Increased Matrix Metalloproteinase-9 Predicts Poor Wound Healing in Diabetic Foot Ulcers

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OBJECTIVE — We studied the relationships of diabetic ulcer wound fluid matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and transforming growth factor-ß1 (TGF-ß1) with wound healing rate.

RESEARCH DESIGN AND METHODS — The ulcers were cleansed to remove exudates, and wound fluids were collected for analysis of MMP-2 and -9, TIMP-1, and TGF-ß1.

RESULTS — At presentation, MMP-9 and the MMP-9–to–TIMP-1 ratio correlated inversely with the wound healing rate at 28 days ($P < 0.001$). MMP-9 and the MMP-9–to–TIMP-1 ratio were lower in the 23 patients who achieved complete healing at 12 weeks versus the 39 who did not. The pro–MMP-9 concentration was predictive of healing within 12 weeks. Addition of cutoffs for TIMP-1 (>480 pg/ml) and TGF-ß (>115 pg/ml) further improved its predictive power (area under the curve 0.94).

CONCLUSIONS — These findings suggest that a milieu with high MMP-9 may be indicative of inflammation and poor wound healing. Measurements of MMP-9, TIMP-1, and TGF-ß in wound fluid may help to identify ulcers at risk of poor healing.

Diabetes Care **32:117–119, 2009**

Diabetic foot ulcers often fail to heal, and the mechanism is not well explained (1). In previous studies of wounds, delayed healing is characterized by an increase in matrix metalloproteinases (MMPs), a decrease in the tissue inhibitors of metalloproteinases (TIMPs), and a reduction in some growth factors, in particular, transforming growth factor-β (TGF-β) (2–9). Both MMPs and TIMPs are secreted by cells involved in wound healing, and their concentrations vary according to the phase of healing (4,6).

Studies of diabetic foot ulcer wounds in humans are limited as a result of the difficulty of obtaining tissue samples. Wound fluid can be obtained noninvasively and could potentially overcome this problem. The clinical relevance of studying wound fluid is supported by our previous report that high bacterial count in diabetic wound fluid has a negative impact on wound healing (10). Therefore, the aim of this study was to measure MMP-9, MMP-2, TIMP-1, and TGF-β1 in wound fluid obtained from diabetic foot ulcers and to examine their relationships with wound healing.

RESEARCH DESIGN AND METHODS

— Patient characteristics are shown in Table 1. The majority of patients had type 2 diabetes ($n = 56$), and their ulcers were classified as neuropathic ($n = 48$), postsurgical ($n = 9$), or neuroischemic ($n = 5$) and graded according to the Texas Grading System (11). The ulcers had been present for 2–10 weeks before presentation. All patients were seen weekly for debridement, offloading, and other treatments during the initial 4 weeks and attended approximately monthly visits thereafter. Antibiotics were prescribed for 83% of individuals. The protocol was approved by the ethics committee of the area.

Wound fluids were collected from the ulcer site at the first clinic visit and after 4 weeks of treatment. Samples were stored at −20°C for quantitation of MMP-2 and -9 by zymography (12) and TIMP-1 and TGF-β1 by enzyme-linked immunosorbent assay. Ulcer size and wound healing rates (WHRs) were determined as previously described (10).

Students *t* test or one-way ANOVA was used for comparisons. Multiple regression analysis was used to determine the relationships between $WHR_{4\ weeks}$, age, duration of diabetes and ulceration, wound fluid pro– and active–MMP-9 and -2, TIMP-1, and TGF-β1. Receiver operating characteristic analysis was used to determine thresholds for pro–MMP-9 in predicting healing within 12 weeks.

RESULTS

— At 4 weeks, none of the ulcers had healed, but by 12 weeks, 23 of the 62 ulcers had completely healed. There were no differences in age, duration of diabetes, or initial size of the ulcer between the healed and unhealed groups (Table 1).

Wound fluid pro–MMP-9 and pro–MMP-9–to–TIMP-1 ratio at presentation correlated significantly with $WHR_{4\ weeks}$ ($r = 0.4538$, $P < 0.001$, and $r = 0.4959$, $P < 0.0001$, respectively). This relationship was not evident for MMP-2 or TIMP-1. The concentrations of pro– and active–MMP-9 in the wound fluid obtained at presentation were significantly higher and those of TIMP-1 and TGF-β1 significantly lower in ulcers that subsequently failed to heal than in ulcers that healed within 12 weeks (Table 1). When the data were expressed as pro–MMP-9–to–TIMP-1 and active–MMP-9–to–TIMP-1 ratios, the difference between the healed and unhealed groups was further enhanced. This pattern of higher MMP-9 was evident in both stage B and stage C

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Received 21 April 2008 and accepted 18 September 2008.
Published ahead of print at http://care.diabetesjournals.org on 3 October 2008. DOI: 10.2337/dc08-0763.

Table 1—*Patient and wound fluid characteristics at the initial visit grouped according to subsequent healing status at 12 weeks*

	Healed ulcers	Unhealed ulcers
Total sample		
Patients	23	39
Sex (men)	17	32
Age (years)	59 ± 11	62 ± 9
Diabetes duration (years)	14 ± 5	16 ± 12
A1C (%)	9.5 ± 1.9	7.7 ± 2.1*
Antibiotic treatment	20	32
After 4 weeks		
Ulcer size (mm^2)	91.7 ± 146.7	213.4 ± 384.4*
WHR (mm/day)	2.7 ± 1.3	0.5 ± 2.1†
At presentation		
Wound duration (days)	61 ± 11	153 ± 49*
Ulcer size (mm^2)	316.9 ± 509.9	329.4 ± 478.4
Pro–MMP-9 (μg/ml)	4.47 ± 2.75	8.19 ± 2.75†
Active–MMP-9 (μg/ml)	1.18 ± 1.21	2.90 ± 1.64*
TIMP-1 (pg/ml)	732.5 ± 270.0	531.2 ± 377.6*
Pro–MMP-9–to–TIMP-1 ratio	7.2 ± 5.24	18.88 ± 6.87*
Active–MMP-9–to–TIMP-1 ratio	2.03 ± 2.47	6.01 ± 1.08*
Pro–MMP-2 (μg/ml)	1.01 ± 1.00	1.46 ± 1.25
TGF-β1 (pg/ml)	231.9 ± 141.9	142.4 ± 119.7†
Subsample with neuropathic ulcers only		
Patients	23	25
Antibiotic treatment	20 of 23	22 of 25
At presentation		
Pro–MMP-9 (μg/ml)	3.92 ± 1.24	9.14 ± 3.84
Active–MMP-9 (μg/ml)	1.20 ± 0.94	2.55 ± 1.24
Pro–MMP-9–to–TIMP-1 ratio	10.17 ± 8.24	34.81 ± 12.87*
Active–MMP-9–to–TIMP-1 ratio	3.33 ± 1.47	8.11 ± 3.08*
TIMP-1 (pg/ml)	683.59 ± 230.0	523.68 ± 150.0†
TGF-β1 (pg/ml)	192 ± 90.6	112.4 ± 59.7†

Data are means ± SD or *n*. *$P < 0.05$ different from group with ulcers healed within 12 weeks. †$P < 0.01$ different from group with ulcers healed within 12 weeks.

ulcers (there were not sufficient numbers for comparison in stages A and D) and remained so if only the 48 neuropathic ulcers were analyzed (Table 1). The pro– and active–MMP-2 concentrations were also slightly higher (1.5-fold) in the unhealed group, but only the ratios of pro– and active–MMP-2 to TIMP-1 (threefold) attained significance. Wound fluid obtained after 4 weeks of treatment also showed the same pattern of higher pro–MMP-9 (mean ± SD unhealed concentration 7.28 ± 4.62 vs. healed concentration 4.73 ± 4.47 μg/ml), higher active–MMP-9 (1.87 ± 3.19 vs. 1.32 ± 1.97 μg/ml), and a higher pro–MMP-9–to–TIMP-1 ratio (23.17 ± 3.81 vs. 8.36 ± 10.95). The protein concentration of the wound fluid was 10.4 ± 5.5 mg/ml in the unhealed and 13.1 ± 6.3 mg/ml in the healed group.

Wound fluid pro–MMP-9, active–MMP-9, and TIMP-1 at presentation accounted for 32% of the variance in healing rate. Duration of diabetes, age, initial wound size, and TGF-β1 were not statistically significant determinants. Measurement of pro–MMP-9 with addition of cutoffs for TIMP-1 at >480 pg/ml and TGF-β1 at >115 pg/ml (respective median values for healed wounds) was the best predictor of wound healing (area under the curve 0.94; $P < 0.00001$). The sensitivity and specificity were 87 and 91%, respectively, and predicted outcome in 94% of cases.

CONCLUSIONS — The results of our study have shown for the first time that high wound fluid concentrations of MMP-9 and high MMP-9–to–TIMP-1 ratios predict poor wound healing in diabetic foot ulcers. The mechanism of increased MMP-9 is uncertain. However, it is likely to be related to increased inflammation because MMP-9 is expressed mainly by neutrophils and macrophages, both cell types important to the inflammatory response (13). This pattern of increased MMP-9 in poorly healing ulcers was observed in varying types of diabetic foot ulcers, suggesting that it is more strongly linked with the healing process rather than with underlying etiology. We have previously shown that high wound fluid bacterial count, even in the absence of overt infection, is also predictive of poor wound healing (10). It is possible that high bacterial count and high MMPs are mechanistically linked in this regard. As such, the results could be affected by antibiotic therapy, which most of our patients were given, but we have not systematically examined this aspect. It is unlikely that the pattern we have observed is due to increased exudates in nonhealing wounds. First, the protein concentration of the nonhealed ulcer group was actually lower than that of the healed group. Second, a simple change in the amount of exudates should not affect the various components such as MMPs and TIMPs differently.

Our study using wound fluid collected by an easy noninvasive technique is an important step to facilitate investigations in this area of study. One of the frustrations for clinicians treating diabetic foot ulcers is that there is not a simple test that can integrate various risk factors to allow prediction of achieving wound healing. Although our results need confirmation, it would be very useful if an index calculated from the measurements of MMPs, TIMP-1, and TGF-β1 at the initial presentation could assist in prognostication.

Acknowledgments— This study was supported by grants from the Diabetes Australia Research Trust and the Medical Foundation of The University of Sydney.

No potential conflicts of interest relevant to this article were reported.

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