Osteonecrosis of the femoral head of growing, spontaneously hypertensive rats

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The femoral head lesions of male spontaneously hypertensive rats (SHRs), which occur naturally and resemble Perthes' disease in man, were investigated radiographically and histologically. In the early phase of growth in SHRs, i.e., a period of 6 to 15 weeks after birth, the femur was shorter than that of control rats, and ossification in the epiphysis of the femoral head was considerably retarded. Osteonecrosis of the femoral head of SHRs appeared at the age of 9 weeks, and occurred in eight out of 12 femoral heads at 15 weeks. We suggest that osteonecrosis in the femoral head of SHRs is due to an abnormality of the cartilage in the epiphysis and metaphyseal growth plate and subsequent enchondral ossification.

Spontaneously hypertensive rats (SHRs), obtained from selective inbreeding of Wister Kyoto rats (WKRs) by Okamoto and Aoki (1963), may be regarded as a model for studies of human essential hypertension (Yamori 1977). Disorders of endocrine systems other than the autonomic nervous system play an important role in the pathogenesis of hypertension in SHRs (Kojima et al. 1976, Braley et al. 1983, Moll et al. 1975, Nosaka 1968, Tsuchiyama et al. 1972). Moreover, it was suggested recently that an osteoporotic bone disorder and abnormality of calcium metabolism are also present (Izawa et al. 1985, Lucas et al. 1986).

In the skeletal survey of SHRs, we observed incidentally that widespread osteonecrosis occurred frequently in the epiphysis of the femoral head and that the histologic findings closely resembled those in Perthes' disease. We report the development of the femoral head lesions in SHRs.

Material and methods

Twenty-four male SHRs (study group) and 24 male WKRs (control group) were obtained when they were 5 weeks old from Charles River Japan Co., Ltd., Kanagawa. All the animals were fed on a standard stock chow diet MF (Oriental Yeast Co., Ltd., Tokyo) under constant conditions at a temperature of 20 °C and a humidity level of 45 percent.

After the measurement of body weight and systolic blood pressure by the tail plethysmographic cuff method (Okamoto and Aoki 1963), the rats were killed under ether anesthesia when aged 6–15 weeks (Table 1). No difference was noticed in the rate of increase in body weight between the two groups of rats. The systolic blood pressure in SHRs increased gradually to more than 170 mmHg at 12 weeks of age.

Both femurs were extracted, and an anteroposterior radiographic projection was obtained using a soft x-ray (Softex Co., Osaka). Then, the length from the top of the major trochanter to the distal end of the femur was measured with a Wakom WT 500 digital analyzer. For histologic examination the proximal femurs were fixed in 10 percent formalin and prepared for paraffin embedding after decalcification. Subsequently, thin coronal sections through the teres ligament were stained with hematoxylin-eosin and Råliš tetrachrome method (Råliš and Råliš 1975), which dyes the osteoid deep blue.

For the statistical analysis, the Mann-Whitney *U*-test was used, and the differences were considered significant when $P < 0.05$

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Table 1. Body weight (g) and blood pressure (mmHg) of Wister Kyoto rats (WKRs) and spontaneously hypertensive rats (SHRs). Systolic blood pressure was measured by the tail plethysmographic cuff method. Median (range)

Age (wk)	WKRs		SHRs	
	Body weight	Blood pressure	Body weight	Blood pressure
6	95 (90–110)	90 (80–120)	100 (90–100)	110 (95–120)
9	225 (210–240)	125 (115–130)	230 (225–235)	150 (130–155)*
12	275 (270–285)	130 (110–145)	270 (260–285)	175 (150–190)*
15	325 (315–350)	125 (110–135)	325 (310–350)	180 (165–190)*

* $P < 0.05$.

Table 2. Comparison of length (mm) of the femur between Wister Kyoto rats (WKRs) and spontaneously hypertensive rats (SHRs). Median (range)

Age	WKRs	SHRs
6	24.6 (24.0–25.5)	24.2 (23.7–24.7)*
9	31.7 (30.8–32.4)	31.0 (30.7–31.6)*
12	34.2 (33.3–34.9)	34.1 (33.6–34.8)
15	36.3 (36.0–36.9)	35.9 (35.0–36.7)*

* $P < 0.05$.

Results

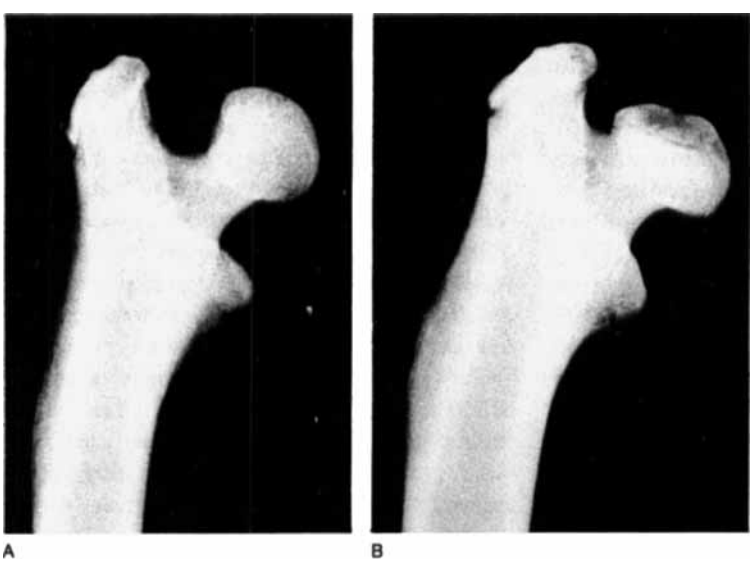
Radiographic findings

The average length of the femur of SHRs was shorter than that of the control rats at 6, 9, and 15 weeks (Table 2). Mottled calcification was seen in the epiphysis of both groups at 6 weeks. Thereafter, in WKRs a bony trabecular pattern appeared in the weight-bearing area of almost all the epiphyses at 9 weeks; bone formation developed steadily and reached completion at 15 weeks. In SHRs at 9 weeks, however, bone formation was seen in only four epiphyses, whereas the other eight epiphyses showed mottled calcification similar to that at 6 weeks. Epiphyseal squeeze and shortening of the femoral neck were recognized in 8 out of 12 femurs at 12 weeks, and these were even more pronounced at 15 weeks (Figure 1).

Histology

No abnormality could be found in the capsule, teres ligament, or articular cartilage by macroscopic examination of the hip joints. Histologic findings of the epiphysis, metaphyseal growth plate, and metaphysis were as follows.

Epiphysis. In terms of maturity, the epiphyses of WKRs and SHRs consisted merely of cartilaginous tissue with hypertrophic chondrocytes, and there were no obvious differences between WKRs and SHRs at 6 weeks. Vascular invasion was seen on the lateral side of a few epiphyses.



A B

Figure 1. The femoral heads at the age of 15 weeks.
A. Wister Kyoto rat.
B. Spontaneously hypertensive rat.

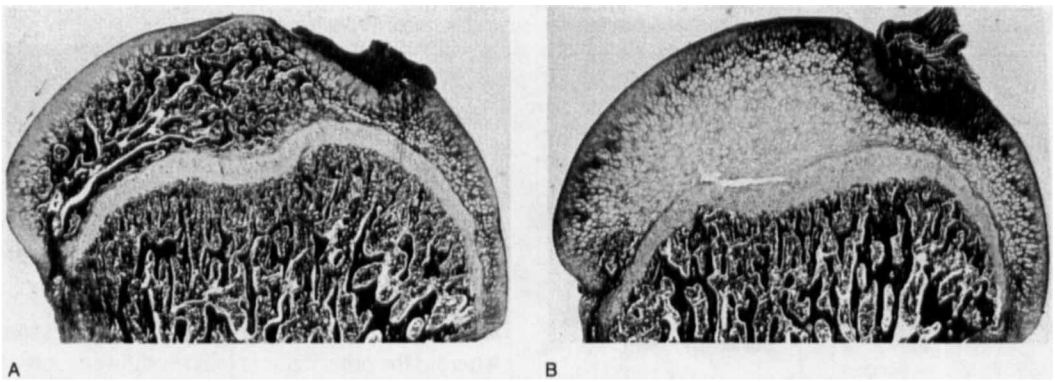


Figure 2. Histologic sections of the femoral head at 9 weeks.
A. Wister Kyoto rat.
B. Spontaneously hypertensive rat. Only minute ossification is seen in the lateral side of the epiphysis.

At 9 weeks, partial ossification in the lateral half of the epiphysis was observed in all the femoral heads of WKRs. Plump osteoblasts rimmed the surface of bone trabeculae, and the intertrabecular space was filled with hematopoietic tissue. Ossification progressed medially, and complete ossification was seen in almost all the epiphyses of WKRs at 15 weeks. In contrast, a smaller degree of ossification was observed in SHRs, and there was no ossification in one third of the 12 epiphyses at 9 weeks (Figure 2). After that, retarded ossification continued; and even at 15 weeks, two epiphyses were almost completely composed of cartilaginous tissue, which showed focal degenerative changes, such as coarse chondroid matrix and loss of chondrocytes in the weight-bearing area. Complete ossification as in WKRs was seen in only four epiphyses.

The degree of epiphyseal ossification was divided into the following four stages (Table 3): (0) no ossification, (1) less than one-third ossification in the epiphysis, (2) between one-third and two-thirds ossification, and (3) more than two-thirds ossification.

As a remarkable change, the findings of osteonecrosis were recognized in two out of all the epiphyses of WKRs and in 10 of those of SHRs (Table 4). In 6 cases – one epiphysis of WKRs at 9 weeks and five epiphyses of SHRs at 9, 12, and 15 weeks – the osteocytes in bone trabeculae of the epiphyseal nucleus became necrotic and the lacunae became empty. No blood supply was seen, and viable hematopoietic cells in the marrow space had disappeared completely (Figure 3). In five of the other six epiphyses, viz., one of WKRs and four of SHRs at 15 weeks, the repair tissue appeared in the necrotic area as follows. The marrow space was invaded by fibrous tissue composed mainly of fibroblastic cells and abundant osteoid matrix, and many dilated blood vessel were prominent. Appositional new bone was laid down in relation to dead trabeculae; as a result, the trabeculae became thicker and irregular (Figure 4). In the remaining epiphysis of SHRs at 15 weeks, the deposition of normal hematopoietic tissue between dead trabeculae with appositional new bone was seen, which suggested the completion of the repair process secondarily to necrosis.

Table 3. The degree of epiphyseal ossification. The number of the epiphyses in each stage is shown

	WKRs, age (wk)				SHRs, age (wk)			
Stage	6	9	12	15	6	9	12	15
0	12				11	4	3	2
1		1	1		1	2	4	5
2		10	2			3	3	1
3		1	9	12		3	2	4

Table 4. The number of the epiphyses with necrosis in each age

Age	WKRs	SHRs
6	0	0
9	1	1
12	0	1
15	1	8

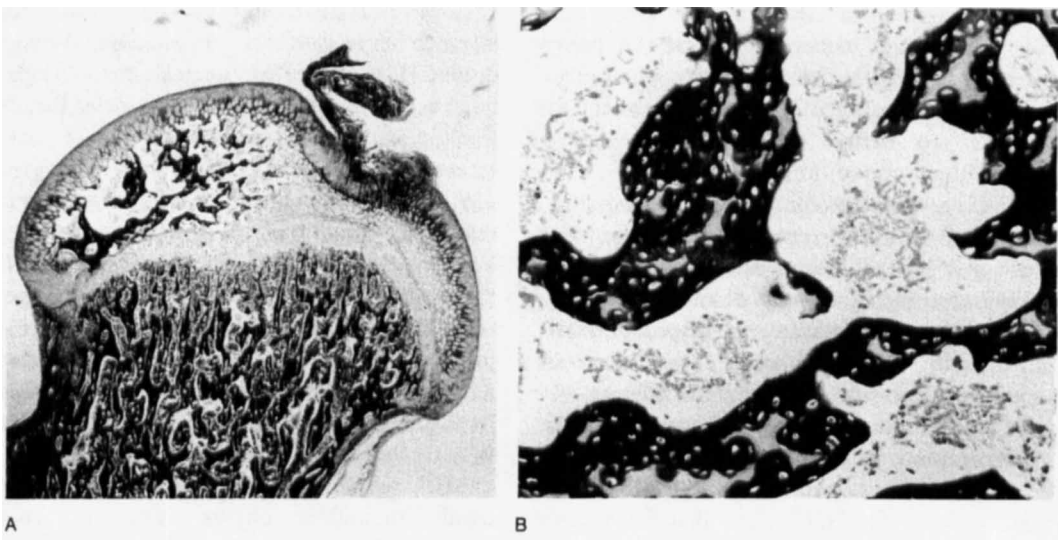


Figure 3.
A. Osteonecrosis in the epiphysis of a spontaneously hypertensive rat at 12 weeks. HE, x13.
B. Necrotic area. Note the disappearance of hematopoietic cells, dead-bone trabeculae, and necrotic osteocytes. HE, x100.

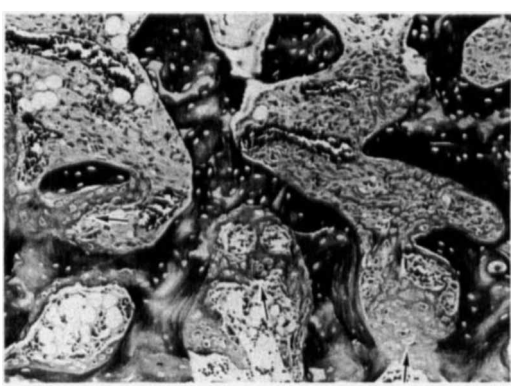


Figure 4. Reparative reaction to the necrosis observed in spontaneously hypertensive rat at 15 weeks. Abundant osteoid formation is seen around dead-bone trabeculae (arrows). HE, x100.

Metaphyseal growth plate and metaphysis. The growth plate was present in the femoral head of WKRs and SHRs even at the age of 15 weeks. Pathologic changes in the growth plate were observed at 9, 12, and 15 weeks in SHRs. The thickness of the plate decreased in the weight-bearing area, and chondrocytes lost the organized pattern observed in the proliferating and hypertrophic zone. The most severely affected plate was seen at 15 weeks; there were defects in the continuity of the plate. Enchondral ossification of the metaphysis was also disorganized in proportion to the disturbance of the metaphyseal growth plate. Bone trabeculae in the primary spongiosa diminished in number, and the thick trabeculae increased conspicuously in secondary spongiosa. No ischemic changes were observed in the metaphyseal region of WKRs and SHRs.

Discussion

In our rats the epiphyseal necrosis appeared before skeletal maturity, and remodeling was accompanied by revascularized granulation tissue and appositional new bone, i.e., the same sequence of events that occurs in Perthes' disease.

As regards the pathogenesis of Perthes' disease, accumulating experience has confirmed that the essential pathological process is necrosis due to infarction and subsequent revascularization of the epiphysis.

McKibbin and Ráliš (1974) described the findings in the femoral head obtained at necropsy from a patient suffering from Perthes' disease. From the findings of abnormally thick dead trabeculae and dead granulation tissue in the epiphysis, they supported the suggestion by Sanchis and Freeman (1973) that Perthes' disease was the result not of one, but of more than one episode of major infarction. Inoue et al. (1976) observed

the histopathologic changes characteristic of double infarction in man by femoral head biopsy specimens of Perthes' disease, and they proposed that the deformation of the femoral head and the chronicity of Perthes' disease might be due to repeated episodes of infarction.

With regard to the constitutional abnormality, Burwell et al. (1978) reported that children who developed Perthes' disease showed a generalized growth disturbance not only of skeletal immaturity, but also short stature and disproportionate growth with relative shortening of the distal bone segments. Recently, Kristmundsdottir et al. (1987) also reported that the mean age at the time of appearance of carpal bones was higher in Perthes' disease as compared with controls. Further, Arie et al. (1986) found that the femoral head on the contralateral side in Perthes' disease was less round than normal and flattened anteriorly. In addition, Rayner et al. (1986) and Burwell et al. (1986) suggested an abnormality of the endocrine system in the pituitary-somatomedin target-tissue axis.

Nevertheless, it is still not possible to fully account for the cause of infarction, because opportunities for histologic examination in the early stage of infarction are rare. Kemp (1981, 1982) demonstrated that changes resembling those in Perthes' disease could be produced by repeated intracapsular tamponade in rabbits and susceptible puppies as a result of venous occlusion, and he suggested that Perthes' disease might also be caused by venous occlusion due to tamponade in susceptible children.

However, a naturally occurring animal model is much more useful in investigating Perthes' disease. It is known that necrosis of the femoral head occurs in certain breeds of puppies that are small in stature and weight at the time of maturation (Hickman and Spickett 1965, Ljunggren 1967), but there have been economic and ethical restrictions in the use of these animals as an experimental model. In osteonecrosis of SHRs, there was no effusion or intracapsular hemorrhage, and no abnormal findings were seen in the joint capsule. Therefore it seemed that infarction did not depend on vascular occlusion resulting from intracapsular tamponade. In respect to the skeletal changes of SHRs, the length of the femur of SHRs was shorter than that of WKRs, and the ossification in the epiphyses was retarded. Those findings were similar to changes in Perthes' disease reported by Arie et al. (1986) and Burwell et al. (1978).

Abnormalities of the metaphyseal growth plate and enchondral ossification in the primary spongiosa were also recognized histologically before the age of 15 weeks, when a high frequency of necrosis was seen. Thus, the occurrence of necrosis seemed to be closely related to the disturbance of the cartilage in the epiphysis and metaphyseal growth plate and subsequent enchondral ossification. These findings may support the hypothesis that Perthes' disease is a constitutional defect (Harrison and Burwell 1981).

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