THE ROLE OF MAST CELLS IN CARBON TETRACHLORIDE INDUCED RAT SKELETAL MUSCLE TISSUE DAMAGE

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Animal models demonstrating skeletal muscle (SM) disorders are rarely investigated, although these disorders accompany liver disorders and can occur during prolonged exercise/training. It is speculated that mast cells, normally present in the interstitial SM tissue, are involved in the pathophysiology of different SM disorders. Thus, the present study aims to analyze, on histopathological level, the involvement of mast cells in acute rat intoxication with carbon tetrachloride (CCl4). Biceps and gastrocnemius muscle were obtained from male Wistar rats acutely exposed to CCl4 (1 ml/kg) and the pathological analysis was performed on Toluidine blue stained tissue sections. The obtained results were statically compared with those from control group using Student’s t-test. In SM tissue obtained from the control group mast cells were found only in the interstitium, while in those that received CCl4 they were located mainly near the blood vessels. Also, in the experimental group treated with CCl4 mast cells were more abundant and were percutenously more degranulated than those found in the control group. Thus, one can say that herein presented model of CCl4-induced SM damage is partially dependent on the activity of mast cells.

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Key words: Carbon tetrachloride, Skeletal muscles, Mast cells

Introduction

Our body is consisted of three distinct types of muscles, from which the skeletal muscles (SM) are the only ones that can contract under our will (deliberately) maintaining body posture and enabling locomotion. Skeletal muscle specific anatomical and physiological organization allows SM to function (1). Each SM is consisted of a large number of myofibrils (muscle cells) that represent the smallest functional unit (1). Body and SM tissue mass can be significantly affected under different underlaying conditions and these changes occur due to tissue protein degradation (2). Changes in SM mass and function can be seen in several liver disorders, as well as under various physiological conditions that involve significant production of reactive oxygen species (ROS) (3,4).

Carbon tetrachloride (CCl4), a synthetic chemical often used in paints, solvents and extinguishers (5), is known to be a useful in inducing ROS mediated tissue damage in laboratory animals. When applied it causes liver, kidneys, brain, muscles, lungs, testis, etc. oxidative damage (6), via trichloromethyl free radicals generated in liver (6,7). The mechanism by which CCl4 damages cell structures is relatively well studied, however the role of mast cells in SM injury caused by CCl4 are still not fully investigated.

Mast cells are mesenchymal cell, stained metachromatically with some blue dyes, that contain numerous granules which contain the majority of the body’s histamine. These cells play a crucial role in body inflammatory and allergic reaction (8). Before their final migration and differentiation in tissue, mast cells (type of leukocytes) circulate in the blood as immature cells derived from hematopoietic progenitor cells. All tissues of the body possess different percent of mast cells, while they are more abundant in tissues that are coming in close contact with external environment (skin, intestinal and airway mucosa) (8).
Aim of the study

Although the toxic effects of CCl₄ on SM tissue have been investigated, the specifics pathogenetic role of mast cells are not completely investigated. Thus, the goal of the present study was to detect and describe on histopathological level the changes in mast cells occurring in rat SM after acute administration of CCl₄.

Material and methods

Animals and housing

Male Wistar rats, weighting 250-300 g, were housed in groups of 6 and obtained from the Vivarium of the Institute of Biomedical Research, Faculty of Medicine, University of Niš, Serbia. The animals were maintained under standard laboratory conditions: temperature 22±2 ºC and humidity 60%, with food and water available ad libitum. All experimental procedures with the animals were conducted in compliance with the declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals (EU Directive of 2010; 2010/63/EU) and were also approved by the local Ethics Committee.

Muscle tissue damage induction

Before the experiment, all animals were divided into two groups of 6 rats each: the control group where the animals were administered only the vehicle (olive oil) in the dose of 10 ml/kg, and the experimental group with CCl₄-treated animals. Acute administration of CCl₄ (1 ml/kg), known to cause significant liver damage (6), was given to rats via an intraperitoneal injection 24 h before the animals were sacrificed by an overdose of ketamine. Skeletal muscle tissue samples collected, using scissors and tweezers, for histological analyses included the left gastrocnemius (GCM) and biceps (BM) muscles.

Histopathological observations

The GCM and BM tissue specimens separated for histopathological examination were fixed in buffered formaldehyde solution (10%, w/v). The fixed tissues were then dehydrated with ethanol solutions of differing concentration (50-100%, v/v), embedded in paraffin, cut into 4-5 μm thick sections, stained with Toluidine Blue (TB) and further examined under an Olympus BH2 light microscope. The average number of mast cells per high-power field (x40) was counted on 10 randomly selected fields for each muscle specimen stained with TB. Also, the percent of degranulated mast cells was counted on each examined high-power field.

Statistical analysis

The results were expressed as mean values ± SD. Statistically significant differences were determined by Student’s t-test (Graph pad Prism version 5.03, San Diego, CA, USA) and the obtained results were tested for correlation as well. Probability values (p) less than or equal to 0.05 were considered to be statistically significant.

Results

The number and % of degranulated mast cells, found in GCM and BM tissue sections stained with TB, were found to be statistically significantly higher in both investigated muscles originating from animals exposed to CCl₄ compared to the control animals (Table 1 and Figure 1).

In the investigated TB stained tissue mast cells granules appeared red-purple (metachromatic granules), while their cytoplasm and background appeared pale blue (orthochromatic). The mast cell shape varied from ovoid to spindle-shaped and one could clearly distinguish preserved (not degranulated) and degranulated mast cells that appeared deep and light blue, respectively (Figure 1). In healthy (control group) animal mast cells were observed only in the interstitial connective tissue that normally separates skeletal muscle into separate fascicles. On the other hand, in animals treated with CCl₄ mast cells appeared in the interstitial connective tissue as well, however they were more frequently found around blood vessels.

Table 1. Number and % of degranulated mast cells obtained for each of the two studied muscles from the two different animal groups

<table>
<thead>
<tr>
<th>Tracked parameter</th>
<th>Average number of mast cells/high power field (x40)</th>
<th>Degranulated mast cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Gastrocnemius muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.6 ± 0.2</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>CCl₄ treated</td>
<td>1.4 ± 0.2*</td>
<td>66.5 ± 16.1*</td>
</tr>
<tr>
<td>Biceps muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.9 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>CCl₄ treated</td>
<td>1.2 ± 0.05*</td>
<td>62.7 ± 14.2*</td>
</tr>
</tbody>
</table>

The results are presented as mean±SD (n=6); *p<0.001 vs. control group.
Figure 1. Microscopic appearance of degranulated and non-degranulated mast cells present in skeletal muscle tissue stained with Toluidine blue (x400).

Discussion

The two muscles evaluated in our study, GCM and BFM, represent important hind-limb muscles that enable animal movement. Biceps femoris muscle is the largest muscle in the hind limb and has multiple functions that involve thigh abduction, hip extension and knee flexion, while GCM is responsible for plantar flexion (9).

Since their discovery, back in 1877, mast cells are considered as normal constituents of interstitial tissue of different vertebrates (10). Later they were recognized as cells important in triggering and/or maintenance of different inflammatory and immunological processes (10). These cells represent the source of inflammatory mediators such as histamine, nitric oxide, proteases tryptase and chymase, and pre-formed tumor necrosis factor-alpha (TNF-α), as well as other toxic mediators by which the muscle tissue can be injured (11, 8). When mast cells are triggered to degranulate, the secretory granules located within the cell are released, exocytosis, resulting in the release of their internal contents (8).

The importance of mast cells in the pathophysiology of muscle disorders was previously shown in the ischemia/reperfusion muscle injury model (11) and the present research proves the involvement of these cells in acute CCl₄-induced muscle damage (Table 1, Figure 1), as well. Also, these cells were found to be significantly increased in different myopathic disorders, e.g. Duchenne muscular dystrophy, where it was suggested that grouped necrosis of extrafusal fibers occurs possibly due to the activity of these cells (10). Thus, the increase in both the number of mast cells and % of their degranulation indicates that their involvement in acute CCl₄-induced muscle damage should not be neglected. Additionally, there is a possibility that a much higher number of mast cells were involved in tissue damage, both resident and mobilized, however due to their degranulation TB staining might not have stained all of mast cells.

The consequence of degranulation can probably be brought in connection with inflammatory cell infiltrate, seen after CCl₄ application, that is comprised mainly of neutrophils. Namely these neutrophils secrete an enzyme, myeloperoxidase, that causes the onset of degranulation (histamine release) form isolated mast cells (12). On the other hand, SM resident mast cells are known to provoke a significant increase in the number of neutrophils in injured SM tissue (13). Similar results were obtained in the study where mast cell membrane stabilizing agent, cromolyn, was administered prior to neutrophil attracting agent, bupivacaine, thus causing a decrease in neutrophil infiltration by 70% (14). All of the results, relating to the presence and state of mast cells, represent an important addition to the overall conclusion concerning the involvement of these cells in muscle tissue damage induced by an acute application of CCl₄.

Also, one can say that the damage caused by CCl₄ seems more significant in GCM than in BM (Table 1), and such slight differences in the extent of CCl₄ injury in GCM and BM are not completely unexpected. Namely, a previous study revealed different degrees of protein catabolism, estimated based on tyrosine release, in various muscle tissues after CCl₄ application (3). Thus, it is not surprising that the two muscles do not suffer identical damage, since their vascularisation, structure (fibers size, mitochondria amount, myoglobin concentration, etc.) and function are not the same.
Conclusion

The herein presented model of CCl₄-induced skeletal muscle damage can be considered a useful model that could mimic both mild ROS-mediated muscle damage seen in strenuous physical exercise and/or muscle damage (waste) that frequently accompanies liver diseases. The results unequivocally demonstrated the involvement of mast cells in skeletal muscle tissue damage caused by CCl₄ after an acute application.

Acknowledgment

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References

ULOGA MASTOCITA U MODELU UGLJEN TETRAHLORIDOM INDUKOVANOG OŠTEĆENJA SKELETNOG MIŠIĆNOG TKIVA PACOVA

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Animalni modeli koji imitiraju oštećenja skeletnih mišića (SM) su retko predmet istraživanja, iako poremećaji SM često prate druge poremećaje organa, kao što su oštećenja jetre. Takođe, oštećenja SM mogu da se javi nakon duge i intenzivne fizičke aktivnosti. Smatra se da su mastociti, deo normalne čelije populacije intersticijuma SM, uključeni u patofiziološki mehanizam nastanaka SM oboljenja. Ova studija ima za cilj da na patohistološkom nivou pokaže ulogu mastocita u akutnom oštećenju SM pacova koji su izloženi ugljen-tetrahloridu (CCl4). Od životinja koje su tretirane akutno CCl4 (1 ml/kg) uzimani su uzorci m. biceps i m. gastrocnemius za dalju patohistološku obradu, bojenje (Toludin blue) i analizu. Dobijeni rezultati su upoređeni korišćenjem Studentovog t-testa. U uzorcima SM koji su dobijeni od životinja iz kontrolne grupe mastociti su bili prisutni najčešće u intersticijumu, dok kod životinja koje su bile izložene CCl4 mastociti su se nalazili većinom u blizini krvnih sudova. Takođe, u eksperimentalnoj grupi životinja tretiranih CCl4 mastociti su bili mnogo više zastupljeniji nego u kontrolnoj, a i procenat onih koji su bili degranulisani je bio statistički značajno veći. Na osnovu rezultata ove studije može se zaključiti da u oštećenja SM koje je indukovan CCl4 delimično zavisi i od aktivnosti mastocita.

Ključne reči: Ugljen-tetrahlorid, Skeletni mišići, Mastociti