Evidence for Mast Cell Activation in Patients with Therapy-Resistant Irritable Bowel Syndrome

Hinweise auf eine gesteigerte Mastzellaktivität bei Patienten mit therapierefraktärem Reizdarmsyndrom

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Schlüsselwörter
- Colon irritabile
- Reizdarm
- Mastzellen
- Mastzellmediator-vermittelte Symptome
- c-kit

Key words
- irritable bowel syndrome
- IBS
- mast cell
- c-kit
- mast cell mediator release syndrome

Zusammenfassung

Neuere Untersuchungen legen eine wesentliche Rolle von Mastzellen in der Pathogenese des Reizdarmsyndroms nahe. Ziel der vorliegenden Pilotstudie war es, die mögliche pathophysiologische Rolle von Mastzellen in Reizdarmpatienten mit einem neuartigen Untersuchungsansatz näher zu beleuchten. An 20 Patienten mit therapierefraktärem Reizdarmsyndrom wurde mit einer validierten Checkliste, die die Identifizierung von Mastzellmediator-vermittelten Symptomen erlaubt, und mit ausgesuchten Surrogatparametern das Vorliegen einer pathologisch gesteigerten Aktivität von Mastzellen mit gesteigerter Mediatorfreisetzung untersucht. Bei 19 der 20 untersuchten Patienten konnten Mastzellmediator-induzierte Symptome festgestellt werden. In Abhängigkeit von der Mastzellaktivität pathologisch veränderte Koagulation- und Finbrinolyseparameter wurden bei 11 von 12 dahingehend untersuchten Patienten gefunden. Ein weiterer Patient hatte einen pathologisch erhöhten Methylhistamingehalt im Urin. Die vorliegenden Befunde geben einen Hinweis auf eine hohe Prävalenz für eine pathologisch gesteigerte systemische Mastzellaktivität bei Patienten mit einem therapierefraktärem Reizdarmsyndrom. Diese Beobachtung passt zu der Vorstellung, dass aktivierte Mastzellen in die Pathophysiologie des Reizdarmsyndroms eingebunden sind. Reizdarmpatienten mit therapierefraktärer Symptomatik könnten demnach therapeutisch von der Gabe mastzellstabilisierender Medikamente oder von Wirkstoffen, die die Mediatorwirkung an den Erfolgsorganen antagonisieren, profitieren.

Abstract

Previous findings suggested an involvement of mast cells in the pathogenesis of irritable bowel syndrome (IBS). The pathophysiological significance of mast cells is defined both by their number in tissue and by their activity. In the present pilot study activity of mast cells in patients with therapy-resistant IBS was investigated for the first time systematically. Twenty patients with therapy-resistant IBS were investigated for the presence of a pathologically increased mast cell mediator release by means of a validated structured interview suitable to identify mast cell mediator-related symptoms and by determing selected surrogate parameters for mast cell activity. Nineteen of the 20 patients presented mast cell mediator-related symptoms. Pathologically increased mast cell activity-related coagulation and fibrinolysis parameters were detected in 11 of 12 patients investigated in that regard. One patient had an elevated level of methylhistamine in urine. The present data provide evidence that in patients with therapy-resistant IBS a pathologically increased systemic mast cell activity may occur with high prevalence. This finding fits to the idea of an assumed contribution of activated mast cells in the pathophysiology of IBS.

received 29.6.2010
accepted 20.8.2010

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DOI http://dx.doi.org/10.1055/s-0029-1245707
Z Gastroenterol 2011; 49: 191 – 194 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0044-2771

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Introduction

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders seen in primary care and specialist practice characterized by abdominal discomfort and/or pain, bloating and disturbed defecation [1, 2]. The etiology and pathophysiology of IBS remain

poorly understood, although there are several physiologic processes that appear altered in IBS: alterations in the motor response of the gut to stimuli; an altered perception of visceral stimuli via the afferent sensory pathway or the brain-gut-axis; an altered perception of non-noxious visceral stimuli as noxious in the brain. In the last decade several findings suggested that in IBS patients mast cells may be involved in the disturbed sensory-motor function [3 – 7]: (1) An increased number of mast cells has been detected in the colonic and ileal mucosa of IBS patients. (2) In the human intestine, mast cells lie in close proximity to mucosal innervation. (3) Mast cell mediators such as histamine, prostaglandins and proteases excited sensory afferent pathways and evoked visceral hyperalgesia in animal models and as recently shown also in human specimen. (4) The clinical picture of IBS resembles that of gastrointestinally pronounced systemic mastocytosis. (5) There are reports about beneficial effects of orally applied sodium cromoglycate, a mast cell stabilizer, ketotifen (a mast cell stabilizing agent with antihistamine potency) and antihistamines in IBS ([8, 9], further references therein).

In the present pilot study, we investigated the potential relevance of mast cells in the pathogenesis of IBS by a novel approach: it was examined whether therapy-resistant IBS was associated with mast cell mediator-related symptoms [10] as an indicator of a pathologically increased mast cell activity.

Study participants and methods

Twenty patients with therapy-resistant IBS (for details, **Table 1**) were consecutively enrolled in the present study between January 2007 and July 2008. The patients were diagnosed with IBS according to the Rome II guidelines [1] (which were the valid criteria at the time of realization of the study). At that time the patients had not been treated with drugs acting at mast cells. After inclusion into the study the occurrence of mast cell mediator-related symptoms was recorded in a standardized form by means of a previously validated structured interview [11, 12] (for the English version of our questionnaire, see supplementary text to [13]). This structured interview in the main comprises the presence of episodic or ongoing symptoms presumably caused by mast cell mediators during a period of the at least last 2 years forming a pattern of symptoms termed mast cell mediator release syndrome (**Table 2**) and that can be identified by calculating a score value. To add to the clinical findings, in all patients the mast cell mediator tryptase in blood serum was determined. In addition, as potential indicators of an increased mast cell activity selected coagulation (factor VIII, prothrombin fragments F1 + 2) and fibrinolysis (plasmin-antiplasmin-complex PAP, D-dimers, α_2-antiplasmin, tissue-type plasminogen activator tPA) parameters were determined in blood which have been shown to be related to an increased mast cell activity [14, 15]. Twelve of the 20 IBS patients gave their consent to this checkup. To exclude that a pathologically increased mast cell mediator release was secondary to a disease, differential diagnoses which could also evoke one or more of the symptoms observed (for details, see supplementary text to [13]) were ruled out by unchanged pathognomonic laboratory parameters, imaging and/or endoscopic methods concerning this matter.

The study was approved by the local Ethics Committee. All patients gave informed consent prior to the investigation according to the guidelines of the local Ethics Committee.

Table 1 Characteristics of the study population.

IBS patients (n = 20)
age (median, range): 43.5 years, 18 – 61 years
male (n = 5)
– diarrhoea predominant (n = 4)
– constipation predominant (n = 0)
– pain predominant (n = 1)
female (n = 15)
– diarrhoea predominant (n = 8)
– constipation predominant (n = 5)
– pain predominant (n = 2)

Table 2 Frequency of occurrence of signs and clinical symptoms ascribed to episodic unregulated release of mast cell mediators as determined by the structured interview in the 20 patients with irritable bowel syndrome of the present study. Specific morphological alterations in the gastrointestinal tract were excluded by the criteria to define irritable bowel syndrome [1].

signs and symptoms	percent
abdominal	95 %
nausea, non-cardiac chest pain, Helicobacter pylori-negative gastritis	
respiratory	60 %
cough, asthma-like symptoms, dyspnoea	
hepatic	60 %
splenomegaly, hyperbilirubinemia, elevation of liver transaminases, hypercholesterolemia	
splenomegaly	10 %
cardiovascular	95 %
tachycardia, blood pressure irregularity, hot flush	
neuropsychiatric	100 %
headache, neuropathic pain, paresthesia, decreased attention span, difficulty in concentration, forgetfulness, anxiety, sleeplessness, tinnitus	
cutaneous	50 %
flushing, efflorescences with/without pruritus, telangiectasia	
abnormal bleeding	40 %
skeletal	15 %
osteoporosis/osteopenia	
constitutional	95 %
fatigue, asthenia	

Results

Ages of patients ranged from 18 to 61 years, with a median of 43.5 years (**Table 1**). Male to female ratio was 1:3 which is in agreement with the gender distribution of IBS in previous reports ([1, 2]; further references therein). In 12 patients (60 %) diarrhoea was the predominant symptom of IBS, 5 patients (25 %) suffered from constipation-predominant and 3 patients (15 %) from pain-predominant IBS.

In addition to the chronic gastrointestinal symptoms abdominal pain, diarrhoea and/or constipation which were part of the inclusion criterion IBS for the study, patients intermittently or chronically presented mast cell mediator-related symptoms to different degree (**Table 2**). According to the structured interview all but one patient were clearly beyond the cut off score (mean ± SEM 20.2 ± 1.6, median 18, range 7.5 - 34; cut off score ≥ 14) indicating the occurence of a mast cell mediator release syndrome, i.e. it had a prevalence of 95 % in our IBS patients sample. Fifteen patients (75 %) reported a contemporaneous

Table 3 Coagulation (factor VIII [FVIII], prothrombin fragments F 1 + 2) and fibrinolysis (plasmin-antiplasmin complex [PAP], D-dimer [DD], α_2-antiplasmin [AP], tissue-type plasminogen activator [tPA]) parameters determined in blood plasma from 12 of the 20 IBS patients investigated in that regard. Those compounds have been shown to be related to a pathologically increased mast cell activity [14, 15].

IBS patient number	pathologically increased parameters
I	PAP, AP, tPA
II	FVIII, PAP, AP
III	PAP
IV	F1 + 2, DD, AP
V	PAP, AP
VI	FVIII, AP
VII	FVIII, PAP, AP, tPA,
VIII	parameters not pathologically altered
X	FVIII
XI	AP
XVII	FVIII, AP
XIX	F1 + 2, DD

sudden onset of the IBS symptoms and their complex mast cell mediator-related discomforts. In six of those patients the initial manifestation of IBS symptoms and the mast cell mediator-related syndrome had been associated with an infective disease. In four patients both syndromes developed slowly but progressively over years.

The level of the mast cell mediator tryptase in blood was in the normal range in all patients. In one patient an increased level of methylhistamine in urine, a metabolite of mast cell-derived histamine [16], was detected. Coagulation and fibrinolysis parameters which have been shown to be related to increased mast cell activity were found to be pathologically elevated in 11 of 12 patients investigated in that regard (**Table 3**).

Discussion

Previous studies revealed an increased density of mast cells in intestinal tissue of IBS patients compared to those of healthy subjects, thereby, indicating a role of mast cells in the pathogenesis of IBS [3–8, 17]. However, the pathophysiological relevance of mast cells is not only defined by their number in tissue but also by their activity; the latter has not been investigated so far. Therefore, in the present pilot study on 20 therapy-resistant patients with verified IBS according to the Rome II criteria [1] the occurrence of an increased mast cell activity was assessed, albeit indirectly, by the presence of mast cell mediator-related symptoms identified by means of a validated structured interview [11, 13]. According to that structured interview all but one IBS patient clearly were afflicted with symptoms due to a pathologically increased release of mediators from mast cells. The result of the structured interview was supported by pathologically increased levels of mast cell activity-related coagulation and fibrinolysis parameters in 11 of 12 patients investigated in that regard and by an elevated level of methylhistamine in the urine in another patient. The level of tryptase in blood was in the normal range in all patients which is in accordance with recent findings [17, 18]. In this context, it is important to recall that normal levels of tryptase in blood do not exclude a pathologically increased release of mast cell mediators. Several reasons can keep the level of tryptase in blood in the normal range despite an increased release of mast cell mediators: (1) In particular, the tight interaction between tryptase and heparin ([19]; further references therein) may attenuate spill over of exocytosed tryptase (and vice versa) into the vascular bed. (2) Mast cells can selectively release components of their stock of mediators by a differential release process [20]. (3) Dependent upon the micro-environmental factors or the nature of a stimulus within a tissue mast cells do not produce tryptase [21].

The receptor tyrosine kinase Kit plays a central role in regulating the activity of mast cells ([22], further references therein). Therefore, we have examined in a another research project whether in mast cells of patients with a mast cell mediator release syndrome functionally activating genetic alterations of the tyrosine kinase Kit were detectable at the level of mRNA. Into that investigation also the 19 IBS patients of the present study who had a mast cell mediator release syndrome were included. In fact, one or multiple complex mutations and/or activating point mutations (such as D419H, D816V) were detected in the tyrosine kinase Kit in 13 of those 19 IBS patients (but not in gender- and age-matched healthy volunteers) additionally substantiating the result of the structured interview that a mast cell dysfunction may be associated with therapy-resistant IBS (Table 7 in [23]: patients # 102–107, 116–119, 121-122; data from 2 patients have not yet been published).

Interestingly, in 15 of 20 patients the contemporaneous sudden onset of the clinical manifestation of both IBS and mast cell mediator release syndrome points to a yet to be defined form of activation of the immune system as one possible trigger for clinical manifestation. Whether here is a link to the pathogenesis of postinfectious IBS [1, 2] remains to be investigated in future studies.

Taken together, the present data extend the knowledge about a potential contribution of mast cells in the pathophysiology of IBS in that not only the number of mast cells is increased in intestinal tissue of IBS patients but also the activity of the mast cells seems to be pathologically increased, at least in patients with therapy-resistant IBS. Hence, those IBS patients may benefit from drugs stabilizing mast cells and antagonizing their mediators [9, 24], thereby improving so far therapy-resistant symptoms.

The study was supported by grants of the Deutsche Krebshilfe, Novartis UK and the Förderclub Mastzellforschung e. V.

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