T230 Dexmedetomidine– Highly Favorable Pharmacokinetic and Pharmacological Features for a CNS Therapeutic Drug

BXCL501: A Therapy for Improved Adaptation to Stress

[1]Michael De Vivo, [1]Subhendu Seth, [1]Sameer Sharma, [1]Dinesh Dhull, [2]Holden Janssens, [2]Julien Roeser, [1]Frank D. Yocca

[1]BioXcel Therapeutics, New Haven CT; [2]Charles River Laboratories, San Francisco, CA

BACKGROUND

- BioXcel Therapeutics Inc. (BTI) **identified agitation in neuropsychiatric diseases as a high unmet need**
- Alpha2-adrenergic receptors are associated with pathways that contribute to the stress response
- These pathways are: (a) activation of the locus coeruleus (LC) causing sympathetic hyper-arousal and (b) activation of the bed nucleus of stria terminalis (BNST) and release of CRF
- Gene polymorphisms, transgenic mice and pharmacological data with other alpha2-adrenergic agonists all indicate that the **alpha2-adrenergic receptors modulate adaptation to stress**
- Dexmedetomidine is a selective and potent full agonist at alpha2-adrenergic receptors (A, B and C).
- BTI created a database of all compounds known to have been tested in clinical studies and scored them for attributes that predict high CNS penetration after dosing. Dexmedetomidine emerged as highly likely to achieve high free brain concentrations after dosing
- Dexmedetomidine possesses poor oral bioavailability as a result of high first-pass metabolism. BTI developed a proprietary sublingual dosing film, BXCL501, that agitated patients are able to easily self-administer
- BXCL501 is being evaluated in diseases in which sympathetic nervous system hyper-arousal and adaptation to stress are significant components including schizophrenia, dementia, opioid use withdrawal and PTSD

Alpha2-Adrenergic Receptors

TYPE	CNS DISTRIBUTION	TRANSGENIC MICE	GENE POLYMORPHISM	PUTATIVE ROLE
2A	Locus Coeruleus Forebrain BNST	Decreased Activation of BNST in Knockdown Mice	Immediate Reward Choice in Cocaine Users	Modulation of Sympathetic Arousal Reduced Response to Stress
2B	Thalamus Cortex	Decrease in Marble Burying in KO Mice	Complusive Behavior	Complulsive Behavior
2C	Basal Ganglia Olfactory Tubercle Hippoocampus	Enhanced Startle, Agression in KO Mice	Emotional Dysfunction in Depression	Aggression and Reactive Depression

References

Fetterly TL, Basu A, Nabit BP, Awad E, Williford KM, Centanni SW, Matthews RT, Silberman Y, Winder DG. α(2A)-Adrenergic Receptor Activation Decreases Parabrachial Nucleus Excitatory Drive onto BNST CRF Neurons and Reduces Their Activity In Vivo. J Neurosci. 2019 Jan 16;39(3):472-484.

Havranek MM, Hulka LM, Tasiudi E, Eisenegger C, Vonmoos M, Preller KH, Mösnaer R, Baumgartner MR, Seifritz E, Grünblatt E, Quednow BB. α(2A) -Adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users. Addict Biol. 2017 Mar;22(2):561-569.

Luhrs L, Manlapaz C, Kedzie K, Rao S, Cabrera-Ghayouri S, Donello J, Gil D. Function of brain α(2B)-adrenergic receptor characterized with subtype-selective α(2B) antagonist and KO mice. Neuroscience. 2016 Dec 17;339:608-621

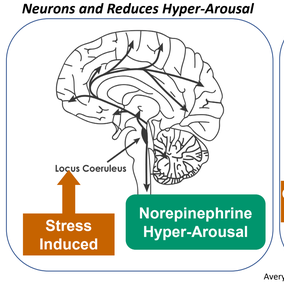
Pupo AS, Minneman KP. Adrenergic pharmacology: focus on the central nervous system. CNS Spectr. 2001 Aug;6(8):656-62.

Rivero G, Martín-Guerrero I, de Prado E, Gabilondo AM, Callado LF, García-Sevilla JA, García-Orad Á, Meana JJ. Alpha2C-adrenoceptor Del322-325 polymorphism and risk of psychiatric disorders: significant association with opiate abuse and dependence. World J Biol Psychiatry. 2016 Jun;17(4):308-15..

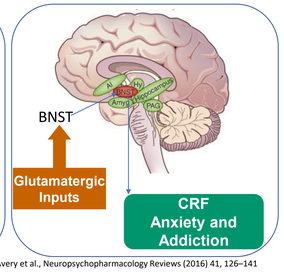
Xie W, Cappiello M, Meng M, Rosenthal R, Zhang W. ADRA2B deletion variant and enhanced cognitive processing of emotional information: A meta-analytical review. Neurosci Biobehav Rev. 2018 Sep;92:402-416.

Pathways Modulated by Alpha 2-Adrenergic Receptors

Modulates NE Release from LC Neurons and Reduces Hyper-Arousal



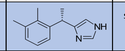
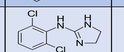
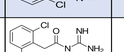
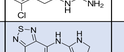
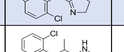
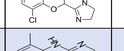
Modulates Excitatory Inputs onto BNST and Reduces CRF Release



Avery et al., Neuropsychopharmacology Reviews (2016) 41, 126–141

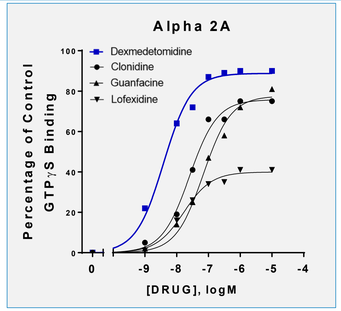
Schizophrenia | Bipolar Disorder | Dementia | Delirium | Opioid Withdrawal

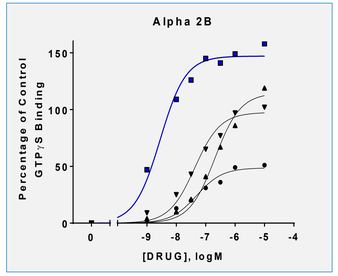
ALPHA2-ADRENERGIC AGONISTS

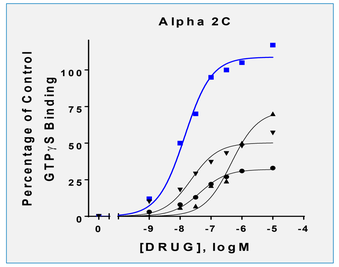
NAME	STRUCTURE	THERAPEUTIC USES
Dexmedetomidine		Semi-arousable Sedation without Respiratory Depression
Clonidine		Hypertension ADHD Anxiety Opioid withdrawal Tourette's
Guanfacine		Hypertension ADHD PTSD
Tizanidine		Muscle Relaxant
Lofexidine		Hypertension Opioid Withdrawal
Xylazine		Animal Tranquilizer Sedation Muscle Relaxant

Key Points:

- Alpha 2-Adrenergic agonists are approved or used off label for many CNS indications. All of these indications are relevant to inadequate response to stress
- These therapeutic actions match the phenotypes associates with transgenic mice and genotypes.
- Clonidine and lofexidine possess the imidazoline structure and are potent agonists at the imidazoline I1 receptor, contributing to cardiovascular effects. Dexmedetomidine does not contain an imidazoline moiety and is 30-fold selective for alpha2-adrenergic receptors over imidazoline I1 receptors







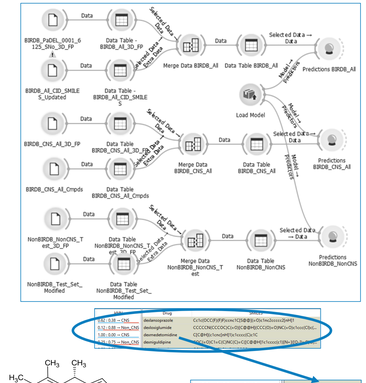
DRUG	Alpha-2A		Alpha-2B		Alpha-2C	
	EC_{50} (nM)	%MAX ACTIVITY	EC_{50} (nM)	%MAX ACTIVITY	EC_{50} (nM)	%MAX ACTIVITY
DEXMED	3.9	89	2.8	147	14	110
Clonidine	25	76	49	49	43	32
Guanfacine	69	78	2010	110	45	72
Lofexidine	17	40	43	97	23	50

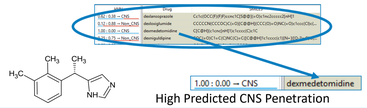
Pharmacology at Human Alpha2 A, B and C Adrenergic Receptors

Key Points:

- Dexmedetomidine is a full agonist at all 3 types of alpha-2 adrenergic receptors
- At therapeutic concentrations, expect activation of all 3 types
- Because all 3 types associated with stress-related sympathetic and glutamatergic activity, **dexmedetomidine may be superior to other agents in this class**
- In case of desensitization of receptors because of high norepinephrine tone, expect partial agonists to become ineffective, but expect dexmedetomidine to retain its efficacy

Fingerprint Analysis: Predicts High Free Brain Levels after Dosing

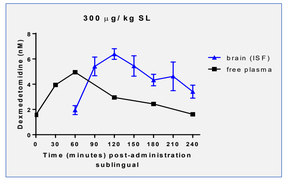


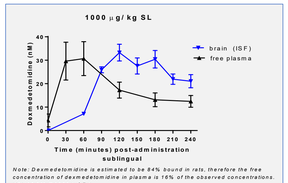


High Predicted CNS Penetration

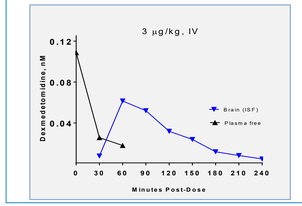
- A dataset of 6,125 compounds available with Broad Institute "Drug Repurposing Hub" (https://clue.io/repurposing) was downloaded
- SMILES representations available for the compounds in the dataset were used to generate energy minimized 3D structures for all compounds;
- Fingerprint and other types of descriptors, such as 1D/2D, 3D, etc., were generated for the compounds;
- A set of ~400 compounds constituted the training set for a supervised M/L based classification model;
- Orange3 application under Anaconda3 environment was used for data analysis and model building; Following iterations with various M/L based methods, such as **kNN**, **Random Forest**, **SVM**, **Neural Network**, etc., and optimization of underlying parameters in each method, several prediction models were developed using only the Fingerprint descriptors;
- Based upon suitable statistical measures, such as **Precision**, **Recall**, etc., the best model so far, BBB-ML3, was identified
- **BBB-ML3** was used to predict CNS/Non-CNS probability for all 6,125 compounds of the Broad Institute dataset

Brain Levels after Sublingual and IV Dosing in Rats





Note: Dexmedetomidine is estimated to be 84% bound in rats, therefore the free concentration of dexmedetomidine in plasma is 16% of the observed concentrations. Values are means ± SEM.



KEY FINDINGS

- Successful CNS drugs achieve high free brain to free plasma ratios after dosing
- Dexmedetomidine predicted to achieve high free brain levels based on favorable structural attributes
- Dexmedetomidine is rapidly metabolized by glucuronidation and CYP-mediated pathways and therefore cannot be administered orally
- Sublingual administration bypasses first pass metabolism and produces sustained (for 2 hours) plasma levels
- Peak CNS free brain levels are approximately equal to Cmax (plasma.free) levels
- Free brain levels were measured by collecting dialysate directly from brain and analysing the content of dexmedetomidine in the dialysate (total brain content was not measured)

Conclusions and Future Perspectives

CONCLUSIONS

- Gene-modified mice, CNS pathway analysis, genotypic variants and experience with therapeutic drugs all indicate that alpha2-adrenergic receptor agonists **modulate the response to stress**
- Stress is major contributor to the emergence of psychomotor agitation and mood disorders in many neuropsychiatric diseases
- Dexmedetomidine predicted to achieve high CNS free brain levels after dosing by using structural analysis of successful CNS drugs; this finding was validated using microdialysis
- The pharmacology of dexmedetomidine indicates it will fully activate all 3 alpha-2 adrenergic receptors in brain for 3 to 4 hours after a single dose with no activation of imidazoline receptors
- Dosing sublingually results in sustained high free brain concentrations

FUTURE DIRECTIONS

- BXCL501 to be tested in clinical studies to demonstrate efficacy in reducing psychomotor agitation, symptoms from opioid use withdrawal, PTSD, and agitation associated with dementia
- Preclinical testing of dexmedetomidine initiated to demonstrate a role in anxiety and depression
- The superior pharmacological and pharmacokinetic properties of sublingual dexmedetomidine, BXCL501, make it an ideal drug to treat diseases in which a maladaptation to stress occurs

Methods

GTPγS Binding: Cells were transfected with alpha2A (Chem-1 cells), 2B or 2C (CHO-K1 cells) adrenergic receptor constructs. Cells grown to confluency in supplemented HAM-F12 media. Cells were harvested in PBS, homogenized centrifuged at 43Kxg for 30 minutes, resuspended, sonicated and frozen in aliquots. Membranes were incubated for 30 minutes at 30° in buffer containing 20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl2, 1 mM DTT, 1 mM EDTA. Incubations were started by the addition of 0.2 nM [35S]GTPγS . Nonspecific binding defined with non-radiolabelled GTPγS. Data were analyzed with GraphPad Prism using a 3-parameter logistic equation. Study was performed by Eurofins Panlabs Discovery Services.

Audinot et al. . Ligand modulation of [35S]GTPgammaS binding at human alpha(2A), alpha(2B) and alpha(2C) adrenoceptors. Cell Signal. 2002 Oct;14(10):829-37. PubMed PMID: 12135704.

***In Vivo* Microdialysis:** Ten male Sprague Dawley rats with a jugular vein cannula were used for this study. Experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Charles River Laboratories. Rats were anesthetized using isoflurane (2%, 800 mL/min O2). Bupivacaine was used for local anesthesia and carprofen for peri-/post-operative analgesia. Animals were placed in a stereotaxic frame (Kopf instruments, USA) and MetaQuant microdialysis probes (PAN membrane, BrainLink, the Netherlands) were implanted into the PFC (4 mm exposed surface). Coordinates for the tips of the probes for the PFC are: antero-posterior (AP) = +3.4 mm from bregma, lateral (L) = -0.8 mm from midline and ventral (V) = -5.0 mm from dura, the toothbar set at -3.3 mm. Microdialysis was performed approximately 24 hrs following surgery. The microdialysis probes were connected with flexible PEEK tubing to a microperfusion pump (Harvard PHD 2000 Syringe pump Holliston, MA). Microdialysis probes were perfused with aCSF containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl2, 1.2 mM MgCl2 + 0.15% BSA, at a slow flow rate of 0.15 µL/min and a carrier flow (UP+0.15% BSA) at a rate of 0.8 µL/min. Microdialysis samples were collected for 30-minute periods by an automated fraction collector (820 Microsampler, Univentor, Malta) into 300 uL polypropylene mini-vials. After stabilization, one baseline sample was collected, and the test compound was given sublingual. Then, eight additional 30-minute ISF samples from the PFC were collected (for a total collection time of 5 hrs). In addition to ISF collection, blood samples were collected via the JV cannula at 0, 30, 60, 120, 180 and 240 minutes after dosing into EDTA anticoagulant vials (~200 uL blood/sample) and kept on ice until processed for plasma (centrifugation at 4°C, 2500g for 10min).

Contact: Frank D. Yocca (CSO) ***BioXcel Therapeutics***
12th Floor, 555 long Wharf Drive New Haven, CT, USA
fyocca@ bioxcelherapeutics.com
Phone: 302 299 3653