Fetal Airway Wound Repair

A New Frontier

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Purpose: Fetal dermal repair is regenerative and scarless until middle to late gestation, when there is a transition to fibrotic repair. Fetal skeletal muscle and tendon undergo repair with fibrosis similar to the process in adults. This study addresses whether fetal mucosal healing is regenerative and scarless.

Methods: Anesthetized pregnant rabbits underwent laparotomy and controlled hysterotomy at 21 to 23 days' gestation (term is 31 days). A midline thyrotomy was made, followed by cricoidotomy and circumferential cauterization of the subglottic mucosa. A similar insult was applied to weanlings. The data were collected in 2 groups. One group was followed to term and killed at 4 weeks. A second group was killed after 6 days (30 days' gestation). The weanlings were killed at similar points. The larynges were harvested and processed for histological and morphometric analysis.

Results: Three litters were followed to term. Of these, 1 was not recovered; in the other two, 7 of 8 manipulated fetuses were found and 3 of 8 were viable. The fourth litter was harvested after 6 days; all 4 injured fetuses were recovered and viable. All animals in the fetal injury groups healed with complete regeneration of the airway mucosa. In contrast, weanlings injured post partum had mucosal inflammation, necrosis, and ulceration; squamous metaplasia and basal cell hyperplasia were also found. There were fibrosis, granulation tissue, and inflammation in the lamina propria; chondritis, cartilaginous necrosis, chondrolysis, and perichondritis were also found.

Conclusions: Fetal airway mucosal healing is regenerative and, thus, scarless. This study provides further support for the thesis that skin and mucosa respond to injury similarly in both the developmental and postpartum stages, and that subglottic stenosis is reasonably thought of as the “hyperplastic scar” of the airway. These results have potential therapeutic applications for mucosal wound management.


SCARRING IS an important medical problem that may lead to poor cosmesis, structure, function, and growth. Otology, laryngology, and bronchoesophagology all have mucosa as their primary building block. Aberrant mucosal wound healing in the middle ear results in conductive hearing loss, eustachian tube dysfunction, adhesive otitis media, tympanosclerosis, and other abnormalities. In the airway, the consequences of scarring are potentially more devastating. The development of nasal, nasopharyngeal, laryngotracheal, and esophageal stenosis may be life threatening. Subglottic stenosis in children, for example, has become increasingly prevalent during the past quarter century because of widespread use of prolonged endotracheal intubation for respiratory support.1 Mortality rates as high as 24%, directly attributable to the tracheostomy in these children, have been reported.2 Not life threatening but, nonetheless, a priority to otolaryngologists are the perturbations in speech and communication caused by laryngeal scarring. Prevention or reduction of scarring remains a major goal of otolaryngologists.

Scarless healing of fetal wounds is the most dramatic observation in modern wound healing research. Although it has long been recognized that wound healing is an age-dependent process, a drastically different repair process has been observed in the fetus.3 Healed fetal skin wounds may be clinically and histologically indistinguishable from unwounded tissue.3 Conversely, healed adult skin wounds are cosmetically and physically different from the nascent tissue from which they are derived.

Despite these remarkable differences in fetal and adult dermal repair, the healing processes in other tissues, such as...
Four pregnant and 10 adult nonpregnant Pasturella-free, New Zealand white rabbits (aged 3–8 weeks) were obtained (Myrtle’s Rabbitry Inc, Thompson Station, Tenn, or Green Meadows Co, Murrysville, Pa). All animals were examined and those not in good overall general health were excluded from the study. All procedures complied with the guidelines of the Animal Research and Care Committee at the Children’s Hospital of Pittsburgh, Pittsburgh, Pa.

The animals were kept 3 to 6 days before surgery to permit adaptation to their new environment in nesting box cages 122×41×61 cm in a quiet area. These measures were believed to decrease the rate of spontaneous abortion.6 Animals were fed rabbit chow (Carnation, Wayne Pet Food Division, Continental Grain Company, Chicago, Ill) and were given water ad libitum. Prophylactic ampicillin (1 mg intramuscular once daily) was administered preoperatively and continued until delivery or cesarean section.

Anesthesia was induced with ketamine hydrochloride (Ketalar, Parke-Davis Co, Morris Plains, NJ) in a loading dose of 35 mg/kg and xylazine hydrochloride (Rompun, Haver-Lockhart, Miles Laboratories, Shawnee, Kan) at 5 mg/kg. Maintenance anesthesia consisted of halothane (1%-1.5%) with oxygen delivered by spontaneous mask ventilation at a rate of 1 L/min, and was supplemented with locally infiltrated 2% lidocaine (Xylocaine, Astra USA Inc, Westboro, Mass).

The animals were divided into 2 groups; the pregnant rabbits (n=4; 32 fetuses, 16 manipulated) comprised the experimental group and the nonpregnant adults (n=10) served as the controls.

The control animals were prepared by clipping the midline neck hair and painting the skin with povidone-iodine. A shoulder roll was then placed to extend the neck. By aseptic technique, a vertical midline neck incision was made. The subglottis was entered via a midline cricoid-diameter incision. The specimens were embedded in paraffin, cut into cross sections, and stained with hematoxylin-eosin. Histological examination was completed by a pathologist (E.C.K.) according to the algorithm outlined above. The adult controls were killed at similar points. The larynges were harvested and processed for routine histological examination. The specimens were embedded in paraffin, cut into cross sections, and stained with hematoxylin-eosin. Histological analysis was completed by a pathologist (E.C.K.) who was unaware of the experimental group.

The mortality for the experimental fetal rabbit surgery arm of the study was as follows. One of the 3 litters (4 manipulated fetuses) was never delivered. Of the remaining 8 manipulated fetuses in the other 2 litters brought to term, 3 survived, although only 7 of the 8 fetuses were found. All 4 of the manipulated fetuses harvested from the final litter by a second laparotomy on the sixth postoperative day were viable. There was no maternal mortality in this study. Regeneration of the airway mucosa was complete in both fetal groups by gross and microscopic examination (Figure 1, left, and Figure 2, left). The only abnormality identified was the incomplete closure of the criciodotomy. This was in striking contrast to the airways injured post partum, which showed marked stenosis (Figure 1, right, and Figure 2, right). Within the mucosa, inflammation, necrosis, ulceration, squamous metaplasia, and basal cell hyperplasia were observed histologically. Within the lamina propria, fibrosis, granulation tissue, and inflammation were identified as prominent features. Finally, in the cartilage, chondritis, necrosis, chondrolysis, and perichondritis were all seen.

For purposes of comparison, a section of uninjured fetal subglottic mucosa was harvested and examined histologically. As is generally the case with fetal tissue,
the overall architecture was similar to that of adult airway mucosa (Figure 3, left). The fetal tissue, however, was markedly more cellular, with less definitive maturation of mesenchymal and stromal components (Figure 3). The epithelium was arranged in a pseudostratified pattern, with cells manifesting a low columnar shape (Figure 3, right). Dark, round to ovoid nuclei composed of evenly disseminated, finely granular heterochromatin were present throughout all levels of the mucosa, but a prominent basilar orientation was evident. Large, clear vacuolar spaces consistent with goblet cells containing mucinous glycoprotein material were frequently noted. Although a distinct ciliated mucosal “brush” border was not visualized, many cells had evident multiple apical blebs, creating a somewhat scalloped appearance. A somewhat poorly defined lamina propria was present subadjacent to the epithelium. This layer was composed of small, dark elongate nuclei within a scanty fibrillar matrix.

The primary thickness of the tracheal wall consisted of focally extensive areas forming the developing cartilaginous plates (Figure 3, left). Closely packed polygonal mesenchymal cells with discrete cellular margins and abundant light-gray, fibrillar-appearing cytoplasm composed these early structures. Mitotic activity was clearly visible within this population. A definitive
band of elongate immature smooth muscle cells formed the trachealis muscle, connecting the developing cartilaginous plates. Around the outer circumference of the airway, a layer of 4 to 6 thin, spindle-shaped cells formed the adventitial surface.

The subglottic areas of the fetal, newborn, and weanling controls along with those of the injured counterparts are listed in the Table. The mean (± SD) fetal subglottic diameter measured 0.11±0.03 mm². The mean subglottic area in those animals injured in utero and killed at 8 weeks of age was 5.80±0.74 mm², with 0% mortality from the injury, compared with 4.70±1.83 mm², with 50% mortality from the injury, for age-matched animals wounded at 4 weeks post partum. The mean subglottic area for age-matched controls was 8.97±1.15 mm². There was no mortality and no discernable morbidity in the group of animals injured in utero, and development and growth appeared to continue normally; however, the luminal area was smaller than that of age-matched controls (P=.01) and not significantly different from the measured luminal airways of the survivors injured 4 weeks post partum (P=.25; Student 1-tailed, unpaired t test).

**COMMENT**

To our knowledge, this is the first experimental model of fetal airway healing, and this model offers a number of beneficial components. Fetal rabbit size and their 31-day gestation consistently allow early third-trimester intervention. We, like others, found that unless the manipulated fetuses are retrieved early (shortly before or immediately after delivery), they are often unable to be found, presumably because of maternal cannibalism. Nonetheless, we were able to retrieve 4 of 8 manipulated rabbits after delivery. We believed, however, that this retrieval rate was unacceptably low, so we delivered the remainder of the animals by cesarean section and intend to use this method in the future.

Most studies of fetal repair have concentrated on the skin. Although early-gestation skin wounds may heal without scarring, incisional wounds made on similarly aged fetal lamb diaphragms heal with scar and without muscle regeneration. This scarring has been shown to be caused by factors other than loss of exposure to amniotic fluid, since marsupialization of the wounded diaphragm and continuous bathing in amniotic fluid does not alter the scarring phenotype. Likewise, gastric wounds in fetal lambs were found to heal with scarring. Fetal lamb bone fractures heal with minimal callus formation and with both intramembranous and endochondral ossification. In fetal rabbits, intestinal repair was found to occur with adhesions, fibrosis, and neovascularization. To our knowledge, this is the first reported investigation of fetal healing in airway mucosa. Although we found that healing occurs in a regenerative rather than a reparative fashion without scar, we studied only 1 point in gestation, namely, 21 to 23 days (term is 31 days). Because different organ systems probably have different windows of gestational age during which healing is scarless, injury at a different point in gestation may yield a different outcome.

Another issue not explored in this study was whether a different scarring phenotype might be found in fetal rabbits of comparable gestational age that are not from an inbred population and, thus, are more genetically dissimilar. Most laboratory animals, such as rabbits and mice, are procured from inbred populations. Sheep, pigs, and opossums, like humans, tend to be more outbred. Studies of the transition from scarless to scar-forming healing in the marsupial, the opossum (Monodelphis domestica), have shown considerable variation in the time of transition and the extent of scarring. If such individual variations exist, they would be important in several ways. First, they could be exploited to investigate causative features in the scarring phenotype, such as inflammatory and cytokine phenotype correlations. Second, defining different populations of animals may permit one to map and possibly pave the road to gene therapy in those genetically predisposed to excessive scarring. Third, if cell therapy is to be considered as a possible prophylactic or therapeutic approach to reconstructive attempts in subjects who form hypertrophic scars or keloids, defining the phenotype of the fibroblasts as scar formers or not is important before an experimental trial.

We were somewhat surprised that airway injury healed without scar because there are a number of structural and environmental differences between fetal integument and fetal airway. Most fundamental are the different derivations of these 2 structures. Fetal skin is derived from ectoderm and mesoderm and has a unique epidermal-dermal architecture. In contrast, the airway is derived from the endodermal lining of the laryngotracheal groove. The cartilage, connective tissue, and muscle are derived from the surrounding splanchnic mesenchyme. Furthermore, unlike integument, the airway is not continually bathed in amniotic fluid. Yet, despite these differences, healing between these 2 structures was similar. This reinforces the point shown by others that amniotic fluid does not account for scarless fetal healing. It further demonstrates that tissue derived from any of the 3 primary embryological germ layers (ectoderm, mesoderm, or endoderm) may heal in a regenerative fashion without scar. Although Longaker et al found that the muscle of fetal lamb diaphragm healed with scar, others found that oral mucosa wounds in the fetal rat regenerated the muscle of the cheek 24 to 72 hours after wounding. Not unexpectedly, the muscle of the airway responded to injury like that of the oral cavity rather than

**Cross-sectional Areas of Subglottic Lumens**

<table>
<thead>
<tr>
<th>Group</th>
<th>Area, mm²</th>
<th>n</th>
<th>Mean±SD</th>
<th>P vs Group 2</th>
<th>P vs Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Injured in utero at 21 d of gestation, killed 6 wk post partum</td>
<td>2</td>
<td>5.80±0.74</td>
<td>.25</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>2. Injured post partum at 4 wk, killed 1-2 wk later</td>
<td>3</td>
<td>4.69±1.83</td>
<td>. . .</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>3. Age-matched controls</td>
<td>4</td>
<td>8.97±1.15</td>
<td>. . .</td>
<td>. . .</td>
<td></td>
</tr>
<tr>
<td>4. 21-d fetal controls</td>
<td>3</td>
<td>0.11±0.03</td>
<td>. . .</td>
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<td></td>
</tr>
</tbody>
</table>

* Ellipses indicate data not applicable.

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like that of the diaphragm. Alternatively, these observed differences may be attributable to such factors as differences in the models or gestation times when the injury occurred. These questions require further study.

Despite these structural and environmental differences between skin and airway mucosa, however, these results do support the findings in the control group and those of a recent study (J. E. Dohar, MD, E. C. Klein, DVM, J. L. Betsch, and P. A. Hebda, PhD, unpublished data, September 1997) that demonstrated that the adult mucosal response to injury is similar to that of the skin. Specifically, the mucosa, analogous to the epidermis in the skin, regenerated to its original structure after insult via cauterity. This was in striking contrast to the connective tissue, namely the lamina propria, which (again, analogous to the dermis in skin) healed by way of a reparative (vs regenerative) fibrotic process. Depth of injury rather than circumferential extent of injury was most predictive of the ultimate healing outcome, ie, the deeper injuries resulted in greater stenosis of the subglottis (J. E. Dohar, MD, E. C. Klein, DVM, J. L. Betsch, and P. A. Hebda, PhD, unpublished data, September 1997). Likewise, it has long been accepted in skin that deeper dermal injuries heal with more scar. With so many similarities between the responses to injury of skin and airway, it is intuitively logical to accept the observation in this study that, like skin, fetal airway injuries heal without scar. This analogy between skin and airway healing may have significant therapeutic implications, since so much more is known about wound healing in the skin.14 It is reasonable, therefore, to expect that some of the therapeutic modalities effective in managing skin wounds might be likewise effective in mucosa.

Although there was no histological evidence of scarring in the animals injured as fetuses, their subglottic areas as measured by image analysis were smaller than those of age-matched controls. The sample size in this pilot study was small, and this may in part account for this observation. An additional explanation might be that, although healing occurred in a regenerative rather than a reparative fashion, a growth center for the cricoid was disrupted by the cricoidotomy, accounting for the ultimate decrease in subglottic/luminal area. The decreased area, although measurable different, did not appear to derive from mucosal thickening (as seen in Figure 2, left). Additionally, it was of no functional importance in that none of the animals experienced any airway compromise. Further study is necessary to understand this observation more fully.

Although there continues to be much debate surrounding the exact mechanisms underlying a scarless fetal wound healing in the skin, some have suggested that the differences between the fetal and adult wound environments may play a crucial role.15 One major extrinsic difference is that fetal skin wounds are continually bathed in warm, sterile amniotic fluid. This theory may be relevant to the management of mucosal wounds of the airway. Occlusive dressings, although not able to recreate the intrauterine environment, can nonetheless retain heat and moisture to a greater extent than nonocclusive dressings or no dressing at all. It has long been accepted that cutaneous wounds dressed occlusively heal better than those that are not so dressed.16 The effects of dressings on mucosal wounds of the airway have not been studied. Clearly, this would be a fairly simple intervention were it shown to be beneficial, and more study is warranted.

As in fetal dermis, fetal airway mucosal healing is regenerative and, thus, scarless. This provides further support for the thesis that skin and mucosa respond to injury similarly in both the fetus and the adult and that subglottic stenosis in the adult airway is reasonably thought of as the “hyperplastic scar” of the airway. Future study should be directed toward substantiating this claim. If it is true, insight might be provided into the application to subglottic stenosis of therapies shown to be effective for these disorders in the skin. Also, studies should explore the differences, at a molecular level, between the fetal regeneration and the adult repair, again in the hope of discovering novel therapies for this condition.

Accepted for publication June 30, 1997.

This work was supported in part by a Children’s Hospital of Pittsburgh Faculty Start-up Grant. Illustrations and line drawings were done by James A. Rosendale.

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REFERENCES