Neuro Detective®
INTERNATIONAL

A Contract Research Network Of Neuroscientists

Effects of Two Proprietary Compounds in a Model of Multiple Sclerosis

DATE

Authentication

This study was conducted under the terms of a Services Agreement between NeuroDetective International Inc. and CLIENT dated DATE.

Sponsor

XXX

Sponsor Study Monitor:

Scientific Supervisor: Dr. Forrest Haun
NeuroDetective International

Study Director:

Testing Facility:

Report approval:

I, the undersigned, hereby declare that the study described in this report was planned, performed and reported under my control as Study Director and that the report provides a true and accurate record of the data generated. I declare further that the original data sheets are archived in my laboratory at the testing facility described above.

Signature: _____ **Date:** _____

Study Director

I, the undersigned, hereby declare that I have reviewed this report and that the interpretations and conclusions drawn from the data are consistent with the results obtained.

Signature: _____ **Date:** _____

Study Sponsor

OBJECTIVE AND DESIGN

In this study, NeuroDetective International Inc. ("NDI") evaluated proprietary compounds supplied by CLIENT ("CLIENT"), designated XXX and YYY, for their ability to reduce symptoms in a model of Multiple Sclerosis (MS) in adult rats.

This study was conducted in the laboratory of _____, under the supervision of NeuroDetective International, Inc.

METHOD AND MATERIALS

1. *DESIGN:*

This study used an experimental autoimmune encehphalomyelitis (EAE) model in adult rats. Two groups of male rats received one of the two test compounds; one group received compound vehicle alone; one group was untreated; and the final (fifth) group was a positive control group that received dexamethasone. Daily motor assessment scores for all animals were obtained. After the last behavior assessment, terminal plasma samples were collected along with the brains, both of which were sent to CLIENT.

2. *ANIMALS:*

Adult male Lewis rats used in this study were purchased from a commercial supplier and housed in a USDA approved laboratory at _____. Animals were maintained on a 12 h/12 h light/dark cycle with food and water available *ad libitum.* All housing and behavior testing facilities, as well as the behavior testing procedures themselves, were approved by the relevant Institutional Animal Care and Use Committee.

3. *EXPERIMENTAL PROCEDURES:*

A solution of myelin basic protein (MBP) was prepared by dissolving 100 µg MBP peptide in 100 µL of PBS (0.1 M PBS, pH 7.3; 50% g/v) then emulsified in 100 µL CFA (10mg/ml M. Tuberculosis; Difco Laboratories, Detroit, MI). Rats were first anesthetized with isoflurane, then received an intracutaneous injection of 0.1 ml of the MBP solution on both sides of the spinal column between the Thoracic 12 and Lumbar 1 spinal regions. This procedure is intended to induce the EAE condition.

On day 7 following the injection of MBP, and on every subsequent day, the animals received twice daily doses (p.o.) of one of the test compounds, vehicle, or positive control. Dosing times were 0800 and 1700 hours. There were 5 experimental groups:

1 – Untreated (no MBP)(n=6)
2 – Vehicle alone (n=11)
3 – Dexamethasone (n=12)
4 – Test compound one, XXX (n=12)
5 – Test compound two, YYY (n=12)

Behavioral testing was performed "blind" to the drug compound administered to the animal. Additional measures to ensure "blindness" included: (1) having one person create and code the drug solutions, randomize animals to drug treatment, and at study end, break the drug code; (2) having another person conduct behavioral testing, as well as perform other daily project-related duties (e.g., daily monitoring of animal health, etc); and finally (3) having a third person conduct data entry and management.

At the end of the study (18 days) blood and brains from the animals were collected and sent on dry ice to CLIENT.

4. *BEHAVIOR TESTING:*

Motor deficits resulting from the EAE condition were evaluated on each dosing day, beginning three hours following the initial dosing, i.e. at 1100 hours. Tail and hind limbs were assessed for degree of motor function/paralysis utilizing the following scale (Pender et al., 1989):

0 = no weakness
1 = weakness of distal part of tail only, the distal tail failing to curl around the examiner's finger/ slight dragging of the toes of the hind foot
2 = weakness of the whole tail but with the proximal tail still being able to be erected vertically against gravity/ severe dragging of the hind foot but not of the rest of the hind limb
3 = severe weakness with only a flicker of tail movement/severe dragging of the whole hind limb
4 = complete flaccid paralysis of the tail/total paralysis of the hind limb

5. *STATISTICAL ANALYSES:*

Statistical analysis was performed using a repeated measures ANOVA, with Day as the repeated factor and Group as the between-subjects measure. Following this ANOVA, the LSD test was used for post-hoc comparisons of group differences. Statistical significance was set at $p < 0.05$.

6. *RESULTS*

Data from a total of 53 adult male Lewis rats were included in this study, 47 of which are all the animals which displayed any of the motor symptoms described above at 13 days post-MBP inoculation, when impairment became most evident (see below). The remaining 6 animals were untreated. The 53 animals were randomly assigned to one of the 5 study groups. The final sample size for each of the four treated groups was 12 animals, except for the vehicle group, which had

eleven animals because one animal was lost during the protocol due to a misplaced injection. The untreated control group had six animals. All animals were maintained on a 12 h/12 h light/dark cycle with food and water available *ad libitum.*

Motor Function Assessment

The motor assessment score for animals in the groups across the test protocol is illustrated in Figure 1. Motor assessment started 7 days post-innoculation with MBP and continued for an additional 11 days. The development of motor impairment was most evident on day 13, as expected. In the vehicle treated group, the severity of motor impairment increased, and was highest on day 17. For statistical analysis, the untreated control group was not included due to a lack of variance (i.e. no animals showed motor impairment). Therefore, the overall repeated measures ANOVA compared motor assessment scores among the 4 treated groups, beginning on day 10. The overall analysis indicated a main effect for group, $F(3,43) = 4.67$, $p < 0.01$, and a significant Group X Time interaction, $F(24,344) = 3.73$, $p < 0.001$. Additional analysis using post-hoc comparisons showed significant group differences beginning on day 14. Specifically, from day 14 to day 18, the dexamethasone and YYY treated animals had significantly less motor impairment compared to vehicle treated animals. On days 17 and 18, the XXX group also had significantly lower motor impairment scores compared to vehicle treated animals.

Body Weight

Body weights were obtained two times per day, immediately prior to dosing. The daily body weights were averaged across test day for each animal. The daily weight of animals in the different groups across the 18 day protocol is illustrated in Figure 2. Untreated animals continued to gain weight throughout the protocol. An overall repeated measures ANOVA comparing weight among the 5 groups across the test protocol showed a significant main effect of Group, $F(4,48) = 15.15$, $p < 0.001$, and a significant Group X Time interaction, $F(68,816) = 33.44$, $p < 0.001$. Post-hoc comparisons showed that significant differences between groups started to appear 2 days following treatment. For instance, a significant difference in weight between the untreated and dexamethasone groups on the one hand, and the untreated and XXX groups on the other hand, appeared on the third day of drug treatment. A significant difference between the untreated group and the YYY group also appeared on the fourth day of treatment. Additional significant differences between the untreated and vehicle groups are evident at 7 days following treatment. Taken together these results show a pattern of all MBP injected animals failing to gain weight normally. The largest loss of weight occurred in the dexamethasone group, while animals treated with either XXX or YYY had significantly less weight loss compared to the dexamethasone treated group.

Side-Effects

All drug treated animals appeared to be in relatively good health throughout the experimental protocol. Although we did not formally quantify additional behavior, *no negative reactions were displayed to any dose of the test compound.* In sum, compared to vehicle control animals, the animals receiving the test compound appeared to be relatively free from negative side effects following a repeated oral dosing of the test compounds, as evaluated using informal observations of the animals' ongoing behavioral repertoire. It should be noted that the administration of the compounds was difficult, which is thought to be due to the nature of the vehicle used.

DISCUSSION

The outcome of the positive control group (dexamethasone) was anticipated, with a robust decrease in motor impairment in the MBP model of MS. The very significant loss of body weight in dexamethasone treated animals is, in general, of relative concern with regard to the use of this compound to treat symptoms of MS. The response pattern of the vehicle group was also as expected. Vehicle treated animals developed a motor impairment, and failed to gain weight normally. Of primary importance for the present study is that, compared to vehicle treated animals, there appears to be a robust YYY drug effect, such that dosing of YYY decreased motor impairment in the MBP model of MS. In addition, both YYY and XXX were associated

with less weight loss compared to dexamethasone treatment.

In summary, it is concluded that YYY treatment is associated with a decrease in motor impairment in the MBP model of MS.

Motor assesment scores for MS animals

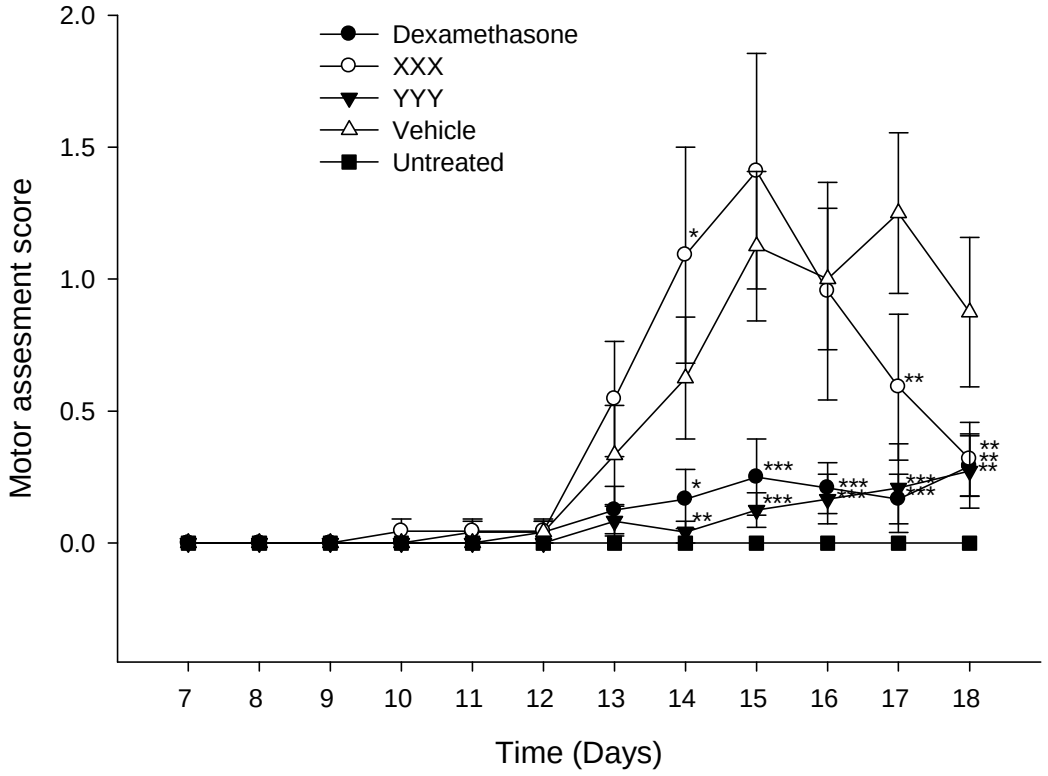

Figure 1: Mean (± SEM) assessment score from day 7 to day 18 following administration of MBP. * = $p < 0.05$; ** = $p < 0.01$;***=$p<0.001$ versus the vehicle control group on each test day. Note: the untreated animals did not receive MBP, and did not have any signs of motor impairment and were therefore excluded from statistical analysis.

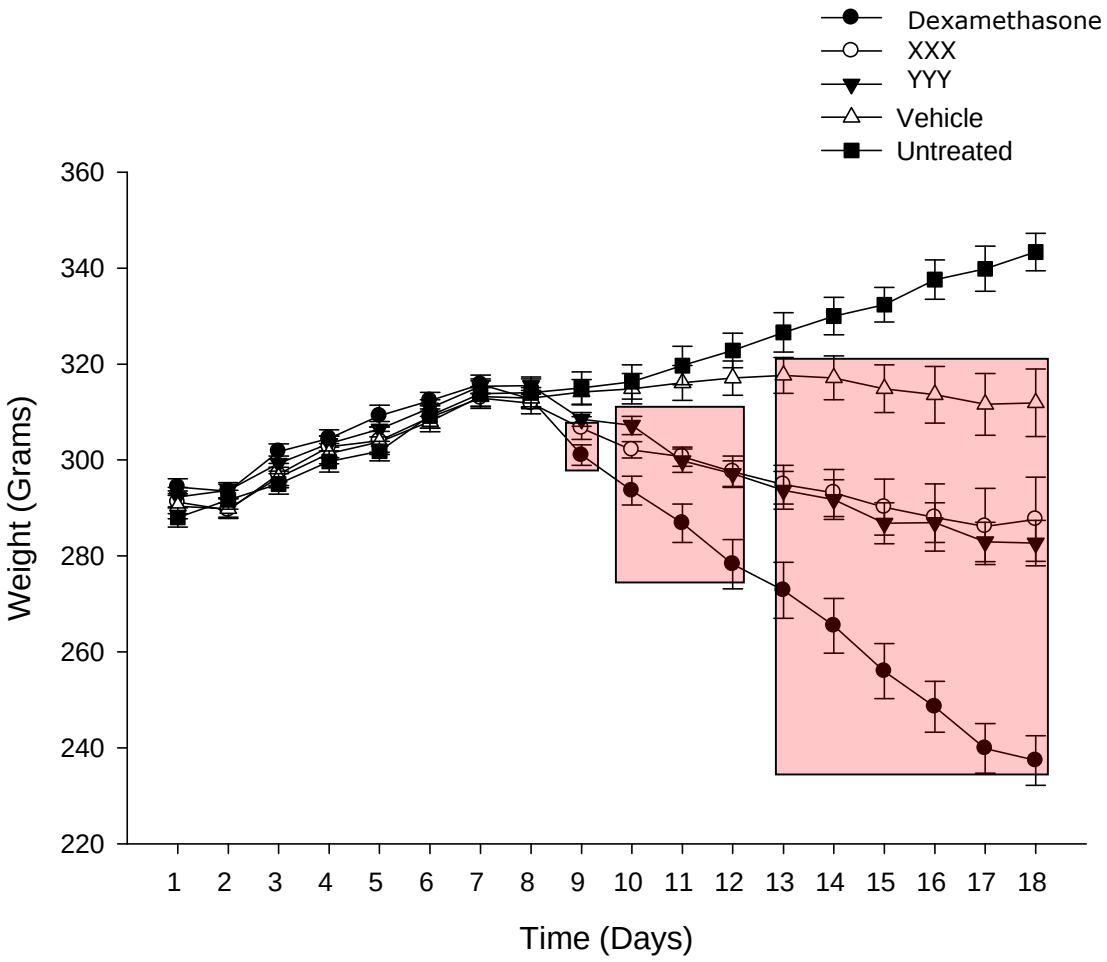

Figure 2: Mean (± SEM) weight from throughout the entire experimental protocol following administration of MBP. The shaded areas indicate group differences compared to the untreated control group.