CORONARY BAND AUTOGRAFTING IN DONKEYS: CLINICAL AND HISTOLOGICAL EVALUATION OF HEALING QUALITY OF HOOF WALL DEFECTS

M. DARADKA, W. ALHAMAD & Z. B. ISMAIL

Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan
University of Science and Technology, Irbid, Jordan

Summary

Daradka, M., W. Alhamad & Z. B. Ismail, 2015. Coronary band autografting in donkeys: clinical and histological evaluation of healing quality of hoof wall defects. *Bulg. J. Vet. Med.*, **18**, No 2, 140–147.

Twelve healthy, adult donkeys of both sexes aged between 2 and 4 years were used to evaluate the feasibility of using coronary band autografts to aid in the healing of deep dermal hoof wall injuries. Wound healing and graft take were monitored visually and evaluated histologically at different time points. Epidermal cells of the coronary papillae survived the process of hoof wall stripping. Almost all areas of the basal membrane were intact and attached to the corium at the site of hoof wall stripping. Grafted basal cells were able to produce new generations of cells (keratinocytes or keratin producing cells) which matured and keratinised, resulting in the production of new stratum medium. In spite of the fact that the new hoof wall was rough and irregular in shape, it was functional and donkeys were no longer lame at 28 days post surgery.

Key words: autograft, coronary band, donkey, hoof wall

INTRODUCTION

The hoof is a modified portion of the epidermal skin (Kainer, 1989; Daradka, 2000; Furst & Lischer, 2006); it originates from the coronary band. Hoof and hoof wall injuries are common in equine practice and their management remains challenging and frustrating for both veterinarians and owners. Hoof wall cracks, hoof keratoma, and coronary band avulsion need a long term and expensive treatment with guarded prognosis (Parks, 1999; Stashak, 2002; Stashak & Christine, 2008).

Injuries to the coronary germinal layer result in hoof wall defects of the hard keratinised stratum medium (Dabareiner *et al.*, 2003; Celeste & Szoke, 2005). The defect moves distally along with the direction of hoof wall growth (Dabareiner *et al.*, 2003; Celeste & Szoke, 2005). Injuries to the hoof wall heal by replacement of the defect by new hoof wall that grows from the intact coronary band (Dollar, 1993; Parks, 1999).

The response of stratum germinativum of the coronary band differs according to the extent of the injury. For example hoof wall injuries involving the dermal portion of the hoof, stratum germinativum produces scar horn rather than proliferation of basal cells (Dabareiner *et al.*, 2003; Celeste & Szoke, 2005). However, in injuries involving the epidermal layer, stratum germinativum reserves its proliferative potential (Pollitt, 1998; Daradka, 2000). Therefore, in order to prevent scar tissue formation, we propose a coronary band autograft for the treatment of coronary band injuries in donkeys as a model for use in horses. Hoof wall striping is well known surgical procedure in equine practice and frequently used to facilitate the repair of cracks and to remove foreign bodies and tumors beneath the hoof wall (Dabareiner *et al.*, 2003; Celeste & Szoke, 2005). The large deficit in hoof wall that results from wall strip surgery heals remarkably well (Pollitt, 1998; Daradka, 2000). In equine practice as well as in the interested scientific community, it is believed that coronary band injuries are unsuitable for various reconstructive surgical procedures that might have been helpful in other tissues. Hence, the primary objective of this study was to evaluate the feasibility of using coronary band autografts to aid in

the healing of deep dermal hoof wall injuries in donkeys as a model for horses.

MATERIALS AND METHODS

The experimental protocol used in this study was reviewed and approved by the Institutional Animal Use and Care Committee at Jordan University of Science and Technology (JUST-ACUC). Twelve healthy, adult donkeys of both sexes, between 2 and 4 years of age and 100 and 150 kg body weight were used in the study. All donkeys were free of lameness and hoof abnormalities. The donkeys were housed at the Veterinary Health Center of the Faculty of Veterinary Medicine in a fenced yard with shaded area available. Animals were divided randomly into 2 groups: Group 1 (n=10) underwent hoof wall striping and autografting of the coronary band. Group 2 (n=2) underwent hoof wall striping and coronary band resection without grafting and served as control.

Hoof wall striping and graft collection

Prior to surgery, all animals were clinically examined. Premedication was obtained by intravenous injection of xylazine (Xylaject, Adwia, Egypt) at 1.1 mg per kg

Fig. 1. Left: donor and recipient site with the dorsal hoof wall stripped and ready for grafting. Right: Coronary band autograft prepared for grafting.

of body weight. Anaesthesia was then induced by intravenous injection of 10% thiopental sodium (Thiotal, Vetoquinol, Canada) at 10 mg per kg body weight. The animal was then placed on lateral recumbency and the distal forelimbs and hooves were clipped and prepared for aseptic surgery. Hoof wall stripping was performed on the middle dorsal aspect of both forelimbs as described previously (Daradka, 2000). Each of the stripped hooves acted as donor and recipient site. Of the stripped part of the hoof, a rectangular full-thickness portion (10 mm wide $\times$ 25 mm long) of the coronary epithelium including the coronary corium and perioplic corium up to the lamellar corium was obtained (Fig. 1). This portion of tissue was incised and peeled off deep to the dermis in preparation for transplantation in the opposite hoof. Each tissue was transferred to labeled Petri dish containing 10 mL normal saline and 1 mL of 10% gentamicin awaiting for grafting.

Tissue grafting

The recipient site was kept moist until the graft was ready for transplantation. Full-thickness coronary autografts were handled gently and sutured to the recipient site of the ipsilateral hoof using four simple interrupted sutures using 2/0 silk. Numerous simple interrupted sutures were made in order to bring full contact of the grafted tissue to the recipient site. The surgical site was then bandaged using sterile non-adherent dressing (Non-adherent dressing, Johnson and Johnson, USA). In control animals, hoof wall stripping and resection of coronary band without grafting was carried and left to heal by second intention. Wound dressing was changed every 48 hours for 7 days. Perioperative antibiotics using 2.2 mg per kg body weight gentamicin and 200,000 IU per kg

body weight of potassium penicillin twice daily intravenously for 5 days. Healing was monitored daily, and photographs of the healing site were taken weekly throughout the experimental period (90 days).

Histopathology

To obtain tissues from different ages of graft transplantation, 2 animals were euthanised at 14, 28, 60 and 90 days after the operation. Control animals were euthanised by the 90th day. Euthanasia was performed using intravenous injection of pentobarbital sodium at 100 mg/kg (Beuthanasia-D; Schering-Plough Animal Health, NJ, USA). Immediately after euthanasia, the middle dorsal hoof wall, coronary and lamellar epidermis and corium were taken using the method of Politt (1996). Collected tissues were transferred to a 10% buffered neutral formalin solution and processed for routine histological examination. Sections of 5 μ m thickness were cut and stained using H & E stain and Periodic acid Schiff (PAS) special stain.

RESULTS

The coronary papillae epidermal cells survived in the grafting bed. Cells in the grafted tissues appeared to proliferate and keratinise producing new keratinocytes immediately after grafting. The tubules and intertubular horn were prominent as early as 28 days post-operatively (Fig. 2 and 3).

Table 1 describes the gross and histological characteristics of the grafted tissues as it appeared at each examination time point. At 7 days old coronary papillae autograft, progressive growth of granulation tissue and revascularisation could be detected (Fig. 2). At 14 days old graft,

Table 1. Gross and histologic description of grafted tissue at the recipient site at different time points post-operatively

Post operative day	Gross description	Histologic description
7	Progressive growth of healthy granulation tissue	Proliferation of epidermal basal cells of coronary papillae
14	Part of superficial layer of grafted tissue was desquamated, while the deep layer was still intact	Granulation tissue formation was evident and new blood capillaries were formed at the site of graft take
28	Beginning of keratinisation of both coronary and lamellar epidermal basal cells	High pigmentation of the proliferating cells of coronary papillae
60	Thickness of keratinised layer was proportional to keratinisation process progression, the thicker the keratinised layer, the more the keratinisation	Keratinocytes began to lose their nuclei adding to stratum corneum of the hoof wall. Collagen fibers were more prominent
90	The coronary keratinised layer became level with the adjacent normal hoof wall	Epidermal basal cells of coronary papillae proliferated and produced new keratinocytes adding to the hoof wall stratum medium. Keratinisation process increased with time

part of the superficial layer of grafted tissue was desquamated, while the deep layer was still intact. At 28 days old graft, the beginning of keratinisation of both coronary and lamellar epidermal basal cells could be detected (Fig. 3). At 60 days old graft, the thickness of both coronary and lamellar keratinised layer increased to 2 mm (Fig. 4). Thickness of keratinised layer was proportional to keratinisation process progression, the thicker the keratinised layer, the more the keratinisation. The more the time passed, the harder the hoof became.

At 90 days old graft, the thickness of both coronary and lamellar keratinised layer increased to 4 mm (Fig. 5). The coronary keratinised layer became level with the adjacent normal hoof wall. The hoof wall became harder than before.

Coronary band transplant did not appear to alter neither the process of keratinisation nor the process of pigmentation.

Normal keratinocytes and normal pigmented horn were produced. The rate of hoof

Fig. 2. A 7 days old coronary papillae autograft. There are different sizes of dermal coronary papillae and proliferated and cornified coronary epidermal basal cells (stratum medium). a, b: dermal coronary papillae, c: intertubular horn, d: coronary cushion. Transverse section, H & E, Bar=50 µm.

Fig. 3. A 28 days old coronary papillae auto-graft. Notice the amount of pigmentation of the proliferating basal cells. a: dermal coronary papilla, b: basal cells, c: intertubular horn. Transverse section, H&E, Bar=50 µm.

Fig. 4. A 60 days old coronary papillae auto-graft showing healed scar at the site of graft take. a, b, c: compressed vasculature, d: condensed fibroblast. Transverse section, H&E, Bar=50 µm.

wall production after grafting was similar to the rate of hoof wall production in control animals (1 cm/month). No complications associated with the procedure were observed.

DISCUSSION

The hoof is a modified portion of the epidermal skin; thus healing of coronary band grafts can be compared with that of a skin graft. Concerning hoof wall injuries

Fig. 5. A 90 days old coronary papillae auto-graft showing dermal coronary papilla, coronary epidermal basal cells proliferated and cornified to produce new hoof wall (stratum medium). a: dermal coronary papilla, b: dense connective tissue of coronary cushion. Longitudinal section, H&E, Bar=100 µm.

in the equine species, it is the stratum germinativum, not basal cells who proliferate in response to deep hoof wall injuries involving hoof horn and dermis to produce scar tissue (Parks, 1999; Stashak, 2002; Dabareiner *et al.*, 2003; Celeste & Szoke, 2005; Furst & Lischer, 2006; Stashak & Christine, 2008). On the other hand, stratum germinativum reserves its proliferative potential for the healing of epidermal injuries (Pollitt, 1998; Daradka, 2000). In this study, we were able to demonstrate that a coronary band graft is well incorporated in the recipient bed, proliferated and produced near normal shape and functional new hoof. In medical literature, grafting of epidermal appendages is limited to hair follicles and cornea of the eye (IJzermans, 2001). Nevertheless, grafting of hard thick keratinised tissues such as the hoof wall is not easy and has never reported before in horses.

In donkeys, hoof wall stripping was performed safely and successfully. Survived grafted coronary papillar epidermal cells kept their ability to proliferate and keratinise in order to produce new hoof

wall, in a way similar to that occurring in hoof wall strip. This agrees with Daradka (2000), who reported the process of proliferation and keratinisation of both coronary and lamellar basal cells in stripped hoof wall in horses.

Immobilisation of the affected hoof using casted material has been proposed as essential for healing of hoof injuries (Parks, 1999; Stashak, 2002; Dabareiner *et al.*, 2003; Celeste & Szoke, 2005; Furst & Lischer, 2006; Stashak & Christine, 2008). In this study, a degree of immobilisation was achieved by suturing the grafted coronary papillae to the recipient bed using numerous simple interrupted sutures. In addition, the donkeys were given limited exercise to limit their movement during the post-operative period. Despite of the limited motion provided, grafted tissues healed satisfactorily. Immobilisation using pressure bandages seemed to be sufficient as was suggested previously to protect the grafting site (Lin *et al.*, 1985).

Degree of contraction of grafted tissue is positively proportional to myofibroblasts production but negatively proportional to the amount of dermis included in the graft (split-thickness or full-thickness) (Van de Berg & Rudolph, 1993). In this study, coronary band (dense connective tissue) contributed to the high number of mesenchymal cells, which could transform to myofibroblasts and caused graft contraction. Contraction was obvious since coronary band grafted tissue was split-thickness graft rather than full-thickness compared to skin but graft take was successful. These findings are in agreement with those obtained by Van de Berg & Rudolph (1993).

Graft take was successful. Healthy granulation tissue was seen grossly as early as 3 days following graft transplan-

tation. Microscopically, channels connecting blood capillaries between dermal part of recipient area and grafted tissue could be detected as early as 7 days post-operatively. Fibroblast proliferation could be detected at the same time. This agrees with Stashak (2002). Desquamation of part of superficial layer of grafted tissue at 14 days old coronary papillae autograft was expected, which agrees with Peacock's statement (1984). Coronary papillar epidermal cells produced tubules and intertubular horn in the same direction of normal hoof wall tubules (vertical to ground surface) but were not parallel to each other (due to graft contraction). The newly formed hoof wall passed over stripped lamellar tissue in proximo-distal direction. A degree of deformity was observed which eventually will disappear in fully grown hoof wall. These findings are in agreement with the findings of Leach (1980), Budras *et al.* (1980) and Daradka (2000).

Histologically, normal coronary papillae were seen in mature hoof wall graft tissue. In transverse sections, the basal cells organised in the same architecture of the normal hoof wall. The basal cells proliferated and keratinised to produce the bulk of the hoof wall (stratum medium). The growth rate of the grafted hoof wall was 1 cm/month which was similar to that in normal hoof wall (Budras *et al.*, 1980; Daradka 2000). The process of proliferation and keratinisation was proportional to time. At 90 days old coronary papillae autograft, hoof wall thickness was 4 mm which is close to the normal thickness of the hoof wall. This agrees with the study of horse hoof keratin by Bertram & Gosline (1987) and hoof wall growth by Budras *et al.* (1980) and Leach (1980).

CONCLUSIONS

The primary objective of this study was to evaluate the feasibility of using coronary band autografts to aid in the healing of deep dermal hoof wall injuries. In equine practice as well as in the interested scientific community, it was believed that coronary band injuries were unsuitable for various reconstructive surgical procedures that might have been helpful in other tissues. Although the researchers in this project do not recommend at this stage using autografts as a treatment of hoof wall injuries, the idea is novel. Our intent was to prove the validity and viability of coronary band grafting using autografts as a preliminary study. In the future, the validity and viability of using allografts are warranted. This will prove a lifesaving procedure for many horses that might be otherwise lost because of devastating hoof wall injuries.

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Paper received 13.01.2014; accepted for publication 25.04.2014

Correspondence:

Mousa Daradka
Department of Clinical Veterinary Sciences,
Faculty of Veterinary Medicine,
Jordan University of Science and Technology,
Irbid, P.O.Box 3030 Jordan.
Phone: +9627201000 ext. 22021
Fax: 00962 2 7095123
E-mail: daradka@just.edu.jo