Reperfusion pattern of the immature femoral head after critical ischemia

A microsphere study in pigs

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The topographic reperfusion pattern of the femoral head after critical ischemia has not yet been investigated. We determined the blood flow of the porcine hip regions with the femoral head epiphysis divided into 24 subregions by the tracer microsphere technique. Blood flow was measured under steady-state conditions, at the end of a 6-hour increase in intracapsular hip joint pressure to 250 mm Hg, and 4 hours after release of the joint tamponade.

Total femoral head epiphyseal blood flow decreased with ischemia and regained steady-state perfusion after tamponade. The reperfusion pattern of the femoral head epiphysis appeared identical with that of the steady state before ischemia. However, 2 of the 11 experimental epiphyses remained ischemic in the reperfusion phase.

We conclude that hip joint tamponade above the arterial pressure level for 6 hours causes global ischemia in the femoral head epiphysis in the immature pig, without regional differences in reperfusion, and that reperfusion occurs at a level like that of the steady state before ischemia.

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The blood supply of the femoral head epiphysis depends exclusively on extraosseous intracapsular vessels in two situations: from the age of 4–10 years and after femoral neck fracture (Trueta 1957). It has been shown in humans that femoral neck fracture can increase intracapsular hip joint pressure by hemarthrosis and that this may be associated with temporary femoral head ischemia (Soto-Hall et al. 1964, Wingstrand et al. 1986). In immature dogs, femoral head necrosis was produced by elevation of the hip joint pressure to 50 mm Hg for 12 hours (Woodhouse 1964). It has been suggested that the "no reflow phenomenon" plays a role in femoral head necrosis induced by temporary ischemia (Urbaniak et al. 1997). The regional reperfusion pattern after critical ischemia of the femoral head has not previously been investigated.

We studied the regional pattern of ischemia of the immature hip with special focus on the subregions of the femoral head during tamponade and studied the reperfusion pattern after critical ischemia for 6 hours.

Animals and methods

Study design

Of 15 immature Danish landrace pigs of both genders, weighing 46–50 kg, and 100 days of age, 11 animals were randomly chosen as an experimental group, and 4 served as controls.

Preparation

The animals were premedicated with 25 mg midazolam and 200 mg azaperon intramuscularly. Intravenous anesthesia was induced by 20 mg etomidate and, after orotracheal intubation, maintained by a combination of 30 mL ketamine 50 mg/mL, 4 mL pethidine hydrochloride, 6 mL midazolam 5 mg/mL, 6 mL pancuron 2 mg/mL, and 4 mL saline at a rate of 20 mL/h. The pig was

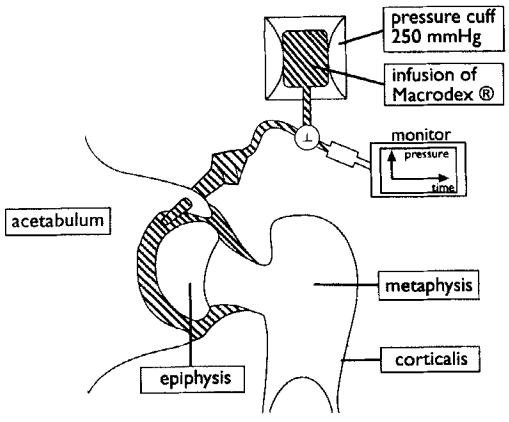

Figure 1. Outline of the unilateral intraarticular hip joint pressure increase and control.

positioned supine and ventilated on a Servo Ventilator 900 with hips in 90° flexion, 20° abduction, and neutral rotation.

Sheaths (Fast-Cath™, Daig Corp., Minnetonka, USA) were placed in both common carotid arteries (7F) and in one jugular vein (6F). Systolic, diastolic, and mean arterial blood pressures were monitored in a carotid artery by a pressure transducer (Uniflow™, Baxter, Valencia, CA, USA) on a CardioMed CM-4008 Physiological TraCe System (Medi-Stim AS, Oslo, Norway), on which ECG and rectal temperature were also monitored continuously. Blood gas analysis was performed half-hourly on an ABL™ 510 (Radiometer AS, Copenhagen, Denmark).

Increasing intraarticular hip joint pressure

The dorsolateral rim of the acetabulum was exposed unilaterally by a parasacral approach with the animal positioned on one side. Perpendicular to the outer acetabular wall, a channel of 5.3 mm diameter was drilled 1 cm medial to the acetabular limb and lateral to the ischiadic nerve. The femoral head (FH) was protected by traction on the limb during drilling. Intraarticular position of the drill was considered to be verified, if release resulted in outflow of synovial fluid. A conic plastic aspirator of 120 mm length (ScandiCare Products, Anderstorp, Sweden) was inserted tightly into the channel and sealed watertight with cyano-acrylate. Direct connection to the intraarticular space was controlled by injection of physiologic saline.

A dextran infusion bag (Macrodex® 60 mg/mL, Medisan, Uppsala, Sweden) was connected to a three-way stopcock mounted on the cannula and pressurized to 250 mm Hg by a pneumatic cuff (Figure 1). The intracapsular hip joint pressure was measured continuously in steady state (phase 1), during 6 hours' pressure increase to 250 mm Hg (phase 2), and 4 hours after pressure release (phase 3), by means of a pressure transducer (Uniflow™, Baxter, Valencia, CA, USA) connected to the stopcock.

Blood flow measurement

Radioactive tracer microspheres (New England Nuclear, Boston, MA, USA) with a diameter of 15 μm labeled with the isotopes tin (^{113}Sn, phase 1), ruthenium (^{103}Ru, phase 2), and cerium (^{141}Ce, phase 3) were used to measure regional blood flow (RBF) of the hip regions and cardiac output in the 3 experimental phases.

For administration of the microspheres, a pigtail catheter (6.0 F, Cook®, Denmark) was advanced into the left ventricle under fluoroscopic control through the sheath in the right carotid artery. Another pigtail catheter (6.0 F) was advanced into the thoracic aorta via the sheath in the left carotid artery.

Microspheres 5.0×10^6 were suspended in 5 mL 10% dextran. Before injection, the batch was shaken for 5 min on a Whirlimixer® (Fisons AG, Loughborough, U.K.). The spheres were injected through the pigtail catheter into the left ventricle over a period of 30s, followed by flushing with 5 mL 37 °C heparin-saline. Reference blood sampling from the aorta was started 30 s before sphere injection and continued until 4 min after the injection (Hansen et al. 1992).

After the last blood flow measurement, the pig was killed with an intracardiac injection of 40 mL potassium chloride solution. The hip and reference regions were removed from the cadaver, cut, and distributed into preweighed counting vials. The FH epiphysis was carefully separated from the growth plate and cut into 24 rectangular columns with articular cartilage as upside and bone bordering on the growth plate down (Figure 2). The reference blood samples and tissue samples were counted by multichannel spectrometry (Packard Cobra™, Packard Instrument Company,

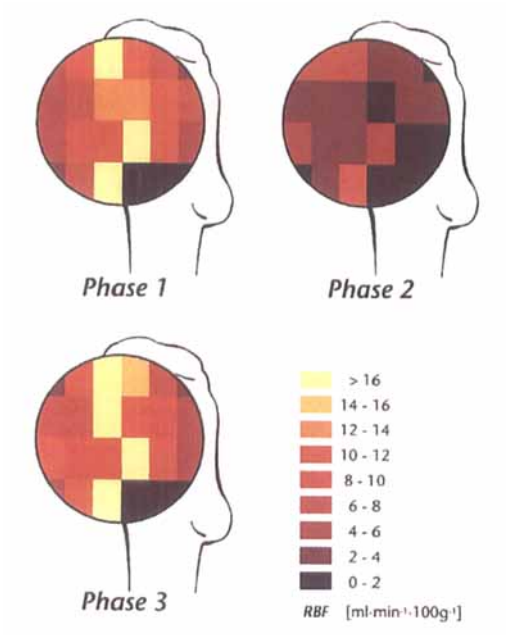

Figure 2. Perfusion of the 24 subregions of the tamponaded FH epiphysis, in a view of the right proximal femur from the medial aspect.

Table 1. Regional blood flow rates [mL*min^{-1}*100g^{-1}] of the right (R) and left (L) hip in the control group of animals (n 4), median followed by first and third quartiles in brackets

Region	Phase 1	Phase 2	Phase 3
Femoral head epiphysis			
R	9 (8–18)	11 (8–17)	13 (12–20)
L	13 (5–21)	13 (9–26)	12 (11–16)
Proximal femoral metaphyseal corticalis			
R	10 (6–14)	13 (8–23)	14 (7–18)
L	8 (6–13)	10 (8–19)	12 (8–15)
Proximal femoral metaphyseal cancellous bone			
R	18 (6–26)	16 (8–33)	18 (13–21)
L	22 (9–29)	21 (13–38)	23 (16–27)
Acetabular bone			
R	6 (1–15)	5 (1–10)	14 (4–21)
L	10 (3–12)	9 (2–16)	8 (3–21)
Hip joint capsule			
R	1 (0–1)	6 (1–9)	4 (1–11)
L	1 (1–5)	2 (1–5)	3 (2–5)
Ligamentum teres			
R	0 (0–1)	3 (0–10)	3 (0–11)
L	2 (0–13)	2 (1–24)	5 (2–19)

Meriden, CT, USA) with the channels set for the three principal emission energies for the isotopes used. The counts in each channel were corrected for background, spill-over, and decay while counting.

Using the Hales method (1974), we determined the regional blood flow of each predefined region (RBF_{biopsy}, mL*min^{-1}*100 g^{-1}) as

$$RBF_{biopsy} = \frac{C_{BIOPSY} * SR * 100}{W_{BIOPSY} * C_{REF}}$$

where C_{BIOPSY} denotes the count rate of a predefined region (cpm),

C_{REF} denotes the count rate of of the reference blood sample from the thoracic aorta (cpm),

SR denotes the sampling rate of the reference blood sample (mL * min^{-1}),

W_{BIOPSY} denotes the weight of the biopsy (g).

Statistics

RBF values are reported as median followed by the first and third quartiles in brackets. Normal probability plot and the Kolmogorov-Smirnov "Goodness of Fit" test of the original and Log10 transformed data revealed a non-normal distribution. The Friedman test for more than two related samples combined with the Wilcoxon signed rank test for paired samples were employed to investigate RBF differences in one hip between the three experimental phases. In this combined test procedure, the overall level of significance was corrected to 0.017 after Bonferroni. RBF differences between the experimental and contralateral hip were examined by the Wilcoxon signed rank test. The Wilcoxon-Mann-Whitney rank sum test was performed to compare hip RBF of the control group with that of the contralateral hip in the experimental animal group. P-values of less than 0.05 were considered significant.

Results

Regional blood flow

No differences in RBF were found between both hips in the control group and the contralateral hip in the tamponade group of animals during the experimental phases (Table 1). The phase 1 RBF of the experimental and the contralateral hip regions

Table 2. Regional blood flow rates [mL*min^{-1}*100g^{-1}] of the experimental (T) and contralateral hips (C), (n 11), median followed by first and third quartiles in brackets

Region	Phase 1	Phase 2	Phase 3
Femoral head epiphysis			
T	12 (10–20)	2 (0–4) [a]	13 (2–19)
C	11 (7–17)	11 (9–21)	10 (7–17)
Proximal femoral metaphyseal corticalis			
T	10 (6–24)	11 (8–24)	15 (9–21)
C	9 (5–17)	10 (6–17)	9 (6–15)
Proximal femoral metaphyseal cancellous bone			
T	18 (13–37)	19 (13–27)	20 (14–35)
C	23 (13–26)	18 (15–32)	20 (13–29)
Acetabular bone			
T	7 (3–9)	1 (1–3)	5 (2–7)
C	7 (4–8)	4 (3–8)	8 (6–13)
Hip joint capsule			
T	1 (1–1)	4 (3–6) [a]	13 (3–30) [a]
C	1 (0–2)	2 (1– 6)	3 (1–8)
Ligamentum teres			
T	2 (1–3)	0 (0–0) [a]	0 (0–2)
C	1 (1–2)	3 (2–6) [a]	4 (2–18) [a]

[a] Significant difference from phase 1 RBF ($p < 0.017$).

Table 3. Femoral head epiphyseal RBF showing non-reperfusion in 2 animals [mL*min^{-1}*100g^{-1}]

Animal No.		Phase 1	Phase 2	Phase 3
4	Test	6.3	0.3	0.3
	Control	7.4	9.6	6.4
10	Test	10.7	4.0	0.4
	Control	11.1	8.8	8.0

Table 4. Ligamentum teres RBF in the 2 animals with FH epiphyseal non-reperfusion [mL*min^{-1}*100g^{-1}]

Animal No.		Phase 1	Phase 2	Phase 3
4	Test	0.89	0.01	0.00
	Control	0.74	0.94	1.35
10	Test	0.02	0.01	0.01
	Control	0.51	1.96	4.74

were similar (Table 2). The median FH epiphyseal RBF on the experimental side decreased to 17% of that of the steady state after 6 hours of 250 mm Hg hip joint tamponade (phase 2) and returned in phase 3 to a level not statistically significantly different from that of the steady state. The subregions of the FH epiphysis all showed ischemia and a reperfusion pattern similar to steady state perfusion (Figure 2). The subregions showed no RBF difference between phases 1 and 3. In one animal, the FH remained ischemic with an RBF of 0.3 mL*min^{-1}*100g^{-1} tissue and in another, RBF decreased to 4.0 in phase 2 and further to 0.4 mL*min^{-1}*100g^{-1} tissue in phase 3 (Table 3).

RBF of the proximal femoral metaphyseal corticalis showed no differences between the experimental and control hips and between the 3 experimental phases. RBF was highest in the proximal metaphyseal cancellous bone which was not statistically significantly different from that of the contralateral hip and it remained unchanged throughout the three experimental phases. RBF in acetabular bone decreased during hip joint tamponade, albeit not statistically significant ($p = 0.06$, n 11), while acetabular RBF in phase 3 was at about the same level as that of the steady state.

Hip joint capsule RBF increased in phase 2 and again in phase 3, compared to phase 2 in the experimental hip, but the contralateral hip joint capsule showed no RBF change.

Ligamentum teres RBF almost ceased during hip joint tamponade and returned to the steady state level during reperfusion. In the 2 animals with FH epiphyseal non-reperfusion, ligamentum teres RBF fell during joint tamponade and remained ischemic after tamponade in one animal and was unchanged low throughout the experimental phases in the other (Table 4). In phases 2 and 3, the contralateral ligamentum teres was hyperperfused.

Central hemodynamics

The blood gases, temperature, and pH remained constant during the experiments. Cardiac output (CO) was 3.2–5.3 L*min^{-1} in phase 1, and 2.9–5.0 L*min^{-1} in phase 2, these values do not differ statistically. However, CO decreased to 1.7–2.9 L*min^{-1} in phase 3 ($p < 0.01$).

Steady state mean arterial blood pressure was 98, SEM 6.7 mm Hg, increased in phase 2, and decreased in phase 3, albeit not statistically significant.

Discussion

The tracer microsphere technique enabled us to do 3 blood flow measurements on the same anesthetized animal giving the RBF for all regions of interest at the same time. The disadvantage of this method is the discontinuous perfusion measurement. The microspheres embolize the microvasculature and reflect peripheral perfusion only at the time of embolization (Hales 1974). The variability of RBF in the same regions between animals was considerable (see Tables 1–3), but this is well known and has been shown to reflect real interindividual variability and not poor reliability of the method (Hoffbrand and Forsyth 1969). The isotopes were not applied randomly, because phase 2 was expected to have a low FH epiphyseal RBF. Here, ruthenium was used because of its high energy emission compared to tin and cerium.

This model was stable with respect to bone hemodynamics, in spite of a reduced cardiac output in phase 3, since bone RBF showed no significant changes throughout the experimental phases in the hips of the control group animals.

RBF of the total FH epiphysis decreased to 17% during 6 hours 250 mm Hg intraarticular hip joint pressure in this study in immature pigs. In this model, ischemia is produced by compression of the intracapsularly running lateral epiphyseal arteries, distal branches from the medial and lateral circumflex femoral arteries and the ligamentum teres artery (Trueta 1957). Circulation of the FH epiphysis during tamponade in our study might be due to a compensatory metaphyseal supply. Ligation of the proximal lateral femoral circumflex artery reduced to 39% the total flow to the FH epiphysis, while ligation of the distal medial circumflex femoral artery reduced flow to 68% of normal in immature pigs (Stuecker et al.1997).

4 hours after tamponade release, overall reperfusion returned to the steady state RBF level. However, in 2 animals FH epiphyses remained ischemic. Thus, these 2 epiphyses had either undergone 10 hours of continuous ischemia or recurrent ischemia following temporary reperfusion, after release of hip joint tamponade. Recently, hip dislocation and ligation of the medial and lateral circumflex femoral arteries and veins for 9 hours in adult dogs resulted in a decrease to 15% in femoral head blood flow determined by the hydrogen clearance method (Nishino et al. 1997). 10 hours' disruption of arterial blood supply might have resulted in FH epiphyseal necrosis in the 2 animals in our study. Thus, joint tamponade and consequent no reperfusion caused by temporary joint inflammation or intraarticular hemorrhage might be early pathogenetic steps in necrosis of the femoral head. 6 hours seems to be the upper limit of ischemia tolerance for the femoral head in this porcine model because 2 of 11 FH epiphyses remained ischemic. 9 hours of ischemia seems to mark the upper limit of ischemia tolerance for the femoral head in the canine model (Nishino et al. 1997).

No reperfusion of the femoral head epiphysis measured by 15 μm tracer microspheres means that the spheres were prevented from reaching the arterioles and capillaries of this region. Endothelial damage and swelling, regional and focal vasospasm, thrombi, and platelet aggregation might morphologically be the barriers in arterioles and capillaries for the microspheres and the microcirculation, respectively. These changes have been observed in a rat cremaster model and have been referred to as the "no-reflow phenomenon" (Urbaniak et al. 1997). In this model, 3 hours of cremaster ischemia were achieved by clamping the vascular pedicle and reperfusion was observed by intravital microscopy. In bone, endothelial dysfunction has been demonstrated during reperfusion in a canine tibia allograft after 24 hours of cold ischemia (Moran et al. 1995). Acetylcholine application failed to increase allograft bone blood flow in this preparation. It was concluded that reperfusion injury inhibited the endothelium from producing smooth-muscle relaxing factors.

Oxygen-derived free radicals are thought to play the major role in reperfusion injury in tissues different from bone (McCord 1985).

The ligamentum teres showed a minor RBF in the 3-month old pigs examined in this study. In humans, it was found that, from birth to about 3–4 years, the vessels of the ligamentum teres do not contribute to FH blood supply (Trueta 1957). The ligamentum teres containing an extraosseous intracapsular artery was not reperfused in 1 of the 2 cases of non-reperfused FH epiphysis (Table 4). The lateral epiphyseal arteries which provide the

major source of intracapsular extraosseous supply might also be occluded in the two cases which caused the epiphyseal non-reperfusion.

Ligamentum teres RBF in the contralateral hip joint increased from phases 1–3, which might be due to systemic neurovascular mechanisms. Capsule RBF of the experimental hip joint increased from phases 1–3. This finding is consistent with the study by Lucht et al. (1983). During acute elevation to 150 mm Hg hip joint pressure, the mean femoral head RBF fell significantly from 47–3.9 $mL*min^{-1}*100g^{-1}$ tissue in mongrel puppies and was hyperperfused afterwards (Lucht et al. 1983).

The clinical relevance of a model of intraarticular hip joint pressure increased to 250 mm Hg by means of pressure infusion has been shown in 2 case reports of traumatic hip joint tamponade (Strömqvist et al. 1985). High intracapsular hip joint pressure is mainly seen in undisplaced femoral neck fractures, where the joint capsule is intact (Wingstrand et al. 1986, Holmberg and Dalen 1987). The intraarticular pressure in the undisplaced Garden grades 1 and 2 fractures averaged 66 (37–145), in the displaced Garden grades 3 and 4 fractures 28 (5–65) mm Hg (Garden 1961, Crawfurd et al. 1988). The low pressures in the displaced fractures were associated with rupture of the capsule shown by ultrasonography. Aspiration of hemarthrosis reduced the intracapsular pressure to zero and postaspiration scintimetry revealed restitution of blood supply to the femoral head (Wingstrand et al. 1986).

However, in a recent study, no difference was found in intracapsular pressure between displaced and undisplaced femoral neck fractures, and development of femoral head necrosis was related to vascular damage, rather than to vascular tamponade (Maruenda et al. 1997).

In conclusion, we found that hip joint tamponade above arterial pressure level caused global ischemia in the femoral head epiphysis of the immature pig. On average, reperfusion of the femoral head reached a level no different from that before ischemia 4 hours after release of joint tamponade, and the regional perfusion pattern was unchanged. Single cases of non-reperfusion may indicate that 6 hours are about the critical ischemia duration in this animal.

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