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Research Article

Redox-active clearance of Highly Glycated RBCs: A new Frontier for the Treatment of Diabetic Vasculopathies

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Highlights

- The chlorite-based drug leads to sustainable reduction of HbA1c values from high to low risk in over 80% of the patients and also reduces the amounts of RBC-derived AGEs.
- This HbA1c- and AGE-reducing effect results from a drug-mediated redox-active clearance of highly glycated RBCs and the restoration of RBC homeostasis in blood.
- The specific effect of the drug on highly glycated RBCs, without direct blood sugar-lowering, is reflected by reduction of HbA1c/FBS values into “non-diabetic” ranges.
- The removal of highly glycated RBCs and the detoxification of hemolytic products leads to termination of endothelial injury and to resolution of diabetic vasculopathy and vasculitis.
- In DFU patients, the therapeutic effect of the drug on diabetic vasculopathies translated to improved and sustainable wound healing and osteomyelitis resolution.

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Abstract

Objective

On the global scale, 24-54% of the diabetic patients do not reach Quality Assurance (QA) target hemoglobin A1c (HbA1c) values, despite Standard of Care (SOC) treatment with glucose-lowering drugs. An alternative approach to lower highly glycated Red Blood Cells (RBCs) directly is needed to reduce the risk for development of diabetic vascular complications. HbA1c > 7.0% (53.0 mmol/mol) is associated with increasing incidence of vascular pathologies and inflammation. We tested an alternative approach to avoid pathological effects of excess glycation via direct redox-active clearance of highly glycated Red Blood Cells (RBCs) with the objective to rapidly terminate the trigger of endothelial damage and thereby to reduce the risk for development/progression of diabetic vascular complications.

Research Design and Methods

We report results from a controlled clinical trial in 20 patients with high HbA1c, elevated Neutrophil-Lymphocyte Ratio (NLR) values and diabetic foot ulcer (DFU), using the chlorite-based drug WF10.

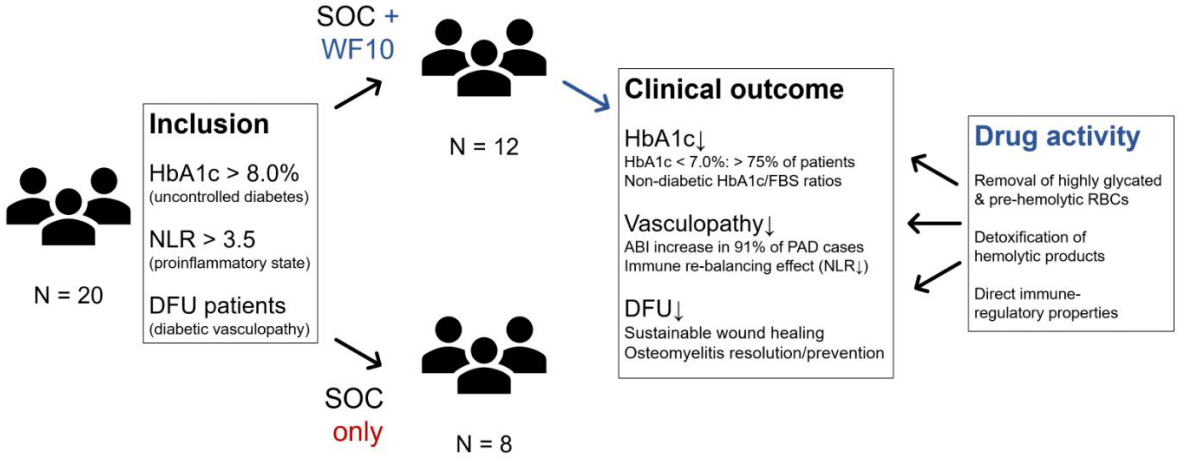
Results

In the treatment group, sustainable HbA1c reduction was observed, with 83% of the patients achieving HbA1c ≤ 7.0% (53.0 mmol/mol), versus 25% in the SOC group. Lowering HbA1c to Fasting Blood Sugar- (FBS-) ratios and changes in hematological markers indicate a removal of highly glycated, pre-hemolytic RBCs by the drug. In patients with peripheral artery disease (PAD), mean Ankle Branchial Index- (ABI-) values improved from 0.83 to 1.01. The median NLR declined from 5.40 to 2.17. Faster and more sustainable DFU wound healing, improved osteomyelitis (OM) resolution and less OM development were observed.

Conclusions

The drug solution WF10 provides the first non-FBS-lowering add on therapy for direct HbA1c reduction in diabetic patients via redox-active clearance of highly glycated RBCs and rapid termination of endothelial injury, which translates into resolution of diabetic vasculopathy and derived micro- and macrovascular diabetic complications. Intriguingly, a NNT of only 10 was sufficient for proof of concept in this study.

Graphical abstract



Keywords: ABI AGEs; HbA1c; NLR; RBC homeostasis; vascular pathologies; WF10

Introduction

High HbA1c, besides being a long-term marker for elevated blood sugar levels in diabetic patients [1], represents an undisputed predictor for the development of late-stage vascular complications [2]. Accordingly, HbA1c became a major therapeutic target as 1.0% reduction translates into 20-40% lower risk for the development of micro- and macrovascular diabetic pathologies [3]. Yet, despite Standard of Care (SOC) glycemic control, with oral anti-diabetics (OADs) and insulin, 24-54% of the patients do not reach target HbA1c values [4].

Thus, many patients still exhibit a high risk for the development of diabetic vascular complications, despite SOC treatment. Furthermore, drastic indirect HbA1c reduction via intensive FBS control bears the risk for the development of severe hypo-glycemia [5] and increased mortality [6]. A novel, direct HbA1c-reducing approach is needed.

HbA1c represents an early-stage Hb glycation product in RBCs. Yet, the Insulin-independent uptake of glucose by these cells also leads to extensive metabolic, functional and structural alterations resulting in the formation of dysmorphic and hemolysis-prone cells [7]. An increased binding of highly glycated RBCs to endothelial cells (ECs), via interaction of RBC-derived advanced glycation end products (AGEs) with corresponding receptors (RAGEs) on endothelial cells (ECs) [8], leads to vascular disturbances, oxidative stress and inflammation [9]. AGE formation correlates with HbA1c and the development of diabetic vascular complications and atherosclerosis [10].

Formation of highly glycated and frail RBCs also causes intravascular hemolysis, reflected by a considerably shortened lifetime of the cells [11]. Hemolytic products, including especially free Hb and free heme, are important contributors to NO depletion, endothelial activation, EC injury, thrombosis, complement system (CS) activation and inflammation at diabetes [12]. HbA1c value ≥ 7.0% (53.0 mmol/mol) are considered a threshold for elevated hemolysis at diabetes [13], which correlates well with clinical QA target ranges.

Diabetic foot ulcer (DFU) represents a severe late-stage diabetic vascular complication characterized by persistent, non-healing and often infected wounds [14]. Both the prevalence and the severity of DFU correlate with HbA1c values [15]. RBCs from DFU patients tend towards self- and vascular aggregation, independent from SOC glycemic control [16]. Elevated NLR values also positively correlate with DFU severity [17], evidencing persistent inflammation as a major factor of impaired wound healing.

Since 2015 the chlorite-based drug solution WF10 is approved in Thailand (Immunokine®) as adjunct treatment for DFU. The drug promotes microcirculation and vascular nitric oxide (NO) bioavailability, reduces inflammation and improves granulation and wound healing [18,19]. Clinical studies also showed distinct HbA1c-reducing effects of the drug [20], apparently based on a removal of highly glycated RBCs [21]. Chlorite, the active component in WF10, promotes physiological removal of these cells [22], especially if they are highly glycated and show impaired anti-oxidative capacity [23].

The objective of this study on DFU patients was to proof the HbA1c-reducing effect of WF10 in a controlled clinical trial (CCT) (primary study endpoint). We also investigated the effect of the drug-derived removal of highly glycated RBCs on the formation of RBC-derived AGEs and further protein modifications. Moreover, this study aimed to reconfirm that WF10-mediated restoration of RBC homeostasis and detoxification of hemolytic products [24,25] also translates to abatement of inflammation (NLR reduction). Finally, this study wanted to show improvement of diabetic vascular pathology under WF10 therapy by evaluating Ankle Branchial Index- (ABI-) values. Effects of WF10 therapy on the clinical outcome were assessed by evaluating wound healing and osteomyelitis (OM) development in the DFU patients.

Research Design and Methods

After registration at the National Medicinal Research Register (NMRR-20-291-53186), and approval by the responsible clinical committee, this prospective controlled single-center clinical trial was performed at University Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur, in compliance with Good Clinical Practice (GCP) and accordance with the Declaration of Helsinki (1964). The trial was also registered at the international ISRCTN registry (ISRCTN12348610). Based on the primary study endpoint (HbA1c reduction), a NNT of 10 was calculated. A total of 20 patients was recruited for the study and randomly assigned to the WF10 group (n = 12) and to the SOC group (n = 8).

Patients (age: 18-80 years) with type 2 diabetes [26], elevated HbA1c values ≥ 8.0% (63.9 mmol/mol, uncontrolled diabetes [21]) active diabetic foot (DFU) problems and elevated NLR values ≥ 3.0-35 were included. Only patients with CKD grade 1-3b (estimated Glomerular Filtration Rate, eGFR ≥ 30) and UACR (urinary albumin to creatinine ratio) categories A1-A2 (≤ 300 mg/g) were included. Major exclusion criteria were ABI values ≤ 0.8, Hematocrit (Hct) values < 27% and Hb values < 9 g/dL.

The overall study duration was 12 weeks, including five weeks treatment period and 7 weeks follow-up. Throughout the study, both study arms received standard of care (SOC) diabetic control and DFU treatment. Patients of the treatment group

additionally received five subsequent weekly infusions of the chlorite-based drug WF10 at a dose of 0.3 mL/kg BW. Blood samples obtained during the study were sent to a laboratory in Germany (Heidelberg University, Prof. Dr. Nawroth, SFB1118) for blinded HbA1c quantification as well as determination of further protein modifications and AGE formation in RBC samples.

Reduction of elevated HbA1c values represented the primary study endpoint, improved DFU wound healing and reduction of elevated NLR values represented major secondary endpoints. DFU assessment included regular wound severity score (WSS) determination [18] and radiographic osteomyelitis (OM) evaluation. Reduction of RBC-derived AGE formation presented a further major secondary endpoint of the study. RBC-derived biomarkers were analyzed to evaluate restoration of healthy erythrocyte homeostasis. Important safety evaluations included monitoring of cardiac safety via electrocardiogram (ECG), renal and hepatic parameters via clinical chemistry and urinalysis.

For calculation of HbA1c/FBS ratios, HbA1c values were converted from % to mmol/mol. The Shapiro-Wilk test was applied for testing normal distribution of the data. For normally distributed data, mean ± SD (Text, Tables) or mean ± SEM (Graphs) are provided, while for not normally distributed data, median ± IQR (Text, Tables) or median ± IQR/$N^{0.5}$ (Graphs) are given. Statistical analysis was performed by using two-sided paired/unpaired t and t-test for inter/intra-group comparison, respectively. Significant changes (*) correspond to $p < 0.05$, highly significant changes (**) to ($p < 0.01$) and extremely significant changes (***) to ($p < 0.001$), respectively.

Results

In STab. 1, baseline (BL) data are provided, which were comparable for the WF10 group (N = 12) and the SOC group (N = 8). About 50% of the patients were adipose (BMI > 27.5%). Further diabetic vascular complications besides DFU as well as hypertension and dyslipidemia were common in the patients.

As shown in Fig. 1a, at BL, there was no significant difference between the mean HbA1c value in the WF10 group (10.19% ± 1.66%, 87.9 mmol/mol) and the SOC group (11.38% ± 1.88%, 100.9 mmol/mol). Yet, in the WF10 group a significant ($p = 0.0186$; 0.0233; 0.0402) stepwise reduction in the mean HbA1c value was observed between BL and Week 06. The lowest value was obtained at Week 08 (6.03% ± 1.04%, 42.4 mmol/mol, primary endpoint). In the SOC group the HbA1c decrease was less pronounced. A group comparison shows (highly) significantly lower mean HbA1c values in the WF10 group between Week 04 and 08 ($p = 0.0453$; 0.0039; 0.0012). Yet, at Week 12, the difference between the groups was no longer statistically significant ($p = 0.0534$).

As shown in Fig. 1b, at BL also the median HbA1c/FBS ratio was comparable in the WF10 group (10.59 L/mol ± 4.79 L/mol) and the SOC group (11.72 L/mol ± 5.93 L/mol). Yet in the WF10 group highly significant lower ratios ($p = 0.0027$; 0.0010; 5.59 L/mol ± 1.82 L/mol and 5.03 L/mol ± 1.67 L/mol) were observed at Week 08 and 12, respectively. These about 50% lower values are solely based on HbA1c reduction, as median FBS levels remained essentially stable (see SFig. 1). In the SOC group, the HbA1c/FBS ratio was lowest at Week 6 (6.80 L/mol ± 2.35), a weakly significant ($p = 0.0469$) and transient reduction from BL. Diabetic patients exhibit HbA1c/FBS ratios > 6.0 (grey line in Fig. 1b, SFig. 2).

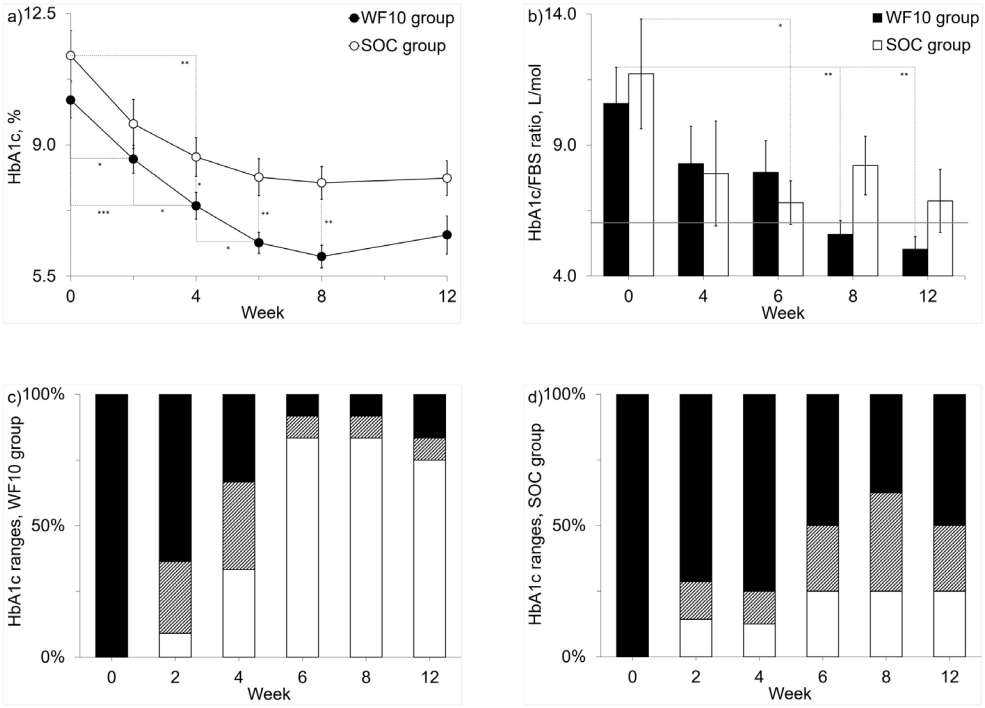


Figure 1: WF10-derived reduction of elevated HbA1c values. (a) shows a quicker and stronger decrease of HbA1c values in the WF10 group (black) as compared to the SOC group (white). (b) shows a significant decrease of the HbA1c/FBS ratio only in the WF10 group (black). The grey line distinguishes between higher ratios in diabetic patients and lower ratios in non-diabetic patients. Categorization of the HbA1c values showed that in the WF10 group (c) the majority of the patients reached HbA1c values ≤ 7.0% (white) after the treatment period. In the control group (d), the majority of the patients exhibited HbA1c values > 8.0% (black) or > 7.0% - ≤ 8.0% (dashed) throughout the study.

N = 12 for the WF10 group and N = 8 for the control group; Mean and SEM are given (normally distributed data) for HbA1c, Median and IQR/$N^{0.5}$ are given for HbA1c/FBS (not normally distributed data); * corresponds to significant changes ($p < 0.05$), ** corresponds to highly significant changes ($p < 0.01$) and *** corresponds to extremely significant changes ($p < 0.001$).

Figs. 1c and d show a patient-specific analysis of HbA1c values in the WF10 group (c) and the SOC group (d). While all patients exhibited HbA1c values > 8.0 (63.9 mmol/mol) at BL (inclusion criterium), in the WF10 group already at Week 04 (end of the treatment period), 8/12 patients had an HbA1c value ≤ 8.0% (63.9 mmol/mol) with 4 of these patients even showing an HbA1c ≤ 7.0% (53.0 mmol/mol). During follow up, 9-10/12 patients reached this target HbA1c range. In the SOC group only 1-2/8 patients reached HbA1c values ≤ 7.0% (53.0 mmol/mol) during the course of the study and 4/8 patients still exhibited HbA1c values > 8.0% (63.9 mmol/mol) at Week 12.

As shown in STab. 2, a blinded RBC sample analysis in Heidelberg, Germany confirmed a stronger HbA1c reduction under WF10 therapy. Thereby however, 1.4-1.5 higher absolute values were obtained due to different analytical methods (IFCCRMP reference method for HbA1c determination versus LC-MS/MS analysis).

The HbA1c-reducing effect of WF10 is based on a removal of highly glycated RBCs and reflected by characteristic changes in RBC-derived biomarkers [21]. Mean Hct values (see STab 3) provide no hint for effects of WF10 on RBCs. Yet, patient-specific analysis (not shown) indicates resolution of pre-anemia (Hct < 35%) and anemia (Hct < 30%) in the WF10 group, which already provides indirect hints for an elevated erythropoiesis in the treatment group.

At BL the number of reticulocytes (RTCs, STab 3) was not significantly different ($p = 0.553$) between the groups (1.25 ± 0.41% and 1.14 ± 0.3%). Yet, at the end of the treatment period (Week 04), an extremely significant ($p = 0.0006$) increase of RTCs (+ 130%) was observed in the WF10 group, which was still

highly significant (p = 0.0011; 0.0025) at Weeks 06-08 but almost returned to BL values at Week 12. In the SOC group, a significant (p = 0.0146; 0.0290; 0.0134) but less prominent (+ 66%) increase in RTCs was observed between Weeks 06-12.

Further RBC-derived biomarkers also illustrate elevated blood rejuvenation in the WF10 group (STab, 3). While MCH values in both groups were not significantly different at BL (p = 0.1527), (highly) significant (p = 0.0180; 0.033) higher values were observed in the WF10 group between Week 04-12. At the same time, MCHC values remained essentially stable (not shown), indicating the formation of novel, bigger and normochromic RBCs.

This study also investigated the effect of WF10-mediated removal of highly glycated RBCs on the AGE formation and protein oxidation and nitration in these cells. As shown in Tab. 1, at BL no significant difference in regard to the investigated protein modifications was found between the groups. Yet, in the WF10 group the mean fructose-lysine (FL) level was 30.4%, still not statistically significant (p = 0.1072), lower at Week 08 as compared to BL. Patient-specific analysis showed FL level reduction in 11/12 patients from the WF10 group. In the SOC group, only 3/7 patients exhibited reduced FL levels at Week 08 and the mean value remained essentially stable. In both groups, FL reduction was always accompanied by HbA1c reduction and lack of HbA1c reduction also meant no FL reduction. However, both for carboxymethyl-lysine (CML) and for carboxyethyl-lysine (CEL), no significant changes in the mean values were observed in either group.

	WF10 group		**SOC group**	
	Baseline	**Week 8**	**Baseline**	**Week 8**
FL, mol/mol Lys	11.43 ± 3.80	7.95 ± 6.09	10.86 ± 7.45	10.63 ± 7.90
CML, mol/mol Lys	1.33 ± 0.26	1,35 ± 0.28	1.40 ± 0.29	1.31 ± 0.31
CEL, mol/mol Lys	9.37 ± 0.45	9.14 ± 1.14	9.03 ± 1.15	9.24 ± 0.63
G-H1, mmol/mol Arg	0.61 ± 0.19	0.47 ± 0.15	0.47 ± 0.13	0.58 ± 0.18
MG-H1, mmol/mol Arg	2.34 ± 1.39	3.54 ± 4.38	2.98 ± 2.17	3.91 ± 4.71
MetSO, mol/mol Met	74.45 ± 50.40	74.35 ± 38.51	119.59 ± 53.86	93.15 ± 62.35
3-NT, mol/mol Tyr	0.66 ± 0.16	0.60 ± 0.20	0.55 ± 0.10	0.64 ± 0.23
N = 12 for the WF10 group and N = 7 for the control group; Mean and SD are given (normally distributed data)				

Table 1: RBC-derived AGEs.

For glyoxal-derived hydroxy-imidazone (G-H1), again an almost significant (p = 0.0576), mean value reduction in the WF10 group by 23.0% was observed between BL and Week 8, based on reduced G-H1 levels in 8/12 patients. In the SOC group, the mean G-H1 value even increased by 23.4%, based on rising values in 4/7 patients. For methylglyoxal-derived hydroxy-imidazone (MG-H1) and methionine sulfoxide (Met-SO), mean values showed no significant change between Baseline and Week 08 in either of the groups. However, in regard to 3-nitro-tyrosine (3-NT), again the mean value showed a, non-significant (p = 0.4258), tendency towards lower values at Week 08 (9.0% reduction) in the WF10 group, based on lower values in 8/12 patients. In the SOC group, a non-significant (p = 0.3611) tendency towards higher mean values (+16.4%) was observed as values increased in 5/7 patients.

We also investigated the effect of WF10-derived removal of highly glycated RBCs on diabetic vasculopathy in the patients by addressing Ankle Branchial Index- (ABI-) values. As shown in

Fig. 2a, mean ABI values were almost identical at BL in the WF10 group (1.06 ± 0.23) and in the SOC group (1.07 ± 0.07). Yet, in the WF10 group, a non-significant (p = 0.0942) increase was already observed at the end of the medication phase (Week 04, 1.19 ± 0.30) and a significantly (p = 0.0262) higher mean value was obtained at Week 08 (1.23 ± 0.09). In the SOC group, no significant ABI change was observed throughout the study.

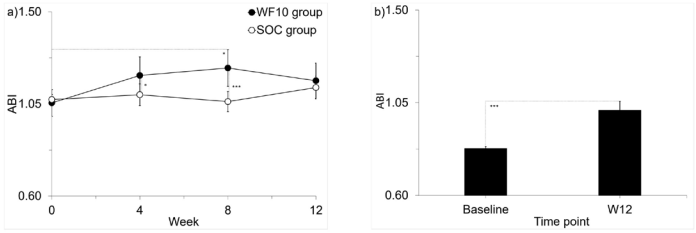


Figure 2: Effect of WF10 on PAD. (a) shows a significant mean ABI increase in the WF10 group at Week 08. (b) shows increase of mean ABI values in PAD patients from PAD- (ABI < 0.9) to non-PAD (ABI > 0.9) under WF10 treatment between Baseline and Week 12 a): N = 12 (11 at Week 4) for the WF10 group and N = 7 (6 at Weeks 4 and 12) for the control group, b): N = 11; Mean and SEM are given (normally distributed data); * corresponds to significant changes (p < 0.05) and *** corresponds to extremely significant changes (p < 0.001).

As patients with ABI values < 0.8 were excluded from the study, only four patients (all WF10 group) exhibited PAD (ABI values < 0.9) at BL. In a previous clinical study under similar conditions [21], seven patients had BL ABI values < 0.9. As shown in Fig. 2b, a pooled analysis of the 11 PAD patients showed an extremely significant (p = 0.0005) increase of the mean ABI value from 0.83 ± 0.03 at BL to 1.01 ± 0.14 at Week 12 under WF10 treatment. Patient-specific analysis shows PAD resolution in 7/11 patients. In further 3/11 patients, ABI values also improved. Only one patient (9.1%), showed a minimal ABI decline from 0.89 to 0.86.

The improvement of DFU wound healing under WF10 treatment represented a major secondary endpoint of the study. As shown in Fig. 3a, at BL an equal overall median wound severity score (WSS) was observed in both groups, including comparable relative contribution of the four aspects infection/inflammation, necrosis, granulation and ulcer depth. However, in the WF10 group an extremely significant (p = 0.0002) reduction of the WSS was already observed at Week 04 (7.0 ± 4.0). At Weeks 08-12, a further significant stepwise reduction (p = 0.0384; 0.0199) yielded a final value of 1.0 ± 3.3, implying fast and sustainable wound healing even after the treatment phase. In the SOC group, a highly significant (p = 0.0057) reduction of the WSS was only observed at Week 8 (3.0 ± 2.0) and the further WSS decrease till Week 12 (1.5 ± 2.3) was not significant (p = 0.1857).

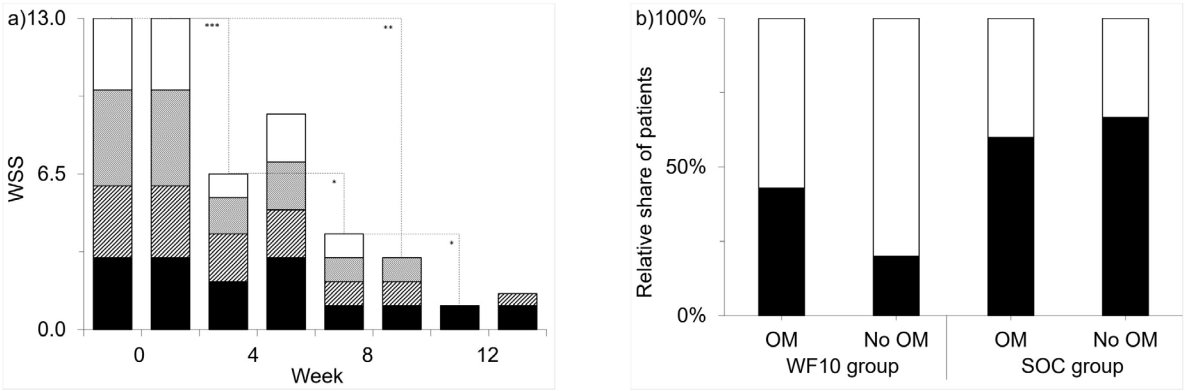


Figure 3: WF10-derived improved DFU wound healing. (a) shows a quicker and more sustained reduction of the WSS (median) in the WF10 group (left) as compared to the SOC group (right). Black corresponds to infection/inflammation, dashed to necrosis, thin dashed to granulation and white to ulcer depth. (b) shows the development of osteomyelitis in patients with (left bar in the groups) or without (right bar in the groups) OM at BL. In the WF10 group (left), considerably more OM resolution (left row, white) and less OM development (right row, black) was observed.

N = 12 for the WF10 group and N = 8 for the control group; Median is given for WSS (not normally distributed data); * corresponds to significant changes ($p < 0.05$), ** corresponds to highly significant changes ($p < 0.01$) and *** corresponds to extremely significant changes ($p < 0.001$).

At Baseline, 7/12 patients from the WF10 group and 5/8 patients from the SOC group exhibited osteomyelitis (OM), which means a comparable relative share (58.3%/62.5%). Yet as shown in Fig. 3b, in the WF10 group OM was resolved in 4/7 cases (57.1%) versus 2/5 cases (40%) in the SOC group. Simultaneously, OM developed in only 1/5 (20%) non-OM patients of the WF10 group but in 2/3 (67%) non-OM patients of the SOC group. During the course of the study, one patient from the SOC group needed a major amputation.

The effect of WF10 therapy on the inflammation at DFU represented a further aspect of the current study. As shown in SFig. 3a, at BL the White Blood Cell (WBC) count was comparable in both groups. Yet, in the WF10 group a highly significant ($p = 0.0031$) and persistent reduction was already observed at Week 04. In the SOC group, significant ($p = 0.0416$) WBC count reduction was only observed at Week 12.

As shown in SFig. 3b, in both groups, leukocytes reduction was paralleled by NLR value decrease. Again, the effect was more pronounced in the WF10 group with a highly significant 59.8% reduction ($p = 0.0032$) between BL and Week 04. At the same time, a non-significant ($p = 0.2592$) 52.3% NLR reduction was observed in the SOC group.

As shown in STab. 5, the median platelet counts were about 30% higher in the WF10 group as compared to the SOC group, while this difference was not statistically significant ($p = 0.8212$). In the WF10 group a continuous decrease of median PLT counts was observed during the course of the study, leading to significantly ($p = 0.0393$) lower values at Weeks 8 and 12 (34% reduction). In the SOC group, a less consistent and not-significant median platelet count decrease was observed.

As also shown in STab. 5, no difference was seen in the prothrombin time (PT) values obtained during the study, neither within not between the groups. In regard to the activated partial thromboplastin time (aPTT), again no significant changes within the groups were observed. However, at Week 12 a significant lower ($p = 0.0143$) median aPTT was obtained in the WF10 versus the SOC group.

In regard to renal safety, median estimated glomerular filtration rate (eGFR) values at BL were considerably, but not statistically significant, lower in the SOC group than in the WF10 group (see STab. 1). No significant change of the median eGFR value was observed in either group during the course of the study (not shown). BL median urinary albumin creatinine-ratio (UACR) values (see S. Tab. 1) were comparable between the groups and also showed no significant changes in either group during the course of the study (not shown).

Regarding hepatic safety, γ-glutamyltransferase (gGT), alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT), while showing higher median values at BL in the SOC (see STab 1), did not significantly change in either of the groups during the course of the study (not shown). One patient from the SOC group developed a persistent infection during the follow-up period, reflected by strongly elevated WBC (16.5 10^9 c/L) and NLR (16.4) values and leading to constantly elevated liver values till the end of the study. In contrast, in the WF10 group one patient with increased hepatic parameters at Baseline due to acute infection (24.1 10^9 c/L WBCs, NLR 7.8) showed good resolution of inflammation (10.7 10^9 c/L WBCs, NLR 1.8), accompanied by normalization of liver values.

Discussion

Elevated HbA1c levels significantly increase the risk for diabetic vascular complications [3], including DFU. HbA1c > 6.5% (47.5 mmol/mol) and HbA1c > 7.0% (53.0 mmol/mol) represent thresholds for micro- and macrovascular complications and higher mortality at diabetes [2].

In this study a quick and persistent WF10-mediated reduction of elevated HbA1c values (> 8.0%, 63.9 mmol/mol) by > 4% was observed, as confirmed by a blinded secondary analysis in Germany. Thereby, > 75% of the patients reached a target range of HbA1c values ≤ 7.0% (53.0 mmol/mol) under WF10 therapy. The WF10-mediated HbA1c reduction was reported before [20,21]. Yet the current study proves the advantage of this drug over SOC therapy only. In this group, 50% of the patients still exhibited uncontrolled diabetes (HbA1c >8.0%, 63.9 mmol/mol) at the study end.

The observed WF10-mediated reduction of HbA1c/FBS ratios, despite stable FBS levels, proves a direct effect of the drug on highly glycated RBCs rather than FBS level-lowering. Considering the adequate interchangeability of FBS and estimated Average Glucose (eAG) levels in diabetic patients [27] and the clinically approved relationship between eAG and HbA1c levels [28], HbA1c/FBS ratios are in the range of 6.0-6.5 L/mol for diabetic patients (HbA1c > 6.0%, 42.1 mmol/mol) versus < 6.0 L/mol in non-diabetic patients (see SFig. 2). WF10 therapy thus led to restoration of "non-diabetic" HbA1c/FBS ratios.

As reported before, the specific HbA1c-reducing effect of WF10 is based on a drug-induced removal of highly glycated RBCs [21]. Evaluation of RBC-derived biomarkers proof this

new pharmacological approach. In line with previous studies [21], RBC homeostasis restoration under WF10 therapy was observed, also including transient erythropoiesis induction and resolution of pre-existing anemia. Anemia is associated with diabetic micro- and macrovascular complications and higher risk for all-cause and cardiovascular mortality [29]. At DFU, anemia is a common co-morbidity and predictive for the clinical outcome [30].

The WF10-derived removal of highly glycated RBCs also resulted in the, yet not significant, reduction of AGEs and further protein modifications in these cells, including FL, G-H1 and 3-NT, between BL and Week 08. Elevated formation of FL, an early glycation product of lysine residues, is associated with diabetic vascular pathologies [31]. However, for the FL-derived AGEs CML and CEL, also suspected to be involved in diabetic vasculopathies [32], no effects of WF10 therapy were observed.

Especially at diabetes, RBCs represent an important sink for peroxynitrite ($ONOO^-$) in blood [33]. Yet resulting 3-NT formation in these cells lead to impaired Hb functionality and promote cell senescence and apoptosis [34]. It may be guessed that WF10-derived restoration of RBC homeostasis also helps to rebuilt the physiological function of these cells as a sink for peroxynitrite. Elevated oxidative and nitrosative stress in diabetic patients and resulting higher $ONOO^-$ and 3-NT levels are highly predictive for atherosclerosis, coronary arterial disease and cardiovascular death [33,35].

We also showed the effect of WF10-mediated removal of highly glycated pre-hemolytic RBCs [21,29] and handling of hemolytic products [12,24], as well as the potential reduction of RBC-derived AGEs by the drug, on diabetic vasculopathy and atherosclerosis [36,37]. A pooled analysis of PAD patients from this and a previous study [21] showed ABI improvement in 91% of the patients with complete PAD resolution in 2/3rd of the cases. DFU is often accompanied by PAD and low ABI values correlate with disease severity, infection, OM development and amputation risk in these patients [38]. Low/decreasing ABI values are highly predictive for atherosclerosis, cardiovascular events and mortality [39]. To date there are only two FDA-approved medications for PAD treatment with only moderate clinical effects [40]. Under WF10 therapy, significant ABI increase/PAD resolution was achieved within 8 – 12 weeks.

The WF10-mediated resolution of diabetic vasculopathy via restoration of RBC homeostasis translated into improved wound healing, better OM resolution and reduced OM development in the patients. Only one patient from the SOC group required an amputation during the course of the study. Direct bactericidal effects of the drug, especially on anaerobic bacteria [41], may also have contributed to the observed clinical outcome.

Both the restoration of RBC homeostasis [21] as well as direct immune-regulatory and anti-inflammatory effects of the drug [42] may have contributed to the restoration of immune homeostasis in the patients (NLR decrease). The obtained normalization of thrombocyte counts under WF10 therapy is also in line with previous observations [21].

Stable eGFR values indicate renal safety of the drug, whereby (non-significantly) increasing median UACR values may even indicate beneficial effects of the drug for renal vascular integrity [21]. Renal ECs are especially vulnerable to hemolytic products, the latter being detoxified by WF10 [12,25]. Hepatic parameters and ECG measurements did not change under WF10 therapy, again showing drug safety. In a patient with elevated liver values at the beginning of the study, WF10-mediated NLR reduction also led to quick normalization of hepatic parameters.

Conclusion

In conclusion, WF10 represents a first-in class medication for efficient and safe reduction of elevated HbA1c values via physiological removal of highly glycated RBCs, thus preventing the pathological effects emerging from excess glycation of these cells. By handling pre-hemolytic RBCs and hemolytic products, the drug provides a causal treatment for diabetic vascular complications, leading to quick regeneration of dysfunctional endothelial layers. Currently approved Glucose-lowering drugs provide neither efficient reduction of elevated HbA1c values nor causal treatment of diabetic vascular complications.

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Conflict of Interest Disclosure

OXO Translational Science GmbH Germany provided the test medication WF10 and was the sponsor of the reported clinical trial. This company is owned by FW Kuehne and J Flemmig is employee at this company. As outlined below, both FW Kuehne and J Flemmig contributed to the study design as well as to collection, analysis and interpretation of the data and to manuscript writing and revision. FW Kuehne holds several patents and patent applications in regard to WF10 and chlorite-based drugs.

The authors declare that there is no conflict of interest regarding the publication of this article. The investigators also declare that there is no conflict of interest to study participants.

Author Contributions and Guarantor Statement

FW Kuehne, MY Bajuri and J Flemmig were involved in the conception and design of the study. MY Bajuri, RZAR Sabudin, AHA Gafor and N Sukor were involved in in the conduct of the study. Z Zainol and J Flemmig collected and analyzed the data. FW Kuehne and J. Flemmig wrote the first draft of the manuscript, and all authors edited, reviewed and approved the final version of the manuscript.

MY Bajuri is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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