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UTILIDAD DE LA ECOGRAFÍA EN EL BLOQUEO ANESTÉSICO DE LA EXTREMIDAD PELVIANA EN EL PERRO

Memoria presentada por

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1. Echeverry DF, Gil F, Laredo F, Ayala MD, Belda E, Soler M, Agut A. Ultrasound-guided block of the sciatic and femoral nerves in dog: A descriptive study. The Veterinary Journal 2010; 186; 2:210-215.

2. Echeverry DF, Gil F, Laredo F, Belda E, Soler M, Agut A. Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog. The Veterinary Journal 2011, doi:10.1016/j.tvjl.2011.06.043.

3. Echeverry DF, Gil F, Laredo F, Belda E, Soler M, Agut A. Ultrasound-guided "two-in-one" femoral and obturator nerve block in the dog: an anatomical study. Veterinary Anaesthesia and Analgesia. Aceptado para publicación.

“Colombia exótica, compleja, espiritual, misteriosa, estimulante, sensual, desconocida, contradictoria, exuberante, llena de color, y exageradamente buena anfitriona. Colombia debe ser el país con la peor reputación sobre la tierra, y sin embargo creo que este país debe ser al que Dios ama más. La naturaleza es sabia. Dios le dio a este país todas, absolutamente todas las cosas buenas que este planeta tiene”.

Emilio Scotto (The longest ride)

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Albert Schweitzer, 1931

RELACION ALFABETICA DE ABREVIATURAS

ESPAÑOL:

AL: Anestésico local

ASI: Abordaje suprainguinal

BNP: Bloqueo de nervios periféricos

MIP: Músculo iliopsoas

NC: Nervio ciático

NCFL: Nervio cutáneo femoral lateral

NE: Neuroestimulación

NF: Nervio femoral

NP: Nervio periférico

NO: Nervio obturador

PL: Plexo lumbar

INGLÉS:

FN: Femoral nerve

IPM: Iliopsoas muscle

LA: Local anaesthetic

LFCN: Lateral femoral cutaneous nerve

LP: Lumbar plexus

NS: Neurostimulation

ON: Obturator nerve

PNB: Peripheral nerve block

ScN: Sciatic nerve

SIA: Suprainguinal approach

US: Ultrasound

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1. INTRODUCCIÓN

Uno de los principales objetivos de la anestesia es el control del dolor. No obstante, antiguamente se consideraba que los animales no sentían dolor o que lo percibían de manera diferente a los humanos; se sugería incluso que el dolor producido por una cirugía o traumatismo era beneficioso para los animales dado a que así se limitaba su actividad. Hoy día está ampliamente establecido que los mamíferos superiores tienen mecanismos de percepción del dolor similares al hombre, y por tal motivo deberán recibir un manejo contra el dolor equivalente al recibido por el ser humano (Hellyer *et al*., 2007; Viñuela-Fernandez *et al*., 2007). La importancia de un manejo adecuado del dolor va más allá de cualquier consideración humanitaria y ética (Jones, 2010). El dolor no controlado tiene una enorme repercusión en la salud y el bienestar de nuestros pacientes. Algunas de las consecuencias negativas del dolor son: tromboembolismo, alteración de la función inmune, incremento del riesgo de sepsis, retardo en la cicatrización de las heridas, automutilación, estrés cardiovascular y convalecencia prolongada entre otros (Muir y Woolf, 2001; Viñuela-Fernandez *et al*., 2007; Muir, 2009). Adicionalmente, el dolor agudo puede favorecer la presentación del síndrome de dolor crónico, el cual una vez establecido es de difícil control (Mathews, 2000).

La estrategia más utilizada actualmente para controlar el dolor perioperatorio en el perro se basa en la aplicación de protocolos de analgesia preventiva multimodal (Muir y Wolf, 2001). Esta práctica analgésica consiste en la administración antes de que ocurra el estímulo doloroso, de una combinación de fármacos analgésicos con acciones a niveles diferentes en la ruta del dolor. De esta manera no solo se consigue potenciar el efecto analgésico y disminuir los efectos adversos de estos fármacos, sino que además, se limita la

presentación del síndrome del dolor crónico (Hellyer *et al*., 2007; Viñuela-Fernandez *et al*., 2007).

El miembro pelviano del perro es objeto de múltiples técnicas quirúrgicas potencialmente dolorosas durante el periodo perioperatorio (Hoelzler *et al*., 2005). La anestesia epidural en combinación con la administración de analgésicos no esteroideos y opioides, continua siendo la técnica de elección para el control del dolor en procedimientos quirúrgicos realizados en el miembro pelviano en esta especie (Jones, 2001; Valverde, 2008). No obstante, esta técnica de analgesia regional puede estar contraindicada en presencia de traumatismos óseos o infecciones cutáneas de la región lumbosacra, hipotensión, sobrepeso, y desórdenes de la coagulación entre otros (Jones, 2001).

En medicina humana el uso de técnicas de anestesia regional mediante el bloqueo combinado de los nervios ciático (NC) y femoral (NF) ha demostrado ofrecer una analgesia de similares características a la epidural, con la ventaja de producir un menor número de complicaciones que las técnicas neuroaxiales (Capdevila *et al*., 1999; Enneking *et al*., 2005). El bloqueo de nervios periféricos (BNP) ha sido realizado mediante el uso de técnicas basadas en marcas anatómicas de superficie, o mediante la obtención de parestesia como métodos tradicionales de localización de los nervios periféricos (NP) (Enneking *et al*., 2005). A finales de los años sesenta gracias a la aparición de la neuroestimulación (NE), fue posible obtener una notable mejora en la localización de los NP (De Andres y Sala-Blanch, 2001). Las técnicas basadas en marcas anatómicas de superficie y NE se consideran actualmente como técnicas “ciegas” dado que no permiten la visualización de los NP ni de la

distribución del anestésico local (AL). Por ello, estas técnicas han sido relacionadas en algunos casos con bajas tasas de efectividad en el BNP y con un mayor porcentaje de complicaciones como punción inadvertida de estructuras nerviosas o vasculares (Marhofer *et al*., 2005a; Hopkins, 2007). Los grandes avances logrados en ecografía, y fundamentalmente para este campo la introducción de transductores de alta frecuencia, han permitido que actualmente sea posible observar los NP, el avance dinámico de la aguja y la distribución del AL. Estos avances han sido útiles para optimizar la eficacia y seguridad de las técnicas de BNP lo que ha incrementado el interés por estas técnicas dentro de los protocolos de anestesia multimodal en medicina humana (Peterson *et al*., 2002; Beekman y Visser, 2004; Marhofer *et al*., 2005a).

Las técnicas de BNP han sido tradicionalmente más empleadas en veterinaria para clínica de grandes animales (Mahler y Adogwa, 2008). Sin embargo, actualmente existe un gran interés por la aplicación de estas técnicas en anestesia canina. Esto se ve reflejado en un aumento de la publicación de trabajos científicos relacionados con el BNP en esta especie (Mihelic *et al*., 1995; Campoy *et al.*, 2008; Mahler y Adogwa, 2008). No obstante, el uso de ecografía como técnica de localización de los NP en el perro no ha sido descrito hasta ahora. En medicina humana, se ha considerado que la ecografía podría ser la técnica de neurolocalizacion del futuro. No obstante la transición a partir de la técnica de NE necesitará aproximadamente unos 10 años o más para completarse (Marhofer *et al*., 2005b).

2. OBJETIVOS

La ausencia de estudios acerca del empleo de ecografía para el bloqueo de nervios periféricos (BNP) en el perro, y los beneficios potenciales que su uso podría aportar a la neurolocalizacion nos ha impulsado a desarrollar esta Tesis Doctoral con los siguientes objetivos:

1. Estudiar desde el punto de vista anatómico las características del nervio ciático (NC) en el perro, con el fin de establecer sus posibles abordajes ecográficos y describir el aspecto ecográfico del mismo, determinando la eficacia de dichos abordajes de cara a su bloqueo anestésico en esta especie **(artículo 1)**.

2. Estudiar las características anatómicas del nervio femoral del perro para determinar posibles abordajes ecográficos que permitan el acceso eficaz a este nervio, describiendo su aspecto ecográfico en los distintos abordajes obtenidos, y evaluando, finalmente, la eficacia de estos abordajes para su bloqueo anestésico **(artículo 1 y 2)**.

3. Evaluar mediante estudio anatómico los principales componentes del plexo lumbar (PL) que intervienen en la inervación del miembro pelviano en la especie canina, con el fin de obtener un abordaje ecográfico efectivo para el acceso a este plexo, describiendo las características ecográficas del abordaje y analizando su eficacia de cara al bloqueo anestésico del mismo **(artículo 3)**.

3. REVISIÓN BIBLIOGRÁFICA

3.1 DESCRIPCIÓN ANATÓMICA DE LA INERVACIÓN DEL MIEMBRO PELVIANO EN EL PERRO

Los troncos nerviosos que intervienen en la inervación del miembro pelviano en el perro forman parte fundamental del plexo lumbosacro, integrado por las uniones de las raíces ventrales de los últimos nervios lumbares y primeros sacros (Sandoval, 1998). Desde el punto de vista anatómico este plexo suele dividirse en dos componentes, lumbar y sacro, que se describen a continuación.

3.1.1 Plexo lumbar

Resulta de las conexiones que establecen las raíces ventrales de los nervios lumbares L3, L4, L5, y L6 (Kitchell y Evans, 1993). Los tres nervios principales de este plexo que inervan estructuras relacionadas con el miembro pelviano son el NF, el nervio obturador (NO) y el nervio cutáneo femoral lateral (NCFL).

3.1.1.1 Nervio femoral

Es el tronco más grueso del PL y en el perro se origina fundamentalmente a partir del quinto segmento lumbar de la médula espinal (raíz ventral de L5). Una vez abandona la médula espinal se interna dentro del vientre del músculo iliopsoas (MIP) para posteriormente alcanzar el miembro pelviano a través de la laguna muscular (Adams, 1988). El NF inerva a los músculos MIP (flexor de la cadera), al sartorio (en algunos perros), al cuádriceps femoral (extensores de la rodilla) y envía una pequeña rama al músculo articular de la cadera (Kitchell y Evans, 1993). El nervio safeno es un ramo del NF, del que se separa en el espesor del MIP y que penetra en el

canal femoral. En algunos perros el músculo sartorio está inervado por un ramo proximal de este nervio. El nervio safeno se sitúa superficialmente conforme progresa en sentido distal con los vastos femorales por el triángulo femoral, cruza la superficie lateral del músculo vasto medial y desciende por la cara medial de la pierna en intima relación con los vasos safenos. Los ramos cutáneos del nervio safeno llegan a la piel de las regiones femoral medial, crural medial y tarsiana dorsomedial. El dedo I (cuando está presente) y el dedo II reciben inervación cutánea de los ramos terminales del nervio safeno (Adams, 1988). El déficit del NF se caracteriza por producir pérdida de la capacidad de extender la rodilla y mantener el peso, generalmente el animal afectado arrastra la extremidad, y el reflejo patelar está disminuido o ausente (Oliver *et al.,* 2003).

3.1.1.2 Nervio obturador

Se origina a partir de las raíces ventrales de los nervios lumbares L4, L5, y L6 aunque existen variaciones individuales (Kitchell y Evans, 1993). Una vez conformado, el tronco queda en el espesor del MIP. Abandona este músculo para posteriormente entrar en la pelvis y prestar inervación a los músculos obturador externo, pectíneo, gracilis y adductor (Kitchell y Evans, 1993).

3.1.1.3 Nervio cutáneo femoral lateral

Se origina principalmente a partir de las raíces ventrales del cuarto nervio lumbar (L4). Inmediatamente, cruza el vientre del músculo psoas menor en compañía de los vasos circunflejos iliacos profundos para luego atravesar la pared abdominal, y finalmente ramificarse en la piel del área de la tuberosidad

coxal. Se trata de un nervio esencialmente cutáneo que inerva la piel de la región glútea craneal, femoral craneal y genual lateral (Adams, 1988; Budras *et al*., 1989).

3.1.2 Plexo sacro

Se forma a partir de las raíces ventrales de los dos últimos nervios lumbares (L5 y L6) y de los tres primeros sacros (S1, S2, y S3). Sin embargo los últimos nervios sacros (nervios rectales caudales y pudendos) escapan del ámbito topográfico del miembro pelviano, ya que inervan territorios relacionados con el periné y genitales externos. Por esta razón el plexo sacro puede quedar reducido al llamado plexo ciático (Sandoval, 1998), integrado en las carnívoros por las conexiones que establecen entre sí las raíces de los nervios L6, L7, S1 y S2. Dichas conexiones forman un grueso cordón nervioso conocido como tronco lumbosacro, origen común de los nervios glúteos, cutáneo caudal del muslo y ciático. Este último, continuación directa del tronco lumbosacro, es el nervio más grueso del organismo y el de mayor importancia en la inervación de estructuras relacionadas con el miembro pelviano.

3.1.2.1 Nervio ciático

El NC, continuación directa del tronco lumbosacro y totalmente extrapélvico, es un nervio mixto compuesto por la unión de dos grandes nervios unidos entre sí por tejido conectivo. Estos dos nervios son el peroneo común y el tibial, e inervan la mayor parte de estructuras intrínsecas del miembro pelviano (aquéllas que no están inervadas por los nervios, NCFL, NF, NO, glúteos y cutáneo femoral caudal). En la mayoría de los perros se forma a partir de las raíces ventrales de los nervios L6, L7 y S1, aunque ocasionalmente

también puede contribuir el S2 (Kitchell y Evans, 1993; Oliver *et al.,* 2003). En su trayecto, el NC contornea caudodistalmente la articulación de la cadera, entre el trocánter mayor del fémur y la tuberosidad isquiática, quedando cubierto por los músculos glúteos superficial y medio, y discurriendo sobre la superficie de los músculos glúteo profundo, tendón del obturador interno y gemelos. A este nivel, el NC emite varios ramos musculares, que inervan a dichos músculos y al bíceps femoral, abductor caudal de la pierna, semitendinoso y semimembranoso, es decir a los músculos implicados en la extensión de la cadera y flexión de la rodilla (Adams, 1988). Una vez en el muslo, el NC discurre sobre la superficie de los músculos cuadrado femoral, aductor mayor y semimembranoso, quedando cubierto por la superficie medial del músculo bíceps femoral. La división de sus componentes principales, nervio peroneo común y tibial, es variable, pudiéndose localizar ya sea proximal a la articulación de la cadera o más distalmente a nivel del espacio poplíteo (Kitchell y Evans, 1993).

Los nervios peroneo común y tibial inervan a los músculos situados distalmente a la región de la rodilla, ubicados en la región de la pierna y proporcionan inervación cutánea a las partes caudolaterales y caudomediales de la pierna, cara lateral de la rodilla y pie, excepto por el dedo más medial que está inervado por ramos del NF (Adams, 1988). Desde el punto de vista motor, el nervio tibial inerva los músculos situados caudalmente en la pierna, que intervienen en la extensión del tarso y flexión de los dedos (Oliver *et al,* 2003). Su déficit se caracteriza en la marcha porque la articulación del tarso cae distalmente cuando el animal anda o intenta sostener su peso. Una vez alcanza la región de la pierna, el nervio peroneo común se divide a su vez en los

nervios peroneo superficial y profundo: el nervio peroneo superficial se ramifica en la cara dorsal de los huesos del tarso y la parte proximal del metatarso para inervar principalmente la parte dorsal de los dedos del pie. El nervio peroneo profundo inerva a los músculos craneolaterales de la pierna: músculos flexores del tarso y extensores de los dedos (Adams, 1988). Durante la marcha, su alteración se caracteriza por déficit de propiocepción, por lo que los dedos estarán flexionados aunque pueden apoyar su peso. El reflejo de retirada estará también ausente. La sensibilidad por debajo de la rodilla se encuentra afectada craneolateralmente por el nervio peroneo y caudalmente por el nervio tibial. Cuando está afectada la función de los dos componentes del NC predominan los déficits motores correspondientes al nervio peroneo (Oliver *et al.,* 2003).

3.2 NEUROFISIOLOGÍA DE LA NOCICEPCIÓN

La nocicepción es el proceso neurofisiológico que consiste en la transducción, transmisión, modulación y percepción de señales generadas en respuesta a un estimulo nocivo (Lamont *et al*., 2000; Lemke, 2004). Este proceso es iniciado en los nociceptores, los cuales son terminaciones libres de las neuronas sensitivas. Estos nociceptores reconocen estímulos nocivos mecánicos, térmicos o químicos y los transforman (transducción) en señales eléctricas (potenciales de acción) (Muir y Woolf, 2001; Power y Kam, 2008). La transducción se inicia con la activación de proteínas de membrana presentes en los diversos nociceptores, lo que conduce a la apertura de canales iónicos para el sodio y a la despolarización de la membrana neuronal. De esta manera se generan potenciales de acción que se propagan a través de los axones de fibras nociceptivas especializadas (mielinicas Aδ y amielínicas C) hacia el

sistema nervioso central principalmente hacia el asta dorsal de la médula espinal (Lemke, 2004; Lemke y Creighton, 2010). Estos axones, en el asta dorsal, pueden hacer sinapsis de manera indirecta con interneuronas excitatorias o inhibitorias (modulación) antes de ser enviada la señal nociceptiva hacia centros supraespinales (Viñuela-Fernández *et al*., 2007; Power y Kam, 2008) o sinapsan de manera indirecta con neuronas de proyección, las cuales conducen el estimulo nervioso hacia centros supraespinales a través de los tractos espinotalámico y espinoreticular fundamentalmente (Muir y Woolf, 2001). En los centros supraespinales los estímulos sensitivos son integrados, reconocidos, identificados (percepción), y transformados (modulación secundaria) a una forma apropiada de experiencia y respuesta motora protectiva y recordada (Muir y Woolf, 2001; Muir, 2009; Lemke y Creighton, 2010).

3.3 ANESTÉSICOS LOCALES

Los AL son un grupo de fármacos que bloquean de forma reversible la conducción de los impulsos nerviosos en las fibras nerviosas. Los AL bloquean los canales del sodio a nivel neuronal, evitando el influjo de estos iones al interior de la neurona, lo que impide la despolarización de la membrana neuronal, y, consecuentemente, la propagación de los potenciales de acción (Mama, 2009). Así, no solo se bloquea por completo la transmisión del dolor en la región desensibilizada, sino que también se limita la aparición de sensibilización central al dolor (Muir y Woolf, 2001; Skarda y Tranquilli, 2007). Los AL son bases débiles que se clasifican como aminoesteres (procaína, benzocaína) o aminoamidas (lidocaína, bupivacaína, Ropivacaína) (Skarda y Tranquilli, 2007; Mama, 2009).

La acción clínica de los AL puede ser descrita en relación a su potencia anestésica, velocidad e inicio de acción, duración de la acción, y tendencia a producir bloqueo diferencial. La potencia anestésica está relacionada con la liposolubilidad del AL. La velocidad e inicio de acción está determinada por el Pk_a (Skarda y Tranquilli, 2007). La duración del efecto se relaciona con la unión a proteínas, la vasoactividad y la liposolubilidad de los AL (Tabla 1). La tendencia a producir bloqueo diferencial (sensitivo-motor) varía según el nervio bloqueado, el diámetro de la fibra, la longitud del nervio expuesto, y el grado de mielinizacion. Las fibras nerviosas amielínicas C y Aδ, de pequeño diámetro, se bloquean antes de las gruesas mielinizadas Aβ (Mama, 2009). Este último tema es controvertido, ya que estudios recientes indican que las fibras C serian más resistentes a los AL y se bloquearían después de las fibras sensitivas y motoras de mayor diámetro (Lemke y Creighton, 2010). Otros autores afirman que el bloqueo motor se inicia primero que el sensitivo debido a que las fibras nerviosas motoras se agrupan en la periferia de los nervios, con lo cual estarían expuestas al AL antes que las fibras sensitivas que se localizan en el interior de los nervios (Carpenter y Mackey, 1992; Mama, 2009).

La actividad de los AL también puede verse influenciada por la vascularización del sitio de inyección, dosis y concentración utilizadas; así, volúmenes altos y concentraciones elevadas de AL se asocian con bloqueos nerviosos más rápidos y densos (Skarda y Tranquilli, 2007; Lemke y Creighton, 2010). Así mismo, algunos AL producen un bloqueo selectivo más eficaz de cara al bloqueo de fibras sensitivas que motoras (ej., bupivacaína, ropivacaína) (Feldman y Covino, 1988).

La toxicidad de los AL se asocia fundamentalmente con alteraciones del sistema nervioso central y del sistema cardiovascular, debida a un bloqueo generalizado de los canales de sodio. Esta es directamente proporcional a la potencia del AL utilizado y se origina por la presencia de concentraciones plasmáticas de AL muy elevadas, debido a inyección intravascular accidental, absorción sistémica masiva o sobredosificación (Skarda y Tranquilli, 2007). En el perro se recomienda no superar la dosis de 6-8 mg/kg y 1.5-2 mg/kg respectivamente para la lidocaína y bupivacaína (Lemke y Creighton, 2010).

Tabla 1. Propiedades físicas, químicas y biológicas de algunos anestésicos locales. Adaptado de Skarda y Tranquilli, 2007.

Droga	liposolubilidad	Potencia *	Pk_a	Unión a proteínas (%)	Inicio de acción	Duración acción (min)
Procaína	1	1	8.9	6	lento	45-60
Lidocaína	3.6	2	7.86	65	rápido	60-120
Mepivacaína	2	2	7.7	75	rápido	90-180
Ropivacaína	14	6	8.07	95	intermedio	180-480
Bupivacaína	30	8	8.1	95	intermedio	180-480
Levobupivacaína	31.1	ND	8.09	>97	intermedio	180-480

Potencia*: relativa a la procaína, ND: no determinada

3.4 ANESTESIA LOCOREGIONAL

Las técnicas de anestesia locoregional están ganado amplia aceptación en el manejo del dolor perioperatorio de los animales de compañía (Lemke y Creighton, 2010). El mejor entendimiento de la fisiopatología del dolor alcanzado en estos últimos años respalda el empleo de estas técnicas en el manejo del dolor perioperatorio (Lemke, 2004). Las técnicas locoregionales pueden ser usadas de manera segura de varias maneras. La aplicación tópica

de AL puede ser usada para procedimientos quirúrgicos menores de la piel y mucosas. La infiltración local de AL puede ser utilizada para desensibilizar la piel y tejidos subcutáneos en procedimientos quirúrgicos o diagnósticos. La aplicación intraarticular, intraperitoneal o intrapleural de AL permite controlar el dolor asociado a procedimientos quirúrgicos o diagnósticos realizados en estas cavidades (Gaynor y Mama, 2009). Las técnicas de anestesia regional intravenosa, el BNP, y las técnicas neuroaxiales, pueden ser usadas para el manejo del dolor tanto en pacientes quirúrgicos como en no quirúrgicos. Las técnicas locoregionales son las únicas capaces de producir un bloqueo completo de la transmisión nociceptiva hacia el sistema nervioso central, prevenir la sensibilización central al dolor y consecuentemente la aparición del dolor patológico (Lemke, 2004; Lamont, 2008). Actualmente estas técnicas son aplicadas en combinación de opioides analgésicos no esteroideos, y anestésicos generales como parte de una estrategia multimodal del control del dolor (Viñuela-Fernández *et al*., 2007).

La anestesia epidural es considerada aún como la técnica de referencia de la anestesia regional para procurar analgesia de cara a procedimientos quirúrgicos realizados en la extremidad pelviana en el perro (Valverde, 2008). No obstante, esta técnica está contraindicada en casos de infecciones cutáneas o anormalidades anatómicas de la región lumbosacra, desordenes de coagulación entre otros (Jones, 2001). Recientemente el uso de técnicas de BNP específicamente el bloqueo combinado de los nervios NC y NF se ha descrito como una alternativa válida a las técnicas neuroaxiales que permite obtener una analgesia similar a la epidural pero asociada a un menor número de complicaciones en humanos (Capdevila *et al.,* 1999; Enneking *et al*., 2005).

3.4.1. Anestesia locoregional del miembro pelviano

En medicina humana existe una gran cantidad de técnicas de neurolocalizacion como la ecografía, la NE, y las marcas anatómicas de superficie (Enneking *et al*., 2005; Deschner *et al*., 2009). No obstante en veterinaria los estudios que describen el bloqueo anestésico de la extremidad pelviana en el perro son limitados al uso de marcas anatómicas de superficie (Mihelic *et al*., 1995; Duke, 2000; Hofmeister *et al.,* 2007) o NE (Rasmussen *et al*., 2006; Campoy *et al*., 2008; Mahler y Adogwa, 2008). Estas técnicas denominadas “ciegas” pueden estar asociadas a una menor tasa de efectividad y a un mayor número de complicaciones durante los bloqueos (Marhofer *et al*., 2005a; Roberts, 2006).

3.5 EMPLEO DE LA ECOGRAFÍA EN EL BLOQUEO DE LOS NERVIOS PERIFÉRICOS

El principal requerimiento para el éxito de la anestesia regional es el de asegurar una optima distribución del AL cerca del nervio diana, esta exigencia se cumple apropiadamente cuando se usa la ecografía como método de neurolocalizacion (Marhofer *et al*., 2005a). La ecografía permite visualizar el nervio diana, guiar el desplazamiento de la aguja de manera segura hacia éste evitando estructuras sensibles, y observar la distribución del AL. Así se mejora la eficacia de los bloqueos y se reduce en número de complicaciones (Hatfield y Bodenham, 1999; Carty y Nicholls, 2007). Para obtener una buena visualización ecográfica de los NP se requiere del uso de transductores de alta frecuencia, debido al pequeño tamaño y localización superficial de algunos de estos nervios (Carty y Nicholls, 2007). La mayoría de los BNP se pueden

realizar con buena resolución usando transductores lineales de 10-14 MHz (Marhofer *et al*., 2005a; Kramer y Hudson, 2008), no obstante a estas frecuencias se disminuye el poder de penetración del haz de ultrasonidos (Hatfield y Bodenham, 1999).

El aspecto ecográfico de los nervios NP pueden variar dependiendo del tamaño del nervio, la frecuencia del transductor utilizada, el ángulo de incidencia del haz de ultrasonidos y de la naturaleza de los tejidos que rodea al nervio (Marhofer *et al*., 2005a). Así, un mismo nervio puede pasar de ser hipoecoico a hiperecoico en función de lo descrito anteriormente (Carty y Nicholls, 2007). Un correcto posicionamiento del transductor es especialmente importante durante el BNP ecoguiado. Los NP son mejor observados cuando el haz de ultrasonidos incide perpendicularmente al nervio. Cuando el ángulo de incidencia es menor a 90° la ecogenicidad del nervio puede verse afectada, este fenómeno se denomina anisotropía (Beekman y Visser, 2004).

Para realizar el BNP, los nervios pueden ser abordados ecográficamente en el eje largo o en el eje corto, de esta manera obtenemos respectivamente un corte longitudinal o transversal de los mismos. Las agujas pueden ser introducidas en plano, cuando se introducen paralelas al transductor, o fuera del plano, cuando se introducen perpendicular a este (Carty y Nicholls, 2007). Las agujas que se utilizan en BNP guiado por ecografía son diseñadas para ser mejor visualizadas ecográficamente y reducir la posibilidad de punción nerviosa (Hatfield y Bodenham, 1999; Schafhalter-Zoppoth *et al*., 2004). El ángulo de introducción de la aguja en relación al haz de ultrasonidos es importante para su adecuada visualización, así ángulos mayores a 45° dificultaran la observación de las mismas (Carty y Nicholls, 2007).

4. RELACIÓN DE ARTÍCULOS

1. Echeverry DF, Gil F, Laredo F, Ayala MD, Belda E, Soler M, Agut A. Ultrasound-guided block of the sciatic and femoral nerves in dog: A descriptive study. The Veterinary Journal 2010; 186; 2:210-215.

2. Echeverry DF, Gil F, Laredo F, Belda E, Soler M, Agut A. Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog. The Veterinary Journal 2011, doi:10.1016/j.tvjl.2011.06.043.

3. Echeverry DF, Gil F, Laredo F, Belda E, Soler M, Agut A. Ultrasound-guided “two-in-one” femoral and obturator nerve block in the dog: an anatomical study. Veterinary Anaesthesia and Analgesia. Aceptado para publicación.

4.1 ARTÍCULO 1

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Ultrasound-guided block of the sciatic and femoral nerves in dogs: A descriptive study

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ABSTRACT

Ten canine cadavers were used to investigate the anatomy and ultrasonographic approaches to the sciatic (ScN) and femoral (FN) nerves and to assess the accuracy of an ultrasound (US) guided technique to locate and block these nerves in the dog. The nerves of four sedated dogs were sought using US, blocked with 1% lidocaine and successful location confirmed by peripheral neurostimulation. The ScN was identified by US in all cases whereas the FN was not located in all cases. This study validates the usefulness of the US-guided technique to locate and block the ScN at the midfemoral level but the acoustic window of the inguinal region was less successful for locating and blocking the FN.

Introduction

Regional anaesthesia (RA) and nerve blocks are widely used in human anaesthesia in order to avoid the need for general anaesthesia and the associated risk, and also to improve intra-operative analgesia and increase patient comfort during the postoperative period (Roberts, 2006). Until recently, nerve block techniques were not commonly performed in small animals (Mahler and Adogwa, 2008) but recent studies suggest an increased interest (Duque, 2000; Moens and Caulkett, 2000; Futema et al., 2002; Wenger et al., 2005; Rasmussen et al., 2006; Hofmeister et al., 2007; Lamont and Lemke, 2008; Mahler and Adogwa, 2008) . However, descriptions and validation of RA techniques in the dog are scarce.

In humans, RA has traditionally been performed using surface anatomical landmarks or peripheral nerve stimulators (PNS) to locate the nerves. However, when these 'blind' techniques are employed, there is a significant failure rate in achieving the block and serious complications are possible (Marhofer et al., 2005a; Roberts, 2006). The key requirement to perform safe and successful nerve blocks is to ensure optimal distribution of the local anaesthetic (LA) around the nerve (Marhofer and Frickey, 2006), as incomplete spread may impair or produce partial or delayed blockades (Marhofer et al., 2005b).

Ultrasound (US) guided peripheral nerve block techniques are considered useful for optimising needle position in relation to the target nerve, and for observing in real time the spreading of LA (Marhofer et al., 2005a). Moreover, the direct visualisation of the nerves and their associated structures may improve the success rate of the block and the safety of these techniques, as potential complications such as nerve puncture can be more easily avoided (Oberndorfer et al., 2007). This could be particularly important when the blocks are performed in anaesthetised patients unable to express signs of discomfort or paraesthesia when the needle is close to the nerve.

In veterinary anaesthesia, peripheral nerve blocks are currently performed by using surface anatomical landmarks or PNS techniques to locate the target nerves (Duque, 2000; Moens and Caulkett, 2000; Futema et al., 2002; Wenger et al., 2005; Rasmussen et al., 2006; Hofmeister et al., 2007; Lamont and Lemke, 2008; Mahler and Adogwa, 2008), but information on US-guided techniques to perform peripheral nerve blocks has not been documented in dogs. The aims of this study were to determine the US approaches to localise the sciatic (ScN) and femoral (FN) nerves, to describe the ultrasonographic appearance of these nerves, and, finally, to assess the reliability of an US-guided technique to block the ScN and FN in the dog.

Materials and methods

Animals

The project was approved by the Animal Care and Ethics Committee of the University of Murcia. It was carried out in three phases. Firstly, 12 pelvic limbs from six fresh canine cadavers were used for the anatomical study. In the second phase

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(the ultrasonographic study) eight pelvic limbs from another four fresh canine cadavers were used; these dogs came from the Zoonoses and Public Health Service and were humanely euthanased for reasons unrelated to pelvic limb lameness. All of the dogs were adult with a mean weight of 21.8 ± 3.01 kg (range 18–27 kg). In the third phase of the study, four live, healthy adult experimental Beagle dogs of mean weight 11. 75 ± 1.26 kg (range 10–13 kg) and mean age 5 ± 0.8 years (range 4–6 years) were used to test the accuracy of an US-guided location and blockade technique for the ScN and FN. The animals had no signs of musculoskeletal or neurological disorders related to the pelvic limbs and were handled in accordance with the University's guidelines for humane care of experimental animals.

Anatomical study

Eight pelvic limbs from four canine cadavers were used. Skin incisions were made from the trochanter major of the femur to the stifle to dissect the ScN. The skin was reflected cranially and caudally from the vertical incision to expose the lateral aspect of the pelvic limb. The biceps femoris was reflected from its insertion and the ScN identified (Fig. 1). For the FN, the skin incisions were made on the medial aspect of the thigh cranial to the pectineus muscle. The skin was reflected cranially and caudally from the vertical incision and the FN identified cranial to the femoral artery. The FN was dissected as far as the iliopsoas muscle (Fig. 2).

Red coloured latex was introduced through the abdominal aorta artery in another two fresh canine cadavers. The cadavers were then frozen at −30 °C. Eight days later, transverse cryosections were made of the pelvic limbs using a high-speed band saw at the desired thickness of 2.5 cm from the hip joint to the stifle. Photographs of each slide were taken to facilitate the interpretation and comparison of the anatomical structures to the corresponding ultrasonographic images.

Ultrasonographic study

Ultrasonographic scans of the ScN and FN were carried out on eight limbs from another four fresh cadavers. The US scans were performed immediately after euthanasia. The hair was clipped from the sacroiliac region to just below the stifle on the dorsal, lateral and medial aspects of the limb. Then the skin was cleaned and coupling gel was applied. A 4–13 MHz linear transducer (MyLab 70, Esaote) was used for the ultrasound.

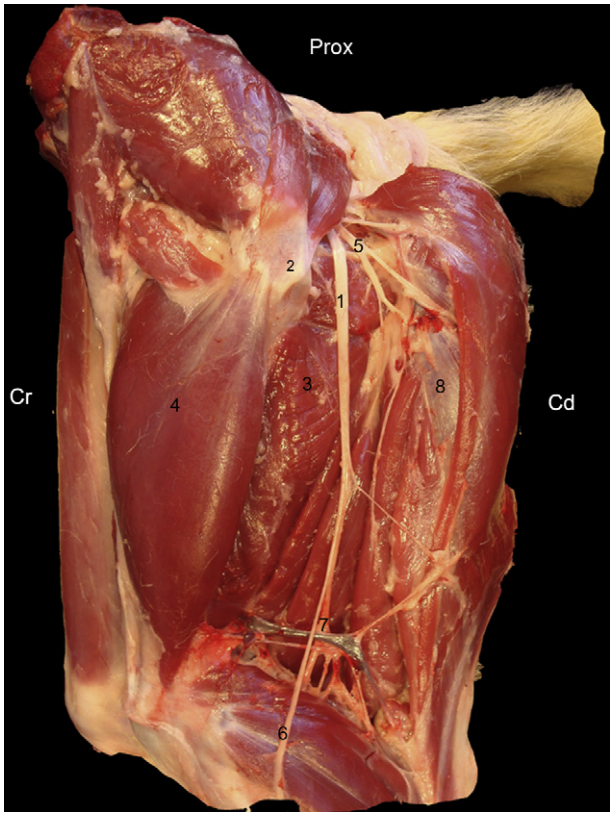

Fig. 1. Gross anatomy of the ScN at the lateral aspect of the thigh. (1) ScN, (2) trochanter major of the femur, (3) adductor magnus muscle, (4) quadriceps femoris muscle, (5) rami musculares of the ScN, (6) peroneus communis nerve, (7) tibialis nerve, (8) biceps femoris muscle reflected laterally. Prox, proximal; Cr, cranial; Cd, caudal.

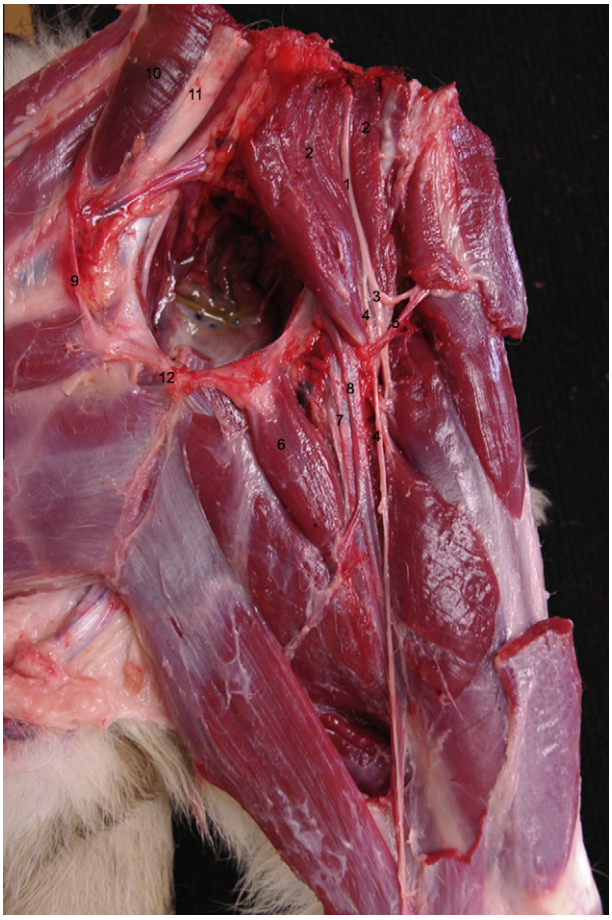

Fig. 2. Gross anatomy of the FN within the psoas major muscle and at the medial aspect of the limb. (1) FN, (2) psoas major muscle (the muscle has been split to show the nerve), (3) rami musculates of the FN into the quadriceps muscle, (4) saphenous nerve, (5) lateral femoral circumflex artery, (6) pectineus muscle, (7) femoral vein, (8) femoral artery, (9) inguinal ligament, (10) psoas major muscle (not split), (11) insertion tendon of the psoas minor muscle, (12) pubis.

The dogs were positioned in lateral recumbency. The transducer was placed in the transverse plane just distal and caudal to the trochanter major and then directed toward the distal aspect of the thigh. In this fashion, the ScN was imaged from the dorsal aspect close to its origin to the point where the peroneus communis and tibial nerves diverge near the stifle. Transverse images were also obtained by positioning the transducer parallel to the dorsal anatomical plane, caudal to the trochanter major of the femur and cranial to the ischiatic tuberosity. Longitudinal images of the ScN were obtained by rotating the transducer 90° from the position used to obtain the transverse images. Several acoustic windows were available to approach this nerve along the lateral surface of the thigh. However, it was decided to select a mid-femur approach in order to standardise the procedures (Fig. 3).

The FN was imaged at the medial aspect of the thigh. The dogs were positioned in lateral recumbency and the transducer placed in the inguinal skin crease, cranial to the pectineus muscle while the contralateral limb remained abducted. The nerve was scanned cranially to the pectineus muscle. This was the only acoustic window found available to approach the FN in this area (Fig. 4).

The structures identified as the ScN and FN were injected with 0.3 mL/kg of black ink under US guidance. A needle (Stimuplex 30 mm 22 G, B-Braun) was inserted using a long axis technique and the nerves approached on transverse images. The insertion sites of the needle into the skin were marked and photographed to serve as reference points to describe the optimal acoustic windows. The injected limbs were then immediately dissected to confirm the accuracy of the US nerve location by the presence of black ink staining the target nerves.

Experimental US-guided nerve location and blockade in vivo

Four dogs were sedated by IM administration of medetomidine (Domior, Pfizer) 10 μg/kg and lactated Ringeŕs solution was given IV at a rate of 5 mL/kg/h during the procedures. On each dog, two experimental procedures were performed with an interval of 2 weeks. On the first occasion, the right ScN and the left FN were blocked. On the second, the left ScN and the right FN were blocked. The location

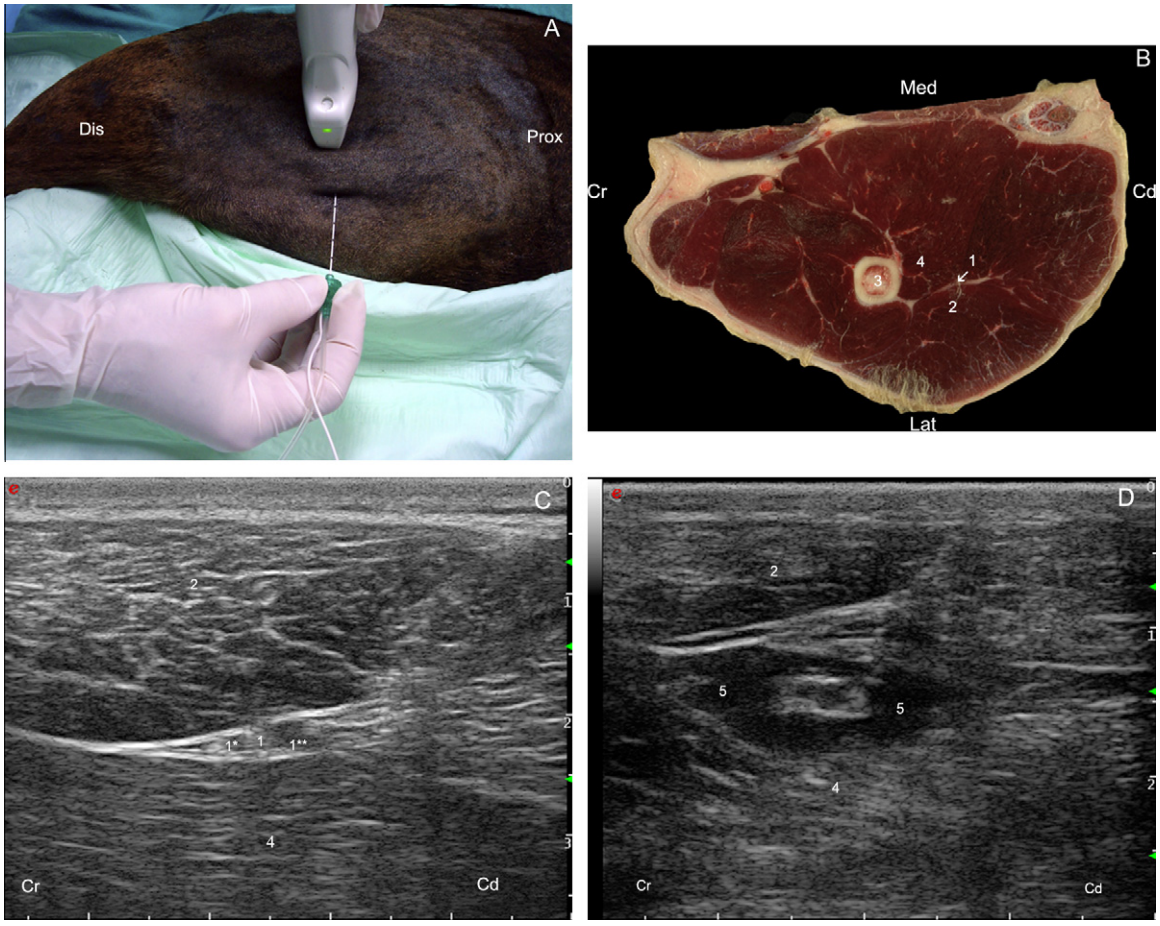

Fig. 3. (A) Position of the ultrasound transducer and needle for a midfemoral approach to the ScN. (B) Cross-sectional anatomical image of the nerve at that level. (C) Corresponding transverse ultrasound image of the ScN. The two components of the ScN are readily distinguished and appear as two hypoechoic tubular structures surrounded by a thin hyperechoic rim. (1*) peroneus communis nerve, and (1**) tibialis nerve. (D) Local anaesthetic solution surrounding the ScN showing the characteristic 'donut sign'. (1) ScN, (2) biceps femoris muscle, (3) femur, (4) adductor magnus muscle, (5) local anaesthetic solution. Prox, proximal; Dis, distal; Cr, cranial; Cd, caudal; Med, medial; Lat, lateral.

of the nerves by US was always performed by the same investigators (DE, AA). The administration of the local anaesthetic (LA) to block the nerves was performed by a single operator (FL).

The skin was clipped and aseptically prepared. The ScN and FN were approached by US using the acoustic windows established earlier. The accuracy of the nerve location was confirmed by peripheral nerve stimulator (PNS) (Stimuplex HNS 11, B-Braun); an insulated needle (Stimuplex 30 mm 22 G, B-Braun) was connected to a PNS delivering a current of 0.5 mA, at a frequency of 2 Hz and a pulse duration of 0.1 ms. The insulated needle was inserted in the long axis (in-plane) of the US transducer. When its tip was seen close to the nerve, the PNS was switch on. A positive EMR, defined by extension or flexion of the tarsus for the ScN and by extension of the knee for the FN, confirmed the accuracy of the US location of the nerve. Then, an initial volume of 1 mL of lidocaine 1% (B-Braun) was slowly injected. Multiple incremental injections of LA were given around the whole cross-sectional area of the nerve to produce a 'halo' of LA surrounding the entire nerve (commonly referred as the 'donut sign'; Marhofer et al., 2005a). A total of 0.3 mL/kg of lidocaine was injected around each nerve. A negative aspiration test was performed before the injection of LA. The dogs were evaluated by one clinician (DE) for 3 days after the experiences to assess for potential complications such as infection, haematoma or neurological deficits.

Results

Phase 1

The gross dissection of the ScN was easily performed in all cases. The nerve exited the pelvic cavity through the foramen ischiadicum majus, continued caudally and then turned distally to pass between the trochanter major of the femur cranially and the ischiatic tuberosity caudally. Then, the nerve passed distally along the lateral surface of the thigh, deep to the biceps femoris, and terminated dividing into two large branches, the tibialis and peroneus communis nerves (Fig. 1).

The gross dissection of the FN was difficult, due to its short pathway within the thigh before it ended by ramifying within the quadriceps femoris muscle. The FN was covered by multiple layers of tissues, and its location is hidden by fat, fascia and muscle. The FN passed through the iliopsoas muscle. It was observed as a white cord inside the muscle when longitudinally split (Fig. 2). The anatomical structures were identified and labelled in the ultrasonographic images and corresponding anatomical section (Figs. 3 and 4).

Phase 2

The ScN was identified correctly in all cadaver pelvic limbs and successfully stained when the ink injected under US guidance was seen to be within the ScN at dissection. The ScN was visible between the muscles of the thigh where it lay medial to the biceps femoris and caudal to the femur. In the transverse images, the two components of the ScN, the peroneus communis and tibialis nerves, were readily distinguished. The two components of the ScN appeared as well differentiated oval-to-round hypoechogenic structures surrounded by a hyperechogenic thin rim (Fig. 3). The cranial structure corresponded to the peroneus communis nerve while the caudal corresponded to the tibialis nerve, always

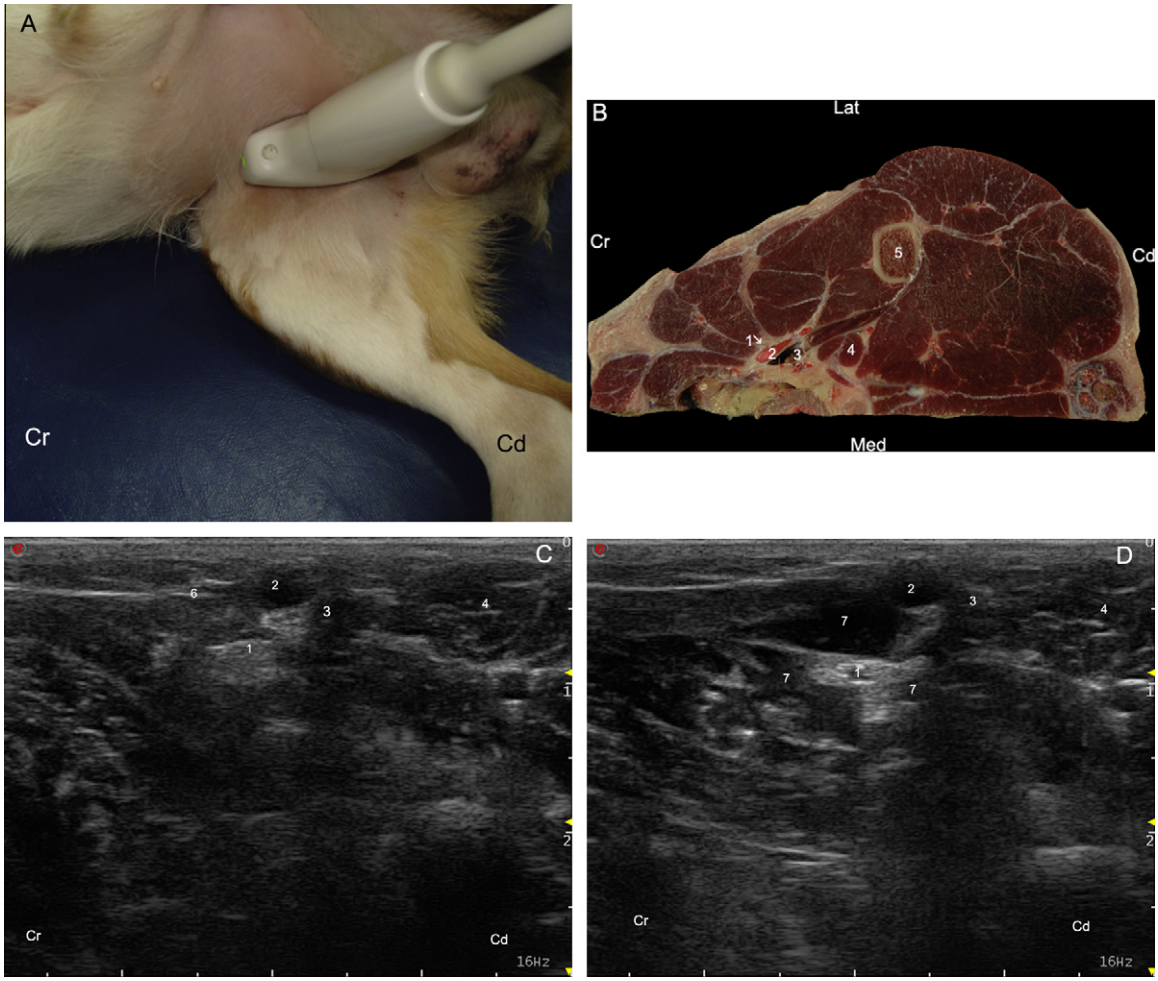

Fig. 4. (A) Position of the ultrasound transducer for the inguinal approach to the FN. (B) Cross-sectional anatomical image of the nerve at that level. (C) Corresponding transverse US image of femoral nerve. (D) Local anaesthetic solution surrounding the FN. (1) FN, (2) femoral artery, (3) femoral vein, (4) pectineus muscle, (5) femur, (6) iliacus fascia, (7) local anaesthetic solution. Prox, proximal; Dis, distal; Cr, cranial, Cd, caudal; Med, medial; Lat, lateral.

appearing slightly greater in diameter than the peroneus communis nerve. The peroneus communis and tibialis nerves were observed to diverge at the level of the popliteal fossa. In sagittal views, the ScN appeared as a tubular hypoechogenic structure delimited by two hyperechoic lines. Several acoustic windows can be used to approach this nerve on the lateral surface of the thigh.

The FN was not easily identified. The dye injected under US guidance was found inside the FN only in 5/8 (62.5%) pelvic limbs at dissection. The FN was located cranial to the femoral artery and appeared as a hyperechogenic triangular shaped structure ventral to the fascia iliaca (Fig. 4). The anatomical features of the FN determined that only one acoustic window could be employed to approach and block this nerve.

Phase 3

In all cases it was possible to identify the ScN and visualise the same structures as could be seen in cadavers. There were no differences between the US appearance of the ScN in living or dead animals. The transducer was placed at the level of the mid-femur to obtain the transverse plane images of the ScN. Then, the needle was introduced into the posterior part of the thigh and inserted in-plane of the US beam. It was possible to follow the movements and the advance of the needle by US in real time in all cases. Once it was considered that the tip of the needle was close to the ScN and the accuracy of this location confirmed by PNS a negative aspiration test was performed. Then, the slow administration of the initial dose of LA commenced producing an immediate abolition of the EMR to the PNS in all cases. Multiple incremental injections of LA were performed around the nerve and in each case the LA spread completely around the nerve and the 'donut sign' was easily observed (Fig. 3).

The FN was not identified in 4/8 (50%) of the pelvic limbs examined in vivo. There were no differences between the US appearance of the FN in living or dead animals. To access the FN, the femoral artery was palpated in the femoral triangle, then the transducer was placed on the inguinal skin crease and oriented to obtain the best possible transverse view of the femoral artery and vein. These vessels were observed as two hypoechoic rounded structures. The caudal structure was identified as the femoral vein and the cranial one as the femoral artery. The transducer was moved cranially until the FN was identified as a hyperechogenic triangular shaped structure (Fig. 4). The block needle was introduced in the plane of the US beam. In this case, it was not possible to see the entire length of the needle because its angle of insertion was steep and the nerve was located superficially. Once it was considered that the tip of the needle was close to the FN and the accuracy of this location had been confirmed by PNS a negative aspiration test was performed. Then, the slow administration of the initial dose of LA commenced producing an immediate abolition of the EMR to PNS. The LA was seen to spread to the adjacent levels along the cranial part of the femoral artery (Fig. 4).

No complications were encountered during the procedure. All the dogs recovered uneventfully from the procedures, and did not show signs of neurological disorders as a result of a nerve injury.

Discussion

In humans there has been an increased interest in the use of US to guide peripheral and central neuraxial blocks (Marhofer et al., 2005b). However, to the author's knowledge, this is the first study investigating the use of US guidance for nerve blocks in dogs. We selected the ScN and the FN to perform this study because these nerves are commonly blocked to produce analgesia during surgical procedures involving the stifle and the distal areas of the hind limb in humans and dogs (Rasmussen et al., 2006).

The anatomical study was undertaken to identify the target nerves and their relevant associated structures to determine the optimal acoustic windows to approach and block these nerves. The length of the ScN allowed us to approach it at different locations. The possibility of approaching this nerve at different levels may be of clinical interest because the ScN could be blocked whilst avoiding areas of skin lesion or infection which could affect the lateral aspect of the thigh in polytraumatised animals. In all dogs, we could clearly identify the ScN at different levels along the lateral aspect of the thigh. Nevertheless, an approach of the ScN at the mid level of the femur was selected for the blocks, as the nerve at this level is unaccompanied by any other major nerve or vascular structures that could complicate or impair the block. Moreover, it was easier to fixate the needle in this region during the approach.

In both cadavers and live dogs, the FN was difficult to identify clearly. It has been documented that the femoral artery is the main anatomical landmark to identify the FN (Mahler and Adogwa, 2008). However, the relations between the femoral artery and the femoral nerve are variable in dogs (Adams, 1998). Based on the anatomical observations found in our study, the inguinal skin crease was the only acoustic window available to approach the FN. However, this area is small due to the proximity of the abdominal wall, thus limiting the transducer motion range. The size of the probe footprint (5 cm) was too large for the area of the acoustic window but this would be resolved by using smaller probes for smaller animals. In addition, the FN was superficial and short at this location and is surrounded by fat tissue, tendon, muscle and fascia structures with similar echogenic properties which hindered detection (Silvestri et al., 1995).

The ultrasonographic appearance of the ScN and its division in the popliteal fossa in dogs has been previously described (Benigni et al., 2007). In our study, there were no differences between the ultrasonographic appearance of the ScN in fresh cadavers and live dogs. In contrast, Benigni et al. (2007) reported that the nerve appeared more uniformly hypoechoic in cadavers and hypothesised that post-mortem degeneration of the nerve may be responsible for the reduction in the internal echotexture of the nerve structures. We believe that these differences may be attributed to the fact that in our study the US scans were performed immediately after euthanasia, while Benigni et al. (2007) employed thawed cadavers. In all dogs, we could clearly identify the ScN. It appeared as an oval shaped hypoechoic core of the nerve fascicles surrounded by the hyperechoic rim representing the epineurium (Benigni et al., 2007).

The FN appeared slightly more echogenic than the surrounding muscle tissue in agreement with previous descriptions of the nerve in humans (Gray et al., 2004; Carty and Nicholls, 2007), but there are no descriptions of the US appearance of the FN in dogs in the veterinary literature. In general, the degree of hyperechogenicity of a peripheral nerve reflects the amount of connective tissue within the nerve. Consequently, the echogenicity of the nerves can change as they pass through different tissues (Carty and Nicholls, 2007). In the current study, the different echogenicities observed between the FN and ScN may be related to differences in the fascial tissues covering each nerve and the anisotropy of the nerve due to the angle of incidence of the US beam (Silvestri et al., 1995).

In our study, the technique of US-guided allowed for an easy and accurate nerve location and blockade of the ScN in all live dogs, which suggests that the block could be easily achieved clinically using an US-guided technique alone. However, the localisation of FN was difficult under US guidance, and the PNS was essential to confirm the nerve location by observing a positive EMR response.

One benefit of using US-guided techniques is the direct visualisation of the spread of LA around of the nerve. An incorrect spreading of LA can be easily corrected by repositioning the needle (Marhofer et al., 2005b) and the injection stopped when the target nerve is surrounded by LA (Oberndorfer et al., 2007). In our study, the spread of LA around the nerve was considered successful when the sonographic image of the 'donut sign' was observed. This was seen around the ScN in all dogs but only observed in 50% of limbs during the FN block. Obviously, these were the cases where the FN was clearly visualised by US and the 'donut sign' is considered a good predictor of a successful block (van Geffen and Gielen, 2006). After the injection of LA in the proximity of the target nerves, an immediate inhibition of the positive EMR was noted (De Andrés and Sala-Blanch, 2001; Futema et al., 2002).

The direct visualisation of the spread of LA around of the nerve seems to result in a more profound blockade using lower volumes of local anaesthetics. Oberndorfer et al. (2007) reported a reduction of the volume of LA by one-third for ScN blocks and by one-half for FN blocks when performed using US compared with nerve stimulator guidance in children. In a study conducted to determine the minimum volume of LA required for an effective blockade of the ilioinguinal/iliohypogastric nerves using US guidance in children, the adequate blockade was achieved with a reduction of the volume of LA to 0.075 mL/kg, compared with the recommended doses from 0.3 to 0.4 mL/kg (Willschkle et al., 2006). The reduction in volume of LA is another advantage of US guidance in regional anaesthesia, since the risk of toxicity is diminished and unwanted side effects reduced. Despite this reduction, the duration of analgesia was not reduced in either report (Willschkle et al., 2006; Oberndorfer et al., 2007).

Another advantage in visualising the needle, the nerves and their surrounding structures, is the ability to detect individual anatomical variations in these structures. In addition, seeing the spread of LA in real time may allow experienced practitioners to decrease the risk of complications even further (Abrahams et al., 2009). In our study, no complications were observed, but it was a study limited to a small number of live dogs. Larger clinical studies are needed to confirm our results.

One of the main drawbacks of the US-guided technique is that the needle must be on the same plane as the transducer to visualise its tip (Marhofer et al., 2005a) in order to prevent accidental nerve puncture. In the current study, this was not a problem during ScN blockade since the transducer was situated in the same plane as the needle (Marhofer et al., 2005a). In contrast, the insertion path was short for the FN and the angle of insertion was steep, such that the needle could only be seen as a white dot (Marhofer et al., 2005a). However, Marhofer et al. (2005a) preferred the short axis technique for most types of nerve block, because the shorter insertion path required was found to be more comfortable for the patient.

Conclusions

This study validates the usefulness of the US-guided technique to locate and block the ScN at the midfemoral level in dogs. The

acoustic window of the inguinal region to locate and block the FN was not useful in all cases. Additional studies are necessary to evaluate different acoustic windows to approach the FN in dogs. This technique offers the advantage of performing blocks with direct visualisation of the needle and LA spread as well as all involved anatomical structures.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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4.2 ARTÍCULO 2

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Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog

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ABSTRACT

This prospective study assessed a ventral ultrasound-guided suprainguinal approach to block the femoral nerve (FN) in dogs. The anatomical features of the FN were evaluated in four canine cadavers. In another five cadavers, the FN was located by ultrasound-guidance and the accuracy of this technique was evaluated by injection of black ink and posterior evaluation of the degree of staining of the nerves. In five live dogs, the FN was blocked with 2% lidocaine.

The distribution of lidocaine around the nerve and the presence of motor deficit were evaluated. The FN was easily located and accurately blocked in all cases. This new ultrasound-guided approach was reliable for blocking the FN and might be a suitable alternative to the traditional approaches described to block the FN in the dog.

Introduction

Epidural analgesia in conjunction with opioids and non-steroidal anti-inflammatory drugs is effective in providing pre-emptive analgesia for orthopaedic procedures involving the hind limbs (Valverde, 2008). However, epidural analgesia is associated with some potential side effects, such as hypotension and urinary retention, complications including technical failure in obese patients, and is contraindicated in cases of bleeding disorders, infectious skin diseases or anatomical abnormalities in the lumbosacral area (Hendrix et al., 1996; Jones, 2001). Blockade of the sciatic nerve (ScN) and femoral nerve (FN) have been described as a practical alternative to epidural analgesia, since it produces a similar analgesic effect with a lower risk of side effects (Marhofer et al., 2005; Campoy et al., 2009; Echeverry et al., 2010b). Peripheral nerve block techniques are not routinely employed in small animal practice, since they are considered to be more challenging than epidural analgesia. The introduction of peripheral nerve stimulators and, more recently, the development of ultrasound (US)-based technology, have improved the accuracy of the location of peripheral nerves (Valverde, 2008).

In dogs, a number of studies have described 'blind' techniques to block the FN using anatomical landmarks and nerve stimulation. In those studies, the FN was accessed by inguinal or by dorsal approaches (Mihelić et al., 1995; Campoy et al., 2008; Mahler and Adogwa, 2008). These techniques have been associated with complications, such as intraneural and intravascular puncture or a failure to achieve satisfactory analgesia (Enneking et al., 2005; Marhofer et al., 2005). In humans, studies have demonstrated that US increases the safety and quality of the blockade of peripheral nerves (Gray et al., 2004; Enneking et al., 2005) because the advance of the needle to the target nerves can be monitored to avoid sensible anatomy. Previous studies in dogs have described the use of US to block the ScN and the FN (Campoy et al., 2010; Echeverry et al., 2010a); the ScN was easily identified and blocked safely at the lateral aspect of the thigh. In contrast, the success rate of the FN block was suboptimal (50–62.5%) using an approach at the femoral triangle (Echeverry et al., 2010a).

The main goal of the present study was to evaluate an US-guided technique to block the FN within the iliopsoas muscle (IPM) using a ventral suprainguinal approach (SIA). The ultrasonographic characteristics of the FN and the feasibility and accuracy of this new approach were investigated in cadavers and in five experimental dogs.

Materials and methods

Animals

The study was approved by the Animal Care and Ethics Committee of the University of Murcia. Four fresh intact dog cadavers were used for the anatomical study. Another five fresh intact cadavers were used for an ultrasonographic study. The dogs were obtained from the local Zoonoses and Public Health Service and were humanely euthanased by the use of an overdose of pentobarbital (200 mg/kg) for

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reasons not related to hindlimb neurological or musculoskeletal disorders. All dogs were adults, with a mean weight of 19.4 ± 3.36 kg (range 16–25 kg).

Five healthy adult experimental beagle dogs with a mean weight of 14.75 ± 1.26 kg (range 12–16 kg) and a mean age of 6 ± 0.8 years (range 4–6 years) were enrolled for the in vivo study. The dogs were handled with guidelines for humane care of experimental animals.

Gross dissection of the femoral nerve

The left and right FN nerves of two cadavers were dissected to investigate the anatomical characteristics of the FN and its related structures. Skin incisions were made on the medial and proximal aspect of the thigh cranial to the pectineus muscle. The skin was reflected cranially and caudally from the vertical incision and the FN identified cranially to the femoral artery, near the inguinal ligament. Then, the IPM was located and split to expose the FN. The FN was dissected in its entire pathway within the IPM (Fig. 1).

Anatomical cross-sectional study

Red latex was injected through the abdominal aorta artery in another two cadavers to evaluate the anatomical features of the FN. The carcases were frozen at −30 °C. Eight days later, transverse cryosections of the caudal abdomen were made using a high-speed band saw at a thickness of 1 cm from the sixth lumbar vertebra to the hip joint. Photographs of each slide were taken to correlate the anatomical structures to the corresponding ultrasonographic images.

In vitro ultrasound nerve study and blockade

Ultrasonographic scans were performed on the left and right FNs of five fresh cadavers (immediately after euthanasia). The hair of the abdomen was clipped and the skin cleaned. A 4–13 MHz broad band linear array probe (MyLab 70, Esaote) was used for the US. The adjustments in depth and gain were made to achieve the optimal ultrasonographic vision of the target nerve. The cadavers were placed in dorsal recumbency and the limb to be scanned was moderately extended. The probe was orientated perpendicular to the midline and slightly cranial to the inguinal nipple, and the orientation marker of the probe was positioned medially (Fig. 2). From this point, the probe was directed cranially, trying to trace the projection of the nerve on the abdomen.

Transverse images of the FN were obtained with the probe placed in this position. Longitudinal images of the nerve were also obtained by rotating the probe 90° from this point and placing the orientation marker of the probe towards the cranial aspect. The structure identified as the FN was injected using insulate needles (Stimuplex 50 mm 22 G, B-Braun) with 0.3 mL/kg black ink under US- guidance by a transverse cross-sectional view. The needles were inserted by an in-plane technique (Fig. 2). The needle insertion sites were marked on the skin and photographed to serve as reference points to select the optimal acoustic window. The limbs were immediately dissected to confirm the accuracy of the US nerve location by the presence of black ink staining the FN. A successful FN block was defined as the staining of the nerve in a length ⩾2 cm (Campoy et al., 2008). The area where the FN was scanned in cadavers constituted the acoustic window used to perform the in vivo blocks.

In vivo ultrasound guided nerve blockade

The dogs were sedated with medetomidine (Domitor, Pfizer) 10 μg/kg administered IM. The right and the left FN of each dog were blocked with an interval of 8 days between procedures. The blocks were always performed by the same investigator, using the technique described above for cadavers and after the aseptic preparation of the puncture site and the probe. An initial volume of 0.3 mL of lidocaine 2% (B-Braun) was injected slowly after confirming the absence of blood in the needle by a negative aspiration test. Then, the remaining dose of local anaesthetic solution (LA) was administered by means of a multiple injection technique until the nerve was completely surrounded by LA, creating a typical anechogenic halo (donut sign) (Marhofer et al., 2005) (Fig. 2). A total dose of 0.3 mL/kg lidocaine 2% (B-Braun) was injected. A positive US-guided block was defined as the observation of the LA around the FN 'donut sign'.

At the conclusion of the procedure, atipamezole (Antisedan, Orion Pharma) 50 μg/kg, IM was administered to reverse sedation, and the blocked limbs were evaluated for the presence of motor or proprioceptive deficits and inability to bear weight. The dogs were also evaluated during the following 72 h to assess potential complications, such as infection, haematomas or nerve injury, after the blocks.

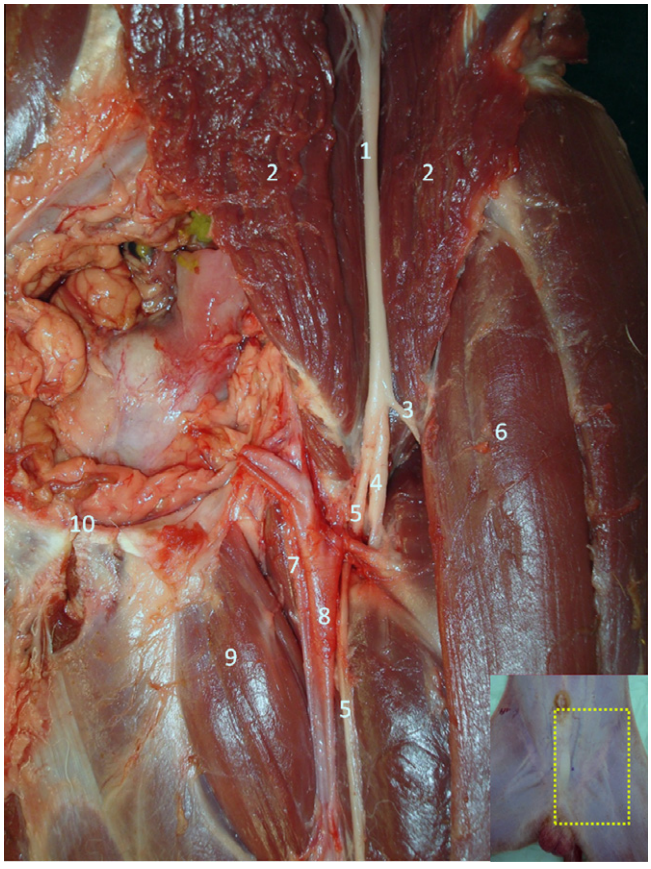

Fig. 1. Gross anatomy of the femoral nerve (FN) within the iliopsoas muscle (IPM) and at the femoral triangle (proximomedial aspect of the thigh). Picture in the insert shows the dissected area (1) FN, (2) IPM (the muscle has been split to expose the nerve), (3) muscular branch of the FN to the sartorius muscle, (4) muscular branch of the FN to the quadriceps femoris muscle, (5) saphenous nerve, (6) sartorius muscle, (7) femoral vein, (8) femoral artery, (9) pectineus muscle, (10) pubis.

Results

Gross dissection of the femoral nerve

The FN descended through the body of the IPM emerging from this muscle at the lower part of its lateral border, through the muscular lacuna, to reach the pelvic limb. It was easily identified during the dissection as a white cord (Fig. 1). At this location, the nerve was devoid of any other vascular or fascial structure. The FN within the IPM (from caudal to cranial) gradually passed from ventral to dorsal and from lateral to medial. The anatomical structures were identified and labelled in the ultrasonographic images and corresponding anatomical sections (Fig. 2).

In vitro ultrasound nerve study and blockade

The FN was easily identified using the inguinal nipple as a landmark. The FN was scanned from cranial to the inguinal nipple to the midpoint between this nipple and the ipsilateral abdominal caudal nipple. It was located at a mean depth of 1.31 ± 0.21 cm (range 1.15–1.55 cm) from the skin. The nerve was successfully stained in a length of 6.2 ± 0.6 (range 5.0–6.8 cm).

The FN appeared as single, well-differentiated, rounded hypoechogenic structure surrounded by a marked hyperechogenic thick rim located within the IPM body on transverse scans (Fig. 2). On longitudinal scans, the FN appeared as a tubular hypoechogenic structure bordered by marked thick hyperechoic borders. Cranial to the selected acoustic window, visualisation of the FN was difficult and it was necessary to apply a variable tilt of the probe to see the nerve. The FN was observed away from relevant vascular,

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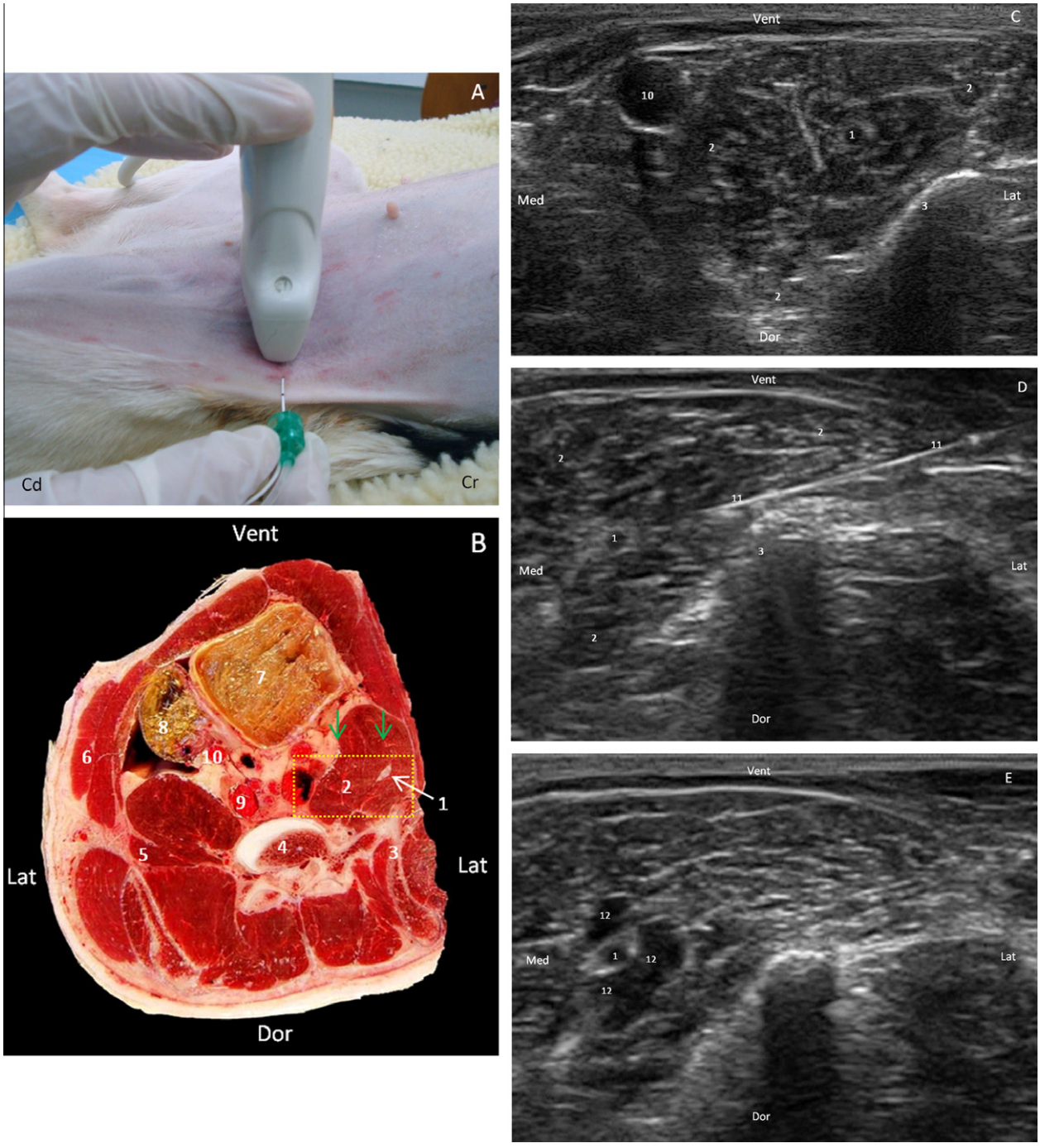

Fig. 2. (A) Position of the probe and the needle relative to the skin for the suprainguinal approach to the femoral nerve (FN). (B) Cross-sectional anatomical image of the FN at that level (the arrows shows the direction of the ultrasound beam). (C) Corresponding transverse ultrasound image of the FN and related structures. (D) Ultrasonographic image showing the approach of the needle towards the FN. (E) Local anaesthetic (LA) agent surrounding the FN showing the characteristic 'donut sign'. (1) FN, (2) iliopsoas muscle, (3) ileum, (4) vertebral body, (5) quadratus lumborum muscle, (6) abdominal wall, (7) urinary bladder, (8) descending colon, (9) common iliac vein, (10) external iliac artery, (11) needle, (12) LA. Cr, cranial; Cd, caudal; Med, medial; Lat, lateral; Vent, ventral; Dor, dorsal.

osseous and abdominal viscera structures at the selected acoustic window. The IPM was observed as an ovoid to triangular hypoechoic structure with an internal pattern of scattering echoes in transverse scans (Fig. 2). On longitudinal scans, its appearance was of a tubular to ovoid hypoechogenic structure with a pattern of internal fine and parallel echoes. There was a good correlation between the ultrasonographic scans and the transverse cryosections.

In vivo ultrasound guided nerve blockade

The FN was accurately blocked in all the cases. There were no differences between the US appearances of the FN in living or dead animals. The FN, the needle and the distribution of LA around the nerve ('donut sign') were easily observed during the procedures (Fig. 2). No signs of discomfort were noticed during the blockade. All the dogs showed motor deficit after the blockade. Clinical signs

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also included proprioceptive deficit and inability to hold their weight and use the blocked limb. The recovery was uneventful and no complications were found.

Discussion

The aim of this study was to evaluate a ventral US-guided SIA to block the FN in dogs. To our knowledge this approach has not been previously reported in dogs. Despite the fact that a sensory test was not conducted, the approach studied here was considered to be successful, since it produced adequate staining of the FN in cadavers and a complete motor blockage in all live dogs.

The FN and the ScN are the main nerve supplies of the pelvic limb in the dog. The ScN is responsible for most of the cutaneous innervation of the lateral, caudal and part of the cranial aspects of the pelvic limb. The FN innervates the iliopsoas and quadriceps muscles and, when it arises from the IPM, it produces the saphenous nerve, which is responsible for most of the cutaneous innervation of the medial aspect of the pelvic limb (Kitchell and Evans, 1993). The merits of the SIA may facilitate the location of the FN by US. In a clinical context, this approach could be highly effective in providing surgical analgesia for procedures carried out on the medial aspect of the pelvic limb and could also be useful when analgesia of the medial and lateral aspects of the limb is required when a simultaneous blockade of the FN and the ScN should be performed.

The anatomical study was useful for establishing an adequate acoustic window to block the FN. The anatomical features of the FN and its related structures within the IPM were similar to previous descriptions (Kitchell and Evans, 1993). In dogs, the FN has been described as a hyperechogenic triangular structure when approached at the level of the femoral triangle (Campoy et al., 2010; Echeverry et al., 2010a).

The ultrasonographic appearance of the FN within the IPM has not been described previously in dogs. In our study, the FN within the IPM was observed as a hypoechogenic oval structure surrounded by a hyperechogenic rim. These differences in echogenicity might be explained by the effect of the tissues covering the FN at different anatomical regions. The FN is covered by multiple facial planes and fat tissue at the level of the femoral triangle (Echeverry et al., 2010a), whereas it is devoid of these tissues within the IPM where the nerve is only surrounded by the muscle mass. The tissues surrounding the FN at the femoral triangle have a similar echogenic pattern to the nerve. In the femoral triangle approach, the FN could be hidden as a result of the lower ultrasonographic contrast between all these structures (Silvestri et al., 1995). In contrast, visualisation of the FN using a SIA may be superior because the hyperechogenic border of the nerve is better contrasted, since it is surrounded by a hypoechoic muscle tissue.

Variable FN block success rates (86–100%) have been reported in the dog when performing blind techniques using dorsal (Mihelić et al., 1995; Campoy et al., 2008) or femoral triangle approaches (Mihelić et al., 1995; Mahler and Adogwa, 2008). It is well established that blind techniques can be associated with inadvertent vascular or nerve puncture and may also exhibit a low sensitivity in the accurate location of the target nerves (Marhofer et al., 2005).

The results from this study suggest that the ventral US-guided SIA may offer some clinical advantages over other approaches previously described. Firstly, the ventral SIA can be performed without the requirement of anatomical landmarks. For example, the location of the anatomical landmarks required to perform a dorsal approach to the FN can be difficult in dogs with spinal abnormalities and in obese patients. Difficulties in locating the FN have also been described between non-chondrodystrophoid and chondrodystrophoid breeds due to anatomical differences (Mihelić et al., 1995). The presence of anatomical variations has been recognised as an important factor for peripheral nerve block failure in humans (Marhofer and Frickey, 2006).

In the ventral SIA, the needle has to cross fewer structures (sartorius muscle, caudal abdominal wall, IPM) to reach the FN than by a dorsal approach (quadrates lumborum muscles, transverse processes of lumbar vertebrae, iliolumbar ligament, IPM). This fact and the absence of osseous structures in the path of the needle during the ventral SIA facilitate the block procedure and permits a more suitable visualisation of the nerve and the needle at all times, thus increasing the safety and efficacy of the FN block. Another consideration is that the distance between the spinal cord and the injection site is greater when the ventral SIA is employed, thus decreasing the possibility of epidural spread of the LA described for the dorsal approach in dogs (Mihelić et al., 1995; Campoy et al., 2008). This complication seems more closely related to the approach method than to the volume of LA injected (Mannion et al., 2005). Finally, when the FN is approached by a ventral SIA, the nerve is located far away from vascular structures and the risk of inadvertent vascular punctures is greatly reduced. These punctures have been reported during the FN blockade in humans using a femoral triangle approach (Berg and Rosenquist, 2007).

The success rate of the FN blockade in this study was 100%, in accordance with a previous report where the FN was approached at the femoral triangle (Campoy et al., 2010). However, another study reported a suboptimal success rate (50–62.5%) employing this approach (Echeverry et al., 2010a) but in this study the dogs were placed in lateral recumbency, thus limiting the movements of the probe and the needle in an already small acoustic window.

The optimal success rate found in our work could be explained by the better visibility of the FN using the ventral SIA. Moreover, the acoustic window in which the FN could be visualised was greater using the ventral SIA than with the femoral triangle approach, thus allowing easier location and blockade of the FN. Moreover, the FN is observed for a greater length, which can facilitate blockade at different points and avoid skin lesions or infected skin areas during the injection. On the other hand, the absence of fascial planes within the IPM could enhance the distribution of the LA around the nerve. Interferences with the adequate distribution of LA have been described by the presence of fascial planes during the FN blockade in humans (Gray et al., 2004) and also during the ScN blockade in the dog (Shilo et al., 2010). Finally, another clinical advantage of the ventral SIA over other approaches is that the FN can be blocked before it branches. Therefore, the distal components of the FN, including the sensitive (saphenous nerve) and motor branches, will be effectively blocked. Partial FN blocks have been reported in humans when approaching the FN at the level of the femoral triangle in humans (Nielsen et al., 2003).

The evaluation of the sensory blockade was not performed in this preliminary study. Nevertheless, the adequate spread of the LA ('donut sign') around the FN observed in all canine cadavers and the motor deficit achieved in the experimental dogs supports the clinical efficacy of the FN block performed by the investigated approach. The spread of the LA around the entire diameter of peripheral nerves has been described as a valid predictor of a successful nerve block (Marhofer et al., 2005). The volume of the staining solution was adequate in cadavers to stain the FN in a length $\geqslant$2 cm in all the cases, although a previous study by Campoy et al. (2010) described FN staining in a length >2 cm using a lower volume of staining solution (0.1 mL/kg). Further studies related to the minimum effective volume of LA to block the FN by the ventral SIA should be performed in the future to confirm the clinical efficacy of the FN block.

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Conclusions

The ventral US-guided SIA may allow a safe, accurate and feasible access to the FN to provide a successful nerve blockade in dogs and could be considered as a suitable alternative to previously described approaches to the FN.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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4.3 ARTÍCULO 3

Ultrasound-guided "two-in-one" femoral and obturator nerve block in the dog: an anatomical study.

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Abstract

Objective: To evaluate the dye extent and distribution at the lumbar plexus (LP) of three volumes of local anaesthetic-methylene-blue solution administered close to the femoral nerve (FN) by the use of a ventral ultrasound (US)-guided suprainguinal approach (SIA).

Study design: Prospective experimental trial.

Animals: Twenty mongrel canine cadavers weighing 17.7 ± 3.8 kg (mean ± SD)

Methods: The left and right LP of 2 cadavers were dissected to identify the FN, obturator nerve (ON) and lateral femoral cutaneous nerve (LFCN). Then, the extent and distribution of dye at the LP of three volumes of injectate of 0.2 , 0.4 and 0.6 mL kg^{-1} administered close to the FN by a ventral US-guided SIA were

studied in another 18 dog cadavers (n=6 per group). Staining of ≥ 2 cm along the target nerves was indicative of sufficient spread to produce a nerve block.

Results: The ventral US-guided SIA allowed the observation of the FN within the iliopsoas muscle (IPM) in a total of 17 cadavers. The assessment of the dye extent and distribution revealed a similar pattern regardless of the injected volume. From the injection site, the spreading of injectate occurred in cranial, lateral and caudal directions. The FN and ON were effectively stained in all the cases. The LFCN was not effectively stained in any case.

Conclusions and clinical relevance: A volume of 0.2 mL kg^{-1} administered close to the FN by a ventral US-guided SIA produced a sufficient distribution of the injectate within the IPM to produce the effective staining of the FN and ON. This US-guided technique may be an appropriate alternative to previously reported techniques based on electrolocation to block the FN and ON in the dog.

Keywords: Regional anaesthesia, dog, lumbar plexus, femoral nerve, obturator nerve, ultrasound.

Introduction

An increasing number of regional anaesthetic techniques can be employed to manage perioperative pain produced by a variety of surgical procedures performed in the pelvic limb in the dog. Epidural anaesthesia is extensively employed for this purpose; however it is contraindicated in cases of infectious skin disease in the region of the lumbosacral area, uncorrected hypovolaemia, bleeding disorders or in patients with lumbosacral abnormalities

(Jones 2001). Recently, techniques of peripheral nerve blockade such as the combined block of the FN and sciatic (ScN) nerves have been described as a valid alternative to epidural in the dog (Campoy *et al*. 2009; Echeverry *et al*. 2010). Nevertheless, the combined blockade of the FN and ScN has been associated in humans with incomplete analgesia during some surgical procedures carried out on the knee (Sakura *et al.* 2010) and hip (Murray *et al*. 2010). As a result, the combined blockade of the LP and ScN may be a more reliable technique when analgesia of the entire pelvic limb is required in humans (Murray *et al*. 2010; Sakura *et al*. 2010). The main nerve components of the LP that innervate structures of the pelvic limb in the dog are the FN (sensitive and motor nerve), ON (motor nerve) and LFCN (sensitive nerve) (Adams 2004).

The LP block can be performed in humans using anterior or posterior approaches, and locating the injection site by the use of anatomical superficial landmarks or nerve stimulation (Capdevila *et al*. 2005; Murray et al. 2010). It has been recently reported that the use of ultrasound (US) may improve the efficacy of this technique (Deschner *et al*. 2009; Christos *et al*. 2010). The success of the LP block is highly dependent on the accurate location of the FN as the location of this nerve is considered the end-point to administer the local anaesthetic (LA) solution (Kirchmair *et al*. 2002; Capdevila *et al*. 2005).

There is only one study regarding the LP (also called psoas compartment) block in the dog. In this study the distribution of three volumes of staining solution was evaluated after performing a LP block using a dorsal approach and employing nerve stimulation to locate the FN (Campoy *et al*. 2008). This study reported a lack of staining for the FN and ON in four dogs in the low dose group, two dogs in the medium dose group, and one dog in the

high dose group. Information regarding the staining of the LFCN was not provided in this description. Recently, a success rate of a 100 % in the visualization of FN within the IPM was reported in the dog by the use of a ventral US-guided suprainguinal approach (SIA) (Echeverry *et al*. 2011). To our knowledge, an US-guided technique with the potential of achieving a complete LP block has not yet been evaluated in the dog

The aim of the present study was to assess the extent and distribution of the dye at the LP of three volumes of injectate (lidocaine/methylene blue solution) administered close to the FN. It was hypothesized that the injection of an appropriate volume of injectate close to the FN may produce its effective diffusion towards the three nerve components of LP either within the IPM or within the facial planes of the local musculature.

Materials and methods

Animals

A total of 20 fresh intact mongrel canine cadavers weighing 17.7 ± 3.8 kg (mean ± SD) obtained from the Local Zoonoses and Public Health Service were employed. All the dogs were humanely euthanized for reasons unrelated to this study. In a first phase, two cadavers were used to conduct an anatomical study of the LP. In a second phase, another 18 cadavers (a total of 36 lumbar plexuses) were used to study the distribution of three volumes of injectate at the LP.

Anatomical study

The left and right LP of two cadavers were dissected by an incision performed in the linea alba from the sternum to the pubis. The skin was reflected laterally and the abdominal viscera were exposed. The abdominal contents were reflected laterally to define the retroperitoneal space. Then, the FN was located at the level of the femoral triangle, and dissected in a cranial direction by blunt dissection until the nerve reached the IPM. Then, this muscle was split to expose the FN, ON, and LFCN which were identified and dissected.

Ultrasound-guided block study

Eighteen cadavers were randomly assigned to three experimental groups to evaluate the spreading at the LP of three volumes of injectate of 0.2 mL kg^{-1} (L, low dose), 0.4 mL kg^{-1} (M, medium dose) and 0.6 mL kg^{-1} (H, high dose). The FN was accessed by the ventral US-guided SIA technique previously described (Echeverry *et al*. 2011). The cadavers were placed on dorsal recumbence and the limb to be injected was moderately extended. The caudolateral part of the abdomen was clipped and the skin cleaned. A 4-13MHz broad band linear array transducer attached to an US machine (MyLab 70; Esaote, Italy) was employed. A frequency of 13 MHz set to perform the scans. Adjustments in depth and gain were made to obtain the optimal view of the FN. The transducer was orientated perpendicular to the midline and slightly cranial to the inguinal nipple, with the orientation marker positioned medially to obtain a cross-sectional view of the FN. From this point, the transducer was directed cranially, trying to trace the projection of the nerve on the abdomen. The inability to identify the FN by US was considered as exclusion criteria for this

study. The injection site was located about the midpoint between the inguinal nipple and the ipsilateral abdominal caudal nipple (Fig. 1) to obtain the most cranial possible distribution of the injectate. Once the FN was identified, an insulated needle (Stimuplex D 50 mm 22 G; B.Braun Medical SA, Germany) was inserted using an in-plane technique to administer the injectate close to this nerve (Fig. 1). A single injection technique was selected in our study to promote a wider diffusion of injectate at the LP. Therefore, circumferential spread around the FN ("donut sign") was not attempted. The injection site was marked and photographed. On each cadaver, the left and right LP were injected. The injectate consisted of a mixture of lidocaine 2 % (Lidocaine chloride 2%; B.Braun Medical SA, Spain) and methylene blue 1% (Methylene blue powder; Panreac, Spain) in a 1:1 proportion. The injectate was always administered slowly and using a "one-hand" injection technique to decrease the risk of applying excessive pressure to the injections. It was established that the administration of injectate would be discontinued and the needle relocated in case any increase in the resistance during the injections was perceived. The US-guided location of the FN was performed by the same two investigators (AA, n=18 and DE, n=18) and the administration of injectate conducted by the same investigator (FL) in all cases. These investigators have significant clinical experience in the blockade of peripheral nerves using conventional techniques and also using US-guided techniques. The LP was dissected in 12 cadavers (n=4 per group) to assess the extent and distribution of the dye. A nerve staining over a length of ≥ 2 cm was considered to be indicative of a clinically effective nerve blockade (Campoy *et al*. 2008). In another 6 cadavers (n=2 per group), red coloured latex was introduced through the thoracic aorta. These

animals were frozen at -80°C for 24 hours. Then, transverse abdominal cryosections of 1 cm in thickness were made from the fourth lumbar vertebra to the hip joint, by a high-speed band saw. The extent and distribution of the dye as well as the occurrence of epidural spread were evaluated in the cryosections. The presence of nerve staining in a minimum of two consecutive cryosections (length ≥ 2 cm) was considered to be indicative of a clinically effective nerve blockade.

Results

Anatomical study

The IPM, the psoas minor muscle, and the quadratus lumborum muscle were covered by the fascia iliaca. The FN emerged at the level of the fifth lumbar segment to run in a caudal direction within the body of the IPM. It left the abdomen through the muscular lacuna to reach the pelvic limb. The ON nerve emerged from the fourth to the sixth lumbar segments to run within the caudomedial portion of the IPM entering into the pelvis canal. The LFCN exited the spinal medulla at the level of the fourth lumbar segment. The trajectory of this nerve was variable passing either the body of the psoas minor or between the psoas minor and IPM. Finally, the LFCN crossed the abdominal wall to ramify in the skin at the level of the tuber coxae.

Ultrasound-guided block study

The ventral US-guided SIA allowed the observation of the FN, the needle and the spread of the injectate close to this nerve in a total of 17 cadavers (Fig. 2). In these cadavers, the FN was observed as an oval to rounded

hypoechogenic structure surrounded by a hyperechogenic thick rim located within the IPM body on transverse scans. The IPM was observed as an ovoid to triangular hypoechoic structure with an internal pattern of scattering echoes in transverse scans. One cadaver of the H group was excluded from this study due to the impossibility to visualize the FN. In this dog, the IPM exhibited a marked increase in its echogenicity. The reason for this increased echogenicity was not investigated. The procedures were completed successfully and no complications were observed during the injections.

The assessment of the extent and distribution of the three volumes of dye revealed a similar pattern regardless of the volume injected. From the injection site, the spreading of the injectate was observed to occur simultaneously in three directions: cranial, lateral and caudal. The most important distribution occurred in the cranial direction and the dye was observed to reach the level of the 6^{th} lumbar vertebra. In the caudal distribution, the dye travelled within the IPM and stained the FN and its branches at the level of the femoral triangle. In the lateral distribution, the injectate was found under the fascia iliaca, between the fascial planes of the IPM and the quadratus lumborum muscle. The FN and the ON were stained over a length ≥ 2 cm in all the cases (Fig. 3). The FN was stained directly by the administration of injectate while the ON was stained by the cranial diffusion of the dye solution. The LFCN was not effectively stained in any case. In one case belonging to the L group, this nerve was stained over a length of 1.0 cm. In another two cases belonging to the M group, the LFCN was stained respectively over a length of 1.2 and 1.5 cm. In these cases, the LFCN was stained when it passed over the IPM and the quadratus lumborum muscles.

The anatomical cryosections showed that the spreading of injectate occurred within the IPM in the same directions described above. The cranial distribution occurred within the muscular fascicles of the IPM as well as within the fold where the FN and the ON runs. The injectate reached the L6 level. There was no evidence of dye distribution within the vertebral canal (Fig. 4).

Discussion

This study evaluated the extent and distribution at the LP of three volumes of injectate administered close to the FN by the use of a ventral US-guided SIA in dog cadavers. This technique was effective to produce a successful staining of the FN and ON, even in the low volume group (0.2 mL kg^{-1}), in all the cases.

The ultrasonographic features of the FN and the IPM observed here were identical to those described in a previous report (Echeverry *et al*. 2011). In the present study, the FN was adequately visualized in all but one cadaver belonging to the H group. In this cadaver, the increased echogenicity of the IPM impaired the visualization of the FN. The contrast difference between the FN and the IPM has been described as the cause of the adequate visualization of the FN in this site (Echeverry *et al*. 2011). The increased echogenicity observed in this cadaver may have been produced by muscular fibrotic changes which might be related to chronic muscle injuries (Breur & Blevins 1997). Difficulty in the visualization of peripheral nerves produced by muscular fibrosis has been reported in humans (Kirchmair *et al*. 2001).

It was hypothesized that the injection of an appropriate volume of injectate close to the FN may produce its effective diffusion towards the three

nerve components of LP either within the IPM or within the facial planes of the local musculature. The results from this study allowed us to confirm this hypothesis for the FN and the ON. The localization of these nerves within the IPM may explain their successful staining in all the cases, as the IPM is the site where the injectate was mainly distributed. Contrarily, the location of the LFCN outside the IPM may have impaired the staining of this nerve. In the H group the injectate was not able to reach the point where this nerve passes over the IPM and the quadratus lumborum muscle. Surprisingly, in 3 cases belonging to the L and M groups the LFCN could be stained, but in a length < 2 cm. These results may suggest that the assessed technique could be reliable to produce a 2-in-1 block of the FN and the ON but not a complete LP block.

There is only one description regarding the LP block in the dog which describes the distribution of injectate after a dorsal LP block carried out by nerve stimulation (Campoy *et al*. 2008). In this study, a lack of effective staining of the FN and ON was found in four out of eight dogs injected with 0.1 mL kg^{-1}, two out of seven dogs injected with 0.2 mL kg^{-1} and one out of seven dog injected with 0.4 mL kg^{-1}. Information regarding the staining of the LFCN was not included in this report. The results from the present study show that the FN and ON were successfully stained in all the cases at the three volumes administered. These differences could be explained by the differences in accuracy of the techniques employed to locate the FN. In our study, the use of US may have allowed a more precise location of the FN.

The results from our anatomical study show that the FN and ON were successfully stained in all the cases, even when a low volume of 0.2 mL kg^{-1} was administered. This volume is lower than the volume of 0.4 mL kg^{-1}

recommended in a previous description to block the LP in dogs (Campoy *et al*. 2008). It has been reported that the minimum effective dose of LA necessary to block a peripheral nerve can be significantly reduced with the use of US-guided techniques (Marhofer *et al*. 2005).

The length of the nerve in contact with the LA is a main factor determining the success of a peripheral nerve block. An in-vitro study has suggested that only a length of 2-5 mm of nerve should be in contact with LA to obtain an effective nerve block (Raymond *et al*. 1989). In the present study, a nerve staining length ≥ 2 cm was selected as compatible with a clinically effective block in accordance with previous reports (Campoy *et al*. 2008). Due to the cadaveric nature of the present study, we might only speculate about the physical distribution of the injectate on the target nerves as one of the elements necessary to obtain an adequate clinical block (Raymond *et al*. 1989).

The results from the present study suggest that the ventral US-guided SIA could offer some clinical advantages in the blockade of the FN and the ON over the technique employing electrolocation previously described. Some of these advantages have recently been reported and are mainly related with its efficacy to locate the FN and to visualize in real time the distribution of LA (Echeverry *et al*. 2011). This may produce a more effective spread of LA within the IPM towards the FN and ON. An epidural distribution of the injectate has been reported in two dogs (8.7 %) when the LP block was carried out by a dorsal approach (Campoy *et al*. 2008). The epidural spread of LA is one of the more frequent complications reported in humans, with an incidence of 1-16%, when this block is performed by a dorsal approach (Parkison *et al*. 1989; Capdevila *et al*. 2005). It can be considered that the increased distance

between the injection site and the vertebral canal when a SIA is used may decrease the risk of a neuraxial distribution of the injectate. The epidural spread of a LA seems to be more closely related with the approach employed than to the volume of LA administered (Mannion *et al*. 2005). Other factors that have been related to the incidence of epidural spread are the pressure and speed of injection during the nerve block procedure (Patel 2009). The injectate was administered slowly and using a “one-hand” injection technique to decrease the risk of this complication in our study. There was no evidence of spread of injectate within the vertebral canal in the cryosections (n=6).

A potential clinical advantage of the studied technique is that the addition of an ON block to a FN or to a combined FN and sciatic nerve block might improve the perioperative analgesia in procedures performed in the pelvic limb, particularly of those involving the knee or the hip joint. The addition of an ON block to a FN (Macalou *et al*., 2004) or to a FN and sciatic nerve block (McNamee *et al*., 2002) decreases the consumption of opioids and pain scores after major knee surgery in humans. The presence of branches of the ON in the stifle joint has been reported in some dogs (Budras *et al*. 2007). This may suggest that the analgesia provided by the exclusive blockade of the FN and the ScN could be insufficient in some cases, possibly due to the presence of some ON innervation in the stifle. Contrarily, others authors have not described the presence of those ON branches in the dog (Kitchell & Evans 1993). There are some descriptions of the ON innervation in the hip joint in humans (Birnbaum *et al*. 1997) and cat (Dee 1969) which may reinforce the hypothesis of the presence of some ON innervation in the hip joint also in the dog. The presence of accessory branches of the ON (10-30 %) may have a negative

effect on the success of the blockade of this nerve for surgical procedures performed in the hip joint in humans (Sim & Webb 2004). Finally, the present technique allows blocking the FN and ON nerves before they are ramified which may be effective to depress the activity of the accessory branches of those nerves.

In conclusion, the ventral US-guided SIA might be suitable to produce an effective and safe 2-in-1 FN and ON block in the dog. Furthermore, a reduced volume of 0.2 mL kg^{-1} is adequate for this purpose. Further research employing live dogs is required to evaluate if the nerve block association and the volumes of injectate employed in this study offer an adequate clinical efficacy.

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FIGURAS ARTÍCULO 3

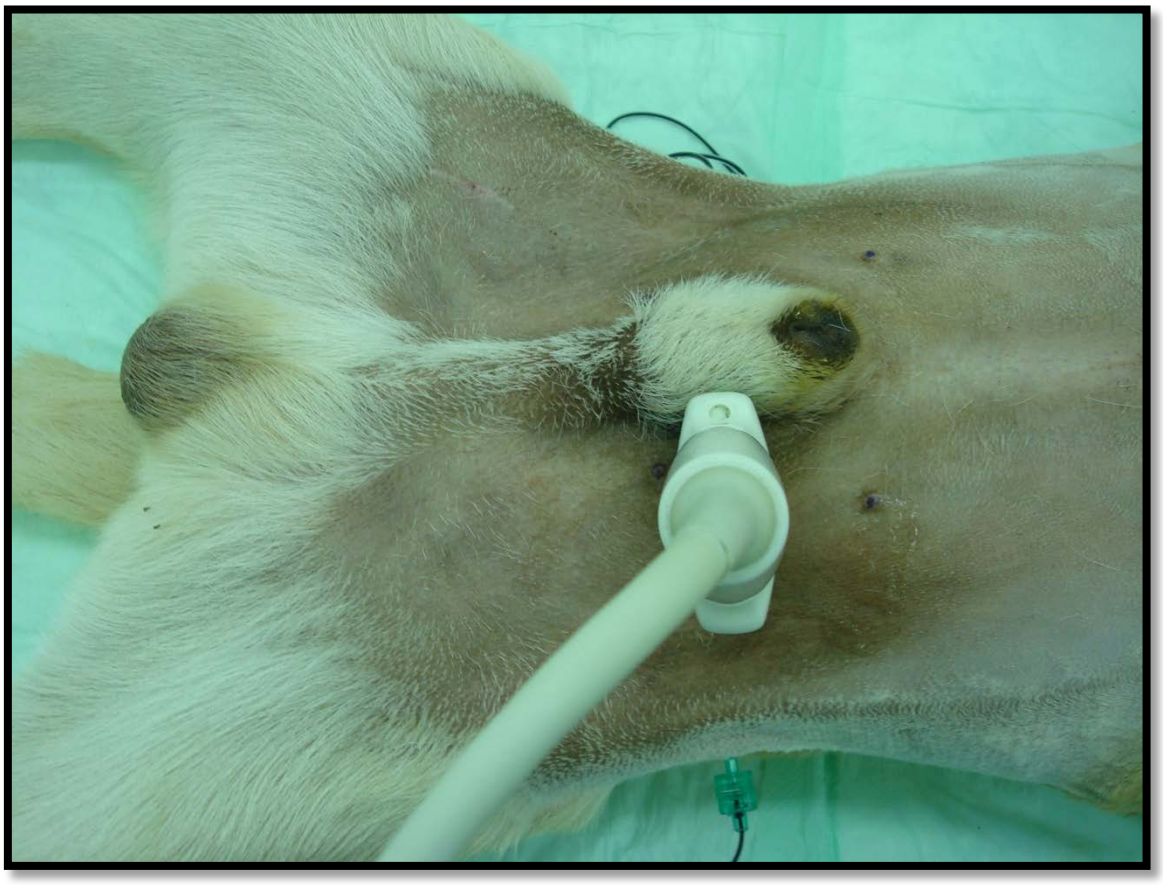

Figure 1. Position of the ultrasound transducer and needle to approach the FN by a ventral SIA in the dog.

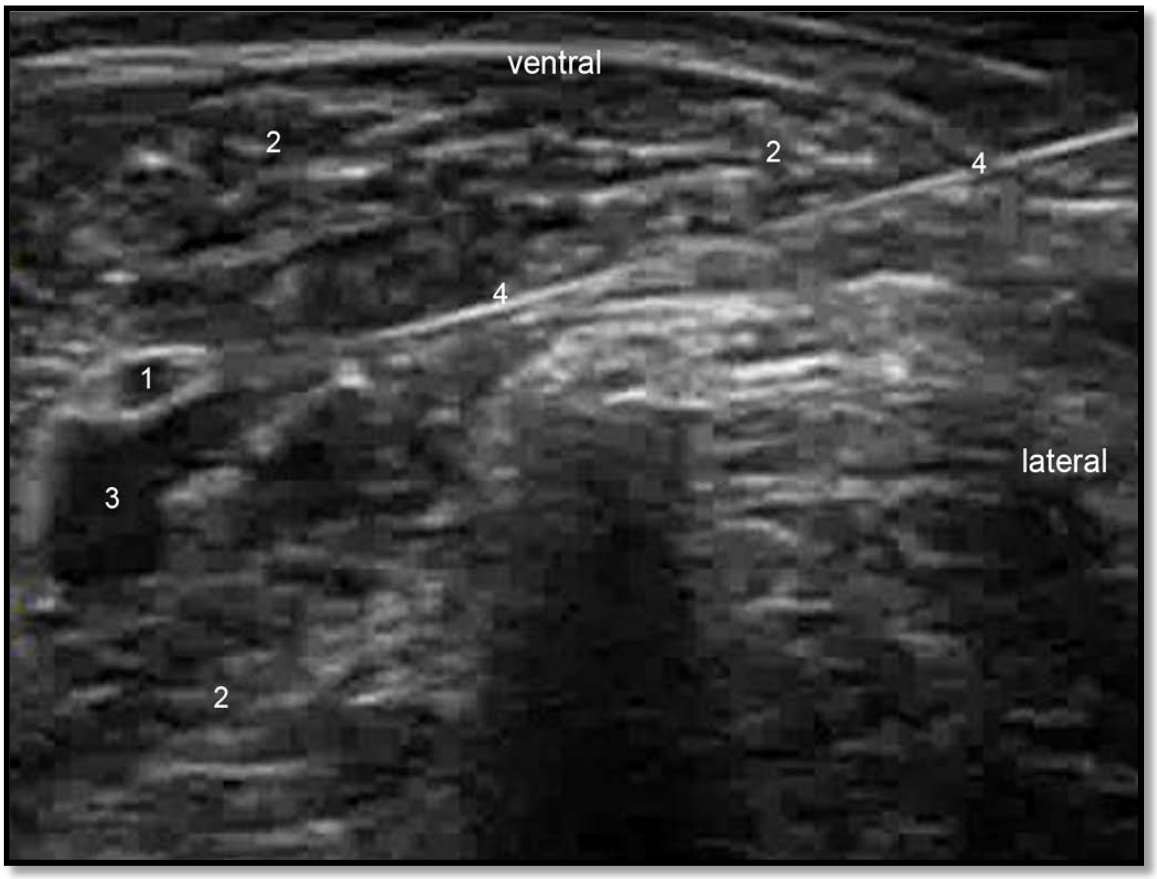

Figure 2. Transverse ultrasonographic image obtained in a dog cadaver by a ventral SIA: (1) FN, (2) Iliopsoas muscle, (3) spread of injectate, (4) needle.

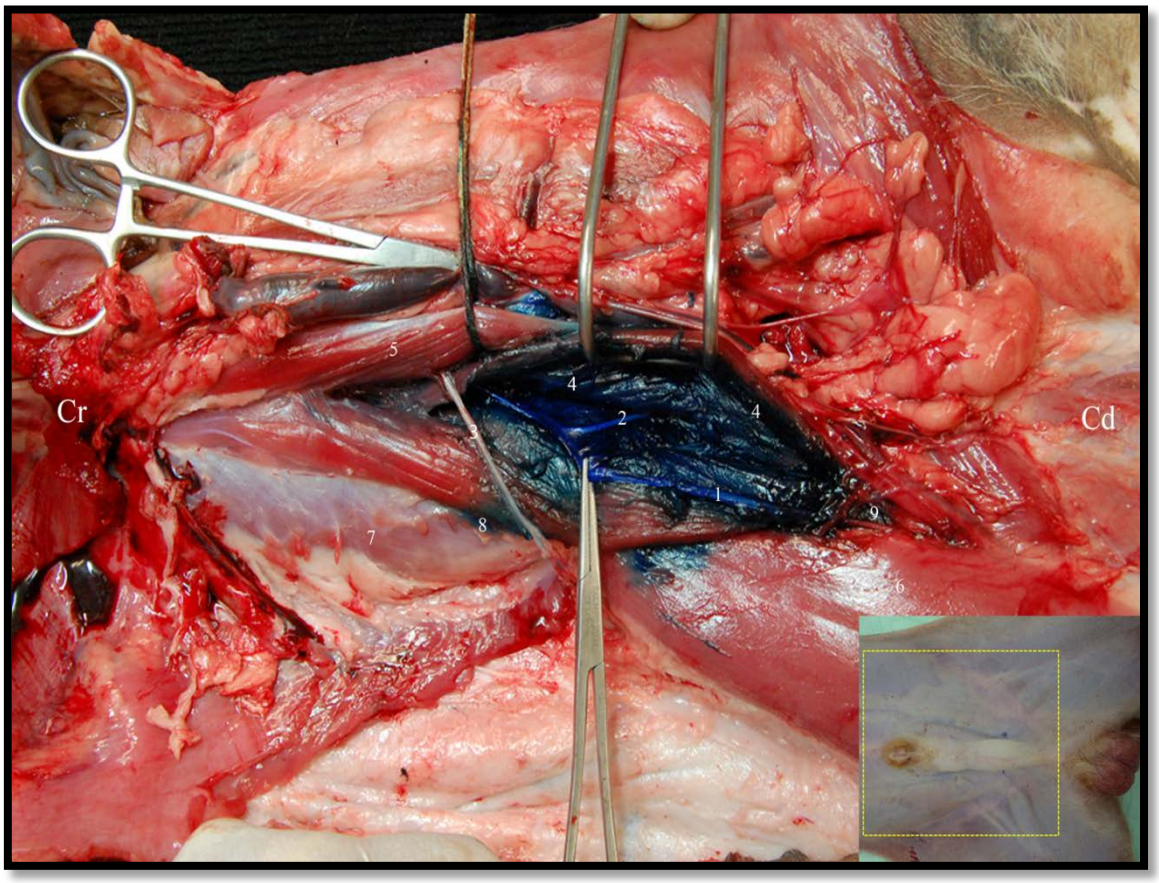

Figure 3. Dissection of the anatomical region of interest showing the spread of the injectate: (1) FN, (2) ON, (3) LFCN arising through the psoas minor muscle, (4) iliopsoas muscle (the muscle has been split to expose the target nerves), (5) psoas minor muscle, (6) sartorius muscle, (7) abdominal wall, (8) detail of the lateral distribution of the injectate between the IPM and the quadratus lumborum muscle, (9) detail of the caudal distribution of the dye solution reaching the femoral triangle.

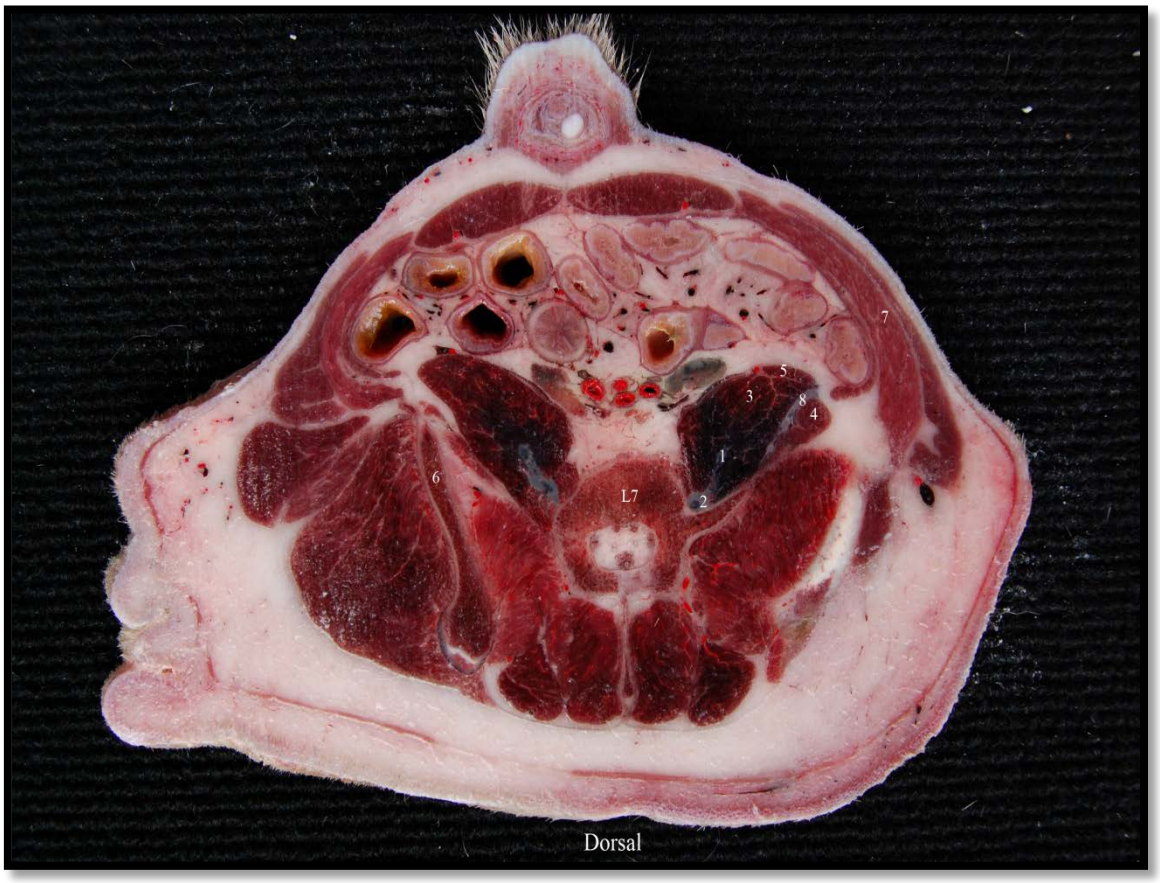

Figure 4. Transverse cryosection obtained after the administration of the injectate: (1) FN, (2) ON, (3) iliopsoas muscle, (4) quadratus lumborum muscle , (5) psoas minor muscle, (6) ilium, (7) abdominal wall, (8) detail of the lateral distribution of the injectate between the IPM and the quadratus lumborum muscle, (L7) 7th lumbar vertebra.

5. CONCLUSIONES

1. La gran longitud del nervio ciático permitió su abordaje ecográfico en varias ventanas acústicas localizadas a lo largo de la cara lateral del muslo. Ecográficamente, en el corte transversal el nervio ciático se observó como una estructura ovoide hipoecoica delimitada por bordes hiperecoicos y en el corte longitudinal, como una estructura tubular hipoecoica con bordes hiperecoicos. El mejor abordaje ecográfico para bloquear el nervio ciático fue establecido en la cara lateral del muslo, ligeramente caudal a la porción media del fémur. Este abordaje ofrece la ventaja de posicionar la aguja en el plano y la visualización simultanea de esta y del nervio. Estos hallazgos validan la utilidad de la ecografía de cara al bloqueo anestésico del nervio ciático en el perro.

2. El abordaje del nervio femoral a través del triángulo femoral, solo permite la obtención de una ventana acústica de reducido tamaño, debido a la escasa longitud que presenta el nervio a este nivel. Por el contrario, en el abordaje suprainguinal la ventana tuvo una mayor amplitud, lo que permitió que el abordaje ecográfico del nervio femoral fuera más fácil. En el abordaje del triángulo femoral, el nervio femoral se observó en sección transversa como una estructura hiperecoica triangular. Sin embargo, en el abordaje suprainguinal el nervio femoral se visualizó como una estructura ovoide hipoecoica con bordes hiperecoicos en su sección transversa. El abordaje suprainguinal podría ser considerado como una alternativa eficaz y segura al abordaje realizado en el triángulo femoral para el bloqueo del nervio femoral en el perro.

3. El estudio anatómico del plexo lumbar reveló que sus componentes principales, los nervios femoral y obturador quedaban situados dentro del vientre del complejo muscular iliopsoas, mientras que el NCFL quedaba fuera del mismo. El abordaje suprainguinal permitió un acceso ecográfico eficaz al nervio femoral dentro del complejo muscular iliopsoas. Mediante dicho abordaje, la inyección de un volumen de 0.2 mL/kg en las proximidades del nervio femoral, permitió la distribución del inyectado dentro del complejo muscular iliopsoas hacia este nervio y hacia el obturador en todos los casos. Por lo tanto el abordaje suprainguinal podría ser una técnica eficaz para el bloqueo clínico dos en uno de los nervios femoral y obturador en el perro.

6. RESUMEN GENERAL

1. INTRODUCCIÓN

El BNP es muy utilizado en anestesiología humana para mejorar la calidad de la analgesia perioperatoria. Recientemente, la introducción de la ecografía como técnica de neurolocalizacion ha mejorado la eficacia y seguridad del BNP en medicina humana (Marhofer *et al*., 2005a). En anestesia veterinaria el BNP se realiza habitualmente localizando los nervios mediante el empleo de marcas anatómicas de superficie o de NE. No obstante, el empleo de éstas técnicas, consideradas “ciegas”, puede asociarse a una menor eficacia y a un mayor número de complicaciones (Marhofer *et al*., 2005a; Hopkins, 2007). Debido a la falta de estudios relacionados con el empleo de la ecografía en el BNP en la especie canina, se propusieron como objetivos de la presente Tesis Doctoral los siguientes: Evaluar desde el punto de vista anatómico las características de los nervios NC y NF y del PL en el perro, con el fin de establecer sus posibles abordajes ecográficos, describiendo su aspecto ecográfico en estos abordajes, y finalmente determinar la eficacia de dichos abordajes de cara su bloqueo anestésico en el perro.

2. MATERIALES Y MÉTODOS

Para los estudios *in vitro* realizados en la presente Tesis Doctoral se emplearon *los* cadáveres de 39 perros mestizos adultos obtenidos del Servicio Local de Zoonosis y Salud Pública, eutanasiados por razones no relacionadas con los objetivos del presente estudio. En el estudio 1 y 2 se utilizaron además 9 perros vivos experimentales adultos clínicamente sanos de la raza Beagle procedentes del animalario de la Universidad de Murcia. Para realizar los

estudios ecográficos se utilizó un transductor linear de banda ancha de 4-13 MHz (MyLab 70, Esaote, Italia).

Artículo 1

En este estudio se utilizaron 10 cadáveres con un peso medio de 21.8 ± 3.01 kg. En 4 de estos cadáveres se realizó la disección anatómica de los nervios NC y NF. En otros dos cadáveres se introdujo látex coloreado a través de la arteria aorta abdominal, tras lo cual se congelaron a -30 °C durante 8 días. Transcurrido este tiempo, se realizaron criosecciones transversales de 2,5 cm de grosor desde la articulación de la cadera hasta la rodilla. Las criosecciones fueron fotografiadas para ser comparadas posteriormente con las imágenes ecográficas obtenidas.

El estudio ecográfico de los nervios NC y NF se realizó en los 4 cadáveres restantes. Para ello, la extremidad pelviana fue depilada, limpiada, y se aplicó gel acústico. Para la evaluación del NC los cadáveres se colocaron en decúbito lateral. El transductor se situó paralelo al plano anatómico dorsal, caudal al trocánter mayor del fémur y craneal a la tuberosidad isquiática. Luego fue dirigido distalmente siguiendo la trayectoria del NC hasta la rodilla. Así, se obtuvieron imágenes transversales del NC desde el aspecto proximal, cerca de su origen, hasta el lugar donde este nervio se divide claramente en los nervios peroneo común y tibial, cerca a la rodilla. Se localizaron varias ventanas acústicas consideradas como aptas para acceder ecográficamente al NC a lo largo de la cara lateral del muslo. No obstante, se seleccionó la ventana acústica localizada ligeramente caudal al tercio medio del fémur con el fin de estandarizar la técnica de bloqueo en este estudio.

El NF se estudió en estos cadáveres tras colocarlos en posición de decúbito lateral con la extremidad a bloquear situada ventralmente y la no intervenida ligeramente abducida. El transductor se colocó en la cresta inguinal craneal al músculo pectíneo. El nervio fue observado en sección transversa, lateral y ligeramente ventral a la arteria femoral. Esta fue la única ventana acústica que se consideró disponible para acceder a este nervio. Posteriormente, las estructuras identificadas como NC y NF fueron inyectadas con 0.3 mL/kg de tinta china usando para ello una aguja específica (Stimuplex 30 mm 22 G, B-Braun, Alemania). Los nervios fueron bloqueados mientras se observaban en sección transversal, usando una técnica en plano de la aguja con relación al transductor. Los lugares de inyección fueron marcados y fotografiados con el fin de describir la ventana acústica óptima para acceder a cada nervio. Las extremidades pélvicas fueron inmediatamente disecadas y la eficacia del bloqueo ecoguiado fue determinada observando macroscópicamente la tinción de los nervios estudiados.

El bloqueo experimental *in vivo* fue realizado en 4 perros de la raza Beagle con un peso medio de 11. 75 ± 1.26 kg y una edad media de 5 ± 0.8 años. Los perros fueron sedados con medetomidina (Domtor, Orion Pharma, Finlandia) 10µg/Kg vía IM. Los bloqueos fueron inicialmente realizados en el NC derecho y el NF izquierdo, empleando las ventanas acústicas y técnicas de inyección seleccionadas en el estudio realizado en cadáveres. Tras un periodo de descanso de dos semanas se bloquearon los nervios contralaterales, en estos mismos animales. Para efectuar los bloqueos, la piel de la zona del abordaje se preparó de forma aséptica. La precisión de la neurolocalizacion ecoguiada de los nervios estudiados fue confirmada mediante el uso de NE.

Para ello la aguja empleada para el BNP se conecto a un neuroestimulador (Stimuplex HNS 11, B-Braun, Alemania). Cuando la estructura identificada como NC o NF se encontraba cerca de la aguja, el neuroestimulador se encendía para administrar una corriente eléctrica de una intensidad de 0.5 mA, con una frecuencia de 2Hz y una duración de 0.1 ms. La observación de respuestas motoras consistentes en la flexión o extensión del tarso para el NC, y de extensión de la rodilla para el NF, confirmaban la eficacia de la neurolocalizacion. Seguidamente, se procedía a inyectar un volumen de 1 mL de lidocaína 1% (lidocaína 2%, B-Braun Medical, España) lentamente para confirmar la adecuada localización de la aguja mediante la desaparición del patrón de contracciones. Finalmente se completaba la administración de lidocaína hasta alcanzar un volumen final de 0.3 mL/kg para cada nervio. Se utilizó una técnica de inyección múltiple tratando de rodear completamente la periferia los nervios con lidocaína hasta crear el típico “signo de donut” (Marhofer *et al*., 2005a).

Artículo 2

En el estudio *in vitro* se utilizaron 9 cadáveres con un peso medio de 19.4 ± 3.36 kg. En la fase *in vivo* se utilizaron 5 Beagles con un peso medio de 14.75 ± 1.26 kg y una edad media de 6 ± 0.8 años.

Los NFs izquierdo y derecho se disecaron en 2 cadáveres para evaluar sus características anatómicas. En otros 2 cadáveres, se inyecto látex coloreado a través de la arteria aorta abdominal. Posteriormente, estos especímenes se congelaron durante 8 días a -30°, para efectuar criosecciones transversales de 1 cm de grosor desde el nivel de la L6 hasta la articulación de

la cadera. Estas criosecciones fueron fotografiadas para ser comparadas posteriormente con las imágenes ecográficas obtenidas.

El estudio ecográfico y el bloqueo *in vitro* del NF fue realizado en otros 5 cadáveres utilizando el NF izquierdo y el NF derecho. El abdomen caudolateral fue depilado, los cadáveres se colocaron en decúbito dorsal con la extremidad a bloquear ligeramente extendida caudalmente. El transductor se colocó perpendicular a la línea media abdominal y levemente craneal al pezón de la mama inguinal. Desde este punto, el transductor se dirigió cranealmente tratando de seguir la proyección del NF en el abdomen, de esta manera se obtuvieron imágenes transversas del NF. La estructura identificada como NF fue inyectada con 0.3 mL/kg de tinta china usando para ello una aguja específica (Stimuplex 50 mm 22 G, B-Braun, Alemania). Los nervios fueron abordados ecográficamente de manera transversal y bloqueados usando una técnica en plano. Los sitios de inyección sobre la piel fueron marcados y fotografiados con el fin de describir la ventana acústica óptima para acceder a cada nervio. Por último, se disecaron todos los NFs y la eficacia de la técnica de bloqueo ecoguiado del NF fue demostrada por la tinción de este nervio en una extensión ≥2 cm.

En el estudio de bloqueo *in vivo*, se emplearon 5 perros de raza Beagle que se sedaron con medetomidina (Domtor, Orion Pharma, Finlandia) 10µg/kg vía IM. La técnica utilizada para bloquear el NF fue la misma descrita previamente en cadáveres. Una vez identificado el NF ecográficamente, se administró un volumen inicial de 0.3 mL de lidocaína al 2% (lidocaína al 2%, B-Braun Medical, España) cerca del mismo. Seguidamente se administró el volumen remanente hasta completar un volumen total de 0.3 mL/kg usando una

técnica de múltiple inyección, con el fin de rodear completamente el nervio con el AL para crear el “signo de donut” (Marhofer *et al*., 2005a). La eficacia de la técnica guiada por ecografía fue demostrada por la presencia del AL alrededor del NF. Además, tras revertir la sedación con atipamezol 50µg/kg vía IM (Antisedan, Orion Pharma, Finlandia) se evaluó el efecto clínico del bloqueo nervioso mediante el análisis de la presencia de déficit motor o propioceptivo y la incapacidad para sostener el peso en la extremidad bloqueada.

Articulo 3

En este último estudio se utilizaron un total de 20 cadáveres caninos con un peso medio de 17.7 ± 3.8 kg. En una primera fase, el PL se disecó en 2 cadáveres para evaluar sus características anatómicas más relevantes para este estudio. En una segunda fase, se asignaron de manera aleatoria 18 cadáveres dentro de tres grupos experimentales para evaluar la distribución dentro del PL de tres volúmenes de tinción de 0.2 mL/kg (B, dosis baja), 0.4 mL/kg (M, dosis media) y 0.6 mL/kg (A, dosis alta) inyectados cerca del NF. El producto inyectado consistió en una mezcla de lidocaína al 2% (lidocaína al 2%, B-Braun Medical, España) y azul de metileno al 1% (Azul de metileno en polvo, Panreac, España) en proporción 1:1. Para el bloqueo del NF se uso el ASI descrito en el artículo 2. El sitio de inyección se localizaba a mitad de camino entre los pezones inguinal y abdominal caudal ipsilateral. La incapacidad para observar el NF bajo ecografía se consideró como criterio de exclusión en este estudio. La estructura identificada como NF fue inyectada de manera ecoguiada con el volumen de inyectado seleccionado, usando para ello una aguja específica (Stimuplex D 50 mm 22 G, B-Braun, Alemania), y empleando una técnica de inyección única. Los sitios de inyección fueron

marcados y fotografiados. Posteriormente, se disecó el PL en 12 cadáveres. En los restantes 6 cadáveres, se introdujo látex coloreado a través de la arteria aorta abdominal, tras lo que se congelaron a -80°C durante 24 horas, para realizar criosecciones transversales de 1 cm de grosor desde el nivel de la L4 hasta la articulación de la cadera. La extensión y distribución del inyectado fue evaluado en las disecciones y criosecciones. Una tinción del NF ≥ 2 cm de longitud se consideró compatible con un bloqueo clínico efectivo de este nervio (Campoy *et al*., 2008).

3. RESULTADOS Y DISCUSIÓN

Artículo 1

En medicina humana existe un renovado interés por el uso del BNP gracias a la aplicación de la ecografía como método de neurolocalizacion (Marhofer *et al*., 2005a). Sin embargo, bajo nuestro conocimiento este es el primer trabajo que investiga el uso de la ecografía para el BNP en el perro.

La gran longitud del NC permitió acceder ecográficamente a este nervio a través de múltiples ventanas acústicas localizadas en la cara lateral del muslo. La posibilidad de acceder a este nervio en diferentes lugares podría ser de utilidad clínica debido a que así se podrían evitar áreas de lesión o infección cutánea que potencialmente pudieran afectar la piel del aspecto lateral del muslo. No obstante, a pesar del amplio acceso ecográfico que permitía el NC, se seleccionó la ventana acústica localizada ligeramente caudal al tercio medio del fémur, ya que en éste área el NC no se acompaña de otras estructuras vasculares o nerviosas que pudieran complicar el bloqueo. Contrariamente, y

por lo que respecta al NF, su escasa longitud en el triángulo femoral permitió obtener una única ventana acústica de tamaño reducido en este abordaje.

En sección transversal el NC fue fácilmente observado ecográficamente como una estructura ovoide hipoecoica con bordes hiperecoicos (Benigni *et al*., 2007). Por su parte, el NF resultó de difícil localización, y se observó en corte transversal como una estructura triangular hiperecoica localizada craneal y ligeramente ventral a la arteria femoral. Esta descripción es similar a otras previamente realizadas en humanos (Gray *et al*., 2004; Carty y Nicholls, 2007). Las diferencias en la ecogenicidad del NC y el NF pueden ser explicadas con base a las variaciones existentes en los tejidos que recubren a estos nervios en distintas localizaciones (Silvestri *et al*., 1995; Carty y Nicholls, 2007).

En el presente estudio el bloqueo del NC fue fácil de obtener y resultó eficaz en el 100% de los casos, lo que sugiere que la ecografía puede ser útil en la práctica clínica como única técnica de neurolocalizacion de este nervio. En el caso del NF, su bloqueo fue difícil de obtener siendo efectivo solamente en el 50-62.5% de los casos. Basados en la observación anatómica, la ventana acústica localizada en la cresta inguinal fue la única disponible para abordar ecográficamente este nervio. La NE resultó esencial para localizar el NF en este abordaje. La mayor eficacia en el bloqueo del NC puede deberse al mayor tamaño de este nervio. Esto permitió su fácil localización y la disponibilidad de una ventana acústica amplia que facilitaba la maniobrabilidad del transductor y la aguja. Contrariamente, el reducido tamaño del NF dificultó no solo la visualización del NF sino que también hizo más reducida la maniobrabilidad del transductor y de la aguja. Así mismo, otro factor que pudo influir en la baja tasa de éxito para el bloqueo del NF fue la presencia de tejido graso y planos

fasciales sobre este nervio. Estos tejidos presentan una ecogenicidad similar al NF lo cual pudo dificultar su visualización (Silvestri *et al*., 1995).

Uno de los principales beneficios de la técnica de BNP guiada por ecografía es la posibilidad de visualizar simultáneamente los nervios diana, el desplazamiento de la aguja y de la distribución del AL (Marhofer *et al.,* 2005a). Esto permite mejorar la eficacia y la seguridad del BNP pues de esta manera se evita lesionar estructuras sensibles y se disminuye la dosis de AL (Marhofer *et al*., 2005a; van Geffen y Gielen, 2006; Oberndorfer *et al*., 2007). En el presente estudio fue posible visualizar en tiempo real todo el procedimiento de bloqueo así como la distribución del AL alrededor del nervio, en todos los casos para el NC pero no para el NF. La distribución del AL alrededor del nervio ("signo de donut") es considerada como un buen predictor del éxito del BNP (van Geffen y Gielen, 2006).

Este estudio valida la utilidad de la ecografía en la localización del NC al nivel del aspecto lateral del muslo en el perro. Se requieren estudios adicionales para obtener una ventana acústica más eficaz para el bloqueo ecoguiado del NF.

Artículo 2

En el primer estudio desarrollado en esta Tesis Doctoral para evaluar la eficacia del abordaje ecográfico al NF en el triangulo femoral, se obtuvo una baja tasa de éxito en su bloqueo. Con el ánimo de obtener un acceso ecográfico más eficaz para el bloqueo de este nervio en un segundo estudio, se evaluó la viabilidad y eficacia del ASI como abordaje alternativo al efectuado en el triángulo femoral. En este estudio se observó que la longitud del NF en el

ASI era mucho mayor que la observada a nivel del triángulo femoral. La mayor longitud del NF permite una localización más sencilla de este nervio y la posibilidad de acceder ecográficamente al mismo a través de una ventana acústica más amplia localizada entre los pezones ipsilaterales inguinal y abdominal caudal. En el ASI, el NF se encuentra alejado de estructuras vasculares, contrariamente a lo que sucede en el triángulo femoral donde este nervio se encuentra cercano a la arteria y vena femoral lo cual puede complicar la seguridad del bloqueo.

El NF fue observado ecográficamente en este abordaje como una estructura ovoide hipoecoica con bordes hiperecoicos en la sección transversa. Contrariamente a lo que sucede con la descripción del NF a nivel del triángulo femoral donde aparece como una estructura triangular hiperecoica. Estas diferencias podrían deberse a la diferente naturaleza de los tejidos que cubren al NF en diferentes localizaciones anatómicas (Carty y Nicholls, 2007). En el triángulo femoral el NF estaba cubierto de múltiples planos faciales y tejido graso, que presentan una ecogenicidad similar al nervio con lo que disminuye el contraste ecográfico (Silvestri *et al*., 1995). En el ASI, el NF solo está rodeado por el MIP lo que mejoraría el contraste del NF y, consecuentemente, la visibilidad de este nervio.

El NF se pudo bloquear fácilmente en todos los casos a través del ASI. Este resultado es similar al obtenido en un estudio previo donde el NF se abordaba ecográficamente a nivel del triángulo femoral (Campoy *et al.,* 2010). Sin embargo, cuando el NF fue abordado en el triangulo femoral en una primera aproximación a este nervio se obtuvo una baja tasa de éxito (50-62.5%). La adecuada tasa de éxito en el bloqueo del NF obtenida en el

presente estudio se debió a la mejor visualización del nervio y al empleo de una ventana acústica de mayor tamaño. Contrariamente la baja tasa de éxito en el abordaje a nivel del triángulo femoral fue relacionada con una difícil visualización ecográfica del NF y un reducido tamaño de la ventana acústica empleada. Además, en este estudio el NF, la aguja y la distribución del AL alrededor del nervio fueron fácilmente observados durante el procedimiento. La visualización de estos elementos durante el BNP está relacionado con un bloqueo anestésico efectivo y seguro (Marhofer *et al*., 2005a). En el ASI, el NF fue bloqueado en un nivel superior bastante anterior a su ramificación. Esto permitiría que sus componentes distales, incluyendo los sensitivos (nervio safeno) y motores fuesen adecuadamente bloqueados. El bloqueo parcial del NF ha sido descrito tras abordar este nervio a nivel del triángulo femoral en humanos (Nielsen *et al.,* 2003). Los resultados del presente estudio sugieren que el ASI permitiría un acceso ecográfico eficaz y seguro para el bloqueo anestésico del NF en el perro.

Articulo 3

La buena observación de NF mediante el ASI permitió plantear como parte final del presente trabajo, el estudio de la difusión de un volumen de AL administrado de manera precisa cerca del NF dentro del PL. Se planteo como hipótesis de trabajo que la administración de un volumen adecuado podría producir una distribución amplia y efectiva del mismo, dentro del MIP, lo que podría producir un bloqueo efectivo de los principales componentes nerviosos del PL: NF, NO y NCFL.

Los resultados de este estudio, demostraron que en todos los cadáveres menos en uno fue posible identificar de manera adecuada el NF. En este cadáver se observó un aumento en la ecogenicidad del MIP lo que disminuyó el contraste del NF impidiendo su adecuada visualización. El incremento en la ecogenicidad del MIP se ha asociado con cambios fibróticos asociados a lesiones musculares crónicas (Breur y Blevins, 1997). Dificultad en la visualización de los NP producida por fibrosis muscular ha sido descrita en humanos (Kirchmair *et al*., 2001).

Los resultados observados mostraron que la distribución del inyectado solo fue eficaz en el caso del NF y NO. La localización de estos dos importantes nervios dentro del MIP, donde el inyectado se distribuyó principalmente, explicaría el éxito de la tinción de estos dos nervios en una longitud adecuada. Contrariamente, la localización del NCFL fuera del MIP impidió su tinción efectiva.

Existe un único estudio previo, donde se evalúo la distribución de tres volúmenes de inyectado en el PL empleando una técnica de NE y un abordaje dorsal (Campoy *et al*., 2008). En este estudio se usaron 0.1, 0.2 y 0.4 mL/kg de inyectado, y la efectividad para el bloqueo del NF y el NO con estos volúmenes fue de 50, 71 y 86% respectivamente. En ese estudio no se describe el bloqueo del NCFL. Los resultados de nuestro estudio demuestran que los nervios NF y NO se tiñeron con éxito en todos los casos. Las diferencias en la eficacia de estas técnicas se pueden explicar por la mayor eficacia de la técnica ecoguiada de cara a la localización del NF, lo que aseguraría la aplicación exacta del inyectado y su distribución más efectiva dentro del MIP hacia los nervios NF y NO. Los resultados del estudio

anatómico evidencian que los nervios NF y NO fueron adecuadamente teñidos incluso con un volumen más bajo inyectado (0.2 mL/kg). Este volumen es menor al recomendado en el estudio previo (Campoy *et al*., 2008). Se ha descrito que la ecografía permite disminuir el volumen de AL necesario para obtener un BNP efectivo (Marhofer *et al*., 2005a).

Los resultados de este estudio sugieren que el ASI ecoguiado, puede ofrecer ventajas clínicas en el bloqueo de los nervios NF y NO comparado con técnicas basadas en electrolocalización descritas previamente (Campoy *et al*., 2008). Algunas de estas ventajas, ya descritas en el segundo estudio, están principalmente relacionadas en una mayor efectividad del la técnica ecoguiada para localizar el NF y monitorizar la distribución del inyectado. Otra ventaja del ASI es la mayor distancia existente entre el sitio de la inyección y la médula espinal, lo cual podría disminuir la posibilidad de una distribución neuroaxial del inyectado.

Estudios demuestran que la adición del bloqueo del NO al bloqueo del NF o del NF y el NC mejora la calidad de la anestesia en humanos sometidos a cirugías de rodilla y cadera (McNamee *et al*., 2002; Macalou *et al*., 2004). La posibilidad de obtener el bloqueo simultáneo del NF y el NO mediante el presente abordaje podría ser de utilidad clínica al mejorar la cobertura analgésica para procedimientos quirúrgicos realizados en la rodilla del perro; toda vez que se ha descrito la presencia de ramas del NO para la rodilla en algunos perros (Budras *et al*., 1989). Los resultados del presente estudio indican que el ASI puede ser útil para producir un bloqueo seguro y eficaz dos en uno de los nervios NF y NO en el perro.

4. CONCLUSIONES

1. La gran longitud del nervio ciático permitió su abordaje ecográfico en varias ventanas acústicas localizadas a lo largo de la cara lateral del muslo. Ecográficamente, en el corte transversal el nervio ciático se observó como una estructura ovoide hipoecoica delimitada por bordes hiperecoicos y en el corte longitudinal, como una estructura tubular hipoecoica con bordes hiperecoicos. El mejor abordaje ecográfico para bloquear el nervio ciático fue establecido en la cara lateral del muslo, ligeramente caudal a la porción media del fémur. Este abordaje ofrece la ventaja de posicionar la aguja en el plano y la visualización simultanea de esta y del nervio. Estos hallazgos validan la utilidad de la ecografía de cara al bloqueo anestésico del nervio ciático en el perro.

2. El abordaje del nervio femoral a través del triángulo femoral, solo permite la obtención de una ventana acústica de reducido tamaño, debido a la escasa longitud que presenta el nervio a este nivel. Por el contrario, en el abordaje suprainguinal la ventana tuvo una mayor amplitud, lo que permitió que el abordaje ecográfico del nervio femoral fuera más fácil. En el abordaje del triángulo femoral, el nervio femoral se observó en sección transversa como una estructura hiperecoica triangular. Sin embargo, en el abordaje suprainguinal el nervio femoral se visualizó como una estructura ovoide hipoecoica con bordes hiperecoicos en su sección transversa. El abordaje suprainguinal podría ser considerado como una alternativa eficaz y segura al abordaje realizado en el triángulo femoral para el bloqueo del nervio femoral en el perro.

3. El estudio anatómico del plexo lumbar reveló que sus componentes principales, los nervios femoral y obturador quedaban situados dentro del vientre del complejo muscular iliopsoas, mientras que el NCFL quedaba fuera del mismo. El abordaje suprainguinal permitió un acceso ecográfico eficaz al nervio femoral dentro del complejo muscular iliopsoas. Mediante dicho abordaje, la inyección de un volumen de 0.2 mL/kg en las proximidades del nervio femoral, permitió la distribución del inyectado dentro del complejo muscular iliopsoas hacia este nervio y hacia el obturador en todos los casos. Por lo tanto el abordaje suprainguinal podría ser una técnica eficaz para el bloqueo clínico dos en uno de los nervios femoral y obturador en el perro.

7. SUMMARY

1. INTRODUCTION

The peripheral nerve block (PNB) is widely employed to improve the quality of the perioperative analgesia in humans. In recent years the employ of ultrasound (US) as neurolocation technique have improve the efficacy and security of the PNB in humans (Marhofer *et al*., 2005a). In veterinary anaesthesia the PNB is usually performed using superficial landmarks or neurostimulation (NS) based techniques to locate the target nerves. However the employ of these “blind techniques” could be associated to a lower efficacy and higher rate of complications in the blocks (Marhofer *et al*., 2005a; Hopkins, 2007). Due to the lack of studies related whit the employ of US for the PNB in the dog, following objectives were proposed in the present Doctoral Thesis: Evaluate the anatomical features of the sciatic nerve (ScN), femoral nerve (FN) and lumbar plexus (LP) with the aim to establishing the more suitable ultrasonographic approach to access to these nerve structures. Describe the ultrasonographic features of these nerve structures at the selected acoustic windows, and finally, to evaluate the efficacy of these approaches for the anaesthetic block of the ScN, FN, and LP in the dog.

2. MATERIALS Y METHODS

For the *in vitro* studies, the cadavers of 39 adult mongrel dogs obtained from the local Zoonoses and Public Health Service, and euthanized for reasons unrelated to the aims of this study were employed. Aditionally, 9 healthy adult experimental Beagle dogs were employed in the studies 1 and 2. All the ultrasonographic studies were performed by the use of a 4-13 MHz linear transducer (MyLab 70, Esaote, Italy).

Article 1

In this study 10 dog cadavers with a mean weight of 21.8 ± 3.01 kg were employed. The anatomical features of the ScN and the FN were evaluated in 4 of these cadavers. Red coloured latex was introduced through the abdominal aorta artery in the remaining 2 cadavers. Then, these cadavers were frozen at -30 °C for 8 days. Later, the pelvic limbs of these cadavers were used to obtain transverse cryosections (2.5 cm Thickness) from the hip joint to the stifle. Photographs of each slide were taken to facilitate the interpretation and comparison of the anatomical structures to the corresponding ultrasonographic images.

Ultrasonographic scans of the ScN and FN were carried out in 8 limbs of another 4 cadavers. The skin of the lateral aspect thigh was clipped and the dogs positioned in lateral recumbency. The transducer was placed parallel to the dorsal anatomical plane just caudal to the trochanter major of the femur and then directed toward the distal aspect of the thigh. In this fashion, the ScN was imaged in a transverse plane from the dorsal aspect close to its origin to the point where the peroneus communis and tibial nerves diverge near the stifle. Several acoustic windows were available to approach the ScN along the lateral surface of the thigh. However it was decided to select the mid-femur approach to standardize the procedures.

The dogs were positioned in lateral recumbency and the transducer placed in the inguinal skin crease cranial to the pectineus muscle (while the contralateral limb remained abducted) to evaluate the FN. This acoustic window was the only one found available to approach the FN in this study. The

structures identified as the ScN and FN were injected with 0.3 mL/kg of black ink. A needle (Stimuplex 30 mm 22 G, B-Braun, Germany) was inserted using a long axis technique and the nerves approached on transverse images. The insertion sites were marked and photographed to serve as reference points to locate the optimal acoustic windows. Then, the injected limbs were immediately dissected to confirm the accuracy of the US nerve location by observing the presence of black ink staining the target nerves.

The in *vivo blocks* were performed in 4 dogs with a mean weight of 11. 75 ± 1.26 kg and a mean age of 5 ± 0.8 years. The dogs were sedated by the administration of medetomidine (Domtor, Orion Pharma, Finland) 10 µg/kg IM. The blocks were performed first in the right ScN and then in the left FN. A resting period of two weeks was observed between the trials. The skin was clipped and aseptically prepared. The ScN and FN were approached by an US-guided technique using the acoustic windows and blocking techniques established in the *in vitro* study. The accuracy of the nerve location was confirmed by NS. An insulated needle (Stimuplex 30 mm 22 G, B-Braun, Germany) was connected to a peripheral neurostimulator (Stimuplex HNS 11, B-Braun, Germany). A current of 0.5 mA was delivered at a frequency of 2 Hz and pulse duration of 0.1 ms in all the cases. The insulated needle was inserted in the long axis (in-plane) of the transducer. When the tip of the needle was seen close to the target nerve, the neurostimulator was switched on. A positive evoked muscular response defined by the extension or flexion of the tarsus for the ScN and by extension of the stifle for the FN, confirmed the accuracy of the US location of the nerve. An initial volume of 1 mL of lidocaine 1% (Lidocaine chloride 2%; B.Braun Medical SA, Spain) was injected slowly. Then multiple

incremental injections of local anaesthetic solution (LA) were given around the whole cross-sectional area of the nerve to create a ‘halo’ of LA around the nerve (“donut sign”). A total LA volume of 0.3 mL/kg was injected around each nerve.

Article 2

For the in *vitro* studies 4 cadavers weighing 19.4 ± 3.36 kg were employed. For the *in vivo* phase 5 dogs with a mean weight of 14.75 ± 1.26 kg and mean age of 6 ± 0.8 years were employed. The left and right FN nerves of 2 cadavers were dissected to investigate the anatomical features of the FN. Red latex was injected through the abdominal aorta artery in another 2 cadavers. These cadavers were frozen at -30 °C for 8 days. After this period, transverse cryosections of the caudal abdomen of 1 cm in thickness were made from the L6 vertebra to the hip joint. Photographs of each slide were taken to correlate the anatomical structures to the corresponding ultrasonographic images.

An ultrasonographic study and the *in vitro* blocks of the FN were performed on the left and right FNs of 5 cadavers. The area of the caudolateral abdomen was clipped. The cadavers were placed in dorsal recumbency and the limb to be scanned was moderately extended. The probe was orientated perpendicular to the midline and slightly cranial to the inguinal nipple. From this point, the probe was directed cranially, trying to trace the projection of the nerve on the abdomen. Transverse images of the FN were obtained with the probe placed in this position. The structure identified as the FN was injected using insulate needles (Stimuplex 50 mm 22 G, B-Braun, Germany) with 0.3 mL/kg black ink using an US-guided technique and a transverse cross-sectional view.

The needles were inserted using an in-plane technique. The needle insertion sites were marked on the skin and photographed. The limbs were immediately dissected to confirm the accuracy of the US nerve location by the presence of ink staining the FN. A successful FN block was defined as the staining of the nerve in a length ≥2 cm. The area where the FN was scanned in the cadavers constituted the acoustic window used to perform the in vivo blocks.

For the *in vivo* blocks 5 dogs were sedated with medetomidine (Domtor, Orion Pharma, Finland) 10 µg/kg administered IM. The blocks were always performed using the technique described for the *in vitro* study. The structure identified as the FN was injected with an initial volume of 0.3 mL of lidocaine 2% (Lidocaine chloride 2%, B.Braun Medical, Spain). Then, the remaining dose of LA was administered by means of a multiple injection technique until the nerve was completely surrounded by LA, creating a typical anechogenic halo ("donut sign"). A total dose of 0.3 mL/kg lidocaine 2% (Lidocaine chloride 2%; B.Braun Medical, Spain) was injected. A positive US-guided block was defined as the presence of the LA surrounding the FN 'donut sign'. Atipamezole (Antisedan, Orion Pharma, Finland) 50 µg/kg, IM was administered to reverse sedation. Finaly the blocked limbs were evaluated to test the presence of motor or proprioceptive deficits.

Article 3

A total of 20 dog cadavers weighing 17.7 ± 3.8 kg were employed. In the first phase of this study, two dogs were employed to perform an anatomical study of the LP. In a second phase, 18 cadavers were randomly assigned to three experimental groups to evaluate the spreading at the LP of three volumes

of injectate of 0.2 mL/kg (L, low dose), 0.4 mL/kg (M, medium dose) and 0.6 mL/kg (H, high dose). The FN was accessed by a ventral US-guided SIA technique previously described in the article 2. The inability to identify the FN by US was considered as exclusion criteria for this study. The injection site was located about the midpoint between the inguinal nipple and the ipsilateral abdominal caudal nipple to obtain the most cranial possible distribution of the injectate. Once the FN was identified, an insulated needle (Stimuplex D 50 mm 22 G, B.Braun, Germany) was inserted using an in-plane technique to administer the injectate close to this nerve. A single injection technique was selected in our study to promote a wider diffusion of injectate at the LP. Therefore, circumferential spread around the FN (“donut sign”) was not attempted. The injection site was marked and photographed. On each cadaver, the left and right LP were injected. The injectate consisted of a mixture of lidocaine 2 % (Lidocaine chloride 2%, B.Braun Medical SA, Spain) and methylene blue 1% (Methylene blue powder, Panreac, Spain) in a 1:1 proportion. The LP was dissected in 12 cadavers (n=4 per group) to assess the extent and distribution of the dye. A nerve staining over a length of ≥ 2 cm was considered to be indicative of a clinically effective nerve blockade. In another 6 cadavers (n=2 per group), coloured latex was introduced through the thoracic aorta. These animals were frozen at -80°C for 24 hours. Then, transverse abdominal cryosections of 1 cm in thickness were made from the L4 vertebra to the hip joint. The extent and distribution of the dye as well as the occurrence of epidural spread were evaluated in the cryosections. The presence of nerve staining in a minimum of two consecutive cryosections (length ≥ 2 cm) was considered to be indicative of a clinically effective nerve blockade.

3. RESULTS AND DISCUSSION

Article 1

In humans there has been an increased interest in the use of PNB due to the introduction of US to locate the target nerves (Marhofer *et al*., 2005b). To the author's knowledge, this is the first study investigating the use of US for the PNB in dogs. The length of the ScN allowed approaching this nerve at different locations at the lateral surface of the thigh. The possibility of approaching this nerve at different levels may be of clinical interest because the ScN could be blocked whilst avoiding areas of skin lesion or infection which could affect the lateral aspect of the thigh. In all dogs, the ScN was clearly identified at different levels along the lateral aspect of the thigh. Nevertheless, an approach of the ScN at the mid level of the femur was selected for the blocks, as the nerve at this level is unaccompanied by any other major nerve or vascular structures that could complicate or impair the block. In contrast, the short length of the FN within the femoral triangle allows only a single small acoustic window.

In cross sectional the ScN was easy to locate and was observed as an ovoid hypoechoic structure with hyperechoic borders (Benigni *et al*., 2007). In contrast, the FN was difficult to locate, and was observed as a hyperechoic triangular structure located cranial and slightly ventral to the femoral artery in the cross section view. A similar ultrasonographic pattern was described previously in humans for this nerve (Gray *et al*., 2004; Carty y Nicholls, 2007). In the current study, the different echogenicity observed between the FN and ScN may be related to differences in the fascial tissues covering the nerves in different locations (Silvestri *et al*., 1995; Carty y Nicholls, 2007).

In the present study, the ScN was easy located and block in all the cases, which suggests that the US could be suitable as a single technique to locate this nerve in the dog. The FN was difficult to locate and effectively blockade in the 50-62.5% of the cases. The NS was essential to locate this nerve in the employed approach. Based on the anatomical results found in this study, the inguinal skin crease was the only acoustic window available to approach the FN. The superior efficacy found in this study to block of the ScN may be due to the greater length of this nerve. This fact allowed an easier location of the nerve and a wider range in the motion of the transducer and needle. In contrast the reduced size of the acoustic window employed to block the FN limited the transducer and needle motion range. In addition, the FN was superficial and short at this location and it was surrounded by fat tissue, tendons, muscles and fascia structures with a similar echogenic properties which hindered its detection (Silvestri *et al*., 1995).

One of the main benefits of the US-guided PNB is the possibility to obtain the simultaneous visualization of the nerve the needle and the spread of the LA which improve the efficacy and security of the PNB (van Geffen y Gielen, 2006; Oberndorfer *et al*., 2007). In the present study it was possible to observe in real time the entire blocking procedure including the distribution of LA around the nerve (“Donut sign”) for the ScN, but not for the FN. The ‘donut sign’ is considered a good predictor of a successful block (van Geffen y Gielen, 2006).

This study validates the usefulness of the US-guided technique to locate and block the ScN at the midfemoral level in dogs. The acoustic window of the inguinal region to locate and block the FN was not useful in all cases. Additional studies are necessary to evaluate different acoustic windows to approach the

FN in dogs. This technique offers the advantage of performing the blocks under direct visualization of the needle, relevant anatomy, and LA spread.

Article 2

In our previous study, a low success rate was observing after blocking FN when approached at the femoral triangle. Therefore, the efficacy of the ventral US-guided SIA approach to block the FN in the dog was evaluated in order to describe a better approach for this nerve. The anatomical study revealed in the present work the greater length of FN at the SIA in comparison to its short length evidenced in the femoral triangle approach. This fact allowed an easier location of the FN and the possibility of approach this nerve in a wider acoustic window which was located between the ipsilateral abdominal caudal nipple and the inguinal nipple. Additionally, the FN at the SIA was far away from vascular structures, whereas at the femoral triangle the FN is closer to the femoral vessels which can complicate the blocks. In the present study the FN was observed in a transverse view as an ovoid hypoechoic structure with hyperechoic borders. This is contrary to our previous description of this nerve where it appeared as a hyperechoic triangular structure. These differences in echogenicity may be explained by differences in the nature of the tissues covering the FN at different locations (Carty y Nicholls, 2007). The FN is covering by several fascial layers and fat tissue at the femoral triangle. These tissues have an echogenic pattern similar to the nerve which may produce a reduction of the ultrasonographic contrast (Silvestri *et al.,* 1995). At the SIA, the FN is only surrounding by the iliopsoas muscle (IPM) and, consequently, the ultrasonographic contrast is better.

In the present study, the FN was easy located and block in all the cases. This finding is agreement to a previous study in which this nerve was approaching at the femoral triangle (Campoy *et al*., 2010). In our previous study, a lower success rate during the block of this nerve was observed employing the same approach. The successful blocking rate found in the present study may be explained because of the better visualization of the FN and also by the use of a wider acoustic window. Contrarily, the lower success rate obtained when the FN was block at the femoral triangle may be explained by the poorer visualization of the nerve and the reduced size of the acoustic window in the mentioned approach. In the current study, the nerve, the needle and the spread of the LA could be monitored during the entire procedure. This fact is related with an effective and safe blocking procedure (Marhofer *et al*., 2005a). In the present study, the FN could be block in a superior level before it is branched. This fact may allow blocking the sensitive and motor components of this nerve more effectively. A partial block of the FN has been described in humans when the FN was approached at the femoral triangle (Nielsen *et al*., 2003). The ventral US-guided SIA may allow a safer and more accurate access to the FN providing a more successfully FN block in the dog. This approach could be considered as a suitable alternative to approaches to the FN previously described.

Article 3

It was possible to identify the FN in a total of 17 cadavers. One dog was excluded from the study because the increased echogenicity of the IPM impaired the visualization of the FN. The contrast difference between the FN and the IPM has been described as the cause of the adequate visualization of

the FN in this site. The increased echogenicity observed in this cadaver may have been produced by muscular fibrotic changes which might be related to chronic muscle injuries (Breur y Blevins, 1997). Difficulty in the visualization of peripheral nerves produced by muscular fibrosis has been reported in humans (Kirchmair *et al*., 2001).

It was hypothesized that the injection of an appropriate volume of injectate close to the FN may produce its effective diffusion towards the three nerve components of LP either within the IPM or within the facial planes of the local musculature. The results from this study allowed us to confirm this hypothesis for the FN and the ON. The localization of these nerves within the IPM may explain their successful staining in all the cases, as the IPM is the site where the injectate was mainly distributed. Contrarily, the location of the lateral femoral cutaneous nerve (LFCN) outside the IPM may have impaired the staining of this nerve.

There is only one description regarding the LP block in the dog which describes the distribution of injectate after a dorsal LP block carried out by nerve stimulation (Campoy *et al*., 2008). In this study, a lack of effective staining of the FN and ON was found in four out of eight dogs injected with 0.1 mL kg^{-1} , two out of seven dogs injected with 0.2 mL/kg and one out of seven dog injected with 0.4 mL/kg. Information regarding the staining of the LFCN was not included in this report. The results from the present study show that the FN and ON were successfully stained in all the cases at the three volumes administered. These differences could be explained by the differences in accuracy of the techniques employed to locate the FN. In our study, the use of US may have allowed a more precise location of the FN. The US-guided

technique makes possible the exact delivery of the injectate and its distribution within the IPM towards the FN and the ON. The results from our anatomical study show that the FN and ON were successfully stained in all the cases, even when a low volume of 0.2 mL/kg was administered. This volume is lower than the volume of 0.4 mL/kg recommended in a previous description to block the LP in dogs (Campoy *et al*., 2008). It has been reported that the minimum effective dose of LA necessary to block a peripheral nerve can be significantly reduced with the use of US-guided techniques (Marhofer *et al*., 2005a).

The results from the present study suggest that the ventral US-guided SIA could offer some clinical advantages in the blockade of the FN and the ON over the technique employing electrolocation previously described. Some of these advantages, previously reported in our other studies, are mainly related with its efficacy to locate the FN and to visualize in real time the distribution of LA. This may produce a more effective spread of LA within the IPM towards the FN and ON. Another advantage of the SIA is the increased distance between the injection point and the medullar channel; this could diminish the risk of an epidural distribution of the injectate.

Some studies demonstrate an improving in the analgesia in humans undergoing surgical procedures performed in the knee and hip (McNamee *et al*., 2002; Macalou *et al*., 2004) when the ON block is added to the blockade of the FN and ScN. The possibility to achieve the simultaneous blockade of FN and ON might offer clinical advantages to improve the analgesic coverage for surgical procedures performed on the stifle in the dog. The presence of some branches of the ON innervating the knee have been reported in some dogs (Budras *et al*, 1989). In conclusion, the ventral US-guided SIA might be suitable

to produce an effective and safe 2-in-1 FN and ON block in the dog. Furthermore, a reduced volume of 0.2 mL/kg is adequate for this purpose.

4. CONCLUSIONS

1. The length of the ScN allows its ultrasonographic approach at several acoustic windows located trough the lateral surface of the thigh. In transverse scans the ScN was observed as a hyperechoic ovoid structure with hyperechoic borders. In longitudinal scans the ScN was observed as a hypoechoic tubular structure with hyperechoic borders. The most suitable ultrasonographic approach to block the ScN was located slightly caudal to the middle portion of the femur in the lateral surface of the thigh. This technique offers the advantage of performing blocks with direct visualization of the needle, the nerve and the LA distribution. This approach might allow a suitable ultrasonographic approach for the ScN facing the clinical anaesthetic block in the dog.

2. The short length of the FN at the femoral triangle approach just allows obtaining a single narrow acoustic window. Contrarily, the largest width of the acoustic window obtained by the SIA allows and easier ultrasonographic approach for the FN. At the femoral triangle approach and in transverse scan, the FN was observed as a hyperechoic triangular structure. However, at the SIA, and in transverse scan, the FN was observed as a hypoechoic ovoid structure with hyperechoic borders. The SIA could be considered as a suitable and safer alternative for the FN block in the dog.

3. The anatomical study of the LP revealed that the main components of the LP namely FN and the ON were located within the IPM body. Contrarily the FCLN was located out of this muscle. The SIA allowed a suitable ultrasonographic approach to the FN within the IPM. The injection of a volume of injectate (0.2 mL/kg) near the FN by the SIA, allowed the injectate distribution within the IPM towards the FN and the ON in all the cases. The SIA might be an effective approach for the clinical block two-in-one of the FN and the ON in the dog

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9. APÉNDICE

Índice de impacto de las revistas

Ultrasound-guided block of the sciatic and femoral nerves in dogs: A descriptive study

Diego F. Echeverry [a], Francisco Gil [b], Francisco Laredo [c], Maria Dolores Ayala [b], Eliseo Belda [c], Marta Soler [c], Amalia Agut [c,*]

Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog

Diego F. Echeverry [a], Francisco G. Laredo [b], Francisco Gil [c], Eliseo Belda [b], Marta Soler [b], Amalia Agut [b,*]

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Ultrasound-guided “two-in-one” femoral and obturator nerve block in the dog: an anatomical study.

Diego F Echeverry[*†], Francisco G Laredo[†], Francisco Gil[‡], Eliseo Belda[†], Marta Soler[†], Amalia Agut[†]

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☐	42	LAB ANIM-UK	0023-6772	1227	1.282	1.280	0.232	56	9.6
☐	43	ACTA VET HUNG	0236-6290	575	1.264	0.980	0.208	48	6.7
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☐	45	VET CLIN N AM-EQUINE	0749-0739	935	1.221	1.163	0.340	47	>10.0
☐	46	COMPARATIVE MED	1532-0820	695	1.205	1.152	0.089	56	6.0

Carta de aceptación articulo 1

Ms. No. YTVJL-D-09-00324R1

Ultrasound-guided block of the sciatic and femoral nerves in dogs: A descriptive study The Veterinary Journal

Dear Dr Agut,

We are nearly there. The referees are now satisfied with the technical aspects of the manuscript. Please read the edited version carefully to ensure I have not mistaken your meaning at any point. You must ensure that all anatomical terminology complies with the WAVA Nomina Anatomica Veterinaria (2005) and the terms should be given in English where possible and the English use is generally recognised. Also, you should advise me which of your figures need to be reproduced in colour in hard copy and whther you are able to contribute to the costs of colour reproduction (all figres will be reproduced on line in full colour free of charge).

Please submit your revision online by logging onto the Elsevier Editorial System for The Veterinary Journal using the following combination: http://ees.elsevier.com/ytvjl/

Kind regards,

Dr Andrew Higgins BVetMed MSc PhD FIBiol MRCVS
Editor-in-Chief
The Veterinary Journal

Carta de aceptación articulo 2

Ms. No. YTVJL-D-11-00276R1

Ventral ultrasound-guided suprainguinal approach to block the femoral nerve in the dog

Dear Dr Agut,

I am pleased to confirm that, subject to editorial corrections, your paper is accepted for publication in The Veterinary Journal and is now In press. The publishers will forward e-proofs to you in due course.

Once the final proof corrections have been made, we will aim to publish it electronically within 6-8 weeks. Your paper will be allocated a digital object identifier, or doi number; and, once on line, the paper may be cited as published using its unique doi. You will be notified by the publisher when the paper is available on line and you can check its status and doi number using the Journal's website www.elsevier.com/locate/tvjl (please click on Volume/Issues and then Articles in Press). The hard copy version of the Journal containing your paper will follow later, but should certainly be within 12 months of acceptance.

Thank you for submitting this paper to TVJL. We hope to receive more papers from you soon!

Dr Andrew Higgins BVetMed MSc PhD FSB MRCVS
Editor-in-Chief
The Veterinary Journal

Carta de aceptación articulo 3

11-Jan-2012

Dear Dr. Laredo,

It is a pleasure to accept, subject to editing, your manuscript entitled "Ultrasound-guided “two-in-one” femoral and obturator nerve block in the dog: an anatomical study." in its current form for publication in Veterinary Anaesthesia and Analgesia.

At VAA we edit your paper then return it to you (via First Look - see below) to agree/defend rejection of any changes. This means that when you get the proofs they are not such as to bear no resemblance to what you wrote (as occurs with some journals). At the moment the time to editing is around 7 months, but we are working very hard to reduce this.

Thank you for your fine contribution. On behalf of the Editors of Veterinary Anaesthesia and Analgesia, we look forward to your continued contributions to the Journal.

Yours sincerely,

Prof. Kathy Clarke
Editor, Veterinary Anaesthesia and Analgesia
kclarke@rvc.ac.uk