EVALUATION OF SKIN WOUND HEALING KINETICS IN A PORCINE DIABETIC DYSLIPEMIA MODEL.

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INTRODUCTION

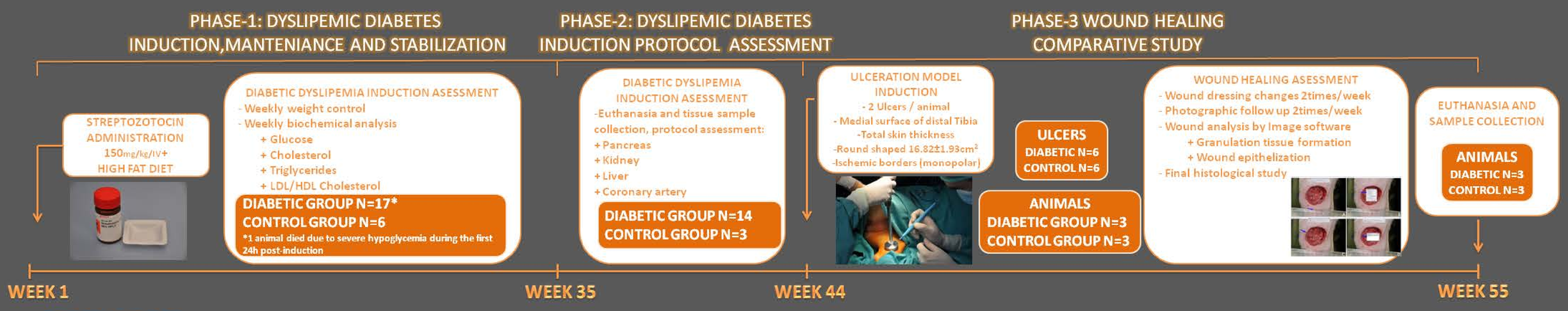
Over 60 million people live with diabetes in Europe[1]. Chronic ulcerations represent a major chronic complication of diabetes, and are responsible of the majority of hospitalizations in diabetic patients. The pig is the one of the most reliable and used model in wound healing research due to its great similarity with human healing processes and kinetics. The pig has also gained interest as a preclinical model in diabetes research because their metabolic and physiological responses closely resemble those observed in humans[2,3]. These facts stand up pigs as a valuable wound healing model for the development of new treatments and therapies. Göttingen minipigs can be very useful in these studies because of their small size, un-pigmented skin and gentle behaviour. The development of a skin wound model in diabetic-dyslipemic Göttingen minipigs would be a valuable platform to support the development of new therapies and treatments for chronic diabetic ulcers.

OBJECTIVE

The aim of this study was to provide basal kinetic data from the healing of a new skin wound model in diabetic-dyslipemic Gottigen minipigs.

MATERIAL AND METHODS

The study protocol was approved by the Institutional Animal Care and Use Committee. 24 adult male Gottingen minipigs were used, 6 of them served as controls while in the other 18 diabetes was induced by Streptozotocin administration. Maintenance feed was supplemented with animal fat until reaching a 15% of fat on the daily ration to establish diabetic dyslipidemia on the diabetic group. Weekly monitoring of glucose, cholesterol and triglycerides were conducted during all the study. After 35 weeks of diabetes stabilization 14 animals from the diabetic group and 3 from the control group were euthanized in order to assess the efficacy of the induction protocol as described by this group before [4]. In the remaining animals skin biopsies and angiographic studies were performed in order to select the best location to mimic the features displayed in human chronic diabetic wounds. After 44 weeks of stabilization, two round-shaped full-thickness wounds of 16.82±1.93cm² were created surgically (using monopolar diathermy in coagulation mode to obtain initial ischemic wound edges) on the hind legs of the six animals remaining for this phase (3 diabetic, 3 control), under general anesthesia, proper analgesia and sterile conditions. Wound surface and granulation tissue formation were assessed from photographs taken two times per week using free license image software (Image-J, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/). Histopathology of major organs and wound samples were performed at the end of the study.



RESULTS

The diabetic-dyslipemic induction protocol produced an intense and irreversible diabetic state increasing also cholesterol and triglycerides levels as published before [4]. On the wound healing comparative study, while control group covered the wounded area with a thick uniform granulation tissue layer during the first week of study, the diabetic group developed two separate granulation tissue tongues that slowly covered the wound area until they meet at the center of the wound approximately 14 days post wound creation (Figure 3). Statistical differences on the granulation tissue formation were observed during the first three weeks of study (Figure 1). The wound epithelization kinetics were similar in both groups, but diabetic group took almost four weeks more to reach 100% because of the osteomyelitic fistulas developed (Figure 4). The histology from skin biopsies did not revealed any significant difference regarding the healing quality between both study groups. Histopathology of major organs from these 6 animals (Figure 5), after 55 weeks of study, revealed advanced diabetic changes in pancreas (almost complete absence of Langerhams islets), liver (lipidosis and glycogenosis) and kidneys (glomerular expansion, basal membrane increased thickness and tubular lipidosis). The Sudan IV staining of the complete aortas showed atherosclerotic initial lesions in diabetic animals (Figure 6 and 7) where calcium deposits were found (Figure 5.- H).

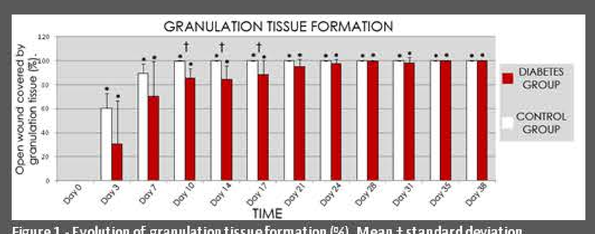


Figure 1.- Evolution of granulation tissue formation (%). Mean ± standard deviation.
ANOVA . HSD-Tukey (p≤0.05). T- Student (p≤0.05) .
*: Significant difference between times respect the basal value;
†: Significant differences between groups.

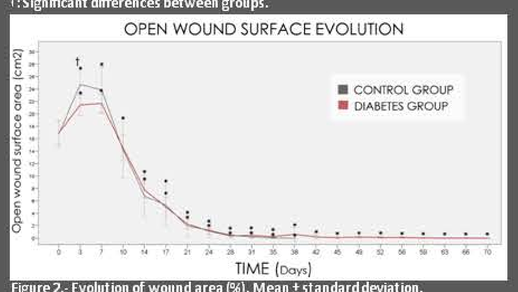


Figure 2.- Evolution of wound area (%). Mean ± standard deviation.
ANOVA . HSD-Tukey (p≤0.05). T- Student (p≤0.05) .
*: Significant difference between times respect the basal value;
†: Significant differences between groups.

Figure 3.- Evolution of wound healing kinetics in one animal from each group.

Figure 4.- Osteomyelitis fistula developed by animals from diabetic group.

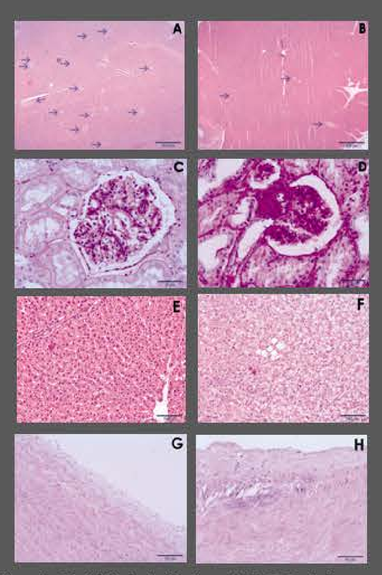


Figure 5.- Main histological features. A,C,E,G: Control group.
B,D,F,H: Diabetic group. A,B: Pancreas 4x; C,D: Kidney; E,F: Liver
G,H: Aorta artery.

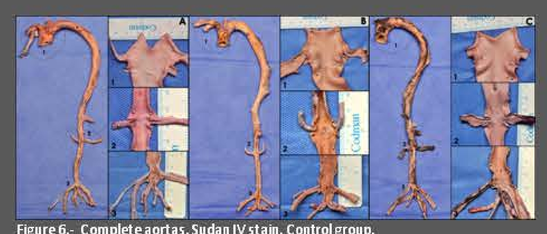


Figure 6.- Complete aortas. Sudan IV stain. Control group.

Figure 7.- Complete aortas. Sudan IV stain. Control group. Red stain indicates fat infiltration.

DISCUSSION

The diabetic-dyslipemic induction protocol produced an intense and irreversible diabetic state and also increased cholesterol and triglycerides levels as published before [4].The statistical differences on the granulation tissue formation were observed during the first three weeks of study, but diabetic animals had a slightly lower bodyweight at the ulceration induction time that may had influence the formation of the granulation tissue. The exposure of the bone cortical during two weeks produced osteomyelitic fistulas that extended the time that diabetic ulcers took to reach total healing. The atherosclerotic lesions observed were categorized as light initial lesions with minimal or no clinical implications. Immunohistochemical analysis from wound samples at different times during the healing process wound would help identifying the factors and mechanisms that determined the slower granulation tissue formation in diabetic group.

CONCLUSIONS

- Although diabetic wounds showed a delay in total wound epithelialization time and a slower granulation tissue formation, healing kinetics were very similar in both groups.
- The proposed model reproduces several clinical features from human diabetic wound, but human diabetic wounds are often a late chronic complication that has is major clinical impact in elderly patients, these facts highlights the importance of age and the duration of exposure to the predisposing factors for obtaining a reliable animal model of this chronic pathology.
-The lack of uniformity on the diabetic dislipemia protocols published and the absence of basal data from diabetic wound healing in Göttingen minipigs makes this study a good first step in the establishment of a reliable and reproducible diabetic ulcer large animal model in order to support the development and test the efficacies of new treatments and therapies.

Bibliography

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