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Systemic effects of intralesional treatment of patients with diabetic foot ulcers using epidermal growth factor (Heberprot-P)

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Palabras clave

diabetes, úlcera de pie diabético, factor de crecimiento epidérmico, estrés oxidativo, inflamación

RESUMEN

Objetivos: La infiltración intralesional con factor de crecimiento epidérmico (EGF) ha emergido como alternativa terapéutica para la cicatrización de las úlceras de pie diabético (UPD). Los objetivos de esta investigación fueron: determinar el perfil molecular de los pacientes con UPD y caracterizar los efectos sistémicos del tratamiento intralesional con EGF en tales pacientes atendiendo a marcadores de balance redox, componentes de la vía de los productos avanzados de glicación (AGE), factores relacionados con la estabilidad de la matriz extracelular (MEC) y marcadores pro-inflamatorios. **Métodos**: Se caracterizaron 13 pacientes con UPD en comparación con diabéticos sin úlceras (compensados y no compensados) e individuos no diabéticos, atendiendo a marcadores de balance redox, componentes de la vía de los AGE y elementos relacionados con la estabilidad de la MEC. La respuesta sistémica de los pacientes con UPD al tratamiento intralesional con EGF se evaluó atendiendo a los mismos parámetros, además de otros marcadores pro-inflamatorios. **Resultados**: Los pacientes con UPD exhibieron un perfil bioquímico muy alterado, con elevado estrés oxidativo, bajas reservas antioxidantes, aumento de los productos de glicoxidación y de las metaloproteasas de la matriz (MMP), con respecto a los diabéticos sin úlceras y a los no diabéticos. La administración intralesional de EGF estuvo asociada a una recuperación significativa de los

parámetros estudiados y a la atenuación sistémica de varios marcadores pro-inflamatorios. **Conclusiones**: Los resultados indican que la infiltración intralesional con EGF se traduce en efectos sistémicos antioxidantes, anti-inflamatorios, anti-degradativos y anti-AGE, que contribuyen a restaurar la homeostasia del paciente diabético.

Efectos sistémicos del tratamiento intralesional con factor de crecimiento epidérmico (Heberprot-P) en pacientes con úlceras de pie diabético

ABSTRACT

Objectives: Intralesional infiltration with epidermal growth factor (EGF) has emerged as a therapeutic alternative for diabetic foot ulcer (DFU) healing. The objectives of this research were: to determine the molecular profile of patients with DFU and to characterize the systemic effects of EGF intralesional treatment in such patients, taking into account redox balance markers, components of the advanced glycation end-products (AGE) pathway, factors related to the extracellular matrix (ECM) stability and pro-inflammatory markers. **Methods**: Thirteen patients with DFU were characterized in comparison with diabetic patients without ulcers (compensated and uncompensated) and non-diabetic subjects, attending to redox balance markers, components of the AGE pathway, and elements related to the stability of the ECM. The systemic response of patients with DFU to the intralesional treatment with EGF was evaluated according to the same parameters, in addition to other pro-inflammatory markers. **Results**: Patients with DFU exhibited the most disheveled biochemical profile, with elevated oxidative stress, low antioxidant reserves, increased glycoxidation products and matrix metalloproteases (MMP), with respect to non-ulcerated diabetic patients and to non-diabetic subjects.The intralesional administration of EGF was associated with a significant recovery of the parameters studied and with the systemic attenuation of several pro-inflammatory markers. **Conclusions**: These results indicate that intralesional infiltration with EGF translates into systemic antioxidant, anti-inflammatory, anti-degradative and anti-AGE effects, which contribute to restoring homeostasis in the diabetic patient.

Key words

diabetes, diabetic foot ulcer, epidermal growth factor, oxidative stress, inflammation

INTRODUCTION

Diabetes Mellitus (DM) type 2 has expanded progressively until becoming a pandemic. It represents 90-95% of all diabetic population [1]. It is a heterogenous and complex group of diseases involving multiple pathogenic factors and multiorgan complications. Hyperglycemia is its most constant and direct marker [2]. Diabetic foot ulcer (DFU) is one of the most feared complications, because it leads to amputation of lower limbs, resulting in disability, social exclusion and early mortality. The diabetic population represents 80% of all non-traumatic amputations of lower limbs worldwide [3]. In the pathogenesis of diabetes, there occurs a multifaceted interaction between the DFU and its host [4]. The diabetic wounds exhibit a complex network of inflammatory cytokines, reactive oxygen species (ROS) and reactive nitrogen species (RNS), advanced glycation end-products (AGE), extracellular matrix (ECM) proteases, and a polymicrobial biolayer which, together, contribute to the senescence of the wound cells, the chronicity of the lesion, and the patient's homeostasis unbalancing [5]. This functional damage seems to be related to the deregulation of the availability and activity of the growth factors [6]. The system composed of the epidermal growth factor (EGF) and its receptor (EGFR) deteriorates as a consequence of diabetes [7], which involves the reduction of the circulating salivary and tissular EGF and the descent of tyrosine kinase activity of the EGFR [8].

EGF is the growth factor most widely studied in relation to healing. The prolonged local bioavailability and the opportune stimulation of the receptors are requisites for a significant impact of the EGF on the healing of the wound [9]. As an alternative to evade the hostile surroundings of the DFU, and to ensure an adequate availability of EGF to its receptor in responding cells, our group has carried out intralesional infiltrations with EGF for longer than a decade [10]. In these studies, the intrinsic capacity of EGF to trigger the necessary biological actions for

the healing of DFU has been demonstrated [11]. Comprehensive studies on pharmacovigilance confirm the clinical efficacy of the infiltration procedure in terms of safety and patients' response, with a reduction of 16% and 71% of absolute and relative amputation risk, respectively, and only 5% of re-ulceration over a follow-up period of 12 months [12, 13].

In this research, the objectives were to determine the molecular profile of DFU patients and to characterize the systemic effects of intralesional treatment with EGF in those patients, taking into account redox balance markers, components of the advanced glycation end-products (AGE) pathway, factors related to the extracellular matrix (ECM) stability and pro-inflammatory markers. This paper includes two independent and extemporaneous studies [14, 15]. The results demonstrate that the intralesional therapy with EGF is associated with the recovery of the systemic redox balance, the attenuation of pro-inflammatory markers, the control of the AGE pathway, and the restoration of the balance between the pro-degradative and pro-synthetic forces of the ECM.

METHODS

Ethical considerations

The protocols of the studies comply with the ethical guidelines written out in the Helsinki Declaration (1975). Besides, these protocols were verified and approved by the ethics committees of the National Institute of Angiology and Vascular Surgery (INACV in Spanish) and the Center for Attending to Diabetics (CAD), both in Havana, Cuba. All the patients and non-diabetic volunteers signed an informed consent document before being recruited for the study.

Populations studied

Patients with moderate and severe neuropathic DFU were included, according to the Wagner scale [16] (n=13 in the first study and n=11 in the second). All the patients take part in the National Program for Integrally Attending to the Diabetic Patient, which includes the intralesional infiltration with human recombinant EGF (Heberprot-P).

Control populations

For the first study, as a reference with respect to the group with DFU, groups of metabolically compensated diabetic patients were included (n=12), and also uncompensated ones (n=12). These patients were recruited at the CAD. The compensation criterion was based on the glycated hemoglobin (HbA1c) ≤7%(17). Additionally, 13 non-diabetic volunteers, apparently healthy, were recruited. Their age was similar to that of the patients. They made up the control group for all diabetic patients.

Human recombinant EGF

The human recombinant EGF (Heberprot-P [HeberBiotec S.A., Cuba]) was obtained at the Center of Genetic Engineering and Biotechnology (Havana, Cuba) [18]. The patients received 75 µg of EGF through intralesional infiltration, three times a week, every other day. The medication and the procedure to administer it have been described in detail previously [19, 20].

Sample collection

10 mL of blood were collected from each individual, on an empty stomach. For the patients with DFU, the collection in "time zero" (T0) coincides with the sample taken before the first infiltration with EGF. The "time one" sample (T1) was collected 3-4 weeks later, when 9-12 infiltration sessions had been completed.

Biochemical determinations

All the biochemical parameters were determined by means of spectrophotometric methods, using reagent sets from the firms Immundiagnostik (Germany), Oxis International Inc. (USA), Abcam (Great Britain), Innovative Research (USA), Cusabio (China), Donglin (China), BlueGene (China), Alpco (USA).The markers of oxidative stress included total oxidant capacity, advanced products of protein oxidation (APPO), total organoperoxides (TOP), malondialdehyde (MDA), and nitrite/nitrate ratio. The markers of antioxidant reserve included: total antioxidant capacity, state of the sulfhydryl groups (SH), and dismutase superoxide (DSO). The circulating levels of AGE, its receptor (AGER), pentosidine (PTD), and HbA1c were assessed. The matrix metalloprotease (MMP) -9 and the tissular inhibitor of the MMP (TIMP) -1 were also quantified. The acute phase reactants were erythrocyte sedimentation and C reactive protein (CRP), while the pro-inflammatory markers included interleukin (IL)-1β, IL-6, and intercellular adhesion molecule 1 (ICAM-1). In each case, the manufacturer's instructions were followed.

Cytokine profile

In the second study, 17 cytokines were determined by means of assays in magnetic beads (HCD8MAG15 K17PMX human [Millipore Sigma Corp., USA]). Samples of the patients were analyzed in T0 y T1, according to the manufacturer's instructions. The quantification was made in a Luminex MAGPIX piece of equipment, using the program xPONENT 4.2 and it was analyzed with the program MilliplexAnalyst (v5.1, Millipore), with a linear regression of five parameters (r^2> 0,99 in all cases).

Statistical analysis

The statistical analyses were conducted by means of GraphPadPrism 6.01. The normal distribution and the variance homogeneity were analyzed through the D'Agostino-Pear-

son and Brown-Forsythe tests, respectively. In case the data meet the normal distribution and the variance homogeneity, the comparisons among groups were performed with one-way ANOVA followed by the Holm-Sidak test of multiple comparisons. In the contrary case, the Kruskal-Wallis test was used followed by theDunn test of multiple comparisons. The comparisons between T0 and T1 were performed by means of analysis of repeated measures. The data with normal distribution were analyzed using the t test of Student for paired data. Otherwise, the Wilcoxon test was performed. The values of $p<0,05$ were interpreted as indicative of statistically significant differences.

RESULTS AND DISCUSSION

This research comprises two independent and extemporaneous studies. In the first study, a group of patients with DFU was characterized, compared with diabetics without ulcers, compensated and uncompensated ones, and non-diabetic individuals, considering redox balance markers, components of the AGE pathway and the elements related to the stability of the ECM. Afterwards, an evaluation was made of the systemic response of the patients with DFU to the intralesional treatment with EGF, considering the same parameters. The second study focused on the acute phase reactants and the pro-inflammatory markers. Additionally, some of the results from the previous study were validated.

Demographic characteristics

In the first study, four experimental groups were evaluated: patients with DFU (n=13), compensated diabetics without ulcers (n=12) and uncompensated ones (n=12), and non-diabetic individuals (n=13). No significant differences were found among these groups in terms of age and the evolution time of diabetes. In the second study, 11 patients with DFU were characterized. Males prevailed in the groups with DFU and among non-diabetic individuals, whereas females prevailed among diabetics without ulcers. The patients were between 30 and 85 years old. The evolution time of diabetes ranged from 2 to 40 years. In the groups with DFU, the evolution time of the wounds ranged from 7 to 270 days.

Characterization of the patients with DFU

Table 1 shows the results of the molecular characterization of the groups studied. Patients with DFU showed levels of oxidant capacity, APPO, and MDA significantly higher than the rest of the experimental groups. With respect to the concentration of TOP, although no statistical differences were detected, patients with DFU exhibited values that tripled those of the other groups.

The parameters of antioxidant reserve showed an opposite behavior: the ulcerated patients exhibited levels of antioxidant capacity and SH groups significantly lower than those of the other groups (Table 1). The SOD activity of patients with DFU was significantly lower than that of non-diabetics, but it was not different from that of diabetics without ulcers.

Coherently with previous studies [21, 22], this one shows that ulcerated patients exhibit the exacerbation of oxidative stress, with the concomitant deterioration of the antioxidant reserve, compared with the rest of the groups studied. The question about whether the oxidative stress is a cause of the ulceration or a consequence of the glucotoxic process remains unanswered [23]. Regardless of this controversy, several studies indicate that the oxidative stress is associated with anomalies in the migration, secretion, proliferation, and polarization of fibroblasts and keratinocytes [24, 25]; they also indicate that the pharmacological manipulation of this biochemical sector improves the healing of the wounds [26].

The AGEs constitute a heterogenous group of molecules that, when interacting with the AGER, lead to the generation of ROS, the overexpression of pro-inflammatory cytokines and adhesion molecules, all of which are involved in the physiopathology of diabetes complications [27].

Statistical differences concerning AGE and RAGE were not detected between the experimental groups. Nevertheless, the levels of pentosidine were significantly higher in patients with DFU compared with diabetics without ulcers (Table 1). The pentosidine is a product of glycoxidation, that is, glycation and oxidation [28], which is coherent with the prevalence of oxidative stress and the low antioxidant reserves observed in the group with DFU. In previous studies, it is noted that diabetic patients with chronic complications exhibit higher concentrations of pentosidine than diabetics without complications [29]. Therefore, the level of pentosidine is considered to be a reliable predictor of DM complications [30].

The HbA1c is another type of AGE. In this case, the patients with DFU and uncompensated diabetics exhibited levels significantly higher than compensated patients and healthy individuals (Table 1). In this regard, it is stated that a high level of HbA1c reflects bigger production of AGE [31].

MMPs and TIMPs are two groups of molecule agonists involved in the physiology of healing, and they require a delicate temporal and spatial balance [32, 33]. The levels of MMP-9 were significantly higher in ulcerated patients compared with the rest of the experimental groups (Table 1), which indicates that the DFU impacts the rise in MMP-9 systemic levels. It is interesting that the values of TIMP-1 exhibited a similar behavior. In patients with DFU, the concentrations of TIMP-1 could have risen as a compensatory physiological response to the

Table 1. Molecular characterization of patients

Marker	DFU	Comp. DM	Uncomp. DM	No DM
	Oxidative stress			
OxidantCapacity (μM)	1471 ± 277[a]	692 ± 349[b]	824 ± 374[b]	421 ± 263[b]
APPO(μM)	40,5 ± 7,7[a]	16,7 ± 4,1[b]	16,9 ± 3,6[b]	18,6 ± 3,5[b]
MDA (μM)	6,44 ± 3,55[a]	5,05 ± 1,69[a,b]	3,87 ± 1,32[b,c]	2,69 ± 0,70[c]
TOP (μM)	26,61 ± 26,50	8,27 ± 6,40	8,46 ± 7,61	9,05 ± 7,67
	Antioxidant Reserve			
Antioxidant capacity (μM)	233 ± 40[b]	318 ± 41[a]	313 ± 40[a]	324 ± 36[a]
SH groups (μg/mL)	91,4 ± 25,4[b]	175,3 ± 37,8[a]	166,2 ± 58,9[a]	179,6 ± 32,2[a]
SOD (% inhibition)	43,2 ± 13,2[b]	54,5 ± 8,6[a,b]	56,5 ± 8,0[a,b]	61,5 ± 9,4[a]
	AGE pathway			
AGE (μg/mL)	8,67 ± 4,02	18,08 ± 12,54	24,93 ± 19,12	10,95 ± 7,63
AGER (pg/mL)	388 ± 168	575 ± 456	438 ± 185	553 ± 106
PTD (ng/mL)	2,873 ± 3,424[a]	0,372 ± 0,074[b]	0,559 ± 0,482[b]	0,465 ± 0,118[a,b]
HbA1c (%)	10,37 ± 0,98[a]	5,96 ± 1,04[b]	8,15 ± 0,92[a]	5,67 ± 0,59[b]
	Elements of the extracellular matrix			
MMP-9 (ng/mL)	1531 ± 933[a]	563 ± 415[b]	589 ± 390[b]	554 ± 484[b]
TIMP-1 (ng/mL)	1163 ± 328[a]	642 ± 102[b]	609 ± 99[b]	531 ± 88[b]

Legends. DFU: diabetic foot ulcer; Comp. DM: compensated non-ulcerated diabetics; Uncomp. DM: uncompensated non-ulcerated diabetics; No DM: non-diabetics; APPO: advanced products of protein oxidation; MDA: malondialdehyde; TOP total organoperoxides; SH: sulfhydryl; SOD: superoxide dismutase; AGE: advanced glycation end-products; PTD: pentosidine; HbA1c: glycated hemoglobin; AGER: AGE receptor; MMP-9: matrix metalloprotease 9; TIMP-1: tissular inhibitor of the MMP. Different letters represent statistically significant differences.

high levels of MMP-9 circulating. Not detecting significant differences between the groups of compensated and uncompensated diabetics with regard to most parameters studied confirms the existence of a level of damage that is independent from the glycemic control. This probably represents the so-called "vicious circle of the metabolic memory" [34].

The DFU patients' response to the intralesional treatment with EGF

The effect of the intralesional administration of EGF to DFU patients was analyzed after 9-12 infiltrations. Although the main objective of this study was not evaluating the clinical efficacy of EGF, it is appropriate to mention that the treatment triggered the accumulation of granulation tissue, the contraction of the edges of the wound, and the re-epithelialization in all patients.

In the first study, the treatment with EGF was associated with significant reductions in the four parameters of oxidative stress (Fig.1 A-D). Accordingly, the antioxidant capacity and the SH groups exhibited significant increments after the therapy (Fig. 1 E, F). With respect to each of these markers, at least 50% of the patients showed a favorable response to the redox balance. Therefore, in addition to the benefits related to the granulation stimulation and the closing of the wound [35-37], the EGF contributes to restoring the circulating levels of several markers of the redox state up to values close to those of diabetics without ulcers and non-diabetic individuals.

Despite the small number of patients studied, this work has the merit of showing for the first time that the healing effects of the intralesional therapy with EGF are, at least partially, mediated by a compensatory antioxidant mechanism. Consequently, we think that the EGF infiltrated is capable of countering the redox unbalance to attenuate the premature senescence, the apoptosis, and the proliferative arrest of the local fibroblasts representative of the DFU [38] and other chronic wounds [10, 39].

In the second study, the result of oxidant capacity was confirmed, with a significant reduction in T1. Besides, the treatment significantly reduced the nitrite/nitrate ratio (Fig. 1G), main oxidation products derived from NO [40]. Several pieces of evidence hint at the nitrosylative stress being closely related to numerous complications of diabetes, organ dysfunction, and even the outcome of patients [41, 42]. The healing of cutaneous wounds is also dramatically influenced by the metabolism of NO [43], which requires a precise adjustment both at the level of the organism and the conditions of the wound for its adequate healing [44].

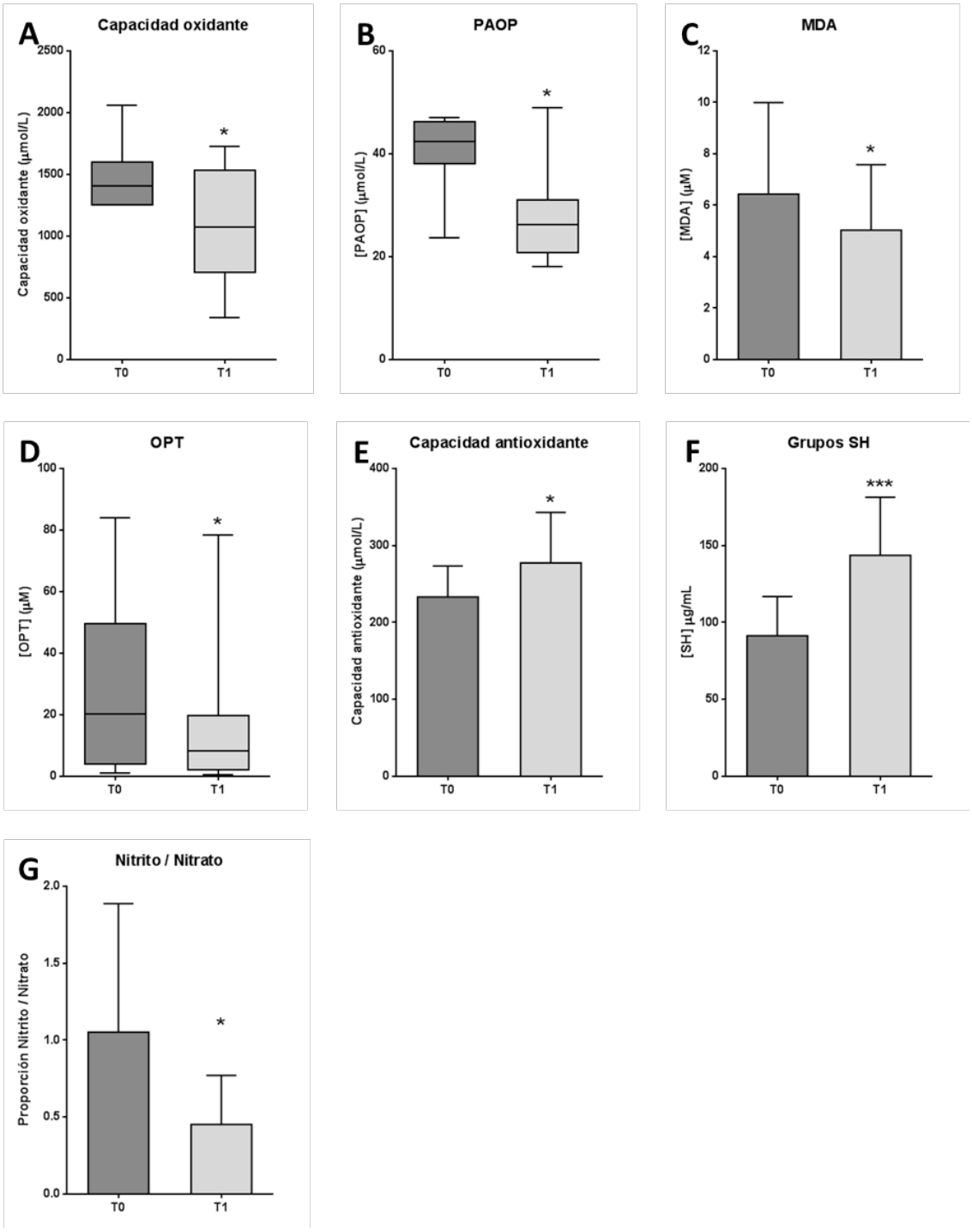

Figure 1. Circulating levels of oxidative stress markers and antioxidant reserve in patients with DFU, before (T0) and after (T1) the intralesional treatment with EGF. A, oxidant capacity. B, advanced products of protein oxidation. C, malondialdehyde (MDA). D, total organoperoxides. E, antioxidant capacity. F, sulfhydryl groups (SH). G, nitrite/nitrate ratio. *p<0,05; ***p<0,001.

The EGF has cytoprotective properties mediated by the agonistic stimulation of the phosphatidylinositol 3-kinase (PI3K) – Akt axis, by the phosphorylation of the EGFR [45]. Various experimental models have documented the ability of EGF to stimulate the survival of cells, tissues, and animals after taking lethal impact, including cytotoxic oxidants [46, 47] and episodes of ischemia/reperfusion [48]. These studies suggest that the re-supply of EGF to tissues is mediated by an antioxidant effect. Furthermore, this is the first demonstration in a clinical context.

With respect to the AGE pathway, in the first study, the circulating levels of AGE did not exhibit statistical differences between T0 and T1 (Fig. 2A). However, the mean dropped 22% after the therapy, which could be biologically relevant. The levels of pentosidine decreased significantly after the treatment, with a median reduction of 42% (Fig. 2B). This effect could have contributed to attenuating the damage associated with the AGE pathway, which include the inflammation of the wound, the arrest, and the apoptosis of the fibroblasts [49]. This result was confirmed in the second study.

The concentrations of AGER were not modified significantly (data not shown). Nevertheless, in the second study, AGER exhibited a significant increase, with a 16% median increment (Fig. 2C). Soluble AGER acts as a "decoy" receptor exhibiting the signaling through the AGE/RAGE axis, thus preventing the amplification of cytotoxic episodes.

The levels of MMP-9 did not vary significantly between T0 and T1 (Fig. 3A). However, the concentration mean in T1 was

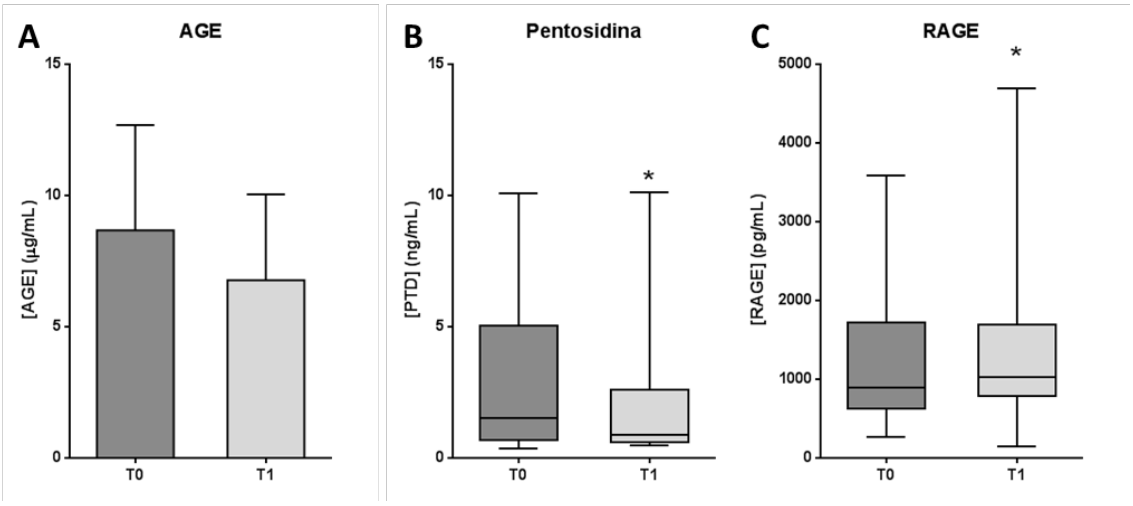

Figure 2. Circulating levels of molecules of the AGE pathway in patients with DFU before (T0) and after (T1) the intralesional treatment with EGF. **A**, advanced glycation end-products (AGE). **B**, pentosidine. **C**, AGE receptor (RAGE in Spanish). *p<0,05.

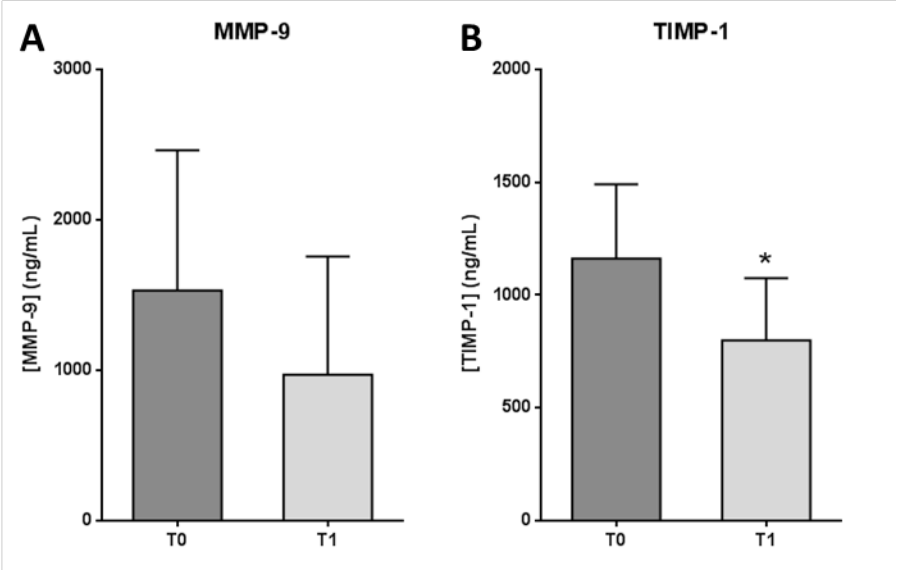

Figure 3. Circulating levels of proteins related to the stability of the ECM in patients with DFU before (T0) and after (T1) the intralesional treatment with EGF. **A**, matrix metalloprotease-9 (MMP-9). **B**, tissular inhibitor of the MMP type 1 (TIMP-1). *p<0,05.

37% lower than T0. The values of TIMP-1 decreased significantly after the therapy (Fig. 3B), with a 31% mean reduction. The results suggest the recovery of the balance between the degradative molecules and their inhibitors, which could lead to the restoration of the balance between pro-degradative and pro-synthetic forces of the ECM.

In the second study, the circulating levels of a group of markers were evaluated, including acute phase reactants and pro-inflammatory cytokines. The treatment with EGF was associated with a highly significant reduction of erythrocyte sedimentation (Fig. 4A), verified in all patients. The CRP did not show statistical differences between T0 and T1 (Fig. 4B). However, the median in T1 was 45% lower than T0. The interpretation of these clinical findings rouses speculation about the systemic exposure to EGF contributing to attenuating the hepato-reactive phenotype [50]. The modulatory effect of EGF on acute phase proteins derived from the liver is documented in previous studies [51]. Other in vitro studies illustrate the effect of EGF on the modulation of acute phase proteins in liver cells [52, 53].

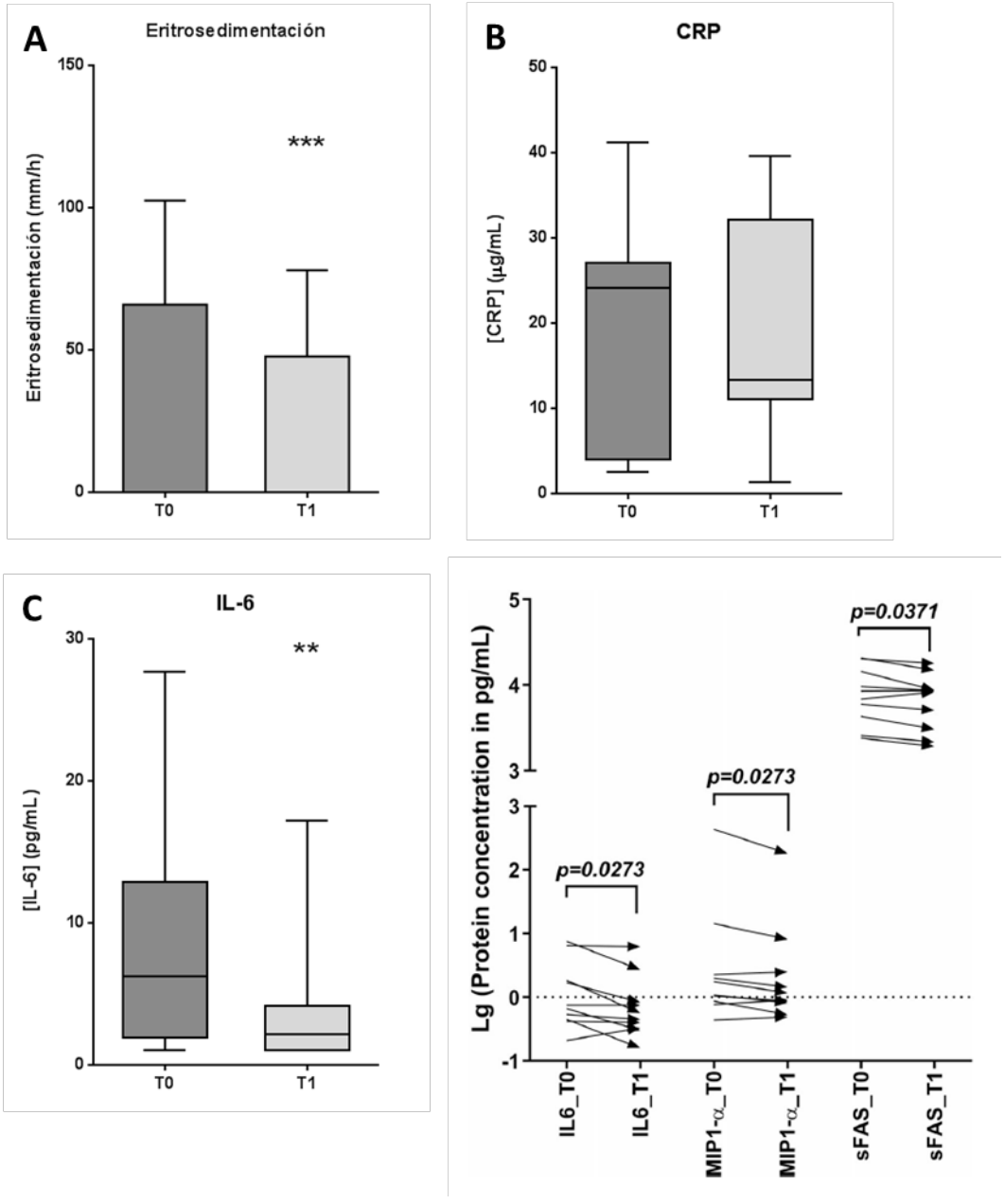

Figure 4. Circulating levels of acute phase reactants and pro-inflammatory markers in patients with DFU before (T0) and after (T1) the intralesional treatment with EGF. **A**, erythrocyte sedimentation. **B**, C reactive protein (CRP). **C**, interleukine-6 (IL-6). **D**, pro-inflammatory cytokines that exhibited significant variations according to the evaluation based on Milliplex: IL-6, macrophage inflammatory protein 1-alpha (MIP1-a) and soluble FAS (sFAS). **p<0,01; ***p<0,001.

The levels of IL-1β and ICAM-1 were not significantly modified after the treatment (data not shown). In contrast, the levels of IL-6 decreased significantly in T1, with a 66% median reduction (Fig. 4C). In addition, a more comprehensive evaluation of pro-inflammatory markers was carried out. It was made by means of an assay with magnetic beads, in which the reduction of the IL-6 circulating levels was validated, accompanied by a significant decrease in the macrophage inflammatory protein 1-alpha (MIP1-a) and soluble FAS (sFAS) (Fig. 4D).

The IL-6 plays a pathogenic role in the appearance, progression, and complications of DM type 2 [54]. sFAS has been linked to resistance to insulin, the appearance of DM type 2, and the subsequent endovascular inflammation, the apoptosis associated with diabetes, and the multi-organ complications [55]. MIP1-a is one of the inflammatory cytokines induced by high levels of glucose [56], and it is pathogenically involved in pre-diabetes and the chronic diabetic inflammation [57]. Together, our findings suggest that the decrease in the circulating levels of these three markers could contribute to restoring the metabolic homeostasis and the pro-anabolic programs of tissues along the process of healing in diabetic patients [58].

The interpretation of these findings suggests that, behind the successful healing achieved through intralesional infiltration with EGF, there exists a broad scope of pharmacodynamic effects, determined by the systemic restoration of multiple molecular events which converge to re-establish a healing physiological trajectory. In addition to its contribution to the recovery of uncompensated soluble mediators, the EGF locally infiltrated has the capacity to counter the premature senescence associated with diabetes [59].

Conclusions

This research contributes the first pieces of evidence confirming the concept that the DFU works as an entity superimposed over a metabolically uncompensated host into which it releases pro-inflammatory, pro-oxidant, glycoxidative, and pro-apoptotic reactants, which amplify the metabolic decompensation and create a circuit of mutual influence that hinders healing. Moreover, for the first time, the systemic effects of an intralesional treatment with EGF are documented in a clinical context: antioxidant, anti-AGE, anti-degradative, anti-inflammatory, and anti-apoptotic effects that translate into the correction of the internal environment of the diabetic organism.

As a whole, the results indicate that this therapy contributes to restoring the diabetic patient's systemic homeostasis; then, the human recombinant EGF (Heberprot-P) could broaden its pharmacological scope toward the prevention and control of other complications of diabetes resulting from the pro-inflammatory and glycoxidative phenomena.

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