Improved Function of Diabetic Wound-Site Macrophages and Accelerated Wound Closure in Response to Oral Supplementation of a Fermented Papaya Preparation

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Abstract

Carica papaya Linn is widely known as a medicinal fruit. We sought to study a standardized fermented papaya preparation (FPP) for its effects on wound healing in adult obese diabetic (db/db) mice. FPP blunted the gain in blood glucose and improved the lipid profile after 8 weeks of oral supplementation. However, FPP did not influence weight gain during the supplementation period. FPP (0.2 g/kg body weight) supplementation for 8 weeks before wounding was effective in correcting wound closure. Studies on viable macrophages isolated from the wound site demonstrated that FPP supplementation improved respiratory-burst function as well as inducible NO production. Reactive oxygen species support numerous aspects of wound healing; NO availability in diabetic wounds is known to be compromised. Diabetic mice supplemented with FPP showed a higher abundance of CD68 as well as CD31 at the wound site, suggesting effective recruitment of monocytes and an improved proangiogenic response. This work provides the first evidence that diabetic-wound outcomes may benefit from FPP supplementation by specifically influencing the response of wound-site macrophages and the subsequent angiogenic response. Given that FPP has a long track record of safe human consumption, testing of the beneficial effects of FPP on diabetic wound–related outcomes in a clinical setting is warranted. *Antioxid. Redox Signal.* 13, 599–606.

Introduction

IN THE UNITED STATES, chronic wounds affect ~6.5 million patients. It is claimed that an excess of US$25 billion is spent annually on the treatment of chronic wounds, and the burden is growing rapidly because of increasing health care costs, an aging population, and a sharp world-wise increase in the incidence of diabetes and obesity (49). More than 23 million people, or 7.8% of the U.S. population, have diabetes. During the period from 2005 through 2007, the total incidence of diabetes increased by 13.5%. It is estimated that, in ≤25% of all diabetes patients, a diabetic foot ulcer will develop. Sixty-seven percent of all lower-extremity amputation patients have diabetes (4).The treatment goals for patients with diabetes have evolved significantly over time, from preventing imminent mortality, to alleviating symptoms, to the now-recognized objective of control of glucose levels, with the intent of forestalling diabetic complications. At present, >100 plant species from a wide range of families containing various chemical classes of compounds are in consideration for the care of diabetes (45). Today, complementary forms of medicines are used extensively over the world, representing >US$60 billion global sales (57).

Carica papaya Linn is widely known as a medicinal fruit (5). Several observations point to the hypothesis that treatment with papaya preparations may help facilitate wound-healing responses (5, 13, 16, 28, 32, 36). Preliminary data suggest that the papaya-derived enzyme papain, when applied topically, may facilitate enzymatic wound debridement (58). The aqueous extract of *C. papaya* fruit seem to help facilitate wound-healing activity in streptozotocin-induced diabetic rats (32). However, the underlying mechanisms remain poorly developed. The study of herbal preparations requires a standardized source as study material. Fermented papaya preparation (FPP) (31) is one such product that is commercialized as functional food in several countries including Japan, the United States, and European countries. FPP is produced by fermentation of papaya fruit by using yeast. FPP possesses antioxidant properties that seem to be helpful against age-related complications (6, 11, 17, 21, 23–24, 37). The

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objective of the current study was to determine the effect of FPP supplementation on diabetic wound healing and to understand the mechanisms of action of FPP.

Materials and Methods

Animals

Adult male obese $Lepr^{db}$ diabetic mice (db/db, 8–10 wk; Jackson Laboratories, Bar Harbor, ME) were group-housed in standard cages with *ad libitum* access to food and water. Mice were maintained in a vivarium with controlled temperature (~20°C) on a 12-h light/dark cycle and were randomly assigned to experimental groups after a 2-week habituation period. All procedures were conducted in accordance with protocols approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

Methods

FPP supplementation. Mice (db/db) were randomly divided in two groups: FPP and placebo control. FPP, commercially known as Immun'Age, was prepared by fermenting *C. papaya* at the Osato Research Institute (Gifu, Japan). To control for the effect of glucose present in FPP, the control group was supplemented with matching amount of D-glucose. Both groups were supplemented with oral gavage (0.2 g/kg) 5 days/wk for 8 weeks. Body weight was obtained at the start of the study and each week thereafter, and dosing was adjusted accordingly.

Blood glucose and lipid profiling. Blood glucose was measured at baseline (before supplementation), and at 8 weeks into the supplementation period. Blood-lipid measurements were also conducted at 8 weeks after supplementation. To collect blood for these analyses, mice were briefly anesthetized with isoflurane, and whole blood was collected by using a standard submandibular bleeding method. Blood glucose was analyzed by using the Glucometer Elite (Bayer, Tarrytown, NY), and lipid profiles were analyzed by using a clinical CardioChek analyzer (Polymer Technology Systems, Indianapolis, IN), as described previously (38).

Secondary-intention excisional cutaneous wound model. For wounding, mice were anesthetized with isoflurane inhalation. Two 6-mm full-thickness (skin and panniculus carnosus) excisional wounds were placed on the dorsal skin [shaved and cleaned with povidone-iodine (Betadine)], equidistant from the midline and adjacent to the four limbs. The wounds were allowed to heal by secondary intention (41–42, 50).

Determination of wound area. Wound planimetry was performed by using images collected by a digital camera (Canon PowerShot G6, Lake Success, NY). The open wound area was determined by using (WoundMatrix software, Chadds Ford, PA) as described previously (41–42, 50). Animal studies were approved by the Institutional Animal Care and Use Committee of The Ohio State University.

Polyvinyl alcohol sponge implantation. Circular (8-mm) sterile polyvinyl alcohol (PVA) sponges were implanted subcutaneously on the dorsum of the mice to match the site of excisional wounds (1). In brief, after anesthesia by isofluorane

inhalation, the dorsal midline was shaved and cleaned with povidone-iodine (Betadine). Two midline 1-cm incisions were made with a scalpel. Next, two small subcutaneous pockets were created by blunt dissection. Two PVA sponges (8 mm) were inserted into each pocket. Incisions were closed with skin staples (9 mm) or suture (3-0 Surgilene, Surgitech, esutures.com, Mokena, IL). Mice were then returned to clean cages and monitored during recovery. During harvest, mice were killed with CO_2 inhalation for retrieval of the implanted sponges.

Isolation of wound macrophages from PVA sponges. Subcutaneously implanted PVA sponges were harvested on day 3 after implantation, and a single-wound-cell suspension was generated from sponges by repeated compression. The cell suspension was filtered through a 70-μm nylon cell strainer (BD Falcon, BD Biosciences, San Jose, CA) to remove all sponge debris. For macrophage isolations, magnetic cell sorting was carried out by using mouse anti-CD11b–tagged microbeads (Miltenyi Biotec, Auburn, CA).

Immunohistochemistry. Formalin-fixed paraffin-embedded or frozen wound-tissue specimens were sectioned. To visualize blood vessels, frozen wound sections (8 μm) were immunostained by using anti-rat CD31 (1:200; BD Pharmingen, San Diego, CA) and the appropriate fluorescence-tagged secondary antibody. Counterstaining was performed with DAPI to visualize nuclei (Molecular Probes, Invitrogen, Carlsbad, CA) (41).

Image quantification. Stained sections were imaged by using a Zeiss Axiovert 200 (Thornwood, NY) imaging microscope equipped with a digital camera. Multiple high-powered images were quantified for each data point from each animal. Quantification was performed by using the Axiovision 4.3 image-analysis software. The fluorescence intensity was quantified per square millimeter area, as described (41).

Determination of intracellular reactive oxygen species. Detection of reactive oxygen species (ROS) in phorbol 12-myristate 13-acetate (PMA, 0.001 mg/ml, 1 h)-activated wound macrophages was performed by using dichlorodihydrofluorescein diacetate (H_2DCF-DA) (Molecular Probes, Invitrogen, Carlsbad, CA), as described (54). In brief, 1 h after activation with PMA, wound macrophages were washed with PBS, centrifuged (500 g, 5 min), resuspended in PBS, and incubated with 10 μM H_2DCF-DA for 20 min at 37°C. To detect cellular fluorescence, fluorochrome-loaded cells were excited by using a 488-nm argon-ion laser in a flow cytometer. Dichlorofluorescein emission was recorded at 530 nm. Data were collected from $\geq$5,000 cells.

Nitric oxide measurement. Nitric oxide production by wound macrophages was assessed by using the Griess reaction that measures nitrite, a stable end product of the reaction of nitric oxide and oxygen. Wound macrophages were plated at a density of 2×10^6 cells and activated with lipopolysaccharide (LPS; 1 μg/ml). After 12-h treatment, supernatants from wound macrophages were collected, and nitric oxide was measured by using Griess reagent, as described previously (19).

Superoxide measurement. Superoxide anion generation by macrophages was measured with the LumiMax superoxide anion detection kit (Stratagene, La Jolla, CA), according to the manufacturer's instructions. In brief, freshly isolated wound macrophages (1×10^6 cells) were treated with PMA ($1 \mu g/ml$), or not, for 10 min. Superoxide was measured at 430 nm with a luminometer (model Lumat LB9507; Berthold Technologies, Bad Wildbad, Germany) at 5-min intervals for a total duration of 30 min.

Isolation of RNA, reverse transcription, and quantitative RT-PCR. Total RNA was extracted by using the mirVana RNA isolation kit (Ambion, Foster City, CA), according to the manufacturer's instructions. Specific mRNA was quantified by real-time or quantitative (Q) PCR assay by using the double-stranded DNA-binding dye SYBR Green-I, as described previously (39–40, 42–44). The primer sets used for the individual genes are listed later. 18S was used as a reference housekeeping gene. The $\Delta\Delta Ct$ analysis approach was used to normalize gene data to 18S ribosomal RNA expression. Primer sets used were as follows:

VEGF-A$_{165}$ TGCCCACTGAGGAGTCCAACAT
CACGTCTGCGGATCTTGTACAAACA
iNOS GTGACAAGCACATTTGGGAATGGA
CTGAGTTCGTCCCCTTCTCCTGTT
CD68 TCCACCCTCGCCTAGTCCAA
GCCCCAAGCCCTCTTTAAGC

Statistics

Results are presented as mean ± SD. The difference between means was tested by using Student's t test or ANOVA, as appropriate. A value of $p < 0.05$ was considered statistically significant.
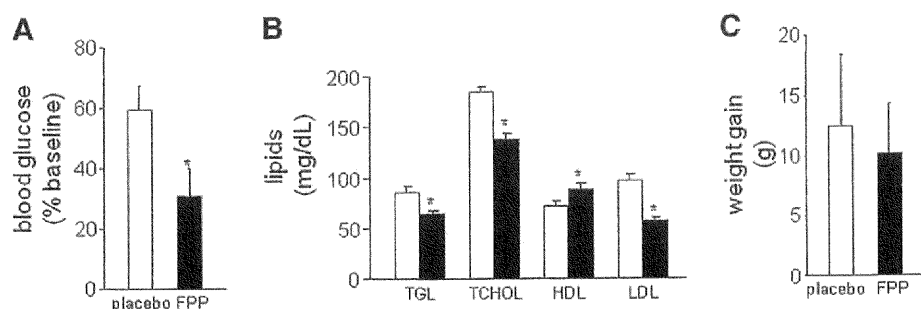
Results

In db/db mice, elevations of plasma insulin begin at 10 to 14 days of age and of blood sugar at 4 to 8 weeks. Thereafter, these mice experienced a sharp and uncontrolled increase in blood sugar. Blood collected from adult db/db mice was assayed for glucose before (baseline) and after 8 weeks of supplementation. FPP significantly ($p < 0.05; n = 10$) blunted the percentage gain
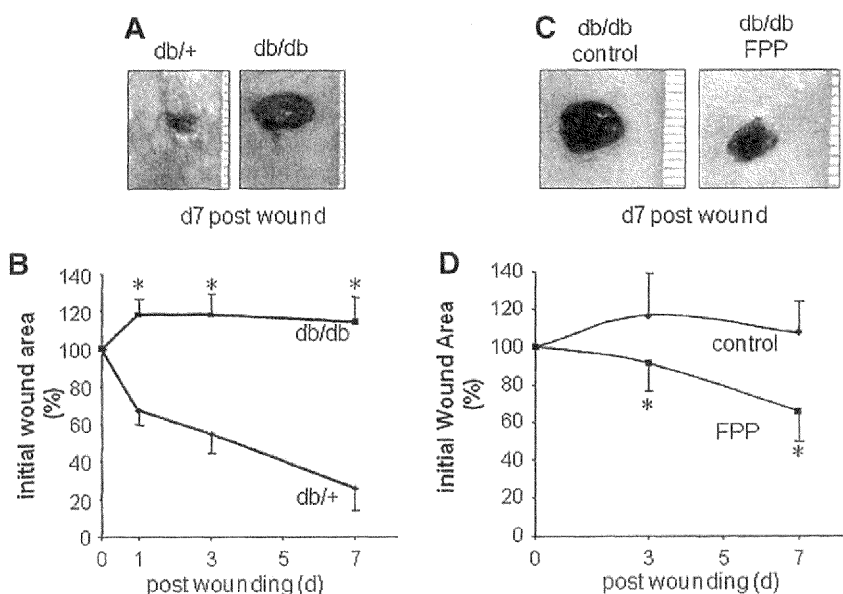
in blood glucose after 8 weeks of supplementation (Fig. 1A). The effect of FPP supplementation on the blood lipid profile also was determined. After 8 weeks of supplementation, total triglycerides (TGL), total cholesterol (TCHOL), and low-density lipoprotein (LDL) levels were significantly ($p < 0.05; n = 10$) decreased in FPP-supplemented mice compared with placebo-supplemented mice (Fig. 1B). High-density lipoprotein (HDL) levels, conversely, were significantly ($p < 0.05; n = 10$) increased in FPP-supplemented mice compared with placebo-supplemented mice (Fig. 1B). FPP supplementation for 8 weeks did not influence weight gain during the supplementation period (Fig. 1C). The supplementation dosage of 0.2 g/kg in our study was derived from a human study in which the subjects were fed 9 g FPP/day (25). Approximating the average weight of a human to be 70 kg will result into a dose of ~0.2 g/kg body weight.

Diabetic (db/db) mice exhibited impaired wound healing compared with nondiabetic (db/+) mice (Fig. 2A and B). To determine whether FPP may influence impaired wound closure in db/db mice, dorsal full-thickness skin wounds were placed on mice either supplemented with FPP for 8 weeks or unsupplemented. FPP supplementation significantly ($p < 0.05; n = 5$) improved wound closure of diabetic mice (Fig. 2C and D). Previously, we demonstrated that oxidants play a critical role in wound healing by supporting redox signaling, leading to beneficial responses, such as angiogenesis (41, 50, 51). Likewise, the deficiency of nitric oxide (NO) is known to complicate diabetic wound healing (61).

Basal and inducible ROS and nitric oxide production by wound macrophages was tested for sensitivity to FPP. Wound macrophages were isolated from placebo or FPP-supplemented diabetic mice after 8 weeks of supplementation. ROS production was measured by using an oxidant-sensitive fluorescence probe DCF and flow cytometry. Basal and PMA-induced ROS production was significantly ($p < 0.05; n = 3$) higher in wound macrophages obtained from FPP-supplemented mice compared with those from control mice (Fig. 3A). Next, specifically to measure superoxide production, a product of NADPH oxidase activity, a chemiluminescence-based assay was performed by using PMA-stimulated wound macrophages. Superoxide levels were increased in macrophages from FPP-supplemented mice (Fig. 3B).

FIG. 1. Oral supplementation with FPP improved blood hyperglycemia and dyslipidemia in diabetic mice. (A) Percentage increase of plasma glucose levels after the 8 weeks of FPP supplementation. Blood glucose was recorded before the start of supplementation (baseline) and then after the eighth week of supplementation. Data are presented as percentage change in 8 weeks compared with baseline. Data are expressed as mean ± SD ($n = 10$); *$p < 0.05$ compared with placebo. (B) Total triglycerides (TGLs), total cholesterol (TCHOL), HDL, and LDL levels in blood were determined after 8 weeks of FPP supplementation. *Open bars*, placebo supplemented; *solid bars*, the FPP-supplemented diabetic mice. Data are expressed as mean ± SD ($n = 10$) *$p < 0.05$. (C) FPP did not have any significant effect on overall weight gain throughout the 8 weeks of supplementation. Data are expressed as mean ± SD ($n = 10$).

A db/+ db/db

C db/db control / db/db FPP

d7 post wound

d7 post wound

B

D

FIG. 2. **Improved wound closure in FPP-supplemented diabetic mice.** Full-thickness (skin and panniculus carnosum) dorsal wounds were created on the male diabetic (db/db) or nondiabetic (db/+) mice by using a 6-mm biopsy punch. The wounds were left to heal by secondary intention. Wounds were imaged on days 0, 3, and 7 after wounding, and the wound area was calculated by using digital planimetry. (**A**) Digital images of representative day 7 wounds from db/db and db/+ mice showing impaired closure in db/db mice. (**B**) Area measurements of wounds from db/db or db/+ mice. Data are expressed as mean $\pm$ SD ($n = 5$). *$p < 0.05$. (**C**) Digital images of representative day 7 wounds from FPP- or placebo glucose-treated db/db mice showing improved closure in FPP-treated db/db mice. (**D**) Area measurements of wounds from FPP- or placebo-treated db/db mice. Data are expressed as mean $\pm$ SD ($n = 5$). *$p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Diabetes is characterized by a nitric oxide deficiency at the wound site (60). Thus, we tested whether FPP may improve NO production by wound macrophages. LPS-stimulated nitric oxide production was significantly higher in macrophages derived from wounds of FPP-supplemented mice compared with those isolated from wounds of control mice (Fig. 4A). Such an increase in NO production was associated with increased *iNOS* gene expression in the wound tissue (Fig. 4B). Furthermore, FPP supplementation significantly increased the expression of CD68 and VEGF transcripts in day 3 wound tissues of diabetic mice (Fig. 5), indicating improved recruitment of macrophages to the wound site and a favorable angiogenic environment.

Finally, to determine whether FPP-dependent increases in ROS and VEGF at the diabetic wound site influence wound angiogenic responses, endothelial cells were stained by using anti-CD31 immunostaining of postclosure day 12 wound tissue. CD31, a platelet–endothelial cell adhesion molecule
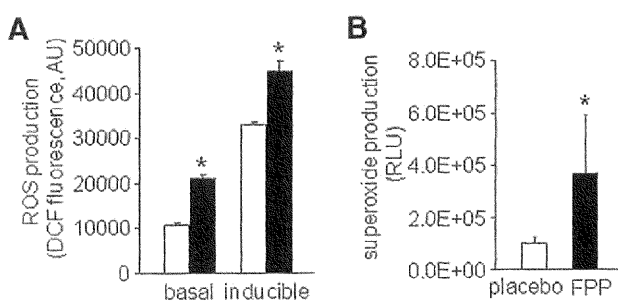
FIG. 3. **Increased oxidant generation by wound macrophages.** Wound macrophages were isolated from diabetic (db/db) animals supplemented with FPP or placebo for 8 weeks. Wound macrophage isolation was performed by using PVA sponges and CD11b magnetic-bead sorting. (**A**) Total oxidant production under nonstimulated (basal) or phorbol ester–stimulated (inducible) conditions was measured by using oxidant-sensitive fluorescence probe DCF and flow cytometry. *Open bars*, placebo supplemented; *solid bars*, the FPP-supplemented animals. Data are expressed as mean $\pm$ SD ($n = 3$), *$p < 0.05$. (**B**) Specifically to identify whether the oxidant species is superoxide, chemiluminescence-based assay for superoxide anion was performed on PMA-stimulated wound macrophages. Data are expressed as mean $\pm$ SD ($n = 4$), *$p < 0.05$.
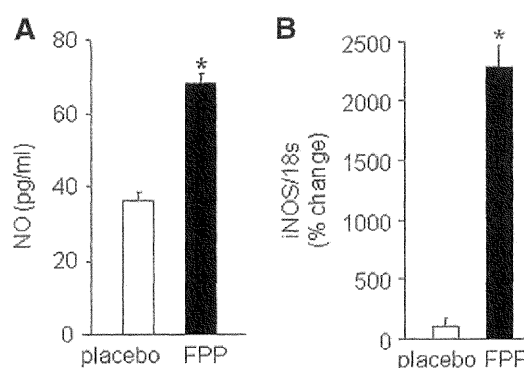
FIG. 4. **Improved nitric oxide production by wound macrophages and elevated iNOS gene expression in wound tissue in FPP-supplemented diabetic mice.** (**A**) Wound tissue was collected on day 3 after wounding from FPP- or placebo-supplemented db/db mice. NO production from wound macrophages was measured by using Greiss reagent. Wound macrophages were isolated from diabetic (db/db) animals supplemented with FPP or placebo for 8 weeks, as described earlier. Data are expressed as mean $\pm$ SD ($n = 5$), *$p < 0.05$. (**B**) iNOS transcripts were quantified in wound tissue by using real-time PCR. 18S was used as a reference housekeeping gene. Data are expressed as mean $\pm$ SD ($n = 4$), *$p < 0.05$.
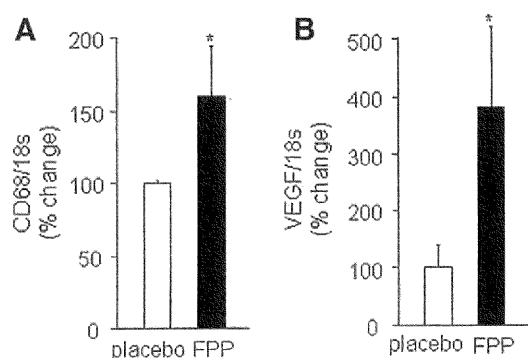
FIG. 5. Elevated abundance of CD68 and *VEGF* gene in wounds of diabetic mice supplemented with FPP. Wound tissue was collected on day 3 after wounding from FPP- or placebo-supplemented db/db mice, and total RNA was extracted. CD68 and VEGF transcripts were quantified by using real-time PCR. 18S was used as a reference housekeeping gene. Data are expressed as mean $\pm$ SD ($n = 4$); $*p < 0.05$.

present at the lateral borders of endothelial cells, was significantly elevated in FPP-treated wounds compared with control wounds (Fig. 6). Increased CD31 staining is suggestive of improved angiogenic response in the granulation tissue in response to FPP supplementation.

Discussion

The current work provides the first evidence demonstrating that oral supplementation with FPP may benefit diabetic wound closure. This observation is consistent with reports demonstrating that FPP possesses immune-modulatory functions *in vivo* (33). FPP administration is suggested as an adjuvant drug with oral antidiabetic therapy in type 2 diabetes mellitus (12). Papaya has drawn the interest of wound care professionals for its topical application in wound debridement. The enzyme papain, obtained from the ripening fruit of *C. papaya*, facilitates debridement. Papaya extracts, in general, are known for their antibacterial effects (13). The literature demonstrates that topically applied papaya extract may favorably influence wound outcomes by influencing inflammatory responses (28, 32); papain is a cysteine proteinase that digests necrotic tissue by liquefying eschar, thus facilitating the migration of viable cells from the wound edge into the wound cavity (58). Papain also is useful in reducing the bacterial burden, decreasing exudates, and increasing granulation tissue formation (14).

NADPH oxidase activity is essential to fight wound infection (48). NADPH oxidase deficiency causes chronic granulomatous disease, which is characterized by impaired healing (47, 48, 53). Hyperglycemia is known to inhibit oxidant production through inhibition of NADPH oxidase activity (8, 35). Compromised respiratory burst has been observed in alveolar macrophages from diabetic animals (29), as well as in neutrophils of diabetes patients (18). Thus, the ability of FPP to preserve NADPH oxidase function of macrophages obtained from the wound sites of diabetic mice could translate to improved efficiency in fighting wound infection, a common problem noted in diabetic wounds.
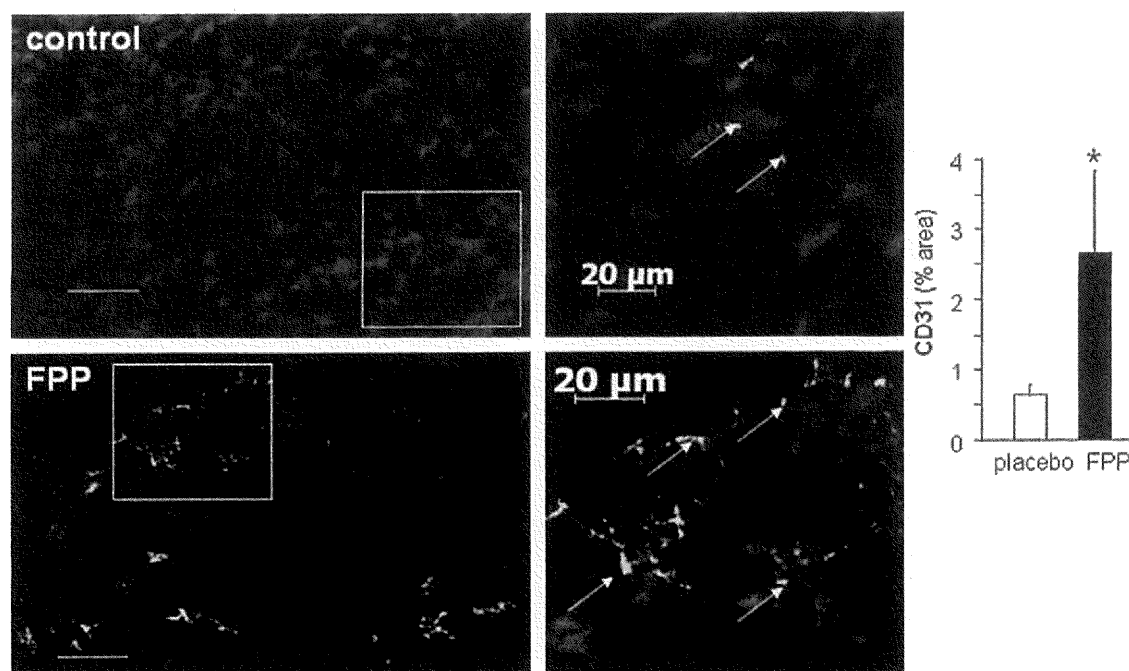
FIG. 6. Greater endothelial cell abundance in the wound tissue of FPP-supplemented diabetic mice. Day 7 wound tissue from FPP- or placebo-supplemented db/db mice was cryosectioned, and a marker of vascularization was estimated by staining for CD31 (red, rhodamine) and nuclei (blue, DAPI). The images in the right panels are magnifications of the white boxed area in the corresponding left panels. Scale bar, 50 μm (left panels). Scale bar, 20 μm (right panels). Bar graph presents image-analysis outcome (mean $\pm$ SD; $n = 3$); $*p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

Nitric oxide (NO) is known to be involved in wound healing through multiple modes of action (2, 7, 10, 59). Diabetes is characterized by limited nitric oxide levels in the wound microenvironment (46). In an acute inflammatory setting, such as the wound, macrophages represent a key contributor of NO (9). Augmentation of NO delivery by FPP is therefore a beneficial effect that could help offset the compromised levels of NO at the diabetic wound site. At a time when NO donors are sought for wound therapies (27, 59), FPP provides the option to bolster the endogenous capability of macrophages at the inflammatory site. The substantial elevation of iNOS levels noted in wound tissue of FPP-supplemented diabetic mice is likely to be contributed by two factors. First, increased recruitment of macrophages at the wound site, under conditions in which it is known that early inflammatory-cell recruitment to the wound site, is compromised in diabetics (34). This hypothesis is supported by data demonstrating a higher abundance of CD68 in the wound tissue of FPP-supplemented mice. CD68 is a glycoprotein that binds to low-density lipoprotein and is expressed on monocytes/macrophages. Second, FPP may have induced iNOS in macrophages of supplemented mice.

The first work demonstrating that FPP regulates macrophage function was based on the study of the RAW cell line. FPP was identified as a macrophage activator that augmented NO and TNF-α secretion, independent of lipopolysaccharides (37). Sustained production of large amounts of superoxide and NO in close proximity will lead to the production of hazardous peroxynitrite ($ONOO^-$), which may have deleterious effect on wound healing (55). Production of peroxynitrite after FPP supplementation requires further investigation.

Myeloid cells, including macrophages, play a key role in adult angiogenesis (30). Macrophage-derived ROS, as well as NO, are known to support wound angiogenesis. The wound site is rich in oxidants, such as H_2O_2, contributed mostly by neutrophils and macrophages. H_2O_2 drives redox signaling (56), which supports the healing response (50, 52, 53). Specifically, at the wound site, H_2O_2 induces VEGF expression (41, 50). Elevated levels of VEGF were noted in the wound tissue of FPP-supplemented diabetic mice. This finding was consistent with results from immunohistochemical staining for endothelial cells demonstrating a higher abundance of endothelial cells at the wound site of FPP-supplement diabetic mice. Wound vascularization is specifically known to be impaired in diabetes patients (26), and therefore, enhanced endothelial cell proliferation at the wound site of FPP-supplemented diabetic mice is a desirable effect.

As a nutraceutical, FPP is known for its antioxidant properties (3, 15, 22, 25). In one study, FPP was separated into low- (LMF) and high-molecular-weight fractions (HMFs) (37). LMFs and HMFs demonstrated different activity patterns in macrophages. NO radical formation, measured in real time with EPR spectroscopy, was higher in the presence of LMF and IFN-γ. However, iNOS mRNA levels were enhanced further with HMF rather than with LMF. Furthermore, in nonstimulated macrophages, TNF-α secretion was enhanced by HMF only. Because FPP is made by yeast fermentation, and (1-3)-β-D-glucans are major structural constituents of the yeast cell wall, it was postulated that some of the immunomodulatory effects of FPP are directly related to (1-3)- β-D-glucans (37). β-glucans have been shown to increase the cellular response to the injury, thus facilitating healing (20).

Clinical studies demonstrated that FPP attenuated hemolysis in a patient with paroxysmal nocturnal hemoglobinuria (15). In patients with severe forms of thalassemia, FPP may alleviate symptoms associated with oxidative stress (3). Oxidative DNA damage occurs as an early event in hepatitis C virus infection and is an indication of the potential for carcinogenesis. FPP has been shown to minimize oxidative DNA damage and to improve cytokine balance in such patients (25).

The blood glucose–controlling effects of FPP noted in the current study is consistent with previous studies demonstrating that oral supplementation with FPP may cause a significant decrease in plasma sugar levels both in healthy subjects and in type 2 diabetes patients (12). This work provides the first evidence that diabetic wound outcomes may benefit from FPP supplementation by specifically influencing the response of wound-site macrophages and subsequent angiogenic responses. Given that FPP has a long track record of safe human consumption, testing of the beneficial effects of FPP on diabetic wound–related outcomes in a clinical setting is warranted.

Author Disclosure Statement

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Abbreviations Used

ANOVA = analysis of variance
DAPI = 4′,6-diamidino-2-phenylindole
DCF = dichlorofluorescein
FPP = fermented papaya preparation
H_2DCF-DA = dichlorodihydrofluorescein diacetate
H_2O_2 = hydrogen peroxide
HDL = high-density lipoprotein
HMF = high-molecular-weight fraction
iNOS = inducible nitric oxide synthase
LDL = low-density lipoprotein
LMF = low-molecular-weight fraction
LPS = lipopolysaccharide
NADPH = nicotinamide adenine dinucleotide phosphate
NO = nitric oxide
$ONOO^-$ = peroxynitrite
PMA = phorbol 12-myristate 13-acetate
PVA = polyvinyl alcohol
ROS = reactive oxygen species
TCHOL = total cholesterol
TGLs = triglycerides
TNF-α = tumor necrosis factor α
VEGF-A = vascular endothelial growth factor A