Local implantation of autologous mononuclear cells from bone marrow and peripheral blood for treatment of ischaemic digits in patients with connective tissue diseases

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Objective. CD34-positive bone marrow mononuclear cells (MNCs) have been successfully used for regeneration of small arteries in Buerger’s disease. The objective of this study is to examine the angiogenetic potential of autologous MNCs from bone marrow and peripheral blood implanted into the ischaemic digits from patients with connective tissue diseases.

Methods. Three patients with systemic sclerosis, two with mixed connective tissue disease, and one with CREST syndrome were enrolled who had painful ischaemic digits with necrosis refractory to several vasodilators including intravenous prostaglandins. MNCs obtained from 7 ml/kg bone marrow blood and 400 ml peripheral blood were implanted into 20 different sites in palms and/or soles. The study was performed open-labelled.

Results. Pain in the numeric rating scale improved remarkably up to 1 month after implantation of bone marrow or peripheral MNCs to the same extent, although no significant differences were found in transcutaneous oxygen pressure and thermogram before and after the implantation. Bone marrow MNCs increased blood flow of the hand determined by intra-arterial digital subtraction angiography, while peripheral MNCs did not.

Conclusions. Implantation of autologous MNCs from peripheral and bone marrow into the ischaemic digits was so effective in pain-relief and more clinical trials would be warranted to see whether this could be a new treatment modality for angiogenesis in connective tissue diseases as in Buerger’s disease.

Key words: Connective tissue disease, Bone marrow mononuclear cell, Peripheral mononuclear cell, CD34-positive cell, Angiogenesis.

Introduction

Damage to the vascular endothelium leading to arteriolar stenosis and occlusion are observed in hands and feet from patients with connective tissue diseases. Without successful treatment, ulceration and necrosis often ensue and a mandatory amputation is not infrequent [1]. Vasodilators such as calcium channel blockers and prostaglandins have been used most frequently for their treatment, although with very limited effects, especially in severe cases [2–4].

A group of angiogenic growth factors, such as basic fibroblast growth factor, angiogenic growth factor, and endothelial growth factor were isolated and their amino acid sequences were determined in the 1980s. In the 1990s, therapeutic trials using these angiogenic factors were performed with great success in experimental models of ischaemic heart disease and limb ischaemia [5–7]. It was shown, subsequently, that functional vascular endothelial growth factor (VEGF) was secreted along with angiogenesis in myocardium and skeletal muscles of rats after implantation of adenoviral vectored VEGF gene [8, 9]. On the other hand, autologous bone marrow-derived mononuclear cells implanted locally were shown very efficacious in improvement of limb ischaemia of Buerger’s disease in the human clinical trial, termed the TACT study [10].

We performed a therapeutic trial on six patients with connective tissue diseases who had severe ischaemia and necrosis in their fingers and/or toes to examine the angiogenic potential of mononuclear cells (MNCs) from their bone marrow and peripheral blood. The results of 1-yr follow-up are reported here.

Patients and methods

Six Japanese patients were enrolled in this trial: case 1, 49-yr-old woman with systemic sclerosis for 27 yrs; case 2, 64-yr-old man with systemic sclerosis for 9 yrs; case 3, 60-yr-old man with mixed connective tissue disease for 4 yrs; case 4, 51-yr-old woman with systemic sclerosis for 18 yrs; case 5, 47-yr-old woman with mixed connective tissue disease for 11 yrs; and case 6, 72-yr-old man with CREST syndrome for 14 yrs. They all had long-standing intractable digital ulcers and finger necrosis with severe pain despite use of vasodilators including intravenous prostaglandins. According to their previous histories, amputation of the ischaemic digits was highly probable if left untreated.

Under general anaesthesia, 7 ml/kg bone marrow blood was aspirated from iliac bones and MNCs were purified by centrifugation on Ficoll-Hypaque (Axis Shield, Oslo, Norway) as reported previously [11]. Purified MNCs were suspended in 20 ml of RPMI1640 and aliquots of 0.5 ml were implanted intramuscularly at 20 different sites in palms and/or soles. Red cells were recovered and returned to the patients.

Peripheral blood of 400 ml was withdrawn from individual patients and MNCs were purified and implanted in the same manner as for MNCs from bone marrow. MNCs from bone marrow and those from peripheral blood were implanted on the opposite side to compare the difference in effectiveness. The side with more severe lesion received bone marrow MNCs, although the patients were blinded on this.
The evaluation of effectiveness included: (1) numeric rating scale for evaluating the improvement in pain made in 11 levels from 0 to 10 [12]; (2) transcutaneous oxygen pressure (TcPO2) by PO-850 (Sumitomo-Hightechs, Tokyo, Japan) for the assessment of peripheral blood flow; (3) thermography by TH3106ME (NEC San-ei Instruments, Tokyo, Japan) to measure the skin surface temperature on hands and feet; and (4) intra-arterial digital subtraction angiography (IADSA) before and 2 months after the implantation. Items (1)–(3) were performed pretreatment, 1 week, 2 weeks, 1 month, 6 months and 1 yr after the implantation. The assessor was different from the implanter, although the blindness between the two was not complete.

This study design was deliberated and accepted by the Jichi Medical University Ethics Committee. Every patient gave a written consent on the purpose of this clinical trial.

Results
An average of $7.7 \times 10^8$ ($5.0 \times 10^8$ to $10.8 \times 10^8$) MNCs was isolated from 7 ml/kg bone marrow blood with an average recovery of $3.5 \times 10^6$ ($2.85 \times 10^6$ to $5.33 \times 10^6$) CD34-positive cells. Likewise, an average of $3.5 \times 10^5$ ($1.0 \times 10^5$ to $4.8 \times 10^5$) MNCs was isolated from 400 ml peripheral blood with an average recovery of $0.14 \times 10^9$ ($0.01 \times 10^9$ to $0.37 \times 10^9$) CD34-positive cells.

Relief of pain as judged by the pain scale was achieved in all but one patient in a week, and continued up to one month in four patients. However, pain continued at the baseline level throughout the study period in one patient (case 5). Bone marrow and peripheral MNCs brought about the similar level of pain-relief; Fig. 1A depicts changes in pain level on the side of the body where bone marrow MNCs were implanted. Pain-relief remained satisfactory in three patients (case 2, 4 and 6) until 1 yr; however, pain gradually returned to the pretreatment level in two patients (case 1 and 3).

As shown in Fig. 1B, TcPO2 slightly increased in two patients (cases 4 and 6) before 2 weeks after implantation of bone marrow MNCs, although the courses thereafter were not obtained. Case 2 gained a gradual increase in TcPO2 beyond 1 month, and kept increased levels until 1 yr. This might be responsible for the perfect pain control observed in him. TcPO2 measurement on the side implanted with peripheral blood MNCs showed an increase similar to that of bone marrow MNCs (data not shown).

Skin surface temperature judged by thermography did not change remarkably throughout the study period except in one patient (case 4). It decreased and stayed lower than before up to 1 yr in the patient (case 2) who completely lost pain by the end of 1 yr. Fig. 1C shows the thermogram on the side of bone marrow MNCs. The peripheral blood MNCs' side showed similar improvements (data not shown).

IADSA performed before and 2 months after the implantation did not change significantly in five patients. In the remaining one patient (case 6), however, IADSA imaged an increased arterial flow up to the tip of fingers (Fig. 2B) on the bone marrow MNCs' side, where the arterial blood flow was recognized only slightly distal to the arcuate artery before the implantation (Fig. 2A). In case 6, implantation of the peripheral blood MNCs did not increase arterial blood flow to the level that could be visualized in IADSA (data not shown).

Discussion
Remarkable pain-relief was obtained in case 4 immediately after the implantation and pain-relief in satisfactory levels was obtained in the remaining four patients within 2 weeks. However, no changes in the pain level were observed throughout the study period in case 5. The difference between bone marrow and peripheral blood MNCs was not appreciable. TcPO2 did not show any constant trends after the implantation. It was kept at a slightly increased level in case 2 whose pain was dramatically improved, while it fluctuated and became even lower than the pre-implantation level 6 months after the procedure in case 6; his finger pain remained in satisfactory levels.

The surface temperature of fingers and/or toes measured by thermography did not increase significantly; it decreased even in the patient in case 2 who gained remarkable reduction in pain.

A point which came as a surprise to us was no difference in the pain scale, TcPO2, or thermography achieved between MNCs from bone marrow and peripheral blood, in sharp contrast to the results in Buerger’s disease in the TACT study. The implantation of CD34-positive bone marrow cells was quite effective in pain-relief and recovery of circulation detected by IADSA in Buerger’s disease in comparison with MNCs from peripheral blood [10]. Because the number of CD34-positive cells from bone marrow blood was much higher than that of those from peripheral blood, they did not seem to be the main cell population effective in rebuilding the vasculature of ischaemic digits in patients with connective tissue diseases. The number of circulating endothelial progenitor cells increases in the early stage of systemic sclerosis, although their number in bone marrow decreases and they are functionally impaired [13]. This could be another possibility and needs to be clarified in the future trial.
In this study, only one patient accomplished an increased vasculature with collateral vessels detectable by IADSA in the hand of the side where bone marrow MNCs were implanted. However, it was not clear whether collateral vessels were newly developed or merely re-opened to recover from a collapsed state. Tateishi-Yuyama et al. [10] reported in the TACT study that collateral vessels were visualized by IADSA in 27 of the 45 (60%) patients with Buerger’s disease; we followed the similar procedure, although the implanted cell number was a little lower in this study. New vascular formation and its maintenance would be more difficult in the arterial obstruction due to connective tissue diseases than Buerger’s disease.

Pain-relief was not apparently accompanied by concomitant increase in TcPO$_2$ or surface skin temperature. Although the precise mechanism remains to be clarified, it is conjectured that even a small increase in microvasculature around nerve endings might be effective for reduction of the pain induced by ischaemia. Such an increase would be too small to be detected by thermography or arteriography, because a visible increase in IADSA was observed in only one patient (case 6). Such newly developed microvasculature may soon be obliterated, since pain returned to the pretreatment level in two patients 1 yr after the implantation. This contrasted sharply with the results of the TACT study, in which pain-relief lasted much longer and re-vascularization was clearly visible in more than half of the patients [10].

The importance of CD34-positive cells in angiogenesis was not ascertained in patients with connective tissue diseases in the present study. MNCs from bone marrow contained more than 20 times higher number of CD34-positive cells than those from peripheral blood. Despite this fact, pain-relief obtained by the implantation of peripheral blood MNCs was almost in the same level as that of bone marrow MNCs. Hence, CD34-positive cells from peripheral blood might be more efficacious than those from bone marrow. We have not obtained the evidence that the implanted cells were functioning at the site of injection or they were involved in new angiogenesis as endothelial progenitors. It is possible that not cells but various cytokines or pro-inflammatory substrates, released by the implantation of CD34-positive and CD34-negative MNCs, might contribute to pain-relief as well as the generation of microvasculature.

This study was performed in the small number of patients and was not blinded in a strict sense. The data obtained were not necessarily uniform. However, clinical effectiveness of pain-relief was satisfactory and a new clinical trial is now under way in our division to examine the effectiveness of repeated implantations of MNCs.

**Rheumatology key messages**

- Mononuclear cells from patients’ own bone marrow and peripheral blood were locally implanted to facilitate angiogenesis in ischaemic digits.
- The pain-relief was satisfactory, although thermogram and angiogram showed inconsistency.

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**References**