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Effect of the Chlorite-Based Drug WF10 on Hemoglobin A1c, Hematological Biomarkers in Uncontrolled Diabetic Patients with Foot Ulcer

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Abstract

Aims: To evaluate degree and kinetics of WF10 effects on HbA1c, associated hematological biomarkers, and wound healing in uncontrolled T2DM patients with DFU.

Methods: In this prospective, interventional, pretest-posttest study, 40 DFU patients with HbA1c > 8.5 % were treated with standard therapy plus five weekly infusions of the chlorite-based drug WF10 within outpatient department. Besides HbA1c kinetics, we studied red blood cell distribution width (RDW-CV) value, neutrophil-lymphocyte ratio (NLR) and wound healing at week (W) 0, 4, 8 and 12.

Results: In 38 PP-treated patients WF10 decreased HbA1c value from 10.48 % at baseline (BL) to 8.06 % at W8 and Wound Severity Score (WSS) from 8.0 to 1.4 (both $p < 0.0001$) at W12. RDW-CV was diminished from 13.5 % to 12.8 % ($p = 0.0021$), NLR decreased from 2.8 to 2.2 (NS) but significantly decreased in patients with NLR > 3.5 at BL, from 6.3 to 3.2. No serious side effect of WF10 was observed.

Conclusion: Standard therapy of DFU plus adjunct WF10 application consistently and long-lastingly decreased high HbA1c values and showed good outcome of wound healing in patients with uncontrolled diabetes. The used treatment protocol is applicable for outpatient treatment.

Keywords: Chlorite-based drug; Diabetic Foot Ulcer (DFU); Diabetes mellitus; HbA1c; Neutrophil-lymphocyte ratio (NLR); Red blood cell distribution width (RDW-CV); WF10; Wound healing

Abbreviations: ABI: Ankle-Brachial Index; ALP: Alkaline Phosphatase; ANOVA: Analysis of Variance; BL: Baseline; BMI: Body Mass Index; BP: Blood Pressure; CKD: Chronic Kidney Disease; CVD: Cardiovascular Disease; DBP: Diastolic Blood Pressure; DCCT: Diabetes Control and Complication Trial; DFO: Diabetic Foot Osteomyelitis; DFU: Diabetic Foot Ulcer; eGFR: estimated Glomerular Filtration Rate; GPAC: Gram-Positive Anaerobic Cocci; HbA1c: Hemoglobin A1c; Hct: Hematocrit; Hgb: Hemoglobin; HSA: Human Serum Albumin; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume; NLR: Neutrophil-Lymphocyte Ratio; NS: Not Significant; NR: Non-Responder; OPD: Outpatient Department; PAD: Peripheral Arterial Disease; PLT: Platelets; PP: Per Protocol; PVD: Peripheral Vascular Disease; RBC: Red Blood Cell; RDW-CV: RBC Distribution Width; R: Responder; RTC: Reticulocytes; SBP: Systolic Blood Pressure; sCr: serum Creatinine; SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic Pyruvic Transaminase; T2DM: Type 2 Diabetes Mellitus; UKPDS: UK Prospective Diabetes Study; WBC: White Blood Cell; WSS: Wound Severity Score

Introduction

Type 2 Diabetes Mellitus (T2DM) represents a global pathologic burden with high prevalence and an increasing share of mortality [1,2]. Especially the number of patients with uncontrolled T2DM is on the rise [2,3]. These patients exhibit HbA1c values > 8.0 %

and a high risk for the development of diabetic complications [4,5]. Not hyperglycemia itself but emerging vascular pathologies are responsible for T2DM-accociated morbidities and elevated mortality [1].

Diabetic Foot Ulcer (DFU) represents the most common and severe diabetic vascular complication [6,7]. The mortality of DFU patients (5 – 10 % within the first year [8,9]), sums up to > 40 % five-year mortality [7,10]. DFU is the major reason for non-traumatic lower-limb amputations in T2DM patients [11] and makes up one third of the overall expenses for diabetic patients [7,10,12,13]. Both the risk for recurrent ulceration [6,7,3] and mortality rates [10] almost double after amputation. About 20 % of DFU-derived wound infections spread to the bone, causing Diabetic Foot Osteomyelitis (DFO) [10].

Traumata are the initial cause for DFU formation [14], facilitated by T2DM-associated neuropathy [15]. Yet, the chronically impaired wound healing [1,16,17] results from a multifaceted pathology [1,6,18], including

1) a chronic systemic pro-inflammatory state [13,16,19]
2) higher susceptibility to wound infection [10,20]
3) vasoconstriction (impaired oxygen/nutrition supply) [1]
4) endothelial dysfunction and
5) Peripheral Vascular/Arterial Disease (PVD, PAD) [11]

Elevated Neutrophil-Lymphocyte Ratio (NLR) values reflect the immunological imbalance in DFU patients [13,20] and correlate with PAD and DFU pathology severity [9,13,19]. About 50 % of T2DM patients exhibit PAD, which explains both the high prevalence of DFU and the elevated risk for cardiovascular diseases (CVD) in these patients [13,21]. RBC Distribution Width (RDW-CV) values are also elevated in T2DM patients [22] and represent a reliable predictive blood marker for the cardiovascular risk and death [23,24].

A multidisciplinary medical approach is needed to meet the multifaceted pathology of DFU [6]. Standard treatment of DFU patients includes

- Wound care with surgical debridement, wound dressing and exudate control
- Infection management with local and systemic antibiotic therapy, and
- Diabetic control with oral anti-diabetics and insulin [6,7,15].

However, clinical DFU management still lacks standardization [7,10] due to the limited knowledge about the underlying pathological mechanisms [1,7,15], and limb amputations due to uncontrolled infections/inflammation are still common [25].

Clinical studies with WF10, a chlorite-based drug already approved in Thailand as adjunct for DFU treatment (tradename: Immunokine), showed manifold positive effects in DFU patients [26-28]. WF10 (1) improved wound healing (decreasing Wound Severity Score, WSS) [29], (2) diminished inappropriate immune activation [30], and (3) improved microcirculation/tissular oxygen supply [31]. The drug also diminishes opportunistic infections [32] and, in T2DM patients, lowers HbA1c values [28].

This study aimed to confirm the extent and the kinetics of WF10-derived HbA1c reduction and concomitant wound healing in uncontrolled T2DM patients with DFU. We also addressed the effect of the drug on hematological biomarker associated with impaired erythrocyte homeostasis, including hematocrit (Hct), MCV (Mean Corpuscular Volume) and RDW-CV values, to gain insights into the mechanisms behind the effect of chlorite-based drugs at diabetic vascular complications. RDW-CV values were also used to monitor drug-derived erythropoiesis induction [24] and, in combination with blood pressure (BP) data, to evaluate cardio-protective drug effects [23,33]. Platelet counts (PLT) [34-36] and eGFR (estimated Glomerular Filtration Rate) values [37] were followed to monitor drug-derived anti-thrombotic activities and effects on renal functionality. Immune-modulatory effects were addressed by determining NLR values [13,20].

Material and Methods

Patient selection

The study was conducted at the HRH Princess Maha Chakri Sirindhorn Medical Centre, Faculty of Medicine, Srinakharinwirot University (SWU), Thailand between July 2019 and April 2020. The clinical research ethics committee approved the study protocol to be conducted as a prospective, interventional, pretest-posttest clinical trial under conditions of an outpatient department (OPD). In brief, 81 T2DM patients with DFU were screened and patients (age: 18 – 80 years) with HbA1c > 8.5 % (uncontrolled diabetes) and Hct values > 30 % were enrolled. Major exclusion criteria were a Karnofsky performance status < 60, severe arterial occlusion (Ankle-

Brachial Index (ABI) < 0.4) and end-stage renal disease. Only patients who provided written consent where included in the study and received WF10 therapy. The baseline characteristics of the enrolled patients are shown in Table 1.

Characteristic	Value
General	
Number of patients	40
Sex (Male/Female)	17/23
Age, years	54.7 ± 8.3
Smoking (≥ 3 cigs/d)	3 (8 %)
Nutritional status	
Body weight, kg	73.4 ± 18.0
BMI, kg/m^2	26.9 ± 5.3
Serum albumin (HSA), g/dL	4.0 ± 0.4
Diabetes	
Duration of Diabetes, years	11.8 ± 7.0
HbA1c, %	10.5 ± 1.5
FBG, mg/dL	248.5 ± 87.6
DFU	
Ulcer duration, weeks	52.2 ± 67.3
Wound severity score (WSS)	8.0 ± 4.4
ABI	1.0 ± 0.2
Previous minor amputation	6 (15 %)
Previous major amputation	1 (3 %)
Wound type	
Neuropathic ulcer	21 (53 %)
Ischemic ulcer	2 (5 %)
Infected ulcer	17 (43 %
Suspected osteomyelitis	12 (30 %)
Comorbidities	
Nephropathy[1]	6 (15 %)
Retinopathy	10 (25 %)
Heart disease	4 (10 %)
Hypertension[2]	26 (65 %)
Dyslipidemia	20 (50 %)
Laboratory findings	
Hct, %	37.5 ± 3.9
RDW, %	13.5 ± 1.5
eGFR, mL/min/1.73 m^2	76.9 ± 29.7
Serum creatinine, mg/dL	1.1 ± 0.4
WBC (x10^9 c/L)	8.6 ± 2.7
NLR	2.8 ± 2.4

[1]Nephropathy: based on medical documentation and reported medication

[2]Hypertension: based on reported anti-hypertensive medication

Abbreviations: ABI: Ankle-Brachial Index; BMI: Body Mass Index; eGFR: Estimated Glomerular Filtration Rate; Hct: hematocrit; FBG: Fasting Blood Glucose; HbA1c: hemoglobin A1c; HAS: Human Serum Albumin; NLR: Neutrophil-Lymphocyte Ratio; RDW: RBC Distribution Width; WBC: White Blood Cell; WSS: Wound Severity Score

Table 1: Baseline demographic characteristics of the enrolled patients.

Treatment protocol

The wounds of the patients were classified into three groups, according to common clinical presentations of DFU, which had essentially different severities and prognose [27]:

(1) Neuropathic ulcers on high pressure plantar area with no or minor degree of infection;

(2) Ischemic ulcers at toe, medial/lateral margin of foot with tissue necrosis/gangrene, and no or minor degree of infection;

(3) Severely infected ulcers with neuropathic or ischemic ulcers that presented with acute severe infection/inflammation (severe cellulitis, necrotizing fasciitis, deep abscess, and/or osteomyelitis).

All patients received DFU wound management according to the current international standards [38] within the surgical Outpatient Department (OPD) for a duration of 12 weeks. After ulcer assessments and photography, sharp surgical debridement was performed to remove the grossly necrotic tissue, if present. A wet dressing was applied once a day using 0.9 % saline-soaked gauze. Thereby, Oxoferin was used, which represents a 5-fold diluted WF10 solution used for topical wound treatment [39,40].

Every patient with active wound infection received antibiotics. For mild infections (infection severity score 1-2), oral antibiotics were used whereas patients with severe infection (infection severity score 3-4) received parenteral antibiotics. All patients were advised to rest and to reduce foot pressure loading by rest and using crutches or walkers and well-fitted standard shoes with soft insoles; however, no custom-molded shoe was used. The general management of diabetes and its comorbidities was also provided for the patients, during and after intervention, with the same drug regimen.

As a major addition to standard of care, all patients included in the study also received WF10 as an adjuvant medication, applied at a dose of 0.3 mL/kg BW diluted in 300 mL physiological saline with infusion in a period of 3 hours. In contrast to previous studies [26-28], a reduced application frequency with five subsequent once-a-week infusions was chosen.

Clinical outcome

A 16-point Wound Severity Score (WSS) system was applied to characterize the DFU wounds with regard to (1) infection/inflammation severity, (2) relative amount of necrotic tissue, (3) relative amount of granulation tissue and (4) ulcer depth [27]. Ulcer depth was addressed via visual inspection and metal probing to bone test while ulcer area was assessed by using a transparency grid overlay. While severe infection/inflammation and tissue necrosis are indicative for impaired wound healing, granulation and decrease in ulcer depth are signs for good wound healing. All four aspects were graded (1-4) and summed up to 0-16 points for healed to most severe ulcers. A WSS of 0-1, 2-4 or > 4 at W12 was considered as good, fair or poor wound healing, respectively. The kinetics of WSS reduction was followed as well and a lowering of >80 %, 20-80% or < 20 % were also considered good, fair and poor wound healing.

Statistical analysis

Statistical analysis was performed by using a two-sided paired t-test for before/after treatment comparison. The statistical test results were considered significant (*) if $p < 0.05$ and strongly significant (**) if $p < 0.001$. One-way ANOVA (analysis of variance, $\alpha = 0.05$) analysis was performed in order to evaluate statistically significant changes of the values during the course of the study. The latter was supplemented by a Scheffé Post-Hoc test.

Results

Main study outcome

While 40 patients were included in the study and completed the WF10 treatment period, two patients were excluded from the analysis due to lost follow-up. From the PP-treated patients (N = 38), 20, 16 and 2 patients exhibited neuropathic, infected or ischemic DFU, respectively. After the treatment period till Week 4 (W4), the patients were followed up until W12 by repeated clinical examinations and wound management.

In the PP-treated patient (N = 38) a strongly significant reduction of HbA1c from BL (10.48 ± 1.46 %) to W8 (8.06 ± 1.55 %) was obtained (Table 2, primary study outcome). The reduction was already/still significant at W4 (8.98 ± 1.54 %) and W12 (8.42 ± 1.76 %), respectively (secondary study outcome), while the latter mean value indicates re-rising HbA1c values. A combined ANOVA and Scheffé post-hoc test analysis shows significant stepwise HbA1c reduction ($F(3, 148) = 16.8278$; $p < 0.0001$) from BL to W8 while the minor increase from W8 to W12 was not significant.

Parameter	N	Baseline	Week 4	Week 8	Week 12
HbA1c,	38	10.48 ± 1.46	8.98 ± 1.54	8.06 ± 1.55	8.42 ± 1.76
%			** (p < 0.0001)	** (p < 0.0001)	** (p < 0.0001)
WSS	38	8.0 ± 4.3	2.7 ± 2.7	2.0 ± 2.3	1.4 ± 1.5
			** (p < 0.0001)	** (p < 0.0001)	** (p < 0.0001)
Abbreviations: HbA1c, hemoglobin A1c; WSS, Wound Severity Score					

Table 2: Primary effects of WF10 in DFU patients treated per protocol (PP).

In regard to the clinical outcome, the WSS (see Table 2) continuously dropped from 8.0 ± 4.3 at BL to 2.0 ± 2.3 at W8 and 1.4 ± 1.5 at W12 in a strongly significant manner. After 12 weeks, 24 (63.2 %), 12 (31.6 %,), and 2 (5.2 %,) patients exhibited good (WSS 0 – 1), moderate (WSS 2 – 4) and poor (WSS > 4) wound healing, respectively. As for wound healing times, WSS reduction became significant at W2 (ANOVA/Scheffé: $F(6,259) = 20.5867$, $p < 0.0001$). Good wound healing (WSS 0 – 1) was obtained after a median time of 4 weeks (N = 26). From the 12 patients with fair wound healing, two patients exhibited wound re-infection after good wound healing (WSS: 1). Thus, the outcome of these patients should actually be considered as good wound healing. The patients with poor wound healing still showed drastic WSS reduction from 14/15 to 5/6 between BL and W12, which should be considered as fair wound healing.

The obtained changes in HbA1c values correlated well with the reduction of fasting blood glucose (FBG) levels. As shown in Table 3, at BL and W12, FBG levels of 250.5 ± 89.0 mg/dL and 199.1 ± 88.0 mg/dL were determined, obviously fitting the corresponding HbA1c values [41]. Yet, while the strongest HbA1c reduction was observed at W8, average FBG levels were lowest at W4 (190.4 ± 74.8 mg/dL) and slightly higher at W8 (212.3 ± 82.3 mg/dL). The same holds for patients with high initial FBG levels (Table 4), where the initial value at BL (334.4± 60.2 mg/dL) dropped almost $^{1}/_{3}^{rd}$ at W4 (225.6 ± 74.0 mg/dL), while at W8 again slightly higher values were observed (242.6 ± 90.7 mg/dL). In this subgroup, a mean FBG value of 216.7 ± 95.3 mg/dL was observed at W12 (Table 4). As shown in suppl. Figure 1, the strongest decrease of urinary glucose secretion was observed at W4, while at weeks 8 and 12 the improvement from BL was less pronounced. These data correlate well with the observed FBG levels but less to the course of HbA1c reduction.

Parameter	N	Baseline	Week 12
FBG,	38	250.5 ± 89.0	199.1 ± 88.0
mg/dL			* (p = 0.0098)
Hct,	38	37.5 ± 3.9	37.5 ± 4.2
%			N.S. (p = 1.0000)
RBC,	38	4.7 ± 0.5	4.5 ± 0.6
$x10^{12}$ c/L			* (p = 0.0011)
MCH,	38	26.7 ± 2.6	27.7 ± 2.5
pg/c			** (p < 0.0001)
MCV,	38	80.4 ± 5.9	83.8 ± 6.0
fL			** (p < 0.0001)
RDW-CV,	38	13.5 ± 1.5	12.8 ± 0.9
%			* (p = 0.0021)
PLT,	38	333.2 ± 126.7	283.4 ± 63.5
$x10^{9}$ /L			* (p = 0.0018)
eGFR,	38	77.6 ± 30.3	78.9 ± 29.0
mL/min/1.73 m^2			N.S. (p = 0.6867)
Abbreviations: eGFR: estimated Glomerular Filtration Rate; FBG: Fasting Blood Glucose; Hct, hematocrit; MCH: Mean Corpuscular Hemoglobin; MCV: Mean Corpuscular Volume; RBC: Red Blood Cell; RDW: RBC Distribution Width; PLT: platelet			

Table 3: Further major effects of WF10 in PP-treated patients.

Parameter	N	Baseline	Week 12
FBG (> 250.5[1]),	17	334.4 ± 60.2	216.7 ± 95.3
mg/dL			* (p = 0.0011)
Hct (< 35),	12	33.5 ± 1.2	34.5 ± 4.0
%			N.S. (p = 0.3432)
RDW-CV (> 13.5),	17	14.6 ± 1.5	13.2 ± 1.1
%			** (p = 0.0007)
PLT (> 450),	7	549.9 ± 91.4	339.0 ± 62.4
$x10^9$ /L			** (p = 0.0005)
eGFR (< 90),	25	60.1 ± 16.3	61.8 ± 18.1
mL/min/1.73 m^2			N.S. (p = 0.3435)
eGFR (< 60),	12	45.8 ± 8.8	46.0 ± 12.4
mL/min/1.73 m^2			N.S. (p = 0.4290)
WBC (> 10),	8	13.0 ± 2.7	8.6 ± 1.5
$x10^9$ c/L			* (p = 0.0078)
NLR (> 3.5)	8	6.3 ± 3.6	3.2 ± 1.7
			* (p = 0.0475)
HSA (NLR > 3.5)	8	3.6 ± 0.4	4.1 ± 0.4
			* (p = 0.0206)

[1]Mean BL value of all PP-treated patients (see Table 3)

Abbreviations: eGFR: estimated Glomerular Filtration Rate; FBG: Fasting Blood Glucose; Hct: hematocrit; HAS: Human Serum Albumin; NLR: Neutrophil-Lymphocyte Ratio; PLT: platelet; RDW: RBC Distribution Width; WBC: White Blood Cell.

Table 4: Effect of W10 in clinically relevant subgroups of PP-treated patients.

Effect on RBCs

As WF10 apparently further decreased the average HbA1c value between W4 and W8 despite stable FBG levels, we addressed additional hematological markers with prognostic value. Table 3 shows that Hct values remained stable between BL (37.5 ± 3.9 %) and W12 (37.5 ± 4.2 %). In patients with anemia at BL (N = 12, Hct < 35 %), even a, yet non-significant, increase of the mean Hct from 33.5 ± 1.2 % to an almost non-anemic mean value (34.5 ± 4.0 % at W12) was observed (see Table 4). These results are in line with a minor but significant decrease in the mean RBC count from 4.7 ± 0.5 x 10^{12} c/L at BL to 4.5 ± 0.6 10^{12} c/L at W12, paralleled by a minimal but strongly significant increase in Mean Corpuscular Hemoglobin (MCH) levels from 26.7 ± 2.6 pg/c to 27.7 ± 2.5 pg/c. (Table 3). At the same time, mean Hgb levels as well as Mean Corpuscular Hemoglobin Concentrations (MCHC) did not change significantly (Table 5).

Parameter	N	Baseline	Week 4	Week 8	Week 12
SBP (> 140),	19	151.1 ± 10.0	136.9 ± 15.6	138.8 ± 14.3	143.9 ± 17.9
mmHg			* (p = 0.0013)	* (p = 0.0013)	N.S. (p = 0.0529)
DBP (SBP > 140),	19	85.5 ± 8.5	76.2 ± 8.9	78.1 ± 9.4	77.3 ± 10.3
mmHg			** (p = 0.0001)	* (p = 0.0058)	* (p = 0.0135)

Abbreviations: DBP: Diastolic Blood Pressure; SBP: Systolic Blood Pressure

Table 5: Effect of WF10 on elevated blood pressure in Per Protocol (PP)-treated patients.

As shown in Tab. 3 both, Mean Corpuscular Volume (MCV) and RBC distribution width (RDW-CV) turned out as further RBC-based biomarkers, which were considerably influenced upon WF10 treatment. While the mean MCV value significantly increased from 80.4 ± 5.9 fL at BL to 83.8 ± 6.0 fL at W12, the RDW-CV value significantly dropped (13.5 ± 1.5 % / 12.8 ± 0.9 %) within the same time. In patients with RDW-CV > 13.5 % (Table 4, N = 17), the mean value even decreased from 14.6 ± 1.5 % to 13.2 ± 1.1 %. The drug apparently selectively replaces older small RBCs by bigger new cells (RBC and RDW-CV reduction, MCV increase), while retaining normochromic cells (stable MCHC) and blood homeostasis (stable Hct and Hgb levels). In fact, in 11 PP-treated patients also the reticulocyte count was determined and showed a significant (p = 0.0104) transient increase in immature RBCs (1.7 ± 0.4 % at BL, 2.7 ± 1.3 % at W4, 1.7 ± 0.7 % at W12).

Vascular effects

The observed RDW-CV reduction also suggests risk reduction in the PP-treated DFU patients in regard to renal disturbances, hypertension and acute cardiac events [23;33]. Accordingly, we addressed further PVD- and CVD-relevant biomarkers, including estimated Glomerular Filtration Rates (eGFR), Systolic/Diastolic Blood Pressure (SBP, DBP) and platelet (PLT) counts. Thereby again both the development within the complete study population (see Table 3 for efficacy data and Suppl. Table 1 for safety data) and in clinically relevant sub-groups (Tables 4 and 5) were analyzed.

Between BL and W12, the mean PLT count decreased significantly from 333.2 ± 126.7 x 10^9 /L to 283.4 ± 63.5 x 10^9 /L (Table 3). In the sub-group with PLT > 450 x 10^9 /L at BL (Table 4, N = 7), the effect was considerably stronger (549.9 ± 91.4 x 10^9 /L / 339.0 ± 62.4 x 10^9 /L). In the sub-group with SBP > 140 mmHg at BL (Table 5, N = 18), both SBP (151.1 ± 10.0 mmHg) and DBP (85.5 ± 8.5 mmHg) (almost) significantly decreased till W12 (143.9 ± 17.9 mmHg / 77.3 ± 10.3 mmHg), while in the overall evaluation these values remained essentially stable (Suppl. Table 1, safety data of PP-treated patients). However, in the hypertensive sub-group WF10 co-treatment led to a (strongly) significant transient decrease of SBP (136.9 ± 15.6 mmHg, reduction by 14.2 mmHg) and DBP (76.2 ± 8.9 mmHg, reduction by 9.3 mmHg) at W4.

The overall analysis (Table 3) showed a slight, yet not significant, improvement in the mean eGFR value between BL (77.6 ± 30.3 mL/min/1.73 m^2) and W12 (78.9 ± 29.0 mL/min/1.73 m^2). As shown in Table 4, in patients with mild (stage 2, eGFR: 60-89 mL/min/1.73 m^2, N = 25) or moderate CKD (stages 3a/b, eGFR: 30-59 mL/min/1.73 m^2, N = 12) this improvement was even more pronounced, yet still not significant. Thus, while the co-treatment of DFU patients with WF10 did not result in improving eGFR values, renal functionality apparently remained stable, especially in patients with already impaired glomerular filtration capacity. Accordingly, also urinary secretion of protein (Suppl. Figure 2) and RBCs (Suppl. Figure 3) did not change over the study period. Still, in regard to the latter parameter, a tendency towards less erythrocyte secretion was observed, which was essentially prominent directly after the WF10 treatment period (W4).

Effects on NLR

As shown in suppl. Table 1, the overall analysis of the PP-treated patients (N = 38) showed a non-significant reduction of NLR from 2.8 ± 2.4 at BL to 2.2 ± 1.2 at W12. However, in the patients with NLR > 3.5 at BL (Table 4, N = 8), the ratio significantly decreased by almost 50 % at W12 (6.3 ± 3.6 / 3.2 ± 1.7). Directly after the treatment period, an even stronger transient NLR reduction was observed (2.8 ± 1.4 at W4). Yet, it has to be clearly stated, that seven out of eight patients in the discussed sub-group exhibited NLR values ≤ 6.2, while in one patient the ratio was 15.1. The latter brings in a significant bias into the BL value, which is also reflected by the large SD values. However, by excluding this patient from the analysis, the NLR value still significantly ($p = 0.0242$) dropped from 5.1 ± 0.8 to 3.1 ±1.8. Regarding mean WBC count, a non-significant decrease from 8.6 ± 2.7 x 10^9 c/L at BL to 7.9 ± 1.6 x 10^9 c/L at W12 was measured in the treated cohort (Suppl. Table 1), while again the effect was significant and more prominent in patients with elevated WBC levels at BL (>10 x 10^9 c/L, Table 4). The sub-group with elevated NLR levels only partially matched the subgroup with elevated WBC counts (3/8).

Safety markers for hepatic function, including Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP) were within the normal physiological range and slightly decreased throughout the study (Suppl. Table 1), significant change in the case of SGPT). Mean serum creatinine levels (sCr) were always found to be below 1.05 mg/dL and did not change during the study (not shown).

Discussion

Elevated HbA1c levels correlate with hyperglycemia at T2DM and thus also reflect both the incidence and the severity of late stage micro- and macro-vascular complications, including DFU [42,43]. Accordingly, large clinical studies (UK Prospective Diabetes Study, UKPDS; Diabetic Control and Complication Trial, DCCT) consistently showed that HbA1c reduction attenuates diabetic vascular pathologies [5,44]. Thereby, a reduction by 1 % already emerged as a promising therapeutic strategy, as e.g. the risk for peripheral vascular diseases was reduced by about 40 % [5]. Yet, an aggressive HbA1c reduction to 6 % may increase the risk for cardiovascular events in T2DM patients [45]. In regard to DFU, diminishing HbA1c values to 7-8 % has been suggested as a good therapeutic strategy to improve wound healing without increasing the mortality risk [45,46].

The adjunct treatment of DFU patients with the chlorite-based drug WF10 led to a constant and long-lasting reduction of HbA1c from uncontrolled to almost controlled values within 4 weeks. Most notably, previous diabetic control was not able to reduce HbA1c

values and medication for glycemic control was either not changed or even reduced under treatment. Five patients did not respond to the treatment (higher HbA1c values at W8/W12). These five non-responders (NR) exhibited extremely high fasting blood glucose (FBG) at W12 (330.8 ± 78.5 mg/dL). The HbA1c-reducing activity of WF10 was already shown before, including stronger drug effects in uncontrolled T2DM patients. [28]. HbA1c levels > 8 % are considered a threshold in regard to significant structural and functional disturbances of RBCs [4].

Moreover, HbA1c reduction was paralleled by good and fast wound healing in many patients, especially in the severely infected ulcer group. These results are in line with previous studies [26-28], although in this study the WF10 infusion frequency was diminished from five consecutive days (in-hospital) to once-a-week for five consecutive weeks (OPD). Good wound healing was observed after a median time of four weeks.

Elevated HbA1c levels correlate not only with hyperglycemia but more importantly with the endothelial adhesion [47], the latter correlates with severity of vascular complications [48]. The swift HbA1c reduction during the WF10-treatment period preceded a slower drop in FBG levels, which suggests direct effects of the drug on highly glycated RBCs. Accordingly, the treatment of DFU patients with the chlorite-based drug influenced several further erythropoiesis-based parameters, including (1) minimal RBC reduction, (2) minor MCH increase, (3) RDW-CV reduction and (4) MCV increase. These observations suggest blood rejuvenation and/or recovery of normal blood homeostasis.

Accordingly, both Hct values and Hgb levels showed a corresponding transient decrease during the treatment period, matching the HbA1c reduction, but recovered to baseline levels at W12 by generation of naïve RBC. The effect of hyperglycemia on RBC indices was already shown before [49]. For the 33 responders, the link between WF10-mediated HbA1c reduction and erythropoiesis-based parameters is illustrated in Suppl. Figure 4. The continuous and persistent HbA1c reduction was accompanied by a transient Hct reduction and RTC increase till W4. As shown in Suppl. Table 2 (efficacy data in the responder-subgroup), a considerable and long-lasting FBG reduction was also achieved in these patients. These results show that a less frequent application of WF10 further improves drug safety while retaining efficacy, confirming preliminary results on T2DM patients without DFU (Paiboon Maraprygsavan, data not shown). In anemic patients, Hct and Hgb levels were even slightly higher at W12 as compared to BL.

The observed MCV increase and RDW-CV decrease may indicate a drug-derived attenuation of microcytotic anemia, a pathological condition of elevated RBC death [50] present at hyperglycemia [51]. In fact, a selective analysis in 11 patients revealed high LDH levels (> 190 U/L) at BL, which may be indicative for elevated hemolysis rates [52]. Highly glycated vaso-adhesive and frail RBCs as well as derived hemolytic products may actively contribute to both immunological disturbances and vascular pathologies at diabetes [14,16], including DFU [1]. Elevated RDW-CV values in patients with T2DM, resulting from higher RBC fragility [53], directly correlate with diabetic vascular complications like renal impairments [54] and the risk for DM2-related adverse events like heart failure and acute myocardial infarction (AMI) [24,55]. Altogether, the results suggest highly glycated RBCs as a potential therapeutic target for the causal treatment of diabetic vascular complication.

In line with the supposed selective removal of highly glycated RBCs, the present study also showed a drug-derived attenuation in regard to PVD and CVD in the DFU patients as (1) a considerable decrease in PLT counts, (2) transiently decreasing SBP and DBP values and (3) stable eGFR values were observed. Elevated PLT counts are common at T2DM and associated with the development of diabetic complications [35,36]. The observed reduction may again be attributable to the removal of highly glycated and hemolysis-prone RBCs [56]. Hypertension is common in T2DM patients and represents a complemental risk factor for cardiovascular events [57]. In regard to kidney function, even at renal impairments WF10 treatment led to no significant changes in eGFR rates, indicating stability of the Chronic Kidney Disease (CKD). The latter is common at T2DM, [11,58], correlates with HbA1c values [59] and may again be derived from hyperglycemic RBCs [60].

The Neutrophil-Lymphocyte Ratio (NLR) displays a simple and reliable predictive marker to evaluate the chronic inflammatory status of T2DM patients both at the development and the progression of the disease [61,62]. Especially in combination with RDW, NLR values exhibit a positive predictive value for renal dysfunction [63] and microalbuminuria [62]. At DFU, NLR values are indicative for PAD severity and predictive for wound healing [13,19,20]. In the study, a significant decrease of NLR was observed in patients with elevated levels (> 3.5) at BL. Previous clinical studies, e.g. in HIV patients, already showed the immune-modulatory potential of the drug [64-66]. Other studies also observed a drug-derived immunological rebalancing at post-radiation cystitis [67,68]. The drug-derived reduction of NLR values matched with elevating HSA values, which are also used to monitor reduced inflammation and improved wound healing in DFU patients [69]. Moreover, the chlorite-based drug is also known for its microbicidal properties, especially in regard to anaerobic bacteria [70,71]. The abundance of Gram-Positive Anaerobic Cocci (GPAC) like Peptoniphilus was recently shown to correlate with impaired DFU wound healing [72].

In summary, T2DM incidence is increasing globally and the clinical management of DFU represents an urgent task, especially in those countries with poor glycemic control [73]. Yet, medical management of DFU patients still lacks standardization due to the multifaceted nature of this pathology [7,15]. Novel therapeutic approaches include, but are not limited to, antithrombotic medication, oxygen supplementation and pro-angiogenic agents [6,74]. The presented study data suggest WF10 as a safe and efficient adjuvant therapy for DFU wound management, reflected by a long-lasting HbA1c reduction by over 2 %. The efficiency most likely results from versatile activities of the drug on the underlying disturbed homeostasis, including (1) a selective removal of highly glycated RBCs, (2) restoration of impaired erythropoiesis, (3) the reduction of thrombosis, (4) vasodilatation and improved oxygen supply, (5) systemic immunological rebalancing and (6) diminishing of wound infection. The versatile efficiency of WF10 can be monitored by using accepted prognostic biomarkers like HbA1c, RDW-CV, PLT, BP, eGFR and NLR. While not addressed in this study, in vitro data indicate that WF10 also inactivates toxic hemolytic products, including Hgb [75] and heme [76], in regard to their vascular toxicity and proinflammatory activity [77-79].

Highlights

- The chlorite-based drug WF10 led to a swiftly flowing and consistent decline of HbA1c values in uncontrolled T2M patients.
- Hematological biomarkers indicate a selective drug-mediated replacement of glycated RBCs by naive RBCs.
- The HbA1c reduction is paralleled by improved and accelerated wound healing in DFU patients.
- The adjusted treatment protocol allows safe and convenient treatment of multimorbid patients under OPD conditions.

Conclusion

The treatment of DFU patients with the chlorite-based drug WF10 decreased consistently and long-lastingly high HbA1c values and removed the pathophysiological blockade for successful wound healing. The treatment protocol used is applicable for outpatient treatment. The presented prospective, non-controlled, one arm, study design allowed comparison of exactly the same wounds pre- and post-treatment. The same study design might limit the meaningfulness of the data.

Clinical Trials.gov: NCT04372355

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References

1. Patel S, Srivastava S, Singh MR, Singh D (2019) Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomed Pharmacother 112108615.
2. Classification of Diabetes Mellitus 2019: World Health Organization (WHO), 2019.
3. Raccah D, Chou E, Colagiuri S, Gaal Z, Lavalle F, et al. (2017) A global study of the unmet need for glycemic control and predictor factors among patients with type 2 diabetes mellitus who have achieved optimal fasting plasma glucose control on basal insulin. Diabetes Metab Res Rev: 33.
4. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK (2016) Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights: 1195-104.
5. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, et al. (2000) Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 321: 405-412.
6. Eleftheriadou I, Tentolouris A, Tentolouris N, Papanas N (2019) Advancing pharmacotherapy for diabetic foot ulcers. Expert Opin Pharmacother 20: 1153-1160.
7. Everett E, Mathioudakis N (2018) Update on management of diabetic foot ulcers. Ann N Y Acad Sci 1411: 153-165.
8. Margolis DJ, Malay DS, Hoffstad OJ, Leonard CE, MaCurdy T, et al. (2011) Incidence of diabetic foot ulcer and lower extremity amputation among Medicare beneficiaries, 2006 to 2008. Data Points #2.

9. Morbach S, Furchert H, Groblinghoff U, Hoffmeier H, Kersten K, et al. (2012) Long-term prognosis of diabetic foot patients and their limbs: amputation and death over the course of a decade. Diabetes Care 35: 2021-2027.

10. Geraghty T, LaPorta G (2019) Current health and economic burden of chronic diabetic osteomyelitis. Expert Rev Pharmacoecon Outcomes Res 19: 279-286.

11. Megallaa MH, Ismail AA, Zeitoun MH, Khalifa MS (2019) Association of diabetic foot ulcers with chronic vascular diabetic complications in patients with type 2 diabetes. Diabetes Metab Syndr 13: 1287-1292.

12. Graz H, D'Souza VK, Alderson DEC, Graz M (2018) Diabetes-related amputations create considerable public health burden in the UK. Diabetes Res Clin Pract: 135158-165.

13. Vatankhah N, Jahangiri Y, Landry GJ, McLafferty RB, Alkayed NJ, et al. (2017) Predictive value of neutrophil-to-lymphocyte ratio in diabetic wound healing. J Vasc Surg 65: 478-483.

14. Sheremet'ev YA, Popovicheva AN, Rogozin MM, Levin GY (2019) Red blood cell aggregation, disaggregation and aggregate morphology in autologous plasma and serum in diabetic foot disease. Clin Hemorheol Microcirc 72: 221-227.

15. Brem H, Tomic-Canic M (2007) Cellular and molecular basis of wound healing in diabetes. J Clin Invest 117: 1219-1222.

16. Armstrong DG, Boulton AJM, Bus SA (2017) Diabetic Foot Ulcers and Their Recurrence. N Engl J Med 376: 2367-2375.

17. Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, et al. (2008) Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. Diabetologia 51: 747-755.

18. Guo S, Dipietro LA (2010) Factors affecting wound healing. J Dent Res 89: 219-229.

19. Teperman J, Carruthers D, Guo Y, Barnett MP, Harris AA, et al. (2017) Relationship between neutrophil-lymphocyte ratio and severity of lower extremity peripheral artery disease. Int J Cardiol 228201-204.

20. Ong E, Farran S, Salloum M, Gardner S, Giovinco N, et al. (2017) Does Everything That's Counted Count? Value of Inflammatory Markers for Following Therapy and Predicting Outcome in Diabetic Foot Infection. Int J Low Extrem Wounds 16: 104-107.

21. Brownrigg JR, Davey J, Holt PJ, Davis WA, Thompson MM, et al. (2012) The association of ulceration of the foot with cardiovascular and all-cause mortality in patients with diabetes: a meta-analysis. Diabetologia 55: 2906-2912.

22. Engström G, Smith JG, Persson M, Nilsson PM, Melander O, et al. (2014) Red cell distribution width, haemoglobin A1c and incidence of diabetes mellitus. J Intern Med 276(2) 174-183.

23. Li N, Zhou H, Tang Q (2017) Red Blood Cell Distribution Width: A Novel Predictive Indicator for Cardiovascular and Cerebrovascular Diseases. Dis Markers 20177089493.

24. Lippi G, Turcato G, Cervellin G, Sanchis-Gomar F (2018) Red blood cell distribution width in heart failure: A narrative review. World J Cardiol 10(2) 6-14.

25. IDF Clinical Practice Recommendations on the Diabetic Foot - 2017: Brussels, Belgium, International Diabetes Federation, 2017.

26. Yingsakmongkol N, Maraprygsavan P, Sukosit P (2011) Effect of WF10 (immunokine) on diabetic foot ulcer therapy: a double-blind, randomized, placebo-controlled trial. J Foot Ankle Surg 50(6) 635-640.

27. Yingsakmongkol N (2013) Clinical outcomes of WF10 adjunct to standard treatment of diabetic foot ulcers. J Wound Care 22(3) 130-136.

28. Maraprygsavan P, Mongkolsuk J, Arnhold J, Kuehne FW (2016) The chlorite-based drug WF10 constantly reduces hemoglobin A1c values and improves glucose control in diabetes patients with severe foot syndrome. J Clin Translat Endocrin 453-58.

29. Hinz J, Hautzinger H, Stahl KW (1986) Rationale for and results from a randomised, double-blind trial of tetrachlorodecaoxygen anion complex in wound healing. Lancet 1(8485) 825-828.

30. Gillissen G, Kuehne FW, Breuer-Werle M, Melzer B, Ostendorp H (1986) Increased resistance towards two systemic experimental infections by tetrachlorodecaoxygen anion complex. Possible implications of cellular and humoral immunity. Arzneimittelforschung 36(12) 1778-1782.

31. Dühmke E (1987) Radiation treatment of advanced malignant tumors under influence of the oxidant tetrachlorodecaoxygen anion (TCDO) - a pilot study; CSS.

32. Raffanti SP, Schaffner W, Federspiel CF, Blackwell RB, Ching OA, et al. (1998) Randomized, double-blind, placebo-controlled trial of the immune modulator WF10 in patients with advanced AIDS. Infection 26(4) 202-207.

33. Dada OA, Uche E, Akinbami A, Odesanya M, John-Olabode S, et al. (2014) The relationship between red blood cell distribution width and blood pressure in patients with type 2 diabetes mellitus in Lagos, Nigeria. J Blood Med 5185-189.

34. Martin-Ventura JL, Madrigal-Matute J, Martinez-Pinna R, Ramos-Mozo P, Blanco-Colio LM, et al. (2012) Erythrocytes, leukocytes and platelets as a source of oxidative stress in chronic vascular diseases: detoxifying mechanisms and potential therapeutic options. Thromb Haemost 108(3) 435-442.

35. Onalan E, Gozel N, Donder E (2019) Can hematological parameters in type 2 diabetes predict microvascular complication development? Pak J Med Sci 35(6) 1511-1515.

36. Szablewski L, Sulima A (2017) The structural and functional changes of blood cells and molecular components in diabetes mellitus. Biol Chem 398(4) 411-423.

37. Low S, Lim SC, Zhang X, Zhou S, Yeoh LY, et al. (2017) Development and validation of a predictive model for Chronic Kidney Disease progression in Type 2 Diabetes Mellitus based on a 13-year study in Singapore. Diabetes Res Clin Pract 12349-54.

38. Clinical Pratice Guideline for Diabetes 2017: Bangkok, Thailand, 2017.

39. WF 10 - Macrokine, TCDO, Tetrachlorodecaoxide (2004) Drugs R D 5(4) 242-244.

40. Weise K, Evers KH (1988) Clinical experiences with tetrachlorodecaoxide in the local treatment of difficult-to-heal wounds]. Aktuelle Traumatol 18(5) 219-225.

41. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, et al. (2008) Translating the A1C assay into estimated average glucose values. Diabetes Care 31(8) 1473-1478.

42. Adler AI, Erqou S, Lima TA, Robinson AH (2010) Association between glycated haemoglobin and the risk of lower extremity amputation in patients with diabetes mellitus-review and meta-analysis. Diabetologia 53(5) 840-849.

43. Zhou ZY, Liu YK, Chen HL, Yang HL, Liu F (2015) HbA1c and Lower Extremity Amputation Risk in Patients With Diabetes: A Meta-Analysis. Int J Low Extrem Wounds 14(2) 168-177.

44. Gallagher EJ, Le RD, Bloomgarden Z (2009) Review of hemoglobin A(1c) in the management of diabetes. J Diabetes 1(1) 9-17.

45. Gerstein HC, Miller ME, Byington RP, Goff DC, Jr., Bigger JT, et al. (2008) Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 358(24) 2545-2559.

46. Xiang J, Wang S, He Y, Xu L, Zhang S, et al. (2019) Reasonable Glycemic Control Would Help Wound Healing During the Treatment of Diabetic Foot Ulcers. Diabetes Ther 10(1) 95-105.

47. Wautier JL, Paton RC, Wautier MP, Pintigny D, Abadie E, et al. (1981) Increased adhesion of erythrocytes to endothelial cells in diabetes mellitus and its relation to vascular complications. N Engl J Med 305(5) 237-242.

48. Wautier JL, Wautier MP (2018) Molecular links between erythrocyte adhesion and vascular dysfunction in Diabetes Mellitus, Polycythemia Vera, retinal vascular occlusion. J Hematol Thrombo Dis 6(2) 1-5.

49. Alamri BN, Bahabri A, Aldereihim AA, Alabduljabbar M, Alsubaie MM, et al. (2019) Hyperglycemia effect on red blood cells indices. Eur Rev Med Pharmacol Sci 23(5) 2139-2150.

50. Lang F, Lang E, Foller M (2012) Physiology and pathophysiology of eryptosis. Transfus Med Hemother 39(5) 308-314.

51. Viskupicova J, Blaskovic D, Galiniak S, Soszynski M, Bartosz G, et al. (2015) Effect of high glucose concentrations on human erythrocytes in vitro. Redox Biol 5381-387.

52. Kucukal E, Ilich A, Key NS, Little JA, Gurkan UA (2018) Red Blood Cell Adhesion to Heme-Activated Endothelial Cells Reflects Clinical Phenotype in Sickle Cell Disease. Am J Hematol .

53. Lippi G, Mercadanti M, Aloe R, Targher G (2012) Erythrocyte mechanical fragility is increased in patients with type 2 diabetes. Eur J Intern Med 23(2) 150-153.

54. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, et al. (2008) Relationship between red blood cell distribution width and kidney function tests in a large cohort of unselected outpatients. Scand J Clin Lab Invest 68(8) 745-748.

55. Fava C, Cattazzo F, Hu ZD, Lippi G, Montagnana M (2019) The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype? Ann Transl Med 7(20) 581.

56. Rother RP, Bell L, Hillmen P, Gladwin MT (2005) The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. JAMA 293(13) 1653-1662.

57. Grossman A, Grossman E (2017) Blood pressure control in type 2 diabetic patients. Cardiovasc Diabetol 16(1).

58. Alicic RZ, Rooney MT, Tuttle KR (2017) Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol 12(12) 2032-2045.

59. Low S, Zhang X, Wang J, Yeoh LY, Liu YL, et al. (2018) The impact of HbA1c Trajectories on Chronic Kidney Disease Progression in Type 2 Diabetes. Nephrology (Carlton).

60. Lee SB, Kim YS, Kim JH, Park K, Nam JS, et al. (2019) Use of RBC deformability index as an early marker of diabetic nephropathy. Clin Hemorheol Microcirc 72(1) 75-84.

61. Duman TT, Aktas G, Atak BM, Kocak MZ, Erkus E, et al. (2019) Neutrophil to lymphocyte ratio as an indicative of diabetic control level in type 2 diabetes mellitus. Afr Health Sci 19(1) 1602-1606.

62. Assulyn T, Khamisy-Farah R, Nseir W, Bashkin A, Farah R (2020) Neutrophil-to-lymphocyte ratio and red blood cell distribution width as predictors of microalbuminuria in type 2 diabetes. J Clin Lab Anal e23259.

63. Kawamoto R, Ninomiya D, Kikuchi A, Akase T, Kasai Y, et al. (2019) Association of neutrophil-to-lymphocyte ratio with early renal dysfunction and albuminuria among diabetic patients. Int Urol Nephrol 51(3) 483-490.

64. Kuehne L, Konstandin M, Samstag Y, Meuer S, Giese T, et al. (2011) WF10 stimulates NK cell cytotoxicity by increasing LFA-1-mediated adhesion to tumor cells. J Biomed Biotechnol 436587-436593.

65. Giese T, McGrath MS, Stumm S, Schempp H, Elstner E, et al. (2004) Differential effects on innate versus adaptive immune responses by WF10. Cell Immunol 229(2) 149-158.

66. McGrath MS, Kahn JO, Herndier BG (2002) Development of WF10, a novel macrophage-regulating agent. Curr Opin Investig Drugs 3(3) 365-373.

67. Veerasarn V, Khorprasert C, Lorvidhaya V, Sangruchi S, Tantivatana T, et al. (2004) Reduced recurrence of late hemorrhagic radiation cystitis by WF10 therapy in cervical cancer patients: a multicenter, randomized, two-arm, open-label trial. Radiother Oncol 73(2) 179-185.

68. Veerasarn V, Boonnuch W, Kakanaporn C (2006) A phase II study to evaluate WF10 in patients with late hemorrhagic radiation cystitis and proctitis. Gynecol Oncol 100(1) 179-184.

69. Irawan H, Semadi IN, Devi A (2018) Effect of hyperbaric oxygen therapy to improve serum albumin for patients with Diabetic foot ulcers. Biomed Pharmacol J 11(1) 569-575.

70. Kuehne HH, Ullmann U, Kuhne FW (1985) New aspects on the pathophysiology of wound infection and wound healing--the problem of lowered oxygen pressure in the tissue. Infection 13(2) 52-56.

71. Stoll P, Huber H, Pelz K, Weingart D (1993) Antimicrobial effects of the tetrachlorodecaoxygen-anion complex on oropharyngeal bacterial flora: an in vitro study. Chemotherapy 39(1) 40-47.

72. Min KR, Galvis A, Baquerizo Nole KL, Sinha R, Clarke J, et al. (2020) Association between baseline abundance of Peptoniphilus, a Gram-positive anaerobic coccus, and wound healing outcomes of DFUs. PLoS One 15(1) e0227006.

73. Yan LD, Hanvoravongchai P, Aekplakorn W, Chariyalertsak S, Kessomboon P, et al. (2020) Universal coverage but unmet need: National and regional estimates of attrition across the diabetes care continuum in Thailand. PLoS One 15(1) e0226286.

74. Rooke TW, Hirsch AT, Misra S, Sidawy AN, Beckman JA, et al. (2011) 2011 ACCF/AHA Focused Update of the Guideline for the Management of Patients With Peripheral Artery Disease (updating the 2005 guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol 58(19) 2020-2045.

75. Pichert A, Arnhold J (2015) Interaction of the chlorite-based drug WF10 and chlorite with hemoglobin, methemoglobin and ferryl hemoglobin. Arch Biochem Biophys 58582-89.

76. Flemmig J, Schlorke D, Kuehne FW, Arnhold J (2016) Inhibition of the heme-induced hemolysis of red blood cells by the chlorite-based drug WF10. Free Radic Res 50(12) 1386-1395.

77. Vallelian F, Schaer CA, Deuel JW, Ingoglia G, Humar R, et al. (2018) Revisiting the putative role of heme as a trigger of inflammation. Pharmacol Res Perspect 6(2) e00392.

78. Alvarado G, Jeney V, Toth A, Csosz E, Kallo G, et al. (2015) Heme-induced contractile dysfunction in human cardiomyocytes caused by oxidant damage to thick filament proteins. Free Radic Biol Med 89248-262.

79. Deuel JW, Vallelian F, Schaer CA, Puglia M, Buehler PW, et al. (2015) Different target specificities of haptoglobin and hemopexin define a sequential protection system against vascular hemoglobin toxicity. Free Radic Biol Med 89931-943.