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Short Communication

Microvascular changes in the lower extremities of paraplegics with heterotopic ossification

S Lotta*[,1], L Scelsi[2] and R Scelsi[3]

[1]*Az USL Piacenza, Rehabilitation Center 'G.Verdi', 29010, Villanova sull'Arda: Pc. Italy;* [2]*Department of Cardiology, University of Pavia and IRCCS S.Matteo Hospital, Pavia, Italy;* [3]*Department of Human Pathology, University of Pavia, Via Forlanini 14, 27100 Pavia, Italy*

Objectives: To investigate the morphological aspects of blood microvasculature of the skin and subcutaneous tissues in subjects with paraplegia with heterotopic ossification (HO).
Methods: In two patients with traumatic spinal cord injury and HO, punch biopsies of skin and hypodermic soft tissue in the region of HO near the hip were studied with histological and ultrastructural methods.
Results: Alterations of endothelial cell and basement membrane of capillaries and small vessels were observed. Hyperactive endothelium, thickening and reduplication of the basement membrane, changes of the perivascular connective tissues and microcalcifications in the subcutaneous fat tissue were also seen.
Conclusions: This present study indicates microvascular changes in the skin and subcutaneous tissue in the region of HO near the hip of two subjects with paraplegia. In our opinion the described vascular changes may induce hypoxiemic alterations of the soft para-articular tissues leading metabolic changes which may contribute to the development of HO. Therefore, it cannot be concluded whether these changes are directly responsible for HO induction.
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Introduction

The inactivity or disuse of extremities of subjects with paraplegia following spinal cord lesions are proposed to lead to skeletal muscle atrophy, disuse osteoporosis and paraosteoarthropathies.

Heterotopic ossification (HO), consisting in bone metaplasia of the soft tissues surrounding peripheral joints, is a frequent complication which occurs in 15% to 53% of patients.[1] In about 15% of these patients, HO induces severe complications such as reduction of the articular movements leading to loss of sitting position, pressure sores, increase in spasticity and pain, which are indications for operative resection of HO.[2,3]

The etiology of HO is still unknown and causes of the disease include bone metabolic changes, traumatic lesions and vaso-motor disturbances.[4–7]

Recent experimental studies demonstrated the increase of the serum osteoblast mitogenic activity together with an increased condroblastic and osteoblastic activity as consequence of a decreased tissue oxygenation in spinal cord injury.[8] Furthermore, the elevation of urinary prostaglandins which has an effect on the formation of lamellar bone, may play a role in the formation of HO.[9]

The hypothesis of a dysmetabolic etiology of HO may be also supported by functional and morphological changes of the lymphatic and blood microvasculature of the paretic legs described in patients after spinal cord injury.[10,11] Since microvascular modifications may induce hypoxia and metabolic alterations in soft tissues, the purpose of this study is to examine, with morphological and ultrastructural methods, the blood microvasculature of the skin and of the subcutaneous tissues in the region of HO near the hip in two subjects.

Patients and methods

Patients
We have studied two paraplegic patients with HO.

*Correspondence: S Lotta, Centro di Recupero e Rieducazione Funzionale 'G. Verdi', via Dante 17, 29010, Villanova sull'Arda, Pc, Italy

M.D., a 33-year-old male, had complete paraplegia at T9 secondary to a traffic accident 18 months before study. The patient had ASIA impairment score A, degree of lower extremity spasticity grade 3 on the Ashworth scale. He had a restriction in flexion of both hips that resulted from HO, the range of movement was 30° of flexion bilaterally.

Laboratory evaluation was unremarkable. CPK and LDH were normal, alkaline phosphatase 237 mU/ml (normal values 60 – 170 mU/ml), erythrocyte sedimentation rate 24 (normal value: 15).

At the time of formation of HO the values of CPK and LDH were increased.

The medical management consisted of a combination of non steroidal anti-inflammatory drugs. Indomethacin 100 mg/day for 1 month, Diclofenac 50 mg/day for 15 days. Anticoagulant therapy was performed for 6 months to prevent the occurrence of deep venous thrombosis. The rehabilitation activity included 1 h/day standing training, slow mobilization of articulation and wheelchair use with regard to limitation of hip range of movement.

P.T., a 38-year-old male, had complete tetraplegia at C6 secondary to a traffic accident 26 months before the present study. The patient had ASIA impairment score A. The Ashworth scale grade 4 at the lower extremity, HO at the hip bilaterally, range of movement 40° of flexion at the hip. Laboratory results were unremarkable: the alkaline phosphatase was 242 mU/ml.

He was on oral anticoagulative therapy for 12 months with deep venous thrombosis, baclofen 25 mg × 3, and disodium etidronate 20 mg/kg/day for a period of 6 months after the occurrence of HO. Rehabilitation activity included daily exercises for arms and lower limbs, standing and training 1 h/day and wheelchair use.

After informed consent, small specimens of skin and hypodermic soft tissue in the region of HO of the hip were obtained by punch biopsy.

Methods

The biopsy samples were fixed in 10% neutral formalin for light microscopy and in Karnovsky fluid for electron microscopy.

Paraffin-embedded serial sections were stained with haematoxylin and eosin and with Van Gieson stains. Intramuscular capillaries and small blood vessels were identified by the Gomori's silver impregnation for reticulin and by immunohistochemical stains for endothelin and for the endothelial marker CD34. Epoxy resin-embedded semi-thin sections were stained with toluidine blue and then observed under a Leitz Laborlux light microscope.

For ultrastructural studies, biopsies were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodilate buffer at pH 7.4 for 4 h at 4°C, post-fixed in 1% OsO4 in 0.2 M collidine buffer at pH 7.4 for 2 h at 4°C, dehydrated and embedded in epoxy resin. Blood capillaries were identified at the light microscope on semi-thin sections stained with toluidine blue. Ultrathin sections from these vessels, contrasted with uranil acetate and lead citrate, were observed and photographed by a Zeiss EM 109 transmission electron microscope. Micrographs were obtained by an automatic Zeiss Laborlux system.

Results

With the light microscope numerous dermal blood vessels of the skin from both patients showed occluded lumen delimited by enlarged endothelial cells surrounded by rarefacted perivascular connective tissue. Ultrastructural findings showed a striking alteration of both endothelial cell and basement membrane of small blood vessels and capillaries. Most of them showed an occluded lumen: endothelial cells were characterised by an enlarged cytoplasmic bulk and by multiple endothelial intraluminal projections. The intercellular contacts were enlarged and rare micropinocytotic vesicles were seen (Figure 1a). Moreover endothelial cells showed a hyperactive cytoplasm characterised by a well developed rough endoplasmic reticulum, free ribosomes, Golgi's apparatus and mitochondria. The basement membrane was thickened and reduplicated: multiple discontinuous layers composed by fibrils of basement-membrane-like material surrounded the endothelial wall. The pericytes showed a scanty cytoplasm fully occupied by numerous micropinocytotic vesicles (Figure 1b).

On the other hand, some capillaries seemed to be normal showing a thin and distended wall (Figure 2a). Endothelial cells were flattened and their cytoplasm was very scanty. Around them reduplications of basement membrane were generally present (Figure 2b).

The perivascular connective tissue was characterized by dissociation and disruption of collagen and elastic fibres. Microcalcifications were sometimes present in the subcutaneous fat tissue.

Discussion

Heterotopic ossification (HO) occurring in paralyzed limbs are an important and frequent complication of traumatic spinal cord lesions, inducing reduction of the articular movements. Morphological and molecular biology studies regarding histogenesis, proliferation and bone transformation of the variant of non-traumatic HO were carried out on soft tissue samples with ossification foci. Phenotyping of osteoclasts in HO revealed their derivation from mature local macrophages and expression intensities of the proliferation markers PCNA and MIB1 suggested a proliferation behavior in peripheral areas of osteogenesis similar to that found in autonomous osseous neoplasias and underlined the reactive-proliferative character of HO.[12]

Prostaglandins and other eicosanoids have been shown to have a role in bone metabolism and to

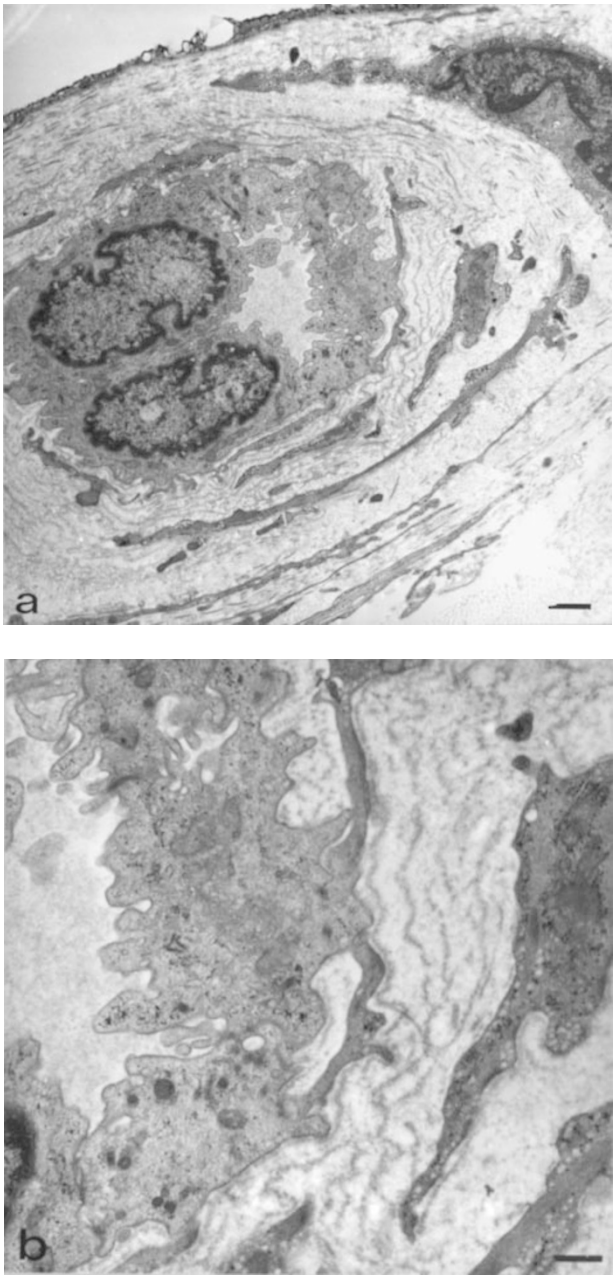

Figure 1 (**a**) Electron micrograph from a blood capillary showing multiple endothelial projections (final magnification: ×7.900; bar 1). (**b**) Detail from (**a**). A swollen and hyperactive cytoplasm characterises the endothelial cells. The basal lamina is thick and reduplicated showing multiple layers. The pericytes show numerous micropinocytotic vesicles (final magnification: ×15.600; bar 0.5)

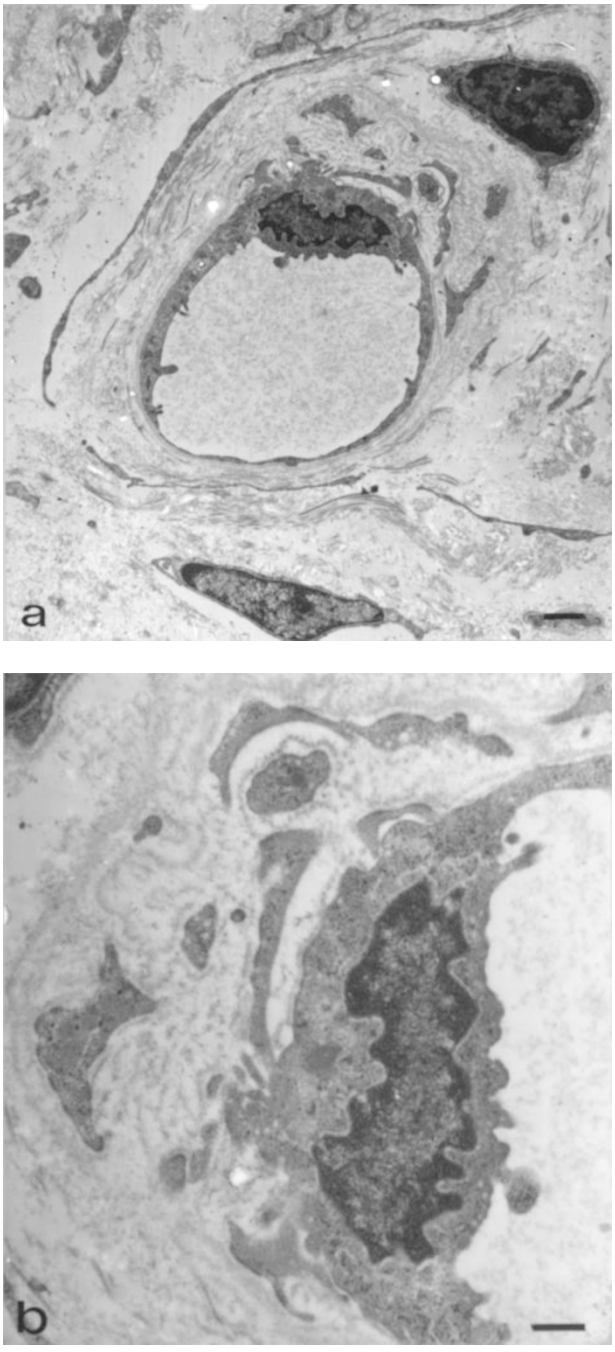

Figure 2 (**a**) Electron micrograph from a blood capillary showing a thin and distended wall (final magnification: ×7.900; bar 1). (**b**) Detail from (**a**). The thickened basal membrane is characterised by multiple layers of basement membrane-like material (final magnification: ×15.600; bar 0.5)

induce HO in experimental animals. Furthermore, elevation of urinary prostaglandin E2 has been demonstrated to herald the formation of HO.[9] Prostaglandins are well known substances regulating locally the vascular tonus and to play a role in inflammation, in addition to their effect on the formation of lamellar bone. Recent literature stressed the hypothesis that HO might be traumatic in origin. Bone scintigraphy, ultrasonography and histological studies of the immature and mature HO in para-articular tissues seem to offer some support for the relationship of HO to microtraumatic lesions either by passive movements or by intensive rehabilitation and transfert activity.[13]

Histological studies demonstrated muscle necrosis, hemorrhage and undifferentiated cellular proliferation in the site of HO, with final differentiation of osteoid matrix and then of mature bone.[14] The histological study conducted by Bodley *et al*[15] indicated in the site of HO small osteoid formations with presence of hemosiderin-loaded macrophages, as consequences of para-articular microhematomas. The authors concluded that microtraumatic lesions might be a causative factor in the occurrence of HO in paraplegics.

Recent morphological and physiopathological studies demonstrated that the cardiovascular system, microvasculature and the venous vascular properties in the legs of subjects with paraplegia have changed. Numerous such subjects show alterations in the cardiovascular performance at rest and during exercise and the vascular responses in the legs during arm exercise are impaired by a lack of sympathetic venoconstriction and the inability to activate the muscle pump in the legs.

Plethysmography demonstrated a decrease in venous distensibility and capacity, and an increase in venous flow resistance.[16] In addition, a lymphatic microangiopathy of paretic legs was detected by fluorescence microlymphography[17] and by morphological investigations.[10]

Dilatation of lymph and blood vessels indicate functional changes probably related to immobilization and bed rest and they are probably the result of vascular adaptation to inactivity and skeletal muscle atrophy.[9,16]

The microvascular changes are most evident in those subjects with thromboembolic disease. In these patients the venous outflow obstruction caused by deep venous thrombosis accompanied by the absence of the ambulatory venous pressure in the paretic leg, determines skin microvascular dilatation and consequent transcapillary diffusion of plasma and lymph material into the perivascular connective tissues.[10]

The present morphological and ultrastructural study indicate microvascular changes in the biopsy of the skin and hypodermal tissue of the hip found in two paraplegics with HO.

Therefore, it cannot be concluded from the present results whether these changes are responsible for HO induction.

In our opinion the alteration of endothelial cells and basement membrane of capillaries in paraplegics with HO may influence the metabolic condition of the soft para-articular tissues with calcium salt deposition and HO induction, through hypoxia, alterations of transcapillary exchanges and modifications of the perivascular connective tissues. These observations suggest the significance of circulatory system protection whether limiting vascular stasis or reducing vascular microtraumas even in rehabilitation management.

References

1 Colachis SC, Clinchot DM, Venesy D. Neurovascular complications of heterotopic ossifications following spinal cord injury. *Paraplegia* 1993; **31:** 51 – 57.
2 Meiners T, Abel R, Bohm V, Gerner HJ. Resection of heterotopic ossification of the hip in spinal cord injured patients. *Spinal Cord* 1997; **35:** 443 – 445.
3 Banovac K, Gonzalez F. Evaluation and management of heterotopic ossification in patients with spinal cord injury. *Spinal Cord* 1997; **35:** 158 – 162.
4 Garland DE. A clinical perspective on common forms of acquired heterotopic ossifications. *Clin Ortho Rel Res* 1991; **263:** 13 – 29.
5 Bravo-Pajno P *et al*. Incidence and risk factors in the appearance of heterotopic ossification in spinal cord injury. *Paraplegia* 1992; **30:** 740 – 745.
6 Chantraine A, Nusgens B, Lapiere CM. Biochemical analysis of heterotopic ossifications in spinal cord injury patients. *Paraplegia* 1995; **33:** 398 – 401.
7 Uebelhart D, Demiaux-Domenech B, Roth M, Chantraine A. Bone metabolism in spinal cord injured individuals and in others who have prolonged immobilisation. A review. *Paraplegia* 1995; **33:** 669 – 673.
8 Renfree KJ. Evaluation of serum osteoblast mitogenic activity in spinal cord and head injury patients with acute heterotopic ossification. *Spine* 1994; **19:** 740 – 746.
9 Schurch B, Capaul M, Valotton MB, Rossier AB. Prostaglandin E2 measurements: their value in the early diagnosis of heterotopic ossifications in spinal cord injury patients. *Arch Phys Med Rehabil* 1997; **78:** 687 – 691.
10 Scelsi L, Lotta S. Microvascular changes in skeletal muscle from paraplegic patients with spinal cord lesion. In: Carraro U, Salmons S (eds) *Basic and Applied Myology: Perspectives for the 90s*. Unipress, Padova, 1995, pp 163 – 170.
11 Scelsi R, Scelsi L, Bocchi R, Lotta S. Morphological changes in the skin microlymphatics in recently injured paraplegic patients with ilio-femoral venous thrombosis. *Paraplegia* 1995; **33:** 472 – 475.
12 Hopman MTE *et al*. Properties of the venous vascular system in the lower extremities of individuals with paraplegia. *Paraplegia* 1994; **32:** 810 – 816.
13 Bosse A. Clinical aspects, differential diagnosis and histogenesis of heterotopic ossifications. *Veroff Pathol* 1997; **146:** 1 – 168.
14 Snoecx M, De Muynck M, Van Laaere M. Association between muscle trauma and heterotopic ossification in spinal cord injured patients: reflections on their causal relationship and the diagnostic value of ultrasonography. *Paraplegia* 1995; **33:** 464 – 468.
15 Cassar-Pullicino VN *et al*. Sonographic diagnosis of heterotopic bone formation in spinal injury patients. *Paraplegia* 1993; **31:** 40 – 50.
16 Bodley R, Jamous A, Short D. Ultrasound in the early diagnosis of heterotopic ossification in patients with spinal injuries. *Paraplegia* 1993; **31:** 500 – 506.
17 Bollinger A, Pfister G, Hoffman U, Franzek UK. Fluorescence microlymphography in chronic venous incompetence. *Int Angiology* 1989; **8:** 23 – 26.