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Histopathological Study on Dysbaric Osteonecrosis (DON) in Tibiae of Sheep with a Hyperbaric Exposure

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Abstract

Previously we proposed a hypothesis that activation of blood coagulability in the intraosseous blood vessels contributes importantly to produce decompression sickness (DCS) including dysbaric osteonecrosis (DON) after a hyperbaric exposure. This paper presents evidence supporting this hypothesis. Crossbred, adult, female sheep were exposed to a three-hour exposure at 2.8-3.2 atm abs, using an experimental hyperbaric chamber. They showed signs of bends in various legs soon after the exposure. Eight weeks after decompression insult, more or less marked and extensive pathologic findings of the bone marrow tissue were noted at the time of autopsy in their tibiae. In three tibiae of seven experimental sheep, a wide spread necrosis was found in the bone marrow, particularly in the upper half of the shaft. In one of them, the large branches including the main ascending branch of the intraosseous nutrient artery were obliterated by organized thrombi at a little above the middle portion of the shaft of the right tibia. This arterial lesion seemed closely related to a widespread necrosis of the bone marrow of upper half of the shaft. Another conspicuous finding was formation of cutting cones, a repair process, observed in the cortical compact bone. Through formation of cutting cones, blood supply of the cortical bone recovered from the periosteum resulting in an active organization of necrotic bone marrow, where active formation of woven bone was observed. These suggest that provocative hyperbaric exposure can cause arterial thrombosis to produce early stage of necrosis of the bone tissues, and the subsequent repair processes can exaggerate the features of DON.

Keywords: sheep tibia, hyperbaric exposure, decompression sickness (DCS), dysbaric osteonecrosis (DON), thrombosis, increased tissue pressure, intraosseous nutrient artery, cutting cone

Introduction

A form of aseptic bone necrosis known as dysbaric osteonecrosis (DON) associated with decompression sickness (DCS) was first reported in 1911 (Bornstein and Plate 1911 & Bassoe 1913), and then various mechanisms, such as bubble embolism, fat embolism, endothelial damage, angiospasm, intravascular coagulation of blood, disturbance of venous flow,

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increased intraosseous pressure, release of inflammatory cytokines, etc. have been proposed regarding the pathogenesis of DON (Kitano 1995).

Kitano and his co-workers have found an extensive thrombus formation associated with air bubble- and fat-embolism in the sinusoidal blood vessels of the bone marrow tissue of divers died of acute DCS or experimental animals after a hyperbaric exposure, and presumed that thrombosis was one of the most important factors for the etiology of DON (Kitano 1995, Kitano et al 1977, Kawashima et al 1977, Kitano et al 1978 & Kitano and Hayashi 1981).

Lehner and his co-workers have succeeded in producing an experimental animal model of DON, and have reported many papers regarding experimentally induced DON in sheep (Lehner et al 1990, Lanphier et al 1990, Lemos et al 1990, Lemos et al 1990[b], Kitano et al 1993 & Lehner et al 1994).

Although it has supposed that various factors contribute to establish the DON lesions, the exact pathomorphogenesis of DON has not yet been fully understood.

This study is based upon autopsy findings in tibiae showing DON lesions in 11 experimental sheep with a three-hour hyperbaric exposure. The purpose of this study is to clarify the relationships between the pathogenesis of early lesions in DON and thrombus formation in the intraosseous branches of the nutrient artery, and between repair processes and further establishment of DON lesions.

Materials and Methods

Animals and hyperbaric exposures

Table 1 shows eleven, three to six year-old, crossbred, female sheep, ranging between 67kg and 109kg in body weight, used in this study. They were divided into two groups. Eight of the 11 sheep were exposed to a three-hour hyperbaric exposure in an experimental hyperbaric chamber. Sheep underwent 2.8, 3.0, or 3.2 atm abs exposure were quickly decompressed to the ambient pressure in approximately 2 minutes (Fig.1). The remaining three animals were used as control. They were not exposed to increased pressure.

One of the eight experimental animals (# 2787-sheep) died soon after the exposure, and remaining seven experimental animals developed marked musculoskeletal decompression sickness, the limb bends. One sheep (# 1896) showed symptoms of cardiopulmonary decompression sickness, the chokes (Table 2), additionally to severe bends in all the limbs.

After the hyperbaric exposure, bone scintigrams of five sheep disclosed abnormalities in the tibiae and radii (Tables 3), (Fig.2).

Eight weeks after the exposure, we euthanized all the animals and performed autopsy.

Pathological examination of the tibiae

The tibiae of the sheep were cut longitudinally along the sagittal direction. One half of the bisected bone was fixed with 10% buffered formalin and the remaining half was fixed in

Table 1. Sheep in three-hour hyperbaric exposure study

	No. of sheep (female, 3～6 years of age)	Body weight (kg)	Pressure (atm abs)
Control Group	0150	87.5	
	2311	67.0	
	2364	70.0	
	Average (S.D.)	74.8 (11.1)	---
Experimental Group	0108	70.0	2.8
	0159	70.5	2.8
	0148	69.5	3.0
	0165	86.5	3.0
	2996	100.0	3.0
	0033	109.0	3.0
	1896	88.0	3.2
	2787	82.0	3.2
	Average (S.D.)	84.4 (14.6)	---

Control group : Three sheep without a hyperbaric exposure.

Experimental group : Eight sheep with a hyperbaric exposure.

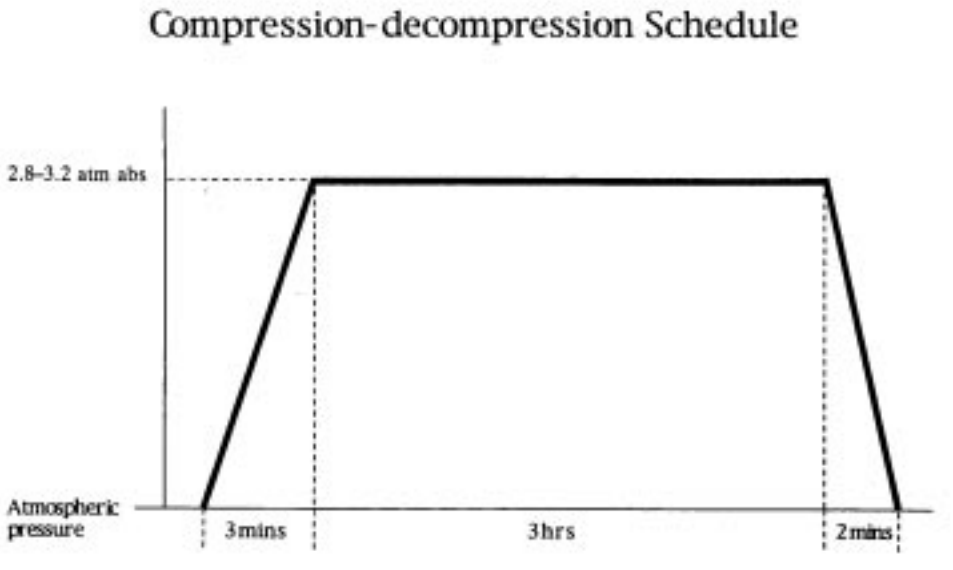

Fig.1. Schedule of compression-decompression procedure.

Table 2. Clinical signs and symptoms of limb bends after a three-hour hyperbaric exposure

	No. of sheep	Left fore limb	Right fore limb	Left hind limb	Right hind limb
Control Grop	0150	−	−	−	−
	2311	−	−	−	−
	2364	−	−	−	−
Experimental Group	0108	+	+	+	+
	0159	+	+	−	+
	0148	+	−	−	−
	0165	+	+	+	+
	2996	−	−	+	−
	0033	−	−	+	−
	1896*	+	+	+	+
	2787§			died	

− : no sign of bends.
+ : positive signs of bends
* : signs of chokes.
§ : sheep died < 1 hour after the hyperbaric exposure

Table 3. Bone scintigraphy seven weeks after a three-hour hyperbaric exposure

	No. of sheep	Left fore limb	Right fore limb	Left hind limb	Right hind limb
Control Grop	0150	−	−	−	−
	2311	−	−	−	−
	2364	−	−	−	−
Experimental Group	0108	+	+	+	+
	0159	+	+	−	+
	0148	+	−	−	−
	0165	+	+	+	+
	2996	−	−	+	−
	0033	−	−	+	−
	1896*	+	+	+	+

− : negative findings.
+ : positive findings.
++ : positive findings, extensive.
* : chokes signs.

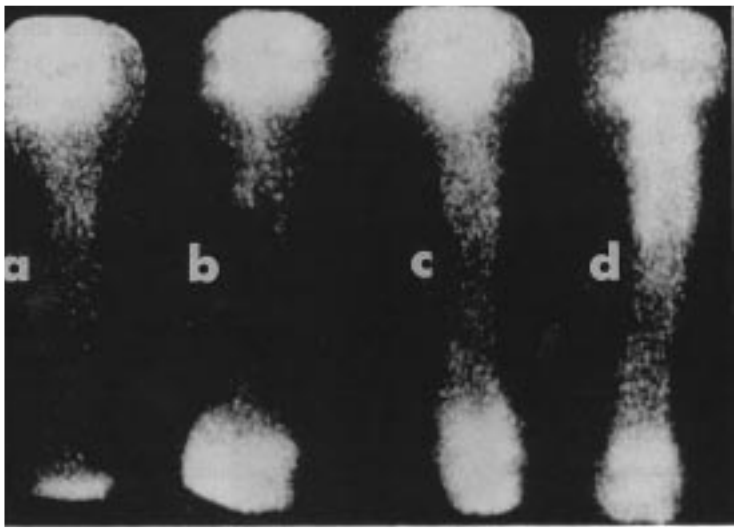

Fig. 2. A time-depending serial bone scintigram of the left tibia of #0033-sheep (a: before the hyperbaric exposure, b: one day after, c: two weeks after, and d: seven weeks after). Abnormal aggregation of Ga-67 citrate is noticeable seven weeks after the hyperbaric exposure.

absolute alcohol.

For microscopical examination, tissue samples were obtained from the various sites of the formalin-fixed tibiae. They were embedded in resin, cut into 50 micrometer, undecalcified sections and stained with fuchsin and methylene blue. Some specimens were decalcified with Plank-Richlo solution, then embedded in paraffin, cut into 5-6 micrometer sections and routinely stained with hematoxylin and eosin.

Evaluation of tissue necrosis

All bone samples from the tibiae were prepared for the necrosis of the bone tissue. Bone marrow necrosis was assessed based on histopathological alterations of cytolysis, karyorrhexis or karyolysis of hematopoietic cells, and loss of either nuclei or cell borders of fat cells. Osteocytic necrosis, including the presence of empty lacunae or pyknotic nuclei of osteocytes, was also assessed. Any evidence of repair, such as bone remodeling composed of resorption of necrotic bone and/or apposition of newly formed woven or lamellated bone on the necrotic bone, and presence of granulation tissue noted in the bone marrow tissue was determined.

Results

Necrotic changes of bone marrow observed in the tibiae of experimental animals were

essentially the same, but the numbers of areas involved and the extent of involvement in each area differed. Three tibiae out of the seven, survived experimental sheep showed a widespread bone marrow necrosis in upper half of the shaft (Table 4 & Fig. 3). Usually, the necrotic foci were surrounded by a reddish-yellowish-white granulation tissue in which aggregation of foreign body giant cells and hemosiderin deposit were remarkable (Fig.4). In some areas of the granulation tissue reactive new bone formation is noted. A granulation tissue area situated a little above the middle portion of the bone marrow in the right tibial shaft of a sheep (# 1896-sheep) revealed a conspicuous and unusual change. Complete occlusion due to vegetation of thrombi was noted in some relatively large branches, including the main ascending branch of the intraosseous nutrient artery (Fig.5a). The thrombi, however, were entirely organized and recanalized (Fig.5b). Regarding this tibia, the bone marrow tissue of the upper half of the shaft was extensively necrotized.

Necrosis of cortical compact bone and trabecular bone was widely noted but the distributions of empty lacunae and osteocytes containing a pyknotic nucleus were irregular, depending on the different cases and the different areas of the affected bone (Fig.6). Moreover, the foci of necrosis were not sharply demarcated from areas of the vital bone tissue. Generally, the degrees of the necrosis were higher in the tibiae of which bone marrow necrosis was extensive than in those of which bone marrow necrosis was not so extensive.

A characteristic feature noted in the cortical compact bone of the shafts at the sites where most lacunae were empty and the remaining osteocytes had a pyknotic nucleus was an irregular and marked widening of the Vollkmann's and Haversian canals (Fig.7). The widened canals, so-called 'cutting cones', seemed composed of a complex of resorption of

Table 4. Summary of bone marrow necrosis in tibiae

	No. of sheep	Left tibia	Right tibia
Control Group	0150	−	−
	2311	−	−
	2364	−	−
Experimental Group	0108	+	+
	0159	+	+
	0148	+	+
	0165	+	+
	2996	+	+
	0033	Upper shaft +++ Lower shaft ++	+～++
	1896*	Upper shaft +++ Lower shaft +	Upper shaft +++# Lower shaft +

\+ : mild, focal necrotic changes in bone marrow and bone.
++ : moderate or marked, focal necrotic changes in bone marrow and bone.
+++ : marked, extensive necrotic changes in bone marrow and bone.
* : signs of chokes.
\# : thrombosis in large branches including the main ascending branch of intraosseous nutrient artery.

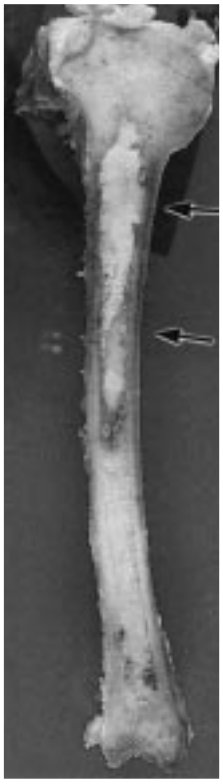

Fig.3. Cut surface of the left tibia of #0033-sheep. The bone marrow tissue of the upper shaft is widely necrotized (arrows), and the necrotic area is irregularly surrounded by a granulation tissue.

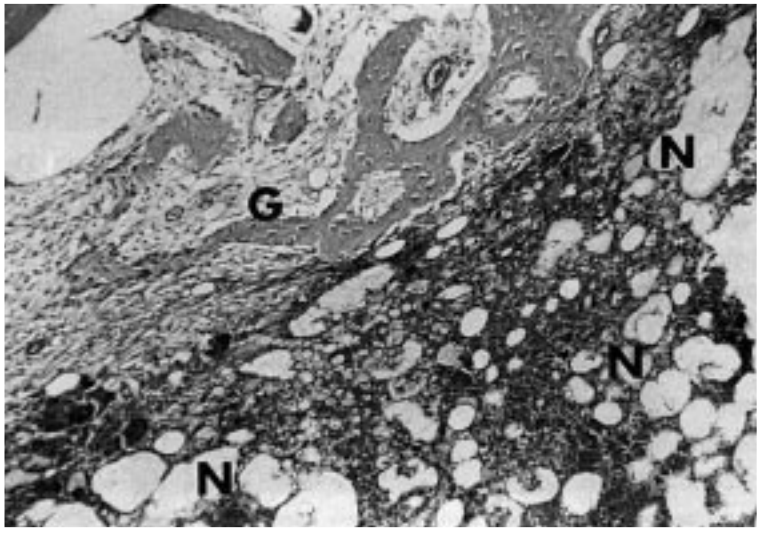

Fig.4. A necrotic marrow tissue(N) surrounded by a granulation tissue(G). Many foreign body giant cells and newly formed bone trabeculae are present in the granulation tissue (Decalcified section stained by H. & E., left tibia of #0033-sheep, x10).

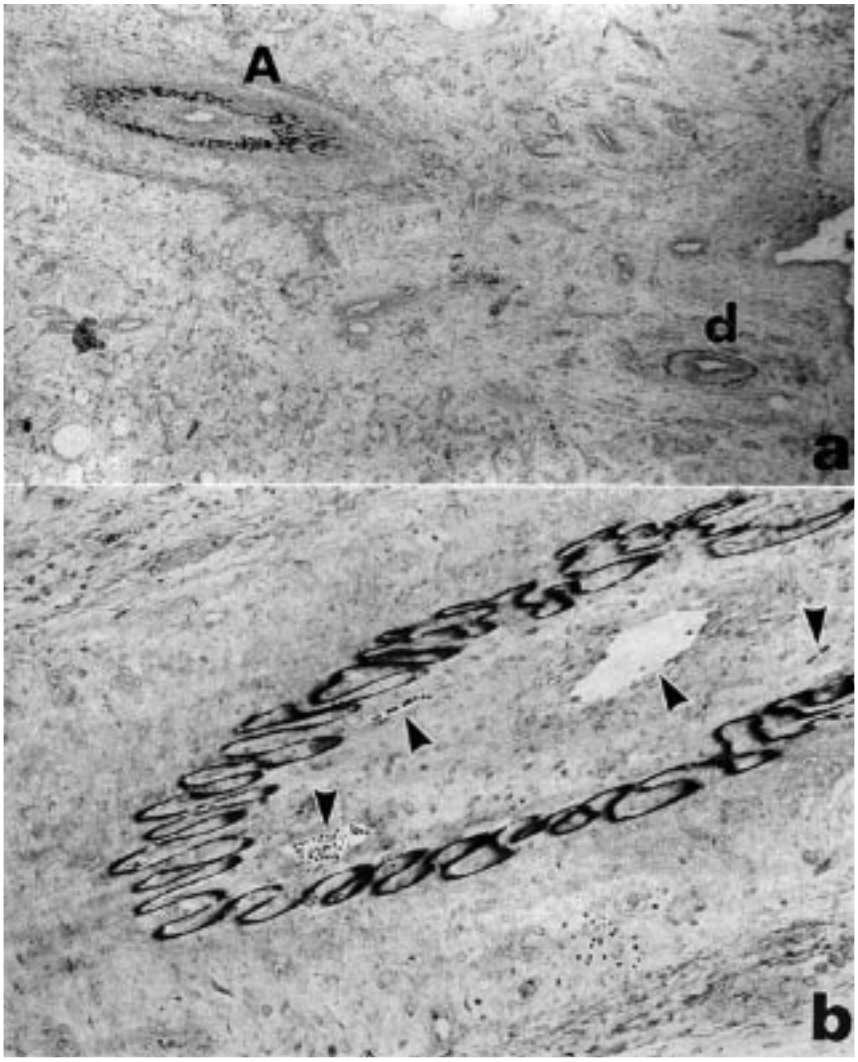

Fig.5a. Intramedullary granulation tissue at the middle portion of the shaft. Thrombozed arterial branches are noted. The bigger one (A) is the main ascending branch and the smaller one (d) is its daughter branch (Undecalcified section stained by Methylene blue and Fuchsin, right tibia of #1896-sheep, x5).

Fig.5b. Larger magnification of Fig.5a. The thrombus within the main ascending branch is completely organized. Recanalization is prominent (arrow heads)(x25).

compact cortical bone by osteoclasts (Fig.8a) and appositional woven bone formation by osteoblasts in the innersurfaces of the widened canals (Fig.8b). Degrees of cutting cone formation were various in case by case. Active formation of granulation tissue, which was sometimes associated with subcortical endosteal new bone formation, was noted in the dead bone marrow at the sites where the cutting cones were extensively formed in the compact cortical bone (Fig.7). It was apparent that the formation of subcortical granulation tissue

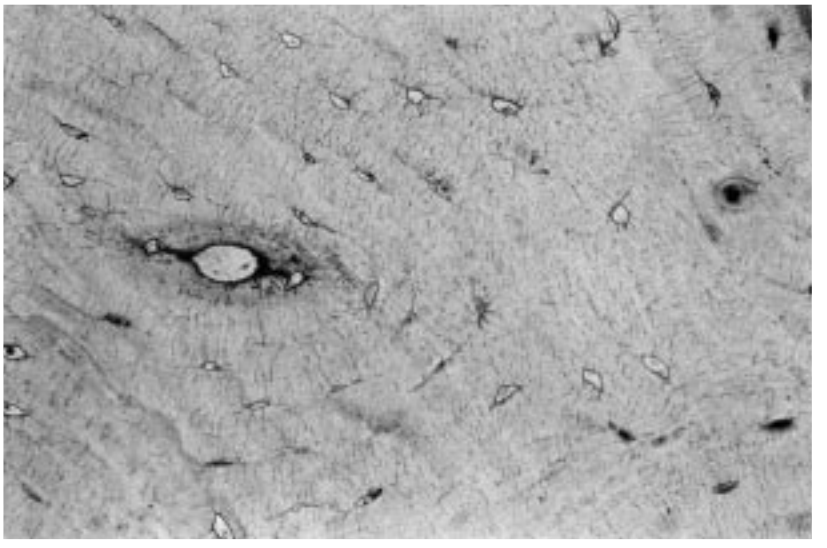

Fig.6. Bone lacunae of the cortical compact bone of the right tibia of #1896. Note some osteocytes are necrotic but some are still vital (Undecalcified section).

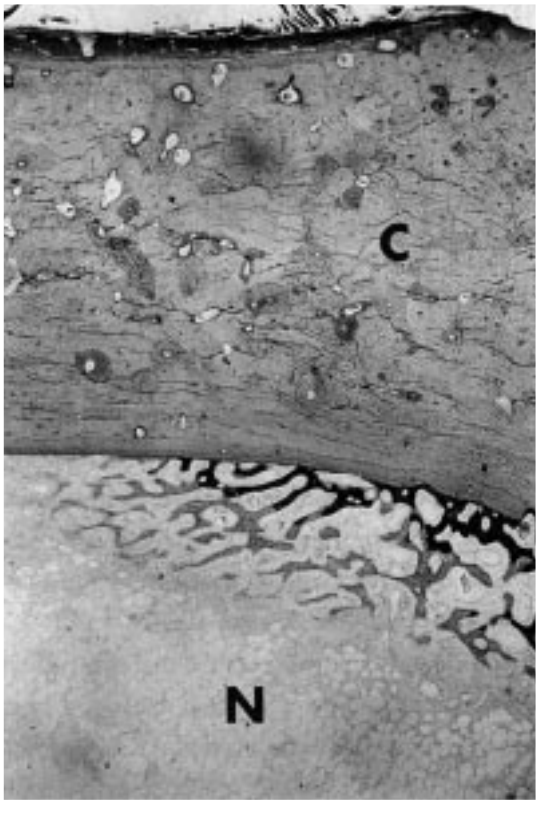

Fig.7. Cutting cones of a cortical compact bone(C) of the right tibial shaft of #1896-sheep. Note many widened canals in the cortical bone. Endoosteal new bone formation is marked and it seems associated with granulation tissue surrounding a necrotic focus(N) (Undecalcified section, x20).

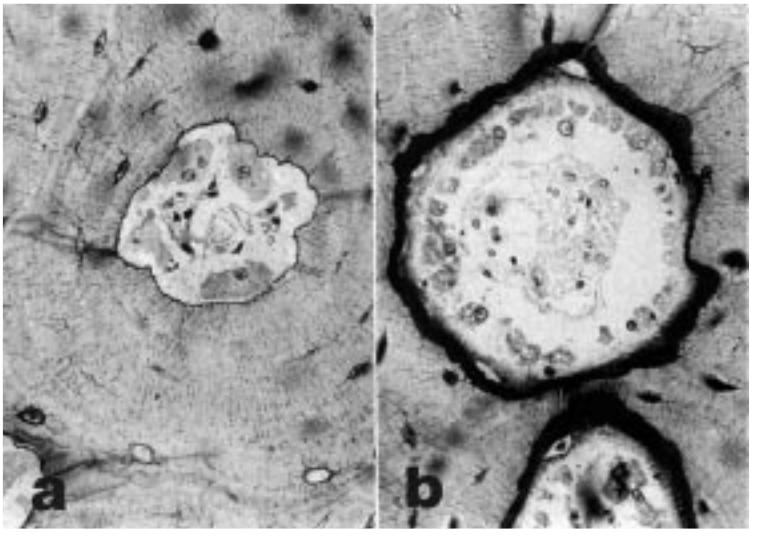

Fig.8a. Cutting cone (right tibia of #1896). Many osteoclasts attach to the innersurface of a cone (Undecaldified section, x20).

Fig.8b. Cutting cone (right tibia of #1896). Osteoblasts line the innersurface of a cone. A woven bone apposition is prominent (Undecalcified section, x20).

seemed closely related to the formation of the cutting cones in the cortical compact bone, because where there was no formation of the cutting cones, subcortical formation of granulation tissue was never seen.

Changes in the articular cartilage of both the upper and lower ends of the bilateral tibiae of all sheep were nonremarkable.

Discussion

Hyperbaric exposure or compression-decompression procedure undoubtedly causes DON associated with DCS in humans and experimental animals. Histopathological investigations have suggested that the bone marrow necrosis induced by hyperbaric exposure was one of the main initial morphological signs of DON (Kitano 1995).

As for the etiology of bone marrow necrosis related to DON, Kitano and his coworkers have pointed out that the exposure lead to activation of blood coagulability resulting in thrombus formation in blood vessels of the bone marrow (Kitano 1995). In this experiment, the autopsy disclosed that one out ot the tibiae of seven experimental sheep had thrombi in the relatively large branches, including the main ascending branch, of the nutrient artery, together with a widespread necrosis of the upper half of the right tibial shaft.

Concerning the intraosseous supply of nutrient arteries of sheep tibia, no detailed anatomical record has been published yet. We dissected some tibiae of normal adult sheep and

our preliminary conclusion was that intraosseous distribution of the nutrient artery of the tibia of sheep was almost same as that of human beings (Menck et al 1992). The nutrient artery, a branch of *A.poplitea,* enters dorsally the cortical compact bone in height of upper two seventh, penetrates it and leaves it into the bone marrow cavity of the shaft at a little above the middle portion of the tibial length, where the artery bifurcates and sends a main ascending branch in the medullary cavity. The nutrient artery itself continues the downward course on the dorsal/dorsolateral side in the medullary cavity (Fig.9a).

It seems appropriate to consider that the necrosis of the upper half of the bone marrow tissue of the tibial shaft of the present case should be essentially caused by a cessation of arterial blood flow due to vegetation of thrombi in the large branches, to include the main ascending branch of the nutrient artery (Fig.9a & b). Three tibiae of seven survived sheep showed a similar feature of extensive bone marrow necrosis of the upper half of the shaft. Histopathologically we could clarify thrombus formation in the ascending branches of the

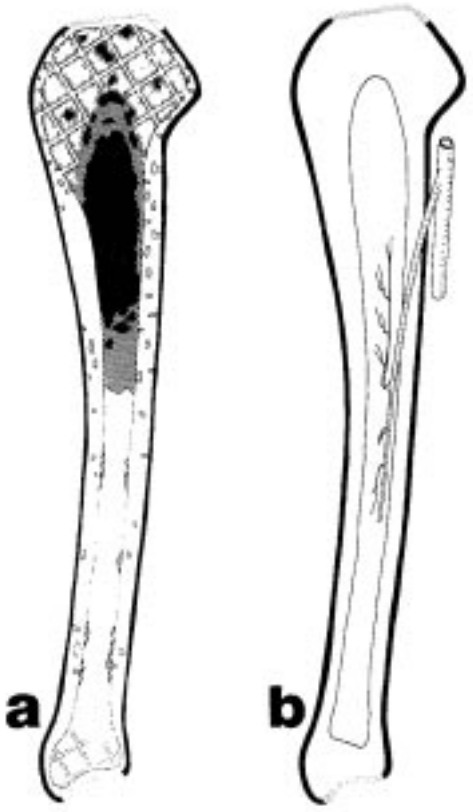

Fig.9a. Schematic drawing of the intraosseous necrotic lesion of the right tibia of #1986-sheep.

Fig.9b. Schematic drawing of the main nutrient artery and its main ascending branch with daughter branches in the bone marrow cavity of a sheep tibia. Distribution and branching of the main nutrient artery of the tibia of sheep seems almost same as those of human beings.

intraosseous nutrient artery in only one of the three tibiae. However, the findings suggests the possible role of thrombosis of these branches in onset of the lesion of DON in the upper shaft of the sheep tibiae. This might point out that the bone marrow necrosis with hyperbaric exposure is essentially caused by cessation of arterial blood flow.

The etiology of thrombus formation that accompanied by DCS is now better understood. Intravascular air bubbles and fat droplets together with tissue disintegration products induced by hyperbaric exposure are considered important for activation of blood coagulability resulting in thrombus formation (Kitano 1995).

Increased tissue pressure after hyperbaric exposure resulting in collapse of the blood vasculature and arterial obstruction may be also important in pathogenesis of DON (Kitano 1995 & Lehner et al 1994). In this study smaller branches of the nutrient artery distributed widely in the upper tibial shaft appeared to be completely or incompletely collapsed and, consequently, marked circulatory disturbances including stasis of blood presumably occurred. It is likely that these events also contributed to thrombus formation.

Most of the osteocytic cells in the cortical bone associated with a wide bone marrow necrosis seemed necrotic but some were still viable, suggesting that the bone itself was in a state of incomplete necrosis, although the degrees of necrosis seemed to be varied depending on the degrees of circulatory embarrassment (Kitano et al 1993).

Features of repair or remodeling in the necrotic bone tissue seemed significantly to participate to the development of the lesions of DON. In the cortical compact bone of the tibial shafts, development of cutting cones, a form of remodeling of bone, was prominent. Remodeling occurring within compact bone, through a sequence of events involves firstly ostoclastic activation with resorption of dead bone, and secondly osteoblastic activation with formation of new bone at the site of resorption. Internal remodeling begins when osteoclasts cut a tunnel through bone, often passing through Vollkmann's and Haversian canal systems. These tunnels are called cutting cones that can create large resorption cavities within cortical bone. Within the cutting cones, groups of spindle to polygonal osteoblasts and small blood vessels, branches of the perforating arteries from the periosteum, follow the advancing group of osteoclasts. Layers of osteoblasts arrange themselves along the surface of the resorption cavity and deposit a successive amount of osteoid matrix. Subsequently, these osteoid layers mineralize. Osteoblasts and new bone matrix narrows the diameters of cutting cones (Buckwalter et al 1995[b]). In the tibial shafts in this study, at the sites where the cutting cones were extensively formed in the cortical compact bone, the necrotic bone marrow tissue in the medullary cavity was pronouncedly organized by a granulation tissue from the subcortical layers, in which formation of endosteal appositional bone was noted (Fig.10).

So, it should be appropriate to consider that the meanings of formation of cutting cones in the cortical compact bone are 1) a repair process of the completely or incompletely dead compact bone itself and 2) a recovery of blood supply through the perforating arteries (Buckwalter et al 1995) into the cortical compact bone itself and also into the bone marrow cavity.

The DON model reported herein is simple and reproducible. Although there may be

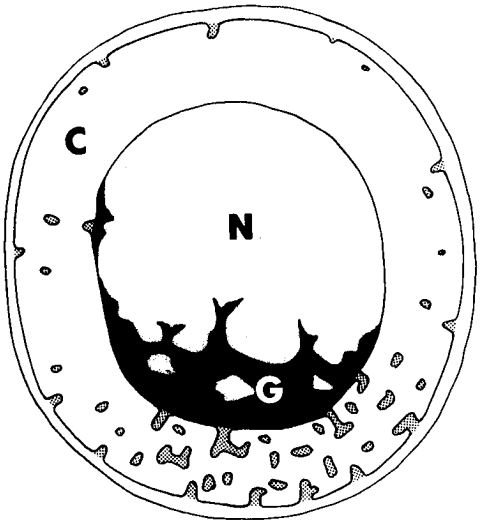

Fig. 10. Schematic drawing of the relationships between cutting cone formation in the cortical compact bone(C) and organization of the necrotic bone marrow(N) by granulation tissue(G).

differences between this model and human beings, animal models can prove useful in clarifying the etiology and early pathogenesis of human DON, and also in drawing medical and pharmacological designs for prevention, and in early treatments including recompression therapy of DON in humans.

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