Acta Orthop Scand 1988;59(3):249–253

Carrageenan-induced coxitis in puppies

Cody Bünger, Kjell Sølund, Frederic Joyce and Oluf Myhre Jensen[1]

We studied the influence of chronic synovitis and joint effusion on the blood supply and growth of the immature femoral head in 12 puppies. Twelve weekly intraarticular injections of Carrageenan solution caused unilateral coxitis with caput magnum formation. Joint pressures in the neutral position were 0.9 kPa in the coxitis hips and −0.5 kPa in the contralateral hips. The joint capsule in coxitis showed hyperemia as measured by tracer microsphere technique. In nonmedicated, awake, standing dogs there was hyperemia of both the femoral head epiphysis and the joint capsule. In all the dogs, acetabular and proximal femoral metaphyseal blood flow was unchanged. In the control group, repeated cannulation and injection of normosaline did not change the hemodynamics, joint pressure, or femoral head size. We found no evidence of femoral head ischemia in coxitis with moderate chronic elevation of resting joint pressure.

The role of transient synovitis and elevated hip joint pressure as a precursor to the development of epiphyseal necrosis has received recent attention (Kallio and Ryoppy 1985, Wingstrand 1986). Scintigraphic studies in Perthes' disease have demonstrated ischemia of the femoral head epiphysis (Sutherland 1981). Animal studies in normal hip joints have attempted to determine a critical joint pressure beyond which the circulation of the femoral head epiphysis ceases (Borgsmiller et al. 1980, Launder et al. 1981, Lucht et al. 1983, Krebs et al. 1986, Kemp 1986). This critical level is probably close to the mean arterial pressure in normal hips, but synovial inflammation may render the epiphyseal blood supply more susceptible to elevated joint pressure (Bünger 1987). Hitherto, animal models of synovitis of the juvenile hip have not been able to maintain measurable increase in hip joint pressure or to induce epiphyseal necrosis (Gershuni et al. 1978).

We created an animal model of chronic coxitis and analyzed the resultant changes in regional blood flow and bone growth.

Materials and methods

Seventeen dogs, initially 8 weeks old, weighing 4 to 5 kg, were divided into two groups: a coxitis group (n 12) and a control group (n 5).

In the coxitis group the hip joint pressures were recorded, growth disturbances of the femoral head epiphysis measured, and joint capsule pathology analyzed. In 7 dogs, regional blood flow determinations were performed under general anesthesia in the supine position, whereas in 5 dogs it was measured in the awake, erect, nonmedicated state.

In the control group the same measurements and histologic investigations were performed after repeated unilateral cannulations and injections of 0.2 ml Isopaque + 1.0 ml normosaline into the hip joint.

Induction of coxitis. In Immobilon^{VET} (etorfin 0.125 mg per ml, acepromazine 0.4 mg per ml),

The Orthopedic Hospital and Institute of Experimental Clinical Research, University of Aarhus, and [1]University Department of Pathology, Aarhus Amtssygehus, Denmark

Correspondence: Dr. Cody Bünger, The Orthopedic Hospital, DK-8200 Aarhus N, Denmark

anesthesia, 1 ml increasing to 2 ml Carrageenan 1 percent solution (Bünger et al. 1984) were injected into the left hip joint weekly for 3 months. Carrageenan is a sulphated mucopolysaccharide derived from seaweed that causes release of lysosomal enzymes and prostaglandins from macrophages resulting in acute and subsequent chronic inflammation (Thompson and Fowler 1981). Prior to cannulation the skin was shaved and disinfected with chlorohexidine 0.5 percent in alcohol. A Terumo 21G cannula was used via an anteromedial approach. The correct position of the cannula tip was controlled by injection of 0.2 ml Isopaque® during fluoroscopy.

Evaluation of growth changes . Bilateral femoral head diameters were determined from monthly radiographs (Kodak X-Omat) of the hips in Lauenstein's position with a film focus distance of 90 cm. Correction for x-ray magnification was made. At the end of the study the frontal and sagittal diameters of the dissected femoral heads were measured with a micrometer screw.

Regional blood flow. Hemodynamic investigations were performed 5 days after the last hip joint cannulation. Under general anesthesia, polyethylene catheters (6F) were introduced via the carotid arteries into the left heart ventricle and into the abdominal aorta for flow measurements with tracer microsphere technique (Hales 1974). NEN-Trac microspheres (New England Nuclear), approx. 1.2×10^7 spheres, 15 $\mu \pm 3$ μ labeled with Sn-113 and suspended in 6 ml 10 percent Dextran with 1 percent Tween 80 added were used for each flow measurement as previously described in detail (Bünger et al. 1983a).

In awake dogs, the blood flow was measured after a recovery period of 48 h with the catheters in situ. The flow was measured in the resting, standing dog after a steady state period of 30 min. In the remainder, flow was measured in intubated, anesthetized (Halothane 0.5–1 percent), mechanically ventilated (O_2/Atm. air) supine dogs. The hips were positioned in 90° flexion, neutral rotation, and 10° abduction. Flow measurements commenced in steady state 1 h after introduction of the catheters. Steady state was controlled before and after flow measurements by means of mean arterial pressure, central venous pressure, and blood gases. Ventilation and oxygen administration were adjusted to maintain an arterial pH between 7.36 and 7.42, a PaO_2 above 12.5 kPa, $paCO_2$ between 4.5 kPa and 6.0 kPa and base excess above −4.

Intraarticular pressure was measured under general anesthesia in all the dogs 30 min after flow determination. The atmospheric pressure at the level of the hip joint served as reference zero pressure. The hip joint puncture was performed with a liquid filled Terumo 23G cannula connected to a three-way stopcock, which was closed during penetration of the tissues. By opening the stopcock, an immediate connection to the pressure recording system (with no perfusion) could be obtained without leakage. The hip joint pressure was measured bilaterally with the hips in 90° flexion, neutral rotation, and 30° abduction.

After the dogs had been killed, the hind limbs were dissected and synovial fluid was cultured from both sides. Blood flow measurements by gamma radiation counting (Bünger et al. 1983a) and histologic studies were made from symmetric tissue biopsies.

Statistics. The Student's *t*-test for paired data was used to compare joint pressure and femoral head diameter. All the flow data were $\log_{10}$ transformed and examined using a two-way analysis of variance with random effects (Searle 1971). The *F*-test was used to compare the flow rates in symmetric locations.

Results

Blood flow (Table 1). In the awake dogs the arthritic hip joint had increased flow both in the capsule and in the femoral head epiphysis ($P < 0.05$), but not in the proximal femoral metaphysis, the trochanter, and the acetabulum. In the anesthetized dogs with coxitis, the blood flow was increased in the joint capsule, but not in the femoral head and other juxta-articular bones. In all the anesthetized dogs the blood flow to the bones of the hip joint was reduced 40–50 percent compared with the values measured in awake dogs.

Repeated weekly injections of normosaline into the left hip joint caused no changes in regional blood flow of the hip compared with the contralateral side.

Table 1. Regional blood flow (ml×100 g^{-1}×min^{-1}) measured by tracer microsphere technique in awake dogs (n 5) and anesthesized dogs with unilteral Carrageenan-induced coxitis (n 7) and normosaline-injected hips (n 5). Mean values (SEM) are given

	Awake dogs		Anesthesized dogs			
	Coxitis group		Coxitis group		Normosaline group	
	E	C	E	C	E	C
Joint capsule	22 (4)[a]	3 (1)	10 (4)[a]	2 (1)	3 (1)	4 (1)
Femoral head epiphysis	21 (5)[a]	14 (5)	10 (2)	8 (1)	9 (2)	8 (2)
Proximal femoral metaphysis	17 (5)	28 (7)	11 (2)	16 (5)	13 (2)	13 (3)
Trochanter	17 (4)	19 (3)	12 (3)	11 (2)	15 (4)	15 (3)
Lateral acetabulum	22 (2)	21 (3)	13 (5)	11 (2)	11 (2)	12 (4)

E experimental hips, C contralateral hips.
[a] compared with contralateral hip $P < 0.05$.

Table 2. Intraarticular pressure (kPa) in Carrageenan-induced coxitis group (n 12) and normosaline control group (n 5). Mean values (range) are given

Coxitis group		Normosaline group	
E	C	E	C
[a]0.9 (0.5–3.0)	−0.5 (−1.6–0.3)	−0.7 (−1.3–0.1)	−0.6 (−0.9–0.1)

E experimental hips, C contralateral hips.
[a] $P < 0.01$ compared with contralateral hip.

Table 3. Radiographic and direct measurements of the femoral head diameter (mm) in Carrageenan-induced coxitis (n 12) and normosaline-injected hips (n 5) after 3 months of repeated injections. Mean values (standard deviation) are given

	Coxitis group		Normosaline group	
Diameter	E	C	E	C
Radiographic	18.9 (2.3)[a]	17.9 (2.2)	17.8 (3.7)	17.7 (3.7)
Direct	20.8 (3.4)[a]	18.6 (3.1)	18.6 (3.1)	18.5 (3.1)

E experimental hips, C contralateral hips.
[a] $P < 0.01$ compared with contralateral hip, $r = 0.68$.

Hip joint pressures (Table 2). The articular pressures in coxitis were all higher than the normal subatmospheric pressure of the contralateral hips ($P < 0.01$). However, all pressure were very susceptible to changes in joint position. In the control group, there were no pressure differences between experimental and contralateral hips.

Growth pattern in coxitis. Radiography after 2 months revealed juxta-articular osteoporosis and enlargement of the femoral head epiphysis (Figure 1). After 3 months of coxitis, caput magnum formation was demonstrated both radiographically and by direct measurements (Table 3). The femoral head enlargement was due to both cartilage and bony overgrowth. No signs of necrosis of the femoral head were encountered.

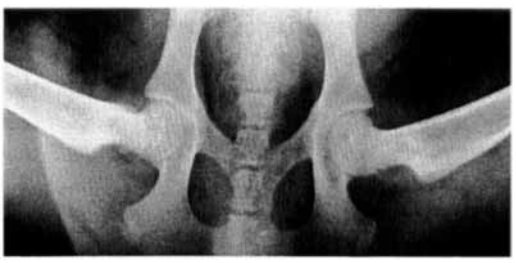

Figure 1. A 6 month puppy with Carrageenan-induced coxitis of the left hip with enlargementof the left femoral head.

Pathoanatomy. The joint capsule in the arthritic hips was thickened and yellowishly discolored. The articular cartilage of the femoral head was pale with no gross defects. Bacterial cultures were all negative. In the control group, all the experimental hips were normal.

Histology of the joint capsule of the arthritic hips showed villous hypertrophy and thickening of the synovial lining. The inflammatory cells were mostly lymphocytes and plasma cells, often in follicular accumulations.

The joint capsule in the experimental hips of the control group showed moderate thickening of the synovial lining in 2 dogs, whereas normal synovial findings were obtained in the other hips. No signs of acute or chronic inflammation were found.

Discussion

Carrageenan-induced synovial inflammation of the immature hip resulted in reproducible caput magnum formation and elevated hip joint pressure, whereas no evidence of femoral head necrosis was found. The induced overgrowth was due to thickening of the articular cartilage and enlargement of the bony epiphysis, probably secondary to synovitis, because the effect of repeated cannulation and infusion of normosaline was negligible. This accords with our observations in experimental arthritis of the immature knee (Bünger 1987).

Juvenile chronic arthritis of the hip in children was accompanied by a mean hip joint pressure of 0.4 kPa in neutral flexion of 45° (Rydholm et al. 1986). In transient synovitis of the hip, higher pressures in slight flexion and neutral rotation have been reported, 2.3 kPa (Wingstrand et al. 1985a) and 8.6 kPa (Kallio and Ryöppy 1985). A strong influence of joint position was demonstrated in all three studies. In comparison of pressure levels between the canine and human hip, the different anatomic relationships between the pelvic axis and the femur may be important. However, comparability may be achieved by reporting the position of minimal pressure, because joint motion in both species will occur around that position.

In 6 of 39 cases of transient synovitis of the hip, a temporarily reduced uptake of ^{99m}Tc-MDP was obtained on scintimetry and interpreted as interruption of the vascular supply of the femoral head epiphysis.(Wingstrand et al. 1985a, b). However, only 1 child developed radiographic evidence of Perthes' disease.

An important observation was our demonstration of unchanged or increased blood supply of the femoral head epiphysis in coxitis at rest in spite of chronic elevation of hip joint pressure. In the normal canine hip, joint pressure between the venous and arterial pressure may hamper the femoral head blood flow (Launder et al. 1981, Lucht et al. 1983). The duration of tamponade leading to irreversible lesions is still unknown, but probably lies between 6 and 12 hours (Woodhouse 1964, Krebs et al. 1986).

The significance of these pressure values has been questioned by recent studies demonstrating a higher vulnerability of epiphyseal blood supply in the presence of unspecific synovitis due to qualitative changes of the subchondral bone vasculature characterized by increased microvascular volume and prolonged transit time of blood (Bünger 1987).

The location of the vascular lesions in Perthes' disease is still obscure. By super selective angiography of the medial circumflex artery using digital subtraction technique, Atsumi et al. (1987) reported an extraosseous vascular lesion of the superior retinacular arteries. They were also able to demonstrate the importance of joint position on the vascular supply; internal rotation alone or combined with abduction resulted in interruption of the superior retinacular arteries at their origin and the blood supply of both the inferior retinacular and the ligamentum teres arteries was markedly decreased. Whether this represents an increase in joint pressure or physical stretch of the vasculature cannot be ascertained.

Another hypothesis on the role of synovitis in Perthes' disease has been suggested by Gershuni and Kuei (1984). They showed in a talcum-induced synovitis in the rabbit hip a loss of mechanical properties of joint cartilage rendering the subchondral bone more susceptible to microfracture. Secondary infarction in Perthes' disease has been found mainly located at the area of load transmission (Salter and Thompson 1984). In our study the entire femoral head blood flow was measured as one unit. Therefore, we were not able to differentiate between blood flow of loaded and unloaded femoral head segments. In the standing dogs the blood supply of the femoral head epiphysis on the arthritic side was higher than in the supine anesthetized dogs. This discrepancy is

probably secondary to a reduction in cardiac output caused by halothane (Katz and Katz 1966). We believe that the demonstrated hyperemia of the femoral epiphysis reflects the presence of hypermetabolism of the epiphyseal bone. Evidence of this has been found in a narrow subchondral rim of the distal femoral epiphysis in arthritis of the knee (Stender-Hansen et al. 1986).

References

Atsumi T, Kuroki H, Sugimori M, Yamano K. A study of the vascular changes of Perthes' disease. Trans Orthop Res Soc 1987;12:250.

Borgsmiller W K, Whiteside V A, Goldsand E M, Lange D R. Effect of hydrostatic pressure in the hip joint on proximal femoral epiphyseal and metaphyseal blood flow. Proc Orthop Res Soc 1980;5:23.

Bünger C, Harving S, Hjermind J, Bünger E H. Relationship between intraosseous pressures and intra-articular pressure in arthritis of the knee. An experimental study in immature dogs. Acta Orthop Scand 1983;54(2):188–93.

Bünger C, Hjermind J, Bülow J. Hemodynamics of the juvenile knee in relation to increasing intraarticular pressure. An experimental study in dogs. Acta Orthop Scand 1983;54(1):80–7.

Bünger C. Hemodynamics of the juvenile knee. Joint effusion and synovial inflammation studied in dogs. Acta Orthop Scand 1987;58(Suppl 222):1–104.

Gershuni D H, Axer A, Siegel B. Experimental synovitis in the hip joint of the rabbit and the resulting biomechanical and regressive cartilage changes. Trans Orthop Res Soc 1978;3:260.

Gershuni D H, Kuei S C. Articular cartilage deformation following experimental synovitis in the rabbit hip. J Orthop Res 1984;1(3):313–8.

Hales J R S. Radioactive microsphere techniques for studies of the circulation. Clin Exp Pharmacol Physiol 1974;(Suppl 1):31–46.

Kallio P, Ryöppy S. Hyperpressure in juvenile hip disease. Acta Orthop Scand 1985;56(3):211–4 .

Katz R, Katz G. Cardiovascular effect of Halothane. Br J Anaesth 1966;38:712–4.

Kemp H B. Perthes' disease: the influence of intracapsular tamponade on the circulation in the hip joint of the dog. Clin Orthop 1981;(156):105–14.

Krebs B, Nue-Møller B, Falstie-Jensen S, Love-Jepsen F, Taagehøj-Jensen F. Scintimetry of hip joint tamponade in dogs. Acta Orthop Scand 1986;57(2): 111–4.

Launder W J, Hungerford D S, Jones L H. Hemodynamics of the femoral head. J Bone Joint Surg (Am) 1981;63(3):442–8.

Lucht U, Bünger C, Krebs B, Hjermind J, Bülow J. Blood flow in the juvenile hip in relation to changes of the intraarticular pressure. An experimental investigation in dogs. Acta Orthop Scand 1983;54(2): 182–7.

Rydholm U, Wingstrand H, Egund N, Elborg R, Forsberg L, Lidgren L. Sonography, arthroscopy and intracapsular pressure in juvenile chronic arthritis of the hip. Acta Orthop Scand 1986;57(4):295–8.

Salter R B, Thompson G H. Legg-Calve-Perthes' disease. The prognostic significance of the subchondral fracture and a two group classification of the femoral head involvement. J Bone Joint Surg (Am) 1984; 66(4):479–89.

Searle S R. Topics in variance component estimation. Biometrics 1971;27:1–76.

Hansen E S, Holm I E, Bünger C, Noer I, Christensen S B, Knudsen V. ^{99m}Tc DPD uptake in juvenile arthritis. Scintimetry and autoradiography of the knee in dogs. Acta Orthop Scand 1986;57(4):299–304.

Sutherland A D, Savage J P, Paterson D C, Foster B K. The nuclide bone scan in the diagnosis and management of Perthes' disease. J Bone Joint Surg (Br) 1980;62(3):300–6.

Tachdjian M O, Grana L. Response of the hip joint to increased intra-articular hydrostatic pressure. Clin Orthop 1968;61:199–212.

Thomson A W, Fowler E F. Carrageenan: a review of its effects on the immune system. Agents Actions 1981;11(3):265–73.

Wingstrand H, Egund N, Carlin N O, Forsberg L, Gustafson T, Sundén G. Intracapsular pressure in transient synovitis of the hip. Acta Orthop Scand 1985;56(3):204–10.

Wingstrand H, Bauer G C, Brismar J, Carlin N O, Pettersson H, Sundén G. Transient ischaemia of the proximal femoral epiphysis in the child. Interpretation of bone scintimetry for diagnosis in hip pain. Acta Orthop Scand 1985;56(3):197–203.

Woodhouse C F. Dynamic influences of vascular occlusion affecting the development of avascular necrosis of the femoral head. Clin Orthop 1964;32:119–29.

Acknowledgements

This study was supported by the Danish Rheumatism Association (grant 233–425), P. Carl Petersen's Fond (grant B 1327), and the University of Aarhus.