Effect of Production of Multiple Keloids on Health of the Animal

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Abstract
Keloids are fibrous tumors developed in the process of wound healing. Keloids are found solely in human species, and not in animals. Keloid animal model has been developed by the authors. Now authors want to investigate if multiple keloids can be produced in one animal without causing any untoward effects on the health of that animal.

Objective: To find out effect of production of multiple keloids on health of the animal.

Methodology: the study was Quasi-experimental trial and settings were Experimental Research/Pathology Laboratories, University of Health Sciences, Lahore. The duration was 12 months. Two rabbits were taken; one as control, and other as interventional. In interventional rabbit eight keloids were produced, four on each ear, using Mannan & Hannan technique.

Results: Eight keloids were produced in one animal without causing any untoward effects on the health of that animal.

Conclusion: Multiple keloids can be produced in one animal without causing any untoward effects on the health of that animal.

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Keywords | Cicatrix; Hypertrophic; Keloid; Models, Animal; Rabbit; Transforming Growth Factor beta 1.

Introduction
Keloids are fibrous tumors developed in the process of wound healing. Keloids are in fact benign tumors of dermis of skin. Hypertrophic scars are of somewhat similar pathology, but they do not erode to adjacent structures². Keloids form as a consequence of trauma. They may occur directly after trauma, or may be deferred for a long time. Keloids are of different sizes, and minor injuries can lead to big keloids in some people³⁴. True cause of occurrence of this disease is not recognized until now. As such, some causes are supposed to be probable explanations. These factors may be inside or outside the human beings⁵⁶.

Keloids are found solely in human species, and not in animals. This is the principal obstacle against planned inquiry concerning keloids, as research on human beings is not feasible, and not appropriate. Studies on keloids have routinely been performed on patients, with low level of scientific evidence. No practicable animal model of this condition has been produced so far. The authors have invented a new technique of production of rabbit ear keloid model, called Mannan and Hannan Technique, in which a pro-fibrotic growth factor, called Transforming Growth Factor Beta 1 is used⁷⁸.
Transforming Growth Factor Beta 1 plays an important role in healing of wounds and formation of normal and abnormal scars; it is a potent chemotactic agent for fibroblasts, and stimulates them to manufacture most important extracellular matrix components like collagen. Transforming Growth Factor Beta 1 itself is manufactured by macrophages, and also by extracellular matrix, thereby producing a vicious cycle. Transforming Growth Factor Beta 1 produces its effect by stimulation of fibroblasts, thereby causing increased collagen assembly. Transforming Growth Factor Beta 1 itself is manufactured in larger quantities in keloids, and keloid fibroblasts react in response to lower levels of Transforming Growth Factor Beta 1 than normal skin fibroblasts. Addition of extraneous Transforming Growth Factor Beta 1 activates fibroblasts, thus escalating fabrication of collagen, and development of keloids.

Use of animals in experimental studies is a major concern with most of the research institutions all over the world. This involves many ethical problems, and need of humane animal research cannot be underemphasized. Improving animal welfare is on international agenda for last half a century. Regarding this, 3R (Replacement, Reduction and Refinement) principles of laboratory animal research were developed by Russell & Burch in 1959, and are now gold standard all over the world. Further, animal husbandry is time-, manpower-, and finance-intensive, and consumes bulk of resources of experimental research laboratories. For research involving internal systems of an organism, whole animal is required. But when research involves only local tissue of an animal, especially skin, multiple lesions can be studied in one animal. In this way one animal can act as multiple subjects.

Previously, most of the animal studies have produced one hypertrophic scar/keloid per animal. This has been due to fears about morbidity and mortality of the animals. Diao et al., in 2013 produced four hypertrophic scars on each ear of rabbit. Xiao and Xi in 2013 produced three hypertrophic scars on each ear of rabbit. Wang et al., in 2015 produced six hypertrophic scars on each ear of rabbit. Multiple hypertrophic scar models have not been reported in other animals. In the present study eight keloids have been produced in one animal.

The objective of this study is to find out effect of production of multiple keloids on health of the animal. The authors want to investigate if multiple keloids can be produced in one animal without causing any untoward effects on the health of that animal.

Methods

It was a quasi experimental trial, carried out at Experimental Research and Pathology Laboratories, University of Health Sciences, Lahore, Pakistan. Its duration was twelve months, from May 2011 to April 2012. Sample consisted of two New Zealand White rabbits. Sampling was done by simple convenience technique.

Approval was taken from institutional Ethical Review Committee. Universal protection was used i.e. surgical gown, surgical cap, surgical mask, surgical goggles, examination gloves, and surgical shoe covers. Surgical instruments were sterilized in hot air sterilizer at 160°C for 90 minutes. Surgeon washed-up with alcoholic surgical hand disinfectant, and wore surgical gloves.

Two rabbits were taken; one as control, and other as interventional. Interventional rabbit was anaesthetized with Ketamine injection (75 mg kg⁻¹ intraperitoneal), and Xylazine injection (15 mg kg⁻¹ intraperitoneal). Rabbit ear was sanitized with sterilized saline gauze and ethyl alcohol swab. Proposed point of keloid was marked on rabbit ear with gentian violet and Castroviejo calliper. In interventional rabbit eight keloids were produced, four on each ear, using Mannan & Hannan technique. Sites of keloids were as follows:

1. Right Ear Ventral Surface; Caudo-Proximal
2. Caudo-Distal
3. Cranio-Distal
4. Cranio-Proximal
5. Left Ear Ventral Surface; Caudo-Proximal
6. Caudo-Distal
7. Cranio-Distal
8. Cranio-Proximal

If bleeding occurred, it was controlled with mosquito artery forceps. If inadvertently dorsal skin was injured, it was stitched with 4/0 surgical silk. General
post-operative care was given. Analgesics were not required. Antibiotics were not used. General wound care was provided. If stitches had been applied, they were removed at one week. Dressing was permitted to drop itself. Keloids were examined at twenty eight days.

Results

Eight keloids were produced in one rabbit without causing any untoward effect on the health of that animal. Scar elevation index was 3.45 at 28 days, and 2.17 at 365 days. The animal was kept under observation regarding signs of discomfort, distress, stress, and suffering. These include pain, fever, anorexia, insomnia, irritability, aggressiveness, and others (Table 1). No change in behavior of our animal was observed (Table 2). No analgesics or antibiotics were needed. Special observation continued for four weeks, and animal remained well during this time.

Table 1: Signs of Distress in Laboratory Animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Posture</th>
<th>Voice</th>
<th>Temperament</th>
<th>Locomotion</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Dormouse</td>
<td>Squeal</td>
<td>Docile</td>
<td>---</td>
<td>Eats Bedding</td>
</tr>
<tr>
<td>Rat</td>
<td>Dormouse</td>
<td>Squeal</td>
<td>Docile</td>
<td>---</td>
<td>Eats Bedding</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td></td>
<td></td>
<td>Quiet</td>
<td>Drags</td>
<td>No Spillage of Food / Water</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Hiding</td>
<td>Squeal</td>
<td>Dozey [sic]</td>
<td>---</td>
<td>No Spillage of Food / Water</td>
</tr>
</tbody>
</table>

Adapted from Morton & Griffiths (1985)

Table 2: Signs of Distress Specific to Rabbits in our Study

<table>
<thead>
<tr>
<th>Sr No</th>
<th>Sign</th>
<th>Yes or No</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eyes open widely</td>
<td>No</td>
<td>No proptosis</td>
</tr>
<tr>
<td>2</td>
<td>Body becomes tense, tail moving up</td>
<td>No</td>
<td>Body relaxed</td>
</tr>
<tr>
<td>3</td>
<td>Ears are laid back tightly</td>
<td>No</td>
<td>Ears elevated</td>
</tr>
<tr>
<td>4</td>
<td>Animal growls or squeaks</td>
<td>No</td>
<td>No squeaking</td>
</tr>
<tr>
<td>5</td>
<td>Animal pushes hand away on touching</td>
<td>No</td>
<td>Cooperative</td>
</tr>
<tr>
<td>6</td>
<td>Animal lacks vitality or interest</td>
<td>No</td>
<td>Animal active</td>
</tr>
<tr>
<td>7</td>
<td>Animal flinches when touched</td>
<td>No</td>
<td>No flinching</td>
</tr>
<tr>
<td>8</td>
<td>Animal breathing becomes rapid</td>
<td>No</td>
<td>Breathing normal</td>
</tr>
<tr>
<td>9</td>
<td>Animal starts biting</td>
<td>No</td>
<td>No biting</td>
</tr>
</tbody>
</table>

Adapted from University of Minnesota Center to Study Human Animal Relationship and Environments (2016)

Keloids produced were considerable on examination. They were of solid consistency. They were of round shape, and their colour was red. They had a smooth surface, and were hairless. Keloids were present even after twelvemonths of follow-up (Figure 1).

Figure 1: Multiple Keloids produced in one Rabbit

On gross examination, keloids were hard in consistency, and were palpated as discrete nodules. They were of red colour and their surface was smooth. They had a gritty feeling on cutting. On cut section, their surface was pale in colour. On microscopic examination, keloids showed glassy, hyalinized, homogeneous, brilliantly eosinophilic collagen. They contained a few fibroblasts, lying in direction of collagen (Figure 2).

Figure 2: Microscopic View of Keloid with Periostin stain

Discussion

The principles of the 3Rs (Replacement, Reduction and Refinement) were developed over half a century ago, as an agenda for humane animal research. These principles are now included in many national and...
international regulations controlling the use of animals in scientific research. Replacement relates to methods to replace animal use with non-animal techniques. Reduction implies approaches to decrease number of animals utilized in one trial. Refinement means ways to reduce distress of animals. Reduction is especially relevant in this study as more information can be obtained from less number of animals, thereby decreasing number of animals used.

There are multiple signs of stress or distress in animals. These signs include diarrhea, inappropriate micturition, increased salivation, decreased salivation, sneezing, coughing, shivering, shaking, restlessness, listlessness, agitation, biting, hiding, and yawning. Signs of distress specific to rabbits include the following: eyes open widely so that sclera becomes visible; body becomes tense with tail moving up; ears are laid back tightly; animal growls or squeaks; animal pushes hand away; animal lacks vitality or interest; animal flinches when touched; animal breathing becomes rapid; animal starts biting. No sign of stress or distress was noted in rabbits in this study.

Hypertrophic scar animal models are usually produced by making one wound per animal. This has been due to fears about morbidity and mortality of the animals. Diao et al., in 2013 produced four hypertrophic scars on each ear of rabbit. They made 10 mm circular excisions of full thickness skin with scalpel blade. They left the wounds exposed to air. They had scar elevation index of 2.07 at 45 days. They did not report any untoward effects on rabbit.

Xiao and Xi in 2013 produced three hypertrophic scars on each ear of rabbit. They used 5 mm biopsy punch, and excised skin and perichondrium. They used polyurethane dressing. They had scar elevation index of 1.73 at 14 days. No side effects were reported on health of animal.

Wang et al. in 2015 produced six hypertrophic scars on each ear of rabbit. They used 7 mm biopsy punch, and excised skin and perichondrium. They had scar elevation index of 2.93 at 21 days. They were no adverse effects reported on health of animal.

In the present study eight keloids have been produced in one animal. Our scar elevation index was 3.45 at 28 days, which is higher than all other studies. Our scar elevation index was 2.17 at 365 days, which has not been reported in other studies. Comparison of our study with previous ones is shown in Table 3. Multiple hypertrophic scar models have not been reported in other animals.

This study will open a further avenue for animal (and human also) research on treatment of keloids in future. This study will also reduce the use of laboratory animals in medical research. No doubt this is a small study, but in future it will lead to bigger studies on laboratory animal research, as well as on keloid therapy. This study also raises the question whether it can be replicated in other laboratory animals like Guinea pigs; only more research will answer this question.

### Table 3: Comparison of Different Studies of Multiple Keloids

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Size</td>
<td>8 mm</td>
<td>10 mm</td>
<td>5 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>Injection</td>
<td>TGFB1</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Instrument</td>
<td>Biopsy Punch</td>
<td>Scalpel Blade</td>
<td>NR</td>
<td>Biopsy Punch</td>
</tr>
<tr>
<td>Tissues Excised</td>
<td>Skin, Ventral Perichondrium, Cartilage, Dorsal Perichondrium</td>
<td>Skin , Skin , Perichondrium</td>
<td>Skin , Perichondrium</td>
<td>Skin, Perichondrium</td>
</tr>
<tr>
<td>Dressing</td>
<td>Hydrocolloid</td>
<td>Nil</td>
<td>Polyurethane</td>
<td>Nil</td>
</tr>
<tr>
<td>Time</td>
<td>28 days</td>
<td>45 days</td>
<td>14 days</td>
<td>21 days</td>
</tr>
<tr>
<td>Scar Elevation Index at 28 Days</td>
<td>3.45</td>
<td>2.07</td>
<td>1.73</td>
<td>2.93</td>
</tr>
<tr>
<td>Untoward Effects</td>
<td>Nil</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Duration</td>
<td>365 days</td>
<td>NR</td>
<td>180 days</td>
<td>NR</td>
</tr>
<tr>
<td>Scar Elevation Index at 365 Days</td>
<td>2.17</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR = Not Reported
TGFB1 = Transforming Growth Factor Beta 1

### Conclusion

Multiple keloids can be produced in one animal without causing any untoward effects on the health of that animal.
Ethical Approval: Given
Conflict of Interest: None
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15. NC3Rs. The 3Rs. Retrieved 08.09.2016, from https://www.nc3rs.org.uk/the-3rs.