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Review

Adjunctive 5-Hydroxytryptophan Slow-Release for Treatment-Resistant Depression: Clinical and Preclinical Rationale

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Serotonin transporter (SERT) inhibitors treat depression by elevating brain extracellular 5-hydroxytryptamine (5-HT_{Ext}). However, only one-third of patients respond adequately. Treatment-resistant depression (TRD) is a major unmet need. Interestingly, elevating 5-HT_{Ext} beyond what is achieved by a SERT inhibitor appears to treat TRD. Adjunctive administration of 5-hydroxytryptophan (5-HTP) safely elevates 5-HT_{Ext} beyond the SERT inhibitor effect in humans; however, 5-HTP cannot be a clinically viable drug because of its poor pharmacokinetics. A slow-release (SR) delivery mode would be predicted to overcome the pharmacokinetic limitations of 5-HTP, substantially enhancing the pharmacological action and transforming 5-HTP into a clinically viable drug. Animal studies bear out this prediction. Thus, adjunct 5-HTP SR could be an important new treatment for TRD. Here, we review the clinical and preclinical evidence for this treatment.

Trends

Clinical and preclinical evidence suggests that elevating brain 5-HT_{Ext} in a sustained fashion beyond the effect achieved by a SERT inhibitor treats TRD. Previous such drug strategies all had safety, mechanism, or pharmacokinetics limitations.

Adjunctive 5-HTP strongly and synergistically augments SERT inhibitor-induced 5-HT_{Ext}-elevation, whereas 5-HTP alone has modest effects. 5-HTP has a good human safety record, but its absorption and elimination is too rapid for a 5-HTergic antidepressant.

Mouse data demonstrate that adjunct 5-HTP SR safely, effectively, and in a sustained fashion elevates 5-HT_{Ext} beyond the selective serotonin reuptake inhibitor (SSRI) effect.

Integrated with a large body of clinical data, these mouse data suggest that an appropriate 5-HTP SR drug would be a safe and effective therapy for TRD.

Current Therapies for Treatment-Resistant Depression Are Inadequate

Depression (see Glossary) is characterized by persistent depressed mood and/or anhedonia in conjunction with other mood and physical symptoms [1]. According to statistics from the USA National Institute of Mental Health, 6.7% of the US population suffer from depression annually. The mainstay of antidepressant therapy remains the **SERT inhibitors** predominantly **selective serotonin reuptake-inhibitors (SSRIs)** and dual serotonin and norepinephrine reuptake inhibitors (SNRIs). SERT inhibitors block the reuptake of **5-HT** from the extracellular space. This causes sustained elevation of brain extracellular serotonin (i.e., **5-HT_{Ext}**), which over time leads to an antidepressant response [2]. Unfortunately, SERT inhibitors achieve remission in only one-third of patients [3]. As such, an estimated 2% of the US population suffers from **TRD** [4]. Current treatments for TRD are of limited benefit [5] and new treatments are needed.

As reviewed below, multipronged clinical data suggest that elevating 5-HT_{Ext} beyond the effect achieved by SERT inhibitor monotherapy is therapeutic in TRD. Hence, a drug that, when administered adjunct to a SERT inhibitor, safely and in a sustained fashion, elevates 5-HT_{Ext}

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beyond the SSRI effect could be a new therapy for TRD. Here, we review the evidence that elevating 5-HT_{Ext} beyond the SERT inhibitor effect could be used to treat TRD. We also present the hypothesis that adjunct treatment with a **SR formulation** of the 5-HT precursor 5-HTP (Figure 1) will be a safe and effective way to elevate 5-HT_{Ext} beyond the SERT inhibitor effect. Furthermore, we highlight three critical points regarding 5-HTP pharmacology, not clearly recognized or articulated previously: (i) 5-HTP by itself only modestly elevates 5-HT_{Ext}, whereas adjunctive 5-HTP strongly and synergistically augments SERT inhibitor-induced 5-HT_{Ext} elevation; (ii) combining 5-HTP with a SERT inhibitor appears safe in humans; and (iii) poor **pharmacokinetics** (i.e., rapid absorption and elimination) prohibits 5-HTP from being a clinically viable drug in its native, **immediate release (IR)**, form. Importantly, convergent data suggest that a SR delivery mode will remedy the pharmacokinetic limitations of 5-HTP and result in a drug with general therapeutic potential in TRD.

The Need for Sustained 5-HT_{Ext} Elevation in Depression Therapy

5-HT_{Ext} elevation remains the best-validated antidepressant mechanism [2]. Furthermore, the evidence indicates that the 5-HT_{Ext} elevation must be sustained to achieve a clinically viable antidepressant effect.

Risk of Relapse

A critical observation supporting the necessity of sustained elevation of 5-HT_{Ext} is that acutely lowering brain 5-HT_{Ext} by eliminating dietary tryptophan, a precursor of 5-HT, precipitates a return of depression symptoms in 50% of patients otherwise remitted on a SERT inhibitor. The relapse occurs within hours [6–8]. In rat models of **tryptophan depletion**, where the procedure lowers plasma tryptophan as in humans (by 80%), brain 5-HT_{Ext} rapidly drops by 50% from the initial SERT inhibitor-elevated level [9]. Thus, it appears that an acute 50% drop in brain 5-HT_{Ext} will trigger acute relapse in 50% of patients with depression otherwise treated to remission with a SERT inhibitor.

Risk of Discontinuation Syndrome

An additional consideration is that lapse of sustained elevation in 5-HT_{Ext} can precipitate specific **adverse events**. Specifically, missing even a single dose of a SERT inhibitor can occasionally precipitate **discontinuation syndrome** [10], characterized by dizziness, nausea, lethargy, and headache. In animals, SSRI-induced 5-HT_{Ext} elevation rapidly reverts to baseline upon SSRI-withdrawal [11]. For the short-acting SNRI venlafaxine [**$T_{1/2}$** = 8 h (average for parent compound and active metabolite)], the discontinuation syndrome is more frequent, and can occur within hours [12]. Given its short $T_{1/2}$, venlafaxine is used predominantly in its SR version. In a head-to-head antidepressant trial, venlafaxine SR was superior to venlafaxine IR [13]. All marketed SSRIs have $T_{1/2}$ >20 h. This leads to <0.3-fold steady-state drug-level fluctuations and, thus, minimal fluctuations in SERT occupancy and essentially stable 5-HT_{Ext}, such that discontinuation does not occur with once-daily dosing [14] (Figure 2).

Thus, for antidepressant therapy, 5-HT_{Ext} elevation must be sustained and cannot drop off, which would otherwise run the risk of relapse and discontinuation syndrome.

Elevating 5-HT_{Ext} Beyond the SERT Inhibitor Effect Has Shown Promise in Treating TRD

Brain 5-HT_{Ext} Levels Are Regulated on Multiple Levels

The SERT is one of several elements controlling 5-HT_{Ext} [15]. 5-HT synthesis [16], degradation [17], neuronal firing [18], conjugations [19], and feedback mechanisms [20] are all important determiners of 5-HT_{Ext} levels. For instance, in the rat brain, levels of glucuronide-conjugated 5-HT_{Ext}, which does not bind to 5-HT receptors, is twice that of free 5-HT_{Ext} [19]. In a naturalistic mouse model of brain 5-HT deficiency, due to a reduction-of-function mutation in tryptophan

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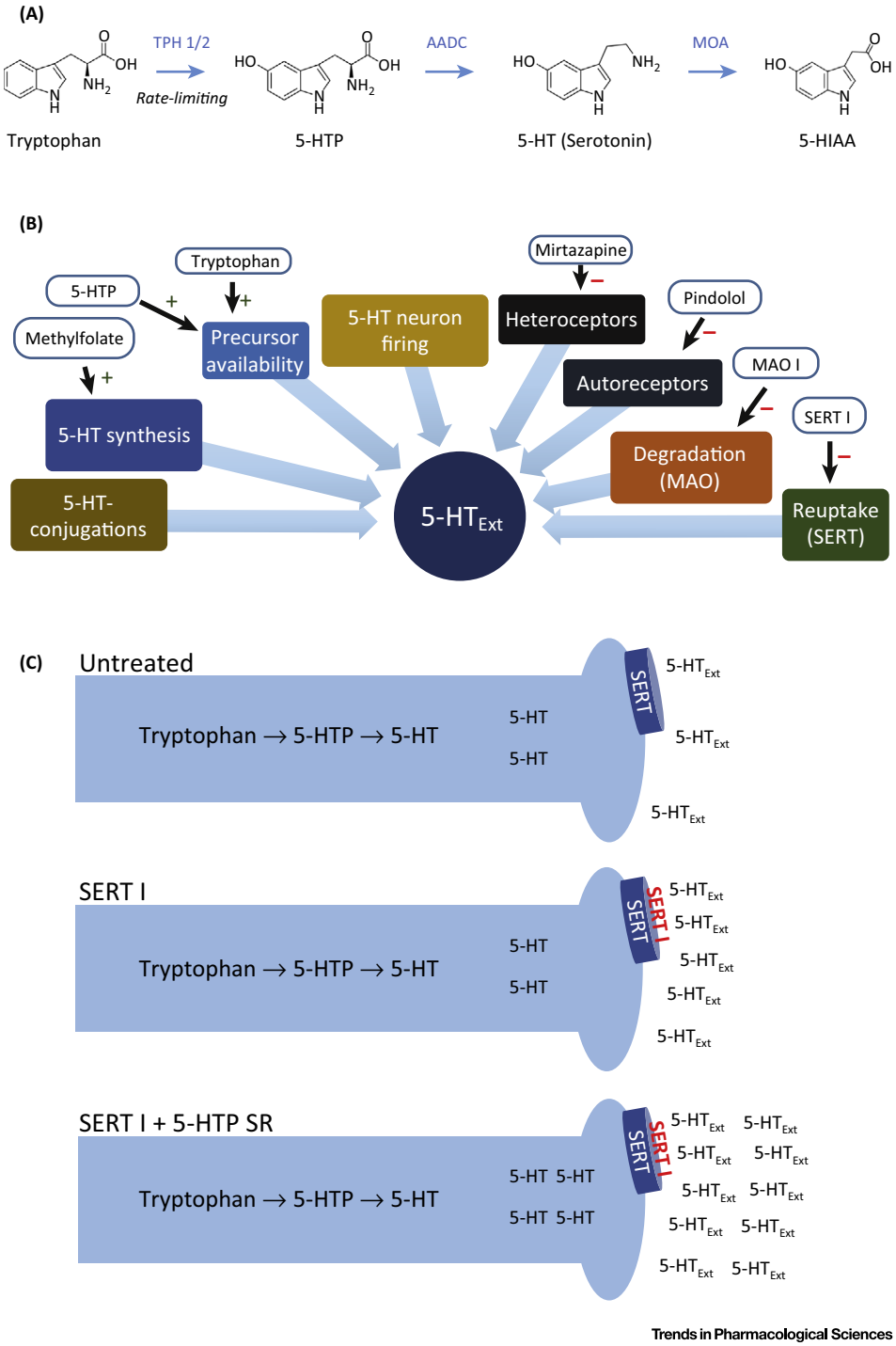

Figure 1. Foundation of Adjunctive 5-HTP SR Therapeutic Rationale. (A) The 5-HT metabolic pathway. Synthesis of 5-HTP from tryptophan via TPH1 (periphery) or TPH2 (CNS) is the rate-limiting step in 5-HT synthesis. 5-HTP is rapidly converted to 5-HT by the ubiquitous enzyme amino acid decarboxylase. 5-HT is metabolized to 5-HIAA, the main metabolite of 5-HT, by monoamine oxidase. (B) Simplified schematic of regulatory elements of CNS 5-HT_{Ext}. Drugs interacting with each element are indicated. (C) Schematic for adjunct 5-HTP SR mechanism of action. Adjunct exogenous 5-HTP increases endogenous 5-HT synthesis, increasing the availability of 5-HT for net release by concomitant SERT inhibitor treatment. Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine (serotonin); 5-HT_{Ext}, extracellular 5-HT; 5-HTP, 5-hydroxytryptopan; AADC, amino acid decarboxylase; CNS, central nervous system; MOA, monoamine oxidase; MOAI, monoamine oxidase inhibitor; SERT, serotonin transporter; SERTI, serotonin transporter inhibitor; SR, slow release; TPH, tryptophan hydroxylase.

Glossary

5-Hydroxytryptamine (5-HT, aka serotonin): signaling molecule in the central nervous system and the periphery.

5-Hydroxytryptophan immediate-release (5-HTP IR): standard, native 5-HTP.

5-Hydroxytryptophan slow-release (5-HTP SR): concept wherein 5-HTP is delivered as SR. In rodents, 5-HTP SR can be modeled using minipumps or dietary administration.

Active pharmaceutical ingredient (API): the compound in a dosage form/drug that exerts the pharmacological action.

Adverse event: undesirable experience associated with use of a medical product.

Blood–brain barrier (BBB): selective permeability barrier separating central nervous system extracellular fluid from the blood.

Cerebrospinal fluid (CSF): brain and spine extracellular fluid.

C_{Max}: the peak concentration of the API following administration.

Depression: mental disorder characterized by persistent feelings of sadness and loss of interest, together with additional symptoms, such as guilt, loss of energy, or suicidal ideation.

Discontinuation syndrome: can occur when 5-HT_{Ext}-elevating antidepressants are stopped abruptly; core symptoms include dizziness, nausea, lethargy, and headache.

Exposure: the API (concentration X time) area-under-the-curve after administration.

Extracellular 5-HT (5-HT_{Ext}): the 'active' 5-HT pool signaling via 5-HT receptors.

Hamilton depression rating scale (HAMD): a questionnaire rating constellations of symptoms to provide an overall depression severity score.

Immediate release (IR): the dosage form delivers the entire API dose instantly.

Peripheral amino acid decarboxylase inhibitor (DCI): penetrates the BBB minimally and, therefore, inhibits conversion of 5-HTP to 5-HT only peripherally. DCI co-administration increases 5-HTP brain exposure five- to 15-fold and doubles the $T_{1/2}$.

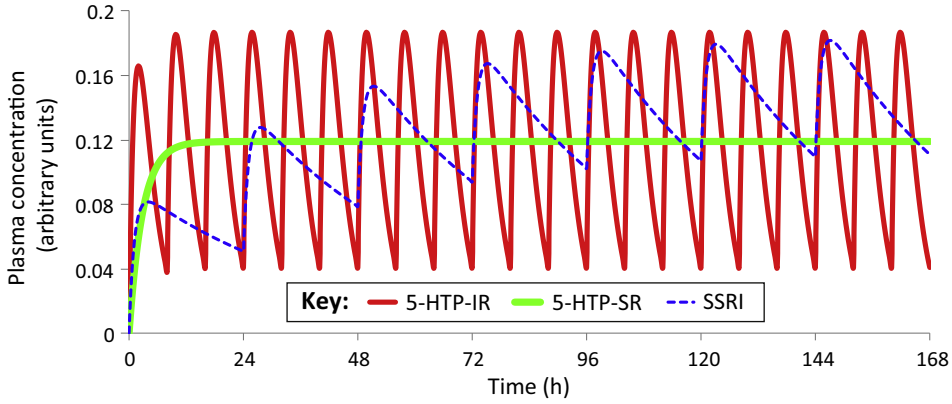

Figure 2. Pharmacokinetics (PK) Simulation Using One-Compartment Modeling and Published Human PK Parameters for 5-Hydroxytryptopan Immediate-Release (5-HTP IR) [61] and the Canonical Selective Serotonin-Reuptake Inhibitor (SSRI) Escitalopram [88]. Even at thrice-daily dosing at 8 h intervals, an unrealistic level of adherence in outpatients, 5-HTP plasma levels will fluctuate fivefold between doses. By contrast, during steady-state once-daily dosing of escitalopram, plasma escitalopram levels will fluctuate only about 0.3-fold. Also shown are 5-HTP plasma levels obtained during steady-state dosing with an ideal 5-HTP slow-release (SR) dosage form producing zero-order, constant, 5-HTP delivery.

hydroxylase 2, the rate-limiting enzyme in brain 5-HT synthesis, chronic SSRI treatment only modestly elevated 5-HT_{Ext} [16,21]. This implies that 5-HT reuptake is a less important determinant of 5-HT_{Ext} under 5-HT deficiency, a putative risk factor in depression [22]. From 5-HT neurobiology, it appears logical that selective SERT inhibition will not realize in all patients the full antidepressant potential of elevating 5-HT_{Ext}. Indeed, substantial preliminary clinical evidence suggests that elevating 5-HT_{Ext} beyond the SSRI/SERT inhibitor monotherapy effect has efficacy in TRD and accelerates antidepressant onset [23–38]. Below, we review key examples of previous pharmacological approaches to this end.

Adjunctive Drugs Elevating 5-HT_{Ext} beyond the Effect of SERT Inhibition Augment the Antidepressant Effect

Adjunctive pindolol elevates 5-HT_{Ext} beyond the SSRI effect for a limited period early during SSRI treatment, by preferentially blocking inhibitory 5-HT_{1A} autoreceptors [39,40]. In double-blind trials, adjunctive pindolol accelerated the antidepressant onset [41,42]. By contrast, pindolol augmentation has limited efficacy in TRD [25], consistent with the fact that chronic SSRI treatment inactivates 5-HT_{1A} autoreceptors over time. Furthermore, pindolol is disadvantaged by a short $T_{1/2}$ of 4 h [43] and a narrow therapeutic window [44]. Adjunctive treatment with the monoamine oxidase inhibitor (MAO I) moclobemide elevates 5-HT_{Ext} beyond the SSRI effect by inhibiting 5-HT degradation [45]. In open trials, adjunctive moclobemide was reported to treat TRD in patients already being treated with an SSRI [27–29]. However, this strategy has the limitation that SSRI + moclobemide cotreatment occasionally triggers serious adverse events [30,31]. In double-blind trials, adjunctive treatment with the 5-HT precursor tryptophan augmented the efficacy of SERT inhibitors [32,33]. However, this approach has the limitations that only a fraction of ingested tryptophan is metabolized to 5-HT [46], and the short $T_{1/2}$ (3 h) of tryptophan [47] necessitates frequent dosing. Likewise, as detailed below, adjunctive treatment with the immediate 5-HT precursor 5-HTP (Box 1) is reported to confer efficacy in TRD already being treated with SERT inhibitors. Recently, in double-blind trials, adjunctive treatment with methylfolate was reported to be effective in patients with TRD who had not responded to SSRIs [34]. Methylfolate enhances the biosynthesis of tetrahydrobiopterin, a co-factor in 5-HT, dopamine (DA), and noradrenaline (N) synthesis [48]. Adjunctive drugs that selectively elevate NA_{Ext} or DA_{Ext} failed to show efficacy in TRD [49,50]. Therefore, methylfolate acts by increasing brain

Pharmacokinetics: the study of how the body disposes of an API.
Selective serotonin-reuptake inhibitors (SSRIs): class of drugs that, at therapeutic levels, selectively inhibits the SERT.
Serotonin syndrome: toxic syndrome caused by excessive 5-HT_{Ext} and characterized by neuromuscular excitation (e.g., clonus), autonomic excitation (e.g., hyperthermia), and altered mental state (e.g., agitation).
Serotonin transporter (SERT): transports back into the neuron cytosol 5-HT released via vesicles to the extracellular space.
SERT inhibitor: a drug that inhibits the SERT; includes SSRIs, dual serotonin-noradrenaline reuptake inhibitors (SNRIs), and certain tricyclic antidepressants (e.g., clomipramine).
Slow-release (SR) formulation: the dosage form delivers the API dose gradually. The result is reduced C_{Max}, delayed T_{Max}, and increased $T_{1/2}$. For APIs with $T_{1/2}$ <12 h, a SR formulation often increases overall clinical effectiveness by decreasing C_{Max}-related adverse events, producing a sustained pharmacodynamics effect, and decreasing dosing frequency.
$T_{1/2}$: the terminal elimination half-life of an API, measured after absorption is complete.
T_{Max}: the time to C_{Max} after dosing.
Treatment-resistant depression (TRD): typically defined as the failure to achieve remission with two or more adequate courses of antidepressants.
Tryptophan depletion: acute administration of a tryptophan-devoid amino acid drink outcompetes uptake of tryptophan via brain amino-acid transporters. The result is an acute drop in brain 5-HT synthesis and in 5-HT_{Ext} (when elevated due to SERT inhibition).

Box 1. 5-HTP Facts

- Stereochemistry: the natural occurring stereoisomer of 5-HTP is L-5-HTP.
- Source: exogenous 5-HTP is usually extracted from the African shrub *Griffonia simplicifolia.*
- Regulatory status: in the USA, 5-HTP is regulated as a food supplement. There is no US Food and Drug Administration (FDA)-approved drug on the market containing 5-HTP as an active pharmaceutical ingredient. To our knowledge, no dosage form of 5-HTP has ever been formally developed as a drug and approved by a regulatory body for the treatment of a disease.
- 5-HTP absorption, distribution, metabolism, and excretion: no metabolic fate for 5-HTP other than decarboxylation to 5-HT is known. In humans, upon oral administration, 5-HTP is rapidly absorbed from the upper intestine, with a T_{Max} of 1.5 h [61]. Elimination is equally rapid, with a $T_{1/2}$ of 2 h [61,89]. The human bioavailability of 5-HTP, when given alone, has not been determined. Whether 5-HTP is passively or actively absorbed is also not known, although, based on rat studies [90], it appears likely that luminal amino acid transporters, also involved in L-DOPA absorption, have a role. In contrast to 5-HT, 5-HTP crosses the **blood–brain barrier (BBB)**, as assessed using radiolabeled 5-HTP tracers [91]. After oral administration of 5-HTP (e.g., of 300 mg/day [92]), enough 5-HTP enters the brain to enhance 5-HT synthesis, as assessed by an increase in **cerebrospinal fluid (CSF)** levels of 5-HIAA, the major 5-HT metabolite. When 5-HTP is co-administered with a peripheral amino acid decarboxylase inhibitor (DCI), which reduces peripheral, but not brain, metabolism of 5-HTP, 5-HTP exposure increased five- to 15-fold, the $T_{1/2}$ doubled to 4 h, and bioavailability was 70% [93]. A few studies have examined the disposition of 5-HTP in animals. In rats, Shindo *et al.* reported that 5-HTP is completely absorbed from the jejunum lumen via an active mechanism. 5-HTP transport into the brain also involves active transport. 5-HTP metabolism in the intestine and liver is three and seven times slower, respectively, compared with L-DOPA [90]. Such slower metabolism could account for the longer human $T_{1/2}$ observed of 5-HTP (2 h) versus L-DOPA (1 h) [94]. In mice, in a study discussed in the main text, the $T_{1/2}$ was determined to be about 12 min [83], ten times faster than in humans, in accordance with the typical mouse:human metabolism and a dose-extrapolation scaling factor of about ten [95].

5-HT levels that are then available for release by SSRI treatment, thus elevating 5-HT_{Ext} beyond the SSRI effect. However, the extent to which methylfolate increases brain 5-HT levels is unknown. Altogether, clinical evidence from the use of five different adjunctive compounds suggests that TRD can be treated by elevating 5-HT_{Ext} beyond the levels achieved by a SERT inhibitor.

Indirect Preclinical Evidence

Several nonserotoninergic adjunctive drugs with varying degrees of evidence for efficacy in TRD in humans elevate 5-HT_{Ext} to varying degrees beyond the SERT inhibitor effect in rodents. These include adjunctive lithium [51], modafinil [52], and atypical antipsychotics [53,54]. Thus, it is reasonable to hypothesize that 5-HT_{Ext} elevation has a role in the therapeutic action in TRD of some adjunctive drugs that do not have direct 5-HT_{Ext}-elevating effects.

Acute Adjunctive 5-HTP Elevates 5-HT_{Ext} beyond the SERT Inhibitor Effect

In rodents, at moderate parenteral doses (10–40 mg/kg), 5-HTP alone only modestly elevates 5-HT_{Ext}. By contrast, adjunctive 5-HTP strongly and synergistically elevates 5-HT_{Ext} beyond the SSRI effect [55,56]. In one acute study in rats, 20 mg/kg 5-HTP or an SSRI elevated 5-HT_{Ext} by 100% and 250%, respectively, whereas 5-HTP plus the SSRI elevated 5-HT_{Ext} by 850% [56]. The same synergism in rats was observed using an acute rise in plasma corticosteroids as a peripheral biomarker of an acute elevation in brain 5-HT_{Ext} [57]. Similarly, in human healthy volunteers, Lowe *et al.* found that oral 200 mg 5-HTP or an SSRI elevated cortisol by 35% and 100%, respectively. However, 5-HTP combined with the SSRI elevated cortisol by 500% [58]. Notably, acute 5-HTP + SSRI and, to a lesser extent, acute SSRI alone, caused rapid-onset vomiting and nausea in some subjects [58], indicating that sudden surges in bodily 5-HT levels are not well tolerated. In patients with depression or obsessive-compulsive disorder (OCD), Meltzer *et al.* also administered acute oral 200 mg 5-HTP and measured the cortisol rise, either before or during chronic SSRI treatment. The cortisol rise after acute administration of 5-HTP was twofold higher during SSRI treatment than before it [59]. No adverse events were observed in this study, conceivably because chronic SSRI administration adapts the gastrointestinal tract to increased 5-HT stimulation. Likewise, in patients with depression, Sargent *et al.* administered acute oral 100 mg 5-HTP and measured the cortisol rise before and during chronic treatment

with an SSRI. The cortisol rise after acute 100 mg 5-HTP was fourfold higher during SSRI treatment than before it. Again, no adverse events were observed [60].

Combined, the published preclinical and clinical data suggest the following: (i) 5-HTP elevates 5-HT_{Ext} more potently when adjunct to a SERT inhibitor than when administered on its own; and (ii) adjunctive 5-HTP can elevate 5-HT_{Ext} beyond the SERT inhibitor effect safely.

5-HTP Has Shown Promising Antidepressant Effects, but Poor Pharmacokinetics Limits Its Therapeutic Potential

The Pharmacokinetics of 5-HTP

Native **5-HTP IR** is a poor serotonergic antidepressant. As discussed above, effective antidepressant therapy requires sustained, minimally fluctuating, 5-HT_{Ext} elevation [6,10]. A $T_{1/2}$ of 2 h means that, even at thrice-daily dosing, 5-HTP plasma levels will fluctuate at least fivefold at steady-state. This contrasts to the less than 0.3-fold steady-state plasma fluctuations of most SSRIs [14] (Figure 2). Furthermore, the fast-onset adverse events of 5-HTP likely result from the rapid absorption and resultant 5-HT spikes upon administration. Co-administering a **peripheral amino acid decarboxylase inhibitor (DCI)** with 5-HTP will modestly extend the $T_{1/2}$, enhance **exposure** several-fold, and not affect $\mathbf{T_{Max}}$ [61]. Including a DCI in a **5-HTP SR** drug could be beneficial, but could complicate formulation development, dosing, and safety.

5-HTP As an Antidepressant

5-HTP has never been formally developed as a drug and optimized dosage forms and dosing regimens are unavailable. Furthermore, all previous 5-HTP trials were small, including at most a few dozen subjects. By contrast, to ensure reasonable statistical power, a typical antidepressant proof-of-concept Phase II trial includes 50–100 subjects per arm [62]. Most trials have used 5-HTP monotherapy, but, as noted above, 5-HTP may be more relevant as an adjunctive, augmentation therapy. For these reasons, previous trials may inherently have underestimated the antidepressant potential of 5-HTP. Nevertheless, most 5-HTP antidepressant reports are positive [46]. Scholarly reviews conclude that 5-HTP has shown promise as an antidepressant, and that more and better trials are warranted [46,63]. Consistent with its pharmacology, the 5-HTP antidepressant effect appears to be more consistent when adjunctive to another 5-HT_{Ext}-elevating antidepressant [46]. Here, we briefly review those adjunctive 5-HTP trials that have been published in English (Table 1).

In a double-blind trial in depressed inpatients, Alino *et al.* [35] found that nialamide (a MAO inhibitor) + 5-HTP (200 mg/day) was superior to nialamide alone. The worst reported adverse event was diarrhea. In an open-label case-series of 99 patients with chronic TRD, most already taking SERT inhibitor therapy, van Hiele [36] found that 5-HTP (average dose 200 mg/day) + DCI treatment induced a 'remarkable recovery' in approximately 50% of patients. Antidepressant responses tended to be all-or-none. Few adverse events were reported, and were mostly nausea. Hypomania occurred in 15 patients, which reversed upon lowering the 5-HTP dose. In a four-arm, double-blind, placebo-controlled trial in inpatients with depression, van Praag *et al.* [37] compared placebo with clomipramine (a SERT inhibitor), 5-HTP (200 mg/day) + DCI, and clomipramine + 5-HTP + DCI. Clomipramine + 5-HTP + DCI was superior to all other arms. Nausea was the most common adverse events. In a double-blind trial in patients with depression, Nardini *et al.* [38] found that clomipramine + 5-HTP (300 mg/day) was superior to clomipramine alone. Adverse events were reported to be few.

These pilot data on the efficacy and safety of adjunct 5-HTP in TRD are encouraging. Similar pilot trials provided initial evidence of the antidepressant efficacy of tricyclic antidepressants and ketamine [64,65]. The data add to the larger rationale supporting adjunctive 5-HTP SR as a novel therapy for patients who respond inadequately to SERT inhibitors (Figure 3).

Table 1. Clinical Trials with Adjunct 5-HTP IR in TRD

Refs	Design[a]	Arms and total daily dose	Dosing	DCI	Duration	Population	Finding	Safety	Comment
[35]	Double-blind; HAMD	Nialamide (MAO I) 200 mg (*N* = 15) versus nialamide + 5-HTP 200 mg (*N* = 15)	BID, at breakfast and lunch	None noted	15 days	Inpatients	Nialamide + 5-HTP superior ($P < 0.05$) to nialamide at day 15	2 patients in nialamide + 5-HTP arm reported diarrhea	Doses of both drugs titrated up over 5 days
[36]	Open-label	Tricyclics (mostly) + 5-HTP (~200 mg/day) (*N* = 99)	TID	Carbidopa, 150 mg/day	Variable	Outpatients; treatment resistant on average for 18 months	Full recovery of 50% of patients	Transient hypomania in 1/3; 'no significant side-effects'	Patients resistant to multiple drug treatments; dichotomous all-or-none response to adjunct 5-HTP
[37]	Double-blind; HAMD	Placebo versus clomipramine 225 mg (tricyclic) versus HTP 200 mg versus clomipramine + 5-HTP (*N* = 10, all groups)	TID	Carbidopa, 150 mg/day, 5-HTP groups only	21 days	Inpatients	Both clomipramine and 5-HTP superior to placebo; clomipramine + 5-HTP superior to other three groups	Nausea	5-HTP doses titrated up
[38]	Double-blind; HAMD	Clomipramine 50 mg (tricyclic) (*N* = 13) versus clomipramine + 5-HTP (300 mg/day) (*N* = 13)	?	None noted	28 days	Inpatients	Clomipramine + 5-HTP superior ($P < 0.05$) to clomipramine at day 28	Few adverse effects	No details on dosing regimen

[a]Abbreviation: HAMD, Hamilton depression rating scale.

5-HTP Has a Good Oral Human Safety Record

Experience from >100 published clinical trials and widespread nutraceutical use suggests that oral 5-HTP, in high milligram to low gram doses, alone or as an adjunct to other serotonergic drugs, with or without a DCI, has a low propensity to cause severe adverse events in humans [46,66][i].

5-HTP Has Not Been Reported to Cause Serotonin Syndrome in Humans

Serotonin syndrome is a toxic state caused by excessive $5\text{-HT}_{Ext.}$ Severe serotonin syndrome is rare, and almost exclusively caused by SERT inhibitor + MAO inhibitor cotreatment [67]. 5-HTP has never been associated with serotonin syndrome in humans. In published reports, >250 humans have been dosed with 5-HTP + a SERT inhibitor, with no serious adverse events [36–38,58–60,68–70]. A MAO inhibitor blocks the metabolic flow through the 5-HT pathway at the point of degradation, which might lead to extreme build-up of 5-HT_{Ext}. By contrast, 5-HTP increases 5-HT synthesis and the dynamic flow through the 5-HT pathway, which might not easily lead to 5-HT_{Ext} build-up.

In rodents, high parenteral acute bolus doses of 5-HTP (e.g., 100 mg/kg) in combination with an SSRI can cause transient serotonin syndrome [71]. However, this is an artifact of preclinical pharmacology methodology (i.e., extreme doses and nonoral routes of administration). Similar high parenteral doses of fluoxetine, methylphenidate, and caffeine often kill rodents [72], whereas, in humans, these compounds are safe in their appropriate oral doses and dosage forms.

Common 5-HTP Adverse Events Are Gastrointestinal

In humans, acute and long-term treatment with 5-HTP, even at high doses, has minimal effects on cardiovascular, hepatic, renal, hematological, or urinalysis parameters (reviewed in [66,73]).

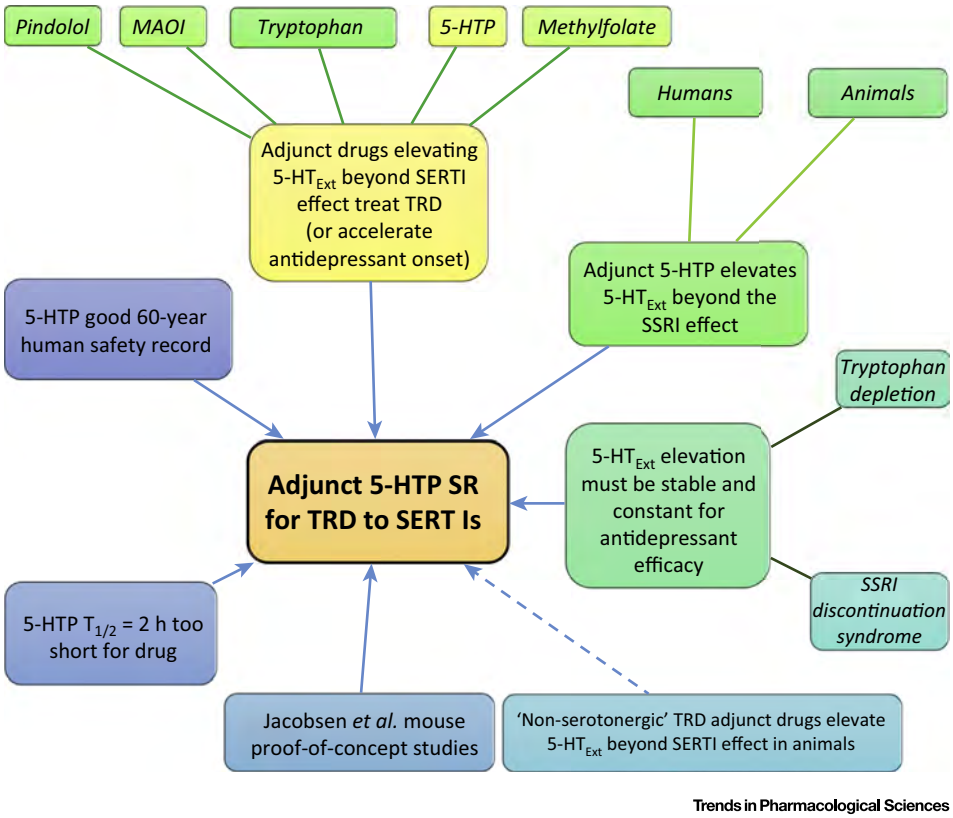

Figure 3. Schematic Summarizing the Multipronged Clinical and Preclinical Data Converging on Adjunct 5-Hydroxytryptopan Slow-Release (5-HTP SR) As a New Therapy for Treatment-Resistant Depression (TRD). Abbreviations: 5-HT_{Ext}, extracellular 5-HT; MOAI, monoamine oxidase inhibitor; SERTI, serotonin transporter inhibitor; SSRIs, selective serotonin-reuptake inhibitors.

Similarly, in rats, a 1-year toxicology study found no effects of oral high-dose 5-HTP (875 mg/kg/day, via the drinking water) on cardiovascular, hepatic, renal, hematological, body weight gain, organ histology, and organ weight parameters [74]. In humans, common adverse events seen with oral 5-HTP are mild to moderate, and gastrointestinal (e.g., nausea or stomach cramps, and less frequently diarrhea and vomiting; reviewed in [73]). Occasional adverse events include hypomania, headaches, lightheadedness, and palpitations. Often adverse event onset is rapid, which is likely due to the rapid conversion of 5-HTP to 5-HT upon dosing with standard 5-HTP IR [36,46,58]. Interestingly, two studies reported that using enteric coated 5-HTP capsules, which delays 5-HTP delivery until the intestine, substantially reduced gastrointestinal adverse events [36,37]. This suggests a direct irritating effect of 5-HTP on the stomach. Most studies do not specify whether they administered 5-HTP with enteric coating. By contrast, vomiting and nausea could be centrally, rather than peripherally, mediated. Evidence for this is that, upon acute bolus 5-HTP administration, DCI cotreatment (which reduces peripheral and increases central 5-HTP conversion to 5-HT) can induce nausea and vomiting at 5-HTP doses (100–200 mg) otherwise devoid of adverse events [61,75,76]. Furthermore, acute cotreatment of 5-HTP IR 200 mg + SSRI causes vomiting and nausea [58], but acute 5-HTP IR 200 mg causes no adverse events when added after 4 weeks of SSRI treatment [59]. In any case, 5-HTP gastrointestinal adverse events lessen or disappear over time [73], as occurs with SSRIs [77]. Overall, the evidence suggests that gastrointestinal adverse events after adjunctive 5-HTP can be greatly reduced if: (i) the 5-HTP $\mathbf{C_{max}}$ in plasma is minimized; (ii) appropriate 5-HTP formulations are used; and (iii) SSRI treatment has lasted several weeks before the start of 5-HTP dosing.

Figure 4. Theoretical and Experiential Superiority of 5-Hydroxytryptopan Slow-Release (5-HTP SR) versus 5-HTP Immediate Release (IR). Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); 5-HT_{Ext}, extracellular 5-HT.

Slow-Release Delivery Will Transform the Therapeutic Potential of 5-HTP

A SR formulation delivers an **active pharmaceutical ingredient (API)** over many hours, thereby delaying the time (T_{Max}) to peak plasma levels (C_{Max}) and increasing the $T_{1/2}$. An SR formulation is particularly beneficial when: (i) $T_{1/2}$ is very short; (ii) sustained API exposure is required; (iii) adverse events are linked to early high peak API levels; and (iv) less frequent dosing than necessary with the IR formulation is required. All points apply to 5-HTP. As parallels, some drugs with fast pharmacokinetics similar to that of 5-HTP are only safe and effective in their SR versions [78,79]. The PK of 5-HTP IR (rapid absorption and short duration of action) is the opposite of that required by a serotonergic antidepressant, where constant and minimally fluctuating drug exposure is mandated [6–8,10]. Unless taken very frequently, 5-HTP in its native IR form will not result in a sustained exposure [61]. At the same time, adherence to more than once- or twice-daily dosing is unrealistic for antidepressants, which are taken for months or years, and mostly by outpatients [80–82]. A SR formulation would produce lower 5-HTP plasma C_{max}, provide the necessary sustained 5-HTP exposure, allow for higher doses, and bring the dosing frequency to a requisite once or twice daily. Thus, in theory, a SR formulation would uniquely improve the therapeutic potential of 5-HTP as an antidepressant in an everyday clinical setting (Figure 4).

In Mouse Models, 5-HTP SR Transforms the Therapeutic Potential of 5-HTP

Directed by the clinical data reviewed above, an adjunctive 5-HTP SR proof-of-concept study was carried out in mice [83]. The authors modeled 5-HTP SR using subcutaneous minipumps, which produce constant (zero-order) SR delivery. Adjunctive 5-HTP SR augmented the 5-HT_{Ext}-elevation induced by chronic SSRI, by 100% in wild-type mice and by 800% in mice with naturalistic 5-HT deficiency [16,21], respectively. No adverse events were observed. Had the minipump capacity not limited the 5-HTP SR dose to 100 mg/kg/day, even stronger 5-HT_{Ext}-augmentation could have been achieved. As expected, 5-HTP SR alone had only modest effects on 5-HT_{Ext}. When modeling adjunctive 5-HTP IR by administering 2 × 50 mg/kg (am and pm) daily subcutaneous bolus 5-HTP injections, large, transient spikes were observed in 5-HT_{Ext}, accompanied by marked gastrointestinal adverse events and mild seizures. Low-dose adjunct 5-HTP IR, 2 × 3.125 mg/kg barely augmenting 5-HT_{Ext}, but still caused adverse events, even while the peak 5-HTP plasma levels were lower than the stable 5-HTP plasma levels resulting from 5-HTP SR 100 mg/kg/day. Recently, it was found that oral adjunct 5-HTP SR ~1000 mg/kg/day enhanced brain 5-HT and plasma 5-HTP levels and, by extension, 5-HT_{Ext}, several-fold stronger than 5-HTP SR via minipumps, and, again, with no adverse events (Jacobsen *et al.*, unpublished, 2016). Thus, in these model systems, as compared with 5-HTP IR, 5-HTP SR potently and safely elevated 5-HT_{Ext} beyond the SSRI effect, and allowed for higher safe 5-HTP exposure. While the contrast may be less stark in the clinic, these mouse data provide proof-of-principle of the therapeutic superiority of 5-HTP SR compared with 5-HTP IR.

Concluding Remarks

Antidepressant drug discovery is hampered by the poor predictability of animal 'antidepressant-like' behavioral models [84]. Optimally, the rationale for a novel antidepressant should rest on strong clinical, as well as preclinical, data. The adjunctive 5-HTP SR therapeutic concept for TRD

Outstanding Questions

Humans: Adjunctive 5-HTP SR to SSRI

Efficacious and safety in TRD?

Daily dose range?

Adverse event profile and therapeutic index?

Best embodiment of a 5-HTP SR drug? Formulation strategy?

Particular relevance for specific patient subpopulations?

Can high-responding patients be pre-identified? Can this be done based on genetic or physiological biomarkers?

Relevance for other central nervous system indications (e.g., anxiety, pain, and neurological disorders)?

Effect on brain connectomics?

Effects on cognitive bias and mood?

Regional intestinal absorption?

Animals: Adjunctive 5-HTP SR to SSRI, Using Clinically Relevant SSRI Dosing

Full behavioral characterization and sequelae in animal behavioral models? Dose–response relations?

Effect on stress responses?

Neurogenetic, epigenetic, cell signaling, and structural effects?

Effect on 5-HT receptor function?

Differential effects under normal and 5-HT-deficient conditions?

Improved modes to model 5-HTP SR in animals (e.g., subcutaneous tablets)?

Mechanism of 5-HTP intestinal absorption? Active and/or passive components?

is founded in 5-HT biology, 5-HTP clinical and preclinical pharmacology, pharmacokinetics, and promising clinical pilot trials in TRD with 5-HTP and four other serotonergic adjuncts. In addition, it has been shown in mice that chronic adjunctive 5-HTP SR safety and robustly elevates 5-HT_{Ext} beyond what is achieved by an SSRI (i.e., an antidepressant augmentation-like effect). Given that 5-HTP pharmacology appears to be similar between rodents and humans, we expect that these mouse data will translate to humans. Based on previous clinical data, we project that the therapeutic dose of adjunct 5-HTP SR will be 500–2000 mg per day [46]. Given the pharmacokinetics, physiochemical properties, and projected dose, realizing a 5-HTP SR formulation drug will be technologically feasible [85]. Many important drugs are specialized formulations of naturally occurring APIs [86,87]. The ideal 5-HTP SR drug would produce essentially stable 5-HTP plasma levels at once- or twice-daily dosing. Defining the pharmacology of 5-HTP SR in clinical and preclinical paradigms opens a new line of inquiry (see Outstanding Questions). Indices of 5-HT deficiency segregate with suicidality, severe depression, and comorbid borderline personality disorder, factors that predict poor SSRI treatment response (reviewed in [22]). Conceivably, adjunct 5-HTP SR will be particularly relevant for such patient populations. SSRIs are also approved for, but only partially effective in, OCD, post-traumatic stress disorder (PTSD), social anxiety, panic disorder, and generalized anxiety. Adjunctive 5-HTP SR could also be therapeutically relevant for these large indications. Furthermore, a 5-HTP SR drug might also be effective as monotherapy, as an alternative to existing antidepressants.

In closing, strong data support that a high-performing adjunctive 5-HTP SR drug will be safe and effective in patients with depression, and potentially with other central nervous system indications, who fail to achieve adequate benefit from SERT inhibitor monotherapy.

Conflicts of Interest

J.P.R.J. and M.G.C. are inventors on US patents pertaining to the adjunct 5-HTP SR method-of-treatment, and hold stock in Evecxia Inc., a company founded to develop a 5-HTP SR drug. A.D.K. and R.R.K. serve on the Evecxia Scientific Advisory Board.

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Resources

[i] http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+4295

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