La bursitis del olecranon es un desorden articular de perros confinados a sueños duros o que sufren traumas repetidos sobre la articulación del codo. Aunque la bursitis del olecranon es un problema cosmético no doloroso, puede ser recurrente y presentarse con algunas complicaciones clínicas, tales como infecciones y fibrosis. Los tratamientos de la bursitis del olecranon mediante aspiración de fluidos y cirugía, son estrategias frecuentemente inefectivas, debido a que el rellenado bursal, las infecciones bacterianas y la dehiscencia de las heridas, son problemas comunes. El plasma rico en plaquetas (PRP), es una fracción sanguínea autóloga derivada del plasma que es rica en plaquetas y factores del crecimiento, los cuales son capaces de promover la reparación de tejidos. El PRP ha sido usado en el tratamiento de lesiones de tejidos blandos y duros. En este contexto, reportamos el caso de una curación de bursitis del olecranon crónica bilateral en un perro tratado con plasma rico en plaquetas autólogo. Tal y como ha sido reportado en humano y equino, nuestras observaciones clínicas indican que el uso de PRP tiene un efecto favorable en la curación de la bursitis in caninos.

Palabras clave: plasma autólogo rico en plaquetas, bursitis olecranon canino

Recibido: 16 de Abril de 2015
Evaluado: 30 de Junio de 2015
Aceptado: 31 de Julio de 2015

USO DEL PLASMA AUTÓLOGO RICO EN PLAQUETAS EN LA CURACIÓN DE LA BURSITIS BILATERAL DEL OLECRANON EN UN CANINO. REPORTE DE CASO.

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USE OF AUTOLOGOUS PLATELET RICH PLASMA IN BILATERAL OLECRANON BURSITIS HEALING IN CANINE. A CASE REPORT.

Yelica López, Eduard Martínez, Juan Carlos White

Abstract

Olecranon bursitis is an articular disorder observed in dogs confined to hard surfaces or suffering repeated trauma on elbow joints. Although olecranon bursitis is a painless cosmetic problem; it can be difficult to resolve and it can concur with some clinical complications, such as infections and fibrosis. Treatments of olecranon bursitis through fluid aspiration and surgery are frequently ineffective approaches, since bursa refilling, bacterial infections, and wound dehiscence are common problems. Platelet rich plasma (PRP) is an autologous blood derived plasma fraction rich in platelet and growth factors, which are able to promote tissue repair. PRP has been used for the treatment of soft and hard tissues injuries. In this context, we report a case of chronic bilateral olecranon bursitis healing in a dog treated with autologous platelet rich plasma. Such as, it has been reported in human, and equine, our clinical observations indicates the favorable effect of using PRP in the healing of bursitis in dogs.

Keywords: autologous plasma rich platelet, olecranon bursitis canine.
USO DO PLASMA AUTÓLOGO RICO EM PLAQUETAS NA CURA DA BURSITE BILATERAL DO OLÉCRANO EM UM CANINO. RELATO DO CASO.

Yelica López1, Eduard Martínez2, Juan Carlos White3

Resumo

A bursite do olécrano é uma desordem articular de cães confinados a pisos duros que sofrem repetidos traumas da articulação do cotovelo. Embora a bursite do olécrano é um problema cosmético indolor, pode ser recorrente e apresentar-se com algumas complicações clínicas, tais como infecções e fibrose. Os tratamentos da bursite do olécrano feitos pela aspiração de fluidos e cirurgias são estratégias frequentemente ineficazes devido ao acolchado bursal as infecções bacterianas e a deiscência das feridas, que são problemas comuns. O plasma rico em plaquetas (PRP) é uma fracção sanguínea autólogo derivada do plasma que é rico em plaquetas e em factores do crescimento, que são capazes de promover a reparação de tecidos. O PRP tem sido usado no tratamento de lesões de tecidos macios e duros. Neste contexto, é relatado o caso de uma cura de bursite do olécrano bilateral crônica num cão tratado com plasma autólogo rico em plaquetas. Como foi relatado em humanos e cavalos, nossas observações clínicas indicam que a utilização do PRP tem um efeito favorável na cura da bursite em caninos.

Palavras chave: plasma autólogo rico em plaquetas, canino, bursite olecrano.
1. - Introduction

Olecranon bursitis, also known as elbow hygroma, is a sub-cutaneous serosa cavity caused by a sustained blunt trauma over the olecranon surface associated with confinement to hard surfaces (Rice, 2001). Although, bursitis is a painless cosmetic problem (Fossum, 2013), can be present with some complications such as bacterial infections (Maxwell, 2011) and chronic recurrence (Pavletic, 2010). A conservative treatment consisting in housing animals in soft floors and using compressive bandage, is recommended (Fossum, 2013). Joint aspiration (Dehn & Asprey, 2013) and surgical treatment are usually ineffective therapeutic approaches due to the risk of wound dehiscence (Pavletic, 2010) and introducing infections microorganism into the bursal cavity (Maxwell, 2011). Platelet rich plasma (PRP) is an autologous blood derived plasma fraction containing an elevated platelet concentration (Civinini, et al, 2013). Recently, platelet rich plasma (PRP) have been used as a rich source of growth factors promoting tissue repair (Amable, et al, 2013), for the treatment of soft and hard tissue injuries, such as ostheoarthritis (Kon, et al., 2011), orthopedic disorders (Hsu, et al., 2013), and soft tissues damages (Mei-Dan, Lippi, Sánchez, Andia & Maffulli 2010). While in human (Anitua, et al., 2015; Vavken, et al., 2015) and equines (Textor, Willits & Tablin, 2013; Bosch, van Weeren, Barnevel and vanSchie, 2011), PRP therapy has shown to be effective for the treatment of skeletal injuries, however to date, the benefits on dog’s chronic olecranon bursitis healing has not be reported. The objective of the present study is to report the effect of PRP on the of chronic olecranon bursitis healing in a dog.

2. - Case report.

The patient’s owner approved the consents regarding to participate in the experimental treatment, as well as for publishing the case report. A 14 month-old, male German shepherd weighing 35 kg was presented to consult with a history of olecranon bursitis recurrence 10 days after olecranon bursa surgical treatment. Medical history in figure 1 summarizes in a timeline the symptomatology and clinical interventions, before and after PRP treatment. Patient was transferred from another clinician, who previously performed the surgical treatment. Thirty five days after surgery, we received the patient.

Upon clinical evaluation, dog was in good condition (body condition scoring of 3.5 on a scale of 5) (Merck, 2012). Two prominent globular masses were observed in both elbows. The transverse diameters of the elbow’s joins were 26 cms in the right left arm and 30 cms in the left one (fig.1).In the right elbow, the mass measured 8 cm length and 12 cm wide, while in the left one was 7 cm length and 10 cm wide. On palpation, the masses appear as soft, painless and fluctuating structures, had a smooth surface, with regular edges and soft consistency. The diagnosis of olecranon bursitis was made from the physical examination.

Figure 1
Laterocaudal images of olecranon bursitis in a 14 months old German shepherd dog, before treatment with autologous PRP.

Font: López, Martínez y White, 2015
Arrows indicate the scars of previous surgical intervention.
To perform synovial liquid aspiration, sedation was induced by administration of acepromazine at 1% (0.01 mg/kg IM); hair was clipped and the skin was disinfected 3 times using sterile gauze impregnated with Betadine® and Gerdex®. The bursal fluid aspiration, was done by two doctors of veterinary medicine (DVM). Once the dog sedative state was verified by muscular relaxation, reduction of body activity and palpebral membrane relaxation, patient was located into a supine position; one DVM supported and flexed the elbow, and stretched the skin on the site of drainage.

The bursal liquid was aspirated using a 22-gauge 1 inch needle. From the right elbow were obtained 35 ml of serous liquid, and from the left one 40 ml; in both cases liquid had a hemorrhagic aspect. Gauze was applied at site of needle withdrawal to reduce bleeding. Since, extensive right elbow wound dehiscence occurred together with a secondary complication with cavitary myiasis and bacterial infection, non-steroidal anti-inflammatory analgesic drug (NSAIA) Carprofen 4 mg/kg orally for 5 days was prescribed, together with antibiotics Cefadroxilo 23 mg/kd/12h orally for 10 days. Recurrence of bursitis was observed 15 days later, in both elbows (Fig. 2).

**Figure 2**
Timeline of clinical history and clinical intervention of a German shepherd dog suffering bilateral olecranon bursitis and treated with autologous PRP infusion.

Once again, in aseptic conditions elbow bursal cavity was drainages. From right elbow, 8 ml of fluid were collected and from left on 10 ml were obtained, in both cases fluid was serose and had hemorrhagic aspect. For diagnostic propose, a small sample of fluid was smeared for synovial liquid cytology analysis.

The slides were air-dried, fixed and stained with Hemacolor® rapid staining kit (Merck Millipore catalog number 111661) (Fig. 3 and 4). The same say, blood sample was collected to prepare autologous PRP, this procedure is described in detail below. Pictures of dog arms were taken before and after treatment (Fig. 5). Ultrasound images of the elbow joint were taken two months after treatment using a Wokan 33-4D B-ultrasound Diagnostic Equipment, multifrequental CA20R 4.5 MHz, 5.0 MHz, 5.5 MHz (C1-8/ 20R/5.0Mhz micro convex probe) (Fig. 6).

**Figure 3**
Synovial liquid sample of the elbow joint from a dog, two months after elbow bursitis surgical treatment.

A) Abundant erythrocytes are present, activated macrophages (arrow) and lymphocytes (dashed arrows) were also observed. B) Undifferentiated mononuclear cell and C) eosinophil. The synovial fluid smear shows general feature of inflammatory exudates. Hemacolor (Merck, 2012) stain, original magnification 1000X
Figure 4.
Synovial liquid sample from the elbow joint from a dog, two months after PRP treatment showing non-inflammatory exudates.

Font: López, Martínez y White, 2015
Arrows indicate echinocytes (spiculated red blood cells), which frequently are artifacts. Hemacolor (Merck) stain, original magnification 1000X.

Figure 5
Right and left elbow joint appearance, before and after PRP treatment.

Font: López, Martínez y White, 2015
Arrows indicate elbow macroscopic aspect: Upper left panel indicates a noticeable swelling of elbow (bursitis), before surgery treatment. Upper right panel shows the recurrence of elbow bursitis at 10 days after surgery. Lower left panel shows the aspect of elbow joint at 21 days after single PRP treatment. Lower right panel shows the elbow aspect 5 months after treatment, no apparent olecranon bursitis was noticed.

Font: López, Martínez y White, 2015
Arrows indicate elbow macroscopic aspect: Upper left panel indicates a noticeable swelling of elbow (bursitis), before surgery treatment. Upper right panel shows the recurrence of elbow bursitis at 10 days after surgery. Lower left panel shows the aspect of elbow joint at 21 days after single PRP treatment. Lower right panel shows the elbow aspect 5 months after treatment, no apparent olecranon bursitis was noticed.

Figure 6
Longitudinal ultrasound images of elbow joint, after PRP treatment.

Font: López, Martínez y White, 2015
Upper panel (A) right elbow, lower panel (B) left elbow (B). Arrow indicates bursa cavity. No echogenic materials or intrarticular effusion were observed into the recess. The synovial membrane was not identified.

2.1.-Blood collection and PRP preparation protocol.
To collect blood, dog was restrained, elbow was extended, and soft pressure was applied back to the elbow to present the vein and maintain it filled with blood. Disinfection of skin was done with gauze impregnated with a mixture of 50% ethanol and 50% betadine®. To obtain PRP we follow the double centrifugation tube method described by Perazzi, Busetto, Martinello et al., (2013) with some modifications. Briefly, 21 ml of blood was collected and placed in two 15 ml centrifuge
tubes (labeled tube A and tube B) (10.5 ml in each one) containing 10 microliters of heparin (1.000 U.I/mL GENCR Pharmaceutical C.A).

Tubes were centrifuged at 3000 rpm during 20 minutes to separate blood components in 3 fractions: red blood cells (bottom), buffy coat (middle) and platelet poor plasma (PPP) (supernatant). From tube A were obtained 5 ml of PPP, whereas from tube B were obtained 5.5 ml of PPP. Gentle PPP was removed and placed in two sterile tubes; these tubes were maintained at 4ºC for use in a later step.

The buffy coat fractions containing mononuclear cells and platelets were carefully collected using a 1000 µL pipette tip, placed in a two 15 ml centrifuge tube (BD Falcon™) and resuspended in 1 ml of the PPP remaining. These tubes were centrifuged at 1300 rpm for 15 min at room temperature (20ºC) in a DINAC® Centrifuge Cat. N° 420093; obtaining a PRP pellet precipitates at the bottom of the tube and PPP on the top. PPP (containing mononuclear cells) was removed from each tube, and remaining PRP pellets were resuspended in 5 ml of PPP mixed and vortexed (Eppendorf Mix Mate® vortex mixer), tubes were maintained at 4ºC until be infused within dog elbow joint.

For PRP infusion, tubes were pre-warmed at 37ºC in an incubator for 10 minutes, then mixed again and drawn up with a syringe for elbow intra-articular injection. All the procedures were performed under sterile controlled conditions. Blood samples were taken before treatment and one month after treatment for complete blood count (CBC), blood smear and blood chemistry analysis, these samples were sent to a private laboratory for analysis (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Hemogram Results</th>
<th>Reference Values</th>
<th>Before treat</th>
<th>After treat</th>
</tr>
</thead>
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<tr>
<td>Hematocrit (%)</td>
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<td>Hemoglobin (g%)</td>
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<td>14.6</td>
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<td>WBC (mm³)</td>
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<td>8600</td>
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<td>Platelets (mm³)</td>
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<td>180,000</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
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<td>Monocytes</td>
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<tr>
<td>Eosinophils</td>
<td>0.58</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Font: white blood cells are reported as the absolute differential value. *Reference values serum biochemistry. (Research Animal Resources, University of Minnesota,, 2009.

2.2. -PRP injection

PRP fractions were injected into single, aseptically prepared surface landmark areas on both elbows. To achieve PRP injection, dog was sedated by administration of acepromazine at 1% (0.01 mg/kg IM). Once the dog sedative state was verified by muscular relaxation, reduction of body activity and palpebral membrane relaxation, patient was located into a supine position; the elbow was holded and flexed, skin was stretched and the target skin area for injection was outlined using a non-toxic marker pen. In each bursal cavity were infused 5 ml of autologous PRP. Soft pressure was done on the site of injection using gauze impregnated with Betadine®. Daily observation post-treatment was done by the Veterinarian and dog’s owner.
To verify screen general health status of the patient, one month after treatment blood sample was taken for complete blood count (CBC), blood smear and blood serum biochemistry analysis (see table 1).

3. -Results

Based on the history, clinical findings, and clinical data results, a diagnosis of chronic olecranon bursitis was made. A time line of clinical events are summarized in figure 1. In figure 2, we show images of appearance of right and left olecranon joints in a German Shepard taken before PRP treatment. Scars of a previous surgical treatment are observed. Blood analysis before and one month after PRP treatment (see table 1) reveals reported values within normal references values for the specie.

The Cytological study of synovial fluid before PRP treatment showed abundant erythrocyes, which is associated with hemarthrosis (see fig 3). Additionally an inflammatory cell infiltration was observed. Isolated eosinophils and undifferentiated mononuclear cells were also seen, infectious organisms were not observed. On the contrary, after PRP treatment, we did not observe inflammatory exudates, however some echinocytes (spiculated red blood cells) and isolated non-reactive lymphocytes were observed (see fig 4).

At day 10 post-treatment, dog showed sensible reduction in bursal size, on palpation and we could not perceive fluid accumulation inside bursal cavity. Over time, the dog recovered satisfactorily, two months after treatment an ultrasound evaluation of both elbows was made. The sonographic study of elbow joint revealed a normal condition of synovial membrane, joint capsule, bone surface and bursa (see fig. 6). More than 5 months after treatment, no evidence of olecranon bursitis recurrence was observed (see fig 5).

4. -Discussion

The use of platelet rich plasma (PRP) has been proposed as a treatment for orthopedic injuries (Hernández, et al., 2012; Murray, 2006; Schneppendahl, et al., 2015). The rationale is the storage within platelets of soluble factors with pro-angiogenesis and anti-inflammatory capacity, as well as factors, which promote homing and proliferation of local progenitors cells, resulting in tissue repair (reviewed in Amable, et al., 2013).

In this report, one single injection of autologous PRP was sufficient to control inflammatory signals and avoid bursitis recurrence. Some locally active growth factors stored as granules within platelets are platelet-derived growth factor(PDGF),vascular endothelial growth factor(VEGF),epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDGDF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1) (reviewed in Lacci & Dardik, 2010).

Once platelets aggregate nearby to injury sites, for at least seven days post-injury, these factors are releasing and promoting cell proliferation, migration and angiogenesis by targeting different cell sub-population including myocytes, endothelial cell, tendon cells, mesenchymal stem cell, chondrocytes, osteoblast and fibroblast (reviewed in Amable, 2013).

In our case, we used PRP as an experimental treatment in a dog with clinical history of recurrence of bilateral olecranon bursitis, after surgical treatment. A limitation in our report is that we did not measure the platelet concentration and amount of growth factors in the fraction of PRP obtained from the patient, and this is important information for interpreting clinical outcomes. However, our preliminary results indicate
that a single dose of PRP directly injected in the injure area is effective to heal chronic bursitis in dogs. Such as, it has been reported in human (Sampson, et al., 2008), and horses (Textor, et al., Willits, & Tablin, 2011), our clinical observations indicates the favorable effect of using PRP in treatment of skeletal injuries in dogs.

5. -Conclusion

Platelet rich plasma (PRP) has emerged as a safe and inexpensive autologous source for tissue repair in several animal model of orthopedic diseases in vivo (Murray, et al., 2006; Hernández et al., 2012; Schneppendahl, Jungbluth, Lögters, et al., 2015), and in this case, resulted in long term relapse of olecranon bursitis symptoms. Because the PRP therapy is performed following a simple protocol, the blood to obtain PRP is collected by using a non-invasive procedure, and there are no risk of cross immunoreactivity; autologous PRP is growing as an attractive therapy for tissue repair; Although, randomized controlled studies are needed, platelet rich plasma might be considered as a candidate for the treatment of dogs with recurrent olecranon bursitis.

6. - Conflict of interest

The Authors declares that there is no conflict of interest.

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