CASE REPORT

Improving ischaemic skin revascularisation by nerve growth factor in a child with crush syndrome

A Chiaretti, M Piastra, E Caresta, L Nanni, L Aloe

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Nerve growth factor (NGF) is the first described neurotrophin that stimulates the growth and differentiation of nerve cells and promotes skin and peripheral tissue regeneration. Recent studies suggest that NGF influences endothelial cell proliferation and angiogenic activity. In view of these proposed regenerative effects, we evaluated the efficacy of subcutaneous administration of highly purified murine NGF in a child with severe crush syndrome of the lower left limb. NGF 10 μg was administered subcutaneously every eight hours for seven days to the extensive ischaemic skin lesion of the calcaneal area. After treatment we observed gradual improvement of the ischaemic area; no side effects were noted. The child was discharged in good clinical condition to await a limited calcaneal escharotomy.

Nerve growth factor (NGF) was the first characterised neurotrophic factor and is structurally related to other growth factors such as brain derived neurotrophic factor (BDNF) and neurotrophins 3 and 4/5.[1] It plays a crucial role in promoting growth, differentiation, and function in sympathetic nerve cells.[1] NGF has been described as a "pleiotropic" molecule, involved in a variety of peripheral actions such as neuropeptide expression regulation, skin physiology, and peripheral tissue regeneration.[2] Moreover NGF has a potent anti-inflammatory effect; it is 10 times more active than dexamethasone and 1000 times more active than non-steroidal anti-inflammatory drugs in animal models.[1] Recent evidence suggests that NGF might influence endothelial cell proliferation and angiogenic activity.[3] The known effects of NGF on connective tissue and endothelial cells support this. It has also been suggested that NGF stimulates the production of vascular endothelial growth factor (VEGF), the most powerful mitogen for endothelial cells that promotes angiogenesis and blood permeability, in peripheral sensory neurones.[3] The action of vascular growth in human tissues proceeds via two distinct pathways, sprouting of capillaries (angiogenesis) and in situ proliferation of pre-existing arteriolar connections into true collateral arteries (arteriogenesis). Whereas angiogenesis produces capillary networks, arteriogenesis is the process responsible for the supply of blood to ischaemic tissues. Recent work shows that NGF promotes enlargement of pre-existing capillaries in the sympathetic ganglia of developing rodents, suggesting that NGF might be implicated in promoting endothelial cell growth within the nervous system.[4] Consequent on these hypotheses we evaluated the efficacy of subcutaneous administration of highly purified murine NGF in a child with a peripheral ischaemic area following a severe crush syndrome.

CASE REPORT

A 5 year old child was referred to our paediatric intensive care unit with crush syndrome following a road traffic accident. On arrival in a peripheral hospital, the child was diagnosed as having hypovolaemic shock as a result of splenic and left kidney rupture, left pulmonary contusion, left femoral fracture, and severe head injury with Glasgow Coma Score 7. After being stabilised, the child underwent splenectomy and left nephrectomy, and was then transferred to our paediatric intensive care unit by heliambulance. On admission the child was intubated and ventilated, with severe persisting hypovolaemic shock and hypoperfused/hypothermic extremities. Lower limb pulses were just detectable, best in the lower left limb. After further circulatory support, cranial and cervical spine computed tomography was performed, showing two small lacero-contusions in the frontal lobes. We undertook our standard approach for severe head injury, including $Paco_2$, intracranial pressure, and cerebral perfusion pressure monitoring and control, early post-traumatic seizure prophylaxis, etc.

x Ray examination confirmed a left pulmonary contusion and left femoral dislocated fracture. Traction was applied to the left thigh, but a few hours after resolution of hypovolaemia, the left lower limb still appeared severely hypoperfused and marbled. Spontaneous mobility was absent even after application of painful stimuli and no arterial pulses were felt. The left foot was generally oedematous with peripheral ischaemic zones. A Doppler ultrasound scan failed to detect any arterial flow at the level of the left popliteal artery. Flow reduction of the superficial femoral artery was shown, with a good collateral circulation. At arteriography the superficial femoral artery was interrupted (fig 1); the interosseal and anterior tibial arteries were seen together with good collateral perfusion. Impaired tactile, heat, and pain sensitivity was detected on electromyography of the left lower limb; absence of voluntary muscle activation with denervated muscle fibres was also present.

On day 2, cyanosis and ischaemia of the left foot worsened and fasciotomy was performed on the gastrocnemius muscle. Limb perfusion did not improve and two days after fasciotomy the lesions of the heel and toes worsened, with likely extensive foot necrosis. We decided to treat her with topical NGF, after obtaining approval of the university's Institutional Review Board and parents' informed consent. We selected the calcaneal region for NGF infiltration because of clearer evidence of a border to the ischaemic area. One mg of highly purified murine NGF was diluted in 5 ml of normal saline; we administered 50 μl of this solution every eight hours for seven days subcutaneously at the junction between healthy skin and the ischaemic area of the heel (fig 2A, arrows). The lesion gradually improved (fig 2B), while untreated areas progressed to necrosis. No side effects were observed during treatment.

Abbreviations: BDNF, brain derived neurotrophic factor; NGF, nerve growth factor; NO, nitrous oxide; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cells

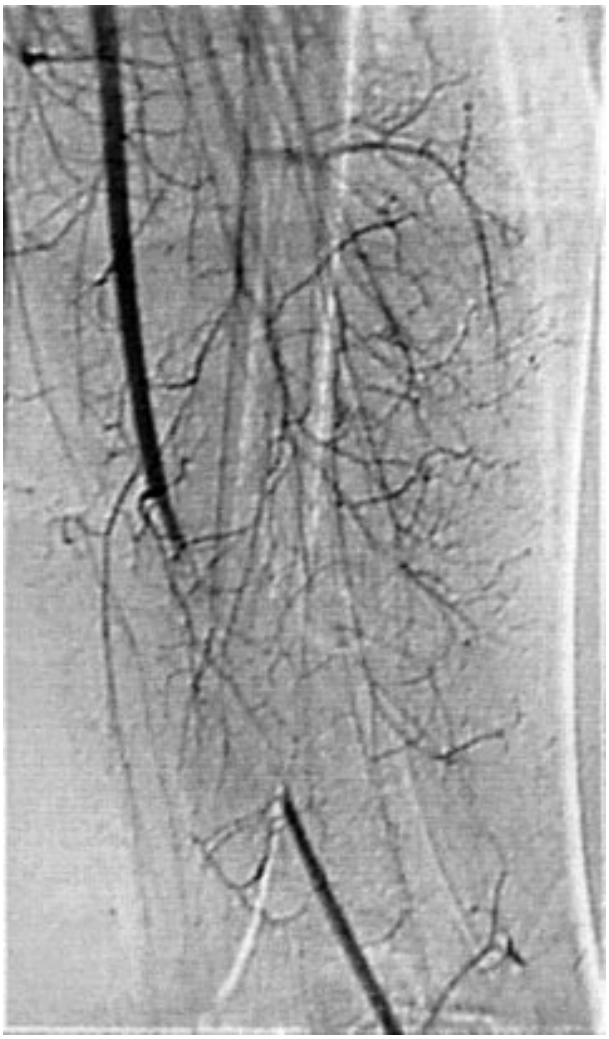

Figure 1 Arteriographic imaging showing superficial femoral artery interruption and collateral circulation.

On day 20 she was discharged in good clinical condition after a femoral osteosynthesis procedure (Glasgow Outcome Score 5). At the time of writing, she awaits a limited calcaneal escharotomy.

DISCUSSION

Angiogenesis, the sprouting of new capillaries from pre-existing blood vessels, is completed around postnatal day 20 and is normally inhibited in mature tissues. However, several non-tumoral conditions can trigger neoangiogenesis in nervous tissue by tilting the balance to activation between angiogenic and angiostatic factors. Angiogenesis also occurs in nerve cell grafts and in spinal cord lesions during peripheral nerve regeneration. Angiogenesis is also triggered by physiological tasks that increase neural function and synaptic activity. Overall, this indicates that migration and proliferation of endothelial cells, remodelling of extracellular matrix, and functional maturation of newly assembled vessels can also be triggered in postnatal non-tumoral nervous tissue. Recent studies suggest that angiogenesis is triggered by an increase of NGF, that promotes neurone induced angiogenesis by stimulating VEGF production.[5] A number of stimuli may induce VEGF expression, including several growth factors, cytokines, hormones, oncogenes, nitrous oxide (NO), and hypoxia through hypoxia inducibile factor.[5] In addition, neuronal VEGF expression has been found to correlate with angiogenesis. Moreover BDNF also seems to exert a direct angiogenic action on endothelial cells in the heart, whereas local NGF administration has proved to increase angiogenesis during sciatic nerve regeneration. The effect of NGF on NO production also supports this hypothesis, because a single NGF injection induces a strong up-regulation of the neuronal isoform of NO synthesis enzyme and of the associated histochemical NAPDH-diaphorase reaction. This is followed by VEGF induced angiogenesis and consequently by a reduction of NO synthesis enzyme production. This pattern of coordinated actions between NO and VEFG has been shown in different tissues under different physiological and pathological non-tumoral conditions. These findings suggest that angiogenesis may be regulated through activation of angiogenic and vasodilatation agents directly produced by neurones and that NGF indirectly stimulates this process. However, the presence of NGF and its receptors in vascular smooth muscle

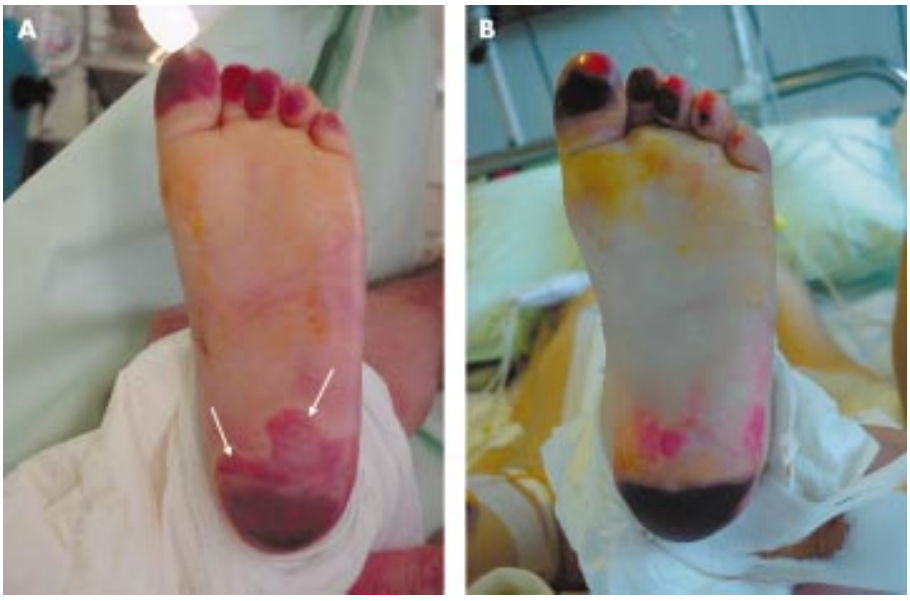

Figure 2 (A) The ischaemic area at the heel, shown by arrows, 48 hours after fasciotomy. (B) Evolution of the necrotic area at the heel (pre-escharotomy), with good revascularisation of pre-existing ischaemic area after one week NGF treatment. Ischaemic lesions of the toes persist unchanged in size, and formation of bullous lesions can be observed.

cells (VSMC) suggests that this neurotrophic factor can play a paracrine/autocrine function in the vascular system and that repeated subcutaneous injections of NGF in the ischaemic hindlimb can accelerate the process of revascularisation, by increasing the number of arterioles.[4] Whether this effect depends on direct NGF action or is secondary to the induction of other growth factors, is still unknown. However, the presence of trk-A (the high affinity receptor for NGF) on VSMC and the evidence that NGF is able to induce cultured VSMC migration, suggests a role of NGF in the development of arterioles. Conversely, since in peripheral sensory neurones NGF stimulates the production of VEGF, it is possible that this neurotrophic factor could directly or indirectly accelerate angiogenic activity.[3] Thus, NGF alone or in combination with other endogenous molecules, can exert its action on endothelial cells and on angiogenic processes. In animal models an increase in length and density of arterioles has been shown in muscle biopsy performed after seven days of subcutaneous NGF administration following induced hindlimb ischaemia.[6]

We administered NGF to our patient after the failure of fasciotomy, with an impending risk of massive foot ischaemia. NGF infiltration was followed by improvement of vascularisation in a broad area of the heel (starting 4–5 days after the first NGF administration), and necrotic evolution ceased. The beneficial effect of NGF is supported by our observation that there was no improvement of the ischaemic lesions of the toes and no decrease in the size of the necrotic area. Moreover, following NGF administration there was good re-epithelisation without any scar formation. Finally, we observed no side effects. Because our case is the first to be described, it is difficult to exclude whether or not NGF anti-inflammatory effect or spontaneous recovery played the dominant role in the marked improvement of the ischaemic lesion. However, we are encouraged in our view that NGF was responsible, in that it has been adopted succesfully for the treatment of human corneal ulcers resistant to steroids and immunosuppressive agents, reducing cell damage and promoting epithelial healing.[7]

In conclusion, we suggest that our experience with NGF treatment should encourage further investigations of its mechanism of action on ischaemic skin and its potential use in other peripheral vasculopathies. The specific effect of NGF on new blood vessel development requires further characterisation, and further studies are needed to elucidate other possible uses of NGF. Nevertheless, the possibility to selectively promote arteriolar formation and epithelial healing may have a role in designing strategies to induce therapeutic angiogenesis, for example in burns and other skin lesions, mostly in the immediate post-injury period.

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