs of clinical or behavioural abnormalities, some of which were CNS related; these included ptosis, photophobia (evaluated during examination of the eye using a slit lamp biomicroscope and an indirect ophthalmoscope), decubitus, pedalling movements, salivation, sedation and biting behaviour.

TEAEs in prucalopride clinical studies that were possibly indicative of abuse potential

To provide data to complement findings from non-clinical studies, TEAEs possibly related to abuse potential that occurred during 18 phase II–IV randomized, double-blind, placebo-controlled clinical trials of prucalopride (0.5, 1, 2 or 4 mg once daily; ≥4 weeks in duration) in patients with CIC were evaluated (ClinicalTrials.gov identifiers NCT00576511,[16] NCT01147926,[17] NCT01424228,[18] NCT00575614,[19, 21] NCT00617513,[20] NCT00487422,[22] NCT00631813,[20] NCT00488137,[23] NCT01116206,[24] NCT00483886,[25] NCT00485940,[26,27] NCT00627692,[28] NCT00596596,[20] NCT00577018,[29] and NCT00598338,[30] and non-registered studies PRU-NED-2,[31] PRU-NED-13 and PRU-FRA-1). During the studies, adverse events were reported by the patient and were recorded by the investigator, from signing of informed consent onwards until the last visit. Adverse events evaluated here include dizziness, feeling abnormal, somnolence, confused state, disorientation, euphoric mood, hallucination, altered mood, mood swings, aggression and elevated mood. These events were selected in accordance with FDA guidelines[15] and were classified as being related to abuse. All events evaluated were identified using Medical Dictionary for Regulatory Activities (MedDRA 16.0) terms.

Results

In vitro receptor binding studies

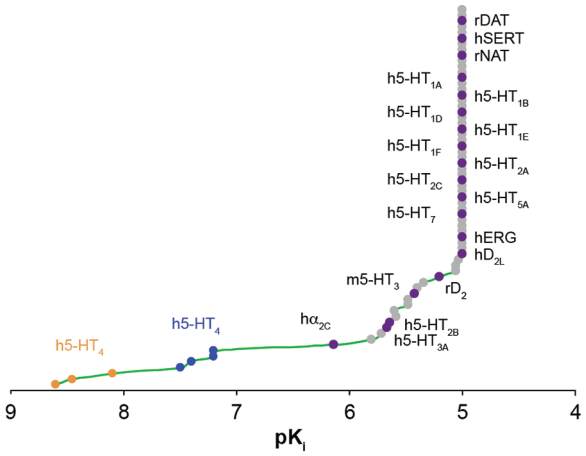
During comprehensive in vitro receptor–ligand binding studies, it was demonstrated that prucalopride had a binding affinity (K_i) of 8 nM for human (h) 5-HT_{4B} receptors and of 2.5 nM for h5-HT_{4A} receptors. When tested for cyclic adenosine monophosphate stimulation of these receptor isoforms, prucalopride was demonstrated to be a potent agonist and exhibited a half-maximal effect concentration of 3 nM and 5 nM on the h5-HT_{4B} and h5-HT_{4A} receptors, respectively. Prucalopride interacted weakly with hD_4 (K_i 1.6–2.4 µM), h5-HT_{2B} (K_i 2.2 µM) and m5-HT_3 (K_i 3.5–3.8 µM) receptor binding sites. Apart from its affinity for the 5-HT_4 receptor, prucalopride did not show any appreciable affinity for the wide range of receptors and monoamine transporters studied at the concentrations tested (Table 1). In addition, prucalopride and its metabolites did not show any appreciable affinity for rat-N-methyl-D-aspartic acid channel, rat-calcium or rat-sodium ion channels (data not shown). Overall, the binding affinity of prucalopride and its metabolites ranged from ≥150-fold to >10,000-fold lower for other receptors than for the 5-HT_4 receptor (Table 1; Figure 1). In one of the three in vitro studies, at concentrations of up to 10 µM (500 times the human therapeutic C_{max}),

Table 1. K_i values of prucalopride and its metabolites for serotonergic and dopaminergic receptors.

Drug/metabolite	K_i (µM)					
	5-HT_{4B}	Rat D_2	Human D_2	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
Prucalopride	0.009	6	14	30	2	41
R112718	0.021	216	207	108	7	103
R104068	0.029	9	14	8	4	76
R107504[a]	0.074	138	235	136	10	211
R129531	0.213	>400	>500	>400	28	148
R112716	0.218	266	207	112	10	115
Drug/metabolite	**K_i (µM)**		**K_i compared with 5-HT_{4B}**			
	5-HT_{4B}	Rat D_2	Human D_2	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
Prucalopride	0.009	678×	1609×	3448×	253×	4713×
R112718	0.021	10 286×	9857×	5143×	310×	4905×
R104068	0.029	310×	483×	276×	138×	2621×
R107504[a]	0.074	1865×	3176×	1838×	134×	2851×
R129531	0.213	NA	NA	NA	131×	695×
R112716	0.218	1220×	950×	514×	44×	528×

All metabolites tested have a binding affinity for 5-HT_4 that is somewhat similar to or less than that of prucalopride.
[a]R107504 is the most abundant metabolite in humans.
×, fold amount greater than affinity for 5-HT_{4B}; 5-HT, 5-hydroxytryptamine; K_i, inhibitory constant.

Figure 1. Binding selectivity of prucalopride.



Binding affinities were obtained from the literature and from experiments performed in the present analysis. Results are shown for binding at human (h), mouse (m) or rat (r) receptors and transporters. Each circle represents a single experimental value; for filled circles, the receptor or transporter studied is indicated. pKi values for the 5-HT4 receptor that were obtained in competitive binding experiments with a radiolabelled antagonist are shown in blue, whilst those obtained in experiments with a radiolabelled agonist are shown in orange.

5-HT, 5-hydroxytryptamine; D, dopamine receptor; DAT, dopamine transporter; ERG, ether-à-go-go related gene; NAT, noradrenaline transporter; pKi, binding affinity, the negative log (base 10) of the Ki value; SERT, serotonin reuptake transporter.

Figure previously published in and adapted with permission from De Maeyer et al. (© 2023 John Wiley & Sons Inc.).[35]

prucalopride did not interact with other receptor sites or monoamine transporter sites, nor did it inhibit the uptake of [^{3}H]-GABA in rat brain synaptosomes (data not shown).

All five metabolites of prucalopride (R104068, R107504, R112716, R112718 and R129531) had a lower affinity for the 5-HT$_4$ receptor with K_i values ranging from 21 to 218 nM, in comparison with prucalopride, which had a K_i value of 8.7 nM.

Chemistry toxicology screening study

Evaluation of prucalopride for structural relationships with controlled drug substances using the ChemAxon Compliance Checker yielded negative results. Furthermore, inspection of prucalopride did not reveal chemical functionality that could reasonably be predicted to be similar to or have pharmacology related to controlled substances.

Tissue distribution in rats

After a single oral dose of ^{14}C-labelled prucalopride 5 mg base-eq./kg, concentrations of total radioactivity were highest in the small intestine (15.6 µg-eq./g, within 30 minutes after dosing), liver (12.5 µg-eq./g, within 20 minutes after dosing) and pancreas (8.8 µg-eq./g, within 30 minutes after dosing) of pregnant and non-pregnant female rats and male rats, respectively (Tables 2–4). In all three studies, very low concentrations of total radioactivity were found in the brain (0.12–0.21 µg-eq./g, within 20–30 minutes after dosing). Findings were similar for the unchanged drug (measured in non-pregnant female rats only), concentrations of which were highest in the small intestine (10.1 µg-eq./g, within 20 minutes after dosing) and lowest in the brain (0.15 µg-eq./g, within 1 hour after dosing) (Table S4). In male and pregnant female rats, concentrations of total radioactivity found in the brain were below the limit of detection within 2–8 hours after dosing (Tables 2–4, Table S4). In non-pregnant female rats, concentrations of total radioactivity were close to the limit of detection 8 hours after dosing, were below the limit of detection within 24 hours (the next measurement time point) and corresponded to <0.1% of the administered dose of prucalopride. In non-pregnant female rats, concentrations of unchanged drug were below the limit of detection within 8 hours after dosing (Table S4).

Across the three studies, the $AUC_{0-8\ h}$ (µg-eq.*h/mL or g) and the $AUC_{0-8\ h}$ tissue to blood ratios for total radioactivity were lowest in the brain (0.33–0.85 and 0.17–0.35, respectively) and spinal cord (0.35 and 0.18, respectively) (Tables 2–4). Considerably higher $AUC_{0-8\ h}$ (µg-eq.*h/mL or g) and $AUC_{0-8\ h}$ tissue to blood ratios for total radioactivity were observed in the liver (38.7–52.1 and 6.5–21.7, respectively), small intestine (23.8–40.4 and 11.0–26.9, respectively) and salivary glands (29.9–122.0 and 8.74–20.5, respectively). Findings were similar for the unchanged drug (measured in non-pregnant female rats only), for which the highest and lowest $AUC_{0-8\ h}$ and $AUC_{0-8\ h}$ tissue to plasma ratios were observed in the small intestine and brain, respectively (Table S4).

In vivo CNS and behavioural observations in pharmacology, safety pharmacology and toxicology studies

Interaction of prucalopride with pentylenetetrazol

In rats, prucalopride administered subcutaneously at 40 mg/kg did not antagonize or potentiate the ability of pentylenetetrazol (at doses up to 160 mg/kg intravenously) to induce tremors, clonic and tonic convulsions

Table 2. Concentrations and $AUC_{0-8\,h}$ of total radioactivity in various tissues of male rats after a single oral dose of prucalopride 5 mg base-equivalent/kg.

Tissue	Concentration of total radioactivity (µg-equivalent/mL or g)						
	0.5 h (*n*=1)	2 h (*n*=1)	4 h (*n*=1)	8 h (*n*=1)	24 h (*n*=1)	$AUC_{0-8\,h}$ (µg-equivalent*h/mL or g)	$AUC_{0-8\,h}$ tissue to blood ratio
Adrenal gland	2.95	1.43	0.91	1.17	≤0.10	10.50	3.07
Blood	1.29	0.43	0.28	0.28	≤0.10	3.42	1.00
Blood vessel	1.51	1.09	0.85	0.74	≤0.10	7.45	2.18
Bone	0.18	≤0.10	≤0.10	≤0.10	≤0.10	ND	ND
Bone marrow	2.56	1.62	0.79	1.13	≤0.10	10.00	2.92
Brain	0.12	≤0.10	≤0.10	≤0.10	≤0.10	ND	ND
Brown fat	0.93	0.56	0.48	0.48	≤0.10	4.30	1.26
Caecum	1.05	1.34	1.80	ND	0.46	5.19[a]	2.25[a]
Epididymis	0.70	1.13	0.77	0.83	≤0.10	6.65	1.94
Oesophagus	1.71	1.96	1.18	0.60	≤0.10	9.90	4.33
Eye	1.30	0.83	0.72	0.55	≤0.10	6.00	1.75
Heart	1.43	0.72	0.48	0.55	≤0.10	5.22	1.53
Kidney	3.28	1.62	1.13	1.52	≤0.10	12.50	3.65
Lacrimal gland	2.88	2.49	1.62	1.46	≤0.10	15.00	4.39
Large intestine	1.45	2.19	2.15	4.44	≤0.10	20.60	6.02
Liver	7.68	6.02	5.41	3.63	0.40	41.70	12.20
Lung	1.66	0.91	0.74	0.73	≤0.10	6.91	2.02
Lymph nodes	2.31	1.12	0.73	0.78	≤0.10	8.02	2.35
Muscle	1.07	0.73	0.41	0.55	≤0.10	4.68	1.37
Nasal tissues	0.41	ND	0.61	0.97	0.10	5.06	1.48
Pancreas	8.85	4.41	3.47	5.01	≤0.10	37.00	10.80
Pineal gland	1.94	0.38	0.68	0.78	≤0.10	6.21	1.82
Pituitary gland	4.66	3.23	2.72	2.50	0.22	23.50	6.87
Preputial gland	3.06	2.57	2.10	3.15	0.36	20.10	5.88
Prostate	1.84	2.49	1.46	3.59	0.16	17.80	5.20
Rectum	2.05	1.94	0.83	0.88	0.26	9.67	2.83
Salivary gland	5.18	5.43	2.90	3.28	≤0.10	29.90	8.74
Seminal vesicles	1.23	1.45	0.95	1.58	≤0.10	9.77	2.86
Skin	0.83	1.66	1.27	1.75	0.13	11.10	3.25
Small intestine	4.97	3.13	8.03	1.60	≤0.10	37.70	11.00
Spinal cord	0.12	≤0.10	≤0.10	≤0.10	≤0.10	ND	ND
Spleen	4.38	2.37	1.16	1.38	≤0.10	14.80	2.89
Stomach	4.29	1.75	1.43	1.31	≤0.10	14.20	4.15
Testicle	0.23	0.47	0.42	0.53	0.15	3.37	0.99
Thymus	1.46	1.17	0.57	0.84	≤0.10	6.88	2.01
Thyroid	3.19	1.61	1.00	1.30	0.12	11.60	3.39

(*Continued*)

Table 2. Concentrations and $AUC_{0-8\,h}$ of total radioactivity in various tissues of male rats after a single oral dose of prucalopride 5 mg base-equivalent/kg. (*Continued*)

Tissue	Concentration of total radioactivity (µg-equivalent/mL or g)						
	0.5 h (*n*=1)	2 h (*n*=1)	4 h (*n*=1)	8 h (*n*=1)	24 h (*n*=1)	$AUC_{0-8\,h}$ (µg-equivalent*h/mL or g)	$AUC_{0-8\,h}$ tissue to blood ratio
Trachea	NA	1.38	1.02	ND	≤0.10	ND	ND
Ventricles	0.47	0.39	0.66	0.36	≤0.10	3.85	1.13
White adipose tissue	0.19	0.13	≤0.10	0.11	≤0.10	0.94[b]	0.27

LOD: 0.1 µg-equivalent/mL or g.
[a]$AUC_{0-4\,h}$ or ratio.
[b]When calculating $AUC_{0-8\,h}$, the level below the LOD was considered as LOD.
$AUC_{0-8\,h}$, area under the concentration *versus* time curve from 0 to 8 hours; LOD, limit of detection; NA, not available; ND, not determined.

Table 3. Concentrations and $AUC_{0-8\,h}$ of total radioactivity in various tissues of pregnant female rats after a single oral dose of prucalopride 5 mg base-equivalent/kg.

Tissue	Concentration of total radioactivity (µg-equivalent/mL or g)					
	0.5 h (*n*=1)	2 h (*n*=1)	8 h (*n*=1)	24 h (*n*=1)	$AUC_{0-8\,h}$ (µg-equivalent*h/mL or g)	$AUC_{0-8\,h}$ tissue to blood ratio
Adrenal gland	5.94	6.86	1.67	<0.14	36.70	6.16
Bladder	1.66	ND	0.72	<0.14	ND	2.14[a]
Blood vessel	2.39	1.76	ND	<0.14	3.71[b]	1.86[b]
Blood	1.25	0.99	0.34	<0.14	5.95	1.00
Bone	0.21	0.21	<0.14	<0.14	0.37[b]	0.19[b]
Bone marrow	4.01	4.05	1.12	<0.14	22.60	3.79
Brain	0.21	0.15	<0.14	<0.14	0.33[b]	0.17[b]
Brown fat	1.79	1.55	0.52	<0.14	9.18	1.54
Caecum	2.67	ND	3.78	<0.14	ND	11.20
Oesophagus	ND	1.88	1.19	<0.14	9.21[b]	2.32[b]
Eye	0.14	0.19	<0.14	<0.14	0.28[b]	0.14[b]
Heart	2.34	1.69	0.55	<0.14	10.30	1.73
Kidney	8.87	8.51	3.62	<0.14	51.60	8.68
Lacrimal gland	6.80	5.48	1.56	<0.14	32.10	5.38
Large intestine	3.53	4.31	2.92	0.37	28.40	4.78
Liver	7.67	5.85	3.04	0.54	38.70	6.51
Lung	2.67	2.06	0.69	<0.14	12.50	2.10
Lymph nodes	3.37	2.93	1.15	<0.14	17.80	3.00
Muscle	1.86	1.94	0.62	<0.14	11.00	1.84
Nasal tissues	0.81	1.35	NA	0.14	1.82[b]	0.92[b]

(*Continued*)

Table 3. Concentrations and $AUC_{0-8\,h}$ of total radioactivity in various tissues of pregnant female rats after a single oral dose of prucalopride 5 mg base-equivalent/kg. (*Continued*)

Tissue	Concentration of total radioactivity (µg-equivalent/mL or g)					
	0.5 h (*n*=1)	**2 h (*n*=1)**	**8 h (*n*=1)**	**24 h (*n*=1)**	**$AUC_{0-8\,h}$ (µg-equivalent*h/mL or g)**	**$AUC_{0-8\,h}$ tissue to blood ratio**
Pancreas	10.50	9.13	5.79	<0.14	62.10	10.40
Pineal gland	4.30	ND	ND	ND	ND	3.44[c]
Pituitary gland	6.87	4.25	2.24	0.17	29.50	4.96
Preputial gland	6.58	10.60	4.09	1.43	58.60	9.84
Rectum	1.65	1.38	3.19	1.21	16.40	2.75
Salivary gland	7.65	25.40	6.28	<0.14	122.00	20.50
Skin	0.77[2]	1.07	0.37	<0.14	5.89	0.99
Small intestine	15.60	4.93	2.09	<0.14	40.40	6.78
Spinal cord	0.21	0.19	<0.14	<0.14	0.35[b]	0.18[b]
Spleen	12.10	8.32	3.53	0.17	53.90	9.05
Stomach	5.28	2.67	0.59	<0.14	17.10	2.87
Thymus	3.74	4.01	1.08	<0.14	22.00	3.70
Thyroid	8.16	3.55	1.44	0.14	25.80	4.33
Trachea	ND	ND	1.09	<0.14	ND	3.23[a]
Ventricles	1.05	1.06	0.65	<0.14	6.95	1.17
White adipose tissue	0.33	0.37	<0.14	<0.14	0.60[b]	0.30[b]

LOD: 0.14 µg-equivalent/mL or g.
[a]8-hour concentration ratio.
[b]$AUC_{0-2\,h}$ value or ratio.
[c]0.5-hour concentration ratio.
$AUC_{0-2\,h,}$ area under the concentration *versus* time curve from 0 to 2 hours; $AUC_{0-8\,h,}$ area under the concentration *versus* time curve from 0 to 8 hours; LOD, limit of detection; NA, not available; ND, not determined.

of the hind and fore paws, or mortality, when compared with solvent (Table S5).

Behavioural observations in pharmacology studies in mice, rats and dogs

In mice and rats at doses up to 40 mg/kg, and in dogs at doses up to 10 mg/kg, prucalopride did not cause tremors or convulsions, respiratory effects (hyperventilation, dyspnoea), cholinergic effects (salivation, lacrimation, diarrhoea), antidopaminergic effects (passivity, sedation, prostration, catalepsy), dopaminergic effects (chewing, licking, rearing or preening in rats; excitation in rats and dogs), adrenergic effects (piloerection, vocalization, aggressiveness), analgesic effects (delayed hot plate reaction time, blockade of pineal and corneal reflexes in mice; vomiting in dogs), or hypnotic or anticonvulsant effects (ataxia, loss of righting, sedation, catalepsy). In addition, no effects on body functions (temperature, muscle tone, pupil diameter) were observed at these doses. At very high doses (>20 mg/kg), prucalopride induced slight palpebral ptosis in mice and rats and decreased vertical motor activity.

Behavioural observations in cardiovascular safety pharmacology studies in dogs

After a single oral dose of prucalopride 0.31 mg/kg (corresponding to a mean peak plasma concentration of 61 ng/mL; approximately eight times the human therapeutic C_{max}), no consistent effects on behavioural parameters, including awakeness, abnormal posture, respiration, sedation, agitation, excitation, aggression, convulsions, relaxation of the lower eyelids, vomiting, urination and defecation, were observed in conscious instrumented dogs, aside from a short period of agitation (30 minutes) in one dog. Similarly, no changes in the aforementioned behavioural parameters were observed in eight out of nine dogs treated with solvent (control group) and in all nine dogs administered a single oral

Table 4. Mean concentrations and $AUC_{0-8\,h}$ of total radioactivity in various tissues of non-pregnant female rats after a single oral dose of prucalopride 5 mg base-equivalent/kg.

Tissue	Mean (SD) concentration of total radioactivity (µg-equivalent/mL or g)								
	20 min (*n*=3)	1 h (*n*=3)	3 h (*n*=3)	8 h (*n*=3)	24 h (*n*=3)	48 h (*n*=3)	96 h (*n*=2)[a]	$AUC_{0-8\,h}$ (µg-equivalent*h/mL or g)	$AUC_{0-8\,h}$ tissue to plasma ratio
Adrenal gland	5.15±1.98	6.46±1.46	3.02±0.56	1.02±0.44	≤0.60[b]	≤0.60	≤0.60	24.30	10.10
Bone marrow	7.41±6.77	10.90±2.30	4.51±2.21	1.38±0.72	≤0.20	≤0.20	≤0.20	37.50	15.60
Brain	0.12±0.03	0.21±0.01	0.11±0.01	0.04±0.02	≤0.03	≤0.03	≤0.03	0.85	0.35
Brown fat	1.43±0.35	2.13±0.66	1.08±0.20	0.45±0.15	0.02[b]	≤0.02	≤0.02	8.47	3.52
Oesophagus	7.48±5.50	3.33±0.23	1.76±0.31	0.66±0.26	0.05±0.02	≤0.02	≤0.02	16.00	6.65
Heart	1.85±0.43	2.33±0.22	1.03±0.14	0.34±0.14	≤0.06	≤0.06	≤0.06	8.50	3.53
Kidney	6.34±1.54	6.63±0.60	3.10±0.40	1.22±0.51	0.11±0.02	0.08±0.02	0.07	25.90	10.80
Lacrimal gland	2.84±1.11	7.14±1.79	3.27±0.37	1.07±0.64	0.07±0.02	0.02±0.01	≤0.01	25.10	10.40
Large intestine	1.45±0.43	2.94±0.72	3.39±0.78	4.84±0.15	0.22±0.06	≤0.03[b]	≤0.03	28.60	11.90
Liver	12.50±2.80	9.54±2.07	6.44±0.32	4.23±0.59	0.77±0.11	0.47±0.02	0.30	52.10	21.70
Lung	3.01±0.92	4.97±0.91	2.32±0.26	0.77±0.43	≤0.04[b]	≤0.04	≤0.04	18.20	7.56
Lymph nodes	3.00±1.13	5.06±1.04	2.06±0.33	0.85±0.15	≤0.07	≤0.07	≤0.07	17.60	7.31
Muscle	0.95±0.30	1.90±0.21	1.05±0.12	0.35±0.11	≤0.03	≤0.03	≤0.03	7.57	3.15
Ovary	2.16±0.76	2.86±0.61	1.25±0.17	0.50±0.18	≤0.02[b]	≤0.02	≤0.02	10.50	4.37
Pancreas	9.23±2.21	11.70±0.70	8.01±0.09	3.55±1.16	≤0.06[b]	≤0.06	≤0.06	57.10	23.80
Perirenal fat	0.22±0.04	0.38±0.07	0.21±0.03	0.08±0.02	≤0.02	≤0.02	≤0.02	1.54	0.64
Pituitary gland	4.46±2.87	11.60±1.80	6.01±1.50	1.76±0.61	≤0.20[b]	≤0.20	≤0.20	43.10	17.90
Plasma	0.46±0.10	0.63±0.09	0.31±0.01	0.10±0.02	≤0.01[b]	≤0.01	≤0.01	2.40	1.00
Salivary gland	5.52±2.26	9.94±0.82	4.45±0.77	5.07±1.55	≤0.10	≤0.10	≤0.10	44.30	18.40
Small intestine	12.40±3.40	12.20±1.40	12.30±4.00	3.93±2.22	0.08±0.01	≤0.03	≤0.03	75.30	31.30
Spleen	7.05±1.75	7.48±2.25	2.66±0.34	1.62±0.58	0.07	≤0.07	≤0.07	26.90	11.20
Stomach	10.30±1.00	7.29±4.37	2.80±0.19	0.77±0.05	0.05[b]	≤0.03	≤0.03	26.60	11.10
Thyroid	8.43±3.55	6.26±1.00	3.20±0.45	1.30±0.73	≤0.20[b]	≤0.20	≤0.20	27.00	11.20
Trachea	3.73±1.62	4.49±0.95	1.91±0.25	0.60±0.21	0.06±0.02	≤0.05	≤0.05	16.00	6.66
Uterus	1.80±0.45	3.16±0.88	1.37±0.13	0.55±0.11	0.03±0.01	≤0.01[b]	≤0.01	11.30	4.69
Vagina	1.16±0.41	2.52±0.07	2.15±1.29	0.74±0.29	0.02[b]	≤0.02	≤0.02	13.30	5.54

[a]One rat was inadvertently dosed twice and data obtained for this animal are thus not reported. [b]Median value.
$AUC_{0-8\,h}$, area under the concentration *versus* time curve from 0 to 8 hours; SD, standard deviation.

dose of prucalopride 2.5 mg/kg (corresponding to a mean peak plasma concentration of 670 ng/mL; approximately 90 times the human therapeutic C_{max}). Trembling was observed in one dog in the control group approximately 25 minutes after administration of solvent.

In dogs administered escalating intravenous doses of prucalopride (0.02–0.31 mg/kg), no changes in behaviour were observed in four out of seven animals. Three dogs briefly vomited immediately after receiving prucalopride 0.04 mg/kg; one of these dogs also vomited after receiving prucalopride 0.08 mg/kg. Mean peak plasma concentrations at prucalopride doses of 0.04 mg/kg and 0.08 mg/kg were 15 ng/mL and 36 ng/mL, respectively (approximately two and five times the human therapeutic C_{max}, respectively).

Behavioural and CNS-related observations in single-dose and repeated-dose toxicity studies in mice, rats and dogs

In single oral dose studies in mice and rats, CNS-related findings included palpebral ptosis and sedation at prucalopride doses ≥160 mg/kg, and tremors, ataxia, clonic convulsions, loss of righting reflex, prostration, hypothermia and salivation at doses ≥320 mg/kg. In addition, in rats, catalepsy and spasms were observed at a dose of 640 mg/kg. These effects were temporary and animals recovered within 3 days of dosing.

In 1-month, repeated-dose studies, ptosis was observed in rats treated with prucalopride ≥80 mg/kg per day (≥800 times the human therapeutic C_{max}). In a 1-month oral toxicity study in beagle dogs, no CNS-related or behavioural abnormalities were noted at prucalopride doses up to 10 mg/kg. At a dose of 20 mg/kg (≥900 times the human therapeutic C_{max}), salivation and appearance of the third eyelid were observed from 0.5 to 4 hours after dosing in all dogs. Ophthalmological examination revealed sporadic instances of bilateral frequent blinking, eyelid tremors and protrusion of the third eyelid. These observations were also noted with increased prominence in dogs administered prucalopride 40 mg/kg, in addition to decubitus and pedalling movements (observed almost throughout the entire dosing period), ptosis and abnormal biting behaviour (from day 14 until study end in three dogs and permanently in one dog).

There were no overt behavioural or CNS-related effects observed in male mice treated with prucalopride up to 80 mg/kg per day or female mice treated with prucalopride up to 20 mg/kg per day for 24 months. In a 24-month oral carcinogenicity study, approximately 50% of male rats (29/59; one rat included in the male group was found to be female; this animal was excluded from the study) treated with prucalopride 80 mg/kg (≥800 times the human therapeutic C_{max}) exhibited ptosis during the first 3 days of dosing only. Ptosis was also observed in four out of 60 female rats treated with prucalopride 40 mg/kg during the first 3 days of dosing only. No additional behavioural or CNS-related changes were observed at doses up to 80 mg/kg in male rats and up to 40 mg/kg in female rats.

In a 12-month, repeated-dose oral toxicity study in dogs, no CNS-related or behavioural abnormalities were noted at prucalopride doses up to 10 mg/kg. Dosing of prucalopride at 30 mg/kg (~900 times the human therapeutic C_{max}) resulted in CNS-related clinical observations, including decubitus (6/8 dogs), slight pedalling movements (5/8 dogs), salivation (slight, 6/8 dogs; moderate, 1/8 dogs) and sedation (slight, 8/8 dogs; moderate, 2/8 dogs). Ocular changes consisted of ptosis (4/8 dogs), protrusion of the third eyelid (1/8 dogs) and photophobia (2/8 dogs).

TEAEs in prucalopride clinical studies that were possibly indicative of abuse potential

In total, 1973 patients with CIC treated with placebo and 3305 patients with CIC treated with prucalopride (0.5 mg, *n*=110; 1 mg, *n*=330; 2 mg, *n*=1516; 4 mg, *n*=1349) were included in this assessment. Patient demographics are shown in Table S6. TEAEs possibly indicative of abuse potential occurred at similar rates in the prucalopride and placebo groups. With the exception of dizziness (which occurred in 1.8% of the placebo group and 3.6% of the prucalopride group), all events possibly indicative of abuse potential occurred in <1% of patients treated with prucalopride or placebo (Table 5). Hallucination and euphoric mood each occurred once in the prucalopride 2 mg group and were not observed in patients who received placebo. Although aggression and elevated mood each occurred once in the placebo group, they were not observed in any patient treated with prucalopride.

Discussion

Given the expression of 5-HT_4 receptors in the CNS, this analysis of behavioural and CNS-related observations made during a series of non-clinical in vitro and in vivo studies aimed to assess the abuse potential of prucalopride. In a comprehensive set of in vitro receptor–ligand binding studies, prucalopride 10 µM to 1 mM (500–50,000 times the human therapeutic C_{max}) did not show any appreciable affinity for a wide array of peptide receptors, ion channels or classical monoamine neurotransmitters. In functional studies investigating the formation of cyclic adenosine monophosphate in cell lines transfected with human 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2c}, 5-HT_{4a} and 5-HT_{4b} receptors, the binding affinity of prucalopride (up to 100 µM, 5000 times the human therapeutic C_{max}) and its metabolites for these receptors was at least 150 times lower than its affinity for the 5-HT_4 receptor. Findings from these in vitro studies demonstrate the selectivity of prucalopride for the 5-HT_4 receptor over other 5-HT receptors.

In tissue distribution studies in rats, very low concentrations of prucalopride and its metabolites reached the brain compared with other organs and tissues. Prucalopride concentrations in the brain were below the limit of detection within 24 hours (and very close to the limit of detection within 8 hours), suggesting that it is not unduly retained in this tissue. Importantly, less than 0.1% of the administered dose was found in the brain. These data suggest that prucalopride would be expected to have minimal effects on the CNS at the concentration tested.

Table 5. TEAEs possibly related to abuse potential that occurred during the on-study period of phase II–IV randomized, double-blind, placebo-controlled studies of ≥4 weeks duration in adults with CIC.

TEAE[a]	Number of patients, *n* (%)					
	Placebo (*n*=1973)	**Prucalopride 0.5 mg (*n*=110)**	**Prucalopride 1 mg (*n*=330)**	**Prucalopride 2 mg (*n*=1516)**	**Prucalopride 4 mg (*n*=1349)**	**Total prucalopride (*n*=3305)**
Dizziness	36 (1.8)	2 (1.8)	9 (2.7)	52 (3.4)	56 (4.2)	119 (3.6)
Feeling abnormal	0 (0)	0 (0)	0 (0)	0 (0)	1 (<1)	1 (<1)
Somnolence	3 (<1)	0 (0)	3 (<1)	2 (<1)	2 (<1)	7 (<1)
Confusion	0 (0)	0 (0)	2 (<1)	1 (<1)	1 (<1)	4 (<1)
Disorientation	1 (<1)	0 (0)	0 (0)	0 (0)	1 (<1)	1 (<1)
Euphoric mood	0 (0)	0 (0)	0 (0)	1 (<1)	0 (0)	1 (<1)
Hallucination	0 (0)	0 (0)	0 (0)	1 (<1)	0 (0)	1 (<1)
Mood altered	0 (0)	0 (0)	1 (<1)	0 (0)	0 (0)	1 (<1)
Mood swings	2 (<1)	0 (0)	0 (0)	0 (0)	1 (<1)	1 (<1)
Aggression	1 (<1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Elevated mood	1 (<1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[a]TEAEs were selected in accordance with FDA recommendations[15] and were identified using Medical Dictionary for Regulatory Activities preferred terms.
CIC, chronic idiopathic constipation; FDA, US Food and Drug Administration; TEAE, treatment-emergent adverse event.

Prucalopride administered at 40 mg/kg did not potentiate or antagonize the ability of pentylenetetrazol to induce tremors, convulsions and mortality, suggesting that it is not expected to have epileptogenic or antiepileptic properties.

In assessments of behavioural and CNS-related changes in mice and rats, prucalopride (<40 mg/kg) had no effects on behaviours or effects that were suggestive of interaction with various receptor types, neurotransmitter uptake, endogenous enzymes, pain perception and body functions. At very high doses (≥20 mg/kg; ≥190 times the human therapeutic C_{max}), prucalopride-treated rats exhibited palpebral ptosis. This effect was also observed in mice administered prucalopride 10 mg/kg or higher. Ptosis has been shown to occur in monoamine neurotransmitter disorders, which are characterized by disruptions in catecholamine and serotonin homeostasis.[33] These disruptions cause weakness in the levator palpebrae superioris muscle, resulting in drooping of the eyelid.[34] At the high concentrations tested, prucalopride may have bound to 5-HT_4 receptors present in the eye, resulting in disruption to normal serotonin binding and leading to the effects observed. At very high, suprathrapeutic doses (≥320 mg/kg), prucalopride induced tremors, ataxia, clonic convulsions, loss of righting reflex, prostration, hypothermia and salivation in mice and rats. These observed effects were temporary and animals recovered within 2–3 days of dosing.

At doses up to 10 mg/kg body weight per day, prucalopride had no effect on behavioural or CNS-related parameters in conscious, instrumented dogs. At a dose of 20 mg/kg (325 times the clinical dose of 2 mg, based on body surface area), prucalopride induced salivation and ophthalmological effects (appearance of the third eyelid), which became more prominent when the dose was increased to 40 mg/kg. CNS-related adverse clinical effects, including decubitis, pedalling movements and sedation, were also observed in dogs administered prucalopride 40 mg/kg.

Taken together, these non-clinical data on the binding selectivity, tissue distribution, and behavioural and CNS effects of prucalopride do not indicate abuse potential for this drug or its metabolites. In addition, most doses of prucalopride tested in these studies significantly exceeded the therapeutic dose used in humans (2 mg oral dose; corresponding to 0.031 mg/kg in a patient weighing 65 kg). These in vitro and in vivo data are corroborated by the low incidence of TEAEs possibly indicative of drug abuse and misuse that occurred during phase II–IV clinical trials of prucalopride in patients with CIC. With the exception of dizziness, all events assessed

occurred in fewer than 1% of patients treated with prucalopride and occurred at similar rates to those treated with placebo. The presence of a euphoria-like response is considered a key observation in the clinical assessment of whether a drug has abuse potential.[15] Although dizziness is listed under euphoria-related Medical Dictionary for Regulatory Activities terms, this adverse event is not by itself considered indicative of abuse potential.[15]

The strengths of this study lie in the wide range of behavioural and CNS assessments performed using both therapeutic and supratherapeutic prucalopride concentrations both in vitro and in vivo in various animal species. In addition, the analysis of TEAEs possibly indicative of abuse potential that occurred during randomized controlled trials of prucalopride in patients with CIC supports the non-clinical findings. A limitation of the study is that the majority of the assessments were performed in animals and the findings may not translate to what is observed in humans. Additionally, owing to a small sample size in many of these studies, no statistical analyses were performed. Finally, though the full range of in vivo studies to assess abuse potential was not implemented, studies were selected for inclusion based on guidance from the FDA.[15]

Conclusions

Findings from this series of in vitro and in vivo studies, which include an assessment of the binding selectivity and tissue distribution of prucalopride as well as the effect of prucalopride on behavioural and CNS-related parameters, suggest a low abuse potential for this drug. This is corroborated by the low incidence of TEAEs possibly indicative of the low drug abuse and misuse that occurred during phase II–IV clinical trials of prucalopride in patients with CIC.

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Disclosure and potential conflicts of interest: KD is an employee of Takeda Development Center Americas, Inc., and is a stockholder of Takeda Pharmaceutical Company Limited. HW was an employee of Takeda Development Center Americas, Inc. at the time of this analysis and is a present employee of Pfizer. ZL was an employee of Takeda Development Center Americas, Inc., at the time of this analysis and is a present employee of H3 Biomedicine. RB was an employee of Takeda Development Center Americas, Inc., at the time of this analysis and is now a self-employed consultant. Portions of this work have previously been presented in the following abstracts: Derakhchan K, Wang H, Papaioannou N, Lou Z, Baughman R, Carey G. Evaluation of prucalopride, a highly selective serotonin-4 (5-hydroxytryptamine)-4 receptor agonist, demonstrates a low potential for abuse liability. *J Pharmacol Toxicol Methods*. 2020;105:106699; Lou Z, Welty D. Investigating the Tissue Distribution of [14C]-Prucalopride Following a Single Oral Dose in Rats. *Am J Gastroenterol*. 2019;S274; and Welty D, Lou Z. P196 – Investigating the Tissue Distribution of [14C]-Prucalopride Following a Single Oral Dose in Rats, 12th International ISSX Meeting; 2019. The International Committee of Medical Journal Editors (ICMJE) Potential Conflicts of Interests form for the authors is available for download at: https://www.drugsincontext.com/wp-content/uploads/2023/01/dic.2022-6-1-COI.pdf

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