Granulocyte-macrophage colony-stimulating factor in foot ulcers

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Introduction

Following encouraging reports on the use of granulocyte-macrophage colony-stimulating factor (GM-CSF) to treat wounds in animals and humans, we conducted a randomised placebo controlled study to establish the usefulness of this drug in treatment of chronic foot ulcers in type 2 diabetes. People who received GM-CSF healed significantly faster than those in the placebo group. We observed no significant side-effects or changes in haematological and biochemical parameters studied. Additional treatment with GM-CSF had further beneficial effects in healing chronic foot ulcers in people with type 2 diabetes.

The diabetic foot is mainly a consequence of peripheral neuropathy, arteriopathy and super-imposed infection. Poor wound healing results from neuropathy, ischaemia and prolonged hyperglycaemia. The care of chronic foot ulcers in diabetes has become a major health problem. Foot ulceration precedes the majority of amputations in diabetes and these ulcers are responsible for more than 50% of major limb amputations (Reiber et al, 1992). Hyperglycaemia is directly related to increased susceptibility to infection (Hostetter, 1990). The polymorphonuclear leucocyte function is abnormal and there is reduced chemotaxis and phagocytic activity.

Successful wound healing represents the sum of a sequence of basic process including inflammation, cell proliferation, matrix formation and remodelling, contraction and epithelisation (Arnold and West, 1992; Clark, 1985). Research has shown that fluids obtained from wounds contain a number of growth factors produced by cells involved in wound repair, such as neutrophils, platelets, macrophages, fibroblasts and endothelial cells (Katz et al, 1991). One of these is granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF causes activation of neutrophils, macrophages and attracts the inflammatory and endothelial cells and inhibits their migration from the site (Witmer-Pack et al, 1987). The assumption that GM-CSF might play a regulatory role in normal wound healing and that its application might improve impaired wound repair led to its experimental use as an exogenous 'healing drug'.

Management of the diabetic foot includes improvement of nutrition. Risk factors like smoking, hypertension, hyperlipidaemia should be under strict control. People should be educated about footwear choice and regularly visit a chiropodist. In recent years, several new treatment strategies have been developed to stimulate wound healing in diabetic foot ulcers. These are topical growth factors, extra cellular matrix products, bioengineered human skin, hyperbaric oxygen therapy and GM-CSF.

GM-CSF is a multipotential haematopoietic growth factor that stimulates proliferation and differentiation of early and late granulocytes, erythroids and megakaryocyte precursor cells. It was originally purified from cultured human cell lines. It is produced in a yeast expression system using recombinant DNA technology (Bertrom et al, 1997). Adverse effects of GM-CSF are generally infrequent and mild, but severe side-effects are seen when high doses are used.

Due to the beneficial quality of the wound healing properties of GM-CSF, we decided to use a perilesional injection of GM-CSF in people with type 2 diabetes and non-healing ulcers.

Material and methods

Selection of participants

A total of 32 people with type 2 diabetes and chronic foot ulcers were randomly selected for the study. After explaining the

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1 GM-CSF is a multipotential haematopoietic growth factor that stimulates proliferation and differentiation of early and late granulocytes, erythroids and megakaryocyte precursor cells.

2 The authors used a perilesional injection of GM-CSF in people with type 2 diabetes and non-healing ulcers.

3 The mean ulcer size decreased more in the study group than in the placebo group after 12 weeks of treatment.

4 Side-effects observed were infrequent, mild and well-tolerated.

5 Although expensive, using GM-CSF is logical considering the cost of hospitalisation and the gains that are achieved if we can prevent amputation.

KEY WORDS

- **GM-CSF**
- **Non-healing ulcers**
- **Amputation**
- **Side-effects**
- **Expense**

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1 Selected participants were evaluated for the presence of microvascular and macrovascular complications such as coronary artery disease, cerebrovascular disease, peripheral vascular disease, retinopathy, nephropathy and neuropathy.

2 The diabetic foot ulcer was assessed by detailed clinical examination; Wagner's classifications were used to classify individual participants.

3 Reconstituted GM-CSF was injected subcutaneously in four perilesional quadrants, 0.5cm from the edge of the wound.

4 In participants with more than one ulcer, all ulcers were treated separately.

5 Treatment was given once a week for 3 consecutive weeks, or until ulcer healing (whichever occurred first).

procedure and theme of the study, informed written consent was taken prior to enrolment. Institutional ethical committee approval was also obtained.

The participants were divided into two groups (n=16). Those who received the injection of GM-CSF were labelled as the 'study group' and those who received the saline injection were labelled as the 'control group'. Diabetes was diagnosed according to the American Diabetes Association revised criteria (ADA, 1998). Blood glucose estimation was carried out by the glucose oxidase method in a venous blood sample.

Patient population

Inclusion criteria:

- Adults aged 30 years and above of both sexes.
- Chronic foot ulcers of more than 3 months duration.

Exclusion criteria:

- Known hypersensitivity to any component of the drug.
- An obvious active infection (systemic, of the ulcers or of the underlying bone). A diagnosis of infection was made if there was purulent discharge and/or two local signs (warmth, erythema, lymphangitis, lymphadenopathy, oedema and pain).
- An active neoplastic disease.
- Immunosuppressive treatment in the last 3 months.
- Any serious pre-existing cardiovascular, pulmonary or immunological disease.
- Pregnant women and lactating mothers.

The selected participants were evaluated for the presence of microvascular and macrovascular complications such as coronary artery disease, cerebrovascular disease, peripheral vascular disease, retinopathy, nephropathy and neuropathy. Body mass index and waist hip ratio was measured in every participant. Neuropathy was diagnosed by history of numbness, paraesthesia, tingling sensation and burning sensation. Neuropathy was confirmed by touch sensation, vibration sense and ankle reflex. Peripheral vascular disease was diagnosed by definitive history of intermittent claudication or if one or more of the peripheral pulses was absent in both feet, and graded according to ankle brachial pressure index (ABPI) by Doppler. HbA_{1c} was measured by high performance liquid chromatography. Lipid profile status was detected using a semiautoanalyser.

We assessed the diabetic foot ulcer by detailed clinical examination and followed Wagner's classifications to classify individual participants (Wagner, 1983). Each eligible participant had a thorough clinical and biochemical examination. The fundamentals points of caring for diabetic foot ulcers are off-loading (i.e. relief of pressure) and frequent dressing. Smoking was not allowed during the study period.

Dosage and administration

The study group received 400 µ g of reconstituted GM-CSF. This was injected subcutaneously in four perilesional quadrants, 0.5 cm from the wound edge. The injected site was compressed to avoid spillage of the drug. The control group received 1 ml of normal saline, administered in the same manner as in the study group.

Reconstitution of GM-CSF: 1 ml of sterilised water was added to the vial of GM-CSF. The vial was gently swirled to dissolve the powder completely. This provided 400 µ g of GM-CSF as an isotonic solution, which was used for subcutaneous administration. In participants with more than one ulcer, all ulcers were treated separately.

Treatment duration: treatment was given once a week for 12 consecutive weeks, or until ulcer healing (whichever occurred first).

Evaluation of response: the two longest perpendicular dimensions of the ulcers were recorded at baseline and weekly thereafter (i.e. on weeks 1–12). Any untoward effects such as local pain, local pruritis, body-aches and leucocytosis were recorded every week.

Primary study endpoints

At the end of the study period, patients were categorised as:

- Complete responders (complete healing of foot ulcers).
- Partial responders (50% or greater reduction in ulcer area of the two longest perpendicular diameters from baseline).
- Non-complete responders (less than 50%

reduction in the ulcer area of the two longest perpendicular diameters from baseline).

- Non-responders (no reduction in ulcer area or increase in ulcer area over baseline).

Results

The demographic data of the study group and control group were similar in age with an average of 58.5 years and 55.6 years for GM-CSF and placebo groups respectively. There was a clear predominance of men in both groups with a male/female ratio of 12/16 and 11/16 in the GM-CSF and placebo groups respectively.

Baseline characteristics of the study group as well as the control group were matched in all the variables including ABPI and Wagner's grading of diabetic foot ulcers and neuropathy except ulcer size area. However, this was unlikely to affect our results because the bigger sized ulcers were present in the study group. There was no difference in haemoglobin levels or biochemical profiles at the beginning of the study, week 1, week 3 or at the end of the study.

In the study group the mean ulcer area decreased from 57.87 cm^2 at the start of treatment to 1.38 cm^2 by week 12. In the control group the mean ulcer area at the start of treatment was 30.93 cm^2 which decreased to 5.09 cm^2 by week 12 (see *Figure 1*).

Table 1 shows the healing response in the study and control group. At the end of the study, there were 14 complete responders and two partial responders in the study group. In the placebo group there were four complete responders and 12 partial responders.

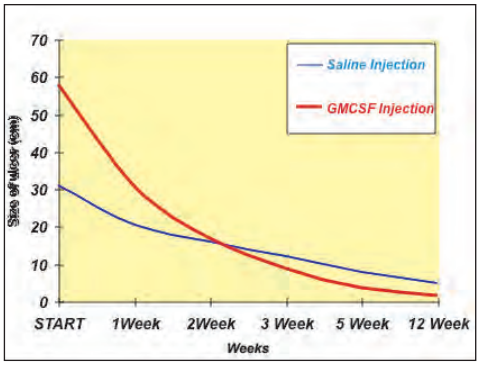

Figure 1. Effect of GM-CSF versus saline treatment on the size of ulcers.

Healing of diabetic foot ulcers may have been adversely affected by duration of diabetes, duration of ulcer and poor glycaemic control (Oyibo et al, 2001; Margolis et al, 2000). However, due to the small size of the participant group used in the study we could not statistically prove these points.

As side-effects, participants reported local pruritis and fever for some days after the injection. Such occurrences were more frequent and lasted longer for people in the GM-CSF treated group. We also observed neutrophilia in the GM-CSF treated group (see *Table 2*).

Discussion

Diabetic foot ulcers cause pain and suffering and may take many months to heal. They lead to loss of working hours, hospitalisation and great expense both to patients and the community. Different treatment regimens have been used to treat diabetic foot ulcers: debridement; different anti-infective wound

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1 The demographic data of the study group and control group were comparable in age with an average of 58.5 years and 55.6 years for GM-CSF and placebo groups respectively.

2 In the study group the mean ulcer area decreased from 57.87cm^2 at the start of the treatment to 1.38cm^2 by week 12.

3 In the control group the mean ulcer area at the start of treatment was 30.93cm^2 which decreased to 5.09cm^2 by week 12.

4 Side-effects were more frequent and lasted longer for people in the GM-CSF treated group.

Table 1. Healing response in study and control group

	Complete responder		Partial responder		Non-complete responder		Non-responder	
	S group	C group	S group	C group	S group	C group	S group	C group
Week 0	0	0	0	0	0	0	16	16
Week 1	1	0	7	4	8	12	0	0
Week 2	2	1	14	6	0	9	0	0
Week 3	8	1	8	10	0	5	0	0
Week 5	9	2	7	13	0	1	0	0
Week 12	14	4	2	12	0	0	0	0

S group = study group C group = control group

Table 2. Distribution of adverse effects in the study and control groups.

Adverse effects	Study group		Control group		t value	p value
	Number	%	Number	%		
Fever/malaise	2/16	12.50	0	0	-1.46	0.154
Local pruritis/burning	4/16	25.0	0	0	-2.24	0.03
Neutrophilia	8/16	50.0	0	0	-3.87	0.0005
Arthralgia/myalgia	1/16	6.25	0	0	-1.00	0.325
Allergic reaction	1/16	6.25	0	0	-1.00	0.325

dressings; antibiotics according to culture and sensitivity; and skin grafting.

Even after using different modes of treatment, the treatment failure rate is very high. Participants who received GM-CSF did better, as healing was quick and significant in comparison to the placebo group. The response of the perilesional injection of GM-CSF was evident even in the first week of treatment, and a significant response was observed by week 12. Of the particpants in the GM-CSF group, 14 (87.50%) achieved complete healing and 2 (12.50%) achieved partial healing. In the control group, only 4 (25%) participants achieved complete healing and 12 (75%) participants had partial healing. Similar observations were made by Da Costa et al (1994), Arnold and Cherry (1995), Marques da Costa et al (1997), Jaschke et al (1999) and Groves et al (2000) in chronic venous leg ulcers of diverse actiology.

Similar results were observed using GM-CSF injection in chronic non-healing ulcers from Pieters et al (1995), Hui et al (1996), Raderer et al (1997) and Borbella-Escoboza (1997). Recently, Viswanathan et al (2003) studied the role of granulocyte colony-stimulating factor (G-CSF) in diabetic foot patients who had extensive cellulitis. They observed a significant (90%) improvement using this treatment. In contrast to these studies, Kastenbauer et al (2003) concluded that additional treatment with G-CSF had no further beneficial effect.

In this study, the mean ulcer size area was greater in the study group than the control group. This may be because of the small cohort (n=16) or due to the Hawthorn effect (when participants self-select themselves to the drug group or they improve because they are looked after well as a consequence of being part of the study). The presence of big ulcers in the study group is not likely to affect the results.

As a side-effect, participants reported itching of the wound area for some days after the injection. We did not observe any significant side-effects with the use of the drug. Those observed were infrequent, mild and well-tolerated by participants.

The results regarding the outcome of GM-CSF injection are very encouraging. The favourable outcome is mediated through increasing the defence mechanism by stimulating and differentiation of early and late granulocytes, erythroids and megakaryocyte precursors cells. GM-CSF is currently an expensive treatment in India and in other countries. However, if we consider the cost of hospitalisation and gains that are achieved if we can prevent amputation, then this treatment seems to be logical. In addition GM-CSF can be safely recommended for chronic non-healing diabetic ulcers.

Conclusion

Additional research is needed to define the specific indications and benefits of GM-CS. Randomised placebo controlled clinical trials in large diabetic populations would further lend credence to the assertion that GM-CSF treatment improves clinical outcomes. It is, however, difficult to conduct a perfectly controlled study of GM-CSF treatment in chronic foot ulcers because of the numerous clinical variables that affect wound healing. ■

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1 Diabetic foot ulcers cause pain and suffering and may take many months to heal; they lead to loss of working hours, hospitalisation and great expense both to patients and the community.

2 Of the particpants in the GM-CSF group, 87.50% achieved complete healing and 12.50% achieved partial healing.

3 In the control group, 25% participants achieved complete healing and 75% participants had partial healing.

4 The favourable outcome is mediated through increasing the defence mechanism by stimulating and differentiation of early and late granulocytes, erythroids and megakaryocyte precursors cells.

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