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REGIONAL BLOOD FLOW IN EXPERIMENTAL MYOSITIS OSSIFICANS

A Microsphere Study in Conscious Rabbits

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In a recent model for heterotopic bone formation (Michelsson et al. 1980), muscular oedema, swelling and necrosis is seen in the quadriceps muscle of rabbit hind limbs immobilized for at least 2 weeks when, from the second week, the immobilized limb is subjected to daily forcible mobilization lasting about 5 min. According to this model, heterotopic calcification develops gradually from the second week of forcible mobilization and is located in the vastus intermedius region. Between the fourth and fifth week of immobilization and forcible mobilization, heterotopic bone formation is seen in virtually all cases. The histological findings are similar to those in human ectopic bone formation.

In the present investigation the labelled microsphere technique was used to study the regional blood flow effects in the early development of myositis ossificans with this model. The results are quite different from those reported by other investigators on immobilization alone and point to a causal relation between regional blood flow and forcible mobilization of the immobilized rabbit hind limb. Prostaglandins as mediators between the traumatic inflammation, a part of the circulatory effects observed and the induction of new bone is suggested.

Key words: blood flow; experimental; heterotopic bone; labelled microsphere technique; prostaglandins; rabbit

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Heterotopic bone formation is a complication after some orthopaedic operations, especially hip replacement (Broker et al. 1972, Charnley 1972, Harris 1972, Nollen & Sloof 1973, Ritter & Vaughan 1977) and is also seen in the soft tissues of the extremities after trauma (Jackson & Feagin 1973, Turek 1977) and in neurological diseases and after intra-cranial traumata with paraplegia (Freiberg 1952, Roberts 1968, Hardy & Dickson 1963, Tibone et al. 1978). Heterotopic bone often causes stiffness of the adjacent joint. The underlying mechanisms are poorly understood and since there is no recognized method of stopping heterotopic bone formation once it has developed, it sometimes constitutes a serious problem to the orthopaedic surgeon. In a recent model by Michelsson et al. (1980), a method of provoking heterotopic bone that is radiographically and morphologically similar to that seen in human myositis ossificans is offered. Immobilization of the rabbit knee in extension progressively leads to osteoarthritis (Langenskiöld et al. 1980, Michelsson et al. 1977), but not, alone, to the development of heterotopic bone formation (Michelsson et al. 1977). When immobilization is combined with daily forcible mobilization for about 5 min according to this model, the stiffness become more severe because of pronounced soft tissue changes and the heterotopic bone formation, which in fact is irreversible. Referring to the soft tissue changes, the first lesions noted are small haematomas adjacent to the knee, then muscular oedoma and necroses ensue. These changes are normally quite severe in the quadriceps muscle after 2 weeks of forcible mobilization. Neoformation of capillaries and granulation tissue follows.

According to several previous works (Imig et al. 1953, Hult & Olerud 1960, Semb 1966, 1968, 1969) arterial blood flow is increased in immobilized extremities of experimental animals. The aim of this work was to study regional blood flow in different tissues and parts of the immobilized and forcibly mobilized hind limbs of rabbits at 1 and at 2 weeks. The labelled microsphere technique was used in the present study of an experimental model of heterotopic bone formation. An attempt was made to elucidate the possible relation between regional blood flow and the macroscopic and microscopic changes observed in different tissues with this model.

MATERIALS AND METHODS

Adult rabbits of both sexes and of different breeds weighing 2.9–5.0 kg were used. One hind limb of each of 12 rabbits was immobilized by means of a plastic splint and an elastic bandage with the knee in extension and the hip unrestricted. All animals were fed with pellets and water ad libitum and were allowed to get accustomed to the immobilization splint for 1 week before the forcible mobilizations were introduced. The splint was then removed once daily (6 days per week) for about 5 min and the knee was passively mobilized through the maximum range, forcibly if necessary.

In six of the rabbits regional blood flow was determined after 1 week and in the other six rabbits regional blood flow was measured after 2 weeks of forcible mobilization of the immobilized hind limb. The animals were prepared under pentobarbital (mebumalum NFN) anaesthesia 1 day before the blood flow measurements. The right carotid artery was cannulated with a polyethylene tubing in distal direction for sampling and blood pressure measurement and the left heart ventricle was cannulated via the same artery in proximal direction (Hierton 1981). A suddenly reduced blood pressure of high amplitude was noted from the proximal tubing each time as its curved end reached the left ventricle. The correct position of the tubing in the left heart ventricle was moreover always confirmed when the animal was dissected. The tubings were filled with a heparin solution to prevent clotting. The tubing was hidden under a nasty tasting plaster to prevent the rabbits from eating it away. On the next day the animal was placed in an open box, 1000 IU heparin was injected intravenously and blood pressure was measured through the tubing. Care was taken not to excite the animal. The regional blood flow was then determined by injecting about 2 ml of a suspension of 103-Ru labelled (in two cases 141-Ce labelled) 15 μm microspheres (NEN G.M.B.H., Dreieich, West Germany) into the left heart ventricle. From the moment of injection and for the next 60 s, blood was collected from the tubing distally in the right carotid artery. The blood flow in the sampling tubing was about 1.9 ml/min and the blood was collected in 10-s samples. The measurement of the blood pressure was then resumed. Each sphere injection contained about 3.4 million microspheres. All animals were calm and seemingly unaffected by the time of regional blood flow determination. Shortly after the injection of spheres, the animal was killed by an overdose of pentobarbital followed by saturated KCL.

Assay

The amount of labelled spheres present in the tissues and blood samples was determined by two-channel gamma-spectrometry and the regional blood flow was calculated as described by Stjernschantz et al. (1976). Statistical analyses of changes were made using the Student's paired t-test.

RESULTS

In the study with six rabbits undergoing forcible mobilization for 1 week, mean arterial blood pressure was 83.0 ± 12.0 mmHg. Macroscopic bleeding in the quadriceps region, oedema and atrophic areas, especially in the lower hind limb, was noted. The knee joint was moderately stiff with arthritic and synovitic changes clearly visible on macroscopic examination. Regional blood flow in the heart muscle (left ventricle) was 4.60 ± 1.24 $g \cdot min^{-1} \cdot g^{-1}$. Table 1 shows that in the upper hind limb there were no appreciable effects on regional blood flow compared to the control side except for a significant 20 per cent decrease of blood flow to the proximal part of the vastus intermedius muscle. However, in the lower hind limb, blood flow was greatly impaired in the tissues examined and several of these reductions were statistically significant.

Table 1. Effects on regional blood flow in an experimental model of heterotopic bone formation in six rabbits after 1 week of treatment, mean ± S.E.M.

Tissue	Flow in the normal extremity $g \cdot min^{-1} \cdot g^{-1}$	Flow in the immobilized extremity $g \cdot min^{-1} \cdot g^{-1}$	
Skin of the thigh	0.01±0.003	0.01±0.003	n.s.
Hamstrings	0.02±0.01	0.02±0.01	n.s.
M. rectus femoris proximal portion	0.06±0.02	0.06±0.02	n.s.
M. rectus femoris middle portion	0.05±0.01	0.06±0.02	n.s.
M. rectus femoris distal portion	0.05±0.01	0.06±0.01	n.s.
M. vastus medialis	0.06±0.02	0.07±0.01	n.s.
M. vastus lateralis	0.05±0.01	0.04±0.01	n.s.
M. vastus intermedius proximal portion	0.10±0.03	0.08±0.03	$P<0.05$
M. vastus intermedius middle portion	0.09±0.02	0.09±0.03	n.s.
M. vastus intermedius distal portion	0.13±0.05	0.10±0.02	n.s.
Femur diaphysis (cross sect. mid.port.)	0.04±0.01	0.04±0.01	n.s.
Femoral bone marrow (cross sect. dist.port.)	0.18±0.05	0.10±0.03	n.s.
Femoral cortex (cross sect. dist.port.)	0.01±0.004	0.01±0.004	n.s.
Caput femoris	0.06±0.02	0.07±0.02	n.s.
Patella	0.01±0.002	0.01±0.01	n.s.
Patellar tendon	0.02±0.01	0.04±0.02	n.s.
Synovial membrane of the knee	0.04±0.01	0.03±0.01	n.s.
Skin of the lower hind limb	0.02±0.01	0.0002±0.00	n.s.
Calf muscle	0.07±0.03	0.0005±0.00	$P<0.05$
M. tibialis anterior	0.14±0.07	0.0002±0.00	n.s.
Achilles tendon	0.01±0.004	0.0006±0.00	$P<0.05$
Tibia and fibula (cross sect. distally)	0.01±0.005	0.0001±0.00	n.s.
Tibial and fibular bone marrow (cross sect. proximally)	0.16±0.005	0.0004±0.00	$P<0.05$
Tibial and fibular cortex (cross sect. proximally)	0.005±0.002	0.0001±0.00	$P<0.05$

n.s. = not significant.

In the study with six rabbits undergoing forcible mobilization for 2 weeks, mean arterial blood pressure was 71.2 ± 9.3 mmHg. Regional blood flow in the heart muscle (left ventricle) was 7.27 ± 1.57 $g \cdot min^{-1} \cdot g^{-1}$. This value was thus somewhat higher than can be expected for resting animals, even when considering that they were conscious. The reason for this is presumably mostly local and circulating metabolic factors, since increased sympathetic tonus was not indicated by alterations of blood pressure or heart rate and the animals were without visible signs of excitement. Moreover it is well known that relative to the local or metabolic factors that affect coronary vascular resistance, the sympathetic and parasympathetic fibres are of far less importance (Rubio & Berne 1978).

Atrophic and necrotic patches were clearly visible in the quadriceps musculature, especially in the vastus intermedius muscle but the necrotic lesions were more pronounced in the lower leg musculature. The knee joints were considerably stiffer in this study and had more clearcut synovitic and arthritic changes. The skin was necrotic juxta-articularly to the knee but there were also patchy areas of soft skin necrosis in the lower leg under which the muscles were dark and firm. Table 2 shows a slight and insignificant increase of the regional blood flow to the skin of the thigh and to the hamstring muscles. In the quadriceps

Table 2. Effects on regional blood flow in an experimental model of heterotopic bone formation in six rabbits after 2 weeks' treatment, mean ± S.E.M.

Tissue	Flow in the normal extremity $g \cdot min^{-1} \cdot g^{-1}$	Flow in the immobilized extremity $g \cdot min^{-1} \cdot g^{-1}$	
Skin of the thigh	0.05±0.02	0.17±0.07	n.s.
Hamstrings	0.09±0.01	0.12±0.05	n.s.
M. rectus femoris proximal portion	0.14±0.03	0.11±0.01	n.s.
M. rectus femoris middle portion	0.16±0.05	0.13±0.03	n.s.
M. rectus femoris distal portion	0.16±0.05	0.13±0.02	n.s.
M. vastus medialis	0.15±0.04	0.14±0.03	n.s.
M. vastus lateralis	0.11±0.02	0.13±0.02	n.s.
M. vastus intermedius proximal portion	0.24±0.07	0.16±0.02	n.s.
M. vastus intermedius middle portion	0.51±0.33	0.18±0.03	n.s.
M. vastus intermedius distal portion	0.69±0.49	0.13±0.05	n.s.
Femur diaphysis (cross sect. mid. port.)	0.26±0.05	0.21±0.05	$P<0.02$
Femoral bone marrow (cross sect. dist. port.)	0.84±0.18	0.82±0.19	n.s.
Femoral cortex (cross sect. dist. port.)	0.04±0.01	0.08±0.03	n.s.
Caput femoris	0.32±0.05	0.31±0.07	n.s.
Patella	0.04±0.01	0.01±0.01	$P<0.02$
Patellar tendon	0.07±0.02	0.03±0.01	n.s.
Synovial membrane of the knee	0.14±0.04	0.07±0.02	n.s.
Skin of the lower hind limb	0.10±0.03	0.001±0.00	$P<0.05$
Calf muscle	0.33±0.22	0.04±0.02	n.s.
M. tibialis anterior	0.18±0.03	0.03±0.02	$P<0.01$
Achilles tendon	0.07±0.03	0.02±0.01	n.s.
Tibia and fibula (cross sect. distally)	0.10±0.03	0.01±0.005	$P<0.02$
Tibial and fibular bone marrow (cross sect. proximally)	0.75±0.22	0.03±0.02	$P<0.02$
Tibial and fibular cortex (cross sect. prox.)	0.04±0.01	0.01±0.003	$P<0.05$

n.s. = not significant.

region there was a slight and insignificant decrease of blood flow. The patellar blood flow was significantly reduced by 75 per cent and regional blood flow to the femur diaphysis (mid portion) was significantly reduced by 20 per cent. In the lower leg, regional blood flow was clearly and significantly reduced.

DISCUSSION

The pathogenesis of heterotopic bone formation is poorly understood (Michelsson et al. 1980) but trauma and bleeding is considered to be of importance in some cases in humans. Referring to the rabbit model in the present study immobilization alone led to osteoarthritis within a few weeks (Michelsson 1977) but immobilization alone did not lead to ectopic bone formation. The first macroscopic and microscopic tissue changes observed for this model are small haematomas after about 1 week of forcible mobilization. After 2 weeks of forcible mobilization, heterotopic calcification is developing gradually (Figure 1).

The method used in this project to measure regional blood flow seems suitable, in particular for examination of inaccessible and minute tissue regions. In fact few data have been found in the literature regarding studies on regional blood flow in cortical bone and tendons as well as different parts of a muscle (Hierton 1981). However, as stated previously it has been shown by

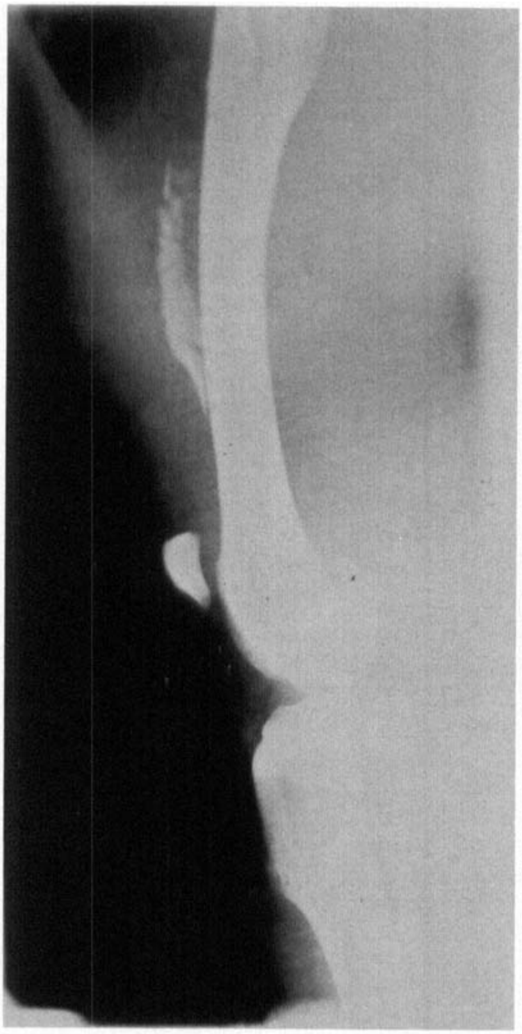

Figure 1. A rabbit knee in extension with radiographically visible ectopic bone formation in the vastus intermedius region after 2 weeks of treatment.

several investigators that blood flow to an immobilized extremity increases. Concerning the works of Semb (1966, 1968, 1969), it is to be noted that although the use of the initial clearance of bone seekers (^{45}Ca, ^{85}Sr) for measuring bone blood flow has been widely accepted, this method has several limitations.

Accordingly, in the work by Schoutens et al. (1979), it was observed that initial bone clearances may underestimate increases in bone blood flow under certain conditions. Nevertheless, Semb reported an increased bone blood flow of immobilized extremities. The results of the present project show on the contrary that regional blood flow to the vastus intermedius was decreased after 1 week and after 2 weeks of forcible mobilization, regional blood flow to the femur diaphysis and the patella was decreased. The regional blood flow to the lower leg was greatly decreased after 1 as well as 2 weeks of treatment. It seems likely, then, that these blood flow effects are related to the forcible mobilization of the immobilized limbs and probably related also to the new bone formation.

The data obtained in this study implies that the specific changes in the hind limbs produced according to the described model have considerable effects on the peripheral circulation in the affected limb as well as on the whole animal. It can thus be assumed that in addition to the direct trauma exerted, the necrotic changes described have a toxic effect on the regulating mechanisms of peripheral arterial resistance. Moreover in some cases a low virulent infection in the affected tissues may have been present. The pathogenesis of the ectopic bone observed and the appreciable alterations of peripheral circulation present in this model can accordingly be anticipated to be linked with mechanical distress of the involved extremities as well as inflammation and possibly latent infection. In clinical situations as the one described, prostaglandins as mediators are believed to play a larger role than under resting conditions (Hierton 1981).

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