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THE ROLE OF MAST CELLS IN INFLAMMATORY REACTION IN BURN INJURY AND POSTBURN REGENERATION

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Summary

Each year, approximately 11 million patients are hospitalized due to burn injuries. Mortality from burns ranges from 1.4% to 18% (Europe). At the same time burn injuries can lead to disability, and full recovery is often impossible. Mast cells can play an important role in burn injuries and regeneration. For a long time these cells had been neglected by scientists. Only recently new data has started to appear about their structure and functional significance in the local homeostasis. The list of substances synthesized by mast cells has significantly expanded, and so has the list of cells which they can influence. A review of studies on mast cells regarding their role in burn injury and its subsequent regeneration is presented in this article.

Rezumat

Rolul celulelor mastocitare în reacția inflamatorie în arsuri și regenerarea post combustională

În fiecare an, aproximativ 11 milioane de pacienți sunt internați din cauza arsurilor. Mortalitatea variază între 1,4% și 18% (Europa). În același timp, aceste leziuni pot duce la handicap fizic, unde recuperarea completă deseori este imposibilă. Mastocitele pot juca un rol important în arsuri și în regenerarea acestora. Pentru o lungă perioadă de timp aceste celule au fost neglijate de lumea științifică. Numai recent au început să apară studii noi privind structura lor și semnificația funcțională în homeostazia locală. Lista de substanțe sintetizate de celulele mastocitare s-a extins în mod semnificativ, astfel influența lor vastă asupra altor celule. Articolul prezintă o sinteză privind celulele mastocitare, cu scopul de a sublinia rolul acestora în arsuri și regenerarea sa ulterioară.

Introduction

Burn injuries occur more than 800 cases per 1 million persons/year. Only motor vehicle accidents have a higher mortality rate than burn injuries [1].

In 2004, 11 million people were victims of burn injuries, 90% of which occurred in developing countries, 50% to 70% in children. About 60% of burn victims are men. The death rate ranges between 1.4% and 18%. The most common causes of death are age of patients, extensive surface burn, multiple organ failure and sepsis. The most common cause of death in the first 48 hours is postburn shock and severe burn of the mucous membranes [2].

Mast cells (tissue basophils) are essential members of connective tissue, i.e. are present anywhere where there is at least a small layer of connective tissue. They represent a great interest because of their multiple functions in the regulation of local tissue homeostasis. These features of mast cells are directly related to their ability to produce a number of cytokines, chemotactic and vasoactive substances, enzymes and proteoglycans. Only in recent years, mast cells have become the center of attention of different researchers. Although to date the morphological features of mast cells are well known, a number of their functions are still not clear. Due to the fact that the activation of mast cells occurs both by immunological and non-immunological triggers (e.g. mechanical) they present an interest in a number of medical and biological fields. Particularly they may have special interest in burn injuries, as well as in the post-burn healing [22, 11].

Role of mast cells in the pathogenesis of inflammation in burn injury

There are several biological processes that have particular importance for the activity of mast cells in burn injuries:

- Tissue heating in burn area. Particularly, 37-41 °C is the physiological temperature for the cells, further its increase leads to their damage. The higher the temperature is above normal level, the faster the tissue damages and more time is needed to lower the temperature. Mechanical damage caused by burn is a stimulus for mast cell activation.
- Damage of mitochondria, leads to inhibition of cellular respiration, i.e. hypoxia. Ischemia strongly affects many cells, but mast cells are relatively resistant to hypoxia. There is a decrease in synthesis of arachidonic acid, but the amount of histamine remains on the same level, as well as cytokines. This fact makes these cells particularly important in areas with low levels of oxygen and glucose [5].
- Damage of the membrane results in depletion of K^+ , Mg^{2+} , Ca^{2+} , Zn^{2+} . This is a chemical stimulus which also actively influences mast cells [9].
- Damage of lysosomes leads to the release of lysosomal enzymes, and tissue damage [24].

For a better understanding of changes in burn injury at the macroscopic level we should mention the systemic inflammatory response syndrome, described by Jackson in 1953.

Thus, he identified three areas of burn wounds:

- The area of primary necrosis and coagulation;

- The area of ischemia and stasis;
- The area of reactive edema [8].

The epidermis and dermis tissues release proinflammatory cytokines and chemokines which increase the capillary permeability. That results in inflammation of this lesioned area. Similar effects appear after histamine is injected into the tissue. This points out the role of this mediator in burn inflammation.

Histamine is a neurotransmitter released by mast cells. Its action appears very fast, within a few seconds after the injury due to rapid degranulation. Particularly histamine is a vasodilator that increases vascular permeability. Among the advantages of this mediator is its rapid breakdown (about an hour), after which, the inflammation, can be maintained by other inflammatory mediators.

Functional characteristics of mast cells

Mast cells are found in almost all tissues. Most of them are located around the blood vessels, particularly in the skin, thymus, bone marrow, and less in the adrenal glands, liver and gastrointestinal tract. Their synthetic activity also varies. Thus, the most active mast cells are found in the connective tissue and the adrenal gland [21].

Mast cells respond to hypoxia, mechanical and chemical factors with the release of biologically active substances. The basic substance in mast cells is histamine, and at the same time it is the marker of mast cells. Moreover they release leukotrienes C₄, D₄, E₄, prostoglandin D, heparin and tryptase. Another important substance is the mast cells collagenase, which has a role in the damage of the connective tissue [22, 23].

O. S. Artashyan differentiates four types of mast cells by:

- Type "1" – small amount of granules in the cytoplasm, located close to the membrane;
- Type "2" - average amount of granules, located diffusely;
- Type "3" - large cells, with dense and diffuse location of granules in the cytoplasm;
- Type "0" - degranulated cells with evidence of disruption of the plasma membrane integrity [21].

There is another mast cell classification which differentiates them into MC_tc, MC_c and MC_t, (MC-mast cell, t-tryptase, c-chymase) depending on the content of the granules. All of them contain histamine. Almost all skin mast cell are MC_tc type, presumably due to their specific function in matrix modulating processes. These mast cells contain tryptase, chymase, carboxypeptidase and a cathepsin G-like proteinase [7].

Migration of mast cells to the site of burn injury

If a tissue is damaged mast cells actively migrate to the area of injury, especially those from the thymus. Mast cells actively respond to hypoxia by degranulation. The density of mast cells during hypoxia, $S = 1\text{mm}^2$ (calculated using light microscopy 400x): Skin (normal conditions) 1326 +/-233, in hypoxia (6h) 541 +/-47, in hypoxia (7 day) 1280 +/-230; Thymus (normal conditions) 798 +/-160, in hypoxia (6h) 432 +/-44, in hypoxia (7 day) 626 +/-107 [21].

In the early stages hypoxia the amount of granules in mast cells decreases and in later stages their amount increases and becomes nearly normal.

Histochemically average ratio of mast cells during hypoxia (J. Astaldi and L. Verga formula): Skin (normal conditions) 2.63 +/-0.04, in hypoxia (6h) 0.91 +/-0.16, hypoxia (7 days) 1.16 +/-0.23;

Thymus (normal conditions) 2.14 +/-0.11, in hypoxia (6h) 0.79 +/-0.09, hypoxia (7 days) 1.97 +/-0.2;

Adrenal glands (normal conditions) 2.39 +/-0.13, in hypoxia (6h) 0.77 +/-0.1, hypoxia (7 days) 1.7 +/-0.04 [21].

During hypoxia mast cells actively migrate to the affected tissue and undergo degranulation. Synthetic activity of mast cells is depleted, but with time they adapt and hardly differ from the physiological norm.

Influence of mast cells on other cells and proteins in burn injury

Mast cells exhibit a regulatory function affecting various cells by bioactive substances and adhesive contact.

Table 1

Mast cell interactions and bioactive substances with their role in the tissue

The substance produced by mast cells	The cells that are affected by the substance	Effects of the substance
Histamine (H1 receptors)	Smooth muscle, endothelium, central nervous system (postsynaptic)	Vasodilation, bronchoconstriction, bronchial smooth muscle spasm, spreads endothelial cells (and, as a consequence, the leakage of fluid in the space around the vessel, swelling), stimulation of hormone secretion by the pituitary gland.
Histamine (H2 receptors)	Located on parietal cells and vascular smooth muscle cells	Primarily involved in vasodilatation, also stimulate gastric acid secretion
Histamine (H3 receptors)	Central nervous, peripheral nervous system	Decreased neurotransmitter release: histamine, acetylcholine, norepinephrine, serotonin
Thromboxane	Platelets, smooth muscle cells	Vasoconstriction, promotes platelet aggregation
Tryptase	Fibroblasts	Promotes fibroblast division, inflammatory, immune response
Chymase	-	Inflammatory, immune response, converts angiotensin I to angiotensin II
Carboxypeptidase A	-	Digestion of proteins
Cathepsin G	-	Digestion of engulfed pathogens, and in connective tissue remodeling at sites of inflammation
Heparin	Platelets, leukocytes	Relieves pain, inhibits clotting and inflammation, restored blood flow, and enhanced healing
Eosinophil chemotactic factor of anaphylaxis	Eosinophils	Eosinophils migration
Leukotriene C4, B4	Smooth muscle cells, leukocytes	Increases capillary permeability, release of lysosome enzymes
Prostaglandin D4, E2	Basophils, eosinophils, T-helper, nerve cells	Vasodilatation, inhibits the release of noradrenaline from sympathetic nerve terminals
Cytokines (IL-1, IL-4, IL-4, IL-5, IL-6, IL-8, IL-13, TNF-a, TGF-b1)	Wide range of cells	Cell growth, cell proliferation, cell differentiation and apoptosis
Nerve growth factor (NGF)	Nerve cells	Maintenance of sympathetic and sensory neurons, peripheral nerve regeneration
Platelet activating factor	Platelets, neutrophils, basophils, endothelial cells	Increases capillary permeability, cell aggregation and degranulation

We should note here several possibilities for the use of mast cell histamine and heparin in thermal injuries.

G.J. Zayas et al., 2007 indicated an increase in survival rate of patients with the use of heparin from 11% to 60%, decreasing mortality from 89% to 40%. The use of heparin resulted in relieved burn pain, significantly reduced mortality and sepsis with fewer procedures, and better cosmetic results (smooth skin) [20].

The studies of M.J. Saliba, Jr., 2001 showed that heparin relieves pain, inhibites clotting and inflammation, restores blood flow, and enhances healing. Heparin preserves lung and improves it function. It preserves intestinal integrity and reduces bacterial translocation. Heparin also enhances collagen restoration, makes the healed skin more smooth. Thus it reduces the need for pain killers, topical antibiotics, resuscitation fluids, blood transfusion, water baths, debridement, surgery and grafts. Thus significantly reducing the cost of treatment [15].

J. Röntfors, J. Cassuto, 2003 have shown in their study that H1, H2 and H3 receptors do not have a considerable role in the regulation of vascular permeability, whereas H3 receptors play an important role by increasing skin blood supply post burn, either by relaxation of vascular smooth muscles and/or by interacting with other inflammatory mediators [13].

The role of mast cells in the post burn regeneration, scarring & keloids

Inflammation and accumulation of collagen, which is synthesized during the process of wound healing often leads to scars. Some in vitro studies have indicated the involvement of mast cells in healing. Y. Nishikori et al., 1998 have tested this theory in the treatment of skin burns in mice. The analysis was performed on 1, 3, 7 and 14 days after the burn. Strong correlations have been found between wound healing and presence of mast cells in the tissue. Minimum mast cells quantity was observed on day 3 in the period of maximum tissue necrosis, but starting from 7th day migration of active cells is observed in the skin. This increases the number of capillaries and collagen fibers, that suggests that mast cells play an important role in wound healing [12].

Another study conducted by M.S. Ribeiro et al., 2004 in which laser beams were used to reduce the number of mast cells in tissues. This showed that reducing the number of mast cells significantly increased the duration of wound healing. The study was conducted on rats with the collection of samples from 3 to 17 days [14].

In addition tissue with lower number of mast cells heals worse, there was a higher percentage of contractures, scars were larger, vascularization was reduced [18].

A hamster model of burn injuries was studied by X. Dong et al., 2013 with the use of ketotifen, a mast cell membrane stabilizer which decreased the local concentration of Ang II, the expression levels of transforming growth factor β 1 (TGF- β 1) and affected collagen I and III formation and the concentration of inflammatory marker interleukin1 β (IL-1 β). These results suggest that mast cell chymase contributes to burn wound healing, indicating that chymase activity provides a promising future therapeutic target to accelerate wound healing [3].

By reducing the content of the granules with protease and elastase scars and general skin deterioration due to inability to break down proteins were marked [19]. Thus, it can be noted that mast cells have a large list of functions in tissue regeneration. Without them the process is more disorganized and the results are worse.

A study performed by N. Harunari et al., 2006 and involved Duroc pigs has shown that scars after 5 months contained 2.4 times more mast cells than uninjured Duroc tissue. Human hypertrophic scar contained 4.2 times more mast cells than uninjured human tissue [6].

The use of moist ointments on rabbits increased the amount of mast cells on day 3-9 resulting in a better results compared with other groups where saline and silver sulfadiazine was used [10].

Besides that mast cells are also at the same time can be the reason of hypertrophic scar formation. Some studies indicated elevated amount of mast cells in scar tissues. It should be noted though that the number of mast cells doesn't indicate the level of their activity [18].

Conclusion

«Millions» of changes occur during thermal injury, which include fluid and electrolyte imbalance, metabolic disturbances, infection and a great amount of complications which can lead to organ failure and death.

Mast cells have a specific role in thermal injury. It is not completely understood rather this role is positive, negative or both, depending on the case. A better understanding of mast cells physiology and potential would indicate what procedures and drugs should be used during burn injury and post burn rehabilitation in the outcome of the patient.

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VARIANTELE ȘI ANOMALIILE DE DEZVOLTARE ALE SPLINEI

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Summary

Variants and anomalies of spleen development

There were studied the anomalies of spleen development: lack and accessory spleen. It has been established by means of macroscopic method that an accessory spleen is most commonly detected near the lienal hilus of the basic organ, it is supplied by the branches of the lienal artery and innervated by the nerve trunks of the lienal plexus.

Rezumat

Au fost studiate anomaliile de dezvoltare splinei: asplenia și splina accesorie. Prin metoda macroscopică s-a determinat că splina accesorie este localizată mai frecvent în regiunea hilului lienal, se vascularizează prin ramurile arterei lienale și este inervată de către nervii plexului lienal.

Actualitatea

Din anomaliile de dezvoltare a splinei se poate numi asplenia – întâlnită cu alte anomaliile de dezvoltare a inimii și sistemului cardiovascular. Lipsa splinei este întâlnită foarte rar: Muir a observat 7 cazuri din 22500 de autopsii.

Splina accesorie (SA) este o anomalie de dezvoltare și se caracterizează prin amplasarea fragmentului lienal departe de splina maternă. Fenomenul SA este cunoscut de mult, dar datele despre frecvența acestuia sunt destul de contradictorii. Splinele suplimentare se depistează în timpul intervențiilor chirurgicale pe organ – de la 10-30% [52] până la 25-40% din cazuri [53]. În cazul splenectomiei deschise, SA se depistează în 15-30% din cazuri, iar la splenectomia laparoscopică – în 0-12% [32]. Prevalența SA a fost raportată la 7,1% din copiii supuși splenectomiei fără extragerea SA. Pe parcurs, poate avea loc hipertrofia SA, ajungând uneori la dimensiunile unei spline normale, cu manifestări clinice similare cu cele de până la operație [38]. Prin tomografie computerizată, s-a depistat SA în 16% din cazuri [33]. Mai frecvent, splinele suplimentare se întâlnesc în unor boli hematologice, consideră autorii citați, ceea ce denotă prezența unor condiții favorabile pentru apariția lor (purpura trombocitopenică, anemia hemolitică microsferocitară moștenită și, într-o proporție mai mică, limfogranulomatoza). În cazul purperei trombocitopenice, frecvența SA a constituit 31%, adică 14 din 45 cazuri: în 6 din ele – câte o SA, iar în 8 – mai mult de una (1 pacient avea 13 SA) [37]. Frecvența medie de