Histological Changes and Testicular Dysfunction in Severely Burned Rats

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Abstract

Background: The immediate and early sequelae of burns such as vascular collapse and wound sepsis have been extensively studied. Some studies now show that burns cause significant changes in most body systems including the male reproductive system. Only few studies have addressed the reproductive consequences of burns in the male however.

Objective: To delineate histologic changes induced in rat testes exposed to major burns.

Setting: Medical School Anatomy department.

Design: Experimental animal study.

Materials & Methods: We induced 3rd degree burns in Wistar rats equivalent to 40% of total body surface area and observed them over several weeks. They were sacrificed at 8 and 16 weeks.

Main Outcome Measures: Epididymal sperm parameters, serum FSH, LH and testosterone (T) were measured. A semi-quantitative evaluation of histo-pathological changes in the testis was also carried out.

Results: Burns caused significant reduction in all sperm parameters (p<0.05). FSH, LH and T were also significantly reduced at 8weeks. By 16 weeks however, only T was still reduced. The key histologic change was germ cell atrophy. In cases with chronic wounds, sloughing left only basal cells such as spermatogonia and Sertoli cells in many tubules.

Conclusion: Burns impair spermatogenesis and damages testicular histology in rats.

Introduction

Burns cause coagulative necrosis of skin due to the dissipation of thermal energy into the tissue. Most burns are classified as minor – involving less than 20% of the total body surface area (TBSA). Burns still occur frequently in many parts of the world, though preventive interventions have significantly reduced burn prevalence in the developed nations [1, 2]. Thermal injury elicits a response in almost all body systems. The immediate response occurs in the vicinity of the burns and involves vascular permeability changes that lead to fluid and colloid loss. In the subsequent days, patho-physiologic changes occur in several body systems including the cardiovascular, [3] neuroendocrine [4] and immunologic systems [5, 6].

Several studies now indicate that testicular dam-
age and impaired spermatogenesis are important aspects of burn sequelae. The effects are affected by the severity of thermal injury [7-9]. The problems of fluid loss, shock, and wound treatment have dominated burn research for decades because they are more apparent and life threatening. Attention has however focused lately on subtle metabolic changes and effects on the male reproductive system. In the process, interesting findings have been made. It has been shown for example that severe burns cause prolonged changes in the body metabolism associated with tissue wasting, bone and muscle loss and a negative nitrogen balance [10].

Studies of these types are to be encouraged because despite the growing evidence from human and animal studies that severe thermal injury can produce significant anti-fertility effects in males, the mechanisms of these effects are poorly understood and detailed studies of the testicular histology in this condition were not found in the reviewed literature. Most studies of histologic changes in the testes of burned men are from autopsy specimens [9]. There is a natural reluctance to obtaining testicular tissue from burn survivors because this will require an invasive process on the testis with an infection risk. We therefore carried out a detailed characterization of histologic changes in Wistar rats exposed to thermal injury. The injuries involved a broad range of severity including cases with chronic wounds and delayed healing.

### Materials and Methods

**Animals and treatment**

Twenty eight mature Wistar rats weighing 280-350 g were obtained from the animal center of the College of Medicine, University of Lagos, and Ladoke Akintola University, Ogbomosho for the studies. They were kept in a well aerated room with hygienic surroundings and with equal and natural light: darkness rhythm. They were fed with food from Livestock Feeds Company and clean tap water was provided ad-libitum. The study design was approved by our College’s Committee for the Care and use of experimental animals.

They were divided into 4 groups of 7 rats each. Group 1 was control and were subjected to sham burn. Group 2 animals were exposed to the scalding liquid for 10 seconds for normal healing. Group 3 animals were treated as in group 2, but with the procedure varied to produce longer lasting wounds, as shown bellow, and were observed for 8 weeks. Group 4 animals were treated in the same way as Group 3, but observed for 16 weeks. At the end of these experimental periods the animals were sacrificed.

**Induction of burn injury**

Each animal was anesthetized with an intra-peritoneal injection of ketamine hydrochloride (20 mg/kbw) and diazepam (0.1 mg/kbw). The skin over the entire dorsum of the rat was dabbed with a liquid containing methyl alcohol and chlohexidine, and shaved.

Burn injury was induced by a procedure based on that developed by Cuttle and others [11]. Briefly, the animal was placed belly up in a shallow perspex bowl with a rectangular hole cut in its floor, with dimensions giving an area equal to the desired percentage of total body surface area to be burned. The dorsum of the rat was pushed into this opening and the bowl was lowered into water at a temperature of 99 °C for 10 seconds. The animal was then removed and the skin dried immediately. In animals intended to develop more debilitating injuries, drying the scalded skin was delayed for several minutes.

Sham-burned animals were anesthetized and shaved. They were then put belly up in cold water for 10 seconds and removed. The animals were observed in separate cages for 8-16 weeks.

Body surface area was calculated using the Meeh-Rubner equation [12].

**Animal care**

All animals tolerated the induction procedure well, recovered and were active and feeding in 6-8 hours. The animals were kept in cages, in a room that was periodically disinfected. The animals were carefully observed for evidence of infection, especially fever and reduced appetite. Though chronic slow-healing wounds, with local sepsis occurred in some animals, systemic evidence of infection was not found in any animal. Animals which developed repeated hemorrhagic wounds were administered topical silver sulfadiazine (Dermazine) ointment and mepyramine maleate (Piriton) cream was applied for itching.

The animals were weighed at the onset of the studies, every fortnight and just before they were sacrificed. One set of animals was sacrificed after 8 weeks and the second after 16 weeks. Blood was withdrawn by cardiac puncture for serum hormone assays. The testes and accessory sex organs were removed and weighed.
Semen analysis

Epididymal sperm concentration was determined according to the method previously described by Yokoi and others [13]. Motility estimation was carried out at room temperature between 24°C and 28°C. The microscopic field was scanned systematically and spermatozoa encountered were assessed as motile or nonmotile. An estimate of the percentage of motile sperm was made [14]. Sperm movement was then classified into whether it was linear and rapid, sluggish or nonlinear based on the predominant quality of motion observed among motile sperm cells [15].

Hormone assays

Serum testosterone (T) concentration was determined by the enzyme-linked immunoassay technique based on the principle of competitive binding between testosterone in the test specimen and testosteronehorseradish peroxidase (HRP) conjugate for a constant amount of rabbit anti-testosterone, as previously described by Tietz [16].

FSH and LH levels were determined using the enzyme-linked immunoassay (ELISA) kit (catalogue number FSH-96 and LH-96 supplied by Teco Diagnostics, Anaheim, CA). The colour development was terminated with the addition of a stop solution. In all the assays absorbance was measured at 450 nm.

Histopathologic evaluation

Paraffin blocks of the testes were cut into 5 µm thick sections, processed and stained with hematoxylin and eosin. A semi-quantitative evaluation of histologic changes in the testes was carried out according to a method described by Sayyim and others [17]. Briefly we examined 100 tubules in each testis and classified them as normal, atrophic, sloughing or degenerative depending on the predominant histologic feature seen in each tubule. The data is presented as mean ± SEM. The results were analysed by ANOVA, and differences between means by the post-hoc Duncan test with p<0.05 as the level of statistical significance.

Results

Weight of animals, testes and accessory organs

Weight of animals, testes and accessory sex organs are presented in Table 1. Burn injury caused a significant reduction in testes weight and volume only in group 4, (p<0.05).

Epididymal sperm parameters

Results for key sperm parameters are presented in Table 2. Burn injury caused a significant reduction in all key sperm parameters. Sperm density, motility and cells with normal morphology were significantly decreased (p<0.05). In group 4 where there was chronic wounds, oligo-zoospermia occurred (p<0.001). Abnormal cell count significantly increased with tail abnormalities being the most common.

Histology of the testis

Results for histopathologic score are presented in Table 4. Severe Burns produced considerable seminiferous tubular damage. In group 2, germ cell...
In group 3 and more so in group 4 where chronic wounds occurred, severe germ cell atrophy affecting all layers occurred. In most tubules in these groups, only basal cells, such as Sertoli cells and spermatogonia, could be identified. In these groups seminiferous tubules showed widespread cyto-architectural disruption (Figures 3 and 4). A severe destruction of all subsets of spermatids trophic in the ad-luminal area was the predominant finding with the percentage of tubules showing atrophy being almost three times higher than that found in control group (p<0.01). Another common histologic finding after atrophy was sloughing of cells. Free mature spermatozoa were absent in the lumen of most tubules.

Table 4: Histopathologic score of seminiferous tubules in all groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Normal Healing</th>
<th>Chronic Wound x 6 week</th>
<th>Chronic wound x 16 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact (%)</td>
<td>89.62 ± 6.12</td>
<td>48.44 ± 3.23$^*$</td>
<td>35.23 ± 2.45$^{10}$</td>
<td>17.44 ± 2.18$^{9}$</td>
</tr>
<tr>
<td>Atrophic (%)</td>
<td>8.52 ± 2.37</td>
<td>38.25 ± 2.44$^b$</td>
<td>12.45 ± 1.86$^{10}$</td>
<td>19.52 ± 2.15$^{10}$</td>
</tr>
<tr>
<td>Sloughing (%)</td>
<td>2.54 ± 0.11</td>
<td>9.24 ± 4.12$^a$</td>
<td>38.41 ± 3.72$^{10}$</td>
<td>43.27 ± 3.18$^{9}$</td>
</tr>
<tr>
<td>Degeneration (%)</td>
<td>-</td>
<td>4.42 ± 0.18</td>
<td>14.12 ± 1.18$^{10}$</td>
<td>20.22 ± 2.24$^{8}$</td>
</tr>
</tbody>
</table>

$^*$- Significant difference compared to control, p<0.01; $^{10}$- Significant difference compared to normal healing group, p<0.01; $^b$- Significant difference compared to normal healing group, p<0.05.

In group 3 and more so in group 4 where chronic wounds occurred, severe germ cell atrophy affecting all layers occurred. In most tubules in these groups, only basal cells, such as Sertoli cells and spermatogonia could be identified. In these groups seminiferous tubules showed widespread cyto-architectural disruption (Figures 3 and 4). A severe destruction of all subsets of spermatids.
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Discussion

In this study severe burns caused a significant reduction in major parameters of epididymal sperm. This is consistent with findings previously reported [7-9]. Reduction in spermatogenesis was also observed to correlate with severity and chronicity of wounds. This is partially consistent with our finding from a previous study in which we showed that a significant positive correlation existed between burn depth and time lapse after injury [7, 18]. Although time lapse post-burn is a factor in testicular damage, significant differences did not occur in some measured parameters between groups 3 and 4 where animals with similar injuries were observed over 8 and 16 weeks duration.

Changes in the levels of reproductive hormones were ambivalent. By 16 weeks, reduction in gonadotropins levels was largely reversed and only a reduction in T level persisted. This is consistent with the findings of Dolecek and others. [8] The reduction in serum T in animals with chronic wounds also agrees with the findings of previous investigators. Bio-available T levels were significantly reduced in ventilator-dependent-men and Niemann has reported that chronic severe illness of whatever etiology was capable of causing hypo-testosteronemia [19, 20].

Endocrine priorities are altered in the immediate post-burn era in mammals, and the hypothalamic-pituitary axis is suppressed [10, 21]. It is believed that in the survival struggle, priority is shifted to the production of stress hormones such as glucocorticoids in the immediate post-burn period. Urinary cortisol has been shown to increase 5-fold and to remain increased for long periods after severe burns. This might contribute to changes in gonadotropin levels and T levels. Jeschke and others reported suppression in T and a rise in estradiol levels in children, which was normalized by the end of the 4th week post-burn [10]. Apparently, chronicity prolongs the T suppression.

Normally a fall in T level induces a feedback increase in LH/FSH levels. An altered hypothalamic-pituitary axis seems to interfere with this response. Both T and gonadotropin levels fell together at least during parts of the duration of this study. A fall in gonadotropins and a profile of androgen suppression and rising estrogenic activity can compromise the endocrine support that drives spermatogenesis and contribute to the fall in epididymal sperm parameters.

Severe histologic changes were induced by burn injury in this study. Our observations of germ cell loss correlated well with reduction in sperm density. We have previously reported severe tubular destruction in men who died from severe burns [9]. The result of this study however differs from that one in that interstitial edema was seldom seen in this study.

In animals with chronic wounds, all evidence of cell association was lost in most tubules. Only basal cells such as spermatogonia and Sertoli cell nuclei were seen in many tubules. Germ cell dissociation and sloughing into the lumen has been previously reported [22]. In some tubules, spermatocytes with multiple nuclei were visible suggesting degenerative transformation. Giant cell degeneration was reported in methotrexate toxicity in rats. Many agents, especially heavy metals and anticancer drugs have been shown to destroy germ cells and tubular cyto-architecture [23, 24]. Multiple pathways may lead to histologic damage found in this study. Germ cell degeneration may result from the cytotoxic effects of locally produced or circulating pro-oxidants. Increased oxidative stress has been found in testicular fluid in conditions associated with testicular damage [25].

In many atrophic tubules, only tail fragments were left in the lumen. Many testiculotoxic conditions have been shown to increase the apoptotic process among germ cells. Apoptosis is a key component of natural spermatogenesis. However a variety of toxic substances including cytokines [6, 10] which are known to increase after burns may prime cells for nuclear degeneration and death.

Cell adhesion is important in the seminiferous epithelium. It is required for the nourishment of differentiating germ cells as they progress to the lumen. Loss of adhesion is another likely factor in cell damage. As Nakai has suggested, impaired production of cadherin, a Sertoli cell product might result from the effect of toxic substances on these cells. Loss of cell adhesion leads to sloughing, distorts histo-morphology and accelerates cell death.

Our study shows that severe burns cause impaired spermatogenesis and histologic damage to seminiferous epithelium. Mass burns are still leaving in their trail many young male survivors in developing

and spermatocytes was apparent in most tubules. Lumen were so empty in many tubules even in group 2, the appearance was like sperm tails had undergone selective lyses. When cells adjoining the lumen showed tails in other tubules, they had a flame-tail appearance.
countries [26]. Although much progress has been made in the last several decades in burn wound treatment, delayed healing from sepsis and other causes still occur. To our knowledge, no clear measures for preventing or mitigating testicular damage in burn patients are in application at the moment though studies of some promising strategies are being carried out in our center. The natural history of suppressed fertility in this condition, including any possible recovery is yet to be delineated. In the meantime therefore, the prevention of chronicity in wounds must be a priority in the care of any young males with major thermal injury.

Acknowledgments

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References


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