Histopathological Study of the Bone Marrow of Rabbit Femora with Experimentally Induced Acute Decompression Sickness

— with consideration of pathogenesis of dysbaric osteonecrosis in diver’s femur —

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Abstract

Experimental data that in an acute stage of decompression sickness (DCS) marked and extensive formation of gaseous bubbles in the sinusoids and extravascular spaces with frequent arterial and arteriolar collapse occurred in the bone marrow of the femora of rabbits is presented.

Discussing the related evidence linking these observations with dysbaric osteonecrosis (DON) in divers and caisson workers, intravascular and extravascular bubble formation may cause marked elevation of intraosseous pressure resulting in arterial collapse especially of the “watershed zones” which include the weight-bearing juxta-articular area and distal shaft area of the bone marrow of human femur. Disturbance in the venous return from the bone marrow due to extensive bubble embolization of the sinusoids should accentuate the elevation of the intraosseous pressure.

*In this paper we use the term “watershed zone”, which is often used in the field of Neuropathology, for the border or boundary zone between the territories of two or more major arteries.

Introduction

Depending upon the autopsy findings of Japanese divers with acute DCS, Kawashima et al. (1976) and Kitano & Hayashi (1981) described that formation of intravascular gaseous bubbles associated with activation of the thrombogenesis, especially of the venous side, was marked and extensive in the bone marrow of the autopsied cadavers, and proposed a hypothesis, the “venous return disturbance theory”, in which circula-

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tory disturbance in the venous side is a very important pathogenetic factor for bone marrow necrosis which is believed to precede DON.

Recently, Lanphier et al. (1990) and Lehner et al. (1990), in conducting experimental sheep DCS, have been paying special attention to the “bone compartment syndrome theory” for the development of DON. When bubble formation from the nitrogen gas dissolved into fat cells occurs rapidly within a compartment, as in the marrow cavity of long bones, an elevation of tissue pressure should occur. They suggest that marked acute elevation of tissue pressure may result in bone marrow necrosis.

This paper is based upon the histopathological findings of the femora of twelve rabbits with experimental acute DCS. The purposes of this study are to clarify the precise pathogenesis of DON and to attempt to determine the correlation between the two theories, the “venous return disturbance theory” and the “bone compartment syndrome theory”.

Materials and Methods

1. Production of DCS in experimental animals.

Six male rabbits, 3.5-4.0 kg in body weight were compressed for six hours at 6 atm. abs. in an experimental hyperbaric chamber (Nakamura), then were quickly decompressed to ambient pressure in five minutes. All of them fell into a dyspneic condition about five minutes after returning to ambient pressure. They all expired five to ten minutes after decompression in shock states.

Another six male rabbits, 3.5-4.0 kg in body weight, were compressed for one hour at 6 atm. abs. and were also decompressed in five minutes to ambient pressure. They exhibited dyspneic conditions and paralysis in the posterior limbs, and expired fifteen to sixty minutes after decompression.

Three male rabbits weighing 3.5-4.0 kg were used as control animals without being subjected to compression-decompression procedures.

2. Histopathological analyses.

All the experimental animals were necropsied for the subsequent histopathological evaluations on various organs, including the femoral bone marrow. The distribution of thrombi in the femoral bone marrow of three of the subjects which had been compressed for one hour was examined using an immunohistological method with FITC-conjugated anti-rabbit-fibrinogen sheep-IgG (CPL).

Results

Numerous, large or small, gaseous bubbles up to 5000 microns in diameter were present in the blood vessels, especially in the sinusoids of the bone marrows of the
femora of which histopathological findings were essentially the same in all the experimental animals. Platelet aggregation was noted in the vicinity of some bubbles while thrombus formation was apparent around many of the bubbles (Figs. 1 & 2). Accumulation of fibrinogen substances around the bubbles in the sinusoids was immunohistologically demonstrated by application of the FITC-conjugated IgG (Fig. 3).

The above-mentioned changes were slightly more predominant in the shaft than the head. Hemorrhage was rather mild and focal in the bone marrow in general.

Moreover, numerous spherical bubbles up to 500 microns in diameter around which no definitive endothelial lining was evident were found in the bone marrow tissue, suggesting development of extravascular gaseous bubbles (Fig. 4).

Another main event in the bone marrow of the femurs was marked collapse of the small arteries and arterioles. This change was particularly prominent near the dilated sinusoids possessing bubbles (Fig. 2 and 5).

Numerous gaseous bubbles were seen in the blood of the venae cavae and the right atrium and ventricle of the heart. The synovial fluid of the knee joints was bubbled. The visceral organs and the brain were markedly congested. The fat tissue of the omentum and mesenterium seemed to be bubbled.

Fig. 1. A longitudinally and laterally dilated sinusoid of the femoral bone marrow of an experimental rabbit possessing many gaseous bubbles entrapped in a fibrinous thrombus (C: Cortical bone of the femoral shaft. Hematoxylin-eosin, x40).
Fig. 2. Dilated sinusoids with gaseous bubbles surrounded by thrombi. Small arteries and arterioles (arrows) in the bone marrow near the dilated sinusoids are collapsed (C: Cortical bone of the femoral shaft. Hematoxylin-cosin, x40).

Fig. 3. Accumulation of fibrinogen substance around the gaseous bubbles. (Immunostaining with FITC-conjugated antirabbit fibrinogen sheep IgG, x100).
Fig. 4. Many spherical spaces in the bone marrow suggesting gaseous bubbles. Some are devoid of apparent endothelial lining (Hematoxylin-eosin, x20).

Fig. 5. Markedly collapsed arterioles (arrow heads) in the vicinity of a dilated sinusoid possessing gaseous bubble (Hematoxylin-eosin, x100).
Discussion and Conclusion

The epidemiological and clinical relation between aseptic bone necrosis and DCS has been well established and is now called dysbaric osteonecrosis (DON). However, the precise etiology of DON is still a controversial subject. Until quite recently, it has been generally accepted that DCS, including DON, is caused by arterial bubble embolization with a common physiological mediation of the basic insult. Previously, Kawashima (1976), Kawashima et al. (1977) and Kitano and Hayashi (1981) proposed their 'venous return disturbance theory' for the etiology of DON because there were numerous gaseous bobbles together with thrombus formation within the sinusoids of the femoral bone marrow of divers who died of acute DCS.

Lanphier et al. (1990) and Lehners et al. (1990), on the other hand, employed another etiology of DON, that is their "bone compartment syndrome theory". The simple mechanical compression-decompression procedures which were undertaken in this study also leave little doubt that the volume of gas which is separated from blood, tissue fluid and fat cells in the bone marrow after the procedures can not be dissipated by tissue compliance in the bone marrow. By "encased" gaseous bubbles extravascular pressure must exceed the pressure of the blood within, causing actual arterial and/or arteriole collapse with subsequent impairment of local circulation.

It is particularly difficult to measure or to find published values for the perfusion of these arteries and arterioles, and this is further complicated by the known sympathetic vasomotor reflex in elevating local perfusion pressure to balance the potential collapsing pressure. The corresponding values in the bone marrow are likely to be appreciably lower, especially in the poorly perfused watershed zones, which are those most prone to decompression injury of the spinal cord (Hills and James, 1982) and cerebrum (Kitano et al., 1991). Thus, encasing pressure has the potential to exceed the perfusion pressure with subsequent vascular collapse resulting in tissue anoxia. This is more likely in long bones where the flow in collapsible blood vessels can be determined more by the transmural arterial pressure than by the arteriovenous pressure difference. The model depicted in Fig. 6 shows probable areas of lowered perfusion pressure in "watershed zones" of the adult human femur.

This model is consistent with two relevant observations. First, there is a clinical finding that incidences of DON in divers and caisson workers are virtually higher in the load-bearing area of the juxta-articular region of the head and the distal shaft area of the femur (Davidson, 1976 and Kawashima, 1976), as these areas have the lowest perfusion pressure and highest nitrogen content resulting from the highest lipid content (Davidson, 1976). Second, anatomical analysis of the arterial circulation (Laing, 1953, Trueta and Harrison, 1953, Okeda, 1967 and Longia et al., 1980) can easily support the concept that there are areas in which arterial branches becomes thinner and arterial flow decreases.

Although it is easy to envisage that large amounts of gas could result in the total bone marrow necrosis sometimes seen (Kitano and Hayashi, 1981), the reason why
dysfunction often does not occur equally throughout the bone marrow of the femur as a total necrosis may be that 1) not all gas is formed equally in various sites of the bone marrow in proportion to distribution of fat cells and hematopoietic cells, 2) where the blood perfusion pressure decreases in the depicted “watershed zones”, it could generate enough differential to cause blood flow to cease. For lesser amounts of gas, therefore, the closure of small arteries and arterioles could be localized, depending upon whether the gas had tracked to produce the bone marrow necrosis.

Studying the femora of the rabbits used in this experiment, it is interesting to see how extensively and markedly dilated, either longitudinally or laterally, the sinusoids possessing large amounts of gaseous bubbles were. Histopathological analyses often revealed tracking of gas, sometimes extending vertically along the long axis of the femora. Aggregation of platelets and thrombus formation around the intravascular
gaseous bubbles also frequently observed. However, it is not feasible that bone marrow necrosis can be introduced from the impairment of sinusoidal circulation alone. If this is correct, hemorrhagic change, which is one of the most prominent features of venous circulatory disturbance, will be more extensive and marked in the bone marrow. Actually, hemorrhage was rather mild and focal in the rabbit femora of this experiment and those of the divers reported by Kawashima et al. (1976) and Kitano and Hayashi (1981).

It is appropriate to consider that the main pathway of dissipation of the elevated intraosseous pressure should be the blood vessels, especially of the venous system, until the perfusion pressure becomes equivalent to the surrounding soft tissues and organs. Thus, tending to retain gas emboli widely within the venous side would not allow reduction of the intraosseous pressure acting directly on the small arteries and arterioles, and raises a possibility of alternative changes due to anoxia and direct mechanical cellular and tissue injuries.

In conclusion, the detailed aspects of bone marrow necrosis and subsequent DON in DCS are too numerous to discuss here, but the salient features would not seem explainable by the "bone compartment syndrome theory" alone, nor by only the "venous return disturbance theory" as shown the basic mechanical experiments including the present study. The primary attribute, however, of the "bone compartment syndrome theory" is its compatibility with the pathological evidence for compromises of arterial blood flow and its ability to explain the high site-predisposition of DON in the femur upon multiple changes of pressure and their consistency upon "watershed zones". The "venous return disturbance theory" may not be the last word on the subject, but it should be taken into consideration in any further research.

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References


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