CanCog Technologies Final Summary Study Report

Assessment of the Effectiveness of GLcNBu as a Therapeutic for Osteoarthritis in the Beagle Dog

Study Number: VRI163-18018-CE
(Full report and data on file))

A. SUMMARY

The objective of this study was to assess the effectiveness of a test compound, N-butyryl glucosamine (GlcNBu), as a therapeutic for Beagle dogs with demonstrated osteoarthritis (OA) in comparison to both carprofen and a leading glucosamine chondroitin sulphate.

Twenty-four Beagle dogs in good general health as determined by baseline veterinary examinations and CBC/biochemistry blood work were included in the study. Selected subjects had radiographic evidence of osteoarthritis in a minimum of 2 joints and presented with positive pain scores on historical pain and function questionnaire assessment records.

At baseline, prior to the start of the treatment phase, the subjects were administered a battery of tests designed to assess aspects of OA, which included the quality of life (QOL) pain and function questionnaire, a T-Maze agility test, Actical® activity monitoring, and an orthopedic evaluation. The baseline data were used to place the subjects into 6 groups of n=4 animals per group. Groups were balanced for total pain and function scores from the QOL and median latencies from T-Maze testing.

The treatment phase followed a blinded Latin Square design with three treatments and three arms, covering all possible orders of testing. Each arm consisted of a 17-day wash-in. Over the next 5 days, subjects were assessed on the quality of life questionnaire (QOL) on 3 occasions and the T-Maze on 2 occasions in an alternating fashion. For the 3 days then animals were assessed for 24-hour behavioral activity using Actical® accelerometer activity monitoring devices. On the last day of each treatment arm, all subjects were administered an orthopedic evaluation by the facility clinical veterinarian. A 10-day washout separated each arm.

Veterinary examinations and blood collection for CBC/biochemistry analysis were repeated at the end of the trial. There were no safety or tolerance issues noted on any of the products.

The results of the study revealed statistically significant treatment effects on the QOL questionnaire, which varied depending on the specific measure. While only carprofen lead to significant overall improvement on the total score, Rejensa (GlcNBu) produced statistically improved performance over baseline on the function component, as did carprofen . Performance under a leading glucosamine chondroitin sulphate did not achieve statistical significance when compared to baseline.

On the orthopedic examination, statistically significant improvement over baseline was seen under the GlcNBu treatment and marginally significant improvement was seen under carprofen There was no treatment effect under a leading glucosamine chondroitin sulphate.

Collectively, these data support the use of GlcNBu as a treatment for osteoarthritis in dogs, confirm the effectiveness of carprofen and suggest marginally significant benefits of a leading glucosamine chondroitin sulphate. The present data also demonstrate differential sensitivity to interventions between the four target variables, with the QOL measure showing the greatest sensitivity; clinical orthopedic exams being the next most sensitive measure, while neither T-Maze running speed nor home pen activity showing any obvious sensitivity.

B. STUDY TITLE

Assessment of the Effectiveness of GLcNBu as a Therapeutic for Osteoarthritis in the Beagle Dog

C. STUDY NUMBER

VRI163-18018-CE

D. OBJECTIVE

The objective of this study was to assess the effectiveness of a test compound, N-butyryl glucosamine (GlcNBu), a synthetic analogue of glucosamine, as a therapeutic for Beagle dogs with demonstrated osteoarthritis (OA) in comparison to both carprofen and a leading glucosamine chondroitin sulphate. This was a non-GLP laboratory study.

E. LOCATION OF FACILITIES

Animal Location:
Vivocore Inc. Canada

Diagnostic Laboratory:
Antech Diagnostics Canada

F. STUDY SCHEDULE

The start date for the study was Thursday, May 3, 2018 and the last date of the in-life phase was Wednesday, August 22, 2018. There were two modifications from the original protocol. First, two additional days were added to baseline for the veterinary examinations and clinical lameness assessments (Addendum #1). Second, blood collection was added on the last day of washout during treatment arm 1 and during treatment arm 2 (Addendum 2).

EXPERIMENTAL MATERIALS

1. Test Product 1

1.1. Identification	N-Butyryl-D-Glucosamine
1.2. Lot Number	Not Provided
1.3. Storage	Room temperature
1.4. Date of Expiration	Not Provided
1.5. Manufacturer	Vibrant Pharma
1.6. Dosage Form	Powder in 2 size 000 gelatin capsules
1.7. Dosage Frequency	SID
1.8. Dose Tested	60 mg/kg

2. Test Product 2

1.1. Identification	(Carprofen)
1.2. Lot Number	210427 (25 mg), 222241 (100 mg)
1.3. Storage	Room temperature
1.4. Date of Expiration	2019-06 (25 mg), 2019-11 (100 mg)
1.5. Manufacturer	Unidentified
1.6. Dosage Form	Tablet (25mg or 100 mg)
1.7. Dosage Frequency	SID
1.8. Dose Tested	4.4 mg/kg (always rounded up)

3. Test Product 3

1.1. Identification for Small-Medium Dogs	a leading glucosamine chondroitin sulphate
1.2. Lot Number	1709062S
1.3. Storage	Room temperature
1.4. Date of Expiration	2021-09
1.5. Manufacturer	Unidentified
1.6. Dosage Form	Tablet
1.7. Dosage Frequency	SID
1.8. Dose Tested	600 mg glucosamine hydrochloride 250 mg sodium chondroitin sulfate 45 mg avocado/soybean unsaponifiables

G. MATERIALS AND METHODS

1. Test System
   Twenty-four (24) Beagle dogs, obtained from the Vivocore Inc. colony, were included in the study. Ages of selected subjects ranged from 6.3 to 13.9 years at study initiation. There were 7 males and 17 females included.

2. Selection and Allocation of Animals
   Animals in good general health as determined by baseline veterinary examinations and CBC/biochemistry blood work were included in the study. Selected subjects had radiographic evidence of osteoarthritis in a minimum of 2 joints and presented with positive pain scores on historical questionnaire assessment records. Following baseline assessments, subjects were ranked in descending order according to baseline QOL total pain and function scores. Subjects were then given a second rank on median latency from T-Maze testing. A rank of 1 represented the highest total QOL score or the highest median T-Maze latency and a rank of 24 represented the lowest total QOL score or lowest median T-Maze latency. The sum of each rank was assigned to a treatment order condition as outlined in Table 3 below. This method ensured that the sum of the ranks was equal for each group to the extent possible.

   Table 3. Method of Group Assignment

Treatment Order Group	Ranking
1	1, 12
2	2, 11
3	3, 10
4	4, 9
5	5, 8
6	6, 7

3. Acclimation and Pre-Treatment of Test System
   All animals involved in this investigation were housed at the Vivocore facility for no less than six months. Therefore, no acclimation period was needed.

4. Administration of Test Articles
   All test articles were administered orally as either tablets or capsules. Each animal was dosed once daily at approximately the same time each day as outlined in the Schedule of Operations. Dosing was performed 1.5 hours prior to testing procedures (±10 minutes).

   GLcNBu was administered at 60 mg/kg as powder split between two size 000 gelatin capsules. The product was provided in bulk by the Sponsor and was prepared in capsules at the test facility based on each animal's most recent body weight measurement.

   Carprofen was ordered by the test facility from a Canadian distributor and was provided in tablet form . Administration was aimed at achieving a dose level as close as possible to 4.4 mg/kg using 25 mg and 100 mg tablets. Calculated dose amounts for carprofen were also determined using each animal's most recent body weight measurement. Doses were as

follows, with animal body weight always rounded up:

Table 4. Dosing of carprofen

Animal Weight (kg)	Dose
7 to 8.5	1 x 25mg + ½ x 25mg
8.6 to 11	½ x 100mg
11.1 to 14	½ x 100mg + ½ x 25mg
14.1 to 17	3 x 25mg

A leading glucosamine chondroitin sulphate was administered as a single tablet for small-medium dogs, containing 600 mg of glucosamine hydrochloride, 250 mg sodium chondroitin sulfate and 45 mg avocado/soybean unsaponifiables.

Additional information on each test product can be found in Section H.

Animals were observed for a minimum of 30 minutes following dosing for signs of vomiting. If vomit was noted within this time frame and an intact capsule or tablet was present, the animal was re-dosed.

5. Housing and Management of Test System

I. Housing

Dogs were kept at Vivocore's animal facility and group housed in pens in compliance with the recommendations of the Canadian Council on Animal Care. All housing areas were cleaned daily according to standard operating procedures.

II. Environmental Management

Environmental management including lighting, ventilation, temperature, and humidity regulation were maintained and controlled according to standard operating procedure. A combination of commercially acceptable fluorescent lighting and natural light was provided for the dogs. The photoperiod was approximately 12 hours (7:00 to 19:00, maintained on a timer), however may have varied due to natural fluctuations of sunlight. Temperature was electronically controlled and was set to maintain the animal housing room in a temperature range of 15°C to 28°C with a relative humidity of 30-70%. The housing room ventilation was designed to provide continual air changes.

III. Feed and Water

All animals were fed according to Vivocore standard operating procedure using a standard commercial diet to maintain body condition – Purina Pro Plan Savor Adult Dog Food, Chicken and Rice Formula. Animals were fed at the end of each day following completion of any assessment procedures. Water

was provided ad libitum. Over the course of the study, food and water consumption was not recorded.

6. Procedures and Data Recorded

I. Animal Health Observations

Animal health observations were conducted twice daily over the course of the study according to standard operating procedures.

II. Veterinary Examinations

Physical examinations were performed by the attending veterinarian on Day -13 and Day 98. The examinations included an evaluation of all body systems including coat/skin, heart, ears, mouth/nose/throat, musculoskeletal, eyes, abdomen, lungs, urogenital system, and nervous system. Examinations were performed according to standard operating procedures.

III. Body Weight Measurements

Animal body weight measurements were performed according to standard operating procedures approximately every 3 weeks. The most recent animal body weight measurement was used to accurately determine dose levels at the beginning of each treatment arm. Weight measurements were performed and scales were operated and maintained according to standard operating procedures.

IV. Blood Collections

Whole blood collections were conducted on Day -13 and Day 98 for the purpose of CBC and biochemistry analysis as well as for stored serum and plasma. At each time point, approximately 6 ml of blood was collected from a suitable vein according to standard operating procedures. Approximately 2 ml of blood was placed into a K_2EDTA tube, 1 ml into a second K_2EDTA tube and 3 ml was placed into a serum separator tube.

The K_2EDTA tube containing 1 ml of blood was centrifuged at 2800-3200 rpm for 10 minutes at 4°C. The resultant plasma was stored as a single aliquot at approximately -80°C for future analysis as required. The second K_2EDTA tube was stored at 2-8°C until sent to Antech Diagnostics for analysis.

The serum separator tube was centrifuged at 2800-3200 rpm for 10 minutes at 4°C. Approximately 0.5 ml of serum was stored as a single aliquot at approximately -80°C for future analysis as required. The remaining serum in the tube was stored at 2-8°C until sent to Antech Diagnostics for analysis.

Additionally, on Day 35 and Day 71 (the final day of each washout period), 3ml of whole blood was collected for the purpose of plasma storage. Blood was transferred to one K_2EDTA tube and inverted gently. Samples were spun

at 2800-3200 rpm for 10 minutes at 4˚C. Plasma was equally divided into two aliquots and stored at approximately -80˚C for future analysis as required.

CBC/biochemistry data can be found in Appendix 3.

V. Quality of Life (QOL) Questionnaire Assessment

The QOL questionnaire is a laboratory-based adaptation of the validated canine brief pain inventory (CBPI) questionnaire, which is a clinical questionnaire used to evaluate pain and function level based on pet owner responses. Modifications to the questionnaire were designed to account for differences between owner pain evaluation of pets and pain evaluation of laboratory dogs by technical staff.

Specifically, the functional ability of each dog to walk, trot, gallop, rear, jump over a low obstacle, climb and descend stairs, jump down from a perch and general activity was evaluated in parallel to subjective evaluation of pain observed during each behaviour. Assessments were performed according to standard operating procedures by the same technician over the course of the study.

The QOL was performed three times during baseline as well as three times during each treatment arm as outlined in the Schedule of Operations. Testing was performed 1.5 hours (±10 minutes) following dosing for all treatment types. Baseline test times were also matched to the treatment phase. Each assessment was video recorded for scoring purposes.

T-Maze Testing

The T-Maze consists of a large apparatus containing a start box, alleyway and two arms leading to a right and left runway. Entrance into either of the arms led to a food reward after the subject passed over a staircase and hoop obstacle. The dependent variable was running speed which was measured by keypress. Subjects were given 60 seconds to traverse the maze, at which point a non-response was recorded.

T-Maze testing was performed twice during baseline as well as twice during each treatment arm as outlined in the Schedule of Operations. Testing was performed 1.5 hours (±10 minutes) following dosing for all treatment types. Baseline test times were also matched to the treatment phase.

VI. Actical® Activity Monitoring

Actical® activity monitoring devices were placed on the animals' collars for 3 days of each treatment arm as well as for 3 days during baseline as outlined in the Schedule of Operations. The devices provided a continuous record of activity including total day time spent active, night time activity, percent time spent sedentary, percent time in light activity and percent time spent in medium activity.

VII. Orthopedic Evaluations

Orthopedic evaluations were carried out by Dr. Shawn Petrik, the Vivocore facility clinical veterinarian at four different timepoints. The variables assessed included: gait analysis and lameness scoring on a scale of 0-5 (with 0=clinically sound and 5=complete non-weight bearing lameness); neurologic examination (including conscious proprioception; cranial nerve evaluation; neck movement and spinal palpation); long bone and muscle palpation; and collateral ligament and joint assessment (including flexion/extension, swelling, crepitus, effusion and pain).

Methods for hind limb palpation:
Flexion and extension of all digits together were performed to assess if a distal limb problem was present, in which case further evaluation of each digit was performed to evaluate for potential collateral ligament injuries or other pathology. The nails, webbing and pads were examined for any injuries, foreign bodies or masses. The sesamoid bones were palpated carefully for swelling and pain. Because there are long and short collateral ligaments in the tarsus, assessment of these was performed by flexion and extension. The insertion of the common calcanean tendon was evaluated carefully for any swelling or pain. The stifle was evaluated for patellar luxation, medial buttress, pain on hyperextension (early sign of cruciate disease), drawer/thrust and a meniscal click. The tibial and femoral diaphysis were carefully palpated for any signs of pain to assess for bone tumors or other pathology. Cruciate disease was ruled out by palpation of the stifle and evaluation of abduction of the hip joint.

Forelimb palpation:
Digital palpation was performed as for the hind limb. The carpus was carefully evaluated for joint effusion. Palpation of the distal radius and proximal humerus was performed. The elbow was evaluated for elbow dysplasia by hyperextending the joint and medial compartment palpation. Shoulder range of motion was evaluated. Abduction of the shoulder was estimated during stance. The biceps tendon was palpated just medial to the greater tubercle. The supraspinatus insertion tendon was palpated at its attachment to the greater tubercle.

One orthopedic evaluation from each treatment arm (including baseline) was videotaped for future reference.

H. DISCUSSION AND CONCLUSIONS

The present study at looked baseline data and at the effects of three different treatments (Carprofen, GlcNBu, and a leading glucosamine chondroitin sulphate) on four different measures of osteoarthritis (OA) in Beagle dogs. In this study, carprofen, a drug widely prescribed to treat OA, served as a positive control. The leading glucosamine chondroitin sulphate is an OA treatment derived from natural products that is commercially available and also widely used. The third treatment, GlcNBu, was the test compound and is a synthetic analogue of glucosamine, that is hypothesized to provide benefits over an above those provided by glucosamine.

The results supported the following conclusions:

1. Carprofen was the only compound that lead to improved performance on the complete quality of life (QOL) test.
2. On the function subtest of the QOL test, treatment with both carprofen and GlcNBu lead to improved performance over baseline.
3. None of the three treatments produced statistically significant improvement on the pain subtest of the QOL test.
4. GlcNBu was the only compound to produce improved scoring on the orthopedic assessment exams.
5. None of the treatments significantly impacted speed of running the T-Maze or normal behavioral activity in the animal's home pens as determined by Actical® activity monitoring.

There were no unforeseen circumstances that affected the quality or integrity of the data.