Selective reduction of bone blood flow by short-term treatment with high-dose methylprednisolone
AN EXPERIMENTAL STUDY IN PIGS
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Treatment with corticosteroids is a known risk factor for the development of femoral head necrosis (FHN) after kidney and heart transplantation and after neurotrauma in both adults and children, but the pathological mechanism is poorly understood. A statistically significant correlation was found between the cumulative dose of methylprednisolone in the first month after heart transplantation and the development of avascular necrosis.

In patients with FHN, uneven blood flow with a lower flow within the necrotic sequester has been described.

Our aim was to investigate in pigs the effect of treatment with high-dose methylprednisolone on blood flow in bone in general and on the pattern of regional perfusion of the epiphysis of the femoral head.

Materials and Methods
We used 30 immature Danish landrace pigs of both genders, weighing 46.0 to 50.0 kg with a mean age of 100 days. They were divided randomly into an experimental group (n = 15) undergoing short-term treatment with high-dose steroids and a control group (n = 15).

The experimental group was treated with intramuscular methylprednisolone (Solu-Medrol; Pharmacia & Upjohn, Copenhagen, Denmark) for 14 days. For the first three days, they received 1000 mg/day, followed by 500 mg/day for the next 11 days. On the 14th day, blood flow was measured using radioactive tracer microspheres.

The animals were premedicated with 25 mg of midazolam (Dormicum; Hoffman-La Roche, Basel, Switzerland) and 200 mg of azaperon (Stresnil; Janssen Pharmaceutica, Beerse, Belgium) intramuscularly. Intravenous anaesthesia was induced by 20 mg of etomidate (Hypnomidate; Janssen Pharmaceutica) and after orotracheal intubation maintained by a combination of 30 ml of ketamine (Ketaminol Vet; Veterinaria, Switzerland) (50 mg/ml), 4 ml of pethidinhydrochloride (Petidin Amino; Amino AG, Switzerland), 6 ml of midazolam (5 mg/ml),
6 ml of pancuron (Pavilon; Organon Teknika, Turnhout, Belgium) (2 mg/ml) and 4 ml saline at a rate of 20 ml/hour. The pigs were positioned supine and ventilated on a Servo Ventilator 900 (Siemens-Elema, Sweden) with the hips in the neutral position.

Sheaths (Fast-Cath; Daig Corporation, Minnetonka, USA) were placed in both common carotid arteries (7F) and in one jugular vein (6F). The systolic, diastolic, and mean arterial blood pressures were monitored in a carotid artery by a pressure transducer (Uniflow; Baxter Healthcare Corporation, Santa Ana, California) on a CardioMed CM-4008 Physiological Trace System (Medi-Stim AS, Oslo, Norway), on which the ECG and rectal temperature were also monitored continuously. Blood-gas analysis was performed every 30 minutes on an ABL 510 blood-gas analyser (Radiometer A/S, Copenhagen, Denmark). Radioactive tracer microspheres (New England Nuclear, Boston, Massachusetts) with a diameter of 15 μm labelled with the isotope $^{113}\text{Sn}$ were used to measure regional blood flow. Two other types of microsphere, labelled with $^{103}\text{Ru}$ and $^{14}\text{Ce}$, were administered afterwards for purposes unrelated to the subject of this paper.

For administration of the microspheres, a pigtail catheter (6.0 F; Cook, Denmark) was advanced into the left ventricle under radiographic control through the sheath in the right carotid artery and another (6.0 F) into the thoracic aorta via the sheath within the left carotid artery. Each vial of microspheres contained 5.0 ± $10^5$ spheres suspended in 5 ml of 10% Dextran. Before injection the vial was agitated for five minutes on a Whirlmixer (Fisons AG, Loughborough, UK). The spheres were injected through the pigtail catheter into the left ventricle over a period of 30 seconds followed by flushing with 5 ml of heparin-saline at 37°C.

Reference blood sampling from the aorta was started 30 seconds before injection of the spheres and continued until four minutes after the injection.

For determination of the cardiac output (CO) the total activity in each vial was measured in an Amersham Calibrator (ARC 120, Capintec Inc, New Jersey) before injection. After the experiment, all the remnants were measured and subtracted to obtain the injected dose in megaBecquerel (MBq). A predetermined MBq quantity of each type of microsphere was suspended in tap water and vortexed, and ten aliquots of 1 ml were withdrawn for determination of the counting efficiency (counts per minute/MBq) of the gamma counter used for the isotopes.

After measurement of the blood flow, the animals were killed by an intracardiac injection of 40 ml of potassium chloride solution. The hip and reference regions were removed, cut and distributed into preweighed counting vials. The epiphysis of the femoral head was carefully separated from the epiphysial plate and cut into 24 rectangular columns perpendicular to the growth plate (Fig. 1). The reference blood samples and tissue samples were counted for gamma activity relating to each type of sphere (Packard Cobra; Packard Instrument Company, Meriden, Connecticut) using spectral analysis and correction for cross-talk, background, and decay during counting.

The regional blood flow of each predefined region (RBF$_{\text{biopsy}}$, ml*$\text{min}^{-1}*\text{100g}^{-1}$) was determined as follows:

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

where $C_{\text{BIOPSY}}$ denotes the count rate of a predefined region (counts per minute, cpm), $C_{\text{REF}}$ the count rate of the reference blood sample from the thoracic aorta (cpm), SR the sampling rate of the reference blood sample (ml $\text{min}^{-1}$), and $W_{\text{BIOPSY}}$ the weight of the biopsy (g).

The CO was calculated from injectates of microspheres (MS$_{\text{inj}}$) and total MS count in the reference blood (MS$_{\text{ref}}$) according to the equation:

$$CO = \frac{MS_{\text{inj}}*SR}{MS_{\text{ref}}}$$

**Statistical analysis.** The normal distribution of the raw data presented as the mean ± SEM was documented by Q-Q-plotting. Homogeneity of variances was achieved by log$_{10}$ transformation of the raw data. Significance was determined by the paired samples t-test when comparing the left and right hips and by the independent samples t-test when comparing the two experimental groups. p values of less than 0.05 (two-tailed) were considered to be significant.

**Results**

No significant differences were found between the right and left hip in either of the groups and therefore the mean value for both hips together was taken (Table I).

In the steroid-treated (CS) group there was a general reduction in bone regional blood flow (RBF) (Table I) compared with the control (NCS) group. The RBF in the epiphysis of the femoral head was 38%, in the proximal femoral metaphyseal spongiosa 32%, in the corticalis 31%, and in the acetabular bone 57% of those of the NCS group. For the humeral diaphysis the RBF in the CS group was 32% of that of the NCS animals (Table II). The absolute RBF of the epiphysis of the femoral head was 1.16 ± 0.16 ml*$\text{min}^{-1}*\text{kg}^{-1}$ in the NCS group and 0.42 ± 0.05 ml*$\text{min}^{-1}$ in the CS animals (p < 0.001). This represents 0.028% of the value for CO (4.2 ± 0.41*min$^{-1}$) in the NCS and 0.014% of that (2.9 ± 0.02*min$^{-1}$) in the CS animals. No significant difference in the RBF was found in the capsule of the hip, the ligamentum teres, the gluteal muscle and skin from the lower limbs in the two groups.

The specific CO (CO/kg/body-weight) was higher in the NCS animals than in the CS animals (92.3 ± 9.4 ml*$\text{min}^{-1}*\text{kg}^{-1}$ body-weight v 64.7 ± 4.8 ml*$\text{min}^{-1}*\text{kg}^{-1}$ body-weight; p < 0.05).

An overall reduction in the RBF of the epiphysis of the
The femoral head was found in the CS-treated animals without a tendency to lower blood flow in the craniomedial aspect (Fig. 1). The four craniomedial subregions were combined and the RBF of these and the combined RBF of the remaining subregions of the femoral head were calculated (Table III). No difference was found between these two regions.

**Discussion**

Osteonecrosis of the femoral head is still common after kidney and heart transplantation and is regarded as an effect of treatment with corticosteroids. In transplantation procedures in children, the incidence of necrosis of the femoral head has been shown to be equal to or even higher than that in adults. Necrosis of the femoral head develops predominantly in patients with a high rate of rejection. Such patients frequently receive a pulsed administration of high doses of corticosteroids containing 1 g of methylprednisolone daily for three days.

Our study has shown a pronounced general reduction of blood flow in bone after two weeks of treatment with high-dose methylprednisolone. The RBF in the epiphysis of the femoral head of CS-treated animals was reduced to half the fraction of the CO compared with that of the NCS animals. There was an overall reduction in the RBF of the epiphysis of the femoral head and that of the proximal femur, acetabular bone, and humeral bone. The RBF of the soft tissue of the hip was unchanged, which suggests that the effect of corticosteroids on the RBF of bone is selective.

Decreased arterial inflow or increased venous outflow resistance can reduce intraosseous blood flow. Based on clinical studies of osteonecrosis of varying aetiology including treatment with glucocorticoids, thrombophilia and hypofibrinolysis have been suggested as the common, major cause of osteonecrosis. Thrombotic occlusion of the venous outflow of the femoral head originating from thrombophilia or hypofibrinolysis is supposed to cause venous hypertension and reduced arterial perfusion of bone. Thrombophilia has recently been discussed as a pathological mechanism in Legg-Perthes’ disease. The blood supply of the immature epiphysis of the femoral head depends exclusively on extraosseous intracapsular vessels because the growth plate imposes a barrier on the intraosseous blood supply at this age. In our study this may make the femoral head more vulnerable to steroid-induced reduction of blood flow.
Wang et al\textsuperscript{16,17} found that the intrafemoral pressures in steroid-treated rabbits were increased by 2.5 times. Hyper trophy of fat cells was found in the femur and humerus of rabbits treated with high doses of methylprednisolone for five months. Recently, in vitro adipogenesis was induced by steroids in a pluripotent cell line from bone marrow.\textsuperscript{18} In the rigid intraosseous compartment, growth of fat cells may cause a rise in intraosseous pressure, and thereby compress the thin-walled sinusoids, with a subsequent decrease in bone blood flow.\textsuperscript{3} We have found, however, decreased blood flow in the cortical bone of the proximal femur which does not contain sinusoids within the vascular bed.\textsuperscript{19}

Degenerative changes in the arteries and arterioles of the capsule of the hip and the femoral head have been found in cadavers of renal transplant patients without clinical hip symptoms. There was thickening of the intima, gross diminution in the number and calibre of the vessels in the arteries of the femoral head and infarcts of subchondral bone.\textsuperscript{20}

The number of stenotic superior retinacular veins was found to be higher in femoral heads obtained at postmortem from steroid-treated patients than in those from patients without steroid therapy.\textsuperscript{21} In seven out of 30 steroid-treated rabbits, proliferation of foam cells was observed in the intima of the ear veins.\textsuperscript{22}

Bünger et al\textsuperscript{23} estimated the total and segmental blood flow of the epiphysis of the femoral head in carrageenan induced coxitis in dogs. Histologically, necrosis has been found to be localised particularly in the craniomedi al zone of the femoral head.\textsuperscript{24} In our study, the epiphysis has been systematically subdivided for the quantitative estimation of blood flow. This subdivision into 24 columns and their relationship to administration and dosage of steroids. \textit{J Bone Joint Surg [Am]} 1994;76-A:1385-8.


