Diabetic foot ulcers are the most severe clinical manifestation of diabetes-related impaired wound healing. Current standard and experimental treatments for these ulcers are largely ineffective. Epicatechin gallate (ECG) is a nontoxic flavonoid previously shown to improve normal wound healing and scar formation. In this study, the neonatal streptozotocin-induced diabetes mellitus (nSTZ-DM) type 2 model in rats was used to investigate the effects of ECG on impaired wound healing and scar formation. Administration of 100 mg/kg STZ induced a significant (P < 0.05) state of mild hyperglycemia in nSTZ-DM type 2 rats, compared to nondiabetic controls. The effects of 0.8 mg/mL ECG on wound healing were then investigated using the full-thickness incisional wound-healing model. ECG significantly improves healing and reduces scar formation in nSTZ-DM type 2 rats (P < 0.05). Biochemical improvements were also found, including significantly increased total nitric oxide synthase activity (NOS; P < 0.001) and inducible NOS (iNOS) activity (P < 0.01). This work highlights ECG as a potential treatment for DM-impaired wound healing.

By 2030, an estimated 366 million people will be diagnosed with diabetes mellitus (DM), of which, approximately 80% will be diagnosed with DM type 2. Diabetic foot ulcers (DFUs) are the most severe clinical manifestation of DM-impaired wound healing and require long-term treatment. The DM type 2 epidemic has generated an increasing need for effective treatment of impaired wound healing. The authors have previously shown that epicatechin gallate (ECG), a polyphenolic flavonoid with antioxidant and anti-inflammatory activity, improves wound healing and scar formation of full-thickness incisional wounds in normal, healthy rats. Herein, the authors utilize the neonatal streptozotocin-diabetes mellitus (nSTZ-DM) type 2 rat model to investigate the effects of ECG on DM-impaired incisional wound healing and scar formation.

**KEYPOINTS**
- The authors utilized a neonatal streptozotocin-diabetes mellitus (nSTZ-DM) type 2 rat model to investigate the effects of ECG on DM-impaired incisional wound healing and scar formation.
Neonatal streptozotocin-induced diabetes mellitus type 2. Diabetes mellitus type 2 was induced in male neonatal Wistar rats on the day of birth (n0) by intraperitoneal (IP) injection of 100 mg/kg STZ in 0.9% saline (n = 30). Nondiabetic controls (NDC) were administered an equal volume of vehicle (0.9% saline; n = 13). Animals with blood glucose levels < 7 mM at the time of wounding were excluded from the study. All experimental procedures were performed in accordance with the University of Otago Animal Ethics Committee guidelines.

Full-thickness incisional wound induction and ECG dosing. Incisional wounds were induced, as previously described,3 and were left uncovered and unsutured. Nondiabetic controls (NDC) were administered an equal volume of vehicle (0.9% saline; n = 13). Animals with blood glucose levels < 7 mM at the time of wounding were excluded from the study. All experimental procedures were performed in accordance with the University of Otago Animal Ethics Committee guidelines.

Assessment of scarring and biochemical analysis. Rats were sacrificed at day 14 PW by CO2 exposure followed by cervical dislocation. Ten µm sections of two wounds were examined for collagen using Van Gieson’s stain and assessed for scarring by six blinded observers, as previously described.3 Micrographs were taken using a Zeiss Axioplan Microscope fitted with an AxioCam HR color digital CCD camera (Carl Zeiss Ltd., Germany). For biochemical analysis, two wounds were homogenized and nitrite level determination, nitric oxide synthase, and cyclooxygenase activity performed, as previously described.3 Bradford assay was used to determine protein concentrations.4

Results

ECG improves healing and reduces scarring in nSTZ-DM type 2 diabetic rats. Van Gieson’s collagen stain was used to demonstrate the quality of healing and the amount of scar formation in the wounds of treatment groups at day 14 PW. NDC and STZ-treatment wounds showed a high proportion of mature (red) collagen fibers that were orientated parallel to the epidermis (Figures 1A and 1C). The parallel orientation of collagen fibers indicates a normal nonscarring healing pattern. Additionally, there was complete wound contraction and the scar tracts were virtually indistinguishable from the surrounding tissue. In contrast, the STZ-vehicle wounds demonstrated high proportions of immature (pink) collagen fibers that were largely disoriented, wide, and with clearly visible wound tracts, indicating poor wound healing or scar formation (Figure 1B). Blinded histological assessment of the wounds identified significantly better healing and less scar formation in STZ-treatment wounds at day 14 PW (P < 0.05) (Figure 1D). Thus, ECG treatment improves scar formation of nSTZ-DM type 2 impaired wounds.
Biochemical effects of ECG on nSTZ-DM type 2 scar formation. Dysregulation of arginine metabolism by the NOS and COX enzyme systems contributes to impaired wound healing.\(^5\) The authors assessed the nitrite levels, NOS, and COX activity in the wounds. No significant difference in nitrite levels was found between the treatment groups (Figure 2A). Total and iNOS activity was significantly higher (\(P < 0.05\)) in the wounds of NDC and STZ-treatment rats, compared with STZ-vehicle rats where NOS activity was undetectable. No significant differences in COX activity were found among the treatment groups (Figure 2C). Therefore, ECG treatment attenuates the reduced iNOS and total NOS activity in nSTZ-DM type 2 impaired wound healing.

Discussion

The DFU wound environment has altered enzymatic activity, which contributes to impaired wound healing and scar formation.\(^6\) The present results show that treatment with ECG attenuates impaired scar formation of full-thickness incisional wounds in nSTZ-DM type 2 rats. Specifically, ECG treatment improves collagen maturation and increases iNOS and total NOS activity in nSTZ-DM type 2 impaired wounds at day 14 PW, which is consistent with ECG treatment of normal incisional wound healing in rats.\(^3\)

Conclusion

The present results suggest that ECG is a potential treatment for wounds in patients with DM type 2.

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