Short Communication

Evaluation of granulocyte-colony stimulating factor (Filgrastim) in infected diabetic foot ulcers

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Abstract

Aims/hypothesis. To re-evaluate the use of Granulocyte-Colony Stimulating Factor (G-CSF) in the treatment of infected diabetic foot ulcers.

Methods. Thirty-seven diabetic subjects were randomised to Granulocyte-Colony Stimulating Factor (G-CSF) ($n=20$) or placebo ($n=17$). The primary endpoint was resolution of cellulitis, which was evaluated clinically and with an infection summary score. Patients were hospitalised for 10 days and received subcutaneously either 5 µg/kg G-CSF or placebo daily. Ulcers were treated with a standard wound protocol and the patients were instructed to stay in bed. All subjects received antibiotics (clindamycin and ciprofloxacin) intravenously until the inflammation had subsided.

Results. Patients who received G-CSF did not have an earlier resolution of clinically defined cellulitis ($p=0.57$). The infection summary score declined, but comparably, in both groups (G-CSF: 29.5 ± 18.4 to 6.7 ± 6.3 $p<0.001$, placebo: 24.2 ± 16.9 to 8.9 ± 7.2 $p<0.001$). The ulcer volume, which was not greater among placebo patients, was reduced by 59% in G-CSF and by 35% in placebo patients.

Conclusion/interpretation. We conclude that antibiotic and non weight-bearing therapy (bed rest) accelerated the resolution of cellulitis in infected foot ulcers. Additional treatment with G-CSF had no further beneficial effect. [Diabetologia (2003) 46:27–30]

Keywords Diabetes, infection, foot ulceration, granulocyte-colony stimulating factor, clinical trial.

Chronic non-healing infected foot ulceration is a major complication in diabetes. Polymorphonuclear leukocytes play a critical role in host defence against bacterial infections, and neutrophil function is commonly impaired in diabetic subjects [1, 2, 3]. Granulocyte-colony stimulating factor (G-CSF) promotes the proliferation and differentiation of neutrophil progeni-

tors and thereby increases host defence. In poorly controlled Type 2 diabetes, neutrophil function can be improved by G-CSF [4].

Two randomised controlled clinical trials have investigated the therapeutic effects of G-CSF in infected diabetic foot ulceration, and yielded contradictory results [5, 6]. The aim of our study was to re-evaluate the potential therapeutic effect of G-CSF in patients with infected foot ulcers. The primary endpoint was “resolution of cellulitis”, specified clinically and by an infection summary score (ISS).

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Abbreviations: G-CSF, granulocyte-colony stimulating factor; ISS, infection summary score.

Patients and Methods

Patients and study design. Of 73 diabetic outpatients (36 were ineligible: 14 non-compliant, 6 PVD, 5 impaired kidney/liver function, 6 improper ulcer diameter, 3 deep infection and

2 acute macrovascular events) 37 were randomised to 2 treatment groups; 20 patients received G-CSF and 17 subjects, placebo. The study was conducted as an in-hospital placebo-controlled, randomised, patient-blinded clinical trial. Since the application of G-CSF results in a considerable increase in leukocyte count, a real blinding of investigators was impossible. To ensure a reliable assessment of cellulitis, an experienced investigator who had not been involved in the clinical decisions, e.g. in antibiotic treatment, evaluated cellulitis.

The study protocol was approved by the local ethics committee and all participants gave written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Inclusion and exclusion criteria. We included diabetic patients who suffered from a moderate-sized (diameter 0.5–3 cm) infected neuropathic (abnormal 10g-monofilament test) foot ulcer of Wagner's grade 2 or 3 [7]. Arterial blood supply was diagnosed by palpability of foot pulses and Doppler ultrasound. An angiography was carried out if the foot pulses were impalpable and sonograms were abnormal. Exclusion criteria were: gangrene, haematological diseases, pancytopenia, neoplasia, impaired kidney/liver function, recent treatment with cytokines or immunoactive drugs.

Treatment. The study was conducted during a 10-day in-hospital stay to ensure that all participants received the same standard of wound care including debridement. The patients had to maintain strict bed-rest. All subjects were treated with i.v. antibiotics (clindamycin and ciprofloxacin) until the inflammation had visibly improved. Oral antibiotics were administered thereafter if necessary.

The patients received daily an initial dose of either 5 µg/kg body weight G-CSF (Filgrastim, Amgen Austria, Vienna) or placebo (0.9% sterile saline solution), injected subcutaneously. Absolute neutrophil and leukocyte counts were measured daily. Treatment was intermitted if the absolute neutrophil count was greater than 50.000/l and the absolute leukocyte count greater than 75.000/l, and was re-installed when neutrophils dropped below 30 000 and the leukocyte count below 50 000.

Clinical foot inspection. Cellulitis, ISS, ulcer volume (volumetric method, using a 1-ml syringe) and Wagner's grade were evaluated daily. The ISS included the following: CRP (absolute values), erythrocyte sedimentation rate (1-h value/10), the presence of erythema (local, dorsal and lower leg; 10 points each) and lymphangitis (20 points), and the difference of circumference (in centimetres) between study compared with control foot at the forefoot, ankle, and lower leg level. The inter-observer variability for the ISS was less than 20%. We also used a clinical definition of cellulitis (erythema, oedema, pus formation and lymphangitis) to compare our results with those of former studies.

The primary endpoint of the study was resolution of cellulitis, defined clinically, evaluated daily, and measured by the ISS.

Data analysis. Data are shown as means ± SD, regardless of their distribution (Shapiro-Wilk W-test). Non-parametric tests were used to compare differences between or within groups (Mann-Whitney U-test, Sign test, Chi-square test). The time course of the clinically defined resolution of cellulitis in both groups was analysed by Kaplan-Meier analysis (log-rank test). The primary endpoint measured by ISS was analysed by intention-to-treat and per protocol. As we found no statistically significant differences between the two methods of analysis, only the intention-to-treat analysis is shown here. A *p* value of less than 0.05 was considered statistically significant.

Results

Demographic data were similar in the two groups (Table 1).

Markers of infection. Treatment with G-CSF resulted in a higher leukocyte count throughout the study (Table 1), and neutrophils increased to nearly 70% of the absolute leukocyte count. The baseline CRP value was comparable between the two groups (*p*=0.79), but was increased by G-CSF throughout the study (expressed as mean CRP from day 2–day 10). Among placebo-treated subjects, CRP declined continuously and the mean CRP was near the normal range (Table 1).

Ulcer evaluation. No differences were found in Wagner's grade of foot ulcers at baseline (*p*=0.59) or at the end (*p*=0.54) of the study; the majority had a grade 2 ulcer, but, contrasting baseline, no subject had Wagner grade 3 at the end of the study (Table 1). The ulcer volume was not greater among placebo patients (*p*=0.20), but did decrease in placebo (by 35%, *p*=0.03) or in G-CSF treated (by 59%, *p*=0.0005) patients (Table 1).

Resolution of cellulitis. Patients who received G-CSF did not have an earlier resolution of clinically defined cellulitis than placebo patients (*p*=0.57). At baseline, the only differences (*p*=0.05) between the two groups were the greater prevalence of local erythema in placebo patients and the higher rates of lymphangitis and erythema in the dorsal forefoot in G-CSF patients (Table 2). At day 10, local, forefoot and lower leg erythema were absent in more than 80% of patients receiving G-CSF or placebo. No subject had lymphangitis at the end of the study, and practically none had any persisting putrid wounds. Peripheral oedema had also resolved in the majority of subjects. The ISS was reduced to a similar extent: from 29.5±18.4 to 6.7±6.3, *p*<0.001 (reduction of 79%) in G-CSF and from 26.0±14.2 to 8.9±7.2, *p*<0.01 (63%) in placebo-treated patients during the study. There was no statistical difference in the ISS between G-CSF and placebo patients at baseline (*p*=0.83) or at day 10 (*p*=0.33; Table 2).

Antibiotic regimen. The antibiotic regimen did not differ between the two groups; 80% of the G-CSF group and 82% of the placebo group were treated with 800 mg ciprofloxacin + 2400 mg clindamycin daily. The remaining patients were treated with 400 mg + 2400 mg and 800 mg + 1200 mg, respectively. The duration of i.v. antibiotic treatment was comparable in the two groups: 5.6±2.5 days in the G-CSF group and 5.8±2.3 days for placebo (*p*=0.87). Only 30% of the G-CSF and 47% of the placebo group were treated with oral antibiotics afterwards (predominantly with 750 mg ciprofloxacin + 1800 mg clindamycin).

Table 1. Demographic data and effect of G-CSF on leukocytes and CRP and changes in ulcer volume and Wagner grade

Date are frequencies or means $\pm$ SD; CRP C-reactive protein
Exact p values given for differences between groups
^a $p < 0.05$, ^b $p < 0.01$ indicates significant changes throughout the study within a group

		G-CSF ($n=20$)	p value between groups	Placebo ($n=17$)
Male (%)		75	0.92	77
Type 2 diabetes (%)		95	0.91	94
Age (years)		60.8 $\pm$ 11.1	0.36	58.2 $\pm$ 8.1
Diabetes duration (years)		14.7 $\pm$ 8.5	0.93	15.5 $\pm$ 10.6
HbA _{1c} (%)		8.9 $\pm$ 1.7	0.65	9.2 $\pm$ 2.6
Leukocyte count ($10^9 \cdot L^{-1}$)	Day 1	8.1 $\pm$ 2.6	0.97	7.7 $\pm$ 1.9
	Day 10	40.8 $\pm$ 16.3 ^b	0.00005	9.3 $\pm$ 8.3
Baseline CRP (mg $\cdot$ dl ⁻¹)	Day 1	1.73 $\pm$ 2.2	0.79	1.71 $\pm$ 2.31
Mean CRP (mg $\cdot$ dl ⁻¹)	Day 2–10	2.14 $\pm$ 2.27	0.06	1.04 $\pm$ 0.63
Wagner grade 1/2/3 (%)	Day 1	0/75/25	0.59	0/82/18
	Day 10	13/87/0 ^a	0.54	7/93/0
Ulcer volume (μ l)	Day 1	203 $\pm$ 203	0.20	358 $\pm$ 395
	Day 10	83 $\pm$ 140 ^b	0.07	233 $\pm$ 235 ^a

Table 2. Course of cellulitis, clinically defined and calculated by the Infection Summary Score

	Grade	G-CSF		Placebo		p value between groups	
		Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Clinically defined cellulitis							
Oedema (%)	none	25	88	24	69	0.92	0.30
	mild	30	6	41	25	0.48	0.10
	mod/sev	45	6	35	6	0.55	0.91
Putrid (%)	none	80	95	88	100	0.50	0.77
	mild	10	0	0	0	0.18	0.99
	mod/sev	10	5	12	0	0.86	0.45
Lymphangitis (%)	none	68	100	82	100	0.24	0.65
	mild	10	0	18	0	0.50	0.99
	mod/sev	22	0	0	0	0.05	0.99
Local erythema (%)	none	0	95	18	81	0.05	0.51
	mild	50	0	47	6	0.86	0.27
	mod/sev	50	5	35	13	0.37	0.45
Erythema of the dorsal forefoot (%)	none	60	95	53	94	0.67	0.77
	mild	20	5	35	0	0.30	0.35
	mod/sev	20	0	12	6	0.05	0.27
Erythema of the lower leg (%)	none	75	100	88	88	0.31	0.50
	mild	15	0	0	6	0.10	0.27
	mod/sev	10	0	12	6	0.86	0.27
Infection Summary Score (points)		29.5 $\pm$ 18.4	6.7 $\pm$ 6.3 ^a	26.0 $\pm$ 14.2	8.9 $\pm$ 7.2 ^a	0.83	0.33

Data are percentages or means $\pm$ SD; mod/sev moderate/severe
Exact p values given for differences between groups

^a $p < 0.01$ indicates significant changes throughout the study within a group

Interventions and events. Of the patients five underwent interventional procedures. The G-CSF group had one peripheral angioplasty, and one peripheral angioplasty + minor amputation of the first ray while the placebo group had two angiographies, and one amputation of two toes due to progressing osteomyelitis from the ulcer site. No foot ulcer had completely healed at the end of the study.

Two G-CSF patients dropped out of the study due to likely G-CSF-related adverse events (worsened liv-

er function, skin efflorescence). One placebo-treated patient dropped out due to osteomyelitis.

Discussion

In a randomised placebo-controlled trial, a beneficial effect of G-CSF on cellulitis was observed in infected diabetic foot ulcers [5]. G-CSF was associated with earlier eradication of pathogens from the infected ul-

cers (4 vs. 8 days), a shorter duration of i.v. antibiotic treatment (8.5 vs. 14.5 days), quicker resolution of cellulitis (7 vs. 12 days) and a shorter hospital stay (10 vs. 17.5 days). In the second trial dealing with this subject, the beneficial effect of G-CSF could not be confirmed. G-CSF had no effect on the time until resolution of infection, nor on the duration of parenteral antibiotic administration or the duration of hospitalisation, despite an increased neutrophil function [6].

In contrast to these two studies, we quantified cellulitis and ulcer volume, ensured that all participants spent the same time period in the hospital, and insisted on bed-rest during the study period in order to guarantee consistent non-weight-bearing conditions for the ulcerated foot.

The term cellulitis reflects tissue damage secondary to the inflammatory process at the surrounding tissue while the resolution of cellulitis is the relevant endpoint in treating infected wounds with G-CSF. We deemed it essential to strictly define and quantify cellulitis, as this would permit a clear distinction between whether the healing process was due to the treatment or due to spontaneous repair during bed rest.

At baseline, the degree of infection was the same in both groups; during the study, cellulitis declined comparably in both. Since the antibiotic regimen was comparable and wound care was standardised throughout the study, other factors than G-CSF had apparently promoted the wound healing process.

Pressure relief itself is the key to healing neuropathic non-infected foot ulcers in diabetes [8]. Besides antibiotic treatment, we suggest that the non-weight-bearing regimen (strict bed rest) is the most important intervention to enhance the spontaneous healing process of infected foot ulcers.

Costly treatment with G-CSF would only be justified if the treatment period and, consequently, the hos-

pital stay could be substantially shortened. Therefore, the use of G-CSF cannot be recommended on a routine basis for diabetic patients with infected foot ulcers.

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