Systemic Host Response Following Skin Burn Injury in Rats: Cytotoxicity and Genotoxicity Evaluation

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Abstract. Aim: The aim of the present study was to investigate whether skin burn injury (BI) can induce cellular changes in skeletal muscle, liver, kidney and blood by means of DNA damage and cytotoxicity. Materials and Methods: Thirty male Wistar rats were distributed into two groups: control (C) and submitted to scald burn (SB), subdivided into three subgroups: 1, 4 or 14 days post-injury. The gastrocnemius muscle and liver were dissected for histopathological evaluation and the single-cell gel (comet) assay was used to investigate damage in skeletal muscle, liver, kidney and blood cells. Results: Histopathological analysis of the muscle in the SB group revealed congested vessels containing inflammatory cells for all periods evaluated post-injury. In liver, the one day post-injury SB group showed sinusoidal congestion, while that of 14 days post-injury exhibited an increased number of Kupffer cells. Conclusion: Despite the histopathological evidence, none of the groups showed any signs of genotoxicity in these target tissues.

Burn injury (BI) has been associated with protein catabolism characterized by a hyper-metabolic response. Patients with burns greater than 40% are always catabolic (1), which will affect their metabolic derangements and persist at least 1 year after injury in most body tissues (2). These burn injuries result in both local and systemic responses distant from the site of thermal injury, such as skeletal muscle (3), bone (4) and liver (5) as a result of systemic host response.

Among all types of burns, the major frequency of this trauma occurs by scald hot liquids, i.e. scald burns tend to be the most common type of thermal injury. In addition, this lesion usually occurs under the age of 5 accounting for over 65% of cases, while fire injury tends to occur in older children accounting for over 56% of cases (6). According to Krishnamoorthy et al. (7) BI in children continues to be a major epidemiologic problem around the world so far.

Generation of DNA damage is considered to be an important initial event in several chronic degenerative diseases (8). DNA damage can be produced by exposure of cells to exogenous environmental (agents including ionizing radiation, sunlight, diet, chemicals and metals) or biological agents such as apoptotic process, hypoxia and inflammatory response. This damage can be related to oxidative stress, which is a direct risk to genome stability that leads to formation of mutations (9).

Tests for detecting DNA damage may have limited use because of complicated technical setup or because they only are applicable to few cell types. The single-cell gel electrophoresis (comet) assay is technically simple, relatively fast, cheap, and DNA damage can be investigated in virtually all mammalian cell types without requirement of a cell culture (8). Particularly, our research group has applied successfully the single gel (comet) assay under different conditions and paradigms to predict genetic instability in rats, such as chronic renal failure (10), myocardial infarction (11), non-alcoholic fatty liver disease (12), cirrhotic liver (13) and sleep deprivation (14). Herein, and due to the absence of relevant reports, it would be interesting to know if, and to what extent, skin BI is able to induce genetic damage in multiple organs of rats as a result of systemic host response (15).

Therefore, the aim of the present study was to investigate whether BI causes changes in multiple organs by means of cytotoxicity and DNA damage in skeletal muscle, liver, kidney and blood cells. Certainly, such data will contribute to a better understanding concerning skin BI on various cellular systems.

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each): control (C) and exposed to scald burn (SB), subdivided into, according to days of euthanasia - 1, 4 or 14 days after injury.

All procedures and protocols were approved by the Experimental Animal Use Committee of University Federal of Sao Paulo (0158/12) and were conducted in accordance with the National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals. Animals were anesthetized (ketamine 50 mg/kg + xylazin 10 mg/kg) and hair on the dorsal and ventral surfaces of the body was removed. A nonlethal scald injury was administered to the 15 animals constituting the SB group by immersing 45% of each rat’s body in a water bath at 48°C for 10 seconds.

Figure 1. Photomicrographs of skeletal muscle cells of controls (left column) and scald burned (right column) rats evaluated at different time points: 1 day, 4 days and 14 days after injury. The control group (C) shows an ordinary appearance. In the scald burn group (SB) congested vessels (arrows) containing inflammatory cells are noted. Stained by H&E; Scale bar=100 μm.
body in 87˚C water as described by Walker and Mason (16). As a result, each animal had 30% of its dorsal and 15% of its ventral area exposed to scald burn for 10 and 3 sec, respectively. An additional 15 rats were not treated and represented the control group (C). Both SB and C groups were injected subcutaneously with the analgesic buprenorphine (0.2 mg/kg) immediately following scald injury, or sham operation, and again 24 h later.

All animals returned to their cages and the status of each animal was checked by recording the body weight and food intake of each daily. One, four and 14 days following the scald burn (SB-1, SB-4 and SB-14) or sham (C-1, C-4 and C-14), five animals from each group were euthanized with an overdose of ketamine and xylazin.

Histopathological analysis. The middle part of the gastrocnemius muscle and a fragment of liver were taken and kept in 10% buffered formalin for 24 h. The specimens were routinely embedded in paraffin blocks and cut in transversal sections (4 μm). The slides were stained with hematoxylin and eosin. The specimens were analyzed with a light microscope (Axio Observer.D1 Zeiss®, Oberkochen, Germany).

Single-cell gel (comet) assay. The protocol used for skeletal muscle, liver, kidney and peripheral blood cells followed the guidelines proposed by Tice et al. (17) with some modifications. Specifically, a volume of 5 μl of peripheral blood or the supernatant (cellular suspension) (10 μl) of the skeletal muscle, liver and kidney was added to 120 μl 0.5% low-melting-point agarose at 37˚C, layered onto a pre-coated slide with 1.5% regular agarose and covered with a coverslip. After brief agarose solidification in the refrigerator, the coverslip was removed and slides were immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris–HCl buffer, pH 10, 1% sodium sarcosinate with 1% Triton X-100 and 10% DMSO) for approximately 1 h. After electrophoresis, slides were left in alkaline buffer (pH >13) for 20 min and electrophoresed for another 20 min at 0.7
Histopathological analysis. The histopathological analysis of skeletal muscle evaluated 1, 4 or 14 days after injury revealed in the control group (Figure 1, left column) normal fibers, identified equidistantly distributed polygonal muscle fibers with peripheral nuclei and muscular fascicles separated by normal blood vessels. By contrast, the SB group exhibited histopathological changes such as muscular fascicles with heterogeneous distribution showing conjunctive tissue with congested vessels replete with inflammatory cells (Figure 1, right column).

The analysis of liver, evaluated 1, 4 or 14 days after injury, revealed in the control group (Figure 2, left column) normal hepatocytes separated by sinusoid space and with a low number of Kupffer cells. On the contrary, the SB group, 1 day after injury, showed sinusoidal congestion that was discontinued after 4 or 14 days. Interestingly, the SB group 14 days after injury showed increased numbers of Kupffer cells when compared with the same group evaluated for the other tested time points and compared with the control group (Figure 2, right column).

DNA damage evaluation. The single-cell gel (comet) assay (Figure 3) was used to measure DNA damage in skeletal muscle, kidney, liver and peripheral blood cells in vivo. None of the groups showed any signs of genotoxicity, i.e. no differences in the tail moment values (Figure 4).

Discussion

BI affects cellular structure and function as a result of systemic host response. The hyper-metabolic and inflammatory responses are associated with protein and amino-acid degradation, insulin resistance, hyperglycemia, lipolysis and multiple organ failure. The progress advances in burn prevention, treatment and rehabilitation depend on the elucidation of disturbance closely related to organ dysfunction syndrome and systemic inflammatory response.

The goal of the present study was to evaluate genetic damage induced in different target organs –skeletal muscle, liver, kidney and blood cells– by experimental BI and to investigate them at different time periods of 1, 4 and 14 days after injury. The experimental approaches used were the single cell gel (comet) assay, as well as histopathological analyses.

According to Chen et al. (18) the advances in burn treatment and rehabilitation are related to comprehension of sepsis and subsequent organ dysfunction syndrome, which originated from systemic inflammatory responses. In the present study the histopathological findings are consistent to signs of systemic inflammatory responses, as detected in skeletal muscle fascicles with heterogeneous distribution showing conjunctive tissue with congested vessels with inflammatory cells. In the liver, the results showed sinusoidal congestion one day after injury, as well as increased number of Kupffer cells 14 days post-BI.

Kupffer cells play a key role in producing systemic changes in host immune response, such as up-regulation of pro-inflammatory cytokines like interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) (19) and thereby contribute to liver injury following severe burning (20, 18).

In a study from 1999 to 2005 investigating the causes of death in patients with severe burns in the Helsinki Burn Center (Finland), there were 71 burn deaths out of which 40% was caused by multiple-organ failure (21). The cause of death involved at least four organ failures per patient with
acute renal failure being noted in all patients with multiple-organ failure. Kraft et al. (22) monitored 821 pediatric burn patients during acute-care hospitalization and found that the highest incidence of organ failure was pulmonary followed by cardiac, liver and kidney collapse. The worst outcome was reported for patients with profound kidney or liver failure. Burn patients with 3 or more organ failures have an extremely poor prognosis.

To further elucidate the role of skin BI on the genetic (DNA damage) basis, we performed experiments using the comet assay. Our results demonstrated that skin BI did not induce genetic damage in blood cells; however, these findings cannot be presently evaluated and/or discussed due to the lack of reproducibility. Nevertheless, independently of the biological mechanism behind this process, we only conjecture that skin BI does not induce genetic damage in rat peripheral blood cells as a result of a systemic host response. Undoubtedly, further studies are necessary to shed light into this complex issue.

Furthermore and according to the in vivo single-cell gel (comet) assay guidelines (17), it is recommended to analyze cells from liver since this organ represents the major site of metabolism. Our results demonstrated that skin BI was not able to induce DNA damage in liver cells. Following the same rationale, our results showed lack of DNA breakage in kidney cells following BI. It is essential to point-out that whether or not DNA damage is repaired or persists, genotoxicity is an important issue to the fate of organs targeted by chemical carcinogens and/or other contributing factors including the production of reactive metabolites, their distribution, and their effect on cell proliferation (23). Nevertheless, no single test is capable of detecting all genotoxic agents. Thus, for a more detailed judgment over the genotoxic potential of chlorhexidine, a battery of tests is feasible. The commonest cause of death in patients suffering from burns in the developed countries or even in developing countries is the multiple-organ dysfunction syndrome and/or the systemic inflammatory response syndrome. In the present study we detected histopathological dysfunction in skeletal muscle and in liver but without damage in DNA in these organs or in kidney and blood. Although important information can be obtained from similar studies, the cytotoxicity and genotoxicity in an experimental model of BI have never been investigated before.

Figure 4. Graphs representing means and stand deviation of tail moment in skeletal muscle (A), liver (B), kidney (C) and blood cells (D) in both control and scald burn-groups evaluated at different time points of 1, 4 and 14 days post-injury.
In conclusion, our data suggest that scald thermal injury induces morphological changes in liver and skeletal muscle related to the inflammatory responses syndrome without, however, genetic damage following skin burn injury in a male Wistar rat model.

Acknowledgements

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