

Chronic effects of autonomic extrinsic denervation on mast cells number in mesenteric tissue of Wistar rats.

Efeitos crônicos da desnervação autonômica extrínseca no número de mastócitos do tecido mesentérico de ratos Wistar.

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ABSTRACT

Introduction: Mast cells are involved in pathogenesis of many digestive tract inflammatory diseases. Moreover, studies have demonstrated an important association between mast cells and nervous system. **Aims:** To demonstrate modification on mesenteric mast cells dynamic following loss of autonomic innervation obtained by extrinsic denervation processes. **Methods:** Male Wistar rats were divided in groups A and B. Animals on group A were submitted to sympathectomy (SS) (n=6) or to simulation surgery (SHs) (n=6); on group B, animals were submitted to vagotomy (VV) (n=6) or to simulation surgery (SHv) (n=6). After period of study, mesenteric windows were prepared for mast cells counting on optical microscope. Means at both groups were compared using Mann-Whitney statistics, and were considered significant differences values of $p < 0.05$. **Results:** Microscope findings demonstrated significant increase on mast cells number in subgroup SS in comparison with subgroup SHs ($p = 0.005$). However, at group B, results did not demonstrated a statically significant difference between subgroups SHv and VV ($p = 0.229$). **Discussion and conclusions:** Significant increase on mast cells on subgroup SS could come from an up-regulation on growth, differentiation, homing or by decrease on apoptosis. However, further studies are necessary to better elucidate many aspects of data shown in this study.

Keywords: Gut, small intestine, mastocytosis, autonomic denervation, vagotomy and sympathectomy.

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RESUMO

Introdução: Mastócitos estão envolvidos em diversas patologias inflamatórias do trato digestivo. Além disso, estudos têm demonstrado importante associação dos mastócitos com o sistema nervoso. **Objetivos:** Demonstrar alterações na dinâmica dos mastócitos mesenteriais provocadas pela perda da inervação autonômica secundária a processos de desnervação extrínseca. **Métodos:** Ratos machos Wistar foram divididos em grupos A e B. Animais do grupo A foram submetidos a simpatectomia (SS) (n=6) ou a cirurgia de simulação (SHs) (n=6); no grupo B, os animais foram submetidos a vagotomia (VV) (n=6) ou a cirurgia de simulação (SHv) (n=6). Após período de estudo, janelas mesentéricas foram preparadas para contagem de mastócitos à microscopia óptica. As médias dos dois grupos foram comparadas utilizando o método estatístico de Mann-Whitney, sendo consideradas significativas as diferenças com $p < 0,05$. **Resultados:** Achados microscópicos demonstraram aumento significativo no número de mastócitos no subgrupo SS em comparação com o subgrupo SHs ($p = 0,005$). Entretanto, no grupo B, os resultados não demonstraram diferença estatística entre os subgrupos SHv and VV ($p = 0,229$). **Discussão e conclusões:** O aumento significativo de mastócitos no subgrupo SS pode ter advindo de uma regulação positiva no crescimento, diferenciação, migração ou pela diminuição da apoptose. No entanto, novos estudos são necessários para melhor elucidar os dados levantados neste estudo.

Palavras-chave: Intestino delgado, jejuno, mastocitose, desnervação autonômica, vagotomia e simpatectomia.

INTRODUCTION

Enteric immune system is compound by a sort of cellular kinds whose are under dynamic equilibrium in response of both intestinal luminal stimulus and systemic pathophysiological states.

Mast cells are an important part of enteric immune system because are involved in pathogenesis of many inflammatory diseases of digestive tract. Moreover, some studies have demonstrated an important association between mast cells, blood and lymphatic vessels and nervous fibers ¹. Indeed, studies have shown that stimulating of vagal nerve fibers in vivo as well as neuropeptides in vitro are capable in causing functional changes on mast cells^{2,3,4}. Even this important act on mast cells, there are no many studies linking direct influence of autonomic nervous system on growth, differentiation, survival or apoptosis of these immune cells.

Thus, the aim of this research was demonstrate modification on mesenteric mast cells dynamic following lost of small bowel autonomic innervation obtained by extrinsic denervation processes.

MATERIAL AND METHODS

In this study were used 24 male Wistar rats, weighting about 150 grams, from General Biotery of Federal University of Mato Grosso do Sul state. During all extension of the study, animals were maintained in "ad libitum" offer of water and food, submitted to controlled temperature, moistness and photoperiod. All norms of Brazillian College of Animal Experimentation were obeyed.

The rats were divided into two groups, A and B; at group A, animals were submitted to surgical procedures of sympathectomy (SS) (n=6) or to simulation surgery (SHs) (n=6); and at group B, animals were submitted to surgical procedures of vagotomy (VV) (n=6) or to simulation surgery (SHv) (n=6). Surgery procedures spent a mean time of 40 minutes, and methodology similar as described by Lachat & Gonçalves (1978) ⁵, readapted by Silva (2004) ⁶, was used.

The sympathectomy was proceeded by section and removal of fragments of splanchnic nerves measuring about 1.0cm, immediately before

entrance in respective celiac ganglions. On the other hand, vagotomy was effectuated on sub diaphragmatic level, involving truncus anterior (right) and truncus posterior (left) of vagus nerve, which was submitted to resection along esophagus immediately before ramifications, and also removal of fragments in the size of approximately 1.0cm was performed. However, on animals of both groups A and B submitted to simulation surgery, only dissection and visualization of the structures were performed.

The animals of group A were studied for 90 days, and confirmation of sympathetic denervation process was performed by detailed analysis of celiac ganglions, for the purpose of recognition of splanchnic nerves remaining sections. Besides, the animals of group B were studied for a period of 30 days, and a detailed analysis of esophagus also was performed aiming to confirm vagotomy.

Mesenteric windows were obtained in the level of jejunum, which were prepared for mast cells visualization using 0.1% toluidine blue in 4% tamponade formalin for 40 minutes. For mast cells counting on mesenteric windows, optical microscopy techniques were used, analyzing 130 non-super-

posed aleatory fields, magnified at 400X, in three different mesenteric fragments. Means at groups A and B were compared using Mann-Whitney statistics and were considered significant differences values of $p < 0.05$.

RESULTS

Anatomical observations of digestive tract in those animals submitted to vagotomy (VV subgroup) demonstrated that all animal had megaesophagus, megastomach and megacolon at the end of experiment, although comparatively there were no decrease in final weight of both groups A and B.

Microscopic findings, also showed in **Table 1**, demonstrated a mean of $809,8 \pm 120,5$ mast cells on subgroup SHs, and a mean of $1331,5 \pm 116,5$ mast cells on subgroup SS. This way, using Mann-Whitney statistics, a significant increase in subgroup SS mast cells were found in comparison with subgroup animals SHs ($p = 0.005$). However, at group B, results from optic microscopy did not demonstrated a statically significant difference between findings on subgroups SHv and SS ($p = 0.229$).

Table 1. Number of mast cells counted in mesenteric tissue of Wistar rats submitted to small bowel autonomic denervation (130 microscopic fields).

	Subgroups	Mean \pm SD	Significance (Mann-Whitney)
Group A (90 days of experiment)	SHs (n=6)	$809,8 \pm 120,5$	$p = 0.005$
	SS (n=6)	$1331,5 \pm 116,5$	
Group B (30 days of experiment)	SHv (n=6)	$1587,0 \pm 153,1$	$p = 0.229$
	VV (n=6)	$1489,8 \pm 342,7$	

SHs – simulation simpatectomy subgroup; SS – simpatectomized subgroup; SHv – simulation vagotomy subgroup; VV – vagotomized subgroup.

DISCUSSION AND CONCLUSIONS

Mast cells are known primarily for their role in inflammatory processes and in allergic reactions, although its ability to produce a variety of cytokines under appropriate conditions suggests that these cells can participate in many immunological processes other than IgE-mediated hypersensitivity reactions. From bone marrow the mast cell progenitors migrate in the circulation prior to entering tissues through an equal cascade of adhesion and de-adhesion events. The tissue-specific localization of mast cells is probably regulated by a certain cytokine milieu and by adhesion of the cells to

extracellular matrix components via specialized cell-surface receptors of the integrin family⁷.

Although mast cells share many characteristics, it has been known since their discovery that they do not represent a homogeneous population. As can be seen in **Table 2**², in rodents, classification subtypes has been based on phenotypical differences between connective tissue mast cells (CTMC), particularly present in the skin and peritoneal cavity, and mucosal mast cells (MMC), particularly present in the intestinal lamina propria. Like rats and mice, humans thus have mast cell subpopulations that differ in neutral protease content, tissue localization and in functional characteristics¹.

Table 2. Rodent mast cell characteristics.

Characteristic	Peritoneal Cavity Mast Cell (CTMC)	Intestinal Mucosa Mast Cell (MMC)
Size	10-20 μm	5-10 μm
Staining	Safranin	Alcian blue
T-cell dependence in development	No	Yes
Protease content	Chymase (RMCP I)	Chymase (RMCP II)
Histamine	10-20 pg/cell	<0.5 pg/cell
Activated by Substance P	Yes	No

RMCP – rat mast cell protease. Modified from reference 1.

Normally, mast cells are preferentially located adjacent to blood and lymphatic vessels, near or within nerves or in skin appendages in mucosal or connective tissue where they release their granule contents or secrete various cytokines upon stimulation⁸.

Evidences for the connection between nervous system and mast cells have been established by ultrastructural and light microscopic studies showing the close contact of both intestinal mucosal mast cell and mesentery mast cells with vagal cholinergic and peptidergic fibers, and the elevated

expression and release of histamine in intestinal and mesenteric mast cells in response to nerve stimulation^{2,3,4}. Studies have demonstrated that half to two-thirds of mast cells are closely apposed to nerves in the intestinal mucosa in both rodents and humans, with many of the small intestinal nerves in close association with mast cells contain substance P and/or calcitonin-gene related peptide (CGRP)^{3,9}. Furthermore, previous reports had also documented a close association between substance P-containing nerves and mast cells in the rat diaphragm and mesentery¹⁰.

A similar degree of mast cell-nerve association documented in the human gastrointestinal tract was found in the skin¹¹. Interestingly, in inflammatory responses such as intestinal parasitic infestation in the rat¹² or psoriatic plaques in humans¹¹, nerve-mast cell contacts were significantly increased as compared with nonlesional areas. Neuropeptides, such as neurotensin and substance P, induce mast cell degranulation, stimulates vascular endothelial cells and induces granulocyte infiltration through mast cell degranulation¹³. Also the calcitonin gene-related peptide (CGRP) is able to induce vasodilatation, at higher concentrations plasma extravasation, and mast cell degranulation¹⁴.

However, mast cell-nerve communication occurs in both directions and stimulation of mast cells also may activate neurons. Many factors produced by inflammatory and resident tissue cells are involved in the regulation of growth and differentiation of mast cells (**Table 3**), and also are essential for nerve fiber development, outgrowth and its maintenance. An example is the neurotrophin nerve growth factor (NGF), which affects mast cell activity and prevent its apoptosis¹⁵, and also sustain and promote nerve sprouting and growth like observed in studies with peptidergic sensory neurons¹⁶.

Table 3. Regulation of mast cell growth and differentiation.

Rodent (reference)	Primate/Human (reference)
Cytokines/Growth Factors	
SCF/c-kit (gain-of-function mutation)	SCF/c-kit (gain-of-function mutation)
IL3, IL4*, IL9*, IL10*, [IL-13]	[IL-3], [IL-4], [IL-6]
[Eotaxin]	Nerve growth factor
[Nerve growth factor]	
Basic fibroblast growth factor	
Transcription Factors/Signaling Molecules	
Tumor growth factor- β **	Granulocyte-macrophage colony-stimulating factor (GM-CSF)**
	Interferon- α and $-\gamma$ **

SCF – stem cell factor; IL – interleukin; * - role in phenotype switching; ** - down regulators; [] - Entries in brackets are cofactors, rather than playing a primary role. Modified from reference 17.

Besides, studies of mouse bone marrow cells cultured in IL-3 gives rise to cultures that consist of 85% or more mast cells by 4-5 weeks¹⁸. Granulocyte-macrophage colony-stimulating factor (GM-CSF), on the other hand, inhibits the differentiation of IL3-dependent mast cells¹⁹ as does interferon- γ (IFN- γ)²⁰.

Although IL3 is known to contribute to murine mast cell, it is unable to induce human mast cell differentiation from cultures of human cord blood or fetal liver, thus a new growth factor was postulated for human mast cells being identified in

both rodents and primates as stem cell factor (SCF) or c-kit ligand^{21,22,23}. The SCF is produced and expressed on the plasma membrane of a variety of murine and human cell types, including fibroblasts, bone marrow stromal cells, epithelial cells, vascular endothelial cells, and tumor cell lines²⁴, and may also play a role as homing and adhesion molecule for hematopoietic progenitor cells and mast cells²⁵.

Several lines of evidence indicate that interactions between the tyrosine kinase receptor c-kit and SCF are essential for normal mast cell development and survival²². For example, mice with

mutations that result in either markedly impaired c-kit function or a marked reduction in the expression of membrane-associated SCF virtually lack tissue mast cells²⁶, and subcutaneous administration of recombinant human SCF can induce mast cell hyperplasia in vivo in humans²⁷. In addition, SCF promotes mast cell adhesion to fibroblasts and to extracellular matrix components²⁸, thus influencing mast cell migration and distribution.

Interleukin-4 has little ability to sustain the proliferation of mouse mast cells in the absence of IL3, but in its presence, IL4 promotes mouse mast cell proliferation and maturation in vitro of both bone marrow-derived²⁸ and peritoneal mast cells²⁹. In humans, IL-4 by itself has no effects on mast cell survival or proliferation, but interestingly, in synergy with SCF, IL-4 strongly enhances mast cell proliferation. Thus, SCF may primarily regulate resident mast cell survival, whereas IL-4 may promote local proliferation of mast cells. Taken together, these data show that the c-kit ligand synergistically interacts with a number of cytokines to directly augment the proliferative capacity of primitive hematopoietic cells.

Overall, the regulation of tissue mast cell number depends both on the rate of production of mast cell precursors and the length of survival of mature mast cells within tissue. Cell death under physiological conditions most often occurs by apoptosis, and intracellular inducers of apoptosis include the tumor suppressor p53, the protooncogenes c-myc and bax. It is now known that mast cells undergo apoptosis upon withdrawal of IL-3, but the apoptotic changes after IL-3 elimination are prevented by the addition of SCF to cell cultures³⁰ suggesting that SCF could suppress apoptosis. Although, it is known that the effect of SCF on mast cell apoptosis is modulated by other cytokines that are too synthesized in tissues like the transforming growth factor- β 1 (TGF- β 1)³¹ that seems to down-regulate the expression of cell surface c-kit³².

Overall, the present study was aimed to demonstrate the effects of chronic sympathectomy

and vagotomy in the number of mast cells of Wistar rat's mesentery. The results have shown a significant increase of these cells only in the group submitted to sympathectomy, with no significant alteration in the number in those animals submitted to vagotomy when compared with control groups. After wide review of knowledge bases, there were no substantial evidences about vagal or splanchnic nerve-derived substances, like neurotransmitters or neuropeptides, that could act directly promoting mast cells growth, development or apoptosis, leading us to more easily explain the data founded. Although it must be consider that many neuropeptides and neurotrophins can provoke functional changes on status of the microenvironment through modulation of immune and vascular systems. Thus, it is plausible that the close relation between peptidergic fibers, i.e. vagal sensitive afferent fibers, associated with the disequilibrium cause by the absence of sympatic tonus could be leading to the mastocytosis observed in group sympathectomized (SS).

Moreover, mechanisms involved in the synthesis of mast cell growth factors like SCF, IL3 e IL4 likely were affected due the small bowel denervation, although there is no actually scientific evidence to confirm this hypothesis.

In conclusion further studies, immunological and histochemical, will be necessary to better elucidate many aspect of data shown in the present study. Overall, this study has demonstrated that chronic sympathetic denervation of small bowel lead to increase in the number of rat mesenteric mast cells ($p < 0,001$). Although, the vagotomy was not able to significantly increase the number of these cells in mesenteric tissue of Wistar rats.

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