

# Selective Serotonergic Properties of Low-Dose Pipamperone May Enhance Antidepressant Effect: Preclinical Evidence

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## Introduction

- It is widely accepted that selective serotonin reuptake inhibitors (SSRIs) are a first-line therapy for major depressive disorder (MDD).<sup>1</sup>
- SSRIs generally have a slow onset of therapeutic effect and low rate of response and remission.<sup>2</sup>
- When response to SSRI monotherapy is unsatisfactory, one approach is to augment therapy with a second medication.<sup>3</sup>
- Drugs antagonizing 5-HT<sub>2A</sub> receptors can provide effective augmentation of SSRI monotherapy<sup>4</sup>; the mechanism may involve inhibition of negative feedback on serotonergic neurons that occurs in response to elevated levels of synaptic 5-HT caused by SSRIs.<sup>5,6</sup>
- Combining a SSRI with a drug that leads to additional and selective inhibition of D<sub>4</sub> and 5-HT<sub>2A</sub> receptors may generate additive and potentially synergistic antidepressant effects by restoring the balance between dopamine (DA) and 5-HT in the limbic system and cortical areas and by increasing both DA and 5-HT tonus while blocking undesired 5-HT<sub>2</sub> receptor activation.
- The neuroleptic pipamperone is a high-affinity antagonist of 5-HT<sub>2A</sub> and D<sub>4</sub> receptors and a low-affinity antagonist of D<sub>2</sub> receptors.<sup>7,8</sup>

## Objective

- To identify a dose of pipamperone that is high enough to have substantial 5-HT<sub>2A</sub> antagonism but low enough to have no relevant D<sub>2</sub> antagonism

## Methods

### Study Design

- To identify appropriate pipamperone doses, a modified version of the apomorphine-tryptamine-norepinephrine (ATN)-test<sup>9</sup> was done in rats.
- Pharmacokinetic and pharmacodynamic modeling was used to predict the binding of pipamperone at relevant plasma concentrations to 5-HT<sub>2A</sub>, D<sub>4</sub>, D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> receptors in patients.
- Protocols were reviewed and approved by the Institutional Animal Review Board and complied with the Declaration of Helsinki.

### ATN-Test

- Male Wiga Wistar rats (Charles River, Germany; n=5 per dose) were injected with pipamperone (0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, or 10 mg/kg subcutaneous) or control (test solvent) and challenged with apomorphine (1.0 mg/kg intravenous [IV]) at 30 minutes, tryptamine (25 mg/kg IV) at 90 minutes, and norepinephrine (1.25 mg/mL IV) at 120 minutes.
- Behavioral and physiologic effects on dopamine, 5-HT, and norepinephrine neurotransmitter systems were scored.
  - After apomorphine challenge: stereotypy (0 = absent, 1 = slight, 2 = moderate, 3 = pronounced) and palpebral opening (1 = one quarter open, 2 = half open, 3 = three quarters open, 4 = wide open, 5 = exophthalmos)
  - After tryptamine challenge: bilateral clonic seizures of the forepaws (0 = absent, 1 = slight, 2 = moderate, 3 = pronounced), palpebral opening (1–5), and hyperemia and cyanosis of the ears (0 = absent, 1 = slight, 2 = moderate, 3 = pronounced)
  - After norepinephrine challenge: α<sub>1</sub> receptor-mediated mortality at 15 minutes (yes/no)

### Pharmacokinetic/Pharmacodynamic Modeling

- In vivo K<sub>d</sub> values for pipamperone binding to the 5-HT<sub>2A</sub>, D<sub>4</sub>, D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> receptors after twice-daily (BID) dosing were predicted from in vitro pK<sub>i</sub> values obtained from literature and corrected based on results of a plasma protein-binding study.
- Assuming sigmoidal binding (using predicted in vivo K<sub>d</sub> and Hill coefficient of 1), occupancy of the 5-HT<sub>2A</sub>, D<sub>4</sub>, D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> receptors after BID dosing was predicted for steady-state plasma concentrations of pipamperone that were determined based on literature values in healthy volunteers and assuming linear pharmacokinetics.

### Statistical Analysis

- Median effective dose (ED<sub>50</sub>) and corresponding 95% confidence limits (CLs) were determined using the Spearman-Kärber estimate<sup>10</sup> with theoretical instead of empirical probabilities to allow tabulation as a function of the log dose-response curve.

## Results

### ATN-Test

- ED<sub>50</sub> values for pipamperone in the ATN-test are shown in **Table 1**.

**Table 1. Median Effective Doses of Pipamperone for Selected Effects in the ATN-Test**

Challenge	ED <sub>50</sub> (95% CL), mg/kg
Apomorphine, 1.0 mg/kg IV	
Inhibition of stereotypy	1.55 (0.96, 2.50)
Decrease of palpebral opening	4.40 (3.30, 8.70)
Tryptamine, 25 mg/kg IV	
Reversal of cyanosis	0.34 (0.22, 0.51)
Inhibition of bilateral convulsions	0.13 (0.08, 0.21)
Decrease of palpebral opening	1.78 (1.10, 2.87)
Norepinephrine, 1.25 mg/kg IV	
Protection against lethality	>10.0

ATN=apomorphine, tryptamine, norepinephrine; CL=confidence limit; ED<sub>50</sub>=median effective dose.

- At very low doses, pipamperone inhibited tryptamine-induced cyanosis (ED<sub>50</sub>, 0.34 mg/kg) and bilateral convulsions (ED<sub>50</sub>, 0.13 mg/kg), indicating blockade of peripheral and central 5-HT<sub>2A</sub> receptors, respectively.
- At higher doses (ED<sub>50</sub>, 1.55–4.40 mg/kg), pipamperone inhibited apomorphine-induced stereotypy (a D<sub>2</sub> receptor-mediated effect) and decreased palpebral opening after the tryptamine and apomorphine challenges (possible α<sub>1</sub> receptor-mediated effects).
- Pipamperone doses of up to 10 mg/kg did not affect norepinephrine-induced lethality.

### Pharmacokinetic/Pharmacodynamic Modeling

- Table 2** shows the in vitro pK<sub>i</sub> values for the 5-HT<sub>2A</sub>, D<sub>4</sub>, D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> receptors and the expected corresponding plasma concentrations of pipamperone required to achieve these values in vivo.
  - These concentrations are above the K<sub>i</sub> value for the 5-HT<sub>2A</sub> and D<sub>4</sub> receptors (**Table 2**).

**Table 2. Expected Plasma Concentrations of Pipamperone Required to Achieve In Vitro pK<sub>i</sub> Values for 5-HT<sub>2A</sub>, D<sub>4</sub>, D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> Receptors In Vivo**

Receptor	pK <sub>i</sub> , M	Pipamperone Total Plasma Concentration, ng/mL
5-HT <sub>2A</sub>	8.2	4
D <sub>4</sub>	8.0	6
D <sub>2</sub>	6.7	117
α <sub>1</sub>	7.2	37
5-HT <sub>2C</sub>	6.9	71
H <sub>1</sub>	5.7	1171

- Predicted receptor occupancy at C<sub>avg</sub> with 2.5–120 mg/kg pipamperone BID is shown in **Table 3**; pipamperone 5.0 mg BID was predicted to produce a C<sub>avg</sub> that would achieve high occupancy of 5-HT<sub>2A</sub> and D<sub>4</sub> receptors and limited occupancy of D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> receptors.
  - Simulations predicted 5.0 mg pipamperone BID would produce a steady-state maximum concentration of approximately 18 ng/mL and a steady-state average concentration (C<sub>avg</sub>) of approximately 11 ng/mL (**Table 3**).

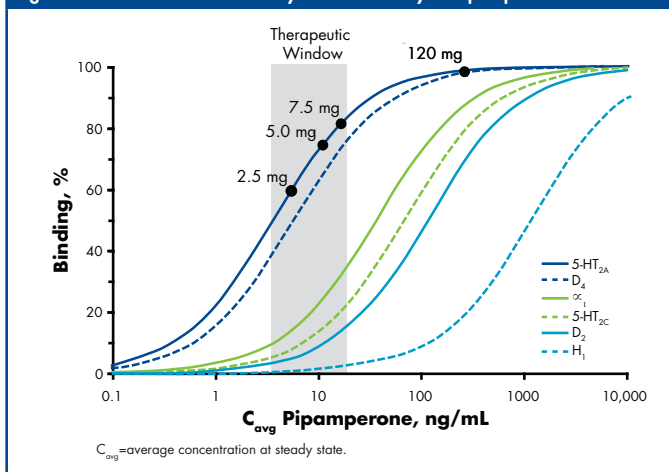
**Table 3. Predicted Binding to 5-HT<sub>2A</sub>, D<sub>4</sub>, D<sub>2</sub>, α<sub>1</sub>, 5-HT<sub>2C</sub>, and H<sub>1</sub> Receptors at Average Plasma Concentrations Achieved With 2.5–120 mg/kg Pipamperone BID**

Pipamperone BID Dose, mg	C <sub>avg</sub> , ng/mL	Binding, %					
		5-HT <sub>2A</sub>	D <sub>4</sub>	D <sub>2</sub>	α <sub>1</sub>	5-HT <sub>2C</sub>	H <sub>1</sub>
120	263	99	98	69	88	79	18.3
7.5	16	82	74	12	31	19	1.4
5.0	11	75	65	9	23	13	0.9
2.5	5	60	48	4	13	7	0.5

BID=twice daily; C<sub>avg</sub>=average concentration at steady state.

- Figure 1** shows predicted efficacy of pipamperone at C<sub>avg</sub> in patients in vivo.
  - Efficacy was predicted with ≥60% occupancy of the 5-HT<sub>2A</sub> receptor and ≥40% occupancy of the D<sub>4</sub> receptor, corresponding to doses ≥2.5 mg BID.
- Figure 1** shows predicted tolerability of pipamperone at C<sub>avg</sub> in patients in vivo.
  - Adverse events were predicted to occur with >10% occupancy of D<sub>2</sub> and H<sub>1</sub> receptors, corresponding to doses ≥7.5 mg BID.
- The optimal dose range of pipamperone for augmenting antidepressant efficacy in patients without neuroleptic effects was predicted to be 2.5–7.5 mg BID.

**Figure 1. Predicted Clinical Efficacy and Tolerability of Pipamperone**



## Conclusions

- At very low doses (ED<sub>50</sub>, 0.13–0.34 mg/kg), pipamperone inhibited tryptamine-induced cyanosis and bilateral convulsions in rats, indicative of blockade of peripheral and central 5-HT<sub>2A</sub> receptors, respectively.
- Inhibition of apomorphine-induced stereotypy, a D<sub>2</sub> receptor-mediated effect, occurred only at higher doses (ED<sub>50</sub>, 1.55 mg/kg).
- Addition of low-dose pipamperone to SSRI monotherapy may improve antidepressant efficacy and accelerate symptom resolution in patients with MDD via selective 5-HT<sub>2A</sub>/D<sub>4</sub> receptor antagonism.
- Pharmacokinetic/pharmacodynamic modeling predicted an optimal pipamperone dose between 2.5 and 7.5 mg BID in patients for effectively blocking 5-HT<sub>2A</sub> and D<sub>4</sub> receptors without significantly affecting D<sub>2</sub> receptors. This dose is well below clinical doses presently used in EU countries<sup>11</sup>; pipamperone is not approved in the United States.

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