EFFECTS OF ORAL AND INTRAMUSCULAR USE OF CHONDROITIN SULFATE IN INDUCED EQUINE ASEPTIC ARTHRITIS.

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Introduction:

Articular pathologies in equines are one of the main causes of poor performances and training loss in sport equines. Osteoarthritis and osteoarthrosis are degenerative articular entities characterized by a variable destruction of the articular cartilage, frequently accompanied by subchondral bone sclerosis and osteophyte formation. Synovitis and hydrarthrosis may, occasionally associate to the disease clinically characterized by pain and disfunction of the affected joint. The destruction of the articular cartilage is the essencial component of a series of facts, sometimes degenerative, others regenerative, which finally affect all joint tissues and structures.(1)

Aseptic articular processes have been associated to varied clinical entities, although it may be said that most of them affect young animals and are related to exercise, compromising principally very mobile joints such as the carpal or metacarpophalangeal joints, or also intertarsal or interphalangeal joints(2). Occasionally, aseptic arthritis may be the result of local or remote infectious processes frecuently observed in colts. In any case, aseptic articular processes constitute a 33% of lameness diagnosed in equines(3).

Authors had paid great attention to the treatment of aseptic arthritis in equines in the last few years (3;4;5;6;7;8). Treatment principles may be divided into areas as follows:

1. Prevention or treatment of the primary cause (fracture, infectious arthritis, etc.).
2. Disease treatment, particularly in soft tissues. This includes rest, immobilization, physical therapy, anti-inflammatory drugs, articular lavages and synovectomy.
3. Treatment of articular degeneration and of changes secondary to arthritis which shall include curettage, osteophytes removal and induction to restoration of damaged articular cartilages.

Some uses of glycosaminoglycans have been reported, specially in the second and third principles before mentioned, with benefits which include decrease of pain and articular movement induction as well as inhibition of protease enzymes present in the synovial liquid during the pathology development, stimulation of the proteoglycans and collagen synthesis, decrease of erosion and articular cartilage growth induction.
It must be pointed out that these benefits have been reported for the intraarticular, intramuscular and oral administration of glycosaminoglycans (3) (6) (11) (12) (13) (14) (15).
Chondroitin sulfate (CS) is a sulphated glycosaminoglycan and is one of the proteoglycans present in the articular cartilage. Chondroitin sulfate is the cartilage major constituent; keratan sulfate and hyaluronic acid are also present in a minor degree. The tridemensional disposition, hydrophylic nature and prominent presence of negative charges give proteoglycans, selective permeability, water retention, elasticity and support to compression, essential characteristics of articular cartilage. In osteoarthritis there is a reduction in proteoglycan concentration due to depolymerization and loss of constituing

GAGS with the subsequent loss of the before mentioned characteristics. Likewise, inflammation causes inflammatory exudates with an increase of the enzymatic activity and of inflammation mediators in the joint, which contribute to increase cartilage degradation. Chondroitin sulfate contributes to keep cartilage regular characteristics through the increase of glycosaminoglycan pool used by the chondrocyte for proteoglycan synthesis. In addition to that restorative effect, the incorporation of CS to the articular cartilage, slows down the inflammatory process acting directly on the enzymes, inhibiting the complement and by antiprostaglandinic activity. These concepts concerning the physiological role of chrondroitin sulfate in the joint, plus the intimate knowledge of the articular disease physiopathology, are the fundamentals of the therapeutic and/or preventive use of CS in degenerative articular pathologies. (16) (20)
The research on different products to treat articular problems has included the use of experimental models to reproduce natural articular pathologies. Models based on induction to articular disease by physical, chemical etc means have been used in target species (equine and others), or in spontaneous articular diseases in different species (17). The use of the injection Adjuvant Complete Freund (ACF) to induce an experimental arthritis belongs to the first mentioned group and has been previously used to study the effects of different glycosaminoglycans in the equine degenerative joint disease (DJD). The induced pathology was characterized by a significant synovitis and capsulitis together with clinical signs such as lameness, increase of articular circumference and less flection of the affected joint as well as increase of the protein content in the synovial liquid and significant subchondral reaction with osteophytes presence.(3).

The purpose of this job was to evaluate the effects of oral and injectable administration of chondroitin sulfate in equines with an induced experimental aseptic arthritis.

Materials and Methods:

This work was carried out on fifteen female equines clinically healthy whose age oscillated between 5 and 10 years old and 350-400 kg. weighed alive. Animals were identified and were divided at random into three groups of five animals each, named Group 1 to 3 respectively. Previous to the test and in order to determine their condition, the animals were clinically and radiologically examined. On the test day, the left carpus joint zone of all animals was depilated and then sanitized and antisepticized. Immediately, each animal was immovilized in order to proceed to the extraction of 2 ml of synovial liquid, inoculating in the same operation 0.5 ml of Adjuvant Complete of Feund (ACF) in the joint, to induce an aseptic arthritis. This operation was carried on in the fifteen animals taking part in the test.

Treatments:

The following treatments were carried on:
Group 1 (n=5): Application of 5 ml of Chondroitin Sulfate[1] via intramuscular each five days (sterile solution at 12%, 120 mg/mL)[2] .
Group 2 (n= 5): Daily application of 10 ml of Chondroitin Sulfate[1] via oral, (solution at 25%, 250 mg/mL)[3].
Group 3 (n=5): No treatment (control group).

Treatments were applied as from zero day up to 30 days after injury induction.

Evaluated Parameters:

A series of parameters were evaluated with the purpose of judging the evolution of the caused injury, to wit:

a) **Articular circumference:** It was taken at the accessory carpal bone level with a flexible metallic ribbon. In order to standardize data, the increase of the articular circumference was taken as percentage (%) of increase.
b) **Strained flexion:** It was made measuring the distance in centimeters (cm) between the foot coronary band and the olecranon bone.
c) **Lameness:** Lameness degree was evaluated making the animals trot immediately after a a minute long strained flexion of the affected joint.

Values were assigned to the observed degrees of difficulty to quantize the parameter:
Degree 4: maximum difficulty; it does not rest the member on the floor.
Degree 3: severe difficulty; it rests slightly in clamps.
Degree 2: moderate difficulty; rests the hoof completely but with some difficulty.
Degree 1: Slight difficulty: good resting but limps in the first steps after flexion.
Degree 0: Normal.

The mentioned parameters were evaluated at zero day (previous to induction) and postinduction on days 2;9;16; 23 and 30.-

d) **Analysis of the synovial liquid:** An extraction of synovial liquid from the left carpus joint by punction of the articular sheath was made on days 0, 15 and 30 of the assay. It was practised to all animals and 2 mL of liquid was extracted to each one. The sample was taken to determine total proteins by Lowry method (9).
e) **Radiological Examination:** Radiographic plates of left carpus joint in all animals were obtained on days 0 and 30 of the assay.

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[1] Chondroitín Sulfate A. Molecular Weight=25,000 D ; Sulfur=6%.Syntex S.A. Argentina.
[2] Artroglycan™ Injectable 12% . Syntex S.A.Argentina.
[3] Artroglycan™ (in some countries Arthroglycan™) Oral Solution 25%. Syntex S.A.Argentina.

f) **Clinical Examination:** Each 48 hours a clinical examination including body temperature (rectal), heart beating and breathing frequency was carried on. On days 0 and 30 a blood sampling by punctioning of the jugular vein was made. The obtained plasm was bound to make a proteinogram and hepatogram

g) **Statistical Analysis:** An Anova was made to the obtained data with a preestablished level of significance of a 5%. Multiple comparisons through a Duncan test with a level of significance of a 5% were made in those parameters in which significative differences were found. The statistical analysis was made through a SPSS version 6.0 statistical package

Results:

Results obtained in the parameters taken into account are shown in figures 1 to 4 where the mean values of different groups throughout the assay may be observed.

a) Articular Circumference (Table 1, Fig. 1) In order to standardize the obtained data, a percentage of increase of the articular circumference was considered. In this parameter we observe that on the second day there is an increase ranging from 14.5 to 22.8 % .This confirms the carpitis effective induction. Significant differences ($p<0.01$) among groups were noticed in all the observations made. Comparison among groups showed that there were differences among groups 1, 2 and 3 on days 2 and 9, while during the rest of the days there only were differences between treated groups (1 and 2) and the Control group (3).

b) Strained flexion: (Table 2, Fig. 2) The strained flexion is an indicator of articular mobility. No differences were observed among groups until day 16. From day 16 and up to day 30, we observed significant differences ($p<0.05$) between treated groups (1 and 2) and the Control group (3). No differences were found between groups 1 and 2 in any other moment throughout the assay.

c) Lameness: (Table 3, Fig. 3) Significant differences ($p<0.05$) between groups 1 and 3 were observed in this parameter throughout the assay. No differences were observed between groups 2 and 3 up to day 16; while from day 23 up to the end of the assay, differences were significative ($p<0.05$). Between groups 1 and 2 differences are noticed on days 2 and 16, while during all other days considered there were no differences.

d) Analysis of synovial liquid: (Table 4, Fig.4). Proteins concentration in the synovial liquid on day 0 was 15 to 17 mg/ml, amount considered normal for the species; there were no differences among the 3 groups. On days 15 and 30 significant differences ($p<0.05$) may be observed between treated groups (1 and 2) and Controles (3).There were no differences between groups 1 and 2 throughout the assay.

e) Radiological Examination: the radiographical evaluation made at the assay end, showed the presence of more condensed areas in animals of group 3, probably due to cartilage fibrosis and to a subchondral bone reaction. These changes are not observed in plates corresponding to animals in groups 1 and 2. Any of the examined animals revealed the presence of osteophytes.

f) Clinical examination: The clinical examination of all animals taking part in the assay does not reveal data different from the species standards.

Table 1: increase % of articular circumference

Day	Injectable	Oral	Control
0	0	0	0
2	14,5 [a]	18,5 [b]	22,8 [c]
9	8,1 [a]	13,8 [b]	24,4 [c]
16	8,2 [a]	11,7 [a]	22,7 [b]
23	7,8 [a]	10,6 [a]	22 [b]
30	5,4 [a]	6,7 [a]	20,3 [b]

a,b,c: Values with different letters in the same line differ significatively ($p<0.05$)

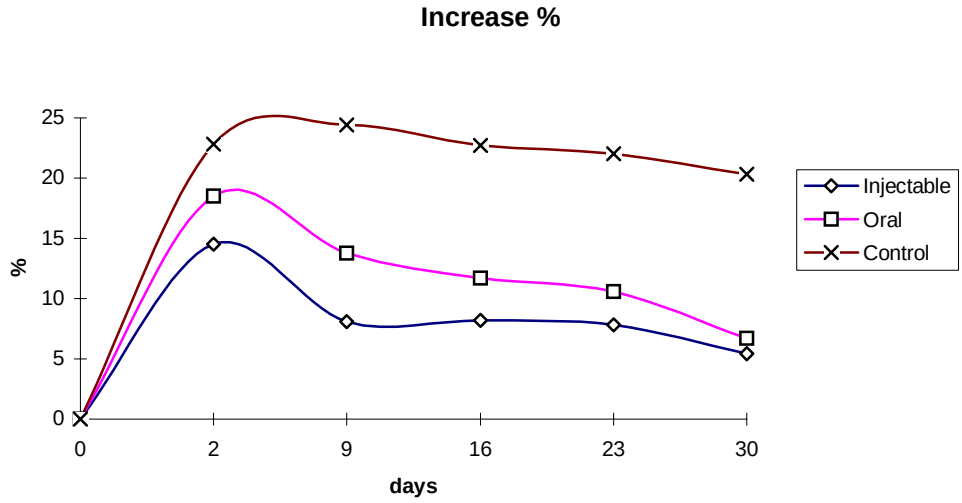

Fig 1: Increase of articular circumference

Table 2: Strained flexion

Day	Injectable	Oral	Control
0	0	0	0
2	28 [a]	20,4 [a]	31,8 [a]
9	15,6 [a]	14,2 [a]	22,8 [a]
16	9,6 [a]	8,6 [a]	28,2 [b]
23	6 [a]	10 [a]	22,8 [b]
30	0 [a]	5 [a]	20,6 [b]

a,b,c: Values with different letters in the same line differ significatively ($p<0.05$)

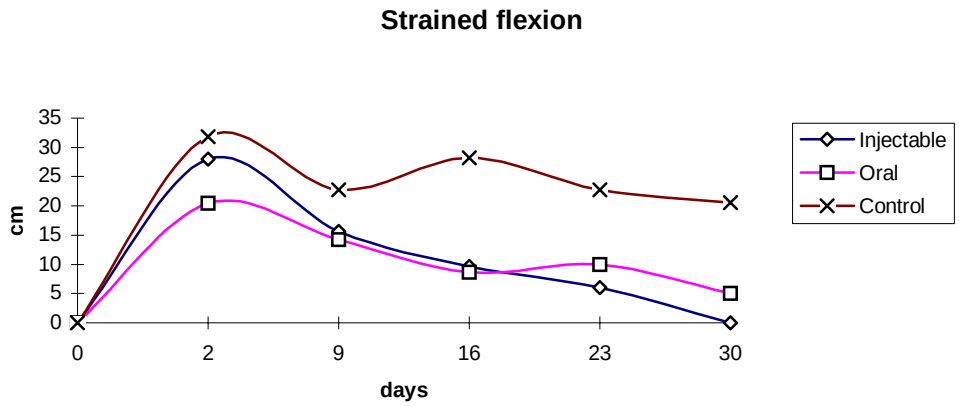

Fig. 2 : Strained flexion

Table 3: Lameness Degree

Day	Injectable	Oral	Control
0	0	0	0
2	2 [a]	2,6 [b]	2,8 [b]
9	0,8 [a]	1,8 [a]	1,8 [a]
16	0 [a]	1,4 [b]	1,2 [b]
23	0 [a]	0,4 [a]	1,6 [b]
30	0 [a]	0 [a]	1,4 [b]

a,b: Values with different letters in the same line differ significatively ($p<0.05$)

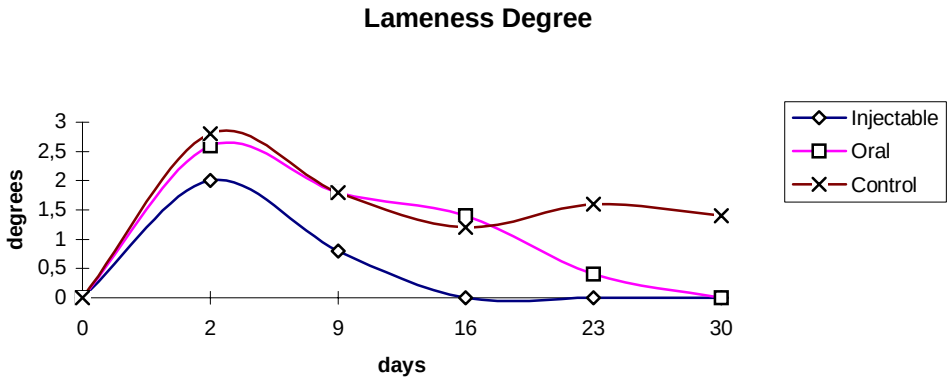

Fig. 3 Lameness degree

Table 4:Proteins in the synovial liquid (mg/mL)

Day	Injectable	Oral	Control
0	14,66 [a]	17,64 [a]	15,9 [a]
15	24,6 [a]	25,9 [a]	47,22 [b]
30	22,8 [a]	23,32 [a]	36,52 [b]

a,b: Values with different letters in the same line differ significatively ($p<0.05$)

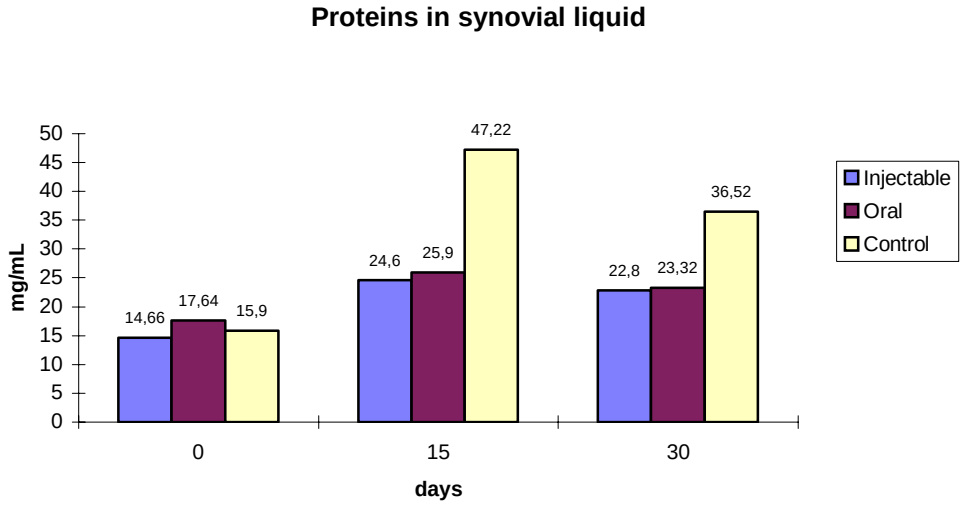

Fig. 4 : Proteins in synovial liquid

Discussion:

The use of experimental models in animals for the study of articular pathologies has been treated in detail by bibliography (17). There is not such a thing as an experimental model faithfully reproducing natural pathology, although the main characteristics of the degenerative articular disease have been reproduced in some of these models.
The experimental carpitis used in this assay showed results similar to those observed in the work of reference (3) for all the parameters taken into account, which help to know better this kind of injuries and their possible solutions.
The results obtained confirmed bibliography data (3-7) which remarks the benefits of the use of glycosaminoglycans in the aseptic articular processes. The increase of the articular circumference was significantly less in treated animals; this suggests a direct antiinflammatory effect of chondroitin sulfate on the injury caused. On the other hand, the differences observed in the response to a strained flexion and the lameness degree, make us think of a significative analgesic effect, which contributes to a fast and better recovery of animals suffering articular injuries. The differences observed as regards the

proteic tenor of the synovial liquid on days 15 and 30 after the injury has been induced, outstands the chondroprotective effect of this kind of products which tend to avoid the inflammatory exudate resulting from the *degradation* of the articular cartilage. The radiographic image makes evident the presence of a subchondral sclerosis plus loss of regular characteristics of the articular capsule present only in Control animals; this reveals a growth-inductive (chondrorepairing) effect of chondroitin sulfate on the cartilage injured by the ACF.

The animals treated with CS via injectable showed a significant evolution 48 hours after the injury induction with respect to some of the evaluated parameters, which is consistent with a bioavailability of the product by this via, showing a plasmatic peak few hours after injection (18). The response observed in the increase of the articular circumference was very good in 48 hours, improving the achievements of the group treated via oral. From day 16 on, the evolution was similar to that observed in animals treated via oral and significantly better ($p<0.05$) than the one observed in Control animals.

The response to strained flexion and the lameness degree showed an important evolution as from day 16, there being no differences among treated groups as opposed to Control animals. All evaluated parameters turned to normality at the assay end in animals treated this via. The response to therapy with CS via IM observed in the experimental model suggests a similar behaviour in natural pathologies; thus making it specially appropriate in acute or subacute pathologies. Likewise, the dosage administered seems to be adequate to treat the induced or natural degenerative articular disease and coincides with that used in similar products. It must be pointed out that the treatment of natural articular pathologies this way has yielded highly satisfactory results in coincidence with findings showed in this work.

CS oral administration yielded satisfactory results in all evaluated parameters. Such improvement start to be observed at day 2 and it was evident on day 9. Up to day 9 significant differences ($p<0.05$) with animals treated via injectable are observed, namely, the reduction of local inflammation (articular circumference). The evolution of this parameter was satisfactory and much better than that of the Control animals, although up to this day the best evolution was shown by animals treated via injectable. It is interesting to note that as from day 16 there are no differences for this parameter among groups treated both vias.

The response to strained flexion and lameness degree show a significative evolution of animals as from day 16, with a practically total remission of injury signs at day 30. There were no significant differences among groups treated both ways as regards the content of proteins in the synovial liquid but such differences did exist between treated and untreated animals ($p<0.05$), which coincides with the rest of the parameters if we consider that the sampling was made on days 15 and 30. During the assay last fifteen days, no significant differences with animals treated via intramuscular were observed in the majority of the parameters taken into account. At day 30, the animals treated via oral exhibited standard values in almost all evaluated parameters. The dosage used was appropriated for the treatment of the induced pathology. It must be pointed out that assays made by us using the same experimental model but different CS dosages showed that for doses superior to that proposed here (20 and 40 mL) there were no significative differences ($p<0.2$) in those parameters being considered (14). The administration of doses lower than 10 mL showed different results. Animals treated with 5 mL showed good evolution in the majority of the evaluated parameters, although such improvement showed up 7 to 10 days after those observed in animals treated with higher doses. These evidences suggest that a 5 mL (1250 mg CS) dose could be used as a probable preventive or maintaining dose. In the same way, doses of 2.5 mL/day were not effective in the

treatment of animals in this experimental model.(Videla Dorna I. et al., not published data). These observations make it possible to adjust to 10 ml. the daily therapeutic dose to be used (2500 mg CS, 5 to 10 mg/kg alive weight) which coincides with doses used in other species, including the man where similar therapeutic results have been observed.
Response to oral administration of CS in this experimental model, let us suppose not only the product bioavailability by this via, fact already confirmed by other authors with respect to other species (18,19) but also its therapeutic efficacy. This coincides with observations made in the evolution of animals with natural articular pathologies which were treated with CS this via and that exhibited an important improvement 7 to 10 days after the beginning of the treatment. Finally, oral administration which did not cause either changes in the physiological constants or digestive alterations in treated animals, constitutes an interesting via of administration for this kind of compounds, specially if we take into account the adverse effects of repeated parenteral applications, specially intraarticular applications. This fact is particularly important for these products which demand a long term administration and let us think of possible prophylactic schemes specially in young animals in training.

Conclusion

The treatment of articular pathologies in human and veterinary medicine has received a great deal of attention on the part of prominent researchers in the matter in these last years. Special attention has been paid to those no conventional treatments such as the use of some latest generation non-steroid antiinflammatories and specially products derived from glycosaminoglycans present in the articular cartilage, which in same cases are identical to the natural ones and in others are obtained by semi-synthesis from natural products; for example, Chondroitin Sulfate (CS) and polysulfated glycosaminoglycans (PSGAG) respectively. Some works have been done (3-8) showing the therapeutic efficacy of PSGAGs, using for that purpose experimental models similar to the one used in the present work.
Some of these authors have intended to demonstrate the inefficacy of products different from PSGAG, administered via oral (21). Their conclussions suggested the necessity of studies with adequate experimental models, to evidence the therapeutic value of these products when administered via oral. Our group has been making assays for some years, to evaluate the effect of the administration of CS by different vias, always using the same experimental model. We can assure then, that we count with a well controlled and standardized experimental model which makes reliable the obtained data, irrespective of the result of the statistical test.
The obtained results are determinative and evidence the CS therapeutic value in the induced pathology, irrespective of the via of administration. Although differences are observed in the product behaviour when administered by different vias, both proved to be effective and lead to normalize the articular condition after 30 days of treatment. The observed differences might be the basis for the elaboration of a possible therapeutic strategy based on the use of CS by different vias in different moments of the articular pathology, although in this election it is also important to consider the kind and condition of the pathology being treated. This shall determine the use of products of a more or less quick effect, as well as the via of administration to be used, that is intra-articular, intramuscular or oral.

The correlation observed among this experimental findings and the multiple observations made by veterinarians in the use of CS through different vias, make it possible to extend the before mentioned conclussions to the field of natural pathologies. In that sense, highly satisfactory results have been reported in the use of 25% CS solution (Artroglycan™, Arthroglycan™) via oral in pathologies such as hydraarthrosis, polyarthritis secondary to omphalophlebitis, arthrosis and arthritis of the metacarpophalangian joint, arthrosis of tarsus with periostitis, navicular disease, articular stress of metacarpophalangian joint, synovitis and capsulitis of the sesamoidal great sheath among others. To this may be added the significant therapeutic success and the world acceptation of pharmaceutical specialties whose active principle is the CS of oral use (Condrosulf™, IBSA, Suiza o Condral™, SPA Italia), in the treatment of different articular pathologies in man (22).

Considering what was previously mentioned and based principally in the experimental results of the present work, the discussion about the viability of the CS via oral or injectable seems to be out of place and inconvenient, in view of the numerous and overwhelming clinical and experimental results. CS, in their varied presentations is then an important therapeutic resource with which veterinarians count for the handling of articular problems.

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