BURNS 41 (2015) 565–574

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/burns

Genetically modified porcine split-thickness skin grafts as an alternative to allograft for provision of temporary wound coverage: preliminary characterization

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ARTICLE INFO

Article history:
Accepted 2 September 2014

Keywords:
Xenograft
Skin graft
GalT-KO
Burn treatment
Wound coverage

ABSTRACT

Temporary coverage of severely burned patients with cadaver allograft skin represents an important component of burn care, but is limited by availability and cost. Porcine skin shares many physical properties with human skin, but is susceptible to hyperacute rejection due to preformed antibodies to α-1,3-galactose (Gal), a carbohydrate on all porcine cells. Our preliminary studies have suggested that skin grafts from α-1,3-galactosyltransferase knock out (GalT-KO) miniature swine might provide temporary wound coverage comparable to allografts, since GalT-KO swine lack this carbohydrate. To further evaluate this possibility, eight non-human primates received primary autologous, allogeneic, GalT-KO, and GalT + xenogeneic skin grafts. Additionally, secondary grafts were placed to assess whether sensitization would affect the rejection time course of identical-type grafts. We demonstrate that both GalT-KO xenografts and allografts provide temporary coverage of partial- and full-thickness wounds for up to 11 days. In contrast, GalT + xenografts displayed hyperacute rejection, with no signs of vascularization and rapid avulsion from wounds. Furthermore, secondary GalT-KO transplants failed to vascularize, demonstrating that primary graft rejection sensitizes the recipient. We conclude that GalT-KO xenografts may provide temporary coverage of wounds for a duration equivalent to allografts, and thus, could serve as a readily available alternative treatment of severe burns.

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http://dx.doi.org/10.1016/j.burns.2014.09.003

1. Introduction

Early excision of burned tissue and replacement with autologous skin grafts is a mainstay of burn treatment, and has been shown to reduce patient mortality by preserving the skin's barrier function, preventing fluid loss and subsequent hypovolemia, electrolyte, temperature and pH imbalances that if untreated contribute to infection, multisystem organ failure, and death. However, the supply of uninjured skin may be limited in severe burns; therefore, alternative means of temporary coverage to preserve barrier function are needed [1–8].

Cultured autologous keratinocytes and various artificial dermal substitutes have been described to this end, however these approaches have significant disadvantages and the outcomes are inferior to allogeneic skin grafts [9–13]. Cultured autologous keratinocytes require weeks to grow before application and yield a thin, delicate graft that is easily injured [10,13]. Artificial dermal substitutes (such as Biobrane™, Transcyte™, and Integra™) are expensive, require vessel ingrowth (a process that can take up to 2–3 weeks) for optimal protection against infection, and still require autologous skin grafts to achieve permanent wound closure [9,14–18]. Surfrasoft™, Mepitel™, and Suprathel™ have mostly been used as biologic dressings to aid in the healing of partial-thickness burn wounds or to aid in healing of split-thickness skin grafts [19–21]. EZ derm, a biosynthetic derived from porcine dermis, has been studied in a few clinical settings as a biosynthetic dressing for partial-thickness burns [22,23] The EZ derm provided wound coverage for about 5–7 days and sloughed as the underlying wound epithelialized [22,23]. Full-thickness wounds still require definitive closure with autologous skin grafts.

The current gold standard for temporary coverage of full-thickness burns is allogeneic cadaver skin. While allogeneic skin grafts predictably reject from the wound bed 7–12 days after placement due to immunologic incompatibility between the burn victim and the cadaver donor, allografts undergo vascularization within 2–3 days in a manner similar to autologous grafts, and are therefore viable and capable of providing a barrier in the early post-burn time frame, when protecting against infection and physiologic insults associated with the loss of skin integrity. Despite their effectiveness, however, allografts also have disadvantages, including cost, limited availability, and the risk of pathogen transmission [24].

Xenogeneic skin grafts provide a potential alternative for temporary wound coverage. Porcine skin has considerable similarity to human skin that makes it an attractive option for temporary wound coverage and maintaining barrier functions in the early post-burn period; including structurally similar rete ridges, papillary dermis, and sparse hair coverage [14,25–28]. In addition, swine share few pathogens in common with humans, thus reducing the risk of disease transmission when compared with cadaveric grafts [29–31]. Furthermore, it would be possible to maintain a herd of swine in a climate-controlled, pathogen-free environment for the purpose of skin graft procurement, an important practical consideration in ensuring consistent availability of high-quality skin suitable for use in medical settings.

Historically, porcine skin grafts have not been a viable option, as they fail to vascularize due to hyperacute rejection, an immediate attack on the endothelium of graft blood vessels mediated by preformed antibodies in humans and Old World primates against the α-1,3-galactose (Gal) moiety present on swine cell membranes [32,33]. Antibody-mediated endothelial injury results in a diffuse thrombotic microangiopathy and subsequent ischemic insult, resulting in quick desiccation and avascular necrosis. Thus, the barrier function of the graft fails after a few days and may even serve as a nidus for bacterial colonization or superinfection.

To avoid the problem of hyperacute rejection, genetically-modified swine have been prepared that do not express the Gal epitope due to selective knockout of the gene encoding α-1,3-galactosyltransferase (GalT-KO) [34]. The availability of these animals now makes it possible to carry out pig-to-primate xenografts without hyperacute rejection mediated by anti-Gal antibodies. Solid organ transplantation from pig-to-primate using GalT-KO swine did not show hyperacute rejection and had prolonged organ survival compared to Gal normal swine [35,36]. Preliminary studies performed in our laboratory have suggested that skin grafts from GalT-KO swine may survive as long as allografts on baboons [25]. Here we have further studied GalT-KO skin grafts to evaluate their performance in comparison with allografts as a potential alternative treatment options for severely burned patients. We demonstrate that skin grafts from GalT-KO miniature swine engraft on primates and provide temporary wound coverage for a period comparable to that offered by allogeneic skin and considerably longer than wild type GalT + porcine grafts.

2. Methods

2.1. Animals

This study was approved by the Massachusetts General Hospital (MGH), Institutional Animal Care and Use Committee (IACUC) and performed in accordance with the guide for the care and use of laboratory animals [37]. Eight baboons (*Papio hamadryas*) were obtained from Mannheimer Foundation, Inc, Homestead, FL. All baboons were aged 2–5 years and weighed 6–10 kg each. The animals underwent routine pathogen screening and quarantine prior to commencement of the studies.

Genetically engineered GalT-KO miniature swine were produced in our own swine facility [34]. The GalT-KO swine herd is monitored using a computerized system to ensure availability and quality control, and housed in a purpose-built facility, which is fully integrated as part of the animal facilities in the laboratory with input and veterinary oversight from the Center for Comparative Medicine of the MGH.

2.2. Skin graft harvest

Swine donors were anesthetized with 2 mg/kg Telazol intramuscular (IM) injection, intubated, and anesthesia maintained using 2% isoflurane and oxygen. The skin surface was disinfected before surgery with 2% (w/v) chlorhexidine