Chronic Social Defeat Mouse Assay for Assessing Anti-Depression Therapies

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PURPOSE OF THE STUDY

The current paradigms for testing anti-depression therapies have little face and predictive validity within only a very narrow range of mechanisms of action and a narrow therapeutic window. The chronic social defeat (CSD) model is ethologically relevant and has robust depressive-like endpoints. Moreover, the paradigm allows for either chronic or acute anti-depressant treatments with any specified dosing paradigm. Depressed patients have evidence of increased microglia. One consequence of this is elevated quinolinic acid - suggesting the induction of microglial indoleamine 2,3, dioxygenase 1 (IDO1). Substantial evidence exists that microglia activating cytokines are elevated in depressed patients and may contribute to symptoms. Therefore, in this study CSD model was assessed for alterations in IDO1 activity, and consequences of IDO1 inhibition.

MATERIALS AND METHODS

Animals

Male C57Bl/6J mice (9-10 weeks) (CR Germany) and male ex-breeder CD-1 mice (CR UK) (age 7-11 months) were used. Animals were housed at a standard temperature (22 ± 1°C) and in a light-controlled environment (lights on from 7 am to 8 pm) with *ad libitum* access to food and water. All animal experiments were approved by the National Animal Experiment Board, Finland. CD-1 mice were housed 1 mouse per cage during the experiments. C57Bl/6 mice were housed in pairs while not subjected to CSD.

Treatment Groups

Following treatment groups were used:

- Group 1: 16 male C57Bl/6J mice as controls – vehicle (Natrosol 0.5 %) treated (p.o., 10 ml/kg) starting from day 13
- Group 2: 12 male C57Bl/6J mice subjected to CSD – vehicle (Natrosol 0.5 %) treated (p.o., 10 ml/kg) starting from day 13
- Group 3: 12 male C57Bl/6J mice subjected to CSD – IDO1, 0.6 mg/kg (p.o., 10 ml/kg) starting from day 13
- Group 4: 12 male C57Bl/6J mice subjected to CSD – IDO1, 3 mg/kg (p.o., 10 ml/kg) starting from day 13

Chronic Social Defeat

First, CD-1 aggressor mice were determined by using the procedures by Golden et al. (2011). After screening and selection of aggressor mice, the chronic social defeat (CSD) paradigm was conducted under dim lighting where CSD mice were subjected to direct exposure with CD-1 aggressor mouse. Behaviour was observed, and the duration of each physical attack was timed; mice remained together until either a cumulative total of 60-sec physical attack had occurred, or 10 min had elapsed. After the 10 min session, CSD mice were exposed to CD-1 mice through perforated transparent divider for 24h. This setup allowed direct (for 10 min) and continued olfactory, visual, auditory exposure for the rest of the day. After each day, a new aggressor mouse was placed in a cage in similar fashion with 10 min direct exposure followed by chronic exposure through divider made from transparent Plexiglas and perforated with multiple holes (Ø = 10 mm). Cycle was repeated for 15 days, replacing the C57Bl/6J mouse with a new one every day until day 15. Thereafter, CSD mice remained caged next to the same CD-1 mice without any further attack sessions or rotations. Control mice were kept in littermate pairs without any exposure.

Open field Motor Activity

OF test was conducted on day 14 by using activity chambers (Med Associates Inc, St Albans, VT; 27 x 27 x 20.3 cm) equipped with IR beams. Testing was done under red light conditions. The mouse was placed in the center of empty arena for 15 min. Following parameters were calculated: distance moved, number of vertical rearings, velocity and % resting time (no movement activity).

Contextual Fear conditioning

CFC test was conducted on day 15 by using a Coulbourn FreezeFrame system (Coulbourn, Whitehall PA, USA). The mouse was placed on the grid floor in the empty arena and challenged with 16 inescapable electro-shocks each of 0.2 mA x 3 sec and delivered at inter-e-shock intervals (ITI) of 50 sec. Mean % time spent freezing during ITIs were calculated.

Two-choice active avoidance Test

AA test was conducted at day 16 by using GEMINI™ active/passive avoidance system (SDI, USA). The mouse was placed on the grid floor in the arena containing a central divider with an opening ("gate") through which mouse could transfer from one side of the arena to the other. The gate was open all the time during the experiment. Training consisted of 40 trials, in which a 10-sec tone (5 kHz, 85 dB, conditioned stimulus, CS) preceded an inescapable e-shock (0.15 mA x 5 sec) with inter-stimulus intervals (ITI) of 40 sec. Avoidance behavior of the mouse was recorded as unsuccessful or successful transfer from one side of the arena to another. The following measures were calculated: total escape failures, total escapes, total avoids, and number of inter-trial crossings.

Mass Spectrometry

Plasma levels of tryptophan (TRP) and its kynurenine (KYN) pathway metabolites were measured using liquid chromatography-tandem mass spectrometry Plasma samples, calibration standards and quality controls were diluted with mobile phase A (2% formic acidin water) and internal standard mix (d5-tryptophan, d4-kynurenine, d3-quinolinic Acid, d5-kynurenic acid). Ice-cold methanol was added for protein precipitation. Subsequently, samples were centrifuged and supernatant was evaporated to dryness under a gentle stream of nitrogen and reconstituted in mobile phase. Additionally, brain samples were lipid depleted by hexane extraction. The LC–MS/MS system used to analyze plasma and brain samples consisted of a CTC HTC PAL Autosampler (CTC Analytics AG) and an Agilent 1290 Series liquid chromatography system (Micro Vacuum Degasser, Binary Pump SL, Thermostatted Column Compartment; Agilent Technologies), coupled to an API 6500™ triple quadrupol mass spectrometer (AB Sciex). Separation of analytes was achieved using a Atlantis® T3 column (3 µm, 50 × 2.1 mm; Waters) with a isocratic elution for 1 min at 100% solvent A and a subsequent linear gradient profile. All standards, solvents and reagents used were of highest purity (LC–MS grade where available; Sigma Aldrich;). Mobile phase A consisted of 0.2% formic acid in water, and mobile phase B consisted of 0.2% formic acid, in acetonitrile.

BODY WEIGHT

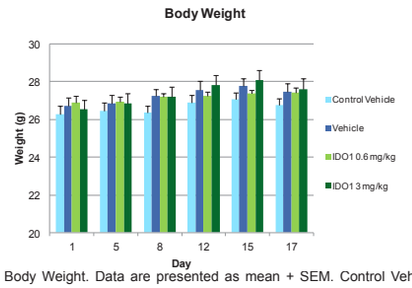

Figure 1. Body Weight. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: no differences.

OPEN FIELD

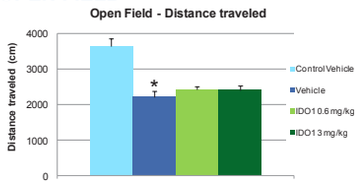

Figure 2. Open field distance. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

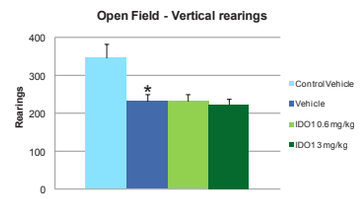

Figure 3. Open field rearings. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

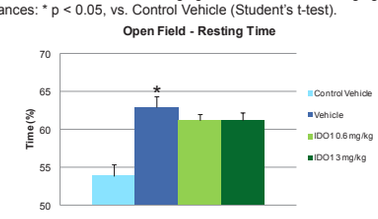

Figure 4. Open field resting time. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

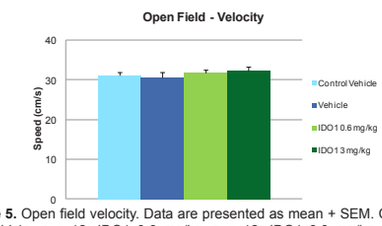

Figure 5. Open field velocity. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: no differences.

CONTEXTUAL FEAR CONDITIONING

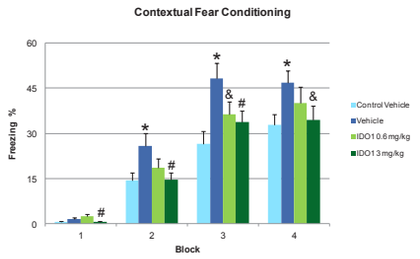

Figure 6. Contextual fear conditioning freezing %. Data are presented as mean + SEM. Control Vehicle, n = 16; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test); #p < 0.05, &p < 0.05 vs. Vehicle (Dunnett multiple comparison).

ACTIVE AVOIDANCE

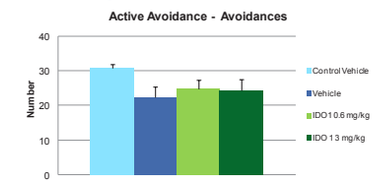

Figure 7. Active avoidance, number of avoidances. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

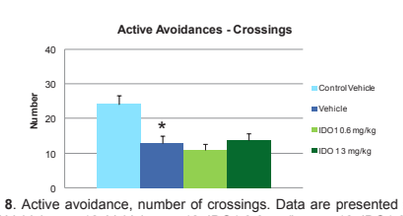

Figure 8. Active avoidance, number of crossings. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

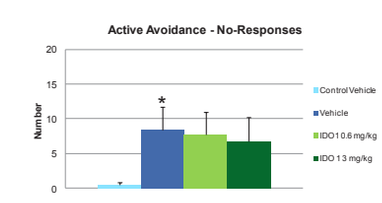

Figure 9. Active avoidance, number of no-responses. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

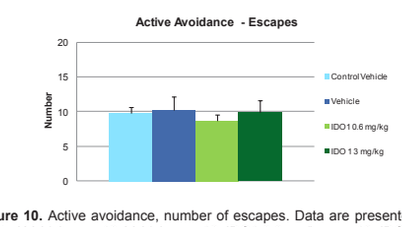

Figure 10. Active avoidance, number of escapes. Data are presented as mean + SEM. Control Vehicle, n = 12; Vehicle, n = 12; IDO1 0.6 mg/kg, n = 12; IDO1 3.0 mg/kg, n = 12. Statistical significances: * p < 0.05, vs. Control Vehicle (Student's t-test).

METABOLIC PATHWAYS

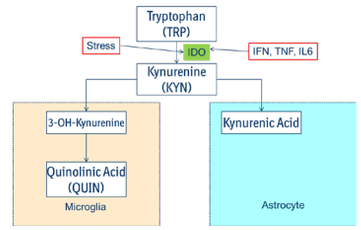

Figure 11. Stress and inflammatory cytokines lead to induction of the IDO1 enzyme, which catalyzes conversion of tryptophan to kynurenine. It was hypothesized that chronic social defeat would lead to upregulated IDO1 in activated microglia, and the consequent increase in quinolinic acid would contribute to the behavioral phenotype.

COMPOUND EXPOSURE LEVEL

Dose	Plasma nmol/L ± SD	Brain nmol/L ± SD	CSF nmol/L ± SD
0.6 mg/kg	100.0 ± 54.5	9.1 ± 9.9	15.3 ± 6.5
3.0 mg/kg	969.8 ± 362.8	40.2 ± 23.5	113.7 ± 42.4

Table 1. The EC50 at mouse IDO1 for this compound is ~63 nM, indicating plasma levels between 0.6 and 6 x EC50 in a cellular assay.

IMMUNOHISTOCHEMISTRY, BIOCHEMISTRY

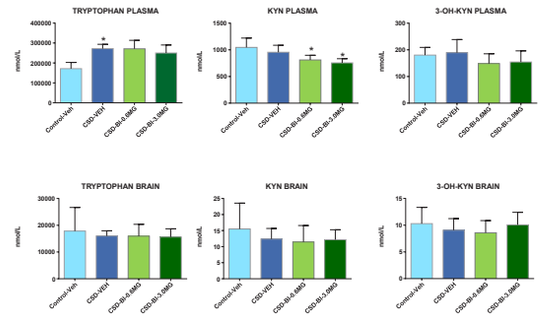

Figure 12. Using whole brain homogenates, no evidence of increased kynurenine or quinolinic acid was detected. These data indicate no IDO1 induction or microglia activation, at least at a whole brain level. Further analysis will investigate microglia activation with immunohistochemistry to identify brain regions that may be particularly susceptible to stress induced inflammation.

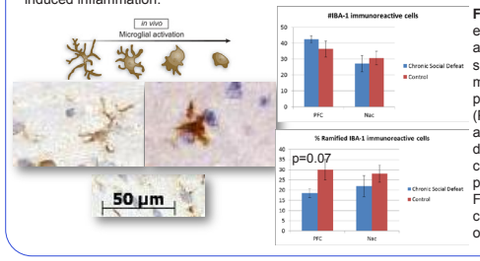

Figure 13. Very early preliminary analysis does not support increased microglia in prefrontal cortex (PFC) or nucleus accumbens, but does suggest a change in phenotype. Further IHC characterization is ongoing.

CONCLUSIONS

- CSD mice showed behavioral alterations in open field, contextual fear conditioning, and active avoidance tests
- Treatment with IDO significantly decreased freezing behavior compared to vehicle treated CSD mice, suggesting some involvement of IDO1 activity in the behavioral phenotype. Validation of this activity is ongoing.

REFERENCES

Golden et al. (2011): A standardized protocol for repeated social defeat stress in mice. Nat Protoc. ; 6(8): 1183–1191. doi:10.1038/nprot.2011.361.