Effect of hyperbaric oxygenation on femoral head osteonecrosis in spontaneously hypertensive rats

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We investigated the effects of hyperbaric oxygenation (HBO) on ischemic osteonecrosis and on ossification disturbance of the femoral heads in growing, spontaneously hypertensive rats (SHR). 10 male SHRs aged 5 weeks (Group A) and another 10 male SHRs aged 8 weeks (Group B) were treated with HBO at 2.8 atmosphere absolute (ATA) for 6 weeks in a total of 30 hours. The control animals, 10 male SHRs (Group C) and 10 male wister Kyoto rats (WKY, Group D), were kept under normal laboratory conditions. All the rats were killed at the age of 17 weeks for microscopic examination. In Group A, there was no evidence of osteonecrosis, and only 2 femoral heads with ossification disturbance were observed. In Group B, there were 2 femoral heads with osteonecrosis and 1 with ossification disturbance. In contrast, there were 6 femoral heads with osteonecrosis and 4 with ossification disturbance in Group C. It was concluded that HBO prevented osteonecrosis and ossification disturbance of the femoral heads in SHR.

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Submitted 91-11-02. Accepted 92-03-24

Hyperbaric oxygenation (HBO) has been known to have a beneficial effect on the hypoxia with various types of tissue ischemia, but has never been found to improve the hypoxia with ischemia of the femoral head.

Femoral head lesions, notably osteonecrosis and ossification disturbance in the epiphysis, are frequently observed in growing male spontaneously hypertensive rats (SHR). They are caused by interruption of the blood supply to the proximal epiphysis.

We have determined the effects of HBO on the femoral head lesions in SHRs.

Materials and methods

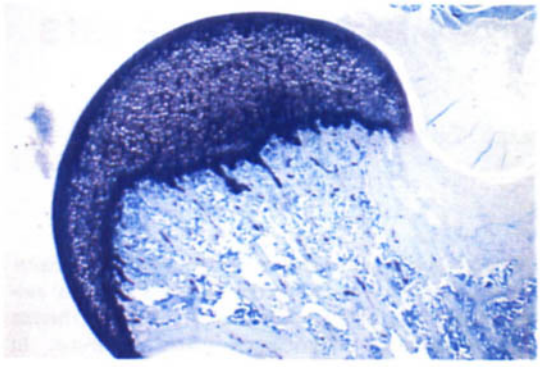
30 male SHRs, aged 4 weeks, and 10 male Wistar Kyoto rats (WKY), aged 4 weeks, were kept in ordinary cages under normal laboratory conditions. These rats were divided into four groups: Group A (n 10) SHRs treated with HBO from the age of 5 to 11 weeks, Group B (n 10) SHRs treated with HBO from the age of 8 to 14 weeks, Group C (n 10) SHRs without HBO therapy, and Group D (n 10) WKYs without HBO therapy. In Groups A and B, HBO was provided for 60 minutes with 2.8 atmospheres absolute (ATA) of pure oxygen in a HBO chamber, 5 days per week for a total of 30 hours. After the treatment with 2.8 ATA for 60 minutes, the pressure was reduced gradually over 30 minutes. All rats were killed at the age of 17 weeks, and both femurs were removed and fixed in 10 percent formalin and embedded in paraffin after decalcification. Thin coronal sections of the femoral heads with the ligamentum teres were stained by hematoxylin-eosin and toluidine blue. Histologic analysis of osteonecrosis and ossification in SHR was performed using the classification of Sagara (1990) (Figure 1). The ossification rate, percentage of the area of the ossification center in the metaphysis of the femoral head, the trabecular density, and percentages of the areas of bony trabeculae in ossification centers were measured (Figure 2).

Results

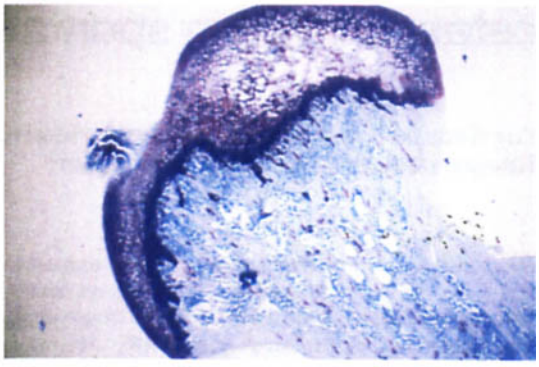
3 rats from Group A, 2 from Group B, 2 from Group C and 1 from Group D were excluded because of unexpected death before the age of 17 weeks. Evidence of bone necrosis, including empty lacunae, loss of hematopoietic cells and thinning or loss of organized trabecula patterns were histologically observed in some specimens in Groups B, C and D (Figure 3).

Ossification disturbance at the age of 17 weeks was defined Stages 0 and 1 because 15 femoral heads in

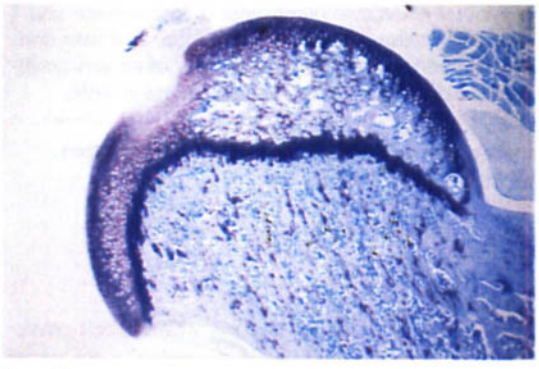
Figure 1. Stages of ossification. Toluidine blue, ×16.



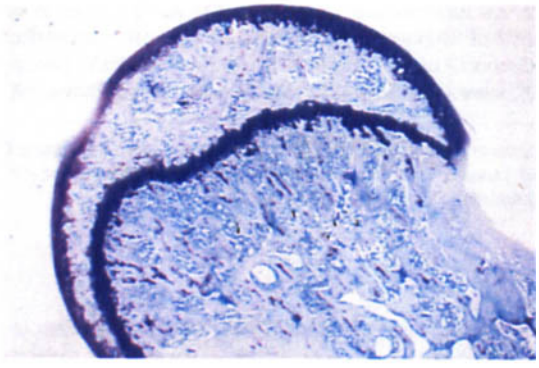
Stage 0.



Stage 1.



Stage 2.



Stage 3.

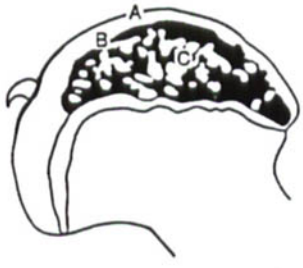


Figure 2. Ossification rate (%) = B/A x 100.
Trabecular density (%) = C/B x 100.

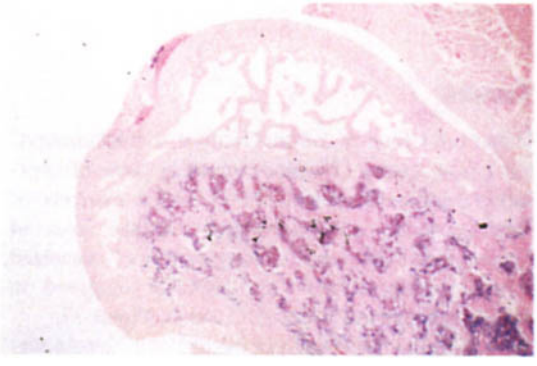


Figure 3. Osteonecrosis of the femoral head in SHR. Empty lacunae, loss of hematopoietic cells and thinning or loss of trabecula patterns were observed. HE, ×16 (left), ×80 (right).

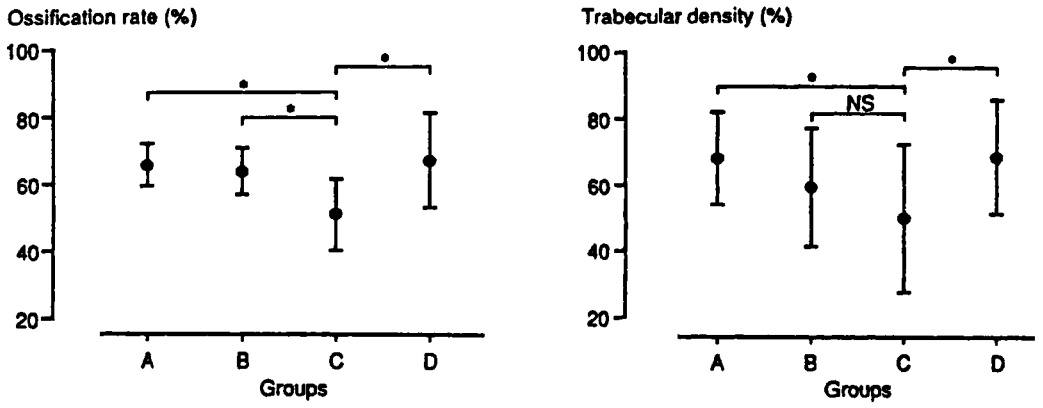


Figure 4. Mean ossification rate and trabecular density. Stars indicate statistical difference ($P < 0.05$), and bars 2 SEM.

Stage 3 out of all 17 heads were in group C. There was no evidence of osteonecrosis, and only 2 heads with ossification disturbance were found in Group A. There were 2 with osteonecrosis and 1 with ossification disturbance in Group B. There were 6 with osteonecrosis and 4 with ossification disturbance in Group C. There were 1 with osteonecrosis and 2 with ossification disturbance in Group D. In contrast to Group C, HBO groups (Groups A and B) showed a decrease of osteonecrosis and ossification disturbance (P 0.01, P 0.04, chi-square).

Group C showed the lowest mean value of the trabecular density in all groups (Group A–C P 0.05, B–C NS, D–C P 0.02; Figure 4) and the mean density in heads with osteonecrosis was only 18 (9.4–31) compared to 68 (54–81) without osteonecrosis. There was no difference between any of the groups in cases without osteonecrosis.

Group C showed the lowest mean rate of ossification in all groups, except the cases with osteonecrosis (Group A–C P 0.01, B–C P 0.02, D–C P 0.04; Figure 4). In addition, there was no difference in the ossification rate between femoral heads with or without osteonecrosis.

Discussion

In SHRs osteonecrosis occurs between the ages of 8 and 15 weeks. The reparative process rapidly takes place and a remodelling of the bony trabeculae ceases at the age of 35 to 40 weeks. The mechanism of hypertension in SHRs is not yet clarified but it is generally agreed that the changes in intracellular calcium concentration are partly responsible for the occurrence of hypertension with abnormal spasms and thickening of the media of the resistance vessels (Mulvany et al. 1981). Blood loss in capillary vessels occurs as a result of an imbalance of cardiac output and vascular resistance. The reparative process of osteonecrosis may take place with increased blood flow by the age of about 18 weeks; cardiac output becomes stable with completion of pathological changes in capillary vessels. Evidence for this hypothesis coincided with the ages in the processes of osteonecrosis and hypertension (Kishi 1990). The progression of osteonecrosis could be prevented if a sufficient blood flow was supplied to capillary vessels between the ages of 6 and 18 weeks, especially between 6 and 11 weeks, because vascular resistance increases rapidly in this period.

Since physically dissolved oxygen increases in relation to atmospheric pressure, animals can theoretically live at 2.8 ATA without hemoglobin (Boerema et al. 1960). But the therapeutic effects of HBO on hypoxia have been debated because of the decrease in cardiac output and the increase in vascular resistance when breathing pure oxygen under high atmospheric pressure. Against this view, Kawamura et al. (1978) reported that HBO had no effect on the normal tissue but had a beneficial effect on the hypoxic tissue in dogs. In summary, HBO can either prevent osteonecrosis or suppress its progression by increasing the blood volume as a result of a decrease in the resistance of the lateral epiphyseal vessel and by increasing oxygen tension.

We think that the ossification rate can indicate progression of ossification more exactly than the previous classification does. The different ossification rates in cases treated with or without HBO suggest that HBO promotes ossification in the femoral head.

Acknowledgements

The authors would like to thank Mr. Y. Miwa and Miss T. Banno for technical support.

References

Boerema I, Meyne N G, Brummelkamp W K, Bouma S, Mensch M K, Kamermans F, Stern Hanf M, Van Aaldern W. Life without blood (A study of the influence of high atmospheric pressure and hypothermia on dilution of the blood). J Cardiovasc Surg 1960; 1: 133–46.

Kawamura M, Sakakibara K, Yusa T. Effect of increased oxygen on peripheral circulation in acute, temporary limb hypoxia. J Cardiovasc Surg (Torino) 1978; 19 (2): 161–8.

Kishi K. Roles of Ca^{2+}-dependent factors in the abnormality of resistance vessels from spontaneously hypertensive rats (in Japanese). Igaku no Ayumi 1990; 153 (7), P. 350–2.

Mulvany M J, Korsgaard N, Nyborg N. Evidence that the increased calcium sensitivity of resistance vessels in spontaneously hypertensive rats is an intrinsic defect of their vascular smooth muscle. Clin Exp Hypertens 1981; 3 (4): 749–61.

Sagara K. Avascular necrosis in the femoral head of spontaneously hypertensive rats—correlation between microangiographic and histological findings (in Japanese). J Jpn Orthop Assoc 1990; 64 (4): 66–74.