Alternative Therapeutic Strategy in Peripheral Arterial Disease of Lower Limbs

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Background: Treatment in peripheral artery disease consists mostly in symptoms management and measures to stop the progression of atherosclerosis. New therapeutic opportunities are opened by current research; they are based on angiogenesis induced by stem cell therapy and growth factor administration. Bone marrow is a rich tissue in cells and growth factors, and it was observed that in bone fracture hematoma, the angiogenesis is induced currently as a vascular response to injury.
Case report: A 42 year-old male patient with peripheral artery disease. The diagnosis was confirmed through angiography, and the surgeon confirmed the impossibility of revascularization. In consequence, the patient was proposed for vasodilatation treatment with prostaglandins. After one month with no improvement, the patient insisted to find a new possibility of treatment, thus we decided to use adult self stem cells from bone marrow. Sixty ml bone marrow aspirate was taken under local anesthesia from the iliac crest and injected intramuscular in the middle outer part of the thigh and gastrocnemian area in 38 injection sites. The patient was followed up for 6 months and we observed an improvement of clinical symptoms, walking perimeter and ankle brachial index.
Conclusion: This is the first case where we tried an innovative therapeutic strategy in a young patient with no other revascularization opportunity, with a benefic result.

Keywords: angiogenesis, fracture hematoma, peripheral artery disease

Received: 2 May 2012

Introduction

Peripheral arterial occlusive diseases are common in smokers or patients with metabolic and nutritional disorders (e.g., dislipidemia, diabetes, etc.) [1–3]. These cases are within the competence of vascular surgery specialists [4, 5]. In advanced situations, where the time of operation was exceeded, their treatment is within the field of internal medicine.

The aim of conservative therapy is to improve symptoms and extend the functionality of the vascular system in the affected segment. These cases often lead to necrosis of the lower limb, when the only therapeutic option is amputation [6–8].

In several cases revascularization of the affected segment can be attempted, using a complex treatment, which consists in the association of vasodilators (prostaglandin) [9] and the injection of autologous bone marrow cells, collected from the patient's iliac crest. This experimental treatment aims to increase peripheral perfusion due to vasodilatation and generation of new vessels, induced by injecting cells from bone marrow aspirate in the affected areas.

Case report

A 42 year-old male patient was admitted to our hospital for pain in his right lower limb. From the anamnesis we learned that a year before a perforating ulcer appeared on his left lower limb, which was treated symptomatically and consequently disappeared. Four months later pain appeared in the patient's right foot and the 2[nd] toe on his right foot was amputated. The patient's family history revealed an aggregation of cardiovascular diseases (father – venous insufficiency, mother – heart disease, etc.). Other aggravating factors were smoking, with an early age of onset (at the age of 12), smoking 40 cigarettes/day for 30 years, and an average consumption of alcohol of 50–75 mg/day.

On physical examination we found: thin and pale skin, right foot ulceration, gangrened toes (II and IV), absence of peripheral pulse in the popliteal, posterior tibial and pedal arteries of the right lower limb, and posterior tibial and popliteal arteries of the left lower limb. Doppler signal was not present at the tibial posterior artery and pedal artery of right limb, without any other abnormal signs concerning arterial impairment.

Following a clinical examination we hypothesized peripheral arterial impairment, and laboratory tests were performed to confirm the diagnosis.

The laboratory results on admission were as follows: hemoglobin: 12.9 g/dl; leukocyte count: 17,990/mm^3; platelet count: 405,000/mm^3; total protein: 8.6 g/dl. Liver enzymes were moderately elevated with the serum alanine transaminase (ALT) at 48 IU/l (normal 9–52 IU/l) and the serum aspartate transaminase (AST) at 37 IU/l (normal 10–55 IU/L). Serum creatinine was 1.04 mg/dl. The ESR was 64 mm/h (normal 1–22 mm/h), showing a slight in-

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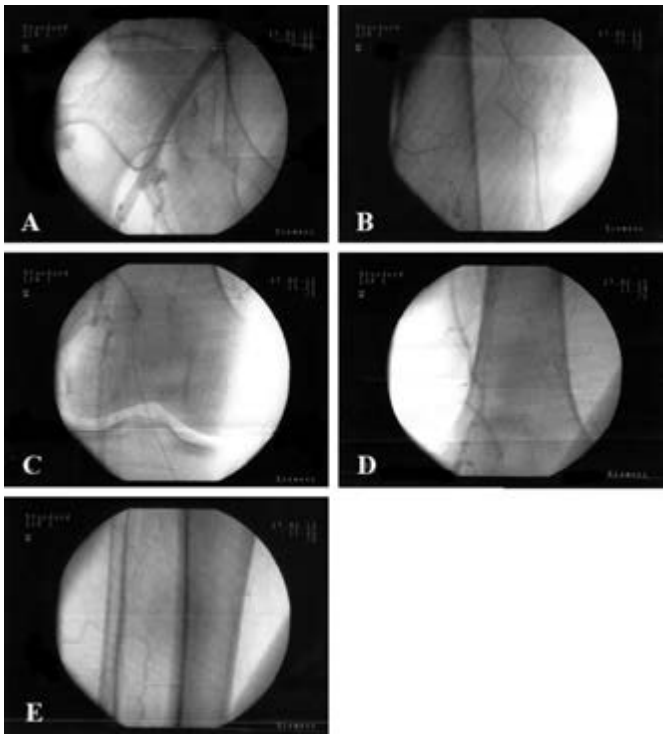

Fig. 1. Digital substraction angiography performed to confirm diagnosis and impossibility of surgical revascularization

flammatory syndrome in association with fibrinogen 414 mg/dl. There were no serological signs of HIV and HCV infection.

Doppler echocardiography, electrocardiography, chest radiograph revealed no other associated pathology. The ankle-brachial index was 0.1 for the right leg and 0.6 for the left leg, with a walk perimeter of 31 m.

Vascular ultrasound revealed atherosclerotic plaques and a 80% stenosis of the femoral common artery, with a flow velocity of 60 cm/s. In the profound femoral artery the flow velocity was 30 cm/s, in the superficial femoral artery there was no Doppler signal in the Hunter channel, the right popliteal artery had no lumen and numerous atherosclerotic plaques, the right genicular artery had a Doppler signal and a flow velocity of 50–60 cm/s.

We performed digital subtraction angiography, establishing the diagnosis of peripheral arterial disease. Superficial femoral artery thrombosis and the distal absence of significant circulation were assessed.

Vascular evaluation confirmed the impossibility to perform a surgical revascularization, and we initiated treatment with Vasaprostan® (alprostadilum) for 28 days, at a dose of 10 μg/day. Because of the worsening of pain, the patient insisted to try a new therapeutic option to stop the progression of ischemia in the distal extremities. After the patient signed a written informed consent, we applied an experimental therapy which involved injecting intramuscular bone marrow aspirate adjacent to the affected areas in order to induce angiogenesis.

The procedure was performed by collecting 60 ml of bone marrow aspirate from the iliac crest in self-prepared heparinized syringes, which was injected intramuscularly in the same session, in the middle outer part of the thigh and gastrocnemian area in 38 injection sites. The procedure was carried out without incidents, the patient reporting specific pain during the intramuscular injection.

The patient was reassessed periodically at 1, 3 and 6 months. In the first month the reevaluation revealed a discreet edema of the right leg, without improvement of the ankle-brachial index and vascular ultrasound. In this period the distal phalanx of finger II has been amputated. In contrast, after 6 months the patient reported reduced pain intensity and the walking perimeter was increased with 24 m. The ankle-brachial index was 0.3 in the right leg and 0.5 in the left leg.

Discussions

The effectiveness of vasodilator prostaglandins is approaching 50% [10,11]. This relatively low percentage mobilized our young patient to try a new treatment option besides prostaglandin.

The most important trials which studied methods to induce angiogenesis were the TRAFFIC study [12] and the RAVE trial for peripheral arterial disease [13]. Their results were variable, due to different methods of administration, administrated human produce etc.

Fracture hematoma is known to induce local angiogenesis. Human fracture hematoma contains the angiogenic cytokine vascular endothelial growth factor and has the inherent capability to induce angiogenesis and thus promote revascularization during bone repair [14], but this repair can also be used to treat other diseases, like peripheral arterial disease.

Bone marrow is constituted mostly of hematopoietic tissue, consists their precursors, adventitial/barrier cells, adipocytes, and macrophages. Hematopoiesis must be supported by a microenvironment that is able to recognize and retain hematopoietic stem cells and provide the factors (e.g. cytokines) required to support proliferation, differentiation and maturation of stem cells along committed lineages. The hematopoietic microenvironment consists of adventitial reticular cells (e.g., barrier cells), endothelial cells, macrophages, adipocytes, possibly, bone lining cells (e.g. osteoblasts) and elements of the extracellular matrix [15–17].

Stem cell therapy is known to be effective for inducing neoangiogenesis and treating peripheral arterial disease. The stem cells differentiate into endothelial cells, passing through a stage known as endothelial progenitor cells [18]. Vascular endothelial growth factor is also known to have a beneficial effect in the treatment of peripheral arterial disease [19].

In our case the patient presented a favorable evolution 6 months after the procedure, with an increasing walking perimeter and ankle-brachial index, associated with pain relief. The favorable evolution could have been confirmed by repeating the angiography, but the patient did not give his consent for the procedure.

Our results are in accordance with studies which attest that the angiogenic effect is mediated by significant concentrations of vascular endothelial growth factor found in the medically induced hematoma and the presence of non-hematopoietic stem cells in the bone marrow aspirate, which are capable to differentiate into endothelial progenitor cells and to stimulate and sustain new vessel formation.

Conclusions

The case we presented demonstrated the beneficial association of vasodilatator prostaglandins and intramuscular injection of bone marrow aspirate, which led to pain relief.

Our results suggest that this method can be safely used in patients who have no other therapeutic possibilities, because the usage of the patient's own cells to simulate a fracture hematoma means there is no rejection risk. The association of prostaglandins can also be useful in increasing blood flow.

This first case opens a new research opportunity in the area of human angiogenesis for the treatment of peripheral arterial disease.

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