Advances in Research in Animal Models of Burn-Related Hypertrophic Scarring

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Skin burn injuries affect approximately 500,000 people per year in France. After deep burns, functional sequelae associated with hypertrophic and retractile scars are an important public health problem. To understand the pathophysiology of sequelae and evaluate new therapeutic approaches, the use of animal models that should be standard tools is necessary. Some pre-clinical models of hypertrophic scars after burns have been described, but the choice of the appropriate and relevant experimental model is crucial to accurately investigate any therapeutic approach. A variety of hypertrophic scar animal models have been described after burn lesions; none of which being totally satisfactory. The most frequently used is the hypertrophic scar model after skin excision of the ear rabbit, but this model does not reflect burn injuries. The red Duroc pig seems to be the more relevant model of human hypertrophic scarring after burns; however, because of costs and the lack of studies evaluating burn injuries in this species, the domestic pig is most commonly used in burn research. Elevated hypertrophic scars are obtained, but they spontaneously resolve within a year. Although mortality in small animals is higher and creates technical difficulties, many models on nude mice are used in research. Indeed, transplantation of human hypertrophic scar tissue or human skin grafts may induce hypertrophic scarring that can last more than a year permitting additional manipulation and experimentation. (J Burn Care Res 2015;36:e259–e266)

Skin burn injuries affect approximately 500,000 people per year in France and are responsible for 10,000 hospitalizations among which 3,000 are managed in burn units. The seriousness of a burn depends on the temperature of the heat source at the skin surface and the duration of exposure. The resulting physiopathology, therapies, and sequelae are different depending on the degree of burn. After deep burns, functional sequelae associated with hypertrophic and retractile scars are an important public health problem. They have been associated with anxiety, social avoidance, depression, a disruption in activities of daily living, the onset of sleep disturbances, and all the resulting difficulties associated with returning to normal life after physical rehabilitation.[1] Hypertrophic scarring following burn injury is more common and severe in children than in adult burn patients, with Bombaro et al reporting an overall incidence of 88% in children compared with 56% in adults.[2] They arise when there is an overproduction of collagen during wound healing and appear more frequently when the healing has been prolonged. Hypertrophic scarring is often associated with poor regulation of the rate of programmed cell death (apoptosis) of the cells synthesizing the collagen or by an exuberant inflammatory response that prolongs collagen production and increases wound contraction.[3] Many therapeutic advances have been made, in particular early skin grafting, compression, and physiotherapy, but none are totally satisfactory.[4] Much remains to be learned about basic aspects of the pathophysiological mechanisms underlining hypertrophic scar formation and a better understanding of these events is needed to optimize or develop new therapeutic options. This will rely on the use of animal models

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that have become standard tools for the study of a wide array of burn wounds.

Some animal models of hypertrophic scarring after burns have been described, but the choice of the appropriate and relevant experimental animal model is crucial to accurately investigate any therapeutic approach. The animal model has to be reproducible and as close as possible to burn lesions occurring in humans, taking into account the different structural properties of the skin between different animal species and humans. Most of the animal models described so far were set up many years ago and are still currently used.

In the present review, we will focus on burn sequelae, such as hypertrophic scarring occurring during the wound healing process and on the different animal models described in the literature that attempt to mimic the human situation.

SKIN CHARACTERISTICS AMONG DIFFERENT ANIMAL SPECIES

A number of animal models have been described to assess the impact of burns on healing after injury. However, the animal model has to be chosen appropriately depending on the applications. Selection of the model should take into consideration the interspecies anatomical and physiological characteristics that reflect the differences in how different types of wounds heal, and analytical techniques to be applied. Models relying on the use of excised human skin should theoretically be more relevant to the clinical situation and represent the "accepted standard" for in vivo experimentation. Their use, however, is limited by skin availability and variability among patients because of differences in age, gender, race, or anatomical site of the sample. Indeed, various animal models from mammals or rodents have been developed as surrogates for human skin; the pig model being the most relevant.

Anatomically and physiologically, pig skin is similar to human skin.[5–8] Nearly 30 years ago, Silverstein reported that deep donor sites in female red Duroc pigs healed with "hypertrophic" scarring with similar characteristics to that occurring with human skin, but in this model the scar spontaneously resolved.[9] Also similarities between domestic pig and human skin were described as sparse hair coat, a thick epidermis with a well differentiated under sculpture, a dermis that has a well differentiated papillary body, and a large content of elastic tissue and abundant subdermal adipose tissue.[10,11] So which breed of pig is the best for an hypertrophic scarring model: the red Duroc pig with pigmented and proliferative skin or the domestic pig with non-pigmented non-proliferative skin?

Zhu et al explored healing in the red Duroc model for similarities to human hypertrophic scarring, studying wound thickness, appearance, healing status at 3 weeks, histology, and immunocytochemical localization of decorin, versican, TGFbeta1 and IGF-1; and examined Duroc skin for cones. They found that healing after deep excisional wounds in Duroc pigs is similar, but not identical, to human hypertrophic scarring. They also found that Duroc skin contains cones. Healing in the female red Duroc pig is sufficiently similar to human hypertrophic scarring to warrant further study so that it can be accepted or rejected as a model of human hypertrophic scarring.[12] Also Liang quantified nerves in red Duroc pig tissue and compared the findings to human hypertrophic scar. The results demonstrate that nerve tissue is increased in Duroc pig scar tissue and is quite similar to that in human hypertrophic scar and to that described in the literature. These data provide additional evidence that the red Duroc pig model may be useful for studying hypertrophic scarring.[13] An interesting study by Engrav showed that the red Duroc scar may be thick at 20 weeks and persist at week 46, demonstrating that the scar had not regressed, and was perhaps even increasing in thickness. The observation that the porcine thick scar has not disappeared at week 46 and, in fact, may be thicker indicates that, as in humans, the porcine scar is long-lived. They also showed that the granulation tissue layer and the scar layer in the Yorkshire breed are much thinner than in the Duroc breed.[14] An increased numbers of myofibroblasts, mast cells, and collagen nodules are reported in red Duroc pig scar similar to that observed in human hypertrophic scar.[15] Based on these studies, it seems that the Duroc pig more closely reflect human hypertrophic scarring. However, to date only excisional wounds have been studied in the red Duroc pig, which are likely to heal differently than burns.

THE PIG AS A MODEL FOR HUMAN WOUND HEALING AFTER BURNS

As described previously, the many similarities between man and pig would lead one to conclude that the pig should make an excellent animal model for human wound healing. Several porcine models of burns have been described.

Heated Bottle Burn Scar Model

A porcine model of hypertrophic scarring after burns was developed by Cuttle in 2006.[16] Partial thickness burns were produced by applying a glass bottle

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containing water at 92°C to the skin of a large white pig for a period of 14 seconds. Animals were kept for 6 and 14 weeks before analysis of the wound healing process, which mainly resulted in contracted, purple, hypertrophic scars. At day 99, the sections of burned skin showed massive hypertrophy of the dermis, and mild hypertrophy of the epidermis. The dermal thickness was because of an increase in the number of fibroblasts in the dermis as well as an increase in the amount of collagen. Collagen fibers, which run parallel to the surface, can be seen in sections from 99 days after the burn injury. This model of hypertrophic scarring after deep dermal partial thickness burn injury has since been widely used in a number of studies on burn healing. A detailed retrospective study described that healing is affected by localization of burns. While some have noted better healing in more caudal wounds, others have noted the opposite.[17] Another report provided evidence that two weeks after injury, the level of alpha smooth muscle actin (α-sma) expression was correlated in vivo with scar contraction and thickness, re-epithelialization and depth of burn.[18] The effect of first aid cooling techniques has also been investigated in this model and current first aid guidelines to use cold tap water (at 15 or 2°C for 20 minutes) were shown to be beneficial in helping to heal the burn wound.[19] The effects of surgical debridement alone were compared to surgical debridement followed by skin grafting or skin substitutes in the porcine burn healing model. In this study, the authors concluded that immediate debridement with an appropriate dressing and without skin grafting may promote wound healing.[20]

A similar model first described by Jandera et al was recently used by Chan et al to evaluate the correlation between time to skin grafting and hypertrophic scarring following an acute contact burn in large white pigs.[21,22] Deep dermal burns were induced with 300 ml of heated water from a kettle at 92°C, which is poured into a bottomless mug. The base of the mug was fitted with a latex membrane and the burn injury induced by contact of the base of the jug with one of the designated burn sites on the animal flank for the duration of 20 seconds. Scar analyses were conducted more than 3 months. Results showed a strong correlation between histological evaluation of the degree of fibrosis and α-sma levels. The increased fibrosis observed in delayed grafting was related to progression of burn depth and infection suggesting that early grafting of deep dermal burns may be preferential.

Heated Aluminum Bar Burn Scar Model

Singer et al evaluated scar formation after burns created on animal's dorsum with an aluminum bar burn model in domestic pigs.[23,24] Burns were created using an aluminum bar preheated to 80°C and applied for 20 seconds resulting in a partial thickness thermal burn extending half way down the dermis. Hour glass shaped scars were noted 28 days after injury. They showed that treatment with a novel TGF-β antagonist speeded re-epithelialization and reduced both scar formation and wound contraction after partial thickness burns. This model has also been used to evaluate wound re-epithelialization after burns as a determinant of wound infection and scarring.[25]

Heated Brass Burn Scar Model

A burn wound model in the Yorkshire domestic pig skin was recently established to investigate wound repair using tissue engineered skin combined with mesenchymal stem cells (MSC).[26] Burns were created using a heated-brass contact injury at 100°C for 20 seconds. Skin substitutes composed of collagen-glycosaminoglycans scaffolds seeded with MSCs were grafted onto the burn wounds. Wound contraction was always observed with this model but not elevated hypertrophic scars. Scar formation was followed until 4 weeks after burn. Better healing and keratinization as well as less wound contraction and more vascularization were observed with MSC providing evidence that epidermal formation was improved.

Radiant Heat Burn Scar Model

Gurfinkel developed an animal model of burns that uses a radiant heater at 400°C for 20 seconds in pigs and rats.[27] In pigs, 16 burns were created on each animal resulting in re-epithelialization of the burns within approximately 3 weeks and hourglass contracted scars in two of three burns within 1 month, but the duration of hypertrophic scarring was not evaluated. Using radiant heat, the authors were able to create consistent burns that maximize safety to the investigators and animals.

Although comparative studies suggest that the Duroc pig model results in hypertrophic scars that most closely resemble human hypertrophic scars, because of the relatively high cost of the Duroc pig, most studies have used the domestic pig. Among the various domestic pig burn models resulting in hypertrophic scars, the "hot water bottle" model is the most commonly reported.[16] Macroscopic, histologic, and biologic criteria were similar with human hypertrophic scar.

However, disadvantages of the porcine models are the cost and difficulty to implement in practice.[8,28]

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This is why small animal models such as rodents have been described and used in a variety of studies.

MURINE MODELS OF BURNS

In the literature, burn models by immersion in hot water account for the largest share of the protocols described in small animals.[29–33] Brass, comb, or aluminum rod burn models were also often described.[34–37] In the different models, dorsal skin is the most frequently used burning zone and rarely the flanks. As an example, Sultan et al used the burn model of a brass rod heated to 100°C in a hot water bath to study the effect of fat grafting on scar fibrosis.[38] In this model, the scar is not grossly elevated and all analyses were based on histology, enzyme-linked immunosorbent assay (ELISA) protein quantification and protein chain reaction (PCR) for vasculogenic and fibrogenic factors. Following a 2-week recovery period, the mice were grafted with 1.5 cm^3 of human fat or saline. Revascularization was evaluated by Doppler scanning immediately before euthanasia 4 and 8 weeks after grafting. Results demonstrated significantly greater vascular flux in fat-grafted animals than saline-grafted animals at 4 weeks. Significant increases in vasculogenic proteins at 4 weeks as well as significant decreases in fibrotic markers at 8 weeks were observed. Although these findings must be reproduced in larger animal models, the murine burn model allowed demonstrating the potential role of fat grafting in wound healing, with notably accelerated revascularization and down-regulation of fibrotic pathways. One limitation of these small animal models is the relatively higher rate of mortality because of anesthetic overdosing or as a consequence of the burn lesion compared with pigs. The size of the lesion is thereby limited. Most of the aforementioned murine models are useful for evaluating burn pathophysiology or burn healing but have limitations for investigating burn scar formation. Actually, the rodent skin is different from the human skin and elevated hypertrophic scars do not develop. In fact, small animals have loose skin and an underlying layer of muscle (panniculus carnosus) that is responsible for wound contraction that is a major determinant of wound closure and contraction that is absent in humans.

THE HUMANIZED MURINE MODELS OF BURNS

Burn Model on Human Skin Grafts in Mice

Because of the limitations of the burn models in rodents, humanized murine models have been developed. The first model using human skin grafted onto the backs of nude mice was described in 1987.[39] With this model, the authors achieved contracture of meshed normal human skin grafts and hypertrophic scar formation in normal human skin that was burned one month after grafting. In 2012, this model was used to evaluate whether the application of silicone gel sheets modified with halofuginone as an antifibrotic agent can modify burn scar formation on full-thickness human skin grafts.[40] After informed consent, full thickness human skin was obtained from patients undergoing abdominoplasty or reduction mammoplasty. Subcutaneous fat was removed, and skin was trimmed into 1.5 × 1.5 cm grafts. Animals had a 1.5 × 1.5 cm wide full-thickness skin wound created on their backs on which a human full-thickness skin graft was applied. Finally, a compression dressing was applied and left until there was complete healing of the wound. In addition, Zeplin et al induced scarring through the controlled application of a partial-thickness burn injury on the full-thickness skin graft using a 10-second application of an 80°C heated copper template. After the grafts had healed and superficial healing of the wounds was complete, scar therapy began with the use of silicone gel sheets. Halofuginone-treated silicone sheets increased the antiscarring effect of silicone gel sheets by deceleration and down-regulation of scar formation.[40] Using this approach, a previous study reported the development of obvious and persistent hypertrophic scars in 90% of cases.[41] The model has been reevaluated to test the reproducibility of the formation of hypertrophic scars since it was not widely accepted.[41] After transplantation and survival of full thickness human skin grafts on the backs of nude mice, a deep second-degree burn was applied. After wound healing, hypertrophic scars similar to those found in humans developed. Obvious and persistent hypertrophic scars, which were red, hard, and elevated above the surrounding skin even 8 months after transplantation were observed. Histologic examinations revealed abundant collagen deposition and inflammatory infiltration in these scars. This model was recently used to evaluate the efficacy of a therapeutic approach.[42] The authors used intra-lesional injections of verapamil and triamcinolone and followed the animals for 4 weeks. They found that verapamil augmented decorin expression spatially correlated with collagen bundle formation.

Human Burn Hypertrophic Scars on Mice

Another burn hypertrophic scar model, using pieces of hypertrophic scars and keloids, was described in

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1989 by Kischer et al.[43] In this model, tissue specimens taken from hypertrophic scars of burn patients are implanted onto the backs of immunologically nude mice. Human hypertrophic scar explants can survive more than a year allowing additional experiments involving this scar. Microvascular anastomosis occurred between host and implant within the first several days and remodeling of the edges of the implant occurred very early. Robb and coauthors were also able to graft human hypertrophic scars, obtained from burn patients onto the mice.[39] However, the grafting technique was poorly described. Furthermore, the healing processes after explantation of human skin into subcutaneous pockets in mice would be expected to be different.[44] In 1998, Polo et al described an in vivo model with explanted human proliferative scars on flaps based on isolated vascular pedicles in congenitally athymic rats.[45] Using these methods, both fibroblastic and epithelial components of explanted scar specimens retained the histologic characteristics of original human scar specimens, for up to 12 months. Over the same time period scar explants continued to express high levels of human collagen type III, compared to those found in the original surgical specimens. The microvasculature of the scar explants demonstrated a double basement membrane, with no staining for human factor VIII in the inner capillary endothelial layer, suggesting that host vessels were growing into ghost vessels of the human donor scar. This model is the first to allow such long-term maintenance and serial evaluation of human proliferative scars on an accessible, isolated vasculature.

DISCUSSION

The objective of the present review was to describe current models of hypertrophic scars with a special focus on burn-related skin fibrosis leading to hypertrophic scarring, which represents most of the burn sequelae in clinical practice. While the variety of available models gives a wealth of valuable information about the pathophysiology and treatment of burn scars, the differences between models make comparisons between studies difficult. Although no consensus currently exists on the most relevant animal model of cutaneous burns, each model has advantages and disadvantages that should be considered. In this context, the validation of an animal model of burns as a reference and its recognition by the research community would be a major advance in the field.

Pre-clinical animal models are of a great importance both as a means for studying the evolution of hypertrophic scars and for evaluating therapeutic modalities. Several models of hypertrophic scarring were described; the most frequently used being the rabbit ear excisional wound model first described in 1997.[46] One of the advantages of the rabbit ear model is that the scars are indeed elevated. However, this model does not reflect burn injuries. While there is no rabbit model to study skin burn sequelae, multiple studies have described the development of pig models for hypertrophic scarring after burn injuries.

Although some studies have shown that the granulation tissue and the scar layers in the Yorkshire breed are much thinner than in the Duroc breed and that the thick scar persists for at least 46 weeks in the Duroc pig,[12–14] domestic pigs are most commonly used because of their low cost.[8,28,47] Furthermore, only excisional wounds have been studied in the red Duroc pig further limiting this model. While many burn models have been described, creation of deep burns is necessary to consistently result in hypertrophic scars. Of the domestic pig models described in the literature, the one used by Cuttle, in which a bottle containing boiling water is used to create burns, is the most common. Analyses have been performed more than 3 months. Although porcine models exhibit many advantages, the cost and difficulty of conducting experiments on these animals stimulated the development of small pre-clinical models of burn associated hypertrophic scarring.

Models of hypertrophic elevated scars in rodent skin do not exist, since rodent skin is very thin with poor collagen content, which prevents hypertrophic scarring. Additionally, rodent skin heals by contraction because of the underlying panniculus carnosus, unlike pigs and humans. One of the first models of hypertrophic scars was described after implantation of human keloidal tissue into subcutaneous pockets in athymic nude mouse.[43] However, in this model, the transplanted tissue was no longer covered with an epithelial layer, which in humans may play a role in the evolution of hypertrophic scars. After that, human hypertrophic scars obtained from humans were explanted onto nude mice. These models were highly reproducible resulting in scars that lasted for at least one year allowing additional experiments to be conducted with them.[45]

Also, models of hypertrophic scarring after burning of human skin grafted in immunodeficient mice have been successfully developed with persistent hypertrophic scar 8 months after skin transplantation. In these models, it has been reported that shedding of the epidermis and upper portions of the dermis of the human full thickness skin grafts in mice not subjected to burn insults may limit their applicability.[41] The causes of the shedding of the

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Table 1. Representative animals models of hypertrophic scars

Animal Species	Model	Depth of Injury	End Point	Ref.
Rabbit ear	Skin excision	Deep	Hypertrophic scar	46
Red Duroc pig	Skin excision	Partial thickness wound	The scars are still thick at 46 wk post wounding further validating the model	12
Red Duroc pig	Skin excision	Varying depth	Nerve tissue is increased in female red Duroc pig scar tissue and is quite similar to that in human hypertrophic scars and to that described in the literature	13
Red Duroc pig	Skin excision	Deep wounds	Healing in the female, red Duroc pig is sufficiently similar to human hypertrophic scarring to warrant further study so that it can be accepted or rejected as a model of human hypertrophic scarring	14
Large white pig model	Heated water at 92°C in a glass bottle applied for 15 sec	Partial thickness burn	Earlier re-epithelialization and thinner scar tissue in caudal than in cranial burns	16
			Burn scar contraction is correlated with the level of alpha-smooth muscle actin expression	18
			Immediate debridement followed by an appropriate dressing and without skin grafting may promote wound healing	20
	300 ml of heated water from a kettle at 92°C for 20 sec	Deep dermal burn	Early grafting of deep dermal burns may reduces hypertrophic scarring	22
Domestic pig	Aluminum bar preheated to 80°C applied for 20 sec	Partial thickness burn	TGF-beta antagonist speeds re-epithelialization and reduces scar formation and wound contraction	23,24
Yorkshire pig	Heated brass contact injury at 100°C for 20 sec	Deep partial thickness burn	Skin substitutes resulted in less contraction, more vascularization, and improved epidermal formation	26
Large white pig	Radiant heat	Deep partial thickness burn	Hourglass contracted scars in two of three burns were obtained within 1 mo	27
Wild-type FVB mice (Friend leukemia Virus B)	Brass rods were heated to 100°C in a hot water applied 10 sec	Full-thickness injury	Fat grafting reduced on scar fibrosis	38
Nude mice	Partial-thickness burn injury on human full-thickness skin grafts using a 10-second application of an 80°C heated 1 cm^2 copper template	Partial-thickness burn injury	Reduction of burn scar formation by halofuginone-eluting silicone gel sheets	39,40
Nude mice	Implanted excised human hypertrophic scars fragments from surgically treated burned patients	No lesion	Both fibroblastic and epithelial components of explanted scar specimens retained the histologic characteristics of original human scar specimens, for up to 12 mo	44,45

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epidermis and upper portions of the dermis of the human skin graft are complex, possibly involving the following factors: 1) strong antigenicity of the skin; 2) remnant T cells in nude mice; or 3) the enhanced compensatory rejection independent of T cells in nude mice. But such results may therefore suggest that hypertrophic scarring may not be because of the burn injury itself but rather as a result of failure of the skin transplant. More investigations need to be done for understanding the mechanisms of human tissue transplant in nude mice. One question still remains incompletely answered: does creating a burn on explanted human skin graft induce hypertrophic scarring?

CONCLUSION

A variety of hypertrophic scar animal models have been described after burn lesions (Table 1); none of which being totally satisfactory. The most frequently used model is the rabbit ear excisional wound model, which does not reflect burn injuries. The red Duroc pig seems to be the more relevant model of human hypertrophic scars but its high cost has led to more frequent use of the domestic pig. One of the disadvantages of these models is that scars remodel and regress over time. Furthermore, only excisional wounds have been studied in the red Duroc pig. Although working with small animals is more technically challenging, and because of their sensitivity to sedatives and anesthetics results in higher mortality rates than in larger animals, models using explanted human scar tissue or skin grafts onto immunologically nude mice offer some advantages and often persist for at least 1 year.

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