Approval Date: May 31, 1988

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

NADA 140-806

B. Sponsor

Fort Dodge Laboratories

C. Proprietary Name

Hyalovet®

D. Established Name

Hyaluronate sodium

E. Dosage Form

Hyalovet (hyaluronate sodium) is a clear, colorless, viscous solution of hyaluronic acid for intra-articular injection.

F. How Supplied

2 mL glass syringes or 2 mL glass vials.

G. Dispensing Status

Rx

H. Dosage Regimen

The recommended dose of Hyalovet is 2 mL (20 mg hyaluronate sodium) in small or medium sized joints (carpus, fetlock) given by intra-articular injection. More than one joint may be treated at the same time. If necessary, the injection may be repeated after one or more weeks but not to exceed two injections per week for a total of four weeks.

Hyalovet (hyaluronate sodium) should be injected using strict aseptic technique. Excess synovial fluid should be removed prior to injection. For best results horses should be given two days of rest or limited exercise before resuming normal training.

I. Indication

Hyalovet (hyaluronate sodium) is indicated for the intra-articular treatment of carpal or fetlock joint dysfunction in horses due to acute or chronic, non-infectious synovitis associated with equine osteoarthritis.

II. EFFECTIVENESS

A. Pivotal dosage-response study in adjuvant arthritis in adjuvant horses.

1. Pharmacological dose titration study, placebo control.

2. Investigator

   Dr. Doyne Hamm
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   Route # 7
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3. General Design

   a. Purpose

      To describe the dosage-response relationship of Hyalovet in horses with arthritis as a means of determining a suitable clinical dosage.

   b. Test Animals

      24 previously healthy, mature horses of the quarter horse type, of both sexes, ranging in age from two to six years, randomly divided into four groups, six animals per group. After initial acclimation to the study environment and initial clinical and pharmacologic evaluation, the left carpal joint was surgically prepared and injected with 0.7 mL Freund's complete adjuvant into the intercarpal space. As previously shown, the above procedure results in acute inflammation of each injected joint (synovitis) which, if left untreated, progresses to chronic degenerative arthritis. The 24 horses thus prepared represented the study population.

   c. Diagnosis

      At the time of treatment (five days after injection of Freund's adjuvant) was acute synovitis as confirmed by lameness, heat, swelling, synovial fluid analysis, measurement of stride length, measurement of flexion of affected joints, and x-rays.

   d. Dosage Form

      Injectable 10 mg hyaluronate sodium (HA) per mL solution in 2 mL glass syringes, identical to the preparation to be marketed.

   e. Controls

      Controls received injections of sterile physiologic saline as a placebo.

   f. Dosage Groups

      Dosage groups included 0 (saline placebo), 5, 20, or 40 mg hyaluronate sodium (HA) per joint given as a single intra-articular injection five days after induction of arthritis.

   g. Test Duration

      Four weeks following treatment.

   h. Pertinent Parameters Measured

Pertinent parameters measured, included angle of joint flexion at rest, maximum flexion permitted, joint circumference, volume of joint, stride length (distance between sequential steps taken with affected limb), and synovial fluid analysis. Additionally, lameness, heat, and pain were evaluated subjectively using a scoring system.

4. Results of dosage response studies

Clinically, all horses developed swelling, heat, pain, and lameness on the affected limbs within five days following arthritis induction. After treatment, horses of the 20 mg and 40 mg dosage group showed marked improvement within one week of treatment and continued to improve throughout the four week follow-up period. Horses of the placebo and 5 mg sodium hyaluronate groups showed much less improvement during this time, as summarized in the following table:

Table 1: Results of Dosage Response Study

Parameter*	Dosage Group	Clinical Scores (mean, n=6)					
		Pre Arthritis	Pre Treatment	1 Week	2 Weeks	3 Weeks	4 Weeks
Lameness (0-4)	Placebo	0	3.0	2.8	2.8	2.7	2.0
	5 mg HA	0	3.0	2.8	2.8	2.2	2.2
	20 mg HA	0	3.0	1.3	0.8	0	0
	40 mg HA	0	3.0	1.2	0.8	0.2	0.2
Joint Pain (0-3)	Placebo	0	2.7	2.5	2.7	2.8	2.0
	5 mg HA	0	2.7	2.7	2.7	2.5	2.2
	20 mg HA	0	2.7	1.7	0.8	0.2	0
	40 mg HA	0	2.7	1.7	1.0	0.5	0
Joint Heat (0-3)	Placebo	0	2.7	2.5	2.7	2.8	2.2
	5 mg HA	0	2.7	2.7	2.7	2.7	2.0
	20 mg HA	0	2.7	1.7	1.0	0.5	0.3
	40 mg HA	0	2.7	1.7	1.0	0.7	0.2

*Lameness: 0=Normal, 1=Slight head bob, 2= Moderate head bob, 3= Markedly Lame, 4 = Non Weight Bearing
Joint Pain and Heat: 0=Normal to 3=Severe

Carpal joint measurements followed the same patterns as noted clinically, with the 20 mg and 40 mg dosage groups showing improvement in all parameters while 5 mg and placebo control groups showed little change:

Table 2: Carpal Joint Measurements

Parameter*	Dosage Group	Carpal Measurements (mean, n=6)					
		Pre Arthritis	Pre Treatment	1 Week	2 Weeks	3 Weeks	4 Weeks
Joint Circumference (Inches)	Placebo	11.3	13.1	13.5	13.9	14.2	14.1
	5 mg HA	11.7	13.4	13.7	14.4	14.3	14.2
	20 mg HA	11.1	12.9	12.8	12.8	12.4	12.4
	40 mg HA	11.7	13.1	13.4	13.2	13.0	12.6
Flexion at Rest (Degrees)	Placebo	0	12	9	8	8	7
	5 mg HA	0	12	8	9	8	7
	20 mg HA	0	10	0	0	0	0
	40 mg HA	0	10	2	0	0	0
Maximum Flexion Allowed (Degrees)	Placebo	117.0	51	56	46	55	59
	5 mg HA	117.0	53	57	50	53	54
	20 mg HA	117.0	52	71	79	90	98
	40 mg HA	117.0	54	74	82	88	96
Stride Length After Rest (Inches)	Placebo	62.5	47	48	44	50	51
	5 mg HA	63.8	47	52	48	51	50
	20 mg HA	62	48	57	60	62	62
	40 mg HA	63.4	48	57	60	61	62
Stride Length, Exercise (Inches)	Placebo	63.2	46	47	42	46	48
	5 mg HA	63.8	45	50	46	49	48
	20 mg HA	62.4	45	58	61	63	63
	40 mg HA	63.7	45	58	61	62	64

Synovial fluid analysis, performed on samples collected at the same times as other parameters, was unrewarding in terms of separating dosage effects. Synovial fluid viscosity, mucin quality, and hyaluronic acid concentration were highly variable before and after treatment. Following arthritis induction, synovial fluid protein and leucocytes increased markedly, then gradually diminished throughout the observation period. Synovial fluid protein was lower in 20 mg and 40 mg Hyalovet groups than in other groups at 21 days and beyond.

Carpal volume was determined by making a mold of each joint before treatment and after four weeks. Horses treated with 20 or 40 mg HA had post-treatment

carpal volume similar to pre-treatment values whereas carpal volumes in 5 mg or placebo treated horses had much larger carpal volumes.

5. Statistical analysis of dosage response data

   All data were subjected to analysis of variance and linear plateau models. The 20 and 40 mg sodium hyaluronate treatments were found to be statistically ($p<0.05$) different from 5 mg and placebo groups, but not different form each other with regard to :

   - maximum flexion permitted
   - lameness
   - joint circumference
   - synovial fluid protei

6. Conclusions

   The results of this study indicated that a single intra-articular injection of Hyalovet at a dosage of 20 mg/joint is effective in alleviating signs of adjuvant arthritis in horses. The 5 mg dosage of Hyalovet and also placebo were ineffective while the 40 mg dose was no more effective than 20 mg.

7. Adverse Reactions

   No adverse reactions were reported in this study.

B. Pivotal well-controlled clinical study

1. Title

   Controlled clinical evaluation of Hyaluronate Sodium in the Horse (double blind evaluation of Hyalovet using an approved active drug control).

2. Investigators

   Doyne Hamm, DVM, Principal Investigator
   Gary White, DVM, Treating Veterinarian
   Donald Dowdle, DVM, Treating Veterinarian

   Research for Animal Health
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3. General Design

   This was a double blind evaluation of the clinical safety and effectiveness of intraarticular injection of Hyalovet in lame horses.

   The study was blinded by providing the test and control articles and a random treatment schedule to licensed veterinarians other than the principal investigator who were designated the treating veterinarians. The treating veterinarians' responsibility was to administer the appropriate drug according to the random treatment schedule without informing the principal investigator of which substance was administered.

a. Purpose

The purpose of this study was to determine the safety and effectiveness of Hyalovet as an intraarticular injection of hyaluronate sodium (HA) at the proposed dose of 20 mg HA for the treatment of arthritis in horses as the target species, under conditions of veterinary practice.

b. Test Animals

Test animals included adult horses of either sex and of any age weight or breed that were presented to the veterinarian for treatment of lameness conditions that met the following criteria:

- clinical evidence of lameness of grade 2 or greater (on a 0 - 4 scale)
- origin of lameness shown to be within a specific carpal joint space
- pretreatment synovial fluid viscosity <10 centistokes or protein >1g/dL

Exclusion criteria were as follows:

- horses that had received intraarticular therapy within previous 2 weeks
- horses that had received nonsteroidal antiinflammatory drugs within 1 week
- cases that were unlikely to respond due to extensive nature of articular damage, severe fracture within the joint, avulsion of ligmentous attachments, osteochondritis dessicans, etc.
- septic arthropathies

c. Type of Control

Type of control was an active drug control. This was a double blind evaluation of the clinical safety and effectiveness of intraarticular injection of Hyalovet in lame horses as compared with HYVISC (Norden) as an approved reference control drug.

d. Dosage Forms.

The investigational drug Hyalovet was supplied as a sterile solution of 10 mg HA/mL in glass vials each containing 2 mL, identical to that to be marketed. The control substance, Hyvisc (Norden), was supplied in sterile syringes, each containing 2 mL of a 10 mg HA/mL solution, as currently marketed. Hyalovet was stored at room temperature as specified in the proposed labeling while the control HA was stored at room temperature in accordance with its approved labeling.

e. Dosages, Route of administration, and Duration

2 mL of either Hyalovet or control HA given by intraarticular injection, repeated after 7 days, with final evaluation 7 days after the last injection. Thus the duration of the test was 14 days in each case.

f. Pertinent Parameters Measured.

The investigator, after carefully examining each candidate horse to determine its eligibility to enter the study and which joint was causing the lameness, evaluated the horse according to the following criteria:

Lameness at a jog
0 = no detectable lameness
1 = subtile lameness without overt head movement, detectable by an expert
2 = easily recognizable, head-bobbing lameness
3 = definite lameness observed at a walk as well as a trot
4 = non weight-bearing on affected limb

Joint heat
0 = none
1 = slight
2 = moderate
3 = marked

Joint circumference measured with a tape measure at a standardized point

Pain on palpation and pain on flexion (evaluated separately)
0 = no response to firm pressure, permits full flexion
1 = digital pressure at site of lesion, or flexion of joint, induces muscle tremors and/or slight avoidance movement
2 = digital pressure at site of lesion, or flexion of joint, induces definite limb withdrawal
3 = light digital pressure, or attempted flexion, induces marked withdrawal

The horse was then transferred to the care of the treating veterinarian who obtained a synovial fluid sample from the affected joint space and administered the appropriate test substance. Samples were mailed to Specialized Assays, Nashville, Tennessee, for determination of viscosity, protein, and leucocyte counts.

Study animals were observed at least 3 times per week by the investigator or his colleagues and any side effects or other noteworthy findings were recorded. All study horses were reevaluated according to the above criteria, including synovianalysis, at 1 week and again at 2 weeks following the initial treatment.

At the time of the last post treatment examination, the investigator evaluated the overall effects of treatment according to the following criteria:

- Excellent no detectable lameness, horse can return to normal work
- Good marked reduction in lameness but horse not completely sound
- Fair only slight reduction in lameness over original condition
- Poor no improvement or horse got worse

4. Results of Pivotal Clinical Study

Case reports on a total of 36 horses were received comprised of 22 quarterhorses, 13 thoroughbreds, and 1 paint and included 12 mares, 4 stallions, and 20 geldings with a body weight range of 975 - 1075 pounds. Ages ranged from 2 to 14 years with the majority in the younger age range. All were listed as race horses, one as a barrel racer. the most frequent diagnosis was carpitis, characterized by

radiographic findings typically of osteophytes, spur formation, and occasionally cartilage erosion, in the left or right radiocarpal or intercarpal joint spaces. All horses met the protocol criteria regarding no previous therapy for the condition within the previous 2 weeks.

Clinically, the horses typically presented with grade 2 or 3 lameness, grade 1 or 2 pain on flexion scores, grade 2 or 3 pain on palpation scores and grade 2 or 3 joint heat scores, and with slight swelling of the joints (mean circumference 12.7 inches), low relative viscosity of synovial fluid of around 3, normal synovial fluid protein, and variable but generally low synovial fluid leucocyte counts. There was no apparent clinical difference between the treatment groups prior to treatment.

a. Clinical scores response.

The horses in both study groups showed a marked improvement in clinical scores for lameness, pain on flexion, pain on palpation, and joint heat within 7 days following the initial injection and continued to show improvement for the 7 days following the second injection. The following table summarizes these results:

Table 3: Mean Clinical Score

Parameter	Test Drug	Pre R_x	Day 7	Day 14	Net Change*
Lameness (0-4)	HYALOVET	2.61	0.94	0.39	-2.22
	Control HA	2.56	0.83	0.39	-2.17
Pain on Flexion (0-3)	HYALOVET	1.94	0.67	0.11	-1.83
	Control HA	1.83	0.56	0.28	-1.55
Pain on Palpation (0-3)	HYALOVET	2.67	0.94	0.39	-2.28
	Control HA	2.39	0.89	0.33	-2.06
Joint Heat (0-3)	HYALOVET	2.28	0.94	0.44	-1.84
	Control HA	2.33	1.17	0.61	-1.72

*Day 14 value minus pretreatment value

b. Joint swelling response.

In addition to the marked reduction in clinical scores noted above, there was also a slight but measurable decrease in joint circumference following intraarticular injection with both Hyalovet or control HA.

c. Synovial analysis response.

All of the horses in both treatment groups met the entry criteria of either synovial fluid viscosity of <10 centistokes or synovial fluid protein >1 g/dL.

Surprisingly, synovial fluid viscosity decreased slightly but rather consistently following intraarticular injection of either Hyalovet or control HA, instead of increasing as previously supposed. Protein and WBC responses were variable but unremarkable.

Table 4: Mean Synovianalysis Values

Parameter	Test Drug	Pre R_x	Day 7	Day 14	Net Change*
Viscosity (ratio)	HYALOVET	2.75	2.45	2.15	-0.60
	Control HA	3.05	2.46	2.41	-0.64
Protein (g/dL)	HYALOVET	1.93	1.83	1.93	0.00
	Control HA	1.77	1.82	1.62	-0.15
Leucocytes (per cmm)	HYALOVET	901.72	824.72	1628.67	726.95
	Control HA	731.50	1503.72	642.00	-89.50

*Day 14 value minus pretreatment value

d. Exercise and Training

Exercise and training consisted of rest in most cases at the time of either HYALOVET or control HA treatment. The following table shows the activity of the horses at each time period by treatment group:

Table 5: Activity of Horses at Each Time Period by Treatment Group

	Pre Treatment		Day 7		Day 14	
Activity Level	Hyalovet	Control	Hyalovet	Control	Hyalovet	Control
Rest or handwalk	17	16	7	5	5	3
Light training	1	2	11	13	7	14
Heavy Training	0	0	0	0	2	1
Racing	0	0	0	0	4	0

From the above data it can be seen that most horses in both groups tended to resume light training within one week after the initial injection, but that a greater proportion of Hyalovet treated horses had returned to racing and heavy training at 14 days than the corresponding control horses, most of which were still in light training.

e. Overall Clinical Response

Overall clinical response was listed as "good" or "excellent" in 17 of 18 (94%) of the Hyalovet treated horses and in all 18 of the control HA treated horses. One horse in the Hyalovet group, the only horse diagnosed as "chronic" carpitis, was given a rating of "fair".

5. Conclusions Drawn from the Study

Intraarticular injection of 20 mg HA as Hyalovet in horses with lameness due to carpitis resulted in marked reduction in lameness and joint heat and pain, and reduction in joint swelling. The response to the approved reference control HA injection was virtually identical in magnitude and direction and was not different from the response to Hyalovet. No side effects were encountered in either group.

It can thus be concluded that Hyalovet is safe and effective for the intraarticular treatment of joint dysfunction in horses due to synovitis associated with equine osteoarthritis.

6. Adverse Reactions

No adverse reactions were reported in either group.

C. Corroborative well-controlled study on cartilage healing

1. Pharmacologic target species model study, placebo control.

2. Investigator

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3. General Design

a. Purpose

Purpose of the study was to determine the effects of a single intra-articular injection of 20 mg Hyalovet (the previously titrated dose) in chronic, experimentally induced osteoarthritis in horses.

b. Test Animals

Test animals were 12 previously healthy mature horses of various breeds and of both sexes. After a suitable acclimatization period, under general anesthesia, one carpal joint of each horse was surgically opened and a small slab fracture of the third carpal bone was created according to surgical techniques previously worked out by the surgeon. The joint fractures thus created are typical of those occurring naturally in race horses, do not in themselves cause appreciable lameness, but do result in sufficient joint instability as to predispose the animal to the development of osteoarthritis with continued use of the joint.

After recovery from surgery the horses were given a paddock exercise daily for a minimum of 12 weeks, during which time the conditions stabilized, with lameness ranging from moderate to almost imperceptible. At this point, the 12 horses were randomly assigned to two groups and the groups randomly assigned to hyaluronic acid treatment or saline as a placebo control. Thus the study population consisted of 12 horses with chronic osteoarthritis secondary to carpal fracture, 6 hyaluronic acid treatment horses and 6 saline control horses.

c. Diagnosis

Diagnosis of chronic osteoarthritis was confirmed by radiographic demonstration of third carpal bone fractures and radiographic lesions consistent with chronic osteoarthritis.

d. Dosage Form, Doasge, Route

Dosage form, dosage, and route was injectable 10 mg sodium hyaluronate per mL solution in preloaded 2 mL glass syringes, given as a single intra-

articular injection into the affected joint, at a dosage of 2 mL per joint (the previously titrated dosage). The preparation used was identical to that to be marketed (Hyalovet).

e. Controls

Controls received a single intra-articular injection of sterile saline as a placebo.

f. Test Duration

Test duration was a six week observation and measurement period following Hyalovet treatment or saline control injection.

g. Pertinent Parameters Measured

Pertinent parameters measured included clinical lameness exams on a standardized 0-5 scale, joint circumference measured with a tape measure, range of flexion measured with a protractor, radiographic evaluation, synovial fluid analysis, and determination of uptake of bone seeking radiopharmaceutical. Radiopharmaceutical uptake provides a means by which relative metabolic activity comparisons in subchondral bone can be made. Uptake increases in osteoarthritis but decreases as cartilage healing occurs.

4. Results of chronic osteoarthritis model studies

Clinically, the arthritic conditions of all horses had stabilized by the time treatment was instituted. There was variable but generally mild lameness, no appreciable swelling, nearly full range of flexion, and stable radiographic and synovianalysis findings in the horses. Consequently there were no striking changes in the above parameters following treatment nor were there detectable differences between Hyalovet treated and saline control groups.

Radiopharmaceutical uptake data, however, revealed a consistent decrease in uptake in Hyalovet treated joints as compared to saline controls as presented in the following table:

Table 6: HA-4, Results of Radiopharmaceutical Uptake Measurements in HYALOVET Treated Joints Compared to Controls

Dosage Group	Pretreatment[a]	Change from Pre Treatment (mean, n=6)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
HA	5.81 ± 0.64	-0.43	-1.00	-1.55	-1.21	-1.15	-0.64
Saline Control	4.65 ± 0.42	0.66	0.54	0.66	0.30	0.42	-0.12

[a] Counts per minute in lesion area/counts per minute in neutral area (mean ± standard error of mean)

5. Conclusions of chronic osteoarthritis model studies

The results of this study revealed that a single intraarticular injection of 20 mg Hyalovet results in significant reduction in subchondral bone activity, a change consistent with cartilage healing. The effectiveness of the previously titrated

dosage of 20 mg per joint was therefore confirmed in chronic osteoarthritis of the carpal joint.

6. Adverse reactions

   Side effects were limited to a mild, transient inflammatory flare observed in two horses following HA injection, which subsided spontaneously within 24 hours.

D. Pivotal Well-Controlled Clinical Studies

1. Title

   Clinical Evaluation of Hyaluronic Acid Horses (well-controlled clinical study using an approved active drug control).

2. Investigators

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   4721 Huntington Blvd
   Hoffman Estates, Il 60195

   Jerry Johnson, DVM, MS
   4400 Briar Hill Road
   Lexington, KY 40511

3. General Design

   a. Purpose

      Purpose of the study was to determine the safety and effectiveness of intra-articular injection of Hyalovet at the proposed dosage of 20 mg per joint for the treatment of arthroses in horses, under conditions of veterinary practice.

   b. Test Animals

      Test animals were horses of either sex and of any age, weight, or breed that were presented for evaluation and treatment of lameness due to joint problems for which the attending veterinarian felt that hyaluronic acid might be indicated. The criterion for placing horses in the study was lameness, the origin of which was shown to be within a specific carpal (knee) or fetlock (ankle) joint. Equally importantly, the exclusion criteria included (1) horses which had received intra-articular therapy within the previous two weeks, (2) horses that had extensive articular damage, (3) horses with avulsion of ligamentous attachments, (4) severe fracture within the joint, (5) osteochondritis dessicans (separation of joint cartilage) and (6) infectious arthritis.

      Once selected for the study, horses were assigned to the treatment or control group by use of a randomized treatment schedule.

   c. Diagnosis

      Diagnosis of conditions suitable for purpose of the study was based on lameness examination, physical examination of the joint, and x-ray evaluation.

d. Dosage Form, Dosage, and Route

Dosage form, dosage, and route was injectable 10 mg sodium hyaluronate per mL solution in preloaded 2 mL glass syringes, given by intra- articular injection into the affected joint a dosage of 2 mL (20 mg) per joint. The hyaluronic acid preparation used was identical to that to be marketed. The investigators were allowed to repeat the injection after 14 days if they felt it was indicated.

e. Controls

Controls were given a similar 2 mL intra-articular injection(s) of an approved sodium hyaluronate product, Hylartin-V.

f. Test Duration

Test duration was 14 to 28 days, depending on whether one or two injections were given.

g. Pertinent Parameters Measured

Pertinent parameters measured, before and after treatment, were as follows:

Lameness (at a trot) was scored on a 0-4 basis where 0=normal, 1=slight, 2=moderate, 3=severe, and 4=non weight-bearing.

Pain on palpation, pain on flexion, and heat were scored on a 0-3 basis where 0=normal to 3=severe.

Angle of maximum flexion permitted, measured with a protractor.

Joint circumference of both the affected joint and the same joint on the opposite side (contralateral), measured with a tape measure.

Synovial fluid (joint fluid) analysis, which included measurements of viscosity, hyaluronic acid concentration, and protein.

4. Results of clinical studies

A total of 75 case reports were received, of which 70 met all protocol criteria and were judged to be acceptable for evaluation. These included 34 horses treated with the Hyalovet preparation and 36 with Hylartin-V, the approved reference sodium hyaluronate as a positive control.

Clinically the most striking effects of both the Hyalovet preparation as well as the approved reference control drug included marked reduction in lameness, joint pain, and joint heat as summarized in the following table:

Table 7: Mean Clinical Score

Parameter[a]	Drug	Pre Rx	Day 0	Day 7	Day 14	Net Change[b]	Improved/Total (%)
Lameness (0-4)	HYALOVET (n=33)	1.7	N/A	0.8	0.4	-1.3	30/33 (91%)
	Control HA (n=34)	1.8	N/A	0.8	0.4	-1.4	32/34 (94%)
Pain on Palpation (0-3)	HYALOVET (n=23)	1.6	1.2	0.3	0.2	-1.4	21/23 (91%)
	Control HA (n=25)	1.6	1.0	0.6	0.4	-1.2	22/25 (88%)
Pain on Flexion (0-3)	HYALOVET (n=29)	2.0	1.7	1.1	0.5	-1.5	28/29 (97%)
	Control HA (n=35)	2.0	1.4	0.7	0.4	-1.6	35/35 (100%)
Joint Heat (0-3)	HYALOVET (n=25)	1.5	1.3	0.4	0.1	-1.4	25/25 (100%)
	Control HA (n=22)	1.7	1.3	0.5	0.4	-1.3	22/22 (100%)

[a] Lameness: 0= Normal to 4 = Severe, non-weight bearing; Pain and Heat: 0 = Normal to 3 = Severe
[b] 14 day value minus pretreatment value

Measurable decreases in joint circumference were found on the treated side in 56% of the Hyalovet treated horses and in 31% of control HA treated horses while contralateral joints usually showed no measurable change:

Table 8: Number of Joints with Decreased Circumference/Total (%)

	HYALOVET		CONTROL HA	
Joint	Treatment	Contralateral	Treatment	Contralateral
Carpus	13/22 (59%)	2/22 (9%)	5/22 (23%)	3/22 (14%)
Fetlock	6/12 (50%)	0/12 (0%)	6/14 (43%)	1/14 (7%)
Combined	19/34 (56%)	2/34 (6%)	11/36 (31%)	4/36 (11%)

Regarding angle of maximal flexion, there were no consistent changes in either direction, nor were there apparent differences in responses between Hyalovet treated horses and approved sodium hyaluronate controls.

Synovianalysis results revealed that approximately half the horses in both Hyalovet and control sodium hyaluronate groups could be classified as having acute synovitis, characterized by low synovial fluid viscosity prior to treatment. Comparison of synovianalysis results before and after treatment are summarized below:

Table 9: Mean ± Standard Error of Mean

	Viscosity (dynes/sec)		HA Concentration (mg/mL)	
	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx
HYALOVET (n=10)	5.06 ± 0.50	12.89 ± 2.36	2.40 ± 0.59	4.23 ± 0.78
Control HA (n-9)	5.80 ± 0.60	10.34 ± 2.17	1.98 ± 0.15	3.36 ± 0.63

An overall satisfactory clinical rating (good or excellent) was given in 29 of 34 (85%) Hyalovet cases compared with 30 of 36 (83%) in the positive control group. The overall clinical ratings were broken down as follows:

Table 10: Overall Clinical Ratings

Rating	HYALOVET (n=34)	Control HA (n=36)
Excellent	17 (50.0%)	19 (52.8%)
Good	12 (35.3%)	11 (30.6%)
Fair	5 (14.7%)	3 (8.3%)
Poor	0 (-)	3 (8.3%)

5. Conclusions drawn from clinical studies

   Hyalovet injected at a dose of 20 mg into the arthritic joints of 34 horses resulted in marked reduction in clinical lameness, pain on palpation, and pain on flexion in over 90% of the cases. The response to control sodium hyaluronate in 36 horses was similar in direction and magnitude. an overall favorable response was given in 85% of the Hyalovet cases compared with 84% of the control sodium hyaluronate cases. In acute synovitis cases in both groups, increases in synovial fluid viscosity and hyaluronic acid concentration were detected following treatment.

   These results support the conclusions obtained from earlier study results that the 20 mg/joint minimum effective dosage is effective in treatment of acute and chronic arthritis in the carpal and fetlock joints in horses, under conditions of veterinary practice.

6. Adverse Reactions

   In the Hyalovet group, no adverse reactions of any kind were reported. In the control sodium hyaluronate group one horse developed joint swelling following injection which persisted for five days.

E. Corroborative Open Clinical Study

1. Title

   Clinical Evaluation of Hyaluronic Acid in Horses (non-controlled clinical study)

2. Investigators

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   Lawrence Shaffer, DVM
   P.O. Box 688
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3. General Design

   This was an open clinical evaluation of Hyalovet in horses, the purpose of which was to obtain additional clinical experience with the drug in a larger population of horses. The study followed the same general protocol and evaluation criteria as in

the pivotal well-controlled clinical studies described above except that no control HA group was included.

Test animals included 62 horses of either sex, actively engaged in training or racing, that were presented for diagnosis and treatment of lameness due to dysfunction in the carpal (37) or fetlock (25) joints.

Dosage form, route, dosage, and duration were Hyalovet as a 10 mg/mL solution of hyaluronate sodium in 2 mL glass syringes, identical to the preparation to be marketed, given by intraarticular injection, at a dosage of 2 mL/joint (20 mg hyaluronate sodium). The duration of the study, including the evaluation period, was 14 days if only 1 injection was given and 28 days if 2 injections were given. (A second injections was given 2 weeks after the first in 19 carpal and 14 fetlock joints).

Pertinent parameters measured included evaluation, before and after treatment, of lameness, joint heat, and pain, according to the following clinical scoring system:

Lameness at a jog:
- 0=no detectable lameness
- 1=subtle lameness without overt head movement, detectable by an expert
- 2=easily recognizable, head-bobbing lameness
- 3=definite lameness observed at a walk as well as a trot
- 4=non weight-bearing on affected limb

Joint heat
- 0=none
- 1=slight
- 2=moderate
- 3=marked

Pain on palpation and pain on flexion (evaluated separately)
- 0=no response to firm pressure, permits full flexion
- 1=digital pressure at site of lesion, or flexion of joint, induces muscle tremors and/or slight avoidance movement
- 2=digital pressure at site of lesion, or flexion of joint, induces definite limb withdrawal
- 3=light digital pressure, or attempted flexion, induced marked withdrawal

Additionally, joint swelling was assessed by measuring the circumference of the affected joint as well as the contralateral joint with a tape measure. Synovial fluid samples were obtained before and 14 days after treatment for determination of viscosity, protein, and hyaluronic acid concentration. Finally, the level of activity (rest, training, racing, etc.) was monitored and, at the time of the last post treatment examination, the investigator evaluated the overall effects of treatment according to the following criteria:

- Excellent no detectable lameness, horse can return to normal work
- Good marked reduction in lameness but horse not completely sound
- Fair only slight reduction in lameness over original conditions
- Poor no improvement or horse got worse

4. Results of Corroborative Open Clinical Study

   a. Clinical response.

   Marked improvement in clinical scores was observed following Hyalovet treatment as summarized in the following table:

   Table 11: Mean Clinical Score

Parameter	Pre Rx	Day 3	Day 7	Day 14	Day 28	# Improved/ Total (%)
Lameness (0-4)	1.7	N/A	1.0	0.5	0.3	62/62 (100%)
Pain/Palpation (0-3)	0.5	0.5	0.2	0.1	0.0	20/20 (100%)
Pain/Flexion (0-3)	1.9	1.8	1.3	0.8	0.5	52/57 (91%)
Joint Heat (0-3)	0.8	0.6	0.3	0.1	0.1	31/34 (91%)

   Essentially no changes were observed in joint circumference measurements of either the Hyalovet treated or contralateral joints. Likewise there were no measurable changes in the angle of joint flexion following treatment.

   Of the 58 horses for which level of activity was reported, 54 (93%) were training or racing at the time of the final evaluation. The response to Hyalovet treatment was judged as "good" or "excellent" in 56 of the 62 cases (90%) with the remaining 6 horses judged as "fair".

   Synovial fluid parameters were highly variable but, on average, viscosity tended to increase, protein showed a very slight decrease, and hyaluronic acid concentration was equivocal, as summarized in the following table:

   Table 12: Synovial Fluid Parameters

Joint	Viscosity (mean, dynes/sec)		Protein (mean, g/dl)	
	Pre Rx	Day 14	Pre Rx	Day 14
Carpus (n=27)*	3.16	6.48	1.7	1.53
Fetlock (n=13)	4.15	5.78	1.38	1.49
Combined (n=40)	3.5	6.24	1.59	1.52

   * Number of cases for which pretreatment and 14 day post treatment analyses were available.

5. Adverse Reactions

   Adverse reactions were limited to only 2 instances of transient joint swelling which subsided within 24 - 48 hours after treatment.

6. Conclusions Drawn From the Study

   The results of this study are consistent with results of previous controlled studies and demonstrate, under field condition, the safety and effectiveness of intraarticular injection of Hyalovet.

F. Other Corroborative Effectiveness Studies

The published literature clearly indicates that sodium hyaluronate is safe and effective as an intra-articular injection for the treatment of joint disorders in race

horses. The following studies are offered as corroborative evidence of effectiveness of the Hyalovet since they were conducted using the same fraction of sodium hyaluronate (Hyalovet) and is identical to that to be marketed (Imported form Trans Bussan, Geneva, Switzerland).

1. Corroborative Open Clinical Studies of the Effect of Hyalovet in Non-Infective Joint disease in Horses in Australia.

   a. Investigators

      Drs. W. L. Whatmore, G. A., Rose, M. S. Wainscott, The Driftway Stables, Londonderry, N.S.W. 2753.

   b. Animals

      Twenty four race horses (14 standardbreds, 10 Thoroughbreds), presented with various arthroses of the fore fetlock or knee joints were selected for treatment with hyaluronic acid based on published criteria (Rose, R. J., New Zealand Veterinary Journal, 2, 1979: 1-2).

   c. Dosage

      Dosage consisted of intra-articular injections of 2 mL (20 mg) sodium hyaluronate as a preparation identical to Hyalovet. Five joints received a second injection and of these two received a third injection.

   d. Design

      The study followed an open design wherein the response in each horse was compared to its pre-treatment condition. Each horse was returned for evaluation at 1 month and again at 2-3 months following treatment.

   e. Other Information

      Other information pertinent to this application are that the study included long term follow-up and included treatment of joints following surgical removal of carpal bone chips and a basal chip from the medial sesamoid.

   f. Results and Conclusions

      Using return to work as the criterion of success, it was found that 19 of the horses (80%) returned to work and ultimately to racing. None of the horses were seen to have suffered adverse effects aside from transient swelling following injection in 4 cases. Hyalovet was thus found to be effective in assisting the return of horses to racing and useful as a follow-up to surgery.

2. Corroborative Open Clinical Studies of the Effect of Hyalovet in the Treatment of Flexor Tendon Sprain (Bowed Tendon) in Race Horses.

   a. Investigators

      Dr. W. L. Whatmore, G. A. Rose, M. S. Wainscott, the Driftway Stable, Londonderry, N.S.W.

   b. Animals

Twenty nine race horses (23 Standardbreds, 5 Thoroughbreds, 1 Stock horse), presented with bowed flexor tendons of the forelimb were selected for treatment with Hyalovet.

c. Dosage

Dosage consisted of injection of Hyalovet placed into the affected tendon and tendon sheath. The preparation used consisted of 2 mL (20 mg) sodium hyaluronate, identical to Hyalovet, which was further diluted by adding 3 mL of 2% mepivacaine solution and 5 mL sterile water for injection. Mepivacaine provided local anesthesia for the pain associated with tendon injection. Four of the horses were injected twice.

d. Design

The study followed an open design wherein the response in each horse was compared to its pre-treatment condition. Historically, however it is well known that bowed tendons are notoriously non-responsive to therapy. Recently developed surgical techniques such as "stab operation", carbon fiber implants and tendon transplants (designed to replace such cruel measures as firing, cautery, and corrosive blisters) have met with mixed success. Thus "historical control" consideration would appear to be applicable to evaluation of clinical data on treatment of bowed tendons.

e. Results and Conclusions

Within the 1-2 month follow-up period, it was found that 24 of the horses (83%) returned to training, of which 15 (52%) returned to racing. It was thus concluded that Hyalovet was therapeutically useful in the treatment of sprain injuries of the flexor tendons of race horses.

3. Corroborative Preliminary Efficacy Trial in Horses with Chronic Osteoarthritis.

a. Investigator

Michael A. Collier, DVM
Department of Clinical Sciences
College of Veterinary Medicine
Cornell University
Ithaca, N.Y. 14853

b. Animals

Four horses with chronic osteoarthritis of the carpal joint (three secondary to surgically induced carpal fracture, one secondary to naturally occurring slab fracture in a race horse) were used in this pilot study.

c. Test Substances and Dosage

Sodium hyaluronate as a sterile 10 mg/mL solution; preloaded in glass syringes, 2 mL per syringe, was used in three horses. A high molecular weight sodium hyaluronate product, similar to the recently approved Hyalartin-V product, was used in one horse as a reference control. Both products were

given as a single intra-articular injection at a dosage of 2 mL (20 mg HA) per joint.

d. Design

Physical examination including gait analysis, radiographic evaluation, synovial fluid analysis, and radionucleotide uptake (bone imaging) were performed on the horses before and at weekly or biweekly intervals for four weeks after treatment with the respective hyaluronic acid products.

e. Results and Conclusions

Favorable responses with regard to improvement in lameness and measurable increases in maximal flexion permitted were detectable in all four horses during the study period. Joint circumference did not change appreciably while synovial fluid viscosity increased, mucin clot quality improved, and protein decreased within the study period, all of which are consistent with improvement in the arthritic condition. Most importantly, radionucleotide uptake decreased markedly by the end of the four week post treatment period, an observation consistent with decreased inflammatory activity in the joints.

There were no apparent differences between the results using the Hyalovet fraction and the higher molecular weight product.

On the basis of the results of this pilot study it was concluded that the carpal chip chronic osteoarthritis model, particularly with radionucleotide uptake measurements, would be a suitable model for definitive effectiveness studies in horses (see results of Corroborative study C, above).

4. Corroborative Long Term Efficacy Study of Sodium Hyaluronate Compared with Intraarticular Corticosteroid Therapy in the Treatment of Equine Arthropathy.

a. Investigator

Iginio Zara
Centro Ippico Scuderia Biosuzzi
Mirano, Italy

b. Animals

Fifty seven performance trotters with traumatic (non-infectious) joint disorders (92 joints) were selected on the basis of expert clinical judgement that intra-articular therapy was indicated.

c. Test and Control Substances and Dosage

Sodium hyaluronate as a sterile 10 mg/mL solution, preloaded in glass syringes, 2 mL per syringe, identical to the Hyalovet fraction was used. Control substances consisted of commercially available preparations of flumethasone and betamethasone.

Dosages given were 2 to 5 mL sodium hyaluronate per joint depending on joint size. The majority of the cases required only one injection but up to

three injections were given in selected cases. Corticosteroid dosages were 0.5 to 2.5 mg flumethasone or 30 mg betamethasone given in up to three injections.

d. Design

The study was a controlled clinical trial wherein horses were allocated to two treatment groups, comparable in age distribution, articular disorder, and clinical conditions. The sodium hyaluronate group included 31 horses (53 joints) while the steroid group consisted of 26 horses (40 joints). Horses of both groups were evaluated for clinical lameness, pain , and performance before treatment, within two weeks after treatment (short term) and two months or more after treatment (long term).

e. Results and Conclusions

The overall clinical improvement was judged as comparable for both treatments in short term evaluation. However, the clinical efficacy had decreased sharply after two months or more in the steroid group while the efficacy rate increased slightly in the sodium hyaluronate group. The tolerance of intra-articular treatment was generally excellent in both groups.

These results are indicative of the long term duration of clinical effect of sodium hyaluronate in arthritic equine joints, in contrast to the short term effects of steroid therapy.

5. Corroborative Clinical Studies of Sodium Hyaluronate in the Treatment of Equine Arthropathy (Proceedings, 6th National Convention of Italian Hippology Society, Salsomaggiore Terme, Italy, 8-9 June 1983).

a. Investigator:

G. Pezzoli
Professor of the Veterinary Surgery Clinic
University of Parma, Italy

b. Materials and Methods:

The investigation was carried out in 76 horses subdivided into two groups. the first group included 60 horses presented for treatment of arthropathy or tenosynovitis. The second group included 16 horses presented for joint surgery, usually for fractures. The diseased joints had already undergone various conventional treatments such as firing, corticosteroids, etc.

Following diagnosis which included clinical, radiographic, and synovial fluid evaluation, intra-articular injections of 2-4 mL (20-40 mg HA) were given along with 10-20 mg gentamicin in a volume equal to the sodium hyaluronate volume. Horses were evaluated both during the first few days and weeks following surgery and/or treatment (short term) and also 2-4 months following treatment (long term). The criteria for effectiveness were: (1) excellent: complete recovery, return to racing activities, (2) good: 90% functional recovery and (3) nil: persistence of symptomatology or only short-lasting remission.

c. Results:

In this study a total of 106 joints were treated with sodium hyaluronate of which 18 were injected following joint surgery. The overall long term results of the study are summarized in the following table:

Table 11: Long Term Results

Diagnosis	No. Joints	Excellent	Good	Nil
Arthritis	40	35 (87.5%)	5 (12.5%)	N/A
Arthroses	40	24 (60%)	12 (30%)	4 (10%)
Synovitis	8	5 (62.5%)	2 (25%)	1 (12.5%)
Post Surgery	18	13 (72%)	5 (28 %)	N/A

Synovial fluid analysis was found to be of limited value both diagnostically and as a means of assessing response to therapy.

6. Corroborative Studies on Therapeutic Experience With Sodium Hyaluronate in Equine Arthropathy.

a. Investigator:

Sergio Orsi
Centro Veterinario Equino
S. Angelo di Piove, Italy

b. Animals:

Twenty six performance trotters with acute of chronic relapsing lameness of variable degree in a total of 40 arthritic joints were evaluated in this study.

c. Test Substance and Dosage:

The sodium hyaluronate preparation used was a sterile 10 mg/mL solution of HA intermediate molecular weight, identical to the Hyalovet product, preloaded in glass syringes each containing 2 mL (20 mg HA) per joint depending on joint size, usually injected with the antibiotic gentamicin as a precaution against infection due to needle contamination. About half the cases were given one injection only.

d. Design:

The study followed an open design wherein the response in each horse was compared to its pretreatment condition and the history of its response to previous therapies. The horses were evaluated for degree of lameness, pain reaction on forced flexion, external morphology of the joint, changes in angle of flexion or extension, appearance of joint fluid, x-rays when possible, and performance in training and racing.

Evaluations were carried out before treatment, two weeks after treatment (short term) and two months or more after treatment (long term).

e. Results and Conclusions:

The majority of the arthroses involved hock joints (24 cases) although stifles, scapulohumeral (shoulder) joints, coffin joints, and fetlocks were also involved. The overall clinical effectiveness was 77% at short term evaluation and 81% in long term evaluation. No local or systemic adverse reactions were observed.

These results are indicative of short term and long term effectiveness of sodium hyaluronate in the treatment of equine arthroses.

7. Corroborative Studies on Activation of Cartilage Repair

a. Investigator:

G. Abatangelo
35031 Abano Terme, Italy

b. Background:

It has been shown in the published literature that sodium hyaluronate (HA) plays a critical role in the structural integrity of cartilage. HA forms the "backbone" of very large aggregates by binding on its threadlike molecule a sequence of high-molecular weight proteoglycan subunits. This interaction confers to cartilage the essential properties of resiliency and stiffness. Dissociation of such aggregates due to increased proteolysis and/or hyaluronate degradation will lead to increased cartilage penetrability and loss of cartilage resiliency. Such a mechanism may be involved in at least some forms of osteoarthritic lesions.

The in vitro interaction of isolated proteoglycan subunits with exogenous HA of various origins has been described in the literature. The purpose of the present study was to determine the ability of the Hyalovet fraction of HA to induce aggregation of isolated bovine cartilage proteoglycan.

c. Materials and Methods:

Deep-frozen bovine nasal septums cartilage, dissected free form adhering non-cartilaginous tissue, was obtained at the local slaughterhouse. While still frozen the cartilage was ground to powder. The proteoglycans were extracted, purified, and dissociated in the laboratory, then incubated with hyaluronidase to ensure complete breakdown and removal of endogenous hyaluronic acid.

Following the above preparation, the proteoglycans thus extracted were subjected to gel chromatography in the presence of sodium hyaluronate and its absence as a control.

d. Results and Conclusions:

The elution patterns displayed by the cartilage proteoglycans in the presence and absence of this specific fraction of HA indicated that interaction had taken place which had increased the molecular weight of the material. The finding illustrates that the HA induces an aggregation of proteoglycans which is measurable by gel chromatography.

The aggregation of proteoglycans in vitro by this specific fraction of sodium hyaluronate is consistent with the view that application of exogenous HA to osteoarthritic cartilage in vivo may also induce proteoglycan aggregation, thus activating tissue repair processes within the joint.

III. TARGET ANIMAL SAFETY

A. Pivotal Subacute Safety Study in Horses

1. Target species exaggerated dosage safety study, placebo control.

2. Investigator

   Stan J. A. Alkemade, BVSc, DVM, MRCVS
   Vetrepharm, Inc.
   69 Bessemer Road
   London, Ontario N6E 2V6
   Canada

3. General Design

   a. Purpose

      Purpose of the study was to determine the local and systemic effects of Hyalovet when given by intra articular injection at multiples of the intended dosage and multiples of the anticipated duration of treatment, in horses as the intended target species.

   b. Test Animals

      Test animals were 16 normal horses, 8 female and 8 geldings, aged 2 to 7 and a half years, weighing between 352 and 475 kg, of various mixed breeds. The horses were assigned to one of four treatment groups, 2 females and 2 geldings per group, to maximize group to group uniformity.

   c. Dosage Form and Route

      Dosage form and route consisted of injectable 10 mg/mL sodium hyaluronate solution in preloaded 2 mL glass syringes, identical to the product to be marketed, given by intra articular injection.

   d. Dosages and Duration

      Dosages and duration consisted of 20, 60, and 100 mg sodium hyaluronate per joint (representing 1X, 3X, and 5X the proposed use rate) injected daily for 4 days followed by twice weekly injections for 4 additional weeks. The control horses received injections of 10 mL sterile physiologic saline and followed the same dosing sequence as HA. The study was terminated 2 weeks after the last injection.

   e. Pertinent Parameters

      Pertinent parameters measured included physical examination (including measurement of stride length, joint circumference, gait analysis, and carpal flexion), hematology, serum chemistry, and synovianalysis (joint fluid

analysis). Additionally one horse from the high dosage group and one form the control group were necropsied for gross examination and histopathologic examination of joint tissue.

4. Results

Repeated intra-articular injection of Hyalovet as well as saline in the horses had no local or systemic toxic effects.

Clinically, all groups gained weight during the study period and remained sound. Stride length, joint circumference,and angle of flexion remained within normal limits in all groups throughout the study period.

Synovianalysis results also remained largely unchanged throughout the study period with the exception of synovial fluid protein and leucocytes which increased slightly in all dosage groups, as a result of the trauma associated with frequent joint injection.

Hematology and serum chemistry determinations remained within normal limits throughout the study period in all treatment groups.

Pathologic examinations of injected joints of a placebo horse and a high dosage sodium hyaluronate horse revealed no evidence of drug induced pathology. In both joints there was evidence of mild inflammation, however, which would be expected as a result of needle trauma.

5. Statistical Analysis

The data were not subjected to statistical analysis since (1) no differences between treatment groups were apparent with regard to any of the parameters, (2) all parameters in all treatment groups remained, for the most part, within normal limits, and (3) with regard to those parameters that did change slightly over time (e.g. synovianalysis), the changes were similar in all treatment groups. Thus, statistical analysis would add no additional insight into the interpretation of the study results.

6. Conclusions

The results of this study demonstrate that the intra articular injection of Hyalovet at the therapeutic dose (20 mg HA per joint) and at 3X and 5X multiples thereof, at multiples of the proposed treatment period, is non toxic both locally within the joint and systemically to the horse. It is thus concluded that the proposed usage of Hyalovet is safe and presents a wide margin of safety in the target species.

B. Pivotal Antigenicity and Local and Systemic Tolerance Study in Horses

1. Type of Study

The sodium hyaluronate product which is the subject of this application (imported from Trans Bussan, Geneva, Switzerland) was tested by the faculty at the University of Berne (Switzerland) to determine its suitability for testing in large scale clinical trials.

2. Investigators

Prof. Dr. H. Gerber, P. Girard, P. Tschudi, M. Diehl
Equine Clinic
University of Berne
Berne, Switzerland

3. General Design

   a. Pupose

      Purpose was to determine whether or not the sodium hyaluronate preparation was antigenic and whether it had adverse effects locally in the joint or systemically in the horse.

   b. Test Animals

      Test animals were 6 clinically normal horses, 3 thoroughbreds and 3 grade horses, 1 mare and 5 geldings, ranging in age from 5 to 18 years and in body weight from 418-483 kg.

   c. Dosage Form

      Dosage form, was a 10 mg/mL injectable solution of sodium hyaluronate preloaded in glass syringes, 2 mL per syringe, identical to the preparation to be marketed. Ringer's solution was used as a placebo control substance.

   d. Dosage, Route, and Duration

      Dosage, route, and duration were 4 mL (40 mg HA) injected intra articularly into the tibio tarsal space of one hock joint of each horse biweekly for six weeks (3 injections). The contralateral hock on each horse was injected with 4 mL Ringer's solution as a control. The observation period continued for three to four weeks beyond the last injection (total of ten weeks).

   e. Pertinent Parameters

      Pertinent parameters measured included physical examination, x-ray evaluation, hematology, clinical chemistry determination, and synovial fluid (joint fluid) analysis. Additionally, antigenicity tests were conducted by injecting sodium hyaluronate into shaved skin and comparing the size of the resulting swelling with that resulting from histamine (positive control) and Ringer's solution (negative control) injections. The skin tests were carried out in each horse before the first injection and again 3-4 weeks after the last injection to determine if the horse had become sensitized to the sodium hyaluronate injection.

      Statistical analysis was not necessary for interpretation of study results since the average of all measured parameters remained within the normal range for the hospital.

4. Results

   Physical examinations conducted daily revealed no abnormal findings in any of the horse throughout the entire duration of the test. Any slight, transient lameness or

swelling was as likely to occur on the control side as on the sodium hyaluronate side.

Hematology and blood chemistry determinations likewise revealed no indication of toxicity during the study period. The average of each parameter for all blood samples remained within the normal range for the hospital at which the study was conducted.

Synovianalysis values also remained, on the average, within the normal range for the hospital in both the sodium hyaluronate treated as well as control hock joints. Of particular interest were the results of determination of alkaline phosphatase, lactate dehydrogenase,and glutamic oxalic transaminase, enzymes that are not routinely measured in synovial fluid in U.S.A., which showed no marked changes over time nor differences between control and sodium hyaluronate treated joints. However, a progressive slight decrease in quantity and viscosity of fluid, slight increase in leucocytes and globulin, and slight increase in lactate dehydrogenase in sodium hyaluronate treated joints, was observed and attributed to mild inflammation due to trauma of repeated joint punctures.

Skin testing results showed that neither Ringer's solution nor sodium hyaluronate in its various dilutions caused skin reactions of the magnitude of the reactions to histamine, indicating that no sensitization had occurred. It was also noted that the more diluted sodium hyaluronate injections disappeared more rapidly from the skin than the full strength (10 mg/mL) injection.

5. Conclusion

   The investigators concluded that repeated intra-articular injections of sodium hyaluronate caused no reactions indicative of an allergic response and no hematological, serum chemistry, synovianalysis, or radiographic evidence of long lasting adverse effects. Although a slight local inflammation of up to 4-7 days duration was sometimes detected clinically, it was likely to occur following injection of Ringer's solution as sodium hyaluronate. Thus they declared the product suitable for clinical testing.

   The results of this study are consistent with results obtained in U.S.A. and support the conclusion that the sodium hyaluronate product is safe under proposed condition of use.

C. Corroborative Safety Studies

The studies summarized below were all conducted using the same fraction of sodium hyaluronate, meeting the same specifications, as HYALOVET.

1. Corroborative Acute Intraarticular Safety Study in Rabbits

   a. Investigator:

      M.P. Liggett
      Huntington Research Centre plc
      Huntington, Cambidgeshire
      England

   b. Design:

Eight New Zealand White Rabbits of both sexes were anesthetized, given a single intra-articular injection of 0.5 mL (5 mg) hyaluronate sodium into the stifle joint and observed for adverse effects. Four of the rabbits were sacrificed 3 days after treatment and the remaining 4 were sacrificed 10 days after injection. The injected stifle joints were examined grossly and histopathologically and compared to the untreated contralateral stifle joints as controls.

c. Results and Conclusions:

No clinical signs of adverse effects were detected in the rabbits within the study period. Gross and microscopic pathologic examination of injected joints revealed no changes that could be attributed to treatment with hyaluronate sodium.

It was thus concluded that intra-articular injection of hyaluronate sodium is well tolerated in rabbit joints.

2. Corroborative 13 Week Intra-articular Toxicity Study in Dogs

a. Investigator:

H. Chesterman
Huntington Research Centre plc
Huntington, Cambridgeshire
England

b. Design:

Twenty four adult beagle dogs of both sexes were divided into 3 dosage groups and further subdivided into interim (4 week) and terminal (full 13 week) subgroups. Dogs were given intra-articular injections into the stifle joint once each week for 4 weeks (interim) or 13 weeks (terminal) as follows:

- physiologic saline (control)
- 5 mg sodium hyaluronate per injection
- 10 mg sodium hyaluronate per injection

The sodium hyaluronate product used was a 10 mg/mL solution of intermediate molecular weight, identical to the HYALOVET fraction.

Clinical signs, ophthalmoscopy findings, hematology and clinical chemistry were monitored before and after drug administration. Post mortem examinations were conducted after the 4th weekly dosage in the interim groups and after the 13th weekly dosage in the remainder of the dogs.

c. Results and Conclusions:

No clinical, hematological, clinical chemistry, pathologic or other evidence of toxicity was detected in the dogs. In dogs of both the control and sodium hyaluronate groups, transient thickening at the injection site,slight swelling, and limping were observed occasionally following intra articular injection. No changes of biological importance in clinical chemistry or urinanalysis parameters were detected. Abnormal findings on post mortem examination

were restricted to subcutaneous hemorrhage of skin covering the injection site.

It was thus concluded that intra-articular injection of sodium hyaluronate is free of toxic effects both locally and systemically in dogs.

3. Corroborative Acute Toxicity Studies in Rats

   a. Investigator:

      Dr. Ferrante Aporti
      35031 Abano Terme, Italy

   b. Design:

      Laboratory rats and mice, five of each sex per dosage group, were given single doses of sodium hyaluronate (identical to the HYALOVET fraction) intravenously at dosages of 12.5, 25, or 50 mg/kg or subcutaneously at dosages of 25, 50, or 100 mg mg/kg and kept under observation for 14 days.

   c. Results and Conclusions:

      None of the animals of either species died or exhibited any adverse effects of sodium hyaluronate treatment by either route of administration. It was thus concluded that the intravenous LD50 of the HA preparation is greater than 100 mg/kg.

4. Corroborative 30 Day Subcutaneous Toxicity Study in Rats

   a. Investigator:

      Dr. Ferrante Aporti
      35031 Abano Terme, Italy

   b. Design: Forty weanling sprague Dawley rats, 20 males and 20 females were divided into 4 groups of 10 animals each and treated for 30 days as follows:

      - Group 1 (5F, 5M rats) - Control (physiological saline) s.c.
      - Group 2 (5F, 5M rats) - Sodium hyaluronate 5 mg/kg daily s.c.
      - Group 3 (5F, 5M rats) - Sodium hyaluronate 10 mg/kg daily s.c.
      - Group 4 (5F, 5M rats) - Sodium hyaluronate 20 mg/kg daily s.c.

      The animals were watched during the first hours of the day throughout the treatment period for behavioral abnormalities, body growth, and food consumption. Laboratory tests included standard hematology and serum biochemistry determinations, urinalysis, and necropsies.

   c. Results and Conclusions:

      Neither the treated animals nor the controls showed any statistically significant modifications of body growth or blood picture in comparative assessments made before and after treatment. No mortality occurred. Likewise, urinalysis findings, serum biochemistry results, and necropsy findings in treated animals did not differ significantly from the corresponding control values.

The results of these experiments show that the daily subcutaneous administration of sodium hyaluronate continued for 30 days is well tolerated by weanling male and female rats of the Sprague-Dawley strain.

5. Corroborative 180 Day Subcutaneous Toxicity Studies in Rats

   a. Investigator:

      Dr. Ferranti Aporti
      35031 Abano Terme, Italy

   b. Design:

      Forty weanling rats fo the Sprague-Dawley strain were divided into 4 groups of 10 each, and treated as follows:

      - Group 1 (10 animals, 5M+5F): Control (physiologic saline s.c.)
      - Group 2 (10 animals, 5M+5F): Sodium hyaluronate 4 mg/kg daily s.c.
      - Group 3 (10 animals, 5M+5F): Sodium Hyaluronate 8 mg/kg daily s.c.
      - Group 4 (10 animals, 5M+5F): Sodium Hyaluronate 16 mg/kg daily s.c.

      Treatment was administered daily, seven days a week, for a total of 180 days (6 months). All animals were maintained in standard laboratory conditions and examined periodically as indicated below. At termination, all test parameters were reassessed and necropsies were conducted.

   c. Results:

      The test parameters assessed periodically throughout treatment were: body growth; hematology (including differential WBC count); SGOT and SGPT, total serum protein content; serum cholesterol; urinalysis. None of these tests revealed any emerging pathology: all parameters were unaffected by the test treatment. Necropsies of treated animals revealed no visible alterations of the liver, kidneys, spleen, heart, lungs, stomach, or intestine: all these structures were consistently normal in size and appearance.

      Histological examinations of suitable specimens of the main organs, revealed no lesions or evidence of adverse influences of the test product.

   d. Conclusions:

      These experimental results show that sodium hyaluronate at the dosages used in this study, has no toxic effects in Sprague-Dawley rats, when administered subcutaneously for 180 consecutive days.

6. Corroborative 30 day Intramuscular Toxicity Studies in Dogs

   a. Investigator:

      Dr. Ferranti Aporti
      35031 Abano Terme, Italy

   b. Design:

Four pairs of adult Beagle dogs (weight 8-11 kg) were treated for 30 days as follows:

- Group 1 - Control, physiological saline daily i.m.
- Group 2 - Sodium hyaluronate 1.5 mg/kg daily i.m.
- Group 3 - Sodium hyaluronate 3 mg/kg daily i.m.
- Group 4 - sodium hyaluronate 6 mg/kg daily i.m.

c. Results:

Body growth of treated animals did not differ significantly from control values. Treatment with the test substance produced no significant changes of blood picture (including differential WBC count), SGOT and SGPT activity, of albumin, glucose, and ketone bodies assayed in the urine. Likewise, total serum protein assays made at termination contributed no abnormal findings.

Necropsies at the end of 30 treatment days revealed no lesions of abnormalities of the liver, kidneys, spleen, heart, lungs, stomach or intestine. Histological examinations of suitable specimens of liver, kidney, heart, spleen, and adrenal glands confirmed the absence of toxic effects due to sodium hyaluronate at the dosage used in this study.

7. Corroborative Fetal Toxicity Studies in Rats

a. Investigator:

Dr. Ferranti Aporti
35031 Abano Terme, Italy

b. Design: Four groups of 10 sexually mature Sprague-Dawley rats were mated with adult males of the same strain, then the female animal were treated as follows:

- Group 1 - Control (physiological saline s.c.)
- Group 2 - Sodium hyaluronate 4 mg/kg daily s.c.
- Group 3 - Sodium hyaluronate 8 mg/kg daily s.c.
- Group 4 - Sodium hyaluronate 16 mg/kg daily s.c.

At the 20th day of pregnancy, the animals were sacrificed and fetuses removed. All fetuses were examined in terms of their number, viability, weight, and malformations; the skeleton was examined after diaphanization of soft tissues and staining with alizarin red. The mother animals' uteri were inspected for evidence of fetal reabsorption sites.

c. Results and Conclusions:

There were no differences between treated groups and the control group in terms of total live and dead fetus count, mean number of fetuses per litter, mean wight of fetuses at birth, or number of reabsorbed fetuses. There were no fetal malformations.

It was thus concluded that the treatment of pregnant Sprague-Dawley rats with sodium hyaluronate did not interfere with the normal course of pregnancy. The number, growth, and viability of fetuses delivered by treated

mothers did not differ from corresponding control values. Analysis of the results, comparing the various frequencies observed in the three groups, failed to reveal any significant differences.

8. Corroborative Fetal Toxicity Studies in Rabbits

a. Investigator:

Dr. Ferranti Aporti
350931 Abano Terme, Italy

b. Design:

Twenty four pregnant New Zealand rabbits were randomly divided into four groups of six animals per group. From the 6th to 18th day of pregnancy the rabbits were treated as follows:

- Group 1 - Control (physiological saline s.c.)
- Group 2 - Sodium hyaluronate 4 mg/kg daily s.c.
- Group 3 - Sodium hyaluronate 8 mg/kg daily s.c.
- Group 4 - Sodium hyaluronate 16 mg/kg daily s.c.

All fetuses removed by cesarean section from sacrificed mothers were examined in terms of their number, viability, weight, and malformations. The skeleton was examined after diaphanization of soft tissues and staining with alizarin red. The dam' uteri were inspected for evidence of fetal reabsorption sites.

c. Results:

At delivery, there were no differences between the three groups in regard to total number of fetuses delivered, mean number of fetuses per litter, number of live fetuses, number of dead fetuses, number of reabsorbed fetuses, or number of malformed fetuses. Statistical analysis of the results, comparing the various frequencies in the four groups, revealed no significant differences.

IV. HUMAN FOOD SAFETY

Data on human safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. The drug is approved only for use in horses that are not to be used for food and is to be labeled: Not for use in horses intended for food. Not for human use.

Human safety relative to possession, handling, and administration: no special caution statement needed.

V. AGENCY CONCLUSIONS

The data submitted in support of this New Animal Drug Application comply with the requirements of Section 512 of the Act and Section 514.111 of the implementing regulations. It demonstrates that Hyalovet (hyaluronate sodium), when used according to the labeled conditions of use, is safe and effective.

The safe and effective use of Hyalovet (hyaluronate sodium) injected intra-articularly in horses requires a veterinarian's knowledge of surgical technique and knowledge of the anatomy of the specific joint. The Agency, therefore, concluded that Hyalovet (hyaluronate sodium) should be provided to the public on prescription basis.

Copies of applicable labels may be obtained by writing to the:

Freedom of Information Office
Center for Veterinary Medicine, FDA
7500 Standish Place
Rockville, MD 20855

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.