UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Escuela de Doctorado

Facultad Veterinaria

PROGRAMA DE DOCTORADO EN INVESTIGACIÓN EN BIOMEDICINA

Uso del Plasma Rico en Plaquetas en Osteoartritis y fracturas en la especie canina: Aspectos funcionales y de seguridad

Sergio López Barbeta

Las Palmas de Gran Canaria 2019

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
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Programa de Doctorado Investigación en Biomedicina

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INFORMA:

Que la Comisión Académica del Programa de Doctorado de Investigación en Biomedicina, en su sesión de fecha 9 de julio de 2019 tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada ***"Uso del Plasma Rico en Plaquetas en osteoartritis fracturas en la especie canina: Aspectos funcionales y de seguridad"*** presentada por el doctorando **D. Sergio López barbeta** y dirigida por el **Dr. José Manuel Vilar Guereño.**

Que la citada tesis doctoral reúne todos los requisitos exigidos por la normativa de este programa de doctorado y de esta universidad, para ser tramitada como tesis doctoral.

Y para que así conste, y a efectos de lo previsto en el Artº 11 del Reglamento de Estudios de Doctorado (BOULPGC 7/10/2016) de la Universidad de Las Palmas de Gran Canaria, firmo el presente informe en Las Palmas de Gran Canaria, a nueve de julio de dos mil diecinueve.

MONTOYA
ALONSO JOSE
ALBERTO -

J. Alberto Montoya Alonso

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
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TESIS DOCTORAL

Uso del Plasma Rico en Plaquetas en Osteoartritis y Fracturas en la Especie Canina: Aspectos funcionales y de seguridad

Doctorando

Sergio López Barbeta

Director

José Manuel Vilar Guereño

Las Palmas de Gran Canaria, 8 de julio de 2019

UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA
Facultad de Veterinaria

José Manuel Vilar Guereño, con **DNI** Doctor en veterinaria, profesor titular de cirugía veterinaria de la Facultad de Veterinaria de la Universidad de Las Palmas de Gran Canaria.

INFORMA:

Que D. **Sergio López Barbeta** Graduado en Fisioterapia, ha realizado, bajo mi dirección y asesoramiento, el presente trabajo de tesis doctoral titulado: **"Uso del Plasma Rico en Plaquetas en osteoartritis y fracturas en la especie canina: Aspectos funcionales y de seguridad"** que considero reúne las condiciones reglamentarias y de calidad científica necesarias, para su presentación y defensa, para optar al título de Doctor por la Universidad de Las Palmas de Gran Canaria.

Lo que firmo, a los efectos oportunos, en Arucas (Las Palmas) a nueve de julio de dos mildiecinueve

José Manuel Vilar Guereño
Profesor titular de cirugía veterinaria

35413 - ARUCAS - LAS PALMAS - ESPAÑA

A mis familiares, en especial a mis padres y abuelos, por darme la educación y valores necesarios para ser un buen hombre.

A mi mujer, por ser el faro que me guía en medio de la tormenta.

AGRADECIMIENTOS

La vida, tal y como la entendemos; se puede resumir en diferentes etapas que conforman un recorrido personal y transmutable, personal porque sólo cada uno de nosotros puede experimentar lo que siente, vive y conoce, transmutable porque cada experiencia vivida (buena y mala) nos trasforma, nos amolda y nos hace avanzar en el largo camino de la vida.

El doctorado, es una de esas experiencias férreas; que marcan un paso en la vida de toda persona que se inicia en el campo del conocimiento, y que busca, de una manera palpable el avance propio y porque no de toda la humanidad, aportando un granito de arena en lo que tiene que ser un movimiento colectivo.

En mi caso, siento que una etapa de mi vida se cierra, pero que otra se abre; que he llegado a poder vislumbrar una luz de conocimiento sin que esta cegara mis ojos, con la firme convicción de seguir subiendo peldaño a peldaño, la sinuosa escalera que me aleja de las sombras de la ignorancia.

Quisiera pues, agradecer de una manera íntima y personal a todas las figuras que han sido y son importantes en esta maravillosa etapa de mi vida; en primer lugar, agradecer a José Manuel Vilar, mi tutor, maestro y compañero que la diosa fortuna tuvo el

beneplácito de poner en mi camino y que sin él nada de esto hubiera sido posible.

A mis padres, Enrique y Tere, por confiar su tiempo, dinero e incluso ilusiones frustradas en que no dejara de avanzar y luchar por conseguir mis sueños.

Por último, a mi mujer Itzel, quién sostiene como un pilar la bóveda de nuestra vida, soportando juntos las enormes cargas que ésta reparte diariamente sobre nosotros, con paciencia, amor y sabiduría a partes iguales, siendo la pieza maestra sin la cual nada tendría sentido.

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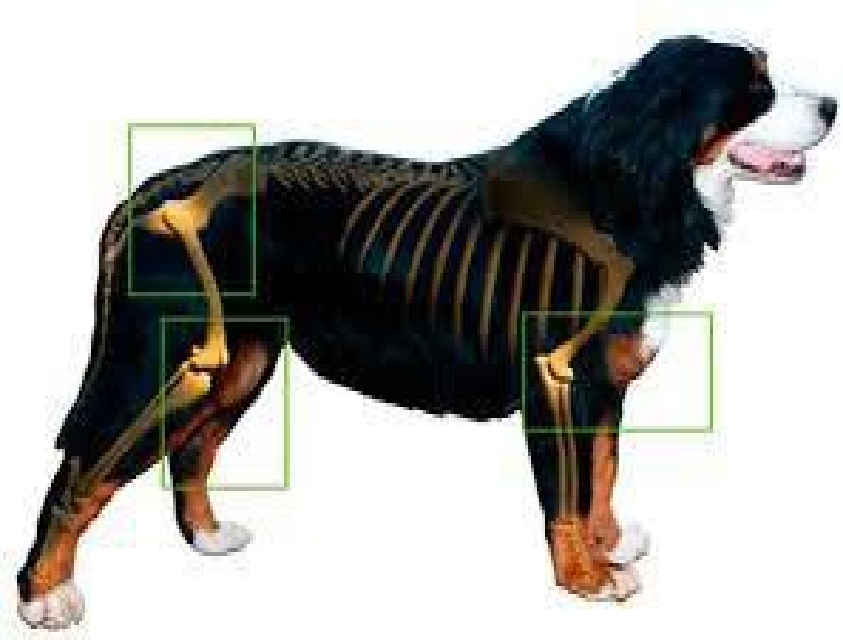


INTRODUCCIÓN

1. INTRODUCCION Y OBJETIVOS

La osteoartritis (OA) es una patología cuya morbilidad en la especie canina aumenta constantemente, sobre todo debido al incremento de la esperanza de vida en esta especie.

La OA causa dolor de intensidad variable lo que se traduce en alteraciones en la locomoción que se traducen en cojera. La evaluación de dicha disfunción suele valorarse tradicionalmente mediante la inspección visual y, por lo tanto, subjetiva.

La radiología ha sido, dentro de los sistemas de diagnóstico por imagen, la técnica más usada. Aunque efectiva, por desgracia no existe una gran correlación entre los hallazgos lesionales por radiología y los signos clínicos. Otras técnicas más actuales como la Tomografía Computarizada y la Resonancia Magnética aportan muchos más datos, sobre todo por sus características tomográficas, pero la correlación sigue sin ser alta.

Para valorar la funcionalidad, se han creado una serie de escalas numéricas visuales que tratan de cuantificar aspectos funcionales, comportamentales e incluso radiológicos. Validadas muchas de ellas, son susceptibles de sufrir variaciones en la valoración dependiendo del observador que las realice, e incluso variaciones intraobservador, por lo que la subjetividad está presente.

En este sentido, cobran especial relevancia las diferentes técnicas biomecánicas para analizar las alteraciones locomotrices. No solo para establecer de forma

objetiva la presencia o no de cojera, sino incluso a la hora de cuantificar la eficacia de un tratamiento en un animal con cojera y poder compararlo con otros en términos de potencia y duración del efecto.

Dentro de las técnicas cinéticas, las fuerzas de reacción del suelo (GRF) están consideradas como el "Gold Standard" en la evaluación del movimiento. Estos datos se han proporcionado clásicamente mediante el uso de la plataforma de fuerza.

En tiempos más recientes, la plataforma de presión, gracias a la presencia de miles de sensores, es capaz de proporcionar muchos más datos cinéticos, entre los que queremos destacar el centro de presiones (COP). El estudio de la trayectoria del COP en los miembros de perros afectados de cojera unilateral por OA constituirá por lo tanto nuestro primer objetivo, y el primer artículo aportado en la presente tesis Doctoral.

Dentro de las estrategias desde el punto de vista médico para tratar la cojera, no solo debida a AO sino también por otras patologías; se han venido utilizando de forma clásica los AINES; no carentes de efectos secundarios. Actualmente, se investigan alternativas terapéuticas lo más inocuas y eficaces posibles. En este sentido, las terapias regenerativas-reparativas están adquiriendo un auge importante en los últimos años. De ellas, las células madre mesenquimales se han postulado como una terapia eficaz en los casos de OA, lo que nos ha llevado a publicar nuestro segundo artículo, en este caso de revisión, en el que se discuten estos aspectos. Otro tratamiento muy actual dentro de las terapias regenerativas-reparativas, es el elaborado a partir de

Plasma rico en Plaquetas (PRP) y sus derivados como el Plasma rico en Factores de Crecimiento (PRGF); Ya utilizado de forma habitual en el tratamiento de OA, su eficacia para acelerar la consolidación de fracturas en la especie humana no ha sido apenas contrastada científicamente, y en el caso de la especie canina, no hay publicaciones al respecto, lo que nos lleva a plantearnos el segundo objetivo, que consiste en valorar su eficacia en fracturas en perros.

Por último, un aspecto que preocupa desde el punto de vista de la presentación de efectos secundarios en animales tratados con PRP, es sobre todo el supuesto efecto ergogénico atribuido a este producto. Dicha cuestión nos lleva a plantearnos como tercer y último objetivo un estudio en el que valorar si a dosis terapéuticas, el PRP presenta dicho efecto.

Definición de osteoartritis.

La osteoartritis (OA) ha pasado a ser una de las enfermedades con mayor prevalencia, y por tanto ha aumentado enormemente su casuística tanto en medicina humana como en veterinaria (Rychel, 2010; Malek y cols, 2012). Este hecho obedece a dos factores principales: uno, el hecho de que la longevidad está aumentando de forma significativa en los últimos años; dos, porque por desgracia la obesidad también lo hace (Lawrence y cols, 2008).

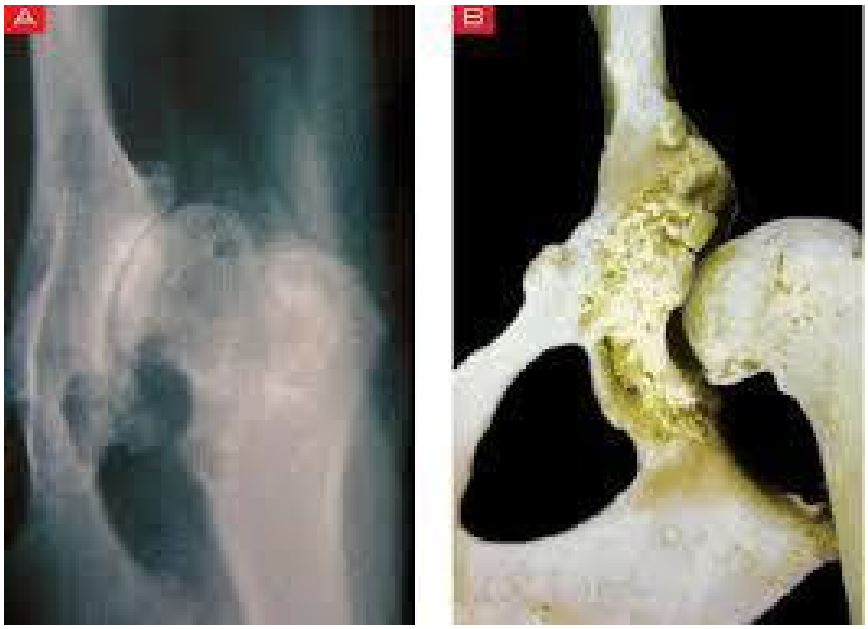
Ya en el campo de la veterinaria, y en concreto en la especie canina, se ha determinado su incidencia en un 20 % en animales adultos, llegando al 80% en geriátricos (Cuervo y cols, 2016).

En los estadios iniciales, el cartílago articular comienza a aparecer rugoso, deja de ser brillante y según va avanzando la enfermedad aparecen fisuras cada vez más profundas llegando incluso a formarse ulceras (Chen y cols, 2013)

Este proceso en última instancia crea fricción entre los huesos; lo cual desencadena un proceso inflamatorio tanto agudo como crónico, presentando los tejidos en ultima instancia un marcado engrosamiento. Todos estos factores determinan una pérdida de funcionalidad (Bland, 2015; Cuervo y cols, 2015).

El inicio del proceso puede ser multifactorial ya que puede originarse por un traumatismo, herida, sobrecarga, desgaste, o bien asociado a un proceso de envejecimiento. Todos estos hechos, cambiaran la composición, estructura y propiedades de los tejidos que forman dicha articulación, limitando el apropiado funcionamiento del cartílago articular (Cuervo y cols, 2015).

El diagnóstico de esta patología se suele realizar mediante parámetros de tipo clínico y por radiología. Desgraciadamente, existe una falta de concordancia entre los hallazgos clínicos y radiológicos, lo que exige el uso de metodologías más objetivas (Dougados, 2005; Martel-Pelletier y cols, 2012).



ARTICULACIONES MÁS FRECUENTEMENTE AFECTADAS DE OA Y PATOLOGÍA MÁS COMÚN

2. ARTICULACIONES MÁS FRECUENTEMENTE AFECTADAS DE OA Y PATOLOGÍA MÁS COMÚN

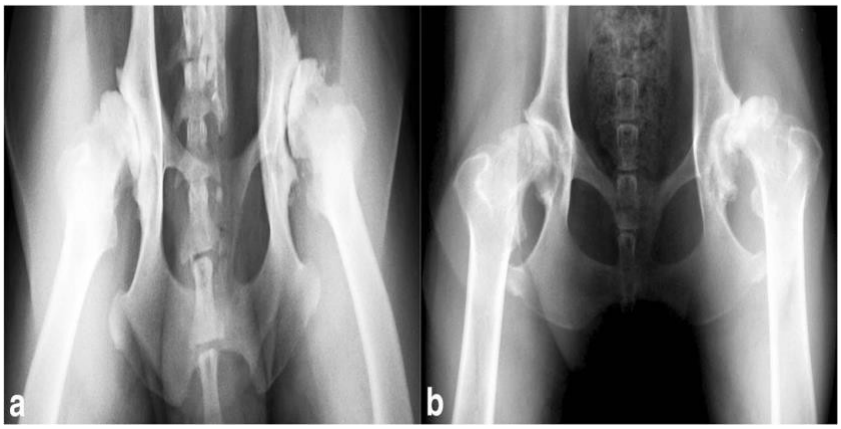
En el ámbito de la clínica de pequeños animales, sobre todo en perros, las articulaciones más afectadas por OA son el codo, la cadera y la rodilla (Malek y cols, 2012). Su origen puede ser múltiple, como defectos del desarrollo o incluso traumatológicos.

2.1. articulación coxofemoral

Displasia cadera

La displasia de cadera (DC) constituye la enfermedad más frecuente en dicha articulación en la especie canina, sobre todo en perros de razas grandes y gigantes (Manera y cols, 2019). Existen aspectos muy importantes en esta patología a considerar, ya que, al ser un problema hereditario, y del desarrollo, los animales es posible que no manifiesten sintomatología hasta que son adultos; esto hace que las medidas para impedir la extensión de la enfermedad excluyendo a los animales descartados como reproductores lleguen demasiado tarde (Rodríguez, 2003).

Esta patología además se caracteriza por una falta de congruencia entre las superficies articulares; por lo que la consecuencia más directa, es que las fuerzas se distribuyan de un modo irregular en la articulación. En definitiva, la articulación se vuelve inestable lo que hace que se instaure un proceso osteoartrítico (Cardinet, 1997).



Radiografías correspondientes a un caso de displasia de grado D (a) y grado E (b). Se puede observar el aplanamiento del acetábulo, cabeza del fémur triangular en vez de esférica, esclerosis en el borde acetabular, y fragmentos óseos (osteofitos).

2.2. articulación del codo

Displasia de codo

La displasia de codo (DC) es un síndrome que engloba diferentes patologías en dicha articulación que en general, supone una incongruencia entre las superficies articulares que la forman, lo cual conlleva una inestabilidad que en último termino provocará la aparición de la osteoartritis como enfermedad articular degenerativa, sobre todo si no se aplica un tratamiento efectivo (Manera y cols, 2019). Las patologías más conocidas que provocan displasia de codo son la fragmentación del proceso coronoides, la osteocondritis disecante y por último el proceso ancóneo no unido (Tobias y Johnston, 2011).

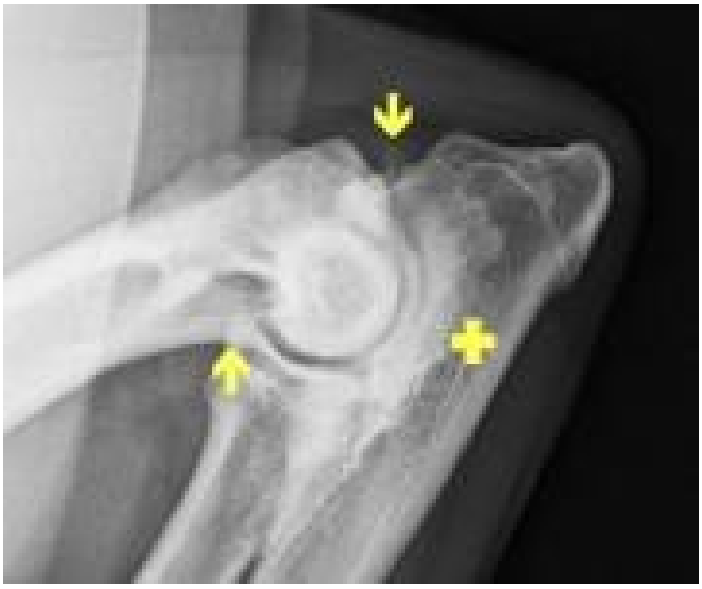
La DC es de carácter hereditario, y multifactorial. Lo cierto es que puede desencadenarse por anomalías en el desarrollo de los centros de osificación,

anomalías en alguno de los huesos largos que forman el codo o una combinación de estos (Durante, 1998; Kirberger y Barr, 1998).

Bassethound con displasia de codo. En esta patología es típica la abducción del miembro afectado.

https://www.basset.net/boards/general-basset-hound-discussion/45337-basset-elbow-dysplasia-surgery-3.html

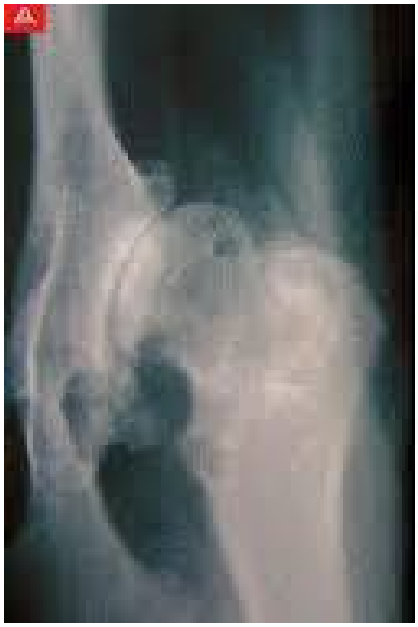
Esta patología es típica de razas grandes y gigantes. Es conveniente resaltar que afecta 3 veces más a machos que a hembras (Durante y Brusa, 1998; Kirberger, 2003; Komsta y cols, 2008).



Osteoartritis avanzada con neoformación ósea (flechas) así como esclerosis bajo las superficies articulares (cruz). https://www.acvs.org/small-animal/canine-elbow-dysplasia

2.3. articulación de la rodilla

Dentro de esta articulación, debemos decir que la rotura del CCL es el principal motivo de cojera de miembro pelviano, y una de las más comunes de las que pueden afectar a la rodilla en la especie canina (Whitehair y cols, 1993; Johnson y cols, 1994; Duval y cols, 1999). Este ligamento proporciona estabilidad en la articulación femorotibial; por lo tanto, su rotura determina inestabilidad, que, como se ha explicado anteriormente, produce en último término la generación de un proceso osteoartrítico (Elkins y cols, 1991; Innes y Barr 1998).



DIAGNÓSTICO DE LA OA

3. DIAGNÓSTICO DE LA OA

3.1. Técnicas de imagen

3.1.1. Radiología

Las técnicas de imagen radiológicas son pruebas de gran utilidad, pues además de realizar un diagnóstico preciso de la dolencias anteriormente citadas, nos permite descubrir en qué fase de la misma se encuentran, ya que; podemos identificar diferentes signos de la osteoartritis (Vilar y cols, 2013; Bland, 2015) y aunque es cierto, que se ha demostrado que la relación entre los signos clínicos y los signos radiológicos no son siempre correlativos, si es muy recomendable en este tipo de patologías realizarlas de manera regular.

Las pruebas de imagen como la RM y el TC nos permiten visualizar de manera más detallada las diferentes estructuras articulares sin que existan superposición de capas de tejido muscular, fascia o tejido ligamentoso, lo cual es una ventaja a la hora de evaluar los cambios en las estructuras óseas o irregularidades articulares (Fossum, 1999).

3.1.2. Artroscopia

La artroscopia es una de las técnicas diagnósticas más utilizadas en displasia de codo; no en vano, aunque sigue siendo una técnica invasiva, es menos agresiva que las artrotomias y proporcionan una visión de alta calidad en las estructuras intraarticulares

(Adrian y cols, 2017; Kim y Joo, 2018) quienes, sin embargo, en lesiones de la articulación femorotibial recomiendan el uso de la ecografía en combinación con la artroscopia para acceder a las zonas articulares de gran interés clínico.

3.2. Técnicas de evaluación funcional

Como hemos podido observar anteriormente, el dolor de intensidad variable es uno de los signos más habituales de la OA. Funcionalmente hablando, este dolor va a provocar una limitación que se traduce en una alteración del paso (cojera) de menor o mayor grado. Como se ha expresado anteriormente, la correlación entre los signos clínicos y radiológicos no son de gran valor por lo que la evaluación de la funcionalidad del miembro o los miembros afectos es de vital importancia (Gordon y cols, 2003).

Si tenemos en cuenta lo que hemos argumentado anteriormente, los métodos para evaluar el estado funcional de los animales se dividen en:

3.2.1. Métodos de análisis funcional subjetivos

La manera tradicional de valorar el dolor en los animales domésticos por parte de los profesionales veterinarios, ha sido teniendo en cuenta de forma subjetiva alteraciones comportamentales, así como la valoración del movimiento en si o incluso mediante la palpación o movilización pasiva. Estos factores (y otros más) han servido a muchos investigadores para elaborar escalas de valoración numéricas, en un intento por cuantificar la discapacidad funcional del aparato locomotor.

En primer lugar, resaltamos el cuestionario denominado Bristol Osteoarthritis in Dogs (BrOAD) (Innes and Barr, 1998) o el Texas A&M Client (Hudson y cols, 2004). Son escalas análogas validadas, cuya cuantificación depende del criterio del propietario del perro. También es muy conocido el denominado Canine Brief Pain Inventory (CBPI) ya que tiene en cuenta una gran cantidad de parámetros (Cimino Brown y cols, 2007.). En línea con esta escala se encuentra también la Helsinki Chronic Pain Index(HCPI), añade parámetros comportamentales (Hielm- Björkman y cols, 2003; Hielm- Björkman y cols, 2009; Walton y cols, 2013).

El HCPI se ha utilizado de forma continua para la evaluación del dolor crónico en perros con osteoartritis de rodilla, codo y cadera (Hielm-Björkman y cols, 2003; Hielm-Björkman y cols, 2009; Wernham y cols, 2011; Hielm-Björkman y cols, 2012; Heikkilä y cols, 2014).

En España, podemos destacar por su utilización, la escala denominada Bioarth®, ya que está basada en aspectos clínicos, radiológicos y aspectos comportamentales que deben ser cuantificados; la valoración obtenida puede aplicarse para medir por ejemplo la efectividad de un tratamiento instaurado en casos de cojera, ya sea de cadera, codo o rodilla (Vilar y cols, 2016).

FUNCTIONAL EVALUATION SCALE OF THE HIP

Pet Name

Owner name

A) FUNCTIONAL LIMITATION

1. CHANGES STANDING STILL: _____ ☐

(0) Standing normally/ (1) Leaning on one side/ (2) Resting the tip of its paw

(3) Non weight bearing on that limb

2. CHANGES STANDING UP: _____ ☐

(0) Standing up normally/ (1) Adopting different positions when standing up/

(2) Difficulty to rise/ (3) Does not stand up

3. LAMENESS AT THE BEGINNING OF EXERCISE: _____ ☐

(0) No lameness present/ (1) The lameness disappears when the dog moves (up to 10 minutes)

(2) The lameness does not disappear

4. LAMENESS AFTER WARM-UP (10 MINUTES): _____ ☐

(0) No lameness present/ (1) Mild lameness/ (2) Severe lameness

(3) Continuous non-weight-bearing lameness

5. LAMENESS DURING THE WALK: _____ ☐

(0) It can walk without difficulties/ (1) It often stops while going for a walk

(2) It can take just very short walks (less than 5 minutes)/

(3) It does not want to go for a walk

6. LAMENESS WHILE RUNNING AND PLAYING: _____ ☐

(0) It can run and play without difficulties/

(1) It runs and plays with difficulties or it gets tired easily

(2) It runs with lots of difficulties under a stimulus

(3) It neither runs nor plays under any stimulus

7. GOING UP THE STEPS: _____ ☐

(0) It goes up without difficulties/ (1) It goes up 16 steps (a flight) with difficulty

(2) It goes up 1 or 2 steps either a kerb, with difficulty

(3) It does not go up the steps

8. SMALL JUMPS 40-50 CM: _____ ☐

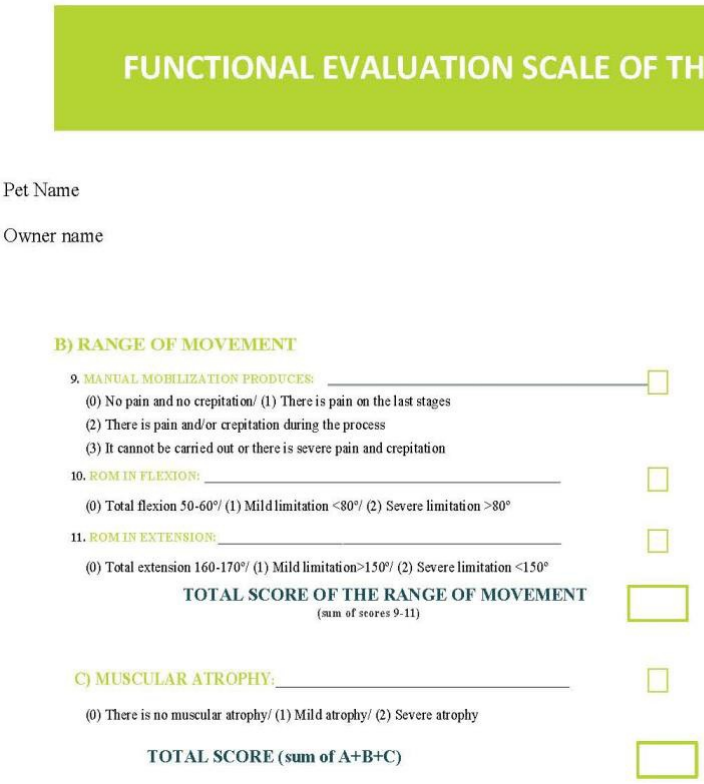
(0) It gets on the sofa or on the car without difficulties

(1) It gets on the sofa or on the car with difficulty

(2) It neither gets on the sofa or on the car

TOTAL SCORE OF FUNCTIONAL LIMITATION ☐

(sum of scores 1-8)



FUNCTIONAL EVALUATION SCALE OF THE HIP

Pet Name

Owner name

B) RANGE OF MOVEMENT

9. MANUAL MOBILIZATION PRODUCES: _____

(0) No pain and no crepitation/ (1) There is pain on the last stages

(2) There is pain and/or crepitation during the process

(3) It cannot be carried out or there is severe pain and crepitation

10. ROM IN FLEXION: _____

(0) Total flexion 50-60°/ (1) Mild limitation <80°/ (2) Severe limitation >80°

11. ROM IN EXTENSION: _____

(0) Total extension 160-170°/ (1) Mild limitation>150°/ (2) Severe limitation <150°

TOTAL SCORE OF THE RANGE OF MOVEMENT
(sum of scores 9-11)

C) MUSCULAR ATROPHY: _____

(0) There is no muscular atrophy/ (1) Mild atrophy/ (2) Severe atrophy

TOTAL SCORE (sum of A+B+C)

Escala Bioarth® para la evaluación de la articulación coxofemoral.

Todas las escalas anteriormente citadas tienen en cuenta en mayor o menor medida factores subjetivos, lo que hace que pueda existir de forma potencial una gran variabilidad intraobservador y/o interobservador que puede alterar de forma significativa la valoración final (Horstam y cols, 2004).

3.2.2. Análisis objetivo (Biomecánica)

Teniendo en cuenta las afirmaciones anteriores, una valoración objetiva sobre el estado alterado del paso (cojera) del animal, así como su diagnóstico y

progresión mientras está siendo tratado son parámetros muy actuales desde el punto de vista científico (Mölsä y cols, 2010).

La metodología de evaluación biomecánica nos ofrece una medición de los procesos que generan la locomoción.

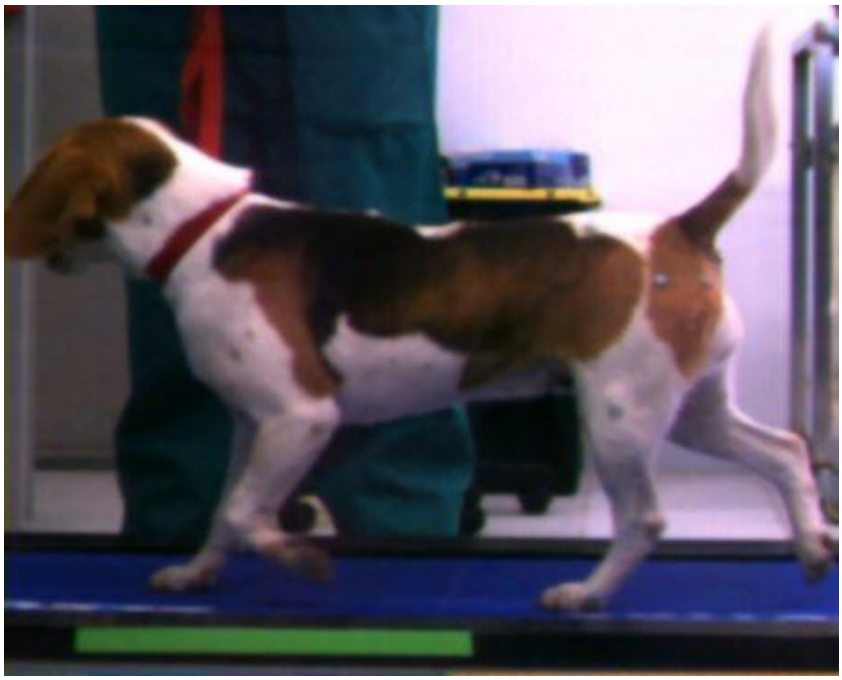
Estos métodos están divididos en métodos cinemáticos y cinéticos.

Sin embargo, debemos hacer hincapié en el uso del tapiz rodante o Treadmill antes de la utilización otras tecnologías, pues supone un complemento muy interesante desde el punto de vista de la estandarización de las condiciones del estudio como velocidad, inclinación, etc. Evidentemente, los animales deben pasar previamente por un periodo de acostumbramiento o habituación. (Khumsap y cols, 2004; Cruz y cols, 2017).

Además, nos permite obtener un registro de pasos consecutivos, muy útil en la valoración final (Drevemo y cols, 1980).

Recientemente, un estudio (Vilar y cols, 2016) ha utilizado el Treadmill para establecer las diferentes características dinámicas, en concreto las características cinéticas y cinemáticas de varias razas de perro con una morfología completamente diferente.

El método consistió en grabar a los animales con una cámara de alta velocidad al paso, para obtener los datos cinemáticos. Para obtener los datos de tipo cinético, se insertó una plataforma de fuerza sobre el tapiz rodante.



Treadmill utilizado para evaluar el movimiento de un perro de raza Beagle. Existe una plataforma de fuerza insertada debajo de la cinta rodante, y para la toma de datos cinemáticos se han aplicado unos reflectantes en los centros de rotación articular.

http://trends.medicalexpo.com/project-45657.html

En esta imagen podemos observar que el Treadmill, también es utilizado en las fases de rehabilitación animal, incluso con los miembros en inmersión.

http://trends.medicalexpo.com/project-45657.html

A pesar de que es una técnica realmente útil y efectiva, presenta una serie de inconvenientes y limitaciones, principalmente en la posible alteración del movimiento natural que el animal realizar sobre un suelo normal.

Cinemáticos

La cinemática se encarga de evaluar el movimiento, pero sin tener en cuenta las fuerzas que lo provocan. Los parámetros obtenidos pueden ser lineares (amplitud de paso, altura del miembro durante la fase de vuelo…), angulares (ángulos de flexión, extensión, rango articular de movimiento, velocidades y aceleraciones angulares…) y temporales para el cálculo de la duración de las diferentes fases de la marcha (apoyo, vuelo, etc.…) así como frecuencias, etc. (Manera, 2019).

Electrogoniometría.

Los electrogoniómetros son instrumentos que se sitúan sobre los centros de rotación articular para obtener los datos angulares de forma simultánea al movimiento del animal mientras se está desplazando. Su utilización en pequeños animales es bastante escasa (Thomas y cols, 2006; Jaeger y cols, 2007).

El electrogoniómetro genera una corriente eléctrica proporcional a la rotación que se esté produciendo, de modo que podremos obtener diferentes parámetros angulares (ángulo, velocidad angular, aceleración angular…).

A pesar de ser un instrumento preciso y de gran interés por sus datos fiables, existen dos factores fundamentales que limitan su precisión: uno es el posible desplazamiento respecto al eje de rotación que se puede producir por el propio movimiento, y el segundo es que hay articulaciones que realizan movimientos

en sentido tridimensional, no pudiéndose detectar por este instrumento (Ratzlaff, 1989).

Colocación de un electrogoniómetro sobre la articulación del codo

Cinematografía de alta velocidad y videografía.

La cinematografía ha quedado superada hoy en día por técnicas más actualizadas como la videografía de alta velocidad; ésta es probablemente el método más usado para la investigación biomecánica en pequeños y grandes animales (Keegan y cols, 2000; Kramer y cols, 2004; Vilar y cols, 2010).

Los principales datos cinemáticos pueden ser obtenidos con esta metodología (ángulos, tiempos de las distintas fases del paso, ángulos articulares en 2D y 3D, etc.).

En dichas determinaciones se utilizan unos marcadores reflectantes fijados a la piel sobre los centros de rotación de las articulaciones. Los datos

anteriormente citados se pueden extraer de forma manual, o existen metodologías actuales que permiten hacerlo de forma automatizada. (Langlois y cols, 1978; Leach y Cymbaluk, 1986; Leach y Dyson, 1988; Kramer y Keegan, 2007). La principal limitación es que, al estar los dispositivos fijados en la piel, el movimiento puede alterar la posición de los marcadores reflectantes (Van Weeren y cols, 1990).

Sensores inerciales o unidades de medición inercial (IMU).

Estos dispositivos obtienen datos cinemáticos como la aceleración lineal (acelerómetros) o velocidad angular (giroscopios). Son de tamaño muy reducido. Los datos registrados se emiten de forma inalámbrica en tiempo real (Martínez-Méndez y Huertas, 2013).

En la especie equina se pueden encontrar un alto número de trabajos de investigación con esta instrumentación, ya que se ha estandarizado su uso para la detección de cojeras (Keegan y cols, 2002; Watanabe y cols, 2011).

Debido a que pueden registrarse simultáneamente los datos de varios de estos dispositivos, su fijación en varias regiones corporales además de los miembros ha contribuido de forma determinante al conocimiento de los mecanismos de redistribución de fuerza compensatorios en los casos de cojera (Maliye y cols, 2013; Rhodin y cols, 2017).

3.2.3. Análisis cinético

La cinética es aquella parte de la biomecánica que se encarga de analizar el movimiento, pero en este caso se ocupa específicamente de las fuerzas que lo

están produciendo. De todas ellas, concretamente las fuerzas de reacción del suelo (GRF por sus siglas en ingles), adquieren una especial importancia por serlas más utilizadas. Además, a partir de dichas fuerzas se podrá analizar el centro de presiones (COP). Su relación se analizará a continuación:

- **El pico de fuerza vertical** (PVF, por sus siglas en inglés) es la GRF más utilizada en los análisis cinéticos y se define como el valor más alto de fuerza (en Newton o quilogramos) que se aplica contra el suelo cuando el miembro está apoyando durante la progresión del movimiento; el impulso vertical (VI, por sus siglas en inglés) es el producto de la fuerza por el tiempo de apoyo. El IV se evidencia en la gráfica que se genera durante el apoyo por ser el área bajo la curva. La objetividad de este parámetro en el diagnóstico de cojeras no está todavía clarificada (Vilar y cols, 2013).

-**El centro de masas (COM) o centro de gravedad (CG)** se define como la suma de las trayectorias de todos los segmentos de fuerza del cuerpo (cráneo/caudal y latero/medial) (Winter y cols, 1991).

-**El COP**, que supondría la posición en el plano de apoyo del CG, sea del cuerpo o de una parte del organismo como por ejemplo un miembro (Winter y cols, 1996); De este modo, el COP representa de forma explícita la fuerza que se está ejerciendo en un área concreta (Baratto y cols, 2002).

Plataforma de fuerza

Las plataformas de fuerza son unos dispositivos que a través de medidores dinamométricos o piezoeléctricos insertados (normalmente 4) en una

estructura de forma cuadrada son capaces de medir la fuerza durante la fase de apoyo. Dichos medidores generaran un impulso eléctrico de forma proporcional a la fuerza ejercida durante la pisada.

Plataforma dinamométrica de fuerza con 4 sensores de la marca Pasco®

Como se había descrito anteriormente, la visualización subjetiva del movimiento tiene como gran limitación la poca discriminación que hace entre cojeras leves o incluso moderadas, e incluso que estas no puedan ser detectadas; esto hace que en estos casos la evaluación del movimiento con las plataformas s de fuerza adquiera una especial importancia (Evans y colsm, 2005). Para poder llevarlo a cabo, los parámetros más utilizados van a ser los citados anteriormente, es decir, el PVF y el VI, considerándose hoy en día el "Gold Standard" (Walton y cols, 2014) para la evaluación del movimiento.

Debido a que las GRF pueden ser modificadas por la velocidad a la que el sujeto se desplaza, los análisis para ser validos deben ser realizados en un

rango muy estrecho de velocidad e incluso de aceleración (Riggs y cols, 1993; Budsberg y cols, 1999).

Otro factor que va a influir en las GRF es el peso, por lo que los estudios científicos, a la hora de obtener valores validados no solo debe hacer acopio de animales con pesos (e incluso conformaciones) muy similares, sino que se hace necesario realizar procesos de normalización; el más utilizado en este campo es el de expresar los valores en relación con el peso corporal (%) (Vilar y cols, 2013).

Normalmente, y de forma genérica se debe decir que en los cuadrúpedos siempre, los miembros torácicos dan valores de PVF más altos que los pelvianos, ya que estos animales el CG está desplazado cranealmente.

En el campo clínico, este dispositivo se viene utilizando de forma regular para evaluar la respuesta a los tratamientos instaurados en animales con osteoartritis derivadas de patologías como la displasia de cadera, codo, e incluso la CCLR (Nelson y cols, 2013; Skinner y cols, 2013; Vilar y cols, 2013; Wurcherer y cols, 2013).

Las plataformas de fuerza presentan como limitación principal que los datos se pueden obtener solamente de un miembro aislado, lo que hace que los exámenes sean largos y por lo tanto dificultosos cuando este estudio se realiza con animales; evidentemente, el hecho de que solo mida la fuerza generada durante la fase de apoyo supone otra gran limitación, ya que en la fase de vuelo también se generan fuerzas. Este dispositivo, debido a su tamaño, tampoco obtiene datos de apoyos consecutivos. (Manera y cols, 2019).

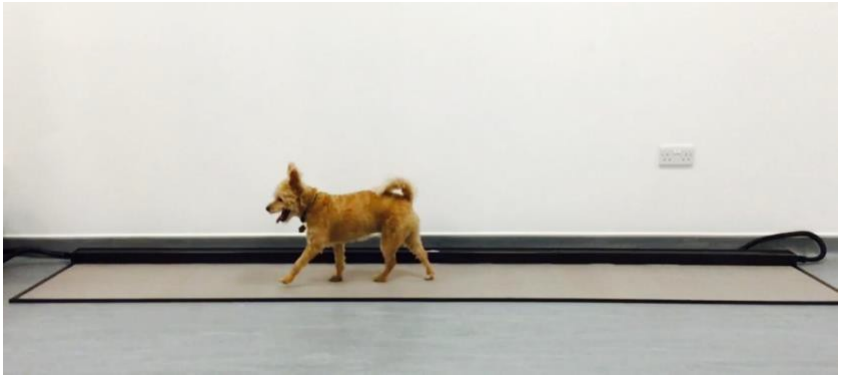
Plataforma de presión

La plataforma de presión es un dispositivo de estudio tanto en el campo clínico como en el investigador que se ha ido añadiendo progresivamente en los últimos años al elenco de dispositivos para obtener GRF. Su principal característica es que dispone de miles de sensores distribuidos por el área de estudio de modo que los datos son específicos de áreas determinadas. Por otro lado, y de forma análoga a las plataformas de fuerza, se puede regular la velocidad de adquisición de datos (Oosterlinck y cols, 2010).

Estos dispositivos no son más costosos que los anteriores, pero adquiere como ventaja importante, el hecho de que pueden ser de grandes dimensiones (hasta 2 metros de largo) por lo que se pueden registrar los datos de varios apoyos.

Otra gran ventaja es que además de los clásicos parámetros (PVF y VI) se pueden obtener otros que cada vez adquieren mayor relevancia como como la distribución del peso en el cuerpo, la presión media y máxima de apoyo, que al estudiarse de forma unificada conforman el análisis podobarométrico (Oosterlinck y cols, 2011; Carr y cols, 2015; Bockstahler y cols, 2016; Schnabl-Feichter y cols, 2017).

Como se podrá ver a continuación, este dispositivo podrá evaluar la evolución del COP durante la marcha (Gomes-Costa y cols, 2015), cuyo análisis en animales que presentan cojera constituye uno de nuestros objetivos principales en la presente tesis.



Las plataformas de presión de gran longitud (hasta 2 metros) permiten el registro de varios pasos consecutivos. Plataforma tekscan.

https://twitter.com/vetbiomechanics/status/666602613715173376

Como viene siendo habitual, muchos de estos dispositivos han venido utilizándose de forma precedente en medicina humana; entre los estudios consultados quisiéramos destacar aquellos en donde las plataformas de presión han servido para determinar cómo patologías como la esclerosis múltiple pueden afectar al mantenimiento postural (Abrantes y Santos, 2012). Pero otro tipo de patologías crónicas como la diabetes también influyen en la postura y por lo tanto en la distribución de la presión durante el apoyo (Anjos y cols, 2010).

Ya en el campo de la veterinaria, es en el mundo del caballo donde estas plataformas se empezaron a utilizar, destacando la profundización en los eventos cinéticos concretos que se producen durante la fase de apoyo del casco (Van Heel y cols, 2005), así como los efectos del recorte del casco en la distribución de presiones en la suela (Moleman y cols, 2006), o la simetría del casco en caballos y ponis sanos (Oosterlinck y cols, 2010a;2010b).

En estos últimos años existe una tendencia hacia el estudio de cojeras, pero en este caso nos basamos en los cambios posturales con el animal inmóvil. La consecuencia (y ventaja) más evidente es que este tipo de determinaciones se pueden realizar en espacios pequeños, ya que no es necesario que el animal realice movimientos de desplazamiento (Gomes-Costa, 2015).

En pequeños animales las plataformas de presión se han empezado a utilizar de forma muy reciente, sobre todo en estudios descriptivos del paso en animales sin patologías locomotrices (Marghitu y cols, 2003; Souza y cols, 2013), en algunos estudios posteriores ya utilizan esta tecnología para estudios en perros con CCLR (Souza y cols, 2014), osteoartritis de cadera (Upchurch y cols, 2016) o en análisis del movimiento en perros a los que se le habían implantado prótesis en dicha articulaciones (Tomas y cols, 2014).

Como decíamos anteriormente, las plataformas de presión permiten obtener datos de diferentes miembros simultáneamente y en pasos consecutivos, lo que ha facilitado el análisis de las cojeras mediante el uso de fórmulas matemáticas encaminadas a obtener los niveles de simetría entre miembros (Carr y cols, 2015).

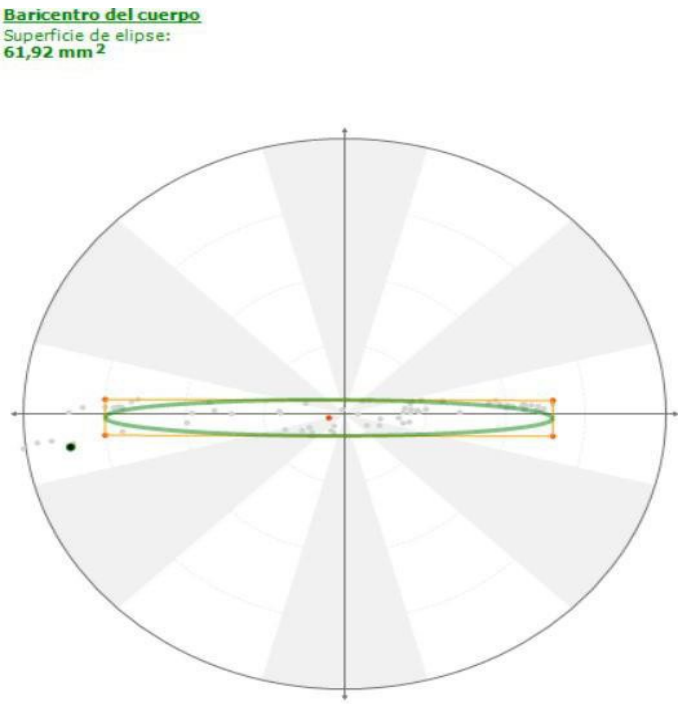
El desarrollo de estos instrumentos hace que se aumente de forma acelerada su potencial, que se consigue fundamentalmente con la producción de dispositivos de tamaños, pero, sobre todo, de resolución diferentes, lo que permite actualmente obtener datos de animales con áreas de apoyo muy pequeñas, como es el caso de los gatos (Schnabl-Feichter y cols, 2017).

Entre las limitaciones que poseen las plataformas de presión cabe destacar la lenta respuesta de los sensores; por otro lado, un estudio realizado en caballos ha revelado que cuando se comparan datos de GRF obtenidos en los mismos individuos de forma simultánea con una plataforma de fuerza y una de presión, esta última arroja resultados más bajos (Oosterlinck y cols, 2010).

Como habíamos adelantado anteriormente, aunque basados en principios físicos diferentes, podíamos asumir que la posición del COP coincidía con la proyección vertical del CG sobre el plano donde los miembros están apoyados (Baratto y cols, 2002). En un animal en la estación, el COP va a describir una trayectoria que se corresponde a los movimientos de desestabilización y recuperación de la postura. Su análisis en los planos X e Y se denomina estabilograma. Esta trayectoria en el plano de apoyo va a ocupar un área determinada, mas pequeña cuanta más estabilidad esté presente; al grafico generado por la trayectoria del COP se le denomina estatocinesiograma.

¿Entonces, cómo se altera la trayectoria del COP cuando existe cojera? De forma experimental, y ya en el campo de la veterinaria, se ha demostrado que en la estación se manifiestan unas alteraciones posturales debido a los esfuerzos por aliviar el dolor transfiriendo la presión hacia el lado sano. Evidentemente su sistema nervioso corregirá de forma totalmente refleja esas alteraciones posturales, creando en definitiva, alteraciones en las características del COP (trayectoria, área del estatocinesiograma, velocidad del desplazamiento del COP, etc.) generándose un ciclo continuo de perturbaciones-correcciones; estas alteraciones, no solo se han evidenciado en la especie

humana sino que, como apuntábamos anteriormente, se ha verificado en canidos y équidos (Buchner y cols, 2001; Oosterlinck y cols, 2010; Manera y cols, 2017). Estos eventos producidos por las desviaciones en el comportamiento del COP, tal y como se evidencia en las referencias anteriormente citadas, han servido desde el punto de vista clínico para la detección de las cojeras en estas especies.



Estatocinesiograma de un perro cojo de la derecha. Aparte de la gran superficie (61,92 mm²), se ve la elipse desplazada hacia el lado izquierdo

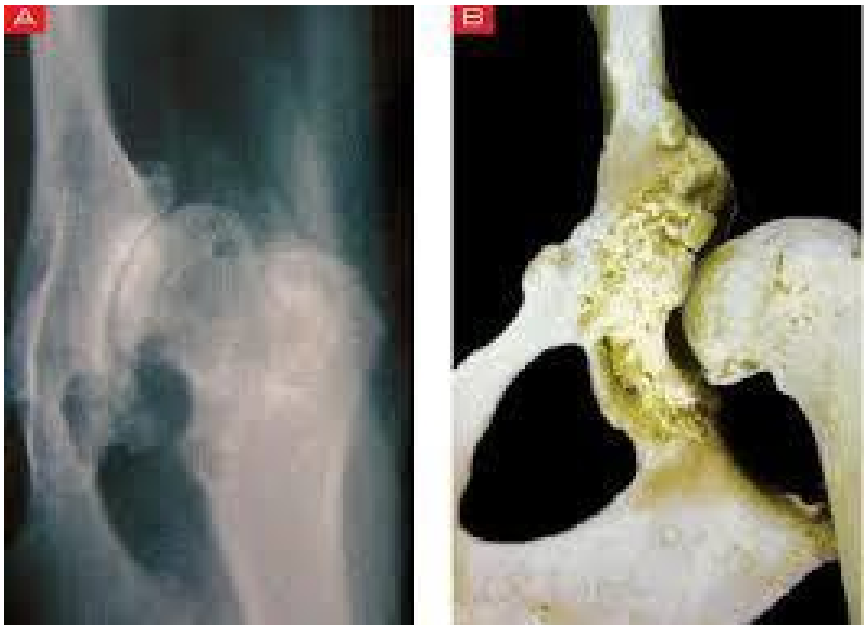
Evidentemente, el COP no solo describe una trayectoria debida a los eventos de alteración-corrección realizados por el SNC tanto en condiciones fisiológicas como patológicas; sino que cuando el individuo está en movimiento, el COP corporal durante toda la fase de apoyo de los miembros, este va a

describir una cierta trayectoria. La trayectoria, o, mejor dicho, las alteraciones de su trayectoria en los miembros ya han servido en la especie humana para detectar las modificaciones en este parámetro en situaciones de cojera (Riskowski y cols, 2013) Parkinson (Kim y cols, 2017) así como en hemiparesia (Robain y cols, 2006)

En las situaciones patológicas anteriores, las principales características derivadas del COP durante el desplazamiento son:

1) **El desplazamiento craneocaudal del COP**; es decir, la medición en un sistema de coordenadas de la distancia entre los puntos inicial y final de la trayectoria (Robain y cols, 2006).

2) **El desplazamiento lateromedial que se va a producir en el COP**; Este índice, denominado en ingles center of pressure excursion index (CPEI) a efectos de normalización, refleja este desplazamiento en relación con la anchura del miembro y se multiplica por 100 para obtener este dato en términos de porcentaje (Yoon y cols, 2010; Riskowski y cols, 2013).



TRATAMIENTO DE LA OA Y ARTÍCULOS

ARTÍCULO #1

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RESEARCH ARTICLE Open Access

Center of pressure limb path differences for the detection of lameness in dogs: a preliminary study

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Abstract

Background: The limb center of pressure (COP) path measures and quantifies the load distribution within a limb in a still or moving subject. Under this premise, the aim of this study was to test whether data derived from this parameter could detect the differences between sound and lame limbs in unilaterally lame dogs with elbow dysplasia.

To accomplish this purpose, ten unilaterally lame dogs of similar conformation were walked over a pressure platform. Next, the COP path, in relation to the position of sound and lame limbs, was measured in a coordinate system over a standard paw template obtained by pedobarography during the whole support phase. To compare variables, force platform data (peak vertical force and vertical impulse) from the same animals were obtained. Sound and lame limb statokinesiograms were also obtained while the animals stood still.

Results: The statistical analysis clearly showed that COP in lame limbs start cranially and were shorter than sound limbs. In addition, the value of the COP excursion index was lower in lame limbs. Finally, the area of statokinesiograms was greater in lame limbs.

Conclusion: This methodology based in limb COP characteristics serves to discriminate between sound and lame limbs in dogs with elbow dysplasia.

Keywords: Balance, Center of pressure, COP, Dog, Statokinesiogram

Background

Various methods to analyze the locomotor status within the veterinary field have been developed in order to generate useful parameters from both kinematic and/or kinetic perspectives. These methodologies should be able to provide accurate and reliable data and, if possible, form a set of parameters that will allow for the normal/abnormal static/dynamic events from a wide perspective. This invariably requires the use of more sophisticated systems [1].

These data should ultimately serve to detect lameness, and, among them, the center of pressure (COP) position may be considered the net output variable of interaction between all of the forces and torques that occur in the body (bCOP) or limb (lCOP) and its inertial properties. The COP position over time is named the COP path. This parameter quantifies the dynamic load distribution under the foot [2]. The lCOP path characteristics obtained in moving subjects provide insights into foot dynamics during the support phase of gait in human and, potentially, in animal species [3–6]. In this sense, it has been able to reliably detect biomechanical modifications due to neurological deficits, such as Parkinson's [7], Hemiparesis [8] or even pain [3], in humans.

The main lCOP pathway characteristics that have been reported as useful are: 1) craniocaudal COP excursion (measured as an initial and final COP relative coordinates) [8]; 2) lateromedial displacement of the lCOP by means of the center of pressure excursion index (CPEI),

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which represents the lCOP path lateromedial excursion relative to limb width and multiplied by 100 to obtain this data in terms of percentage [3, 9].

The COP path can be also obtained in a standing position and records its resultant area during a determinate period of time. This parameter is named statokinesiogram, and its value shows body or limb balance [10].

In the veterinary field, previously published studies only examine the bCOP path [11–13]; more recently, the bCOP path's efficacy for the detection of lameness in ponies at walk has been settled [14]. In dogs, bCOP modifications in unilaterally lame animals with elbow dysplasia (ED) have also been reported [15].

Regarding ED, this is a complex syndrome, where different factors could lead to a growth incongruence between the radius and ulna. Over time, ED causes joint damage, pain, and lameness [16, 17].

The hypothesis of this study was to prove that certain lCOP path characteristics are different in lame and sound limbs in dogs at walk and while standing still. For this reason, the aim of this study was to set a number of lCOP pathways –derived data that could serve to detect lameness in dogs with unilateral ED.

Methods

Animals

This study utilized 10 client-owned, adult dogs with similar conformation (2 rottweiler, 3 labrador retriever, 1 golden retriever, 2 german shepherd, 2 belgian shepherd). The body weight of the enrolled dogs ranged from 30 to 41,8 kg, and the ages were from 3 to 9 years.

Inclusion criteria comprised of the presence of weight-bearing unilateral forelimb lameness due to OA secondary to elbow dysplasia. The lameness of every dogs reached a score of 3–4 in a scale of 0–5 [18].

Furthermore, no medication could have been administered 1 month prior to the analysis.

To confirm or rule out OA, three standard radiographic views of both elbow joints (a lateral extension, lateral flexion, and a 15° oblique craniomedial caudolateral) [19] were taken under sedation with dexmedetomidine 10 ± 20 μg/kg (Dexdomitor, zoetis, Spain). Standard radiographs of stifle and hip joints were also taken in order to exclude other reasons for the observed clinical signs.

A complete clinical evaluation (physical examination, including vital signs and neurologic and orthopedic exams) assured that general health was otherwise normal.

Pressure platform study

A Pressure platform (EPS/R1, Loran Engineering, Bologne, Italy) was used for this study. This device contains a total of 2096 pressure sensors of 1 cm2 distributed in an area of 48 × 48 cm. The range of pressure was set from 30 to 400 kPa.

The procedure for the dynamic and static pressure platform analysis has been previously published [15, 20]; briefly, dogs were leash guided by their owners over the pressure platform at a walk (velocity 1.2 ± 0.2 m/s; acceleration ± 0.2 m/s^2). Velocity and acceleration were measured with a motion sensor (PS-2103A, Pasco®, California, USA) placed within the dogs trajectory. Three trials were recorded at a sampling frequency of 100 Hz from each dog. A trial was considered valid when the studied limb fully supported over the pressure platform and when the dog walked next to the owner without pulling on the leash and without head turns. The pressure platform was interfaced with a dedicated computer using Biomech® (Loran Engineering, Bologna, Italy) software. Once the images were isolated, the paws' length was normalized to a fixed value of 9 cm, and width was then proportionally modified. Measurements were taken with a reference to an X-Y coordinate system.

Statokinesiograms were obtained while the dogs were placed in a quiet stance with their thoracic limbs over the pressure platform, perpendicular to the ground. The dog's owner remained in front of the animal to attract the dog's attention at a close distance. Three trials of 20-s recordings were obtained from each animal. A trial was considered valid when the animal remained with immobile limbs, tail and head during the whole 20 s recording procedure.

The following were the obtained measurements (Fig. 1):

1. Caudal margin (Cm): defined as the distance between the most caudal limit of the paw print and the most caudal limit of the lCOP path.
2. lCOP pathway length (e): the length of the line that joins the recorded points of the lCOP trajectory. Measured in cm.
3. Craniocaudal index (CrCI): determines the COP length (b) related to the paw length (a). This is obtained with the following formula: % = (b / a) × 100. Expressed as a percentage.
4. Center of the pressure excursion index (CPEI): determines the lateromedial excursion of the COP (c) related to the paw width (d). The formula was the following: % = (c / d) × 100. Expressed as a percentage.

Higher values of all the above parameters are associated with better limb support [3, 8, 9].

5. statokinesiograms: defined as the area determined by an ellipse that contains 90% of the recorded points of the COP trajectory [10]. Measured in mm2, a lower value means more stability [15, 21].

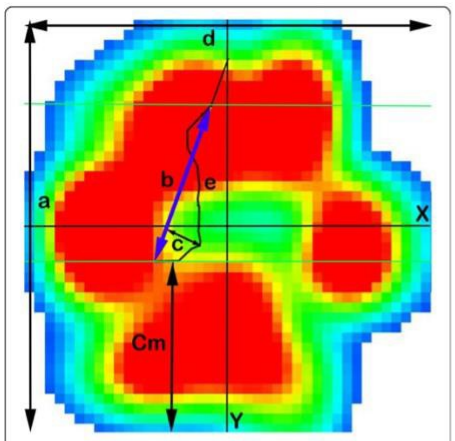

Fig. 1 Paw podobarographic print with coordinate system and measurements made. X: X coordinate; Y: Coordinate; a: paw length; b: COP length; c: ICOP width; d: paw width; e: COP path length; Cm: caudal margin

Force platform analysis

A force platform (Pasco, California, USA) was placed adjacent to the pressure platform in such a way that recordings from animals were performed in the same session. DataStudio software (Pasco, California, USA) was used to obtain PVF (N) values from three valid trials. Mean values were normalized to body weight (%BW).

Statistical analysis

For the data analysis, a linear mixed effects model was considered: for each response variable (COP Length, CPEI, etc), the status of the limb (lame/sound) is a fixed effects factor, while the dog is a random effects factor.

The model is as follows:

$$y\_ijk = \mu\_i + b\_j + \varepsilon\_ijk, i = 1, \ldots, 2 \;\; j = 1, \ldots, 10, \;\; k = 1, \ldots, 3$$

$$b\_i \approx N(0, \sigma\_b) \quad \varepsilon\_ijk \approx N(0, \sigma)$$

where:

1. y_ijk is the k-th measure (k = 1,2,3) on the limb i (i = sound/lame) of the dog j (j = 1...10)
2. μ_i is the (fixed) effect of limb status i. This parameter represents the mean value of the variable in the sound (lame) limb.
3. b_j is the (random) effect of dog j. Values of b_j are supposed to be normally distributed with mean 0 and standard deviation σ_b, so σ_b is the variability in the response of the dogs.
4. ε_ijk is the residual in the measure ijk. This variable is assumed to be normally distributed with the mean 0 and standard deviation σ.

Statistical analysis was performed with 'R' statistical language and environment, version 3.3.2. (https://www.R-project.org/). For assessing the validity of the model, a Shapiro-Wilk test is applied to test the normality of the residuals, and a Levene test is used to test homoscedasticity.

Results

Mean weight (± SD) was 37.08 ± 3.76 kg, and age was 5.80 ± 1.99 years. The mean (± SD) values and 95% CI of all obtained parameters are shown in Table 1. All data were normally distributed and homoscedastic ($p \geq 0.25$ and $p \geq 0.12$, respectively).

Significant differences between LL and CL were found in all cases (< 0.0001); concretely, a higher value of Cm and a lower COP Length, COP Path Length, and CrCI values in LL were observed when compared with CL. In the same manner, CPEI in LL were also lower than CL (Fig. 2, Additional file 1).

In agreement with the data shown above, PVF and VI values also showed significant differences between LL and CL ($p \leq 0.0001$) (Table 1). PVF and VI data were also normally distributed and homoscedastic ($p \geq 0.64$ and $p \geq 0.51$, respectively).

Table 1 Mean ± SD, 95% confidence interval and difference between LL and CLs for CM, Cop Path Length, CrCI, PVF, VI and statokinesiograms. [a] means significant difference

	LL	CL	Difference
Cm (%)	44.85 ± 5.12	31.13 ± 7.61	[a]13.72 ± 0.94
	40.86, 48.84	27.14, 35.12	11.83, 15.61
COP Path Length (%)	42.00 ± 4.94	55.68 ± 9.92	[a]13.69 ± 0.97
	37.05, 46.95	50.73, 60.63	11.74, 15.63
CrCI (%)	31.07 ± 4.49	44.01 ± 6.75	[a]12.94 ± 1.23
	28.08, 34.06	41.02, 47.01	10.47, 15.42
CPEI (%)	4.57 ± 1.65	9.30 ± 1.78	[a]4.73 ± 0.35
	3.65, 5.49	8.38, 10.22	4.02, 5.44
PVF (%)	32.72 ± 4.66	71.12 ± 3.57	[a]38.40 ± 0.78
	31.13, 34.31	69.53, 72.71	36.84, 39.96
VI (%)	13.49 ± 1.32	22.93 ± 1.58	[a]9.44 ± 0.24
	12.80, 14.18	22.24, 13.62	8.96, 9.92
Statokinesiogram (mm^2)	16.18 ± 6.10	5.70 ± 3.43	[a]10.48 ± 0.75
	13.16, 19.19	2.68, 8.71	8.98, 11.98

Cm Caudal margin, *CrCI* Craniocaudal index, *CPEI* Center of pressure excursion index

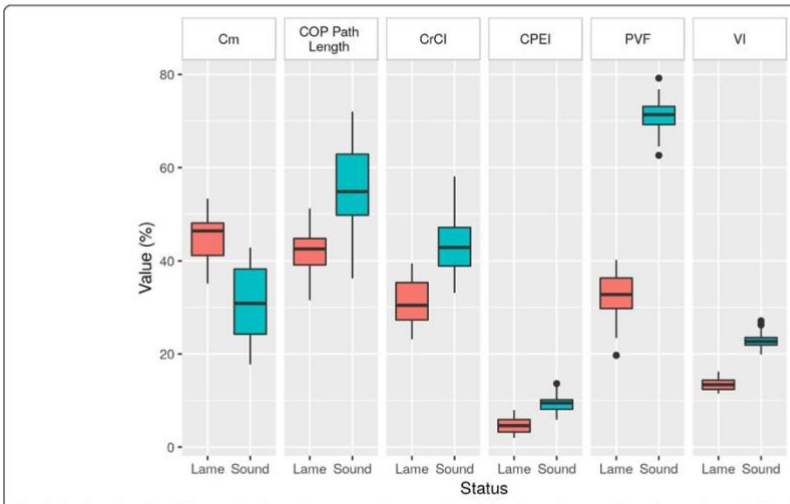

Fig. 2 Boxplots showing differences in dynamic parameters between LL and CL. As can be seen, Cm values are lower in CL, while COP path Length, CrCl and CPEI indexes are higher when compared with LL. This also occurs in PVF and VI values

Finally, the area from the statokinesiograms showed a higher value in LL (Fig. 3, Additional file 2). Additionally, a craniomedial COP slope was observed in both LL and CL when COP length was measured (Fig. 1, blue arrow).

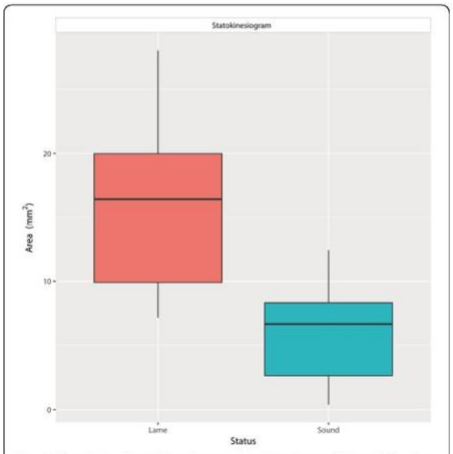

Fig. 3 Boxplots of statokinesiogram (static) values of LL and CL. Area of LL is higher than LL i.e., more instable

Discussion

Our results provide a novel insight into the adaptive changes in lCOP characteristics in unilaterally lame dogs with ED.

To the best of our knowledge, no other previous studies exist regarding the clinical implications of dynamic and static lCOP path characteristics in lame dogs.

Limb weight load amount could be influenced by the gait speed or cadence and, consequently, could alter COP path patterns [22]. Acknowledging this possibility, we performed the study in a narrow range of velocity and acceleration and tried to enroll similarly sized animals in order to minimize severe cadence discrepancies.

Once the data were obtained, we assumed that measurements on caudocranial and mediolateral COP displacement would provide four basic differences between LL and CL regarding:

1) *The extent of net forward lCOP path progression.* Based in our results, lCOP path in LL is shortened and cranialized compared with CL. This is in concordance with other authors' findings [8]. As made evident by the data, a larger Cm directly implies a shorter COP path length. This is invariably due to a shortened swing phase by a lack of limb extension, meaning the limb lands more vertically at the start of the braking phase [23]. This event prevents the metacarpal pad to exert a correct load absorption, expanding with the

increase of weight-bearing when the limb lands [24, 25]. The impact shock could be, in the last instance, potentially transferred to muscles higher up the limb [5].

2) *Net mediolateral lCOP deviation*. As reported in previous research [26], a higher CPEI in CL is determined by an increased pad deformation, given that pad expansion is a direct response to weight loading. This effect has also been observed in human feet [9] and equine hooves [27].
3) *Statokinesiograms*. A greater area determines more instability [15]. This finding, although previously in reference to the body, remains true for limbs as well, since the area was greater in LL.
4) *The lCOP direction of progression in both sound and lame limbs*. As stated above, lCOP path described a certain angle (slope) as it pursued craniomedially with respect to the longitudinal axe of the paw. A possible explanation for this finding may be that the lCOP path follows the direction of the body's center of mass and not the craniocaudal paw axe, which corresponds to other reports in humans [28].

Another interesting finding was that the lCOP caudocranial displacement is constant during the support phase, but velocity is not (Additional file 1), which coincides with reports in human research regarding sound limbs [8]. In the present study, this characteristic was evident not only in CL but also in LL.

In humans, longitudinal COP displacement corresponds to 83% of foot length and 18% of foot width [28]; their equivalent values in CL of our study with dogs were about 44% (CrCI) and 9% (CPEI), respectively, which is approximately half. Two facets could explain these differences: 1- that humans have plantigrad support, which starts in the calcaneus bone, whereas in dogs the support is digitigrade; 2- human bipedalism determines full load transfer to the support limb when walking, whereas dogs walk with two (or even three) limbs simultaneously sharing the load support.

The following are some limitations in our study:

1. The lCOP path patterns in sound limbs cannot be extrapolated to limbs from sound dogs. As in lame dogs, sound limb patterns are showing compensatory movements. For the same reason, data from unilaterally lame limbs should not be extrapolated to bilateral lameness.
2. Compensatory weight redistribution in lame dogs not only implies to the contralateral limb, as has been well established in dogs and horses [29, 30]; thus, it would be useful to obtain hind limb lCOP path values in a subsequent study. Moreover, it should be determined if any correlations exist between the lCOP path values with the lameness degree or lameness origin. Unfortunately, the relatively large dog sizes impede the simultaneous analysis of more than two limbs, and a larger platform pressure mat would be essential.
3. Parameters, such as Cm and CPEI, need to be qualitative and not quantitatively considered, given that cut-points were not defined in our study, although significant differences were found in our study between CL and LL. To establish an accurate limit value for soundness or lameness, a higher number of patients with the same characteristics (weight, conformation, or even breed) are necessary, as reported by others authors in similar human studies [4].
4. Finally, the number of lCOP characteristics assessed could represent a "signature" diagnosis of ED, where the kinetic parameters to detect it have been previously proven [23]. This also means that COP patterns in other musculoskeletal and neurodegenerative disorders could be quite different, which needs further investigation.

Conclusion

This study showed that the lCOP path in LL is shorter, cranialized, and with smaller mediolateral excursion when compared with SL in dogs with unilateral ED. In addition, the lCOP path follows a craniomedial direction and not the paw longitudinal axe in both LL and CL. Its progression velocity is not constant.

Additional files

Additional file 1: Video S1. Limb and body statokinesiograms from a dog with a left limb lameness. As can be seen, the area of ellipse (18.28 mm^2 Vs 8, 33mm^2) in the left (red) LL is greater than the right (blue) CL. In the center (green) the body statokinesiogram can also be seen. (MP4 3152 kb)

Additional file 2: Video S2. Simultaneous videosequence of support phase in a CL (left) and LL (right). The lCOP (black point) path in LL starts more cranially and therefore shortened. (MP4 650 kb)

Abbreviations

bCOP: Body Center of Pressure; CL: Sound limb; Cm: Caudal margin; COP: Center of pressure; CPEI: Center of pressure excursion index; CrCI: Craniocaudal index; ED: Elbow dysplasia; lCOP: Limb Center of Pressure.; LL: Lame limb; PVF: Peak vertical force; VI: Vertical impulse

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Availability of data and materials
All data supporting our findings are included in the manuscript. If readers need additional information and/or data sets, they will be provided by the corresponding author upon reasonable request.

Authors' contributions
JMV, JS, and JMC conceived and designed the experiments; MR, ED and DC performed the clinical and radiological analyses; JMV and SL performed the pressure platform analysis; AS analyzed the data; all authors read and approved the final manuscript.

Authors' information
Not applicable

Ethics approval and consent to participate
The research protocol was revised and authorized by the Ethical Committee of Animal Welfare at the Instituto Universitario de Investigaciones Biomédicas y Sanitarias of the Universidad de Las Palmas de Gran Canaria (IUIBS 14/2017) in compliance with the Directive 2010/63/EU of the European Union. Dog owners were informed of the study and signed a consent to participate in the study, including all performed procedures.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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4. TRATAMIENTO DE LA OA

4.1. Tratamientos convencionales

La estrategia terapéutica ha tratado, de forma clásica, de frenar o al menos ralentizar el proceso inflamatorio que en definitiva determina la aparición del dolor y la alteración funcional.

En primer lugar, debemos destacar aquellas medidas higiénicas y dietéticas, como la reducción de peso, el ejercicio de bajo impacto, así como la fisioterapia, láser de baja intensidad, acupuntura y ultrasonidos entre otros (Gudbergsen y cols, 2012; Cakir y cols, 2013; Fang y cols, 2013).

Desde el punto de vista del tratamiento basado en principios activos, los antiinflamatorios no esteroideos, así como los esteroideos de larga duración han formado de manera amplia el elenco de medicamentos clásicos a usar en estas situaciones. También los complementos nutricionales como los glucosaminoglicanos de origen diverso han demostrado su utilidad (Merashly y Uthman, 2012; Reid y cols, 2012; Godley y cols, 2013).

La evolución de los tratamientos clásicos, fundamentalmente los analgésicos no esteroideos han ido encaminando a conseguir moléculas con menos efectos colaterales, así como de mayor comodidad de administración. En este sentido cabe resaltar el uso de un inhibidor COX-2, el Mavacoxib (Trocoxil™, Pfizer, MY, USA) (Penning y cols 1997). Este compuesto ha sufrido una serie de modificaciones bioquímicas que han conseguido hacerlo muy estable, y por lo tanto una vida media muy alta (Cox y cols, 2010)

Con eso se consigue por un lado mucha comodidad en su administración (una vez al mes), y por otro debido a su bajo índice de aclaramiento, que la dosis sea muy baja para obtener un óptimo efecto terapéutico. (Paulson y cols, 2001).

De modo general, debemos decir que las terapias anteriormente descritas solo obtienen un efecto sintomático, pero no van a impedir la evolución de la OA (Lawrence y cols, 2008; Cuervo y cols, 2016). Por este motivo, las terapias regenerativas están enfocadas en la aportación de células con capacidad de generar cartílago, así como también factores que promuevan la reparación tisular (Williams y cols, 2012).

4.2. Terapias regenerativas

Como decíamos anteriormente, estas terapias suponen una revolución terapéutica pues se adquiere una nueva perspectiva de tratamiento al buscar no solo la mejoría sintomática, sino que además aporta y estimula los factores y procesos presentes en el propio organismo de modo que, como mínimo, frenen o ralenticen el efecto degenerativo sin efectos nocivos al ser sustancias o células autólogas. (Wu y cols, 2007; Singh, 2012).

4.2.1 Células madre.

Las células mesenquimales o, más concretamente las células mesenquimales adultas son capaces de diferenciarse en las diversas variantes de tejido conectivo (adiposo, óseo, cartilaginoso) (Fortier y Travis, 2011; Diekman y Guilak, 2013). Estos dos últimos adquieren gran importancia desde el punto de vista clínico al formar parte de los componentes articulares.

Estas células mesenquimales se extraen principalmente del tejido adiposo (Black y cols, 2008; Yarak y Okamoto, 2010), aunque también se pueden obtener de medula ósea y/o hueso. Las células mesenquimales, y en concreto su eficacia en la OA está siendo objeto de múltiples estudios en la actualidad.

ARTÍCULO #2

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Review

Adipose-Derived Mesenchymal Stem Cells: Are They a Good Therapeutic Strategy for Osteoarthritis?

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Abstract: Osteoarthritis (OA) is a major cause of disability in elderly population around the world. More than one-third of people over 65 years old shows either clinical or radiological evidence of OA. There is no effective treatment for this degenerative disease, due to the limited capacity for spontaneous cartilage regeneration. Regarding the use of regenerative therapies, it has been reported that one option to restore degenerated cartilage are adipose-derived mesenchymal stem cells (ASCs). The purpose of this review is to describe and compare the efficacy of ASCs versus other therapies in OA. Methods: Recent studies have shown that ASCs exert paracrine effects protecting against degenerative changes in chondrocytes. According to the above, we have carried out a review of the literature using a combination of osteoarthritis, stem cells, and regenerative therapies as keywords. Results: Conventional pharmacological therapies for OA treatment are considered before the surgical option, however, they do not stop the progression of the disease. Moreover, total joint replacement is not recommended for patients under 55 years, and high tibia osteotomy (HTO) is a viable solution to address lower limb malalignment with concomitant OA, but some complications have been described. In recent years, the use of mesenchymal stem cells (MSCs) as a treatment strategy for OA is increasing considerably, thanks to their capacity to improve symptoms together with joint functionality and, therefore, the patients' quality of life. Conclusions: ASC therapy has a positive effect on patients with OA, although there is limited evidence and little long-term follow-up.

Keywords: osteoarthritis; mesenchymal stem cells; regenerative medicine

1. Introduction

Osteoarthritis (OA) is a progressive degenerative disease of the joint characterized by gradual degradation of hyaline articular cartilage and sclerosis of bone. This cartilage is composed by type II collagen and proteoglycans. An alteration in the replacement of the proteoglycan and type II collagen network leads to the loss of function of the cartilage [1]. This disease, worldwide, is considered to be the fourth leading cause of disability [2] and the second cause of inability to work in men [3]. OA is the most common articular disease in adults, and knee OA is the most common location. Although, OA also affects other large-weight-bearing joints, such as hip, hands, feet, and spine [4]. Hip and knee

OA are leading causes of disability worldwide [5]. The disease is characterized, at first, by a molecular derangement (alteration of joint tissue metabolism) followed by physiologic/anatomic damages (cartilage degradation, bone remodeling, osteophyte formation, joint inflammation), culminating in a loss of normal joint function [6].

In the United States, 27 millions of people suffer from clinical OA, and the treatment costs 185.5 billion dollars per year [7]. On the other hand, this pathology is the fourth leading cause of disability in Asia [2]. In addition, this chronic degenerative disease of articular cartilage has a current prevalence of 12% in the population over 60 years old, which will increase in the next 20 years [8,9]. It has also been reported that its incidence has doubled in women, and tripled in men, in recent years [10].

Several agents have been associated with a higher risk of suffering OA, such as genetic predisposition, obesity, previous trauma, and age. It has been demonstrated that the risk of developing post-traumatic OA increases by up to four times in people over the age of 50 [11]. As one ages, the chondrocytes, that contribute to 5% of the volume of the articular cartilage, decrease their regenerative response, leading to progressive loss of the articular surface resulting in cartilage degeneration with loss of matrix (which confers the biomechanical properties to the articular cartilage and constitutes the 95% of the tissue), which can result in a complete loss of joint surface. Moreover, chondrocytes produce mediators of inflammation (cytokines, chemokines, and proteolytic enzymes) that induce serious damage [12].

Pain is one of the first symptoms, leading to movement disability and impaired quality of life [9,13]. Synovial inflammation, cartilage breakdown, and bone remodeling are associated with OA chronic pain, and the mechanism responsible for pain involves structural changes and alterations in peripheral transduction and central processing of painful sensory inputs [14]. Consequently, ideal treatment should obtain analgesia, stopping progression of chondral degeneration; modify cartilage structure and revert damage; and finally, improve joint function [15].

While conventional therapies, such as physical therapy, glucosamine and chondroitin sulfate supplementation, arthroscopic surgery, or biological therapies, such as chondrocyte implantation, have little significant results, regenerative medicine (RM), has been demonstrated to be a great option in articular cartilage regeneration [16]. On the other hand, various surgical procedures have been performed to regenerate articular cartilage but have achieved limited success, including abrasion arthroplasty, subchondral drilling, and microfracture [17]. Mesenchymal stem cells (MSCs) are considered to be a promising candidate for cartilage regeneration, due to their ability to differentiate towards cartilage and bone cells and secrete trophic factors with regenerative functions [18]. The paracrine effect and anti-apoptotic, anti-inflammatory and anti-aging functions of these stem cells, is fundamental for the regeneration process. Recently, an anti-aging effect of the conditioned medium of adipose-derived mesenchymal stem cells (ASCs) on OA chondrocytes has been reported, featured by downregulation of senescence markers induced by inflammatory stress [19]. Stem cells promote biological processes, such as vascularization, cell proliferation, differentiation, and modulation of the inflammatory process [20], and can be isolated, among others, from bone marrow, adipose tissue, umbilical cord blood, and placenta [21,22]. It is currently admitted that there are MSCs within the connective tissue of virtually all organs [20]. In humans, ASCs showed a greater capacity for proliferation than the rest of the human MSCs [23], moreover, these cells maintain the differentiation potential after a longer time of culture [24], and the age of donors has less effect on the proliferation of them; this is important in elderly patients with osteoporosis [25].

ASCs were first identified in the early 2000s, and demonstrated to have self-renewal ability and multilineage differentiation potential [26]. These cells have a several benefits: faster and easier expansion in culture, more passage cells that retain stem cell phenotypes, pluripotency [27], less susceptibility to age, and less morbidity of patients [28], furthermore, compared with bone marrow-derived mesenchymal stem cells (BMMSCs), ASCs do have an equal potential to differentiate into cells and tissues of mesodermal origin, such as adipocytes, cartilage, bone, and skeletal muscle.

On the other hand, the easy and repeatable access to subcutaneous adipose tissue and the simple isolation procedures provide a clear advantage [29].

To establish the efficacy of treatment with this regenerative therapy and assess, in the case of OA, the quality and thickness of the cartilage, long-term patient controls are needed [30]. Different studies have shown that the application of MSCs as therapy in the treatment of OA has improved the symptoms suffered by patients, particularly after more than 6 months of follow-up [10,30,31].

Recent studies have focused on BMMSCs for chondrogenesis, but the clinical use of these cells has presented disadvantages, such as, donor site morbidity, pain and low cell number upon harvest [32]. On the other hand, ASCs are a positive alternative treatment for OA treatment as in vitro studies have proven they contain CD73, CD90, CD105, and CD106 markers, which are necessary for cell differentiation into cartilage, and moreover, in vivo studies have also reported good results [33]. For all of the abovementioned reasons, the aim of the present study is to review the application of MSCs in OA, with particular emphasis on the use of ASCs versus other therapies.

2. Results

The prevalence and incidence of OA have increased globally, but pharmaceutical or surgical therapies have limited efficacy in halting OA progression. Among conservative therapies, nonpharmacological treatment (physiotherapy, weight management), systemic pharmacological treatment (analgesics, nonsteroidal anti-inflammatory drugs, glucosamine, and chondroitin sulfate) and injections of intra-articular (IA) therapies are described. Local delivery of corticoids and hyaluronic acid (HA) are approved treatments by United States Food and Drug Administration (US FDA)/European Medicines Agency (EMA), however, some adverse effects have been described, such as inflammation or pain and septic arthritis at the site of injection [34].

When OA advances, some surgical treatments can rebuild the degenerated cartilage, but they do not stop the articular inflammatory process established. These treatments are arthroscopic debridement, microfracture/osteoplasty, and chondrocyte implantation techniques, such as autologous chondrocyte implantation and matrix-induced autologous chondrocyte implantation [35]. The latter is the only cell therapy approved by the FDA [36].The application of such interventions remains limited, due to the necessity of additional surgery for harvesting the donor autograft cartilage, and the poor integration of the grafted defect with the surrounded cartilage [37].

Due to the limitations regarding OA conventional therapies, for example, the pharmacological therapy could produce serious gastrointestinal, renal, and cardiac adverse effects, and some of them can be a threat to life or they can leave a permanent disability. On the other hand, the surgical option, such as microfracture, has been used for the last 20 years, but hyaline cartilage has a limited capacity for regeneration. In recent years, the interest for new therapies, such as IA injection, autologous blood therapies, and MSCs is increasing considerably. Autologous conditioned serum (ACS) is a cell-free treatment obtained by incubating venous blood in a specialized syringe, where blood cells release anti-inflammatory cytokines and growth factors (GFs), such as transforming growth factor-β (TGF-β) [38]. It has been demonstrate that ACS therapy is more effective than HA injection [39] and the treatment with ACS and physiotherapy reduce chronic pain in knee OA [40]. Plasma rich in growth factors (PRGFs), is a type of platelet rich plasma (PRP) preparation. It is an autologous product with a moderate concentration of platelets, multitude of GFs, and absence of leukocytes. It provides an anabolic effect on the resident cells, and due to its potential to inhibit inflammation, relieves OA symptoms [41]. When PRGF is injected IA, it provides a three-dimensional network in the joint composed of fibrin that contains binding sites for cell adhesion, as well as proteins that form the microenvironment leading to different adhesion molecules and cells that help biological cartilage repair [42]. Recently, it has been reported that PRGF injection is an effective option to decrease pain and improve function in patients with symptomatic mild to moderate knee OA, in the 6 month follow-up [43]. In recent years, the release of ASCs with PRP has been reported to improve the

proliferation and chondrogenesis of this type of stem cells [44,45], suggesting new applications in RM for the management of osteochondral defects.

Currently, RM, that aims to promote regenerative or reparative phenomena over the degenerative processes, is in full swing. Among these therapies are the already mentioned PRGF and the MSCs.

2.1. Mesenchymal Stem Cells

MSCs are defined as those cells that meet the criteria established by the International Society of Cellular Therapy. These criteria include an ability to adhere to plastic, the expression of a number of cell markers, including CD105, CD73, and CD90 while undergoing multilineage differentiation, and the ability to self-renew [46]. Those multipotent adult stem cells synthesize mediators (cytokines, neuroregulatory peptides, trophic factors) which participate in tissue repair and regulate inflammatory and immune responses [47]. These adult stem cells could be induced to differentiate exclusively into the adipocytic, chondrocytic, or osteocytic lineages. It has been identified that individual stem cells, when expanded to colonies, retained their multilineage potential [48].

MSCs can be obtained from various adult tissues, for example, bone marrow, umbilical cord, skeletal muscle, synovial capsule, and adipose tissue. This last origin, has a number of advantages over the others, because adipose tissue is abundant and easy to obtain, and can be obtained in large amounts, using local anesthesia and causing minimal discomfort [49]. Subcutaneous fat tissue is the most accessible source, but in recent studies, other sources for obtaining autologous ASCs as the supra- and infrapatellar fat pads have been described [50]. Bone marrow aspirate has a paucity of MSCs, comprising 0.001–0.02% of the mononucleated cell population, in comparison to ~1–7% of the mononucleated cell population within adipose tissue [51,52]. Moreover, adipose tissue is considered a primary source, because it contains 500 times more MSCs than the same volume of bone marrow [53]. Furthermore, bone marrow harvested from the iliac crest is painful, and increases the risk of infection [54]. Recently, some reports demonstrate that IA injection of allogenic ASCs combined with HA could stop OA progression and promote cartilage regeneration [55]. Additionally, a successful management of a post-traumatic chondral defect using IA autologous ASC therapy has been suggested [35].

The FDA regulates the use of adult stem cells. This agency adopted 21 CFR 1271, which modified its jurisdiction over human cells and tissues to include any "transfer into a human recipient". Previously, the code was specified transfer "into another human," excluding autologous cells. Since then, cells that are more than "minimally manipulated," even if they are intended for autologous use, and are subject to similar regulations as manufactured drugs [56]. More research studies on the use of MSCs in OA treatment would allow the FDA and physicians to provide patients with a more confidently alternative, minimally invasive treatment options that may significantly slow disease progression.

2.1.1. The Role of Mesenchymal Stem Cells in Osteoarthritis

The mechanism by which MSCs cause cartilage regeneration is not clear. It has been postulated that these cells may act on subchondral bone, forming the primary repair cartilage [57]. The injection of MSCs in joint cavity is a novel therapy that improves OA symptoms due to their ability to stimulate local repair and regeneration of damaged joint tissues, and to reduce inflammation and associated pain [16]. MSCs modulate the inflammatory response by causing the suppression of inflammatory T-cell proliferation and inhibition of monocyte and myeloid dendritic cell maturation [58]. Moreover, the anti-inflammatory capacity can be stimulated by various pro-inflammatory cytokines (IL-6, tumor necrosis factor and interferon gamma) [59]. Furthermore, these stem cells secrete reparative cytokines, including TGF-β, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF), which are responsible for a trophic effect that produces the local tissue repair [60]. Additional in vitro studies have demonstrated that GFs, such as TGF-β and insulin-like growth factor 1 (IGF-1), can stimulate MSCs towards chondrocytes. These chondrocytes, derived from MSCs, have the same

expression of type II collagen that mature adult chondrocytes, moreover, this type II collagen provides tensile strength in the joint [61].

It has been demonstrated that after IA injection of MSCs (Table 1), these cells were found in the synovial membrane and they expressed molecules with anti-inflammatory and chondrogenic properties. MSCs could help to establish a regenerative microenvironment at the site of release, which would improve the recruitment, activation, and differentiation of endogenous stem cells with the potential to repair the articular cartilage [62].

With the aim of achieving a prolonged regenerative activity in the OA joint, several studies propose using MSCs activated with biomolecules to potentially improve chondral and osteochondral lesion repair. The need to use bioactive scaffolds was proven by the fact that in the knee joint of experimental mice, only 15% of ASCs injected were detectable at 1 month post-injection, and this number decreased to 1.5% in 6 months [63]. This type of MSC co-delivery with scaffolds could have better retention, aggregation, and viability of these cells; moreover, the proliferation, migration, and chondrogenic differentiation improve in scaffolds with large pore size [64].

Some in vitro studies have demonstrated the beneficial effects of scaffolds (CDM, poly l-glutamic acid/chitosan, TGF-β1-conjugated chitosan hydrogel), such as regeneration of hyaline cartilage, enhancing ASC chondrogenesis and reparation of full-thickness cartilage defects [65–67]. More scaffolds include fibrin, gelatin and collagen (protein-based scaffolds), and alginate or agarose, among others (carbohydrate-based scaffolds). Other scaffolds that maintain a three-dimensional structure include the hydrogel family and hydrophilic polymer [36].

Table 1. Research on osteoarthritis with MSC-based therapy.

Study	Model	MSCs Type	OA Location	Results
Garay-Mendoza et al., 2018 [15]	Human	BMMSCs	Knee	Improvement in knee pain and quality of life since first evaluation until the last one at 6 months
Sun et al., 2018 [68]	Rabbit	ASCs + TGF-β3 poly-lactic-co-glycolic acid Microspheres	Knee	Promoted cartilage regeneration and lessened the severity of OA in vivo
Desancé et al., 2018 [69]	Equine	UCBMSCs	In vitro	High proliferative capacity and differentiated into osteoblasts and chondrocytes. Have a great potential for cartilage tissue engineering
Freitag et al., 2017 [35]	Human	Arthroscopy with removal of a chondral loose body + ASCs	Post-traumatic chondral defect of the patella	Complete regeneration of hyaline-like cartilage within the defect and improvement of the pain and function
Abbas 2017 [70]	Human	BMMSCs + cartilage fragments	Osteochondral bone samples from patients with total knee arthroplasty and a central drill defect (human ex vivo osteochondral defect model)	Improvement in chondrogenic differentiation and positive staining for type II collagen antibodies
Murphy et al., 2017 [71]	Human	BMMSCs	First Carpometacarpal joint	Functional and symptomatic relief for the patients
Pers et al., 2016 [72]	Human	ASCs	Knee	Patients treated with ASCs experienced significant improvements in pain levels and function knee compared with baseline.
Rich et al., 2015 [73]	Human	BMMSCs	Knee	Significantly improved the knee injury and Osteoarthritis Outcome Score and knee cartilage thickness (measured by magnetic resonance imaging), indicating that they may enhance the functional outcome as well as the structural component

Table 1. *Cont.*

Study	Model	MSCs Type	OA Location	Results
Jo et al., 2014 [74]	Human	ASCs	Knee	Improve function and pain of the knee joint without causing adverse events, and reduce cartilage defects by regeneration of hyaline-like articular cartilage
Wu et al., 2014 [75]	Rat	SMSCs + fibrin/chitosan scaffold + TGF-β3	Temporomandibular Joint	Fibrocartilage formation with deposition of ColI and Col2
Chen et al., 2013 [76]	Rabbit	BMMSCs	Temporomandibular Joint	Enhance the regenerative process of cartilage repair at the early stage of Temporomandibular joint OA

MSCs mesenchymal stem cells, BMMSCs bone marrow mesenchymal stem cells, OA osteoarthritis, TGF-β3 transforming growth factor β3, UCBMSCs umbilical cord blood mesenchymal stem cells, ASCs adipose-derived stromal cells, SMSCs synovial mesenchymal stem cells, Col1 and Col2 type 1 and type 2 collagen.

2.1.2. Mesenchymal Stem Cells Exosomes in Osteoarthritis

Exosomes are a type of secreted membrane vesicles produced by different cells. Some types of exosomes have been shown to confer immunosuppressive effects in different disease models, among others, rheumatoid arthritis [77]. MSC exosomes are accepted as the principal therapeutic agents present in MSC secretion, and are adequate to mediate the many reported therapeutic options of MSCs [78]. Zhang et al., (2016) have reported that human MSC exosomes promote cartilage regeneration in an immunocompetent rat osteochondral defect model, so MSC exosomes help to regenerate the damaged articular cartilage in OA. The mechanism of action of MSC exosomes in that study were accelerated neotissue filling and enhanced matrix synthesis of type II collagen and sulphated glycosaminoglycan [79].

Another study demonstrates that weekly IA injections of human embryonic MSC exosomes induced a regeneration cartilage and subchondral bone over a period of 12 weeks in an adult immunocompetent rat model [79]. Definitely, the efficacy of MSC-based therapies has been assigned to the paracrine secretion of trophic factors, and exosomes have a fundamental role in mediating tissue repair, thus, exosomes represent a novel therapeutic option for OA.

3. Discussion

ASCs have a series of advantages over other types of cells, because adipose tissue is abundant and easy to obtain. On the other hand, these cells have a high in vitro proliferation capacity and fibroblastic morphology, and they can adhere well to the culture plate. Furthermore, they have a low risk of rejection [80]. ASCs can be isolated from the stromal vascular fraction (SVF) of adipose tissue. The cells are obtained by liposuction, followed by collagenase digestion, centrifugation, and dilution. SVF includes ASCs, as well as other cells, including pericytes, vascular adventitia cells, fibroblasts, preadipocytes, monocytes, macrophages, and red blood cells. The SVF product has 500,000 to 2,000,000 cells per gram, of which 1 to 10% are considered ASCs [81].

It has been calculated that there are approximately $1 \times 10^{5-6}$ ASCs in 1 ml of lipoaspirate, while there are 50–675 BMMSCs in 1 mL of bone marrow aspirate [82]. Another alternative for OA treatment with MSCs are human umbilical cord-derived MSCs (hUC-MSCs). These cells enhance the proliferation of OA chondrocytes and downregulate the expression of inflammatory cytokines, moreover, the co-culture of hUC-MSCs and OA chondrocytes may to be a therapeutic option for OA [22]. hUC-MSCs could be an alternative to BMMSCs for clinical applications, due to their easy preparation and low risk of viral contamination. They can differentiate into the three germ layers that promote tissue and organ repair and modulate immune responses [83]. In this review, we have reported the different types of treatment that are used conventionally in joint degenerative disease. Our discussion focuses on comparing ASCs with others stem cells most commonly used in the OA treatment in recent years.

3.1. Adipose-Derived Mesenchymal Stem Cells

In human medicine, it has been shown that MSCs are a clinical promise for articular cartilage regeneration. Several authors reported studies that demonstrate the effectiveness of MSCs in OA treatment. In relation to ASCs, the first case report was published in 2001 [21]. During the last decade, these cells have attracted great interest because they have been demonstrated to be safe and efficient for articular cartilage regeneration in several trials. In recent years, IA injection of ASCs in knee OA showed clinical, radiological, arthroscopic, and histological evidence at 6-month follow-up [74]. Among other studies, the IA injection of these stem cells (isolated from abdominal subcutaneous fat tissue) in severe knee OA, reported that clinical outcomes (pain, function knee, return to sport) of the low- and medium-dose groups tended to deteriorate after 1 year, while those of the high-dose group tended to plateau after 1 year, until 2 years [10]. Recently, Spasovski et al. (2018) have demonstrated that the use of ASCs from subcutaneous fat in knee OA improves clinical symptoms and reduces pain at 3 months, obtaining the best results at 6 months [30]. ASC therapy in OA has shown chondrogenesis potential, both for the infrapatellar- and suprapatellar-derived ASCs [50,84]. A greater chondrogenesis potential has been reported by infrapatellar ASCs compared to suprapatellar in vitro and in vivo [84,85]. In addition, the suprapatellar-derived ASCs transplantation in a severe knee OA mouse model diminished inflammation and cartilage degenerative grade, increasing the synthesis of glycosaminoglycan and inducing endogenous chondrogenesis [50]. These effects may be due to ASCs-mediated reduction of pro-inflammatory cytokines and chemokines, apoptosis of chondrocytes, hypertrophic and fibrotic chondrocyte phenotypes, and collagenases [86].

One of the limitations of the studies that describe the use of ASCs in OA is the short follow-up period, Joe at al. (2014) and Pers et al. (2016) reported the efficacy of IA injections of these cells for the treatment of knee OA, but their follow-up period was only 24 weeks [72,74]. However, Song et al. (2018) have reported the first study that has demonstrated the efficacy of ASCs therapy in knee OA with long-term follow-up of 96 weeks with repeated injections. These patients showed improvement in pain, function, and cartilage volume of the knee joint with repeated IA injections of these cells [87].

3.2. Bone Marrow Mesenchymal Stem Cells

Pittenger et al. (1999) isolated MSCs from bone marrow adult cells and since then they have been used to treat chondral defects [48]. Some studies in patients with OA treated with BMMSCs obtained good results, reducing the symptoms and, therefore, increasing patient satisfaction. In a study carried out in 24 patients with knee OA infiltrated with BMMSCs, histological and arthroscopic improvement was observed [88]. Another report by Kuroda et al. (2002) concluded that the transplantation of autologous BMMSCs promotes the repair of large defects of focal articular cartilage in young and active patients [89]. Recently, safety of IA injection of BMMSCs was confirmed in 12 OA patients. They showed pain relief and improvement of cartilage quality at 2 years post-treatment [90]. Regarding the regulation of the inflammatory process in OA, Zhang et al. (2016) reported that the co-cultivation of BMMSCs with chondrocytes from patients with OA increases cell proliferation of chondrocytes and inhibits inflammatory activity in OA [91]. In a study of knee OA treatment in 13 patients with in vitro expanded BMMSCs at 12 months, a significant improvement in the thickness of knee cartilage in the femoral and tibial plates was shown [73]. Good results have also been reported with the application of BMMSCs in large [92] and small joints [71] with microfracture.

3.3. Human Umbilical Cord-Derived Mesenchymal Stem Cells

Some studies have demonstrated that chondrocytes secrete the same cytokines and induce human stem cells to differentiate into chondrocytes [93,94]. It has been reported that the hUC-MSCs improve the viability of OA-degenerated chondrocytes. A study carried out in Shangai suggested that the secretion of hUC-MSCs enhanced chondrocyte proliferation and showed that these stem cells increased expression of chondrogenic genes (*aggrecan, sox-9, collagen II*), indicating chondrocytes

promoted chondrogenic differentiation of hUC-MSCs, compared to the control group. They also postulated that hUC-MSCs inhibited inflammatory activity in OA chondrocytes [22]. In the same line, Zheng et al. (2013) reported the chondrogenic differentiation of hUC-MSCs by co-culture with rabbit chondrocytes [95]. In other studies, hUC-MSCs were reported to inhibit the expression of some inflammatory factors [96,97]. Definitely, hUC-MSCs could regulate inflammatory activity and the proliferation of chondrocytes in OA.

Therapeutic options of OA depend on each individual case and multiple factors, such as the disease progression, degeneration degree of articular cartilage, affected joint, and patient's expectations. The gold standard in the treatment of OA is total joint replacement, but surgery is not suitable for patients under 55 years [98], however, HTO is intended to transfer the mechanical axis from medial to slightly lateral, to the midline of the knee, to decrease the load and subsequently delay OA [99] in 40–60 years old patients [100]. Some studies showed that the regenerative process began after realignment [99], but different complications have been described, such as aseptic nonunion and deep infection [101]. MSCs may be a safe and alternative treatment strategy for OA, due to that this therapy has different advantages: it is applied in all joints, the injections are repeatable, and it is minimally invasive [12].

4. Materials and Methods

The authors searched PubMed English languages articles using a combination of "osteoarthritis", "stem cells", "regenerative therapies", and "adipose-derived mesenchymal stem cells" as keywords. After the first selection of the main articles based on OA and conventional treatments, studies of OA and stem cells were selected, and there were a total of about 900 articles in the last decade. Special attention has been drawn to original analyses and studies, around 300 studies, of which we chose about 80 articles dedicated to ASCs and were published in the last 10 years. Other searches were executed using bibliographies of articles found in the primary and secondary search. One limitation in this review, is the fact that our methods, while rigorous, did not follow any formal guidelines for a systematic review (e.g., the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines).

5. Conclusions

OA is a major health problem, especially in the elderly population. Cellular therapy is an emerging modality for the treatment of OA, even the combination of conventional treatments with the application of MSCs is a therapeutic option to improve the quality of life of patients with OA. In recent years, the interest in MSCs as a therapeutic option in OA is due to the facility of harvesting, preparation, and implantation without surgery, the capacity to stimulate local repair and regeneration of damaged joint tissues, and the ability to reduce inflammation and associated pain. ASCs have different advantages, including easy cryopreservation, faster expansion in culture, more passage cells that retain stem cell phenotypes and pluripotency, and less susceptibility to aging together with lower morbidity of patients. Moreover, the obtention of adipose tissue is much less expensive than bone marrow, with less invasive intervention and available in greater quantities.

ASCs in OA may offer an exciting possibility to improve function, pain, and cartilage volume of the joint, suggestive of a good therapeutic strategy for OA. Despite this, further long-term studies are needed to prove and evaluate the effectiveness of ASCs in OA treatment and their safety capacity. However, it is important to establish a standardized therapeutic protocol for this biological therapy, and assess each patient and each pathology individually.

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Abbreviations

ACS	Autologous conditioned serum
ASCs	Adipose-derived mesenchymal stem cells
EGF	Epidermal growth factor
EMA	European Medicines Agency
FDA	Food and Drug Administration
GFs	Growth factors
HA	Hyaluronic acid
hUC-MSCs	Human umbilical cord mesenchymal stem cells
IA	Intra-articular
IGF-I	Insulin-like growth factor-I
MSCs	Mesenchymal stem cells
OA	Osteoarthritis
PRGFs	Plasma rich in growth factors
PRP	Platelet rich plasma
RM	Regenerative medicine
TGF-β	Transforming growth factor-beta
VEGF	Vascular endothelial growth factor
HTO	High tibia osteotomy

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4.2.2. Plasma rico en Plaquetas

El plasma rico en plaquetas (PRP comenzó a utilizarse a principios de los 90 en cirugías cardiacas (Ferrari y cols, 1987), para posteriormente ampliarse su espectro de acción sobre todo en la cirugía maxilofacial y estética, y más adelante comenzó a utilizarse también en traumatología (Floryan y Berghoff 2004; Sampson y cols, 2008)

El PRP es un producto de origen biológico, que se obtiene mediante el procesado de la sangre del propio paciente. En este procedimiento se obtiene un derivado que contiene una concentración más o menos variable de plaquetas, así como factores plasmáticos de crecimiento (Marx, 2001).

4.2.2.1. Eficacia en Aparato locomotor

La eficacia de las terapias con PRP o sus derivados a la hora de la reparación de defectos óseos, condrales, musculares y/o tendinosos en animales ha quedado comprobada (Serra y cols, 2013; Thor y cols, 2013; Cugat y cols, 2017). Sin embargo, en el caso concreto de las fracturas, y el tratamiento de sus complicaciones en la consolidación ósea las publicaciones en medicina humana son muy escasas (Zhang y cols, 2003; Seijas y cols, 2010) y, en el campo de la medicina veterinaria, ausentes.

ARTÍCULO #3

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Assessment of the Efficacy of Platelet-Rich Plasma in the Treatment of Traumatic Canine Fractures

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Abstract: The role of platelet-rich plasma (PRP) in promoting the healing of bone fractures has not yet been clearly stated. The aim of this prospective clinical study was to evaluate the effectiveness of plasma rich in growth factors (PRGF, a PRP derivate) in the treatment of naturally-occurring bone fractures in dogs. With this objective, sixty-five dogs with radius/ulna or tibia/fibula bone fractures were randomly divided into two groups (PRGF and saline solution (SS) groups) and checked at days 0, 7, 14, 21, 28, 35, 42, 49, 56, 60, 63, 70, 120, and 180. All the fractures were treated with an external skeletal fixation, and pain was controlled with Carprofen. Healing was evaluated by physical examination, limb function, radiography, and by a Likert-type owner satisfaction questionnaire. A faster fracture healing was observed in the PRGF group, with statistically significant differences with respect to the SS group. Swelling at the fracture site was significantly greater at day 14 and 28 in animals injected with PRGF, and more pain on palpation was found in the area at day 28. The injection of PRGF in acute bone fractures accelerates bone healing.

Keywords: PRGF; Carprofen; dog; fracture; bone healing

1. Introduction

Plasma-rich growth factors (PRGF) are currently being used to promote bone healing in reconstructive surgeries [1–3]. In canine models, several experimental studies have published the effect of this platelet rich plasma derivate in osteoarthritis with differing results [4–7]. Platelets are very important in the wound healing process [1]; they rapidly arrive at the wound side and begin the coagulation process. In addition, they release multiple wound-healing growth factors and cytokines within 10 min [1–3]. Platelets are viable for seven days and will continue to release growth factors into the tissue during this time [8].

The use of PRGF is based on the assumption that higher platelet concentrations release significant quantities of growth factors, which aids in bone healing [9–11]. Specifically, growth factors are thought to be a contributing factor in bone regeneration and in increasing vascularization, which are vital features of the bone-healing process [6].

Treatments with PRGF have given excellent clinical results in oral and maxillofacial surgery in humans [9,12], and in bone and cartilage healing in animal studies [7,13,14]. Growth factors have also

been used in the treatment of large wounds and skin defects in burn patients [15–17]. However, some controversial results can be found in the cited literature; therefore, the effectiveness of this technique requires further research.

To the authors' knowledge, articles discussing fresh fractures and delayed fracture healing are very scarce [18,19]. In the veterinary field, no publications were found regarding the use of PRGF in fractures.

In this study, the dogs used were clinical patients, but also clinical animal models. In the present study, we hypothesize that treating canine bone fractures with PRGF would accelerate bone healing. Thus, the aim of this clinical trial is to evaluate the use of PRGF in the treatment of naturally occurring bone fractures in dogs.

2. Results

A total of 68 dogs were initially evaluated; however, only 43 met the necessary requirements to be included in the study.

The dogs were randomly assigned to either PGRF or SS groups. Twenty dogs were included in the PRGF group (47%) and 23 in the SS group (53%). The results for each dog belonging to either the PRGF or the SS groups are summarized in their respective tables (Tables 1 and 2).

The mean weight for each group was 16.27 kg for the PRGF group and 13.07 kg for the SS group. Mean age was 40.85 and 57.17 months, respectively, with no statistical differences between groups in these parameters ($p \geq 0.08$).

During the study, all the animals received Carprofen as a rescue analgesia at least one time during the first seven days except for 2 and 4 patients in the PRGF and SS groups, respectively, with no statistical differences between groups ($p \geq 0.05$).

The time (mean ± SD) for implant removal was 41.3 ± 11.73 days in the PRGF group and 49 ± 12.12 days in the SS group. This difference was statistically significant ($p = 0.03$) (Figure 1).

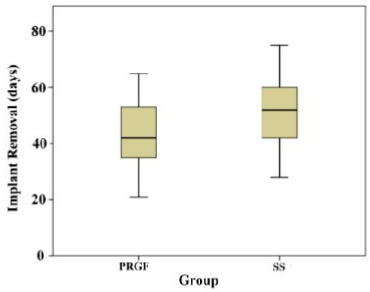

Figure 1. Boxplot corresponding to the days of implant removal for both PRGF and SS groups. Mean time was significantly higher in the SS group.

The time when full weight support was detected was 22.1 ± 13.64 days and 25.47 ± 14.9 days in the PRGF and SS groups, respectively; however, this difference was not statistically significant ($p = 0.45$). All animals were sound within six months post-surgery.

Swelling in the fracture site was present in both groups up to day 14 without statistically significant differences between the groups. Between days 14 and 28, swelling was still present in the PRGF group ($p < 0.048$).

The joint movement evaluation showed almost 100% joint mobility without differences between groups in any of the checking periods.

The evaluation of pain on palpation showed statistically significant differences at day 28 between groups, where pain was still present in the PRGF group ($p = 0.041$).

Table 1. Individual data and main results for PRGF group.

Dog #	Breed	Gender	Weight (kg)	Age (months)	Fracture L	Configuration	Weight Support	Time I Removal (days) *	Complications	Analgesia
1	G DANE	M	53	11	U/R	TYPE IIB 2X3	7	35 (39)	NO	Y
2	CROSSBREED	F	4	24	U/R	TYPE IIB 1X2	7	42 (45)	NO	N
3	CROSSBREED	M	17,8	36	U/R	TYPE IIB 2x4	21	28 (32)	NO	Y
4	CROSSBREED	F	8,5	96	U/R	TYPE IIB 2X3	14	56 (60)	GE	Y
5	CROSSBREED	F	4	3	T/F	TYPE IIB 2X3	7	21 (21)	NO	Y
6	CROSSBREED	M	4	36	U/R	TYPE IIB 2X3	28	63 (63)	NO	Y
7	CROSSBREED	M	7	48	T/F	TYPE IIB 2X3	21	35 (40)	PL	Y
8	CROSSBREED	F	6	6	T/F	TYPE IIB	21	28 (30)	NO	Y
9	CROSSBREED	F	2,3	60	U/R	TYPE IIB 2X3	21	42 (42)	PL	Y
10	CROSSBREED	F	22	12	U/R	TYPE IIB 2X3	14	35 (36)	NO	Y
11	CROSSBREED	F	72	20	U/R	TYPE IIB 2X3	60	56 (56)	NO	Y
12	CROSSBREED	M	6,3	48	U/R	TYPE IIB 2X3	60	42 (45)	NO	Y
13	CROSSBREED	M	4	12	U/R	TYPE IIB 2X3	7	56 (56)	NO	Y
14	BELG SHEPH	F	16	96	U/R	TYPE IIB 2X3	21	49 (49)	PL	Y
15	CROSSBREED	M	4,5	24	U/R	TYPE IIB 2X3	21	28 (30)	NO	Y
16	PODENCO	M	22	56	U/R	TYPE IIB 2X3	28	28 (28)	NO	Y
17	CROSSBREED	M	6	70	U/R	TYPE IIB 2X3	21	63 (65)	NO	Y
18	RAT VAL	F	2	24	U/R	TYPE IIB 2X3	21	49 (50)	NO	Y
19	MASTIFF	M	52	36	T/F	TYPE IIB 2X3	28	35 (40)	NO	Y
20	CROSSBREED	M	12	99	U/R	TYPE IIB 2X3	14	35 (35)	NO	N

* The first number references the checking day when stage 4/5 was reached radiographically and the implant was ready for removal; the number in parenthesis refers to the day the implant was removed. RAT VAL: Ratonero Valenciano.

Table 2. Individual data and main results for SS group.

Dog #	Breed	Gender	Weight (kg)	Age (months)	Fracture L	Configuration	Weight Support	Time I Removal (days) *	Complications	Analgesia
1	SIB HUSK	M	27	15	U/R	TYPE IIB	60	63 (69)	PL	Y
2	RAT VAL	F	1,7	6	U/R	TYPE IIB 1X2	21	35 (35)	NO	N
3	CROSSBREED	F	5,5	12	T/F	TYPE IIB1.5X2	7	28 (34)	NO	N
4	CROSSBREED	F	5,5	12	U/R	TYPE IIB1.5X2	7	28 (31)	PL	N
5	AM STAFFORD	M	30	72	U/R	TYPE IIB 2X3	21	35 (36)	NO	Y
6	CROSSBREED	M	4,5	60	U/R	TYPE IIB 2X3	7	56 (58)	NO	Y
7	CROSSBREED	M	20	72	U/R	TYPE IIB 2X3	21	70 (75)	NO	Y
8	GRIFFON	M	15	50	U/R	TYPE IIB 2X3	28	42 (42)	NO	Y
9	GER SHEPH	F	34	70	T/F	TYPE IIB 2X3	21	42 (44)	NO	Y
10	W HIGH W TERR	F	5,6	48	U/R	TYPE IIB 2X3	21	42 (42)	GE	Y
11	CROSSBREED	M	18	48	U/R	TYPE IIB 2X3	21	42 (45)	NO	Y
12	CROSSBREED	F	12	60	T/F	TYPE IIB 2X3	21	42 (45)	NO	Y
13	CROSSBREED	F	3	24	U/R	TYPE IIB 2X3	21	56 (57)	NO	Y
14	MALTESE	F	9	192	T/F	TYPE IIB 2X3	60	56 (60)	NO	Y
15	RAT VAL	F	5	111	T/F	TYPE IIB	21	63 (68)	NO	Y
16	BELG SHEPH	M	34	86	T/F	TYPE IIB	28	63 (65)	NO	N
17	BELG SHEPH	F	9	20	T/F	TYPE IIB 2X3	28	63 (64)	NO	Y
18	YORKSHIRE	F	1,5	35	U/R	TYPE IIB 2X3	21	35 (38)	PL	Y
19	POODLE	F	8	122	U/R	TYPE IIB 2X3	21	49 (50)	NO	Y
20	CROSSBREED	M	25	75	U/R	TYPE IIB 2X3	21	56 (60)	NO	Y
21	DALMATIAN	M	22	46	T/F	TYPE IIB 2X3	21	49 (52)	NO	Y
22	YORKSHIRE	F	1,5	24	U/R	TYPE IIB 2X3	60	56 (60)	NO	Y
23	CROSSBREED	M	4	55	U/R	TYPE IIB 2X3	28	56 (60)	NO	Y

* The first number references the checking day when stage 4/5 was radiographically reached and the implant was ready for removal; the number in parenthesis refers to the day the implant was removed. SIB HUSK: Siberian Husky; RAT VAL: Ratonero Valenciano; AM STAFFORD: American Staffordshire Terrier; GER SHEPH: German Shepherd; W HIGH W TERR: West Highland White Terrier; BELG SHEPH: Belgian Shepherd.

No significant differences were found in the assessment of owner satisfaction at implant removal, with a satisfaction between 4 (24% in PRGF, 25% in SS) and 5 (76% in PRGF, 75% in SS).

Complications were recorded. One dog suffered gastroenteritis, and three dogs had pins become loose in the PRGF group. The same number of complications occurred in SS group (Tables 1 and 2).

3. Discussion

In the present study, the beneficial effect of PRGF in acute ulna/radius and tibia/fibula fracture healing has been proven, achieving a faster healing compared with controls. However, in all cases, a primary and non-complicated healing was present.

To the authors' knowledge, there is no published clinical research discussing the use of PRGF in fractures in a canine model. Experimentally, some studies proved there was faster bone regeneration when PRGF or other autologous platelet concentrates were applied [20,21]. In human medicine, there was only one clinical study evaluating the healing of fresh fractures using PRGF with no positive effect [18]. On the contrary, a clinical case with a delayed union fracture treated with autologous PRGF showed a favorable healing and concluded to be a safe technology for patients [19].

PRGF has also been used by other authors in combination with other therapeutics, showing positive results. Ya-dong Zhang et al. [22] proved that the use of PRGF combined with a degradable bioactive borate glass promotes functional bone repair. On the other hand, other authors [4] found no effect of PRGF on non-grafted implants in dogs; nevertheless, we cannot compare these results with our study because a different process was used to obtain the PRGF: using thrombin (100U/mL) to stimulate growth factor release rather than calcium chloride.

It is known that Carprofen is suitable alone or in combination with other NSAIDs for the control of pain and swelling in dogs [23,24]. Gastrointestinal inflammation and ulceration are among the most common side defects reported in the literature [25]. In our study, there were only two animals with gastroenteritis, and they responded positively to the conventional treatment.

In the present study, it has been observed that the surgical application of PRGF at the fracture site is associated with increased swelling and oedema during the first days, probably due to the activation of angiogenesis and cell activation [26]. The enhancement of the arrival and formation of blood vessels increases heat, pain, and redness of the area. This swelling associated with oedema has been effectively treated with oral Carprofen.

In any case, increased swelling did not affect the animals' gait nor the functional ability of the joint. In this sense, some papers reported the inhibitory effect of interleukins, which may be attributed to PRP [27]. This effect may be related to a reduction of acute pain in the fracture site, even though the activation of angiogenesis may cause an increased perception of discomfort and inflammation [26]. Thus, even if the application of PRGF increases oedema and swelling on the area, the limb's function was minimally affected during the first days.

Good results have been obtained using PRP to accelerate bone fusion [28]. In our case, the group receiving the PRGF injection presented an earlier implant removal, which is in agreement with those who state that chemotactic and mitogenic effect on mesenchymal cells (stem cells) and osteoblasts accelerate bone healing [29,30].

A rapid return to functionality is crucial for quick and correct healing; when the limb bears weight, a transmission of forces takes place that stimulates osteoinduction. Likewise, early activity boosts vascularization and avoids muscle atrophy, which are factors that clearly activate bone healing. Moreover, the Carprofen helped to control pain and acute swelling at the fracture site, facilitating an earlier return to functionality [29]. This shows that swelling control and post-surgical analgesia are fundamental for early functionality of the affected limb and represent an important parameter to be assessed by the pet owners.

Regarding external fixation, all the animals showed limb weight bearing 48 h after surgery. Very few complications arose in relation to the use of external skeletal fixation. One animal presented

a secondary infection, which is a usual side effect, and only six animals presented pin loosening. Other studies show a larger number of cases presenting pin loosening as the most frequent complication [31].

The present study has three main limitations. First, the use of dogs with a wide weight range potentially limited results that are more accurate. A narrow weight range could provide more reliable and accurate results, at least for a specific weight range. Second, a biomechanical analysis of gait could provide full objective results regarding limb function. Third, statistical analysis of the variable "swelling at the fracture site" could provide more accurate results if it is considered a continuous variable instead of categorical, avoiding detection, performance, and reporting biases; however, the presence of hematoma or callous formation at the fracture site could potentially hinder precise measurements.

4. Materials and Methods

A multicentric study was designed and formed by four surgeons in four different veterinary clinical centers.

4.1. Animal Model

A total of 68 dogs were evaluated. The follow-up of the animals took place until six months after treatment. The inclusion criteria required the presence of a fresh, single, closed fracture and the absence of significant muscular soft tissue damage or abrasions.

The exclusion criteria for the present study were the following:

- Animals presenting concurrent systemic disease (*Leishmania* spp., *Ehrlichia* spp., etc.).
- Animals with hematological disorders.
- Animals with multiple fractures.
- Animals with internal lesions due to traumatism.
- Animals with open fractures or with significant damage to the surrounding soft-tissue.
- Animals with a significant weight loss or functional disabilities due to the treatment or other non-related causes.
- Animals needing different concurrent fixation methods due to the nature or clinical features of the fracture.

Fractures were classified according to the affected bone. In order to acquire similar healing conditions during the study, only tibia/fibula and radius/ulna fractures were included because of their poor vascularization due to their small surrounding muscular mass. The individual data of each dog for the PGRF and SS groups are summarized in Tables 1 and 2, respectively.

4.2. Fracture Treatment

All fractures were treated with conventional open or closed reduction and external fixation. The external skeletal fixation configuration frame was the most appropriate for each fracture, using type IIa or type IIb [32]. In all cases smooth pins of different diameters, connecting bars, and Meynard clamps were used.

After an initial clinical examination, animals were randomly assigned to one of the following groups depending on the treatment received:

- PRGF group: A single infiltration of PRGF in the fracture site during the surgery.
- SS group: A single infiltration of saline solution in the fracture site during the surgery.

All groups were treated with morphine (0.2 mg IM every 6 h), and Carprofen 4 mg/kg IV (Rimadyl®, Zoetis®, Spain) for 24 h. Cephalexin was administered as a post-surgery antibiotic.

After 24 h, the owners were allowed to give Carprofen (4 mg/kg/day) as a rescue analgesic if their pet presented clear signs of distress or discomfort. This fact should be reported during the clinical follow-up.

4.3. PRGF Preparation

For the present study, the extraction, isolation, activation, and administration model of the PRGF was standardized in all clinics following Anitua´s technique [9]. Briefly, 20 mL of blood were aseptically collected in four 4.5 mL citrate tubes, then centrifuged during 8 min at 460 G. Care has to be taken to avoid the buffy coat. Before the infiltration, the PRGF was activated with 5% of its volume with 10% calcium chloride. This obtained PRP derivate is enriched in platelets 2-fold over peripheral blood and less than 0.2 leucocytes $\times$ 10^6/mL.

4.4. Evaluation

The limb function was evaluated on days 0 (pre-surgery), 7, 14, 21, 28, 60, 120, and 180 after the treatment began. This parameter was assessed by the same researcher evaluating animals when standing (1: weight-bearing; 2: no weight-bearing; or 3: no limb support), by observing swelling on the fracture site (0: presence or 1: absence), pain on palpation (0: presence or 1: absence), and joint movement (1: <40%; 2: 40–70%; 3: 70–90%; or 4: >90%).

The same radiologist, unaware of the group of treatment, patient, and surgeon involved, examined all radiographs. Each radiograph was evaluated by a stage score of 1–5 points (1: not visible callus formation; 2: barely visible callus formation; 3: scattered, not homogeneous callus; 4: uniform, mature callus formation; 5: very active, hyperthrophic callus formation). Radiographical examination started for each dog at day 21 and for every two weeks thereafter until the animal reached stage 2; beyond this period, radiographs were taken weekly, coinciding with the checkpoints for the other parameters. When a final score of 4/5 was achieved, implant removal was performed and recorded. The researcher who performed the evaluation of limbs and who read the radiographies were blind to the given treatment (PGRF or SS).

The use of the rescue analgesic and the presence of side effects were registered by the owner. The level of owner satisfaction with the clinical outcome of their pets during the first 28 days and at implant removal was evaluated with the following questionnaire referring to the level of satisfaction measured with a Likert-type scale (Table 3).

Table 3. Likert-type questionnaire of satisfaction for dog owners at time of implant removal.

How do you consider the lameness of (name of the pet) has progressed?				
Excellent	Good	Average	Fair	Poor
5	4	3	2	1
Do you think the treatment given to (name of the pet) has been effective?				
Strongly agree	Agree	Neutral	Disagree	Strongly disagree
How Do You Think (Name of the Pet) has Responded to the Treatment?				
Excellent	Good	Average	Fair	Poor
5	4	3	2	1

4.5. Statistical Analysis

Statistical analysis was performed with the computer program SPSS 18® for Windows® (IBM Co., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant. The descriptive study of the population was shown as the mean $\pm$ SD. To determine the differences between the groups for non-categorical variables (weight, age, and total doses of Carprofen), a Kruskal–Wallis and Mann–Whitney test was done. To determine the effect of PRGF on implant removal time, a Kaplan–Meier curve and a log-rank test were used. The impact evaluation of total doses of Carprofen, age, weight, and bone fractured, within time to implant removal, a multivariable analysis was made using a Cox regression. Categorical variables (evaluation when walking, evaluation when standing, swelling, pain on palpation, joint movement, use of the recue analgesic, owner satisfaction, and

presence of side effects) were assessed using crosstabs with chi square, contingency coefficient, or the Fisher's exact test used when necessary in each variable.

The experimental procedure was approved by the ethics committee of the Research Institute in Biomedical and Health Sciences (ULPGC, Spain). The owners were informed about the aims of the study, and a written consent was required before including their pets in the study.

5. Conclusions

The use of PRGF for bone repair accelerates fracture consolidation and simultaneously promoted healing, achieving clearly shorter implant removal times.

Author Contributions: M.R., E.D., J.J.S., and J.M.C. performed and designed the experiment; S.L. and D.C. wrote the manuscript and participated in performing the experiment; M.R. performed statistical analysis; B.C. and S.L. participated in performing the experiment; and J.M.V. proofread the manuscript and gave approval of the final version.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

PRGF	Plasma rich in growth factors
SS	Saline solution
Fracture L	Fracture location
U/R	Ulna/radius
T/F	Tibia/fibula
Time I removal	Time for implant removal
GE	Gastroenteritis
PL	Pin loosening
Y	Yes
N	No

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4.2.2.2. Seguridad del PRP

Dentro de los efectos beneficiosos que produce el PRP, una parte significativa de ellos es atribuible a uno de los factores de crecimiento, en concreto al IGF-1. Este mediador de la hormona del crecimiento interviene de forma relevante en los procesos anabólicos del organismo (Philippou y cols, 2007). Teniendo en cuenta lo anterior, potencialmente podría producir un efecto ergogénico que, en teoría, si no estuviera conveniente regulado, podría desencadenar un proceso canceroso (Grimberg y Cohen, 2000; Grimberg, 2003; Renehan y cols, 2004; Lim y cols, 2015; Maniscalco y cols, 2015). Su efecto, tanto desde el punto de vista carcinogénico como sobre todo el ergogénico es objeto de discusión en la actualidad y, de hecho, este factor está incluido por este motivo entre la lista de sustancias prohibidas de la World Anti-Doping Agency (WADA).

Por lo tanto, se puede deducir que altos niveles de IGF-1 circulante serían los causantes del afecto a distancia, es decir, general, de los productos que contengan esta molécula como en este caso el PRP, utilizado con fines y a dosis terapéuticas. En este sentido, conviene destacar el trabajo de Vilar y cols (2017) donde se concluye que dosis terapéuticas de PRP no conllevan un aumento significativo de IGF-1 en sangre y, por lo tanto, es un producto seguro.

¿Y a nivel local? Este punto adquiere una enorme importancia ya que la mayoría de los tratamientos de PRP, especialmente aquellos que se usan en el aparato locomotor, se administran de forma parenteral. Por lo tanto, planteamos el siguiente estudio:

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Can Plasma Rich in Growth Factors Be Safe for Parental Use? A Safety Study in the Canine Model

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Abstract: Low invasiveness is the main goal of modern surgery. The use of platelet-rich plasma (PRP) is known to be effective in a variety of applications, such as oral, maxillofacial, orthopedic, dermatologic and cosmetic surgeries. However, a potential ergogenic and carcinogenic effect of PRP derivatives by means of the insulin-like growth factor-1 (IGF-1) pathway has been suggested. Because of this notion, the purpose of this study is to assess the effect of a commercially available PRP-derivative intramuscular injection in the lumbar muscular tissue (local effect) and to determine the IGF-1 blood concentration (systemic effect) on healthy beagle dogs. Local effect was evaluated by computed tomography (CT) scan and echography, and systemic effect was calculated by blood testing on days 0, 14, 28, 42 and 56. No statistically significant changes were observed; thus, PRGF could be considered safe when using therapeutic doses.

Keywords: platelet-rich plasma (PRP); plasma rich in growth factors (PRGF); insulin-like growth factor-1 (IGF-1); canine

1. Introduction

The main goal of modern surgery is to reduce invasiveness and increase the healing process. Regenerative medicine is now one of the most attractive and interesting disciplines that aims to regenerate or repair damaged tissues [1]. Platelet-rich plasma (PRP) is currently used in different medical fields and involves a minimum risk of immune reactions and transmission of diseases [2]. The first descriptions of the development and use of PRP were in the early 1970s in the hematology field, followed by maxillofacial and oral surgery [3]. Subsequently, PRP has been used in a wide variety of disciplines, such as aesthetic dermatology [4], including alopecia [5] and skin rejuvenation [6]; the musculoskeletal field [7]; oral and maxillofacial surgery [8]; and ophthalmology [9].

In recent years, the use of regenerative therapies, such as plasma rich in growth factors (PRGF), a PRP derivative, is also gaining interest to promote healing in muscle injuries, and consequently, to enable the patient to resume daily and sports-related activities quickly without relapse. A considerable number of authors have reported that growth factors (GFs) and fibrin matrix are crucial for the muscle repair and regeneration process by promoting myogenesis, angiogenesis and fibrogenesis [10,11], and promising results have been proven with this novel biological approach in managing musculoskeletal pathologies [12].

Besides the possible beneficial effects of PRP derivatives, several concerns have been raised regarding undesirable side effects. Some authors describe a potential carcinogenetic effect related to the insulin-like growth factor-1 in humans (IGF-1) [13–16]. In veterinary medicine, some studies demonstrated IGF-1 receptor expression and its role in canine osteosarcoma [17] and mammary gland carcinoma [18]. In this sense, there are different IGF-1 isoforms, such as IGF-1Ea, IGF-1Eb and IGF-1Ec. The IGF-1Ec isoform has an important role in physiology and cancer biology through its Ec peptide. After the tissue is damaged from mechanical stimuli, IGF-1Ec isoform and the Ec peptide levels are induced in the muscle, tendon and bone, and its secretion produces cellular proliferation [18–20].

Multiple GFs are secreted during muscle repair and hypertrophy, but only IGF-1 and its isoforms participate in muscle proliferation, differentiation and regeneration [21]. It has been demonstrated that IGF-1, at the onset of the mechanical stress on human skeletal muscle cells, increases IGF-1Ec isoform [22]. Moreover, the application of the Ec peptide in a rat model provided an increase in the expression of myofibroblasts in wound healing [23]. Overall, published scientific research supports that GFs included in PRP are unlikely to trigger a potent ergogenic effect. Regarding IGF-1, the doses in PRP are subtherapeutic, only 1% of the total IGF-1 is biologically available. IGF-1 in plasma has a low half-life (20 h) in humans [24], and although we could not find published data, dogs should be similar. To demonstrate this controversy, the use of PRP intramuscular injections in athletes was prohibited by the World Anti-Doping Agency (WADA) in 2010, despite the use of these biological therapies as the "gold standard" for muscle injury treatment, but again permitted in 2011 due to the limited evidence for systemic ergogenic effect of PRP. Nevertheless, the use of individual GFs in athletes continues to be prohibited under Section S2 of the 2018 WADA Prohibited List [25], particularly fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF-1), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), because of concerns regarding their abuse as ergogenic substances [26].

For these reasons, along with the multiple applications of these therapies and several pieces of evidence for specific ergogenic (local) and carcinogenic (systemic) effects, the use of PRGF in muscle tissue remains of great interest.

Based on this, the purpose of our study was to evaluate (a) the local effect, measuring the cross-sectional area (CSA) of the lumbar muscles by using imaging systems, and (b) the systemic effect by blood IGF-1 determination in healthy beagle dogs, which were submitted to a PRGF intramuscular lumbar injection.

2. Results

2.1. Animals

The ages of the animals included in the study were (mean ± SD) 73.5 ± 38.8 months in the control group, 76.5 ± 38.7 months in the PRGF group, and 79.4 ± 38.9 months in the triple dose (HPRGF) group. Their weights were 16.9 ± 3.3 kg in the control group, 14.8 ± 3.1 kg in the PRGF-treated dogs, and 14.9 ± 2.5 kg in the HPRGF group.

2.2. IGF-1 Evaluation

No statistically significant differences were observed along the studied times nor between groups in IGF-1 serum concentrations. As a result, IGF-1 serum concentrations remained stable throughout the study, showing the inability of PRGF intramuscular injection to have a systemic effect (Table 1).

Table 1. IGF-1 measurements (ng/mL) in the three study groups.

Time	Group	Mean	SD
Baseline	Control	166.3	31.1
	PRGF	132.7	49.1
	HPRGF	95.6	31.5
14 days	Control	174.7	25.1
	PRGF	114.5	40.2
	HPRGF	117.1	30.4
28 days	Control	157.9	26.4
	PRGF	133.8	46.7
	HPRGF	127.5	34.0
42 days	Control	134.6	20.0
	PRGF	143.9	48.5
	HPRGF	126.2	33.1
56 days	Control	155.6	24.3
	PRGF	139.4	49.9
	HPRGF	117.2	38.5

IGF-1: Insuline Growth Factor-1; PRGF: single dose of Plasma Rich in Growth Factors; HPRGF: triple dose of Plasma Rich in Growth Factors.

Regarding external factors affecting IGF-1 serum concentrations, in the three studied groups, weight influenced IGF-1 serum concentrations. In this way, the control group showed higher serum concentrations due to a larger weight in the animals during this phase. Conversely, older animals had lower IGF-1 serum concentrations compared to younger animals.

2.3. *Computed Tomography and Echography Evaluation*

Both CT-scan and echography images were carried out between the three studied groups and along the studied times. A correlation test was also realized between the two measures. No statistically significant differences were obtained between groups nor along the studied times in the studied anatomic level (L5) (Tables 2–4). As a result, no local effect and, therefore, no muscular hypertrophy were observed after PRGF injection.

Table 2. Ultrasound measurements of the muscular area at left L5 level in the three study groups.

Time	Group	Mean	SD
Baseline	Control	12.8	3.6
	PRGF	12.5	3.1
	HPRGF	12.8	3.5
14 days	Control	13.4	3.3
	PRGF	12.6	3.0
	HPRGF	13.1	3.3
28 days	Control	13.1	3.2
	PRGF	12.7	3.0
	HPRGF	13.3	3.1
42 days	Control	13.0	3.1
	PRGF	12.6	3.1
	HPRGF	13.0	3.1
56 days	Control	13.0	3.1
	PRGF	12.4	3.1
	HPRGF	12.8	3.2

L5: fifth lumbar vertebra.

Table 3. CT-scan measurements of the muscular area at left L5 level in the three study groups.

Time	Group	Mean	SD
Baseline	Control	13.3	2.6
	PRGF	12.7	2.4
	HPRGF	12.8	2.9
14 days	Control	12.9	2.7
	PRGF	12.2	2.5
	HPRGF	13.2	2.8
28 days	Control	12.5	2.5
	PRGF	12.3	3.3
	HPRGF	12.8	2.6
42 days	Control	12.4	2.5
	PRGF	12.1	2.7
	HPRGF	13.1	3.1
56 days	Control	12.9	2.6
	PRGF	11.8	2.4
	HPRGF	12.4	3.1

Table 4. Pearson correlation between ultrasound and CT-scan measurements.

Correlations			
		L5 US	L5 CT scan
L5 US	Pearson correlation	1	0.928 *
	Sig. (2-tailed)		0.000
	N	210	105
L5 CT scan	Pearson correlation	0.928 *	1
	Sig. (2-tailed)	0.000	
	N	105	105

* Correlation is significant at the 0.01 level (2-tailed).

3. Discussion

The aim of the present study was to determine if an intramuscular injection of PRGF increases circulating levels of the potentially ergogenic growth factor IGF-1 and thus induces skeletal muscle hypertrophy and, in the last instance, cancer.

Assuming that injections of PRGF within the injured muscle enhance healing and functional recovery [27], the question remains as to what is the correct dosage. In humans, Hamilton et al. (2010) demonstrated that a single injection of 3 mL PRP was effective for grade II semimembranosus strain injury, with a full recovery after 17 days post-injection [28]. Moreover, Hamid et al. (2012) used the same dose after a grade II hamstring injury, and the time needed to return to play in participants was 16 weeks [29]. In this sense, we decided to use a single injection of 1 mL of PRGF (normal-dose PRGF; PRGF group) and 3 mL of PRGF (high-dose PRGF; HPRGF group), taking into account the differences in size and weight between humans and dogs.

Particular attention is drawn to IGF-1 due to its potential ergogenic [14] and carcinogenetic effects [27]. In our study, a single intramuscular PRGF injection in healthy beagle dogs has no effect on circulating IGF-1 values, even when the standard PRGF concentration was increased three-fold. In reference to this systemic anabolic action of PRGF and in concordance with our results, several studies have also demonstrated that different commercial PRP systems do not increase IGF-1 concentrations over normal circulated blood levels [28–30]. Moreover, further scientific research supports the opinion that PRP is unlikely to promote an ergogenic effect in patients. This is due to subtherapeutic doses of IGF-1 in PRP. The isoform of IGF-1Ec in PRP is the isoform that causes muscular hypertrophy [31]. The unbound IGF-1 has too-short a half-life to exert systemic effects, and only 1% of IGF-1 is biologically available and active [32].

The last undesirable effect of IGF-1 suggested by other authors is a potential carcinogenic effect in humans [16,33] and in veterinary medicine [17,18]. Some authors [31,34] have suggested that growth factors, acting only on cell surface receptors, do not access the cell and do not promote cell DNA mutation. In agreement with Schippinger et al. [19], in our study neither the PRGF or HPRGF intramuscular injection showed an increase in IGF-1 serum concentrations. This suggests that PRGF application can be considered a safe method of treatment after 14 days, 28 days, 42 days and 56 days post-injection. Although long-term effects of multiple injections of PRGF were not examined in our study, the HPRGF used in one of the groups contained three-times the dose of normal PRGF, and no statistically significant differences were shown, suggesting that several applications over time would not alter IGF-1 circulating levels [35].

In reference to the effect of weight on serum IGF-1 concentrations, a positive correlation was observed [36], where animals from the control group had higher IGF-1 levels due to a greater weight. In the same way, high IGF-1 concentrations have been shown in obese dogs, which return to normal levels after weight loss [37]. Moreover, regarding the influence of age on IGF-1 circulating levels, with the exception of the control group, older animals show lower systemic IGF-1 concentrations. Moreover, our results are in agreement with other studies in humans [38] and veterinary medicine [39].

To assess the evolution of muscular fiber size after a PRGF injection, an imaging study was carried out with infiltrated lumbar muscles by echography and CT scan. No statistically significant differences were found between groups regarding muscle area measurements. As a result, the muscle size was similar in both infiltrated areas after intramuscular PRGF, HPRGF or saline solution, showing that intramuscular PRGF does not exert an anabolic effect even when injecting high doses.

4. Materials and Methods

4.1. Animal Model

A total of 24 healthy adult Beagle dogs were used in this study and were divided into three groups of eight dogs, five males and three females in each group, with ages ranging from 3–4 years and weights from 10–18 kg. Complete physical examination, haematology, and serum biochemical analyses were performed to ensure that animals were healthy.

The study protocol was approved by the Ethics Committee for Animal Welfare at the University CEU-Cardenal Herrera of Valencia (CEBA/2013).

4.2. Plasma Rich in Growth Factors (PRGF) Preparation and Infiltration

PRGF®-Endoret® technology (BTI Biotechnology Institute, Álava, Spain) was followed to obtain an autologous preparation of PRP [29]. Briefly, blood was collected from the external jugular vein of each dog under sterile conditions in Vacutainer sodium citrate 3.8% tubes (Blood-Collecting Tubes®, BTI Biotechnology Institute, Álava, Spain). The tubes were centrifuged at 460× *g* for eight minutes (PRGF® System III, Biotechnology Institute, Álava, Spain) to separate the different blood phases. The fraction located immediately above the buffy coat (white fraction) corresponded to PRGF, which was activated by adding 5% of calcium chloride ($CaCl_2$ 10%) just before infiltration to activate platelets for GF release.

After obtaining PRGF, the platelet concentrations and the presence of leukocytes between whole blood, PRGF, and plasma poor in growth factors (PPGF) were compared on the initial day of each of the 3 study groups. Regarding the concentration of platelets, in the 3 study groups, the authors observed an increase in the number of platelets between the blood, the PPGF, and the PRGF, showing PRGF platelet values of 1.5–2-times higher than the concentration in blood and PPGF, according to what has been previously described [29]. With regard to the concentration of leukocytes, there are statistically significant differences between blood, PRGF, and PPGF in the three groups of the study. These results confirm the absence of white blood cells after the centrifugation of the blood and the separation of the different types of plasma. These results coincide with a previous report [30], which defends the absence of leukocytes in the PRGF.

Every dog was injected in the left lumbar muscles (lumbar multifidus, latissimus dorsi lumbar, and iliocostal lumbar muscles) at the 5th lumbar vertebrae level with the following treatments:

- Treatment 1: single dose of 1 mL sterile saline solution activated with 0.05 mL $CaCl_2$ 10% (control group) [40].
- Treatment 2: single dose of 1 mL PRGF activated with 0.05 mL $CaCl_2$ 10% (PRGF group).
- Treatment 3: single dose of 3 mL PRGF activated with 0.15 mL $CaCl_2$ 10% (HPRGF group).

4.3. Determination of IGF-1 Concentrations

Under sterile conditions, blood samples were collected from the external jugular vein after intramuscular sedation with medetomidine (0.01 mg/kg), morphine (0.2 mg/kg), and midazolam (0.2 mg/kg). Samples were obtained at baseline, and 14 days, 28 days, 42 days and 56 days after injection of intramuscular PRGF. IGF-1 was analyzed by automated immunoassay system (Immulite 1000 IGF-1 assay; Diagnostic Products, Los Angeles, CA, USA) previously validated in dogs [41].

To evaluate the local effect of the intramuscular PRGF injections, ultrasound and CT-scan studies were performed.

4.4. Muscle Tissue Evaluation by Echography

Following the previous suggestions by other authors that echography has equal sensitivity to MRI for acute muscle injury (hamstring muscle) especially when performed within 2 weeks following injury [42], the ultrasound study was performed for each group (Control, PRGF, HPRGF) at baseline, and 14 days, 28 days, 42 days and 56 days after injection of the corresponding treatments.

The ultrasound images (Esaote mylab60, Genoa, Italy) were taken at left L5 level (midpoint 5th lumbar vertebrae) to calculate the muscular area average (Figure 1).

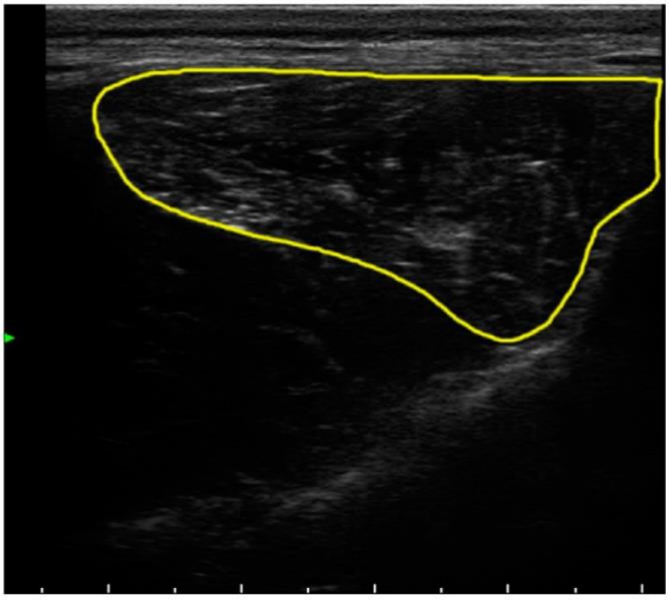

Figure 1. Ultrasonogram of one dog. The approximate contour of the measured lumbar area is delineated in yellow.

4.5. Muscle Tissue Evaluation by Computed Tomography

A CT scan (CT-max, General Electric, Madrid, Spain) was performed every 14 days within the study; therefore, measurements were taken at baseline, 14, 28, 42, and 56 days (i.e., the same as ultrasound examination) under sedation with medetomidine (0.01 mg/kg), morphine (0.2 mg/kg), and midazolam (0.2 mg/kg).

CT-scan images were performed at the same anatomic level as the ultrasound study, and three corresponding measurements were taken from lumbar muscles at left side (Figure 2).

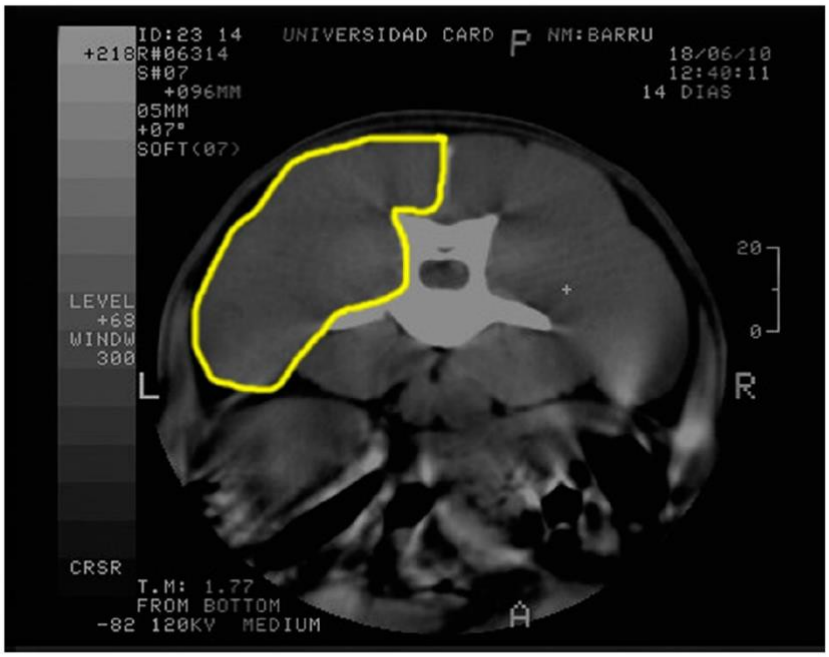

Figure 2. CT scan of one dog. The approximate contour of the measured lumbar area is delineated in yellow.

4.6. Image Processing

Once the ultrasound and CT-scan images were collected, the contours were traced. Measurements from the designated muscular lumbar area were determined via quantitative morphometry using Image Pro Plus software (for Windows 2000, Silver Spring, MD, USA). The median value of the three measurements was considered as long as the measurements differed <10%. When the difference was >10%, new measurements were obtained.

4.7. Statistical Analysis

The data were processed using the SPSS 15.0 for Windows (Chicago, IL, USA). A descriptive study of the mean, standard deviation, and confidence intervals was made for each variable. A value of $p \leq 0.05$ was considered significant. The result of each parameter was evaluated with a nonparametric Kolgomorov–Smirnov test for normality and log transformed if necessary. ANOVA repeated-measures and post-hoc Tukey tests were performed to assess differences with the baseline. A one-way ANOVA

was conducted each time, to assess differences between groups, and a post-hoc Tukey test was carried out when necessary. A Pearson correlation between echography and CT-scan measures was obtained.

5. Conclusions

A single intramuscular application of PRGF does not significantly increase systemic IGF-1 levels nor increase muscle mass, even when three-times the normal dose in canine species was used. Therefore, in the canine species, a single application of PRGF is safe for parental use with respect to local and systemic IGF-1 levels and cancer risk. Despite this, further studies are needed to prove and evaluate the safety of this therapy in humans.

Author Contributions: M.R., E.D., J.S. and J.M.C. performed the experiment and designed it; D.C. and E.D. wrote the manuscript and participated in performing the experiment; M.R. performed statistical analysis; B.C. and S.L. participated in performing the experiment; J.M.V. proofread and gave final approval of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

PRGF	plasma rich in growth factors
HPRGF	high-dose plasma rich in growth factors
PRP	platelet-rich plasma
IGF-1	insulin-like growth factor-1
CRP	C-reactive protein
WADA	World Anti-Doping Agency
VEGF	vascular endothelial growth factor
FGF	fibroblast growth factor
HGF	hepatocyte growth factor
PDGF	platelet-derived growth factor

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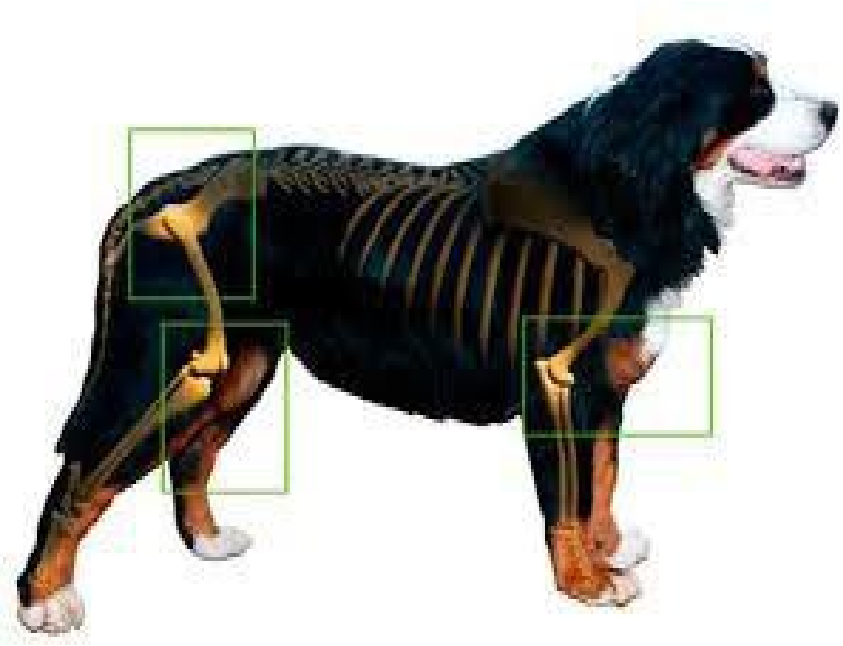


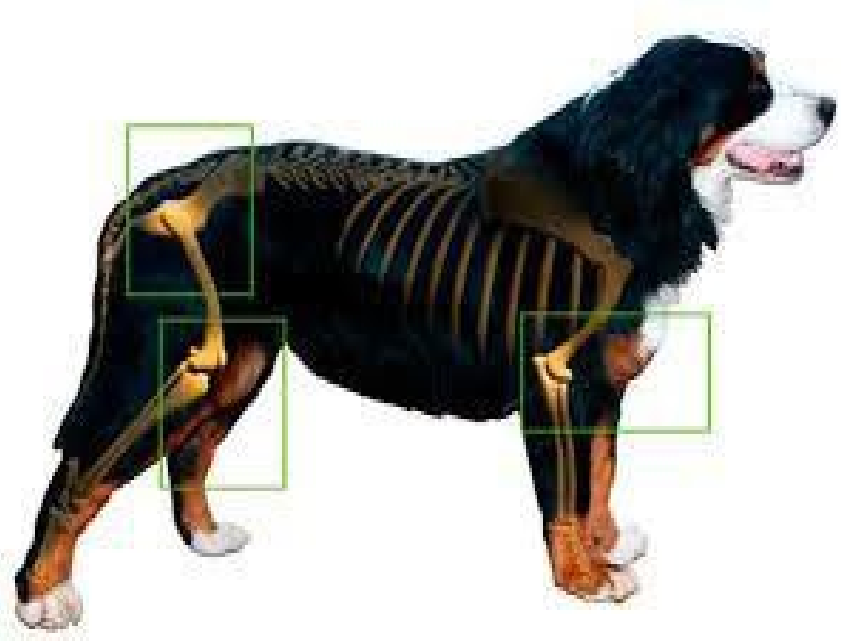
CONCLUSIONES

6. CONCLUSIONES

1) El análisis de la trayectoria del COP en los miembros de animales que presentan cojera unilateral por OA de codo ha permitido demostrar que en los miembros con cojera dicha trayectoria es más corta, está situada en posición más craneal y con menor desplazamiento mediolateral respecto a los miembros sanos; también se ha demostrado que en ambos miembros el COP describe una trayectoria craneomedial y no el eje longitudinal del miembro. Por último, la progresión del COP no se desarrolla a una velocidad constante durante la fase de apoyo.

2) El PRGF acelera el tiempo de consolidación ósea, por lo que acorta el tiempo de retirada de los implantes, en este caso fijadores externos.

3) La aplicación intramuscular de PGRF a dosis terapéuticas, e incluso triplicando esta, no incrementa los niveles de igf-1 en sangre ni incrementa la masa muscular. Esto determina que es segura en términos de no provocar efectos locales ni generales (cáncer) a través de aumentos significativos de los niveles de IGF-1 en sangre.



RESUMEN

7. RESUMEN

La osteoartritis (OA) constituye hoy en día una de las patologías del aparato locomotor de mayor morbilidad no solo en la especie humana, sino también en el campo de la veterinaria. Por lo tanto, a la hora de su diagnóstico en las especies animales, el principal caballo de batalla es el de establecer de una manera objetiva el déficit funcional que el animal está padeciendo para, si un tratamiento es instaurado, conocer de forma igualmente objetiva el verdadero nivel de eficacia.

Por todo ello, en este campo las técnicas de evaluación (diagnóstico) basadas en la biomecánica han ido adquiriendo especial relevancia en los últimos años. Entre ellos, los análisis con las plataformas de presión proporcionan multitud de parámetros que en conjunto pueden dar una visión global de las características y alteraciones de la locomoción tanto en animales sanos como en los que presentan cojera o, incluso, déficits neurológicos.

De este elenco de parámetros, el estudio de la trayectoria del centro de presiones (COP) está presente en estudios actuales, tanto en humana como en veterinaria.

Dentro de las estrategias para el tratamiento de la OA adquieren especial relevancia en la actualidad las terapias regenerativas-reparativas, principalmente a partir de células madre mesenquimales y plasma rico en plaquetas. En este último caso, su uso es muy variado en campos como la dermatología, oftalmología, y el aparato locomotor, aunque no existenestudios

de su eficacia en fracturas en veterinaria. La terapia a base de PRP, a pesar de ser autóloga, es objeto de polémica en la actualidad por su supuesto efecto ergogénico e incluso carcinogénico por vía de uno de los factores de crecimiento, en concreto el IGF-1. Siendo de un uso muy frecuente en los campos anteriormente citados, parece procedente realizar estudios directos e indirectos sobre su seguridad.

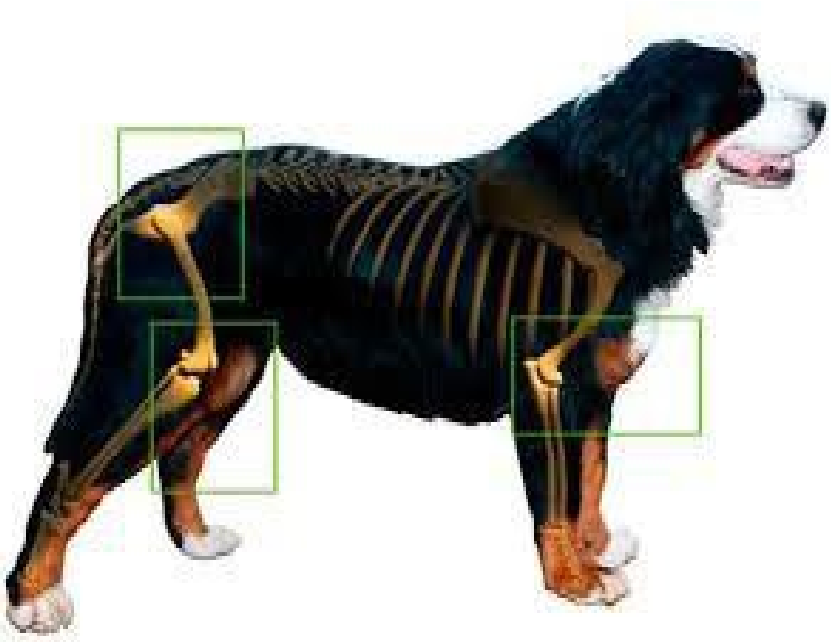
Por todo esto, el objetivo común de esta tesis doctoral consiste en estudiar aspectos de diagnóstico, y tratamiento en animales con osteoartritis y fracturas. Y por este motivo, se han diseñado tres estudios diferentes:

- Un primer estudio utilizando 10 perros con OA unilateral de codo para valorar la eficacia del estudio del COP en la detección de cojeras utilizando una plataforma de presión.

- Un segundo estudio utilizando 65 perros de diversas razas con fracturas en huesos largos, para comprobar la eficacia del PRP en la aceleración de la consolidación ósea.

- Por último, en tercer estudio sobre la seguridad del PRP a dosis terapéuticas, utilizando 24 perros de raza Beagle a los que se les inyecto PRP por vía intramuscular y se evaluó los niveles de igf-1 en sangre, además de técnicas de imagen para evaluar si existía desarrollo muscular.

Adicionalmente, se aporta un estudio de revisión sobre la eficacia de las células madre mesenquimales en el tratamiento de la OA.



SUMMARY

8. SUMMARY

Osteoarthritis (OA) is today one of the pathologies of the locomotor system of greater morbidity not only in the human species, but also in the field of veterinary medicine. Therefore, at the time of diagnosis in animal species, the main workhorse is to establish in an objective manner the functional deficit that the animal is suffering for, if a treatment is established, to know equally objectively the true level of effectiveness.

For all these reasons, in this field the evaluation techniques (diagnosis) based on biomechanics have acquired special relevance in recent years. Among them, the analyzes with the pressure platforms provide a multitude of parameters that together can give a global view of the characteristics and alterations of the locomotion in healthy animals as well as in those that present lameness or, even, neurological deficits.

From this list of parameters, the study of the trajectory of the pressure center (COP) is present in current studies, both human and veterinary.

Among the strategies for the treatment of OA, regenerative-reparative therapies are especially relevant today, mainly from mesenchymal stem cells and platelet-rich plasma. In the latter case, its use is very varied in fields such as dermatology, ophthalmology, and the locomotor system, although there are no studies of its effectiveness in veterinary fractures. PRP therapy, despite being autologous, is currently controversial due to its supposed ergogenic and even carcinogenic effect via one of the growth factors, specifically IGF-1. Being ofa

very frequent use in the aforementioned fields, it seems appropriate to carry out direct and indirect studies on its safety.

For all this, the common objective of this doctoral thesis is to study aspects of diagnosis, and treatment in animals with osteoarthritis and fractures.

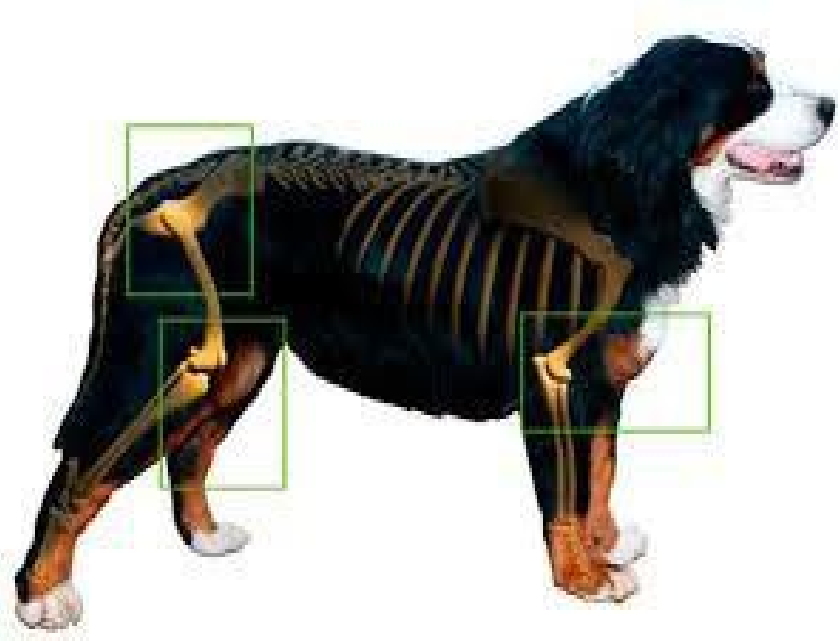
For this, three different studies have been designed:

- a first study using 10 dogs with unilateral elbow OA to assess the effectiveness of the COP study in the detection of lameness using a pressure platform.

- A second study using 65 dogs of different breeds with fractures in long bones, to check the effectiveness of PRP in the acceleration of bone consolidation.

- Finally, in the third study on the safety of PRP at therapeutic doses, using 24 Beagle dogs to which PRP was injected intramuscularly and blood igf-1 levels were evaluated, in addition to imaging techniques for evaluate if there was muscle development.

Additionally, a review study on the efficacy of mesenchymal stem cells in the treatment of OA is provided.



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