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Corticosteroid Reduces Blood Flow to Femoral Heads in Rabbits

Key Words
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Laser Doppler velocimeter

Abstract
Avascular necrosis of the femoral head is one of the common problems in orthopedic practice in Taiwan. The subchondral bone loses its blood supply which weakens its biomechanical support. Steroid overuse is one of many possible etiologies in reducing blood flow to the femoral head. Laser Doppler velocimeter is a precise monitor of regional blood flow of bone which is expressed in perfusion units (PU). In the control group the rabbits were injected with normal saline and there were no statistical differences between blood flow to the right hip (39.26±5.64 PU) and left hip (38.58±4.35 PU). In group B a weekly injection of methylprednisolone into rabbits for 6 weeks demonstrated the reduction of blood flow of femoral head (24.74±3.13 PU) by the laser Doppler velocimeter. The flow decreased further (15.93±2.33 PU) by 12 weeks of steroid treatment. In group C after a weekly injection of steroid for 6 weeks the flow became 31.63±4.79 PU. The steroid was then discontinued for 3 weeks and the flow was 34.6±1.34 PU. In group D the blood flow was 25.89±4.01 PU after 6 weeks of steroid treatment and we stopped the steroid for 6 weeks, the blood flow became 29.86±2.59 PU. The merit of our experiment established a model of study in avascular necrosis of the femoral head in rabbits.

Avascular necrosis of the femoral head remains a challenging problem in orthopedics. Around 20% of total joint replacements are due to late stage of avascular necrosis of femoral heads [13], so an early diagnosis and treatment are important.

One of the etiologies of avascular necrosis of the femoral head is the overuse of steroid [5, 10]. Fisher et al. [2] in their experiment on rabbits demonstrated that long-term use of steroid induced massive necrosis of osteocytes and deposition of fatty tissue in the femoral head. Our clinical experience also supported the factor of steroid in human avascular necrosis of the femoral head. Laser Doppler velocimeter (LDV) is a precise monitor of regional blood flow [6–8, 14]. Swiontkowski et al. [15, 16] proved it to be a useful tool in measuring subchondral blood flow.

This is a study using LDV to investigate the effect of steroid on the blood perfusion of the femoral head in rabbits.

Principle and Technique of Laser Doppler Velocimeter

LDV is an advanced technique using laser beams to measure regional perfusion of the body (fig. 1). A narrow beam of monochromatic light generated by a low-power laser (2 mW He-Ne laser in our experiment) is carried by an optical fiber probe to the tissue being studied. The light is diffusely scattered and partly absorbed within the illuminated tissue volume. Light hitting moving blood cells will

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undergo a slight Doppler shift (change in wavelength), but light hitting static structures will be unchanged. The magnitude and frequency distribution of the Doppler shift are directly related to the number and velocity of blood cells, but virtually unrelated to the direction in which they move. The measurement probe also picks up illuminating beam back-scattered from the tissue to photodetectors where it is converted into electronic signals. The back-scattered light consists of a mixture of Doppler-shifted and nonshifted wavelengths. The Doppler-shifted components are extracted from the laser noise and other interfering frequencies by the special double-channel sensor in the LDV. The background noise is thus effectively suppressed to produce a cell-motion correlated signal called the 'flux' which is defined as: Perfusion or Blood cell flux = Number of blood cells moving in the measured volume × Mean velocity of these cells [7, 9].

The flux value reflects the transport of blood cells through the microvascular network from the arterial to the venous side, so that it is integrated over the entire measure volume and not a single vessel, which means that the flow value is the integrated speed of all red blood cells moving within the measuring area (i.e., a radius of 1 mm in our experiment). LDV can be applied to most tissues of the body as long as the microcirculation of that part is measured [1, 3, 6, 17]. The expression of microcirculation by LDV is a perfusion unit (PU) which is arbitrarily assigned by the machine without significant measuring units. The measurement of PU is relative in nature, thus the same machine should be used to measure all samples.

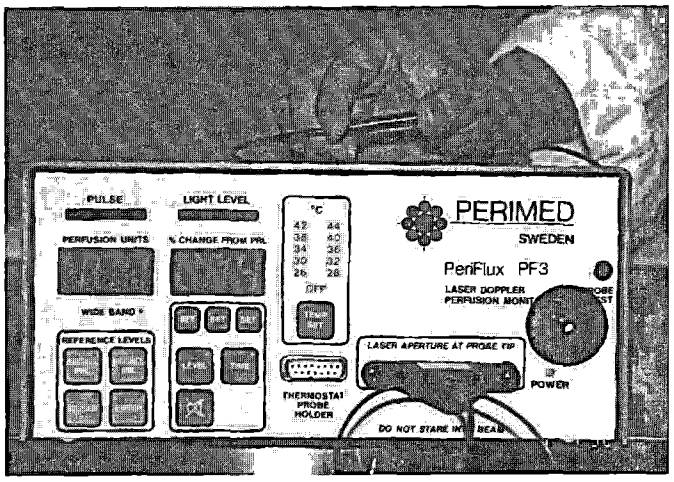

Fig. 1. The LDV monitor used in the present study.

Table 1. Blood flow to the femoral head measured by LDV (in PU; mean ± SD)

Group	Duration of therapy		
	6 weeks	9 weeks	12 weeks
A	39.26 ± 5.64	38.58 ± 4.35	
B	24.74 ± 3.13		15.93 ± 2.33
C	31.63 ± 4.79	34.60 ± 1.34	
D	25.89 ± 4.01		29.86 ± 2.59

Materials and Methods

Adult New Zealand rabbits weighing 2.5–3.5 kg were used in this experiment. The animals were anesthetized with ketamine (25 mg/kg b.w.) and chloropromazine (25 mg/kg b.w.). The brachial artery of the forelimb was cannulated and blood pressure was monitored during the experiment. The rabbit was then placed laterally on the operation table. A longitudinal incision was made over the hip joint. After deepening the wound, we carefully opened the hip joint by a longitudinal incision on the capsule. The joint was rinsed with normal saline and any bleeding was cauterized. A laser Doppler needle probe was placed on the femoral head and perfusion was measured which was expressed in PU. The animals were divided into the following 4 groups: (1) Control group (5 rabbits): the rabbit received an intramuscular injection of 2 cm^3 normal saline every week. Six weeks later the right hip was measured with LDV. Nine weeks after weekly injection the left hip was also measured. (2) Steroid-treated group (15 rabbits): the animals were injected with 12.5 mg methylprednisolone (Upjohn Co.) intramuscularly every week. Blood flow of the right hip was measured at 6 weeks and blood flow of the left hip was done at 12 weeks. (3) Discontinued steroid treatment group I (10 rabbits): the animals received a weekly injection of 12.5 mg methylprednisolone intramuscularly for 6 weeks. The injections were then discontinued. The right hip was measured at 6 weeks and the left hip was measured at 9 weeks with LDV. (4) Discontinued steroid treatment group II (10 rabbits): the animals received a weekly injection of 12.5 mg methylprednisolone intramuscularly for 6 weeks. The injections were then stopped. The right hip was measured at 6 weeks and the left hip at 12 weeks with LDV.

Results

Blood flow to the femoral heads was measured by the LDV in terms of PU (table 1). Student's t test was used for statistics and a probability < 5% was considered statistically significant. All of the corticosteroid-treated rabbits in groups B, C and D lost weight. Loss of body weight ranged from 20 to 50%. There were also high mortality rates in

steroid-treated rabbits. Only 11 rabbits in group B completed the experiment with a mortality rate of 36%. In group C, after 6 weeks of steroid injections, 8 rabbits survived, but only 7 rabbits completed the 9-week experimental course (mortality: 30%). In group D, 8 rabbits completed the 12-week experiment (mortality: 20%). The control group showed equal perfusion on bilateral femoral heads, although they were measured 3 weeks apart, but in the other groups after 6 weeks of steroid therapy, blood perfusion to the femoral head decreased significantly ($^{*}p<0.05$, groups B, C, D vs. group A in 6 weeks). It reduced further if the steroid therapy continued to 12 weeks in group B ($^{**}p<0.01$, in group B: 6 vs. 12 weeks). In group C, the perfusion decreased after 6 weeks of steroid administration. The drug was then stopped and perfusion gradually returned in 9 weeks, but statistically the recovery was not significant ($p>0.05$, in group C: 6 vs. 9 weeks). The same phenomenon was observed in group D, i.e., perfusion decreased significantly after 6 weeks of steroid administration. The steroid was discontinued for 6 weeks, but the recovery of perfusion was not statistically significant ($p>0.05$, in group D: 6 vs. 12 weeks).

Discussion

Avascular necrosis of the femoral head is a special problem in orthopedic practice. The subchondral bone dies from deprivation of blood supply. There are many possible etiologies of avascular necrosis of the femoral heads in humans, e.g.: (a) alcohol overconsumption [12], (b) steroid administration [5], (c) collagen disease, (d) dysbaric disease, (e) blood problems [4], (f) trauma [11] and (g) iodiopathic. The problem in establishing an animal model of avascular necrosis lies in the difficulties of measuring the blood flow in the subchondral bone of the hip. Wang et al. [18, 19] injected steroid into rabbits for 6 weeks and demonstrated the reduction of femoral head blood flow by radioactive microsphere methods. However, they had to sacrifice the animals after injection of the radioactive substance, so a long-term study was not possible. Thus the radioactive microsphere method was a cross-sectional study. LDV is a precise monitor of regional perfusion; by placing the probe on the cartilage firmly, we can measure the subchondral bone circulation accurately [15].

In this study we confirmed the effect of steroid on the reduction of blood flow to the femoral head. In the control group there was no statistical difference of perfusion measured between bilateral hips on different dates, which implies that LDV is very reliable as long as the same machine and same approach are used in the study. Our study showed after 6 weeks of steroid therapy that the blood flow to the femoral heads had reduced markedly. It became more severe if steroid therapy was continued, which implies that the decrease of blood flow to the femoral head is proportional to the period of steroid therapy. But even if the steroid was discontinued for 3 weeks in group C and for 6 weeks in group D, the perfusion did not return to the normal state. The recovery of blood flow to the femoral head might take more than 6 weeks. It can be explained that after steroid therapy the average diameter of the marrow fat cells increased in size. It increased tissue pressure in the closed chamber of the femoral head, thus the perfusion was diminished due to the compression on the capillaries. After 6 weeks of steroid treatment, the structural changes in the rabbit take more than 6 weeks to recover.

LDV is a precise and reliable method with which to measure subchondral circulation. The merit of this experiment is that it provides twice the measurement of blood flow to the hips. In this way it can be a longitudinal study in contrast to the cross-sectional study of the microsphere method.

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