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WJO covers topics concerning arthroscopy, evidence-based medicine, epidemiology, nursing, sports medicine, therapy of bone and spinal diseases, bone trauma, osteoarthropathy, bone tumors and osteoporosis, minimally invasive therapy, diagnostic imaging. Priority publication will be given to articles concerning diagnosis and treatment of orthopedic diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJO*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

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ORIGINAL ARTICLE

Basic Study

Vascular endothelial growth factor for the treatment of femoral head osteonecrosis: An experimental study in canines

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Author contributions: Dailiana ZH participated in the inception and design of the study, the acquisition and the interpretation of the data, and wrote the manuscript; Stefanou N critically reviewed the findings and wrote the manuscript; Khaldi L performed the qualitative histological estimation, quantitative bone histomorphometry and the photography of sections, wrote the histological/histomorphometrical materials and methods and the histological figure legends; Dimakopoulos G performed the statistical analysis; Bowers JR and Fink C participated in the acquisition and interpretation of the data and helped draft the manuscript; Urbaniak JR participated in the inception and design of the study, critically reviewed the findings, and revised the manuscript.

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Abstract

AIM

To evaluate the treatment of osteonecrosis of the femoral head (ONFH) with the use of vascular endothelial growth factor (VEGF).

METHODS

In 30 mature beagles (6 groups of 5 beagles) ONFH was induced cryosurgically and one of the following solutions was administered locally in the femoral head (FH) in each group: Single injection of 500 μg VEGF (t-VEGFμ group); single injection of 500 ng VEGF (t-VEGFn group); continuous delivery of 500 μg VEGF through osmotic micropump (t-VEGFpump-μ group); continuous delivery of 500 ng VEGF through osmotic micropump (t-VEGFpump-n group); single injection of 0.9% sodium chloride (t-NS group), while one group that served as control group did not receive any local solution (No-t group). FHs were retrieved 12 wk postoperatively, underwent decalcification and hematoxylin/eosin and toluidine blue staining. In two canines per group, one half of FH was processed without decalcification and stained with modified Masson Trichrome. Histological sections were observed by light microscopy and measured with a semi-automatized bone histomorphometry system and Bone Volume/Total Volume (BV/TV), Marrow Volume/Total Volume (MaV/TV), and Trabecular Thickness (TbTh) were assessed. Standard and robust tests (Welch, Brown Forsythe) of analysis of variance along with multiple comparisons, were carried out among the categories.

RESULTS

The untreated (No-t) group had signs of osteonecrosis, whereas the VEGF groups revealed reversal of the osteonecrosis. Statistical analysis of the decalcified specimens revealed a significantly better BV/TV ratio and a higher TbTh between the VEGF treatment groups (except the t-VEGFn group) and the No-t group or the control t-NS group. Single dose 500 μgVEGF group had significantly better BV/TV ratio and higher TbTh when compared to the No-t group (50.45 ± 6.18 *vs* 29.50 ± 12.27, $P = 0.002$ and 151.44 ± 19.07 *vs* 107.77 ± 35.15, $P = 0.161$ respectively) and the control t-NS group (50.45 ± 6.18 *vs* 30.9 ± 6.67, $P = 0.004$ and 151.44 ± 19.07 *vs* 107.14 ± 35.71, $P = 0.151$ respectively). Similar differences were found for the prolonged VEGF delivery/pump groups of 500 μg and 500 ng. Analysis of the totality of specimens (decalcified/non-decalcified) enhanced the aforementioned differences and additionally revealed significant differences in the comparison of the TbTh.

CONCLUSION

In an experimental model of ONFH in canines it was found that local treatment with VEGF leads to bone tissue remodeling and new bone formation.

Key words: Osteonecrosis; Vascular endothelial growth factor; Avascular necrosis; Femoral head; Osteogenesis; Animal model

Core tip: Osteonecrosis of the femoral head (ONFH) is a painful disorder which usually results in hip joint destruction. Although the pathogenic process is poorly understood, ON is the final condition that completes an already precarious microcirculation of the FH by traumatic and non-traumatic causes. Since vascular endothelial growth factor (VEGF) regulates numerous cellular events associated with angiogenesis and osteogenesis, we evaluated, in an experimental model of ONFH in canines if the local treatment with VEGF leads to bone tissue remodeling and new bone formation at the necrotic site, and subsequently to reversal of ON.

Dailiana ZH, Stefanou N, Khaldi L, Dimakopoulos G, Bowers JR, Fink C, Urbaniak JR. Vascular endothelial growth factor for the treatment of femoral head osteonecrosis: An experimental study in canines. *World J Orthop* 2018; 9(9): 120-129 Available from: URL: http://www.wjgnet.com/2218-5836/full/v9/i9/120.htm DOI: http://dx.doi.org/10.5312/wjo.v9.i9.120

INTRODUCTION

Osteonecrosis (ON), also known as avascular necrosis (AVN), is defined as a pathologic process that results from a crucial disruption of blood supply to the bone and elevated intraosseous pressure. Ischemic injury subsequently leads to the degradation of the organic elements of the bone and the marrow and usually results in a collapse of subchondral bone in the femoral head (FH)[1-3]. Also, numerous studies have emphasized the association of multiple risk factors, including alcohol consumption, glucocorticoids, trauma, autoimmune diseases, thrombophilia, genetic and metabolic components with secondary ON[4,5]. A process of repair is initiated at the necrotic - adjacent intact trabeculae interface by fibrovascular tissue invasion and osteoclasts activation followed by a temporary osteoblastic activity, but unless the lesion is small, this repair procedure is usually ineffective. The structural collapse of the osteonecrotic segment indicates the progressive course of the disease, leads to osteoarthritis of the hip joint in young adults and up to now total hip replacement is predestinate in the long term[6].

Early diagnosis and management aims to suspend the process of joint destruction through enhancement of bone repair and bone renewal. In the early stages of ON there are surgical alternatives to restrain the progressive destruction of the subchondral bone such as core decompression, osteotomy, non-vascularized or vascularized bone grafting, which might be enhanced

with the use of growth and differentiation factors[6-12]. Recently, scientists introduced the use of cell-based strategies to enhance osseous regeneration by the application of multipotent mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), and osteochondral auto- and allografts in a variety of in vivo and *ex vivo* processing[10-11,13]. Furthermore, promising osteogenic growth factors, investigated for their bone healing potential, such as bone morphogenetic proteins (BMPs), transforming growth factor-β1 (TGF-β1), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF)[10,14,15], received attention the past decades.

As bone is a highly vascularized tissue, angiogenic cytokines are essential components during the healing process of the necrotic FH. Among them, VEGF has shown its vital role, in a paracrine and autocrine manner, as a specific endothelial cell mitogen and a promoter of angiogenesis[16,17]. It is the major endothelial cell survival factor that is required for effective coupling of angiogenesis and osteogenesis in the bone micro-environment. Reconstructing local microcirculation and increasing blood vessel density are essential parameters for effective bone regeneration, the major objective of bone tissue engineering[18,19]. Furthermore, it has been suggested that the inhibition of the VEGF downregulates the extracellular matrix remodeling and bone formation in animal models[20]. Thus, it is not surprising that a recent meta-analysis clarified an association between VEGF polymorphisms and the risk of the FH necrosis[21].

It seems that several molecular pathways regulate the balance between osteoclasts and osteoblasts and determine the rate of bone remodeling. Thus, considerable effort, using *in vitro* and *in vivo* models including animal models, has been directed towards understanding the effect of growth factors and transcription factors on bone resorption and formation. Through the last decades it was well established that VEGF is a crucial part of the wide network of the molecules regulating osteoinduction on the femoral head like BMPs, leptin, hypoxia - inducible factor (HIF) and their target genes[22-25]. Under these considerations, an experimental model of cryosurgically-induced ONFH in canines was used to assess the power of our hypothesis that VEGF could be a crucial therapeutic factor for bone tissue remodeling and reversal of osseous degradation during the treatment of ON.

MATERIALS AND METHODS

After approval from the Institutional Animal Care and Use Committee, cryosurgically-induced ON of the right FH was established in 30 mature beagles (6 groups with 5 specimens)[26]. The canines were sedated with Acepromazine and anesthetized with *iv* injection of Thiopental. Anesthesia was maintained with endotracheal intubation and inhalation of isoflurane, while a Fentanyl patch provided analgesia during the procedure and postoperatively. Under absolute aseptic conditions a cryoprobe (CMS Accuprobe 620, Cryomedical Sciences Inc, Rockville, MD) was inserted in the FH through a drill hole extending from the lateral subtrochanteric region to the subchondral bone of the FH. A freezing lesion was created by a freeze (-180 °C) - thaw cycle[26]. Subsequently, the necrotic area of the FH was either left untreated or treated with local delivery of VEGF (rhVEGF, Genentech Inc, South San Francisco, CA) or 0.9% sodium chloride (normal saline, NS). NS was injected in a single dose of 1 mL, while VEGF was either injected in a single dose of 500 μg (in 1 mL) or 500 ng (in 1 mL) or administered continuously in the necrotic area with the use of an osmotic micropump (ALZET osmotic pumps, ALZA Corporation, Palo Alto, CA), in a dose of 500 μg or 500 ng, delivering 5 μL/h over a period of 14 d.

The beagles were assigned to 6 groups of 5 canines each: Untreated ONFH (No-t group), ONFH treated with NS (t-NS group), ONFH treated with a single injection of 500 μg VEGF (t-VEGFμ group), ONFH treated with a single injection of 500 ng VEGF (t-VEGFn group), ONFH treated with 500 μg VEGF delivered through a pump (t-VEGFpump-μ group), ONFH treated with 500 ng VEGF delivered through a pump (t-VEGFpump-n group). The procedure lasted less than one hour and was tolerated very well by the animals which were weight - bearing the first postoperative hours.

The canines were euthanized at 12 wk. All FHs were retrieved and fixed in 10% neutral buffer formalin for 24 h, cut in half in the frontal level using low-speed diamond sawing machine (IsoMet-Buehler). One half underwent decalcification with neutral EDTA solution pH 7, dehydrated and embedded in paraffin. Sections (5 μm thick) were stained with hematoxylin and eosin (H and E) (Figures 1A, 2A and 3A), as well as toluidine blue (TB) (Figures 1B, 2B and 3B). In two canines per group (with the exception of the t-NS group) the other half of the retrieved FH was processed without decalcification, dehydrated, embedded in methylmethacrylate, sectioned with Polycut Model microtome (Leica, Heidelberg, Germany), and stained with modified Masson Trichrome (MT). Histological sections were observed by light microscopy and measured with a semi-automatized bone histomorphometry system using OsteoMeasure software (Interactive measure system for bone histomorphometry, Osteometrics, Atlanta, GA) and the following values were assessed: Bone Volume/Total Volume (BV/TV), Marrow Volume/Total Volume (MaV/TV) and Trabecular Thickness (TbTh), at the subchondral area (above the tip of the tunnel created for the insertion of the cryoprobe)[27]. The 4 groups treated with VEGF (t-VEGFμ, t-VEGFn, t-VEGFpump-μ, t-VEGFpump-n) were compared with each other, with the group treated with normal saline (t-NS) and with the untreated group (No-t).

Statistical analysis

Means and standard deviations were used to describe the values of BV/TV, MaV/TV and TbTh across the categories studied. Standard, as well as robust tests

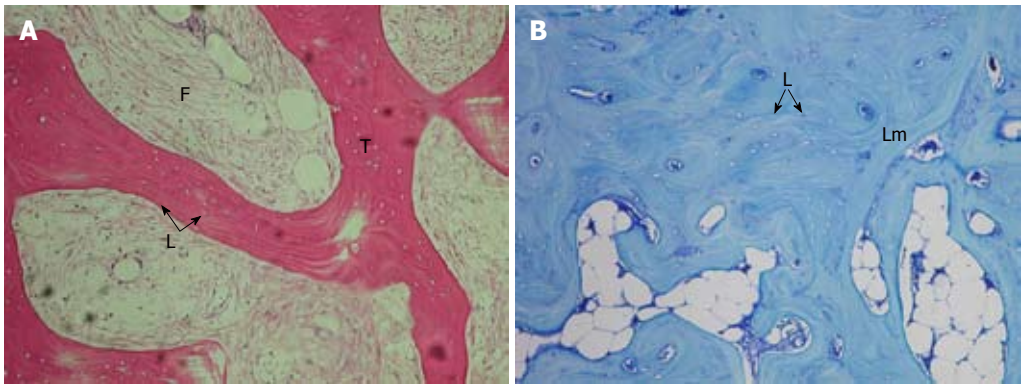

Figure 1 Cryosurgically-induced osteonecrosis of the femoral head (No-t group). The specimen was retrieved 12 wk postoperatively. A: Cancellous lamellar bone (T) with empty bone lacunae (L) marked with arrows, and fibrotic/loose connective tissue marrow space (F), indicating osteonecrosis (Obj. × 10, H and E); B: Lamellar compact bone (Lm) of cortical type, in between spaces filled with adipose tissue (A). The absence of nuclei in osteocyte's lacunae (L) (marked with arrows) indicates that the bone is necrotic (Obj. × 10, TB).

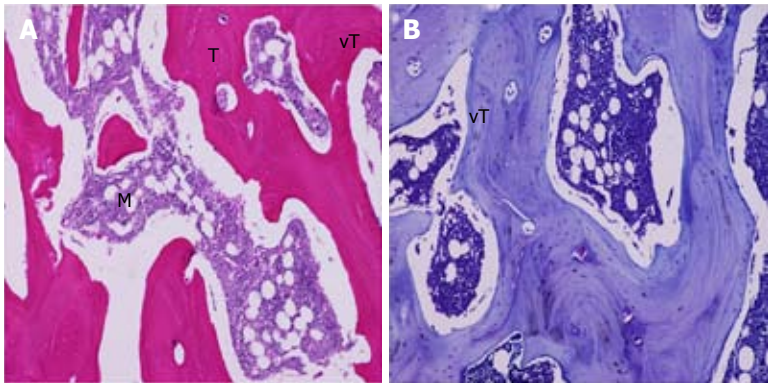

Figure 2 Osteonecrosis of the femoral head treated with a single injection of 500 μg vascular endothelial growth factor (t-VEGFμ group); specimen retrieved 12 wk postoperatively. A well-formed cancellous bone network with thickened lamellar trabeculae is visible, while the in between spaces are filled with normal marrow cells (M). A: Newly formed (vT) were noticed on the surface of the trabeculae (T) (Obj. × 10, H and E). B: Almost all trabeculae's (T) surface is covered by newly (vT) formed bone (Obj. × 10, TB).

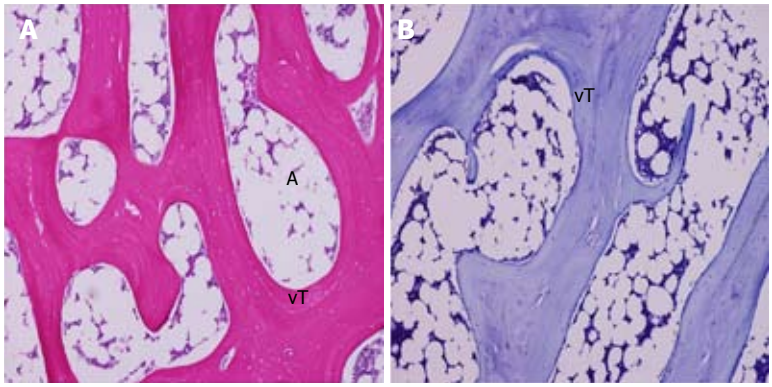

Figure 3 Osteonecrosis of the femoral head treated with prolonged delivery of 500 μg of vascular endothelial growth factor through a pump (t-VEGFpump-μ group). At 12 wk postoperatively, a well-formed trabecular network is visible, with slightly thickened lamellar trabeculae, and normal marrow cells and adipose tissue (A) in between. A: Few trabeculae are covered by newly formed bone (vT), the presence of nuclei in lacunae is noticed (Obj. × 10, H and E); B: New bone (vT) formation is observed on the surface of some trabeculae (Obj. × 10, TB).

(Welch, Brown Forsythe), of analysis of variance along with multiple comparisons under the Tukey's HSD criterion, were carried out to detect statistically significant differences among the categories. Statistical significance was set at 0.05 and the SPSS v21.0 was used for the analysis of the data.

RESULTS

The untreated group had signs of osteonecrosis (Figure

Table 1 Bone volume/total volume and trabecular thickness mean values across the different groups (decalcified specimens)

	N	mean	SD	*P*-value *vs* No-t	*P*-value *vs* t-NS	*P*-value *vs* t-VEGF-n
BV/TV						
No-t	5	29497	12265	-	NS	NS
t-NS	5	30902	6672	NS	-	NS
t-VEGFn	5	35220	5256	NS	NS	-
t-VEGFpump-n	5	48250	6869	0.006	0.013	NS
t-VEGFpump-μ	5	47077	4677	0.011	0.023	NS
t-VEGFμ	5	50450	6181	0.002	0.004	0.036
Total	30	40233	11039			
TbTh						
No-t	5	107776	35146	-	NS	NS
t-NS	5	107149	35711	NS	-	NS
t-VEGFn	5	108195	19986	NS	NS	-
t-VEGFpump-n	5	142307	28107	0.380	0.361	NS
t-VEGFpump-μ	5	156875	21965	0.088	0.082	NS
t-VEGFμ	5	151445	19065	0.161	0.151	0.168
Total	30	128958	33361			

Statistically significant differences for BV/TV are provided as estimated after the Tukey's HSD criterion while non-significant differences and denoted as "NS" or not shown. The same *P*-values are reported for TbTh for comparative reasons. BV/TV: Bone volume/total volume; TbTh: Trabecular thickness; VEGF: Vascular endothelial growth factor; NS: Not significant.

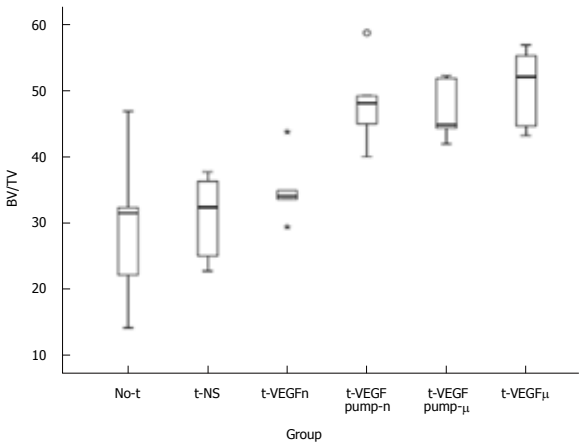

Figure 4 Comparative boxplot of the bone volume/total volume values across the different groups for the decalcified specimens. BV/TV: Bone volume/total volume; VEGF: Vascular endothelial growth factor.

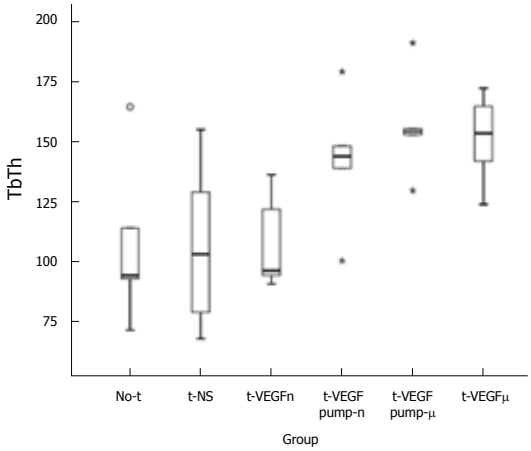

Figure 5 Comparative boxplot of the trabecular thickness values across the different groups for the decalcified specimens. Higher box and whiskers stand for higher TbTh values. TbTh: Trabecular thickness; VEGF: Vascular endothelial growth factor.

1A and B), whereas the treatment groups revealed reversal of the osteonecrosis (Figures 2A and B, 3A and B) except the group treated with NS, that served as control[26]. Statistical analysis was performed in the decalcified specimens (5 samples per group). An additional analysis was performed in the totality of specimens (5 decalcified and 2 non-decalcified; 7 samples per group except of the t-NS group where only 5 decalcified were included). Analysis of the decalcified specimens revealed a significant difference in the BV/TV and MV/TV ratio (BV/TV and MV/TV ratios are complementary) between the VEGF treatment groups (apart of the t-VEGFn group) and the untreated (No-t) group or the t-NS control group (Table 1). A non-significant difference of the trabecular thickness (TbTh) was observed between the VEGF (apart of the t-VEGFn) treatment groups and the untreated (No-t) group or the t-NS control group (Table 1).

Group t-VEGFμ had significantly better BV/TV ratio and higher TbTh when compared to the untreated group (No-t) (P = 0.002 and P = 0.161 respectively) and the control t-NS group (P = 0.004 and P = 0.151 respectively) (Table 1, Figure 2A and B). Analogous differences were found for the groups t-VEGFpump-μ (P = 0.011 and P = 0.088 respectively for comparison with the No-t group; P = 0.023 and P = 0.082 respectively for comparison with the t-NS group) (Figure 3A and B) and t-VEGFpump-n (P = 0.006 and P = 0.380 respectively for comparison with the No-t group; P = 0.013 and P = 0.361 respectively for comparison with the t-NS group). The only group with different behaviour was the t-VEGFn group with similar responses to the No-t group for the BV/TV ratio and the TbTh (P = 0.823 and P = 1 respectively) and the control t-NS group (P = 0.937 and P = 1 respectively) (Table 1, Figures 4 and 5). When the different VEGF treatment groups were compared to each other, there was a hierarchic response concerning the BV/TV ratio as following:

Table 2 Bone volume/total volume and trabecular thickness mean values across the different groups on the totality of specimens (decalcified and non-decalcified)

	N	mean	SD	*P*-value *vs* No-t	*P*-value *vs* t-NS	*P*-value *vs* t-VEGF-n
BV/TV						
No-t	7	26739	11135	-	NS	NS
t-NS	5	30902	6672	NS	-	NS
t-VEGFn	7	38853	7591	NS	NS	-
t-VEGFpump-n	7	47485	7009	< 0.001	0.011	NS
t-VEGFpump-μ	7	44634	6638	0.002	0.053	NS
t-VEGFμ	7	48005	6573	< 0.001	0.008	0.27
Total	40	39863	11013			
TbTh						
No-t	7	97891	33316	-	NS	NS
t-NS	5	107149	35711	NS	-	NS
t-VEGFn	7	120315	26799	NS	NS	-
t-VEGFpump-n	7	147738	28295	0.020	0.145	NS
t-VEGFpump-μ	7	149884	22642	0.014	0.111	NS
t-VEGFμ	7	148194	16770	0.018	0.137	0.42
Total	40	129597	33360			

Statistically significant differences are provided as estimated after the Tukey's HSD criterion while non-significant differences and denoted as NS or not shown. The same *P*-values are reported for TbTh for comparative reasons. BV/TV: Bone volume/total volume; TbTh: Trabecular thickness; VEGF: Vascular endothelial growth factor; NS: Not significant.

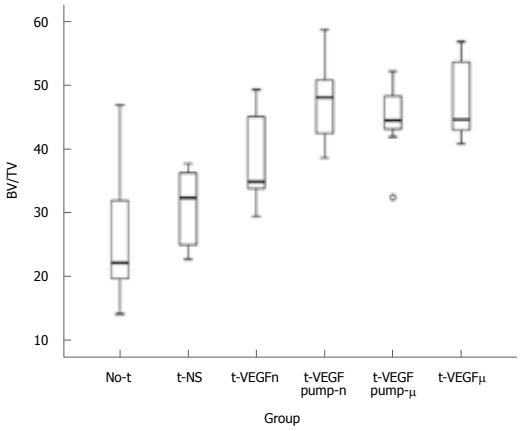

Figure 6 Comparative boxplot of the bone volume/total volume values across the different groups for the decalcified and non-decalcified specimens. BV/TV: Bone volume/total volume; VEGF: Vascular endothelial growth factor.

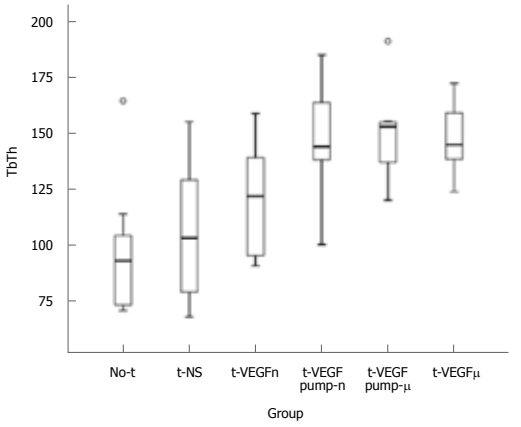

Figure 7 Comparative boxplot of the trabecular thickness values across the different groups for the decalcified and non-decalcified specimens. Higher box and whiskers stand for higher TbTh values. TbTh: Trabecular thickness; VEGF: Vascular endothelial growth factor.

t-VEGFμ > t-VEGFpump-n > t-VEGFpump-m > t-VEGFn, with a significant difference between the t-VEGFμ and t-VEGFn groups (P = 0.036) (Table 1). Analysis of the totality of specimens enhanced the aforementioned differences and also revealed significant differences also in the comparison of the TbTh (Table 2).

Group t-VEGFμ had significantly higher BV/TV ratio and TbTh when compared to the untreated group (No-t) (P = 0.000 and P = 0.018 respectively). When compared to the control t-NS group the differences were higher or significantly higher (P = 0.008 and P = 0.137 respectively for the BV/TV and TbTh) (Figure 2A and B). Analogous differences were found for the groups t-VEGFpump-μ (P = 0.02 and P = 0.014 respectively for comparison with the No-t group; P = 0.053 and P = 0.111 respectively for comparison with the t-NS group) (Figure 3A and B) and t-VEGFpump-n (P = 0.000 and P = 0.020 respectively for comparison with the No-t group; P = 0.011 and P = 0.145 respectively for comparison with the t-NS group). The t-VEGFn group had with similar responses to the No-t group for the BV/TV ratio and the TbTh (P = 0.067 and P = 0.649 respectively) and the control t-NS group (P = 0.520 and P = 0.962 respectively) (Table 2, Figures 6 and 7). The hierarchic responses were the same with the decalcified analysis (Table 2).

DISCUSSION

Osteonecrosis of the FH is a multifactorial disease affecting mostly young individuals (average age 35-50), leading to a subchondral bone infarct and to FH collapse and finally to the destruction of the hip joint. Among all controversial pathogenic mechanisms of ON, ischemic injury, which results from interruption of the blood supply and lack of oxygen, appears to be the most convincing. Decreased blood flow is followed by death of

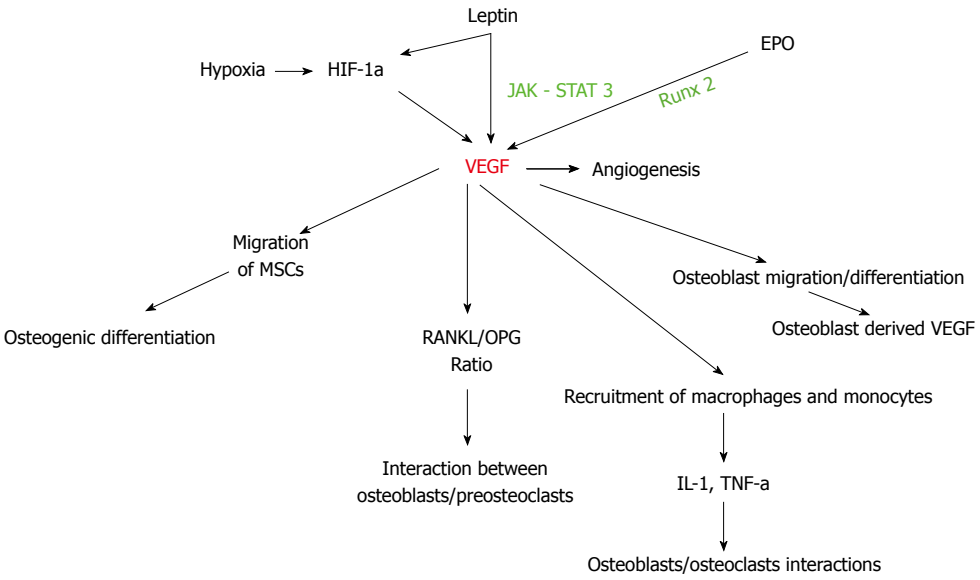

Figure 8 Diagram summarizing potential interactions of vascular endothelial growth factor related to bone tissue healing in femoral head osteonecrosis. VEGF: Vascular endothelial growth factor; HIF: Hypoxia–inducible factor; EPO: Erythropoietin; TNF: Tumor necrosis factor; IL: Interleukin.

bone and marrow elements. A process of repair is then initiated by fibrovascular tissue invasion and osteoclasts activation, followed by a temporary osteoblastic activity, but unless the lesion is small, this repair process is usually ineffective leading to eventual collapse of the architectural bony structure of the FH and loss of hip joint function[1,2,4].

Core decompression is considered the gold standard technique for the treatment of pre-collapse, early stage ON (ARCO Ⅰ-Ⅱ) of the FH and reveals superior clinical outcome in comparison with non-operative treatment alternatives[6,10]. Other surgical options may include rotational osteotomy, non-vascularized and vascularized bone grafting, which might be enhanced with the use of growth and differentiation factors and anterograde osteochondral reconstruction in advanced, post-collapse, stages of ON (ARCO Ⅲ-Ⅳ)[6-11,28]. As the treatment of osteochondral defects in advanced stages of ON remains an unresolved issue in orthopedic surgery most young patients usually resulting in the terminal option of the total hip arthroplasty[6,12].

In order to overcome the disadvantages of traditional therapies, scientists promote bone tissue engineering techniques for achieving effective bone regeneration and successful osteoinduction. It has been well established that the growth, development and maintenance of bone are highly regulated processes, which at the cellular level involve the coordinated regulation of osteoblasts and osteoclasts. Several molecular pathways regulate the balance between osteoclasts and osteoblasts and determine the role of bone remodeling. Osteoclastic activity appears to be modulated by cytokines released by osteoblasts. Numerous osteogenic growth factors such as BMPs, TGF-β1, erythropoietin (EPO), platelet-derived growth factor (PDGF) and granulocyte colony-stimulating factor (G-CSF) have been proposed as target molecules for enhancing the osteoinduction[10,11,29,30].

Blood supply is undoubtedly the absolute goal for any tissue engineering manipulation of the musculoskeletal system and osteonecrotic bone[22,31]. It is well understood that the reconstruction of the local microcirculation is prerequisite for effective bone regeneration[18]. Since angiogenesis and osteogenesis are highly coupled and VEGF is one of the most important growth factors for the regulation of vascular development we can easily recognize it as a pivotal regulator of bone repair[32]. VEGF as a proinflammatory, angiogenic and osteogenic cytokine regulates osteoblastic activity by stimulating crosstalk between endothelial, osteoblastic and hematopoietic cells in a paracrine manner and it directly affects osteoblast functions *via* autocrine mechanisms[18,33]. Moreover, the multipotent role of the VEGF in the bone environment includes the regulation of maturation and differentiation of osteoclasts, the recruitment of MSCs and osteoprogenitor cells, the promotion of the osteogenic differentiation of MSCs and finally cartilage formation and resorption[22,34]. All these studies demonstrate that the bone regeneration process in the osteonecrotic microenvironment could be affected by manipulation of VEGF levels (Figure 8).

In the present study the influence of the commonly accepted as an angiogenic factor-VEGF on osteogenesis was investigated in an experimental model of ONFH, in mature beagles. Our results indicate that the experimental model of osteonecrosis is reliable and leads to uniform and reproducible osteonecrosis of the FH in the canines[26]. The surgical technique is relatively easy and minimally invasive, leading to decreased duration of the procedure and for that is well tolerated by the animals. Other benefits of this canine animal

model are the low costs of rearing, the nature of the species as a representative of mammals, their body size that allows accurate surgical treatment and radiographic guidance or surveillance and the fact that can be achieved histological patterns which are similar to those of humans.

Moreover, we demonstrated that the use of the VEGF affects in a positive and dose dependent manner the necrotic bone and induces the process of osseous regeneration. Since now it is well established by numerous studies that there are strong indications of a cellular and molecular pattern of angiogenic-osteogenic coupling[18,19,22]. The restoration of bone vascularity is an absolute parameter in the process of osteoinduction, which is the target of any therapeutic agent against osteonecrosis in the FH. As VEGF acted in a dose dependent manner we conclude that the optimal amounts of VEGF are critical for the therapeutic outcome and probably depend on the size of the necrotic area. The levels of exogenous VEGF for its adequate activity are probably determined by both the type of cells in which it acts and the production of endogenous VEGF in them.

Taking into account that the analysis of the totality of specimens (decalcified and non-decalcified) magnified the differences between the subgroups we conclude that the healing interaction of the VEGF over the osseous tissue refers not only to the organic but to the inorganic fraction of the bone matrix too. This is important as the bone matrix serves as a reservoir for osteogenic growth factors which are pivotal collaborators of VEGF during bone repair[10,35]. It is essential for every therapeutic agent against ONFH to achieve a good quality of bone tissue during osseous regeneration.

Traditional administration of growth factors is limited by their relatively short half-lives and potential side effects[34]. VEGF has a short half-life of 6-8 h, which means that controlled and more sustained delivery could be required to ensure its efficient activity[19]. Under all these considerations we administered VEGF continuously in the necrotic FH with the use of an osmotic micropump over a period of 14 d but without transcendent outcome in comparison with single dose injection. It seems that a high single, initial dose is superior for the bone defect repair in this animal model and that may reveal that VEGF has a nodal role at early stages of the osteonecrotic bone healing procedure. The optimal delivery model of VEGF needs to be further studied. Utilizing a well-designed delivery system, like specific slow release scaffolds or gene delivery projects may better achieve bone regeneration and improve its therapeutic effect by stabilizing it against rapid degradation[22,35-37].

The results of previous studies supported that the vascular network induced by VEGF alone is immature[29,38]. There is obviously a wide network of molecules regulating bone regeneration such as BMPs, leptin, HIF, TGF, IGF and EPO. Even if VEGF is sufficient to improve revascularization, recently scientists introduced the use of a combination of growth factors and manipulated progenitor cells to enhance bone repair and bone renewal[15,22-24,35,39,40].

In summary, in an experimental model of ONFH in mature beagles it was found that the treatment with VEGF leads to bone tissue remodeling and new bone formation at the osteonecrotic site and subsequently to reversal of ON. This study however, has some limitations; it is an experimental study in canines, and in addition, the induction and treatment of ONFH (with local infusion of VEGF) are almost simultaneous procedures. Although local VEGF administration is known to promote angiogenesis and enhanced new bone formation in ONFH in our study, future studies should further investigate, in a variety of experimental conditions, the role of VEGF as a key molecule and essential player for therapeutic strategies targeting bone reconstruction, so that an even transition to clinical trials may be achieved.

ARTICLE HIGHLIGHTS

Research background

Numerous studies have emphasized the association of multiple risk factors, including alcohol consumption, glucocorticoids, trauma, autoimmune diseases, thrombophilia, genetic and metabolic components with secondary osteonecrosis (ON). ON of the femoral head (FH) is a debilitating disease that usually leads to osteoarthritis of the hip joint in young adults and up to now total hip replacement is predestinate in the long term.

Research motivation

Early diagnosis and management aims to suspend the process of joint destruction through enhancement of bone repair and bone renewal. In the early stages of ONFH there are surgical alternatives to restrain the progressive destruction of the subchondral bone such as core decompression, osteotomy, non-vascularized or vascularized bone grafting. This study extended the prospect of use growth and angiogenic factors for the process of repair at the necrotic trabeculae of the FH.

Research objectives

The main aim of this research project was to evaluate the treatment of ONFH with the use of vascular endothelial growth factor (VEGF).

Research methods

An experimental model of cryosurgically-induced ONFH in canines was used to assess the power of our hypothesis that VEGF could be a crucial therapeutic factor for bone tissue remodeling and reversal of osseous degradation during the treatment of ON. VEGF (2 different doses of 500 μg and 500 ng) was either injected in a single dose or administered continuously in the necrotic area with the use of an osmotic micropump, while in a control group 0.9% sodium chloride (NS) was injected in the necrotic area.

Research results

The untreated group had signs of ONFH, whereas the treatment groups with VEGF revealed reversal of the osteonecrosis, except the group treated with NS, that served as control. These findings demonstrate that the bone regeneration process in the osteonecrotic microenvironment could be affected by manipulation of VEGF levels.

Research conclusions

We demonstrated that the use of the VEGF affects in a positive and dose dependent manner the necrotic bone and induces the process of osseous regeneration. Since now it is well established by numerous studies that there are strong indications of a cellular and molecular pattern of angiogenic-

osteogenic coupling. The restoration of bone vascularity is an absolute parameter in the process of osteoinduction, which is the target of any therapeutic agent against ONFH.

Research perspectives

In a reproducible experimental model of ONFH in mature beagles it was found that the treatment with VEGF leads to bone tissue remodeling and new bone formation at the osteonecrotic site and subsequently to reversal of ON. Besides that, the optimal delivery model of VEGF needs to be further studied. Utilizing a well-designed delivery system, like specific slow release scaffolds or gene delivery projects, may potentially lead to better bone regeneration and improve VEGFs therapeutic effect by stabilizing it against rapid degradation. Even if VEGF is sufficient to improve revascularization, recently scientists introduced the use of a combination of growth factors and manipulated progenitor cells to enhance bone repair and bone renewal. Although local VEGF administration is known to enhance new bone formation in ONFH in our study, future studies should further investigate, in a variety of experimental conditions, the role of VEGF as a key molecule and essential player for therapeutic strategies targeting bone reconstruction, so that an even transition to clinical trials may be achieved.

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