Synergistic effects of high-dose D-cycloserine(DCS) and lurasidone in rodent models of depression, anxiety and PTSD: An optimized, multi-targeted pharmacotherapy approach

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ABSTRACT

Background: N-methyl-D-aspartate (NMDAR) antagonists such as ketamine produce potent anti-depressive effects in humans. In addition, such compounds may be effective in the reduction in symptoms in individuals with chronic PTSD (Feder et al., JAMA psychiatry, 2014). However, available NMDAR antagonists such as ketamine or S-ketamine are limited by psychotomimetic side effects and abuse potential, along with lack of oral bioavailability. D-Cycloserine (DCS) is a clinically marketed anti-tuberculosis agent (Seromycin®) that fortuitously acts as an NMDAR antagonist at high daily doses (>750 mg/d). DCS differs from ketamine in that it acts as a mixed agonist/antagonist at the NMDAR-associated glycine-modulatory site. DCS when used at doses of >750 mg has shown efficacy in small-scale clinical trials targeting treatment-resistant depression (TRD), bipolar disorder and suicidality. Here we evaluated effects of DCS in animal models of depression, anxiety and PTSD alone and in combination with other medications (e.g. antidepressants, antipsychotics) frequently used in treatment of depression/PTSD, to explore PK/PD relationships and potential synergy among compounds.

Methods: DCS effects were assessed in animal models of depression (Forced swim test, FST), anxiety (Elevated plus maze, EPM) and PTSD (Wistar Kyoto Rat feat conditioning, WKY FC) relative to doses associated with clinical response. Studies were performed both alone and in combination with atypical antipsychotics such as lurasidone and quetiapine.

Results: DCS significantly reduced immobility in the FST at a dose of 300 -, but not 30-mg/kg. Effects persisted in the present of lurasidone. In the EPM, both lurasidone and quetiapine significantly reduced % open arm entries, consistent with clinical liability to induce akathisia symptoms. DCS increased % open arm entries consistent with potential anti-anxiety and anti-akathisia effects. In stress-sensitive WKY rats, DCS at 300 mg/kg significantly enhanced unlearning of FC responses, leading to highly significant between group differences in fear-related (freezing) behavior.

Discussion: Although NMDAR antagonists such as ketamine produce reproducible reductions in both depression and anxiety that may be relevant to PTSD, usage is severely limited by lack of orally available formulations and high abuse potential of NMDAR channel blocking agents, such as ketamine. DCS is a clinically available anti-TB agent that has been marketed for over 50 years with a well-documented safety profile and low abuse potential. Anti-depressive effects were first noted over 50 years ago, and have been replicated over recent years. In addition, effects have been observed not only on treatment resistant symptoms of depression, but also on associated suicidal symptoms.

Here, we demonstrate significant anti-depressive, anti-anxiety effects in animal models, similar to those reported previously for ketamine. Moreover, significant synergy was observed with atypical antipsychotic agents that may also be beneficial for PTSD. In WKY rats, DCS significantly increased extinction of previously learned fear responses, suggesting reduced re-experiencing of traumatic memories and enhanced unlearning. These findings support clinical studies of DCS, in combination with atypical antipsychotics such as lurasidone or quetiapine, for treatment of persistent PTSD symptoms.

Overview: Clinical symptoms in Depression/PTSD

- Mood disorders, including unipolar/bipolar depression and PTSD are significant independent causes of morbidity, disability and morality
- In addition, up to 80% of individuals with PTSD suffer from co-morbid depressive disorders, depending upon age and setting (Haviland et al., 2016)
- PTSD is associated with a significant elevated suicide risk both alone (~6x), and in combination with depressive disorders (~9x) (Bryan et al., 2016)
- Currently approved agents for PTSD with or without accompanying depression
  - Sertraline, paroxetine
- However, the medications are only partially effective against depressive symptoms and have limited effect on suicidality
- Newer treatment approaches are needed

Overview: NMDAR antagonists in depression/PTSD

- The recent focus on use of NMDAR antagonists in depression derives from the clinical observations regarding anti-depressant (Berman et al., 2000) and anti-suicidal (Price et al., 2014; Murrough et al., 2015) effects of ketamine
- Beneficial acute effects of ketamine also reported in PTSD (Feder et al., 2014)
- However, repeated use of ketamine is limited by psychotomimetic side effects, abuse liability, neurotoxic potential and poor oral biovailability
- D-cycloserine is an orally available FDA-approved medication that fortuitously serves as an NMDAR antagonist with reduced psychotomimetic side effects relative to ketamine
- Initial rodent behavioral studies investigated potential synergistic effects of D-cycloserine with serotonergic agents (e.g. lurasidone) approved for treatment of bipolar depression
- More recent studies investigated the potential beneficial effects of D-cycloserine in an animal fear-conditioning (FC) model of PTSD.

Rodent depression model: Forced swim test (FST)

The Model

- Mice when forced to swim in a restricted space will rapidly cease attempts to escape and become immobile.
- The Forced Swim test (FST) is an indicator of depression and measures the amount of time the mouse swims before "giving up" and becoming immobile.
- Reduction of immobility time in this assay predicts anti-depressant effect across a range of compound types (Cryan et al., 2005)
- Effects of D-cycloserine were assessed both alone (**Fig. 5**) and in combination with lurasidone (**Fig. 6**)

Results

- When administered alone (**Fig. 5**), D-cycloserine produced a significant, dose-dependent reduction in immobility, with effects equivalent to the active comparator (sertraline)
- Lurasidone had no significant effect on its own in this assay system (**Fig. 6**)
- Beneficial effects of D-cycloserine persisted even in the presence of lurasidone (**Fig. 6**)

Methods: Mice (8 weeks) from Jackson laboratories were used. Mice were acclimated to the test room at least 1 hour prior to commencing the test. The forced swimming test consisted of one 6-minute session of forced swimming in individual opaque cylinders (15 cm tall x 10 cm wide, 1000 ml beakers) containing fresh tap water at a temperature of 23 ± 2 °C and a depth of 12 cm (approximately 800 ml) for each test animal. The time the animal spent immobile was recorded over the 6 min trial.. After the swim test, each animal was placed in a pre-heated cage with a heating pad and allowed to dry.

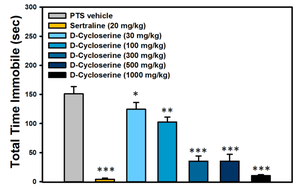
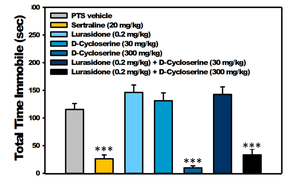


Figure 5: Dose-response effect of D-cycloserine in the FST: *D-cycloserine significantly reduced immobility in the rodent FST in a dose-dependent fashion (F=39.9, p<.0001). Effects were significantly greater following treatment with high (antagonist-level) vs. low (agonist-level) doses of D-cycloserine. Effects of high-dose D-cycloserine were equivalent to those of the active comparator (sertraline).*
** p<.05, **p<.01, ***p<.001*

Figure 6: Effects of D-cycloserine in combination with lurasidone: *Immobility time was significantly lower following treatment with high-dose D-cycloserine alone (▬) or combined high-dose D-cycloserine and lurasidone (▬) than under control conditions. Low-dose D-cycloserine was without significant effect either alone or in combination with lurasidone. * p<.0001*



Rodent anxiety/akathisia model: Elevated plus maze (EPM)

The Model

- The Elevated Plus Maze (EPM) is a standard test for the assessment of anxiety.
- This maze has two opposite open arms and two closed arms.
- Mice tend to first enter and spend most of the test time in the closed arms. Animals will sometimes explore and spend time i0n the open arms.
- The time in the open arms and the number of entries in the open arms are interpreted as a measure of anxiety.

Results

- Treatment with lurasidone alone significantly reduced % time in the open arms (**Fig. 7,** *left*), consistent with its known liability to produce akathisia during clinical treatment
- Treatment with high-dose D-cycloserine prevented the behavioral alterations induced by lurasidone in this assay (**Fig. 7**, *right*)
- D-cycloserine also prevented behavioral alterations induced by other 5-HT2AR antgonists (quetiapine, MDL100907, **Fig. 8**) suggesting that combination treatments may be optimized for specific disorders.

Methods: The maze (Hamilton Kinder) consists of two closed arms (14.5 h x 5 w x 35 cm length) and two open arms (6 w x 35 l cm) forming a cross, with a square center platform (6 x 6 cm). All visible surfaces are made of black acrylic. Each arm of the maze is placed on a support column 56 cm above the floor. Antistatic black vinyl curtains (7' tall) surround the EPM to make a 5' x 5" enclosure. Mice (C57Bl/J6) are placed in the center of the elevated plus maze facing the closed arm for a 5-min run. All animals are tested once. The time spent, distance traveled and entries in each arm are automatically recorded by the computer.

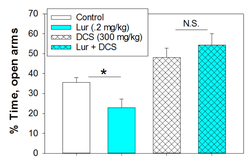
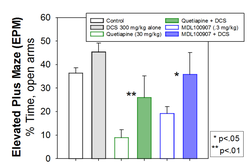


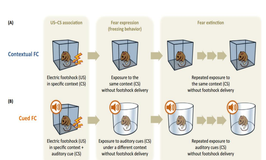
Figure 7: Effects of D-cycloserine in combination with lurasidone: *In the absence of D-cycloserine (open bars), lurasidone significantly reduced % time in the open arms, consistent with clinical liability for akathisia. In the presence of high-dose D-cycloserine (cross-hatch bars), lurasidone no longer induced significant alterations in behavior. Given links between akathisia and suicide, this effect may contribute to anti-suicidal efficacy. *p<.05*

Figure 8: Effects of D-cycloserine in combination with other 5-HT2A antagonists: *High-dose D-cycloserine significantly reversed anxiety-like effects of other 5-HT2AR antagonists including quetiapine (D2/5-HT2AR antag) and MDL100907 (selective 5-HT2AR antag), suggesting generalizability of the DCS effect. *p<.05; **p<.01*



PTSD: Rodent WKY fear extinction model

The Model



From Fores et al., 2015

- Pavlovian fear conditioning (FC) test is a widely-used behavioral assay in rodents for measurement of aversive learning and memory relevant to PTSD
- In the Cued FC paradigm, a tone is paired with a mild electric shock. Once the association is established, the conditioned stimulus (CS) induces a fear response ("freezing") in a similar manner to unconditioned stimulus (US).
- However, if the CS is presented repeatedly without combination with the US extinction occurs in normal animals.
- Wistar Kyoto (WKY) rats show perseveration of avoidance after FC and are used as models of stress
- Effects of high-dose D-cycloserine were assessed in the WKY model

Methods: Male WKY and Wistar rats (Charles River Laboratory) were used in this study.
Fear Conditioning (FC) Training: Animals are exposed to 5 CS-US pairings of a 10 sec tone (70dB conditioned stimulus/CS) co-terminating with a brief electric shock (0.5 mA for 1.0 sec unconditioned stimulus/US). The 5 CS-US pairings are separated by a 60 sec interval with the first CS at 120s. The rats stage in boxes for one minute after the last shock before being moved out.
Extinction training (ET): Cued FC extinction training was conducted 24 and 72 hr post FC training. The extinction training was conducted in a "cylinder", which is a changed context from the boxes of FC training. The extinction training lasts 34 min, including 8 identical bins. Each bin includes a 2 min tone period followed by a 2 min no-tone period (inter-trail interval , ITI). D-cycloserine or saline were administered 30 min prior to each ET session

Results

- Normal Wistar rats, show lower freezing rates than WKY rats at the beginning of ET, and a rapid decrease during ET training (not shown)
- WKY rats, show paradoxical increases in freezing during ET, suggesting that the tone alone may be sufficient to "reactive" the traumatic memory (**Fig. 9A**)
- WKY rats treated with high-dose D-cycloserine (**Fig. 9B**) showed progressive reductions in freezing during ET, leading to significant differences between saline and DCS treated animals (**Fig. 9C**)

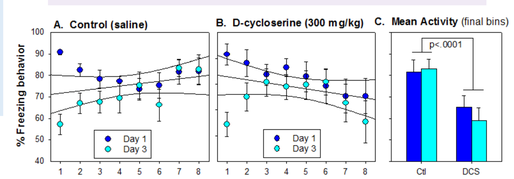


Figure 9: Effects of D-cycloserine during Extinction Training in WKY rats. *A.* Control treatment, *B.* DCS treatment, *C.* Between-group difference.

D-cycloserine (DCS): Mechanism of action

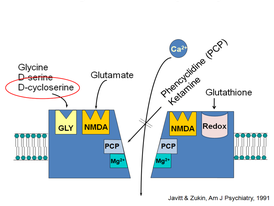


Figure 2: *Schematic diagram of the N-methyl-D-aspartate-type glutamate receptor (NMDAR), showing the site of action of D-cycloserine (DCS)*

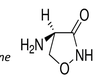


Figure 1: *Chemical structure of D-cycloserine*

- **D-cycloserine (DCS)** is an anti-TB medication that fortuitously cross-reacts with the glycine binding site of the NMDAR-type glutamate receptor
- **Partial agonist ("Mixed agonist/antagonist")**
  - Net agonist at human doses of <100 mg/d
  - Net antagonist at human doses of >500 mg/d
- **Equivalent rodents doses**
  - Agonist dose: ≤30 mg/kg
  - Antagonist dose: ≥300 mg/kg

Clinical effects of DCS in major depression

- Anti-depressant doses of DCS were first reported in the late 1950's in individuals receiving DCS for TB
- Anti-depressant effects in pre-clinical models were demonstrated in the late 1990's (Skolnick, 1999)
- Clinical anti-depressant effects of high-dose DCS were reconfirmed in a recent clinical study in treatment-resistant depression (**Figure 3**) (Heresco-Levy et al., 2013)
- Open-label beneficial effects were also observed in treatment-resistant bipolar disorder (Kantrowitz et al., 2015).
- Orally available
- Reduced psychotomimetic effects, abuse liability and neurotoxicity relative to ketamine

Hamilton depression rating scale (HDRS)

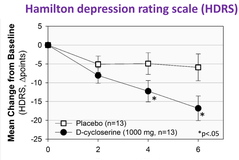


Figure 3: *Effect of high-dose (1000 mg/d) D-cycloserine added to anti-depressants (e.g. TCAs, SSRIs) in treatment resistant major depression.*
From Heresco-Levy et al., 2013

Rationale for combination of DCS with luarasidone in bipolar disorder

Hamilton depression rating scale (HDRS)

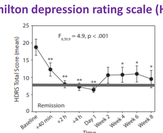


Figure 4: *Effect of high-dose (1000 mg/d) D-cycloserine added to D2/5-HT2A antagonists in treatment resistant bipolar depression. From Kantrowitz et al., 2015*

- **Lurasidone** is one of 3 approved compounds for treatment of depression in bipolar disorder
  - Other approved compounds: quetiapine, olanzapine/fluoxetine
- 50% response/50% non-response rate when used in treatment resistant bipolar disorder (Loebel et al., 2014)
- No significant benefit against suicidality
- In a pilot, open label study (n=8) a significant reduction in depression was observed within 40 min, and persisted through 8 weeks (**Fig. 4**)
- **Mechanism of action**: Combined D2/5-HT2A antagonist
- **Rationale for multi-targeted therapy**
  - Additive anti-depressant/anti-suicidal effects
  - Lurasidone prevents psychotomimetic effects of D-cycloserine
  - D-cycloserine prevents 5-HT2A antagonist-induced akathisia

Results

- High dose (300 mg/kg) D-cycloserine showed highly significant activity in animal models related to depression (FST, Fig. **5,6**), anxiety (EPM, **Fig. 7,8**) and PTSD (WKY FC, **Fig. 9**)
- In the FST, significant (p<.0001) effects persisted in the presence of lurasidone, suggesting additive benefit from the multi-targeted 5-HT2AR/NMDAR approach
- In the EPM, D-cycloserine significantly reversed pro-anxiety effects of lurasidone (p<.05), suggesting potential reductions in akathisia during clinical use, which may reduce suicidal potential especially in younger individuals
- In the WKY fear conditioning (FC) model, D-cycloserine significantly (p<.0001) prevented "re-activation" symptoms during tone alone extinction training (ET), leading to enhanced unlearning of conditioned aversion
- These findings support potential utility of NMDAR antagonists, such as D-cycloserine, in combination with serotonergic agents for pharmacotherapy of both depressive disorders and PTSD
- Ideal combinations for specific disorders need to be determined

Implications: Depression in PTSD

- Combined D-cycloserine/lurasidone (NRX-101, "Cyclurad") is currently under development for treatment of acute suicidal ideation and behavior in bipolar depression ("ASIB"). Hypotheses are that:
  - D-cycloserine/lurasidone will prolong the initial beneficial effects of ketamine,
  - Lurasidone will prevent potential psychotomimetic effects of D-cycloserine alone
  - D-cycloserine will prevent potential akathisia induced by lurasidone alone
  - Combined D-cycloserine/lurasidone will therefore have superior efficacy and reduced side effects compared to either treatment alone
  - As compared to repeated ketamine, D-cycloserine is orally available, has a well-established long-term safety profile based upon use an an anti-TB agent, and has no known abuse liability or neurotoxic potential
- In the present rodent preclinical studies, beneficial effects of D-cycloserine alone and in combination with anti-depressants were observed in relevant animal models, including the FST and EPM
- These findings support continued clinical development of multi-targeted NMDAR/serotonergic drugs for treatment-refractory depressive disorders, alone or in combination with PTSD
- Anti-suicidal effects associated with NMDAR antagonists as a class (e.g., ketamine) may be particularly relevant for co-morbid depression/PTSD

Implications: Core symptoms in PTSD

- The only FDA approved medications for PTSD at present are the SSRI-type anti-depressants sertraline (Zoloft®) and paroxetine (Paxil®). The SNRI venlafaxine (Effexor) is also considered a first line treatment
- Nevertheless, both core symptoms of PTSD (intrusion, avoidance, negative alterations in cognition and mood, alterations in arousal and reactivity)and depression may persist despite best-available treatment
- Low ("agonist") dose D-cycloserine (50 mg/d) has been extensively studied for enhancement of exposure therapy both in PTSD and phobia disorders, based upon the desire to stimulate NMDAR-mediated neurotransmission in order to enhance plasticity. However, effect sizes to date have been modest (e.g., Ressler et al., 2004; Averill et al., 2016; Mataix Cols et al., 2017)
- The present findings in WKY rats suggest an **alternative hypothesis** – i.e. that excessive plasticity may lead to reactivation of the affective component of the traumatic event even in the absence of a negative reinforcers
- To the extent that this hypothesis is correct, use of NMDAR antagonists such as **high-dose D-cycloserine** may be beneficial to reduce avoidance behavior and alterations in arousal/reactivity.
- The potential utility of NMDAR antagonists for treatment of core symptoms of PTSD is supported by studies of ketamine in both humans (Feder et al., 2014) and rodents (Gigenti et al., 2017)

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Acknowledgements and Disclosures

- All assays were performed by Psychogenics, Inc., Tarrytown, NY
- Funding, NeuroRx Pharma

COI: Dr. Javit holds IP for use of DCS in depression, anxiety and PTSD and holds equity in Glytech, Amino Acid Solutions, and NeuroRx Pharma