

DYSBARIC OSTEONECROSIS (DON) IN THE FEMUR OF AN EXPERIMENTAL DOG: PATHOPHYSIOLOGICAL ASPECTS

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Abstract

A 32 months old male beagle dog developed a sign of limb bends in the left posterior limb after a single 24-hour hyperbaric exposure to 3.2 atm abs. with acute decompression. It showed transient MRI lesions in the left femur. Eight weeks after the hyperbaric exposure, histopathological examination disclosed an apparent lesion of dysbaric osteonecrosis (DON), which consists mainly of a cicatrized fibrous tissue including many small necrotic foci in the bone marrow of the left femoral lower shaft. This study supports the potential use of the dog as a model for human DON. The present case and a review of the literature suggest that as for the pathophysiological aspect of DON, changes of the intraosseous nutrient artery and its peripheral microvasculature under stasis of bubbling blood with activation of both Hageman factor and blood platelets are very important.

Key words: Beagle dog, Femur, Hyperbaric exposure with acute decompression, MRI, Dysbaric osteonecrosis (DON), Decompression sickness (DCS), Limb bends, Hemostasis, Hageman factor, Thrombosis, Plasmin, Kallikrein, Complements, Kinins, Vascular permeability, Inflammatory cascade

Introduction

Dysbaric osteonecrosis (DON), a form of aseptic bone necrosis associated with decompression sickness (DCS), is still a significant occupational hazard among divers and caisson workers in Japan as well as in other countries. Various mechanisms, such as bubble embolism, fat embolism, endothelial damage, angiospasm, intravascular coagulation of blood, disturbance of venous flow, increased intraosseous pressure, release of inflammatory mediators including some cytokines, etc. have been proposed regarding the pathogenesis of DON.

For elucidation of the precise pathogenesis of this bone disease, experimental analysis using some animal models is indispensable. Since 1990 Lanphier and Lehner have established an experimental model using sheep.¹⁻⁵ Sheep is a particularly suitable animal to human DON model, and we can observe apparent necrotic changes of the long bones, such as femurs and tibiae.⁶⁻⁸ In 1997 we found a definitive vegetation of thrombi in the branches of the intra-osseous nutrient artery of a sheep tibia which was affected by extensive necrotic changes in bone marrow tissue and cortical bone tissue.^{7,8} Our conclusion was that certain kinds of intra-osseous vascular diseases are very important as a cause of DON.

In Japan, unfortunately, there are no facilities for sheep experiment. Thus, the dual purposes of this study are to clarify whether a dog can be evaluated as a possible model for human DON, and to find evidence of mechanisms relating to the development of DON.

Material and Method

This study was based on one of the eight male beagle dogs which we have described



Fig.1. Sign of limb bends observed in the left hind limb.



Fig.2. Abnormal findings in MRI in the lower shaft of the left femur, 4 weeks after the hyperbaric exposure.



Fig.3. Schematic drawing of distribution of the left femur lesions.

previously (TAYA, et al., 1997, 1998).^{9,10} The experimental procedure used in this study also has been described previously.^{9,10} Briefly speaking, we put the dog, 32 months old, 12.2 kg body weight, into a hyperbaric chamber at JAMSTEC, with the atmospheric pressure raised to 3.2 atm abs (ata) and kept the dog there for 24 hours, at the end of which the chamber was decompressed quickly to ambient pressure. The rates of compression and decompression were approximately

10m/min. At maximum pressure, the PCO₂ level in the chamber air was maintained at <5 torr by CO₂ absorption (SODASORB), and air temperatures were kept at approximately 24°C.

The dog developed marked limb bends 20 minutes after the 24-hour hyperbaric exposure in the left posterior limb, which persisted for two days (Fig. 1).

Non-invasive diagnostic techniques using magnetic resonance imaging (MRI: Toshiba Medical Systems Co. FREX-ART) were used to detect the early developmental stages of DON. MRIs of long bones were taken one week before the hyperbaric exposure, and 1, 2, 4 and 8 weeks after hyperbaric exposure. MRIs revealed a dyaphyseal lesion in the left femur after 2, 4 and 8 weeks (Fig. 2).

We euthanized the dog with an intravenous injection of pentobarbital sodium eight weeks after the hyperbaric exposure. His femurs were cut longitudinally along the sagittal plane and fixed with 10% buffered formalin for subsequent histopathological survey.

Histopathological Analysis on the Femurs

Histopathological analysis of the left femur disclosed a characteristic lesion of bone necrosis in the shaft, while the right femur did not reveal any obvious pathological changes. The bone lesion was relatively well defined in the lower half portion of the shaft marrow, forming



Fig. 4. Irregular-shaped cicatrized regions in the lower shaft of the left femur. The cortical bone shows irregular widening of the canal system (cutting cones).

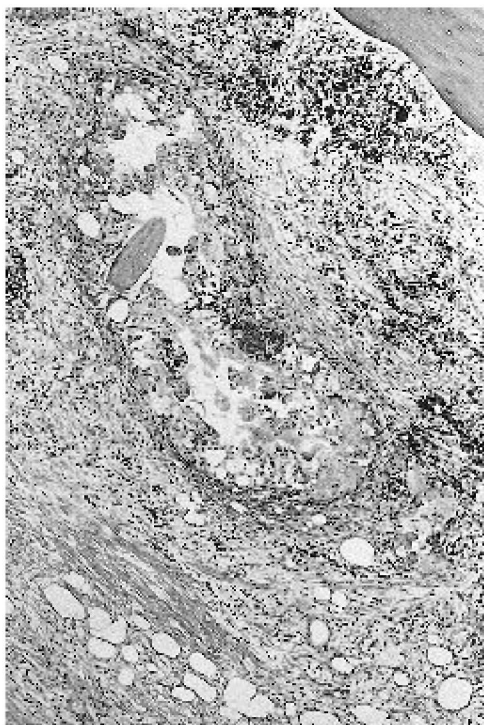


Fig.5. A necrotic focus with foreign body giant cells in the cicatrized layer.

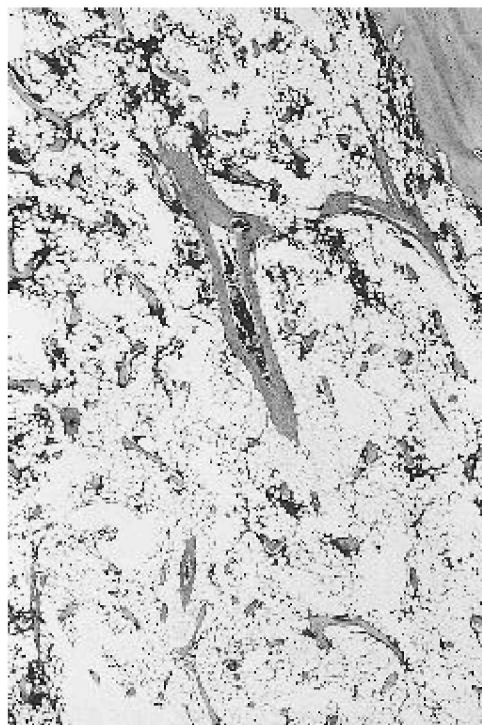


Fig.6. Descending branches of the intra-osseous nutrient artery without any evidence of thrombosis.

cicatrized areas where many small foci of necrotic debris were buried (Figs.3 & 4). Some of the foci were composed mainly of multinucleated giant histiocytic cells with a foamy cytoplasm surrounding small amounts of necrotic debris (Fig.5). Small spaces composed of fat necrosis were surrounded by flat multinucleated giant cells. Formation of reactive new bone trabeculae was observed in the cicatrized areas.

The cortical bone near the cicatrized areas revealed features of cutting cones as a result of active resorption and formation of bone at the inner surfaces of the Haversian and Volkmann's canal systems, perhaps due to cortical bone necrosis.

The upper and lower articular cartilages and juxta-articular bone tissue were almost normal.

There were neither apparent vascular alterations nor thrombotic changes in the lumina of the intra-osseous nutrient artery and its branches (Fig.6).

Discussion

This study supports the potential use of the dog as a model for human DON by a single hyperbaric exposure to 3.2 atm abs which produced a persistent sign of limb bends of the left hind limb and induced abnormal MRI changes in the left femur shaft. At the time of autopsy, we detected an apparent change of bone marrow necrosis in the lower shaft of the left femur. We already pointed out that the lower shaft of the femur was a predisposing site of DON in sheep as well as humans.^{6,11}

We have mentioned that intravascular coagulation of blood with thrombus formation is the

most important factor for the etiology of DON and DCS-associated tissue injuries of various organs.^{7,8,11-13} In the present case, therefore, we examined the intra-osseous nutrient artery and its branches very carefully, but failed to find any thrombotic changes in the left femur.

Whether extensive necrotic changes occur even without an intra-osseous arterial thrombosis is a very important point to discuss. During decompression nitrogen gas bubbles may create in intra-, and extravascular spaces. The formation of extravascular gas bubbles should directly result in the destruction and alteration of cells and intercellular tissue matrices, to some extent. Fat embolism, which has been widely observed in the cadavers of DCS victims^{14, 15} seems caused by fat cell disruption by direct mechanical stress of creation of nitrogen gas bubble in their cytoplasm. Fat droplets, other tissue disintegration products and extravascular gas bubbles may enter the blood circulation system. They all may cause blood stagnation (hemostasis) with activation of Hageman factor¹⁶ and aggregation of activated blood platelets.¹⁷

Hageman factor is activated by exposure to negatively charged surfaces, such as basement membranes, proteolytic enzymes, bacterial lipopolysaccharide and foreign materials such as nitrogen gas bubbles. The activated form of Hageman factor, namely XIIa, is a protease that initiates subsequent interactions among the other factors involved in the intrinsic pathway. These include prekallikrein, factor IX, and factor VIII. The activation of Hageman factor, on the other hand, results in the activation of several additional plasma proteases, which lead to the following: 1) conversion of plasminogen to plasmin, 2) conversion of prekallikrein to kallikrein, and 3) activation of the alternative complement pathway.

Plasmin generated by activated Hageman factor induces fibrinolysis. In turn, the products of fibrin degradation (FDPs) augment vascular permeability in both veins and arteries including blood capillaries. Plasmin also cleaves to components of the complement system, thus generating biologically active products, including the anaphylatoxins C3a and C5a. C3a and C5a increase vascular permeability both directly and indirectly. In addition, anaphylatoxins recruit and activate polymorphonuclear leukocytes (PMN) through their chemotactic action. Activated PMN amplifies tissue damage by releasing cytotoxic substances such as granule-bound enzymes which may degrade the extracellular matrix, active oxygen metabolites and arachidonic acid products (prostaglandins, thromboxane A₂, leukotrienes). They all cause endothelial damage resulting in increase of microvascular permeability. WARD et al. (1990)¹⁸ demonstrated in rabbits that depletion of complement proteins reduced the probability of complement activation and the incidence of DCS. STEVENS et al. (1993)¹⁹ reported that an increased plasma level of C3a and C5a correlated with the occurrence of DCS after a saturation dive. HUANG and LIN (1997)²⁰ investigated the involvement of the complement system and PMN activation in the air bubble-induced injury of the rat lung.

Plasma kallikrein, generated by activated Hageman factor,²¹ cleaves to high-molecular-weight kininogen, thereby producing several vasoactive peptides of low molecular weight, collectively referred to as kinins. Bradykinin is the best characterized of these vasoactive kinins. When injected into skin, bradykinin elicits reversible changes of the endothelium that lead to edema. Many kinins are under the tight regulatory control of specific inactivating enzymes. For instance, plasma carboxypeptidase N (kininase I) selectively cleaves to the carboxyterminal peptide of bradykinin. A dipeptidase known as kininase II cleaves to the dipeptides of bradykinin. Kininase II is also termed angiotensin-converting enzyme (ACE), because it converts angiotensin I to angiotensin II. The action of both kininases renders bradykinin biologically inactive.

On the other hand, the circulating cellular element most intimately involved with confusion

of both the blood itself and the vascular endothelium is the blood platelets.¹⁷ Activation of platelets, platelet adherence, aggregation, and degranulation, occur when platelets come in contact with such foreign surfaces as injured blood vessel walls, gas bubbles, etc. The platelets are important sources of inflammatory mediators, including potent vasoactive substances and growth factors that modulate mesenchymal cell proliferation. Degranulation is associated with the release of serotonin (5-hydroxytryptamine), which directly induces changes in vascular permeability. In addition, the arachidonic acid metabolite thromboxane A₂ (TxA₂) is produced by platelets. TxA₂ not only plays a key role in the second wave of platelet aggregation but also mediates smooth muscle constriction. On activation, platelets, as well as phagocytic cells, secrete cationic proteins that neutralize the negative charges on endothelium and promote increased permeability.

Thus, it is appropriate to conclude that activation of Hageman factor and blood platelets may generate a range of biologically active products resulting in widespread vascular and/or vasculogenic lesions, such as vasodilatation, increase of permeability with severe edema, and even inflammatory cascades. Although this is only speculation, the femoral changes of the present dog case may have been caused through the activation of both Hagen factor and blood platelets without a detectable thrombosis in the vascular channels at the time of autopsy. For confirmation of the mentioned hypothesis, further pathophysiological studies on the development and progression of DON are necessary.

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