Cyclobenzaprine (CBP) and its Major Metabolite Norcyclobenzaprine (nCBP) are Potent Antagonists of the Serotonin Receptor 2A, Histamine H1 and α-Adrenergic Receptors: Mechanistic and Safety Implications for Treating Fibromyalgia Syndrome by Improving Sleep Quality

Bruce Daugherty, PhD; Leland Gershell, MD, PhD; Seth Lederman, MD
TONIX Pharmaceuticals, Inc., 509 Madison Ave. Suite 306, New York, NY 10022

Funded by Tonix Pharmaceuticals, Inc.

Presentation Number 960

BACKGROUND

Bedtime cyclobenzaprine (CBP) improves fibromyalgia symptoms (pain, fatigue, tenderness, and mood) and improves sleep quality (decreases Cyclic Alternating Pattern Type A2 + A3) (1). CBP is metabolized by the hepatic P450 isoforms CYP1A1/2 and CYP3A4 into desmethyl, or norcyclobenzaprine (nCBP)(2), but plasma nCBP has only been detected in cases of overdose (3-5). Although CBP has been shown to interact with both the serotonergic (6,7) and noradrenergic (8,9) receptor systems, the functional interactions of CBP with isolated receptors are not fully characterized and those of nCBP are unknown. Therefore, plasma nCBP was measured in healthy subjects after ingesting CBP and the binding and functional activity of CBP and nCBP was studied on a set of CNS targets with potential relevance to CBP actions.

METHODS

Plasma CBP and nCBP were measured over 168 hr in ten healthy, fasting subjects who received 5 mg PO immediate release CBP-HCl. Area under the curve (AUC), C_{max}, T_{max}, and $T_{1/2}$ were calculated. CBP and nCBP were screened on a broad panel of receptors, channels, enzymes and transporters. Equilibrium receptor binding assays were performed on cell lines expressing select recombinant human serotonin, adrenergic, histamine, and muscarinic receptors. Select receptors were also analyzed for functional antagonism in ligand-induced intracellular calcium mobilization and β-arrestin signaling.

CONTACT

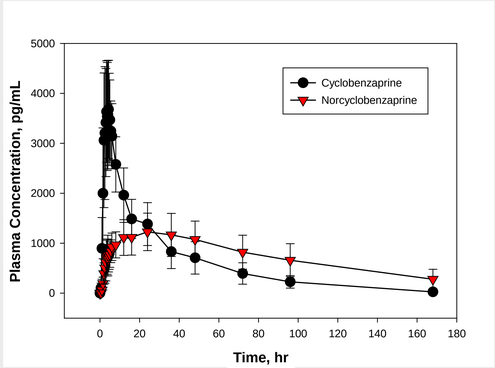
Bruce Daugherty, PhD
bruce.daugherty@tonixpharma.com
212-980-9155 x105
Leland Gershell, MD, PhD
leland.gershell@tonixpharma.com
212-980-9155 x104
Seth Lederman, MD
seth.lederman@tonixpharma.com
212-980-9155 x103

www.tonixpharma.com

Poster Design & Printing by Genigraphics® - 800.790.4001

RESULTS

Figure 1. Pharmacokinetic Profile of CBP and nCBP in Humans



Mean cyclobenzaprine and norcylobenzaprine plasma concentration-time profile (0-168 h) for 1 x 5 mg Cyclobenzaprine-HCl (N=10) administered under fasting conditions

Table 1. Pharmacokinetic Parameters of CBP and nCBP in Humans

	CBP	nCBP
$AUC_{0\text{-}72}$ (pg•hr/mL)	92,227 ± 29,913 (32.43)	91,218 ± 31,691 (34.74)
$AUC_{0\text{-}\infty}$ (pg•hr/mL)	103,076 ± 35,844 (34.77)	169,506 ± 94,277 (55.62)
C_{max} (pg/mL)	4,121 ± 937 (22.73)	1,273 ± 371 (29.18)
T_{max}* (hr)	3.5 (2.0 – 5.5)	24.0 (6.0-36.0)
$T_{1/2\ el}$ (hr)	30.95 ± 7.18 (23.19)	72.75 ± 27.71 (38.09)

Mean ± SD (CV%); *Median (Min-Max)

Figure 2. TNX-102 SL: Sublingual CBP Tablet

- Faster Absorption
- Bypasses "First-Pass" Metabolism

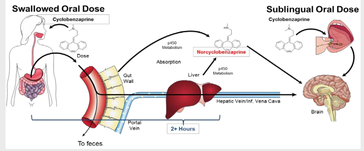
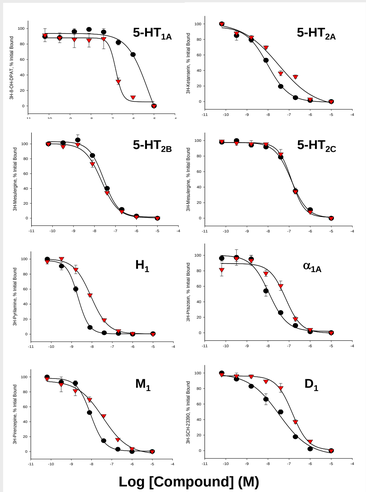
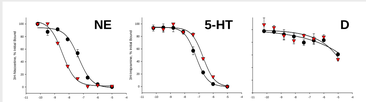


Figure 3. Binding Studies of CBP and nCBP on Receptors Expressed in the CNS of Humans



Equilibrium binding of CBP (black circles) and nCBP (red triangles) to cells expressing recombinant human receptors: Competition against [^{3}H]-ligands. 5-HT: serotonin; H_1: histamine H_1; α_{1A}: adrenergic α_{1A}, M_1: muscarinic M_1; D_1: dopamine D_1

Figure 4. Binding Studies of CBP and nCBP on Transporters Expressed in the CNS of Humans



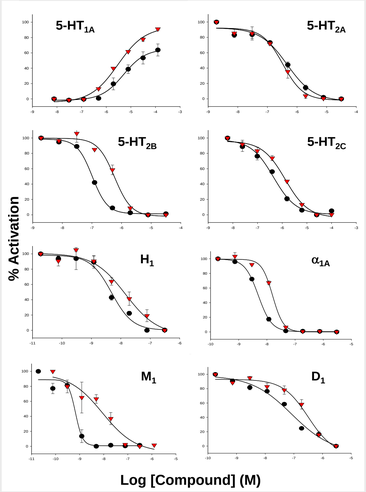
Equilibrium binding of CBP (black circles) and nCBP (red triangles) to cells expressing recombinant human transporters: Competition against [^{3}H]-ligands. NE: norepinephrine transporter; 5-HT: serotonin transporter; D: dopamine transporter

Table 2. Binding and Functional Potency of CBP and nCBP on Targets Expressed in the CNS

	K_i (nM)		IC_{50} (nM)	
Target	CBP	nCBP	CBP	nCBP
5-HT_{1A}	1100	76	5300*	3200*
5-HT_{2A}	5.2	13	230	140
			99**	181**
5-HT_{2B}	15	12	100	580
5-HT_{2C}	43	43	444	1220
5-HT_{5A}	730	1600	-	-
5-HT_6	480	1400	2000	2800
5-HT_7	67	140	-	-
H_1	1.3	5.9	5.2	16
			2.7**	6.1**
α_{1A}	5.6	34	4.9	16
α_{1B}	9.1	11	530	790
			144**	173**
α_{2A}	360	1800	4300	6400
α_{2B}	21	150	-	-
α_{2C}	25	48	-	-
M_1	7.9	30	0.71	8.7
			81**	266**
M_2	250	76	3.3	33
D_1	12	57	65	300
D_{2S}	120	410	-	-
D_3	34	98	-	-
$D_{4.4}$	180	250	-	-
D_5	60	280	-	-
NE-TP	35	2.6	-	-
5-HT-TP	29	91	-	-
D-TP	>10000	>10000	-	-
Sigma 1	120	790	-	-
Sigma 2	480	2000	-	-

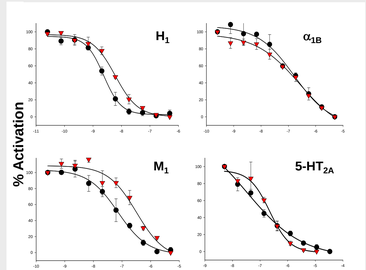
IC_{50} represent antagonist values (calcium flux): * agonist (EC_{50}); ** antagonist (β-arrestin)

Figure 5. Functional Studies of CBP and nCBP on CNS Receptors. G-protein Dependent Signal Transduction: Intracellular Ca^{2+} Mobilization



CBP (black circles); nCBP (red triangles); 5-HT: serotonin; H_1:histamine H_1; α_{1A}: adrenergic α_{1A}; M_1: muscarinic M_1; D_1: dopamine D_1

Figure 6. Functional Studies of CBP and nCBP on CNS Receptors. G-protein Independent Signal Transduction: β-Arrestin Signaling



CBP (black circles); nCBP (red triangles); H_1: histamine H_1; α_{1B}: adrenergic α_{1B}; M_1: muscarinic M_1; 5-HT_{2A}: serotonin 5-HT_{2A}

The oral bioavailability of CBP was similar to published results (C_{max} = 4.12 ng mL^{-1}, t_{max} = 3.5 h, $T_{½}$ = 31.0 h, $AUC_{0\text{-}\infty}$ = 103.1 ng hr mL^{-1}), but plasma nCBP was unexpectedly high and persistent (C_{max} = 1.27 ng mL^{-1}, t_{max} = 24.0 h, $T_{½}$ = 72.8 h, $AUC_{0\text{-}\infty}$ = 169.5 ng hr mL^{-1}). Unlike CBP, nCBP does not form a stable N^+-glucuronide, which may affect its clearance. In vitro, CBP and nCBP exhibited high affinity binding (K_i) to receptors: 5-HT_{2A} (K_i = 5.2 and 13 nM, respectively), 5-HT_{2B} (15 and 12nM), and 5-HT_{2C} (43 and 43 nM), adrenergic α_{1A} (5.6 and 34 nM), α_{1B} (9.1 and 11 nM), α_{2B} (21 and 150 nM) and α_{2C} (25 and 48 nM,); H_1 (1.3 and 5.9 nM); and M_1 (7.9 and 30 nM). CBP and nCBP are functional antagonists at 5-HT_{2A} (IC_{50} = 230 and 140 nM), 5-HT_{2B} (100 and 580 nM), H_1 (5.2 and 16 nM), α_{1A} (4.9 and 16 nM), M_1 (0.71 and 8.7) and M_2 (3.3 and 33 nM) via Ca^+ mobilization. In contrast, both CBP and nCBP are functional agonists on 5-HT_{1A} (EC_{50}= 5.3 and 3.2 μM). CBP and nCBP are also functional antagonists at 5-HT_{2A} (IC_{50} = 99 and 181 nM), H_1 (2.7 and 6.1 nM), α_{1B} (144 and 173 nM), and M_1 (81 and 266 nM) via β-arrestin signaling.

CONCLUSIONS

CBP is metabolized to nCBP which persists in plasma at biologically relevant concentrations after 5 mg oral CBP in healthy subjects. CPB and nCBP bind to and antagonize multiple receptors expressed in the CNS. These two molecules are also antagonists on both G-protein dependent and G-protein independent signaling. Antagonists of 5-HT_{2A} and H_1 are known to have effects on sleep and sleep maintenance. Adrenergic antagonists may have effects on autonomic dysfunction. CBP's antagonist activity on 5-HT_{2B} is consistent with the lack of any association with heart valve pathology. Dry mouth associated with CBP usage is likely explained by anticholinergic effects via antagonism at muscarinic receptors. The accumulation of biologically active nCBP without N^+-glucuronidation may affect responses to CBP therapy in a chronic bedtime dosing regimen.

REFERENCES

1. Moldofsky H et al, (2011) J Rheum 38: 2653-2663
2. Wang R et al, (1996) Drug Metab Dispos 24: 786-791
3. Hucker HB et al, (1978) Drug Metab Dispos 6:659-72
4. Hucker HB et al, (1977) J Clin Pharmacol 17:719-27
5. Wong EC et al (1995) J Anal Toxicol 19:218-24
6. Kobayashi H et al, (1996) Eur J Pharm 311: 29-35
7. Honda M et al, (2003) Eur J Pharm 458: 91-99
8. Barnes C et al, (1980) Neuropharm 19: 221-224
9. Commissiong JW et al, (1981) Can J Phys Pharm 59:37-44