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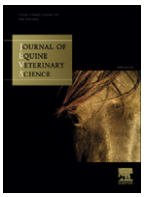

Short Communication

Physiological Concentrations of Acute-Phase Proteins and Immunoglobulins in Equine Synovial Fluid

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ABSTRACT

Synovial fluid (SF) is capable of reflecting infectious, immunological, or inflammatory joint conditions in horses by altering its composition and appearance. Although plasma and SF compositions are quantitatively different, this latter compartment reflects changes in plasma macromolecules. Therefore, changes in serum immunoglobulin protein concentrations tend also to alter intracapsular levels. Therefore, it is necessary to know the physiological concentrations of proteins present in SF. The aim of this study was to determine the levels of total protein, albumin, transferrin, haptoglobin, α1-acid glycoprotein, ceruloplasmin, and immunoglobulins A and G in SF of six healthy horses. The synovial proteinogram was obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The SF proteins reached a maximum of 25% of serum concentrations, varying inversely with molecular weight of the protein, except for the ceruloplasmin.

1. Introduction

Synovial fluid (SF) is mainly a dialysate of plasma that contains proteins, electrolytes, and hyaluronic acid, where the latter is produced by the synoviocytes present in the intimal layer of the synovial membrane and therefore is present in much higher concentration in the SF compared with plasma. Analyses of SF in cases of joint diseases show increasing concentrations of proteins compared with physiological conditions [1].

Joint damage induces the production of cytokines such as interleukin 1, interleukin 6, and tumor necrosis factor α by type A synoviocytes [2], which trigger the acute-phase response (APR) and production of acute-phase proteins (APPs) by hepatocytes [3].

In horses, serum amyloid A is considered a major positive APP, as its concentration increases about 10 times during APR. On the other hand, haptoglobin, C-reactive protein, α1-acid glycoprotein, ceruloplasmin, and fibrinogen are examples of moderate positive APPs because their concentrations increase between 1 and 10 times during APR, whereas albumin and transferrin are examples of negative APP, whose concentrations tend to decrease during APR [4].

Electrophoresis is one of the most reliable methods for identifying proteins in biological fluids such as plasma, serum, or SF. Electrophoresis fractionation has been applied to identify and quantify the proteins in SF of humans with temporomandibular joint disorders, and it is described as a reliable, reproducible, and specific method [5].

From a clinical perspective, the quantification of APP and immunoglobulins (Igs) in the SF of healthy horses

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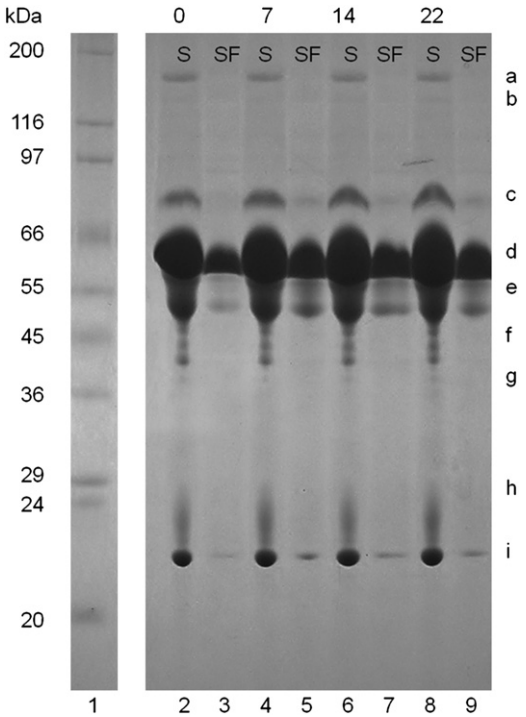

Fig. 1. Typical results of sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of serum (S) and synovial fluid (SF) proteins. Lane 1 refers to the markers used. Lane 2, S from day 0; Lane 3, SF from day 0; Lane 4, S from day 7; Lane 5, SF from day 7; Lane 6, S from day 14; Lane 7, SF from day 14; Lane 8, S from day 22; Lane 9, SF from day 22. The bands marked as a, b, c, d, e, f, g, h, and i indicate IgA, ceruloplasmin, transferrin, albumin, IgG heavy chain, haptoglobin, α_1- acid glycoprotein, IgG light chain, and an unknown 23-kDa protein, respectively.

Table 1
Mean protein concentrations of serum and synovial fluid, collected at four different times, of healthy horses

Protein	Time (d)	Serum (g/dL)		Synovial Fluid (g/dL)	
		Mean	SD	Mean	SD
Total protein	0	6.938	0.841	1.336	0.696
	7	6.303	0.402	1.232	0.461
	14	6.548	0.445	1.045	0.385
	22	6.773	0.571	1.303	0.673
Albumin	0	3.736	0.408	0.863	0.430
	7	3.715	0.321	0.816	0.298
	14	3.825	0.248	0.680	0.231
	22	3.971	0.310	0.838	0.423
Transferrin	0	0.428	0.127	0.075	0.044
	7	0.426	0.061	0.053	0.030
	14	0.393	0.086	0.053	0.022
	22	0.435	0.130	0.058	0.043
Ceruloplasmin	0	0.003	0.002	0.001	0.001
	7	0.004	0.004	0.001	0.002
	14	0.004	0.001	0.002	0.001
	22	0.004	0.002	0.002	0.001
Haptoglobin	0	0.048	0.017	0.004	0.004
	7	0.022	0.013	0.004	0.002
	14	0.027	0.009	0.004	0.002
	22	0.034	0.010	0.007	0.004
α1-Acid glycoprotein	0	0.009	0.005	0.003	0.002
	7	0.014	0.002	0.003	0.001
	14	0.014	0.003	0.003	0.001
	22	0.012	0.007	0.004	0.002
IgA	0	0.085	0.044	0.004	0.005
	7	0.002	0.005	0.004	0.004
	14	0.058	0.046	0.001	0.001
	22	0.072	0.059	0.004	0.002
IgG	0	1.591	0.500	0.200	0.113
	7	1.431	0.174	0.179	0.070
	14	1.362	0.211	0.165	0.086
	22	1.527	0.387	0.217	0.131

According to Tukey test, there were no significant variations ($P > .05$) between sampling times.

could be a suitable auxiliary tool for interpreting the evolution and the therapeutics of equine joint diseases. Thus, the objective of this work was to determine the concentrations of total protein, albumin, transferrin, haptoglobin, α1-acid glycoprotein, and ceruloplasmin, owing to the role they play in the APR, and the Igs A and G (IgA, IgG) present in the SF of healthy horses.

2. Materials and Methods

The present study was approved by the Animal Welfare Ethics Committee of the School of Agricultural and Veterinary Sciences Faculdade de Ciências Agrárias e Veterinárias, Univ Estadual Paulista (FCAV-UNESP) under protocol number 028427-08.

After careful clinical examination, six healthy riding-school horses that perform low-level jumping exercises for 1-2 hr/d, without either history of joint diseases or swelling of metacarpophalangeal joint, with clean and clear appearing SF, and without manifestations of joint pain or lameness were submitted to arthrocentesis of the metacarpophalangeal joint through the lateral collateral ligament for collection of approximately 2.0 mL of SF. The joint puncture was preceded by cleaning and local antiseptic treatment, that is, previous washing using neutral detergent, three germicide procedures with iodine antiseptic, and rinsing with 70% alcohol, precluding the need for shaving. The samples were collected at 0, 7, 14, and 22 days. At the same time points, approximately 10 mL of jugular venous blood was drawn for serum samples, immediately centrifuged under refrigeration (Multispeed refrigerated centrifuge PK121R, NJ4, ALC, Princeton, NJ), and frozen. All samples remained frozen until further analysis.

The SF and serum samples were stored in sterile Eppendorf tubes at −20°C until time of assays. Total protein was determined by the Biuret method, and the serum and synovial proteinogram were obtained by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis [6]. The electrophoretic fractionation was performed by a unidimensional electrophoresis system on 4%-10% gradient acrylamide gels. The separating gel consisted of 15 mL of distilled water, 3 mL of 2*M* Tris−1*M* glycine (pH 9.0), 7.5 mL of 30% acrylamide, 1.5 mL of 2% bis acrylamide, 1.5 mL of glycerol, 0.6 mL of 0.5*M* ethylenediaminetetraacetic acid (pH 8.3), and 0.6 mL of 20% SDS. It was polymerized by adding 15 μL of tetramethylethylenediamine and 0.3 mL of 10% ammonium persulphate. The stacking gel was polymerized by adding 20 μL of tetramethylethylenediamine and 0.1 mL of 10% ammonium persulphate. The upper electrode buffer contained 30 m*M* Tris base, 150 m*M* glycine, 0.1% SDS, and sufficient water to make 1 L; the lower buffer contained 75 m*M* glycine, 0.1 SDS, and

Table 2
Comparison between the protein concentrations (mean ± standard deviation) of serum and synovial fluid of healthy horses

Protein	Molecular Weight (Da)	Serum (g/dL)	Synovial Fluid (g/dL)	Synovial/Serum Concentration
Total protein	–	6.641 ± 0.602 (5.660-8.460)	1.251* ± 0.584 (0.220-2.750)	19%
α1-Acid glycoprotein	36,000	0.012 ± 0.005 (0.002-0.021)	0.003* ± 0.002 (0.001-0.007)	25%
Haptoglobin	40,000	0.033 ± 0.015 (0.007-0.078)	0.005* ± 0.004 (0.000-0.015)	15%
IgG	25,000 and 55,000	1.478 ± 0.333 (0.824-2.530)	0.192* ± 0.102 (0.0301-0.438)	13%
Albumin	66,400	3.812 ± 0.322 (3.369-4.511)	0.812* ± 0.363 (0.150-1.687)	21%
Transferrin	76,000	0.420 ± 0.099 (0.230-0.605)	0.063* ± 0.037 (0.010-0.164)	15%
Ceruloplasmin	130,000	0.004 ± 0.002 (0.001-0.010)	0.001* ± 0.001 (0.000-0.004)	25%
IgA	150,000	0.054 ± 0.052 (0.000-0.137)	0.003* ± 0.004 (0.000-0.016)	6%

The values within parentheses represent the minimum and maximum obtained for each protein.
* According to the paired *t* test, significantly ($P < .01$) less than the corresponding value in serum.

sufficient water to make 1 L. Both buffers were adjusted to pH 8.5. The SF and serum samples (5 μL) were prepared in 40 μL of Dulbecco phosphate-buffered saline solution and 10 μL of gel mix (10% water, 2% SDS, 5% 2-mercaptoethanol, 10 m*M* ethylenediaminetetraacetic acid, 20 m*M* Tris phosphate [pH 7.4], 5% glycerol, and 0.001% bromophenol blue as the dye). The electric current for the 8 × 8-inch vertical gel electrophoresis system was programmed at 35 and 50 mA, while samples were in the stacking and running gel, respectively. After fractionation, the gel was stained in 0.2% Coomassie brilliant blue solution for 10 minutes. Next, the gel was destained in a solution containing 250 mL methanol, 100 mL acetic acid, and 650 mL water until protein fractions appeared clear. The concentrations of these protein fractions were determined, as described by Fagliari et al. [7], using a digital densitometer (9301PC Shimadzu, Tokyo, Japan). Proteins were identified using reference markers (Sigma Chemical Co., St. Louis, MO) with molecular weights of 200,000; 116,000; 97,000; 66,000; 55,000; 45,000; 36,000; 29,000; 24,000; 20,000; 14,200; and 6,500 Da. In addition, the purified proteins (Sigma Chemical Co., St. Louis, MO) albumin, haptoglobin, ceruloplasmin, and transferrin were also used.

The results of the four collection times were statistically evaluated by the repeated-measures variance analysis at each time, followed by Tukey test at 5%. The means were compared by the paired *t* test at 1%.

3. Results and Discussion

This is the first study to determine the synovial proteinogram (Fig. 1) of healthy horses. The concentrations of the serum and SF proteins determined over time are given in Table 1. The values obtained were not significantly different during the sampling period. Table 2 shows that the mean protein concentrations of the SF were lower compared with the serum fraction and thus were considered suitable to determine the mean physiological concentrations in serum and SF of each of the proteins identified.

Table 2 displays the percentages of each of the proteins identified in SF in relation to the serum concentrations of the horses. It was noted that, under normal conditions, as high as 25% of some proteins such as α1-acid glycoprotein and ceruloplasmin cross the synovial membrane to the interior of the joint capsule. This finding may be explained by Iwanaga et al. [8], who reported that the greater the molecular weight of a plasma protein, the lower its capacity to cross the synovial membrane.

The data presented in this study confirm that heavier proteins, such as IgA, appear in lower relative concentrations in the SF of healthy joints. However, ceruloplasmin, a protein considered heavy (130,000 Da), showed a relative concentration equivalent to that of α1-acid glycoprotein with a molecular weight of approximately 3 times less. The reasons for this differentiated diffusion into the intracapsular medium shall be considered in future studies.

Ceruloplasmin is an APP responsible for the transport of approximately 95% of plasma copper, with each molecule carrying eight atoms of this mineral element [9]. Knight et al. [10] found that copper deficiency is responsible for problems of conformation, flexural and angular deformities, osteochondrosis, and physeal dysplasia in horses. Biochemical studies have shown that copper helps to form the cross-link between cartilage and subchondral bone tissue, where its deficiency can predispose to osteochondral fragmentations [11].

The need for copper for adequate intra-articular metabolism along with molecular conformation might explain the fact that ceruloplasmin, despite being a large molecule, is present in relatively high concentrations in the SF of healthy horses.

4. Conclusions

The analysis of the proteins present in horse SF gives relevant information to help in the diagnosis of joint diseases. Therefore, it is necessary to establish the physiological values of these substances to be able to identify altered states with high accuracy.

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