Closing the Preclinical Gap to Derisk the Potential for Serotonin Toxicity: Was that a Twitch I See?

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1 Abstract

Serotonin syndrome can be potentially life threatening and is precipitated by overactivation of both peripheral and central postsynaptic 5-HT receptors, most notably 5-HT2a receptors. Activation of 5-HT2a receptors is reported to induce a characteristic head twitch response (HTR) in mice, which can be utilized preclinically to assess serotonergic activity of test compounds. 5-HTP, which freely crosses the blood-brain barrier, is converted to 5-HT (serotonin) without biochemical feedback; serotonin does not cross the blood-brain barrier. Carbidopa is a general decarboxylase inhibitor that inhibits peripheral synthesis of the centrally acting monoamines such as serotonin. Therefore, it was expected that administration of carbidopa would prohibit the conversion of 5-HTP to 5-HT in the periphery and thus increase the amount of 5-HTP centrally active and available to bind with 5-HT2a receptors. Additionally, pargyline and linezolid (established monoamine oxidase inhibitors [MAOI] with serotonergic activity) administered at high enough doses were anticipated to increase the levels of 5-HT by inhibiting monoamine oxidase (the enzyme responsible for serotonin metabolism), thereby allowing increased activation of 5-HT receptors to potentiate the HTR when compared to a negative control (saline).

The objective of this study was to assess a murine model of central nervous system serotonergic activity via a characteristic HTR elicited following administration of carbidopa and DL-5-hydroxytryptophan (5-HTP). Additionally, two administration routes (Oral and IP) of linezolid were assessed in comparison to the historically used subcutaneously administered pargyline for clinical relevance based on quantifiable HTR.

Consistency between dose routes would permit greater flexibility of preclinical study designs, allowing for a comparison to intended routes of administration and anticipated exposure in humans. Head twitches were recorded for at least 20 minutes (15-35 minutes) following administration of different control articles in male mice to evaluate the potential HTR.

Our results show HTRs were appropriately potentiated by administration of the positive control selected. The comparison of oral and IP dosing of linezolid exhibited similar head twitch potentiation results. These results suggest the allowance of multiple routes of comparison for clinical relevance, thereby potentially closing the gap between preclinical and clinical data to derisk serotonin toxicity.

2 Background

The synaptic enhancement of monoamine levels, predominantly 5-HT (serotonin), is the key pharmacological mechanism of conventional anti-depressants. Through post-marketing surveillance, a potential life threatening condition termed serotonin syndrome has been identified. This condition is caused by a build-up of intrasynaptic concentrations of serotonin and overstimulation of serotonin receptors in the central nervous system. To measure the potential serotonergic activity of a test compound, the head twitch assay was developed. The central activation of 5-HT2a receptors produces a characteristic head twitch response in mice, while in rats a myriad of observations, including limb clonus, is produced; therefore, the mouse is the ideal rodent species in which to conduct this assay.

3 Materials and Methods

Male CD-1 mice were received from Charles River Laboratories, Inc., Raleigh, NC. Mice were 8 weeks old and weighed between 30 and 39 g at the initiation of dosing which was conducted according to the following table:

Group Number	Treatment	Pre-treatment[a] and Dose Time	Compound 1 (Dose Route) -Time 0-	Compound 2 (Dose Route) and Dose Time	Compound 1 Dose Level (mg/kg)	Compound 2 Dose Level (mg/kg)	Dose Volume (mL/kg)[b]	Number of Males
1	Positive Control	NA	Pargyline (SC)	5-HTP (IP) +30 minutes	75	10	10	6
2	5-HTP Negative Control	NA	PBS (IP)	5-HTP (IP) +30 minutes	0	50	10	6
3	MAOI Negative Control	NA	Pargyline(SC)	PBS (IP) + 30 minutes	75	0	10	6
4	Decarboxylase inhibitor control	Carbidopa (IP) -15 minutes	PBS (IP)	5-HTP (IP) +30 minutes	0	50	10	6
5	Decarboxylase inhibitor positive control	Carbidopa (IP) -15 minutes	Pargyline (SC)	5-HTP (IP) +30 minutes	75	50	10	6
6	Linezolid (IP) Control	NA	Linezolid (IP)	5-HTP (IP) +15 minutes	50	50	10	6
7	Decarboxylase inhibitor Linezolid (IP) Control	Carbidopa (IP) -15 minutes	Linezolid (IP)	5-HTP (IP) +15 minutes	50	50	10	6
8	Linezolid (PO) Control	NA	Linezolid (PO)	5-HTP (IP) +15 minutes	50	50	10	6
9	Decarboxylase inhibitor Linezolid (PO) Control	Carbidopa (IP) -15 minutes	Linezolid (PO)	5-HTP (IP) +15 minutes	50	50	10	6

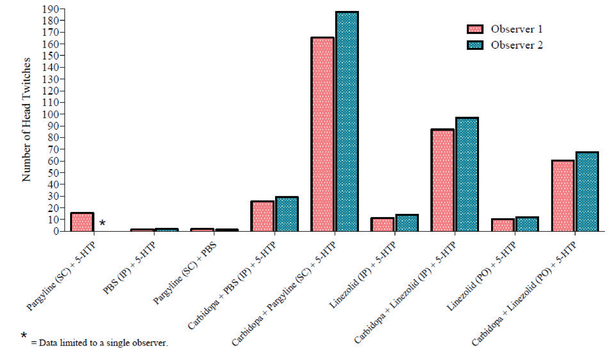
[a] Dose level of carbidopa pretreatment will be 10 mg/kg (IP dose route).
[b] Dose volume is applicable to each compound to be administered. Each animal individually will not be administered more than 3 doses at a dose volume of 10 mL/kg.
N/A = not applicable, PO = oral, IP = intraperitoneal injection, SC = subcutaneous injection

The mice were observed for episodes of characteristic head twitches for a period of at least 20 minutes (15-35 minutes post-dosing) within the first 60 minutes following Compound 2 administration.

The head twitch response (HTR) resembled a strong pinna reflex involving the whole head of the animal; however, unlike the pinna reflex, the HTR occurs without tactile stimulation. It can also be described as a short burst of rapid left-right head shaking that is distinct from grooming behavior. Instances of head twitches within this period were quantitatively recorded for each individual animal.

The same observer quantitatively measured all HTRs while a second technician verified and recorded all observations. Technicians were blinded to treatment condition.

4 Results



* = Data limited to a single observer.

The use of two observers allowed for confirmation of interobserver consistency. The lack of interobserver variance allows for appropriate use of a single observer to evaluate HTRs in future assays.

Based on the results of this study, the carbidopa pretreatment was selected in combination with 5-HTP administration to elicit the baseline HTR. Using an additional compound as a negative control (saline), positive control (linezolid), and investigatory test article would allow comparisons of response to determine levels of potentiation following administration of each. The use of Linezolid proved more successful than pargyline in that the prevalence of associated clinical observations elicited by pargyline in combination with the pretreatment and 5-HTP precluded HTR identification (i.e. head searching, tremors, circling). Administration of linezolid via IP or PO dose routes did not produce markedly varied HTRs; therefore, these results suggest that the intended clinical route of administration (e.g., PO) can be utilized for a test compound for comparison to a negative control.