

The Role of Mast Cell Degranulation in Ischaemia-reperfusion-induced Mucosal Injury in the Small Intestine

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Abstract

The role of the intestinal mast cell system in the pathophysiology of postischaemic mucosal lesions is not understood. The present goals were to investigate the contributions of mast cells and mast cell-derived vasoactive mediators to mucosal injury caused by arterial occlusion. We evaluated the intestinal ischaemia-reperfusion-induced local morphological changes in mast cell-depleted anesthetized dogs. Animals subjected to complete segmental intestinal ischaemia and reperfusion served as controls. The selective mucosal-type mast cell degranulator Cremophor-El and the nonselective mast cell depleter Compound 48/80 were used to investigate the involvement of mast cells in reperfusion-induced tissue reactions. Ileal biopsies taken at the end of 120 min of ischaemia and after 120 min of reperfusion were evaluated histologically. The number of mast cells was determined and the degree of mucosal damage was evaluated according to the 0 to 5-grade Chiu scale. Mucosal histidine decarboxylase activity was measured in tissue biopsies and the rate of release of histamine was determined from the venous effluent of the segment. In the control group, 120 min reperfusion induced a severe tissue injury. In the Compound 48/80 and Cremophor-El-pretreated groups, the reduction in the baseline number of mast cells was 37% and 53%, respectively, and the basal mucosal histidine decarboxylase activity was significantly increased. In these groups, the ischaemia-reperfusion-induced release of histamine was significantly decreased, and the degree of damage of the intestinal mucosa was significantly reduced. Mucosal mast cell degranulation plays an important role in the initiation of tissue injury after intestinal ischaemia-reperfusion. Depletion of mast cells prior to ischaemia decreases the severity of mucosal damage, probably in consequence of the stimulation of mucosal histidine decarboxylase activity.

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Introduction

Although a number of data support the notion that mast cell (MC) degranulation is an important component of the tissue response elicited by ischaemia-reperfusion, the role of the gastrointestinal MC system in the pathophysiology of postischaemic mucosal barrier lesions is still poorly understood. The mucosal MCs (MMC) of the small intestine are a unique cellular source of both preformed and *de novo* synthesized inflammatory mediators, and are located around host-environment interfaces from which they can influence local tissue reactions.¹ Previously, we demonstrated that even a short interval of intestinal ischaemia induces the release of a variety of MC-derived inflammatory compounds, and results in a spectrum of injury ranging from reversible permeability changes to structural

mucosal damage.²⁻⁴ Our results revealed that the intestinal MC system is very sensitive to hypoxia, with the majority of MMC in the ileal villi already involved in the response to ischaemia after 30 min of arterial occlusion.⁵

The purpose of our present investigation was to determine the impact of MC degranulation on reperfusion-induced mucosal damage. We addressed the possibility that MC depletion prior to ischaemia would attenuate ischaemia-reperfusion-induced destructive tissue reactions. Since the gastrointestinal MMCs are different from the MCs of the connective tissue,^{1,6} an additional objective of the study was to compare MMC and peritoneal-type MC degranulator therapies separately for effectiveness. In our studies, we used selective MMC and peritoneal-type MC depleter

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pretreatments to investigate the role of MC in the mucosal structural alterations and haemodynamic changes induced in the small intestine by a standardized ischaemia-reperfusion challenge in dogs.

Materials and Methods

Experimental Protocol

The experiments described in this article were performed in adherence to the National Institutes of Health guidelines on the use of experimental animals. Approval was obtained prior to the experiments from the Animal Welfare Committee of Szent-Györgyi Albert Medical University.

Three separate groups of experiments were performed on a total of 17 mongrel dogs (average weight 18 kg) under sodium pentobarbital anaesthesia (30 mg/kg iv). Small supplementary doses of pentobarbital were administered when necessary. In the control group ($n = 7$), the left femoral artery and vein were cannulated for the measurement of mean arterial pressure and for blood sampling, respectively. A midline laparotomy was performed and an approximately 30-cm long segment of the terminal ileum was selected. The ileal artery supplying the segment was dissected free from the surrounding mesentery, whereas the accompanying mesenteric vein was left intact to avoid venous congestion. An electromagnetic flowprobe (outer diameter 8 mm) was placed around the vessel to measure ileal blood flow. Small enterotomies were performed antimesenterically at the border of the segment, and two hard polyvinyl tubes were inserted into the intestinal lumen. The tubes were secured with threads to block the intramural collateral blood flow and to maintain the continuity of the bowel. The marginal vessels were divided and ligated, and the mesentery was divided to eliminate any possible connection with the systemic circulation other than the isolated ileal artery branch. Small tributaries of the ileal artery and ileal vein supplying the segment were cannulated to measure local mesenteric arterial and venous pressure, respectively. A silastic balloon catheter (TGS Tonomitor, Tonometrics Inc, Worcester, Massachusetts, USA) was introduced into the lumen of the intestinal segment through a small enterotomy.

Following surgery, a control tissue biopsy was taken from the intact part of the ileum, and ischaemia was then induced by occluding the artery perfusing the segment with an atraumatic vascular miniclip (Mehdorn Clip, Aesculap AG, Germany). At the end of the 120-min ischaemic period, just before the removal of the arterial clamp, a circular section was taken from the oral part of the segment. The arterial occlusion was then released, and subsequent tissue samples were taken from the reperfused intestine after 30 min and 120 min of the reperfusion period.

Blood samples were taken from the venous effluent of

the segment immediately before ischaemia, and after 1, 5, 15 and 30 min of the reperfusion.

In Group 2 ($n = 5$), the experimental protocol was identical to that described above, except that the animals were pretreated with the nonspecific MC depleter Compound 48/80 (Sigma Chemicals) 48 h (0.5 mg/kg iv), 24 h (1 mg/kg iv) and 1 h (0.5 mg/kg iv) before the experiments.⁷

Group 3 ($n = 5$) was pretreated with Cremophor-El (Sigma Chemicals) 48 h (0.2 ml/kg iv), 24 h (0.4 ml/kg iv) and 1 h (0.2 mg/kg iv) before surgery to degranulate MMC.⁸ In these groups, segmental intestinal ischaemia was induced and maintained for 120 min as well. Reperfusion was achieved by removing the arterial miniclip, and blood samples were collected at the beginning (1 min), and then after 5, 15 and 30 min of the reperfusion period. The haemodynamics were monitored for 120 min during reperfusion. Biopsies were taken from the ischaemic and ischaemic-reperfused segments and the animals were killed with an overdose of pentobarbital.

Measurements

Arterial blood samples taken from the femoral artery were analyzed for pO_2 , pCO_2 , HCO_3^- and pH with a blood-gas analyzer (BMS 3Mk2 Blood Micro System, Radiometer, Copenhagen, Denmark). Arterial and venous pressures were measured with Statham P23 Db transducers. Segmental ileal blood flow was measured with an electromagnetic flowmeter (Carolina Medical Electronics 501) and the values were referred to 100 g wet tissue weight (ml/min/100 g). Pressure and flow signals were recorded on a Beckman R611 recorder. Segmental vascular resistance (SVR) was calculated from inflow (arterial) and outflow (venous) pressure differences and blood flow (mmHg/ml/min).

Histamine Assay

Plasma histamine level was measured by means of the slightly modified radioenzymatic method of Beaven et al.⁹ Histamine N-methyltransferase was purified from rat kidney by a modified method of Verburg et al.¹⁰

Histidine Decarboxylase Assay

HDC activity was measured in intestinal biopsy samples by means of a slightly modified method of Watanabe et al.¹¹ Mucosal scrapings were homogenized in 0.05 M phosphate buffer containing 1 mM phenylmethylsulfonyl fluoride, 1 mM N-ethylmaleinimide and 1 mM aminoguanidine (Sigma Chemicals). After homogenizing and centrifugation (1000 g for 30 min at 4°C), the sample was dialyzed against 100 vol 0.001 M phosphate buffer pH 6.5. The dialyzed sample (50 μ l) was incubated with 20 μ l 0.01 M pyridoxal-5-phosphate and 20 μ l 2.5 mM L-histidine at 37°C for 180

min. The reaction was terminated by immersion in boiling water for 30 s, and the histamine content formed in the sample by the reaction was measured by the radioenzymatic method of Beaven et al.⁹ The protein concentrations of the samples were measured according to Lowry et al.¹² HDC activity was given as pmol histamine/ml/mg protein/h.

Intestinal Intramucosal pH (pHi)

pHi was measured by a minor modification of the method of Fiddian-Green.¹³ The method is based on the principle that the $p\text{CO}_2$ in the fluid within a semipermeable balloon attached to a catheter equilibrates with the $p\text{CO}_2$ in the lumen of a hollow organ, and in turn with that in its wall. pHi can then be calculated, provided the arterial HCO_3^- value is known. The HCO_3^- concentration in the wall of the gut is assumed to be the same as that of the arterial blood. The tonometer balloon was filled with 2.5 ml 0.9% saline. After a 30 min equilibration, the $p\text{CO}_2$ of the saline from the tonometer sample was determined. An average of three measurements was used. An arterial blood sample was taken simultaneously and pHi was then calculated by a modification of the Henderson-Hasselbalch equation.

Histological Techniques

Intestinal biopsy samples for light microscopy were rapidly placed into ice-cold Carnoy's fixative and trimmed along the longitudinal axis, and the fixed tissue was attached to a hard cardboard backing to ensure the optimal longitudinal direction of the section. After dehydration with ethanol and chloroform, the samples were embedded in paraffin, sectioned (6 μm), and stained with haematoxylin-eosin and toluidine blue. MCs stained positively were quantitated in villus-crypt (VC) units. All cell counting was performed at X 400 optical magnification by one investigator. Mucosal damage was assessed according to the standard scale of Chiu et al.¹⁴ The grading was performed with the following criteria: grade 0, normal mucosa; grade 1, development of subepithelial Gruenhagen's space at the tip of the villus; grade 2, extension of the space with moderate epithelial lifting; grade 3, massive epithelial lifting with a few denuded villi; grade 4, denuded villi with exposed capillaries; and grade 5, disintegration of the lamina propria, ulceration and haemorrhage.

Statistical Analysis

The Wilcoxon test was used for the estimation of stochastic probability in intergroup comparisons. The Friedman test followed by Dunnett's method was applied for multiple comparisons with a control. Median and mean values, and 25th and 75th percentiles are given. *P* values less than 0.05 were considered significant.

Results

MMC Numbers

MCs were quantified separately in the mucosal villi and in the crypts. There was a statistically significant fall in the number of MCs in both treated groups in the villi under control conditions: the decrease was 37% and 53% for the Compound 48 / 80 and Cremophor-El-pretreated groups, respectively. Ischaemia-reperfusion induced identical changes in MC distribution in both regions of the mucosa, but the decrease was more profound in the villi ($P < 0.01$ in all groups). The total MMC count was statistically significantly decreased below the control level in all groups after 120 min ischaemia and 30 min reperfusion. The number of MCs in the VC units was not significantly altered following 180 min reperfusion as compared with 30 min reperfusion.

pHi Changes

In the control group, the mean basal pH of the arterial blood was 7.42, and pHi was 7.3. For the pretreated groups, similar pH values were observed under control conditions. In Groups 1, 2 and 3, the onset of 120 min arterial occlusion was associated with a progressive decrease in pHi to mean values of 6.78, 6.62 and 6.54, respectively. In Group 1, restitution of the mesenteric blood flow resulted in a slow increase in pHi to 6.95, a value not significantly different from the baseline. In Groups 2 and 3, pHi remained significantly lower than the corresponding value for the control group.

Plasma Histamine Levels

In Group 1, a sudden increase in histamine level was observed at the onset of reperfusion. The mean plasma histamine concentration rose to 10.6 nM. Pretreatment with the nonspecific MC degranulator Compound 48/80 (Group 2) suppressed the rise in plasma histamine level, resulting in a mean peak value of only 5.1 nM at the onset of reperfusion. Almost the same effect was observed (2.65 nM peak value) in Group 3, when MMC degranulation was induced before mesenteric ischaemia.

Tissue HDC Levels

In the nontreated Group 1, the average mucosal HDC activity was 4 pM/mg/h. A significant, 3.5-fold increase was observed in control samples in Groups 2 and 3, pretreated with 48/80 and Cremophor-El, respectively. In both pretreated groups, the HDC level in the 30 min reperfusion samples differed significantly from that for the control group. The average HDC activity increased to 22 pM/mg/h and 31 pM/mg/h, respectively, during the 30 min reperfusion. The elevated tissue HDC activity was decreased toward the baseline level in the 120 min reperfusion samples, but remained higher than that for control samples in both groups (Fig. 1).

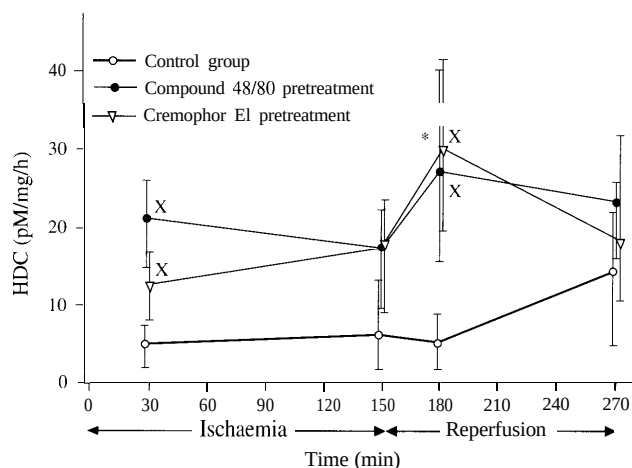


Fig. 1. Median levels of intestinal tissue histidine decarboxylase (pM/mg/h) during intestinal ischaemia-reperfusion. Effects of Compound 48/80 (closed circles) and Cremophor-El pretreatment (open triangles). *: $P < 0.05$, Dunnett test against 0 min sample in the control period. X: $P < 0.05$ in Wilcoxon test against identical sample of control group.

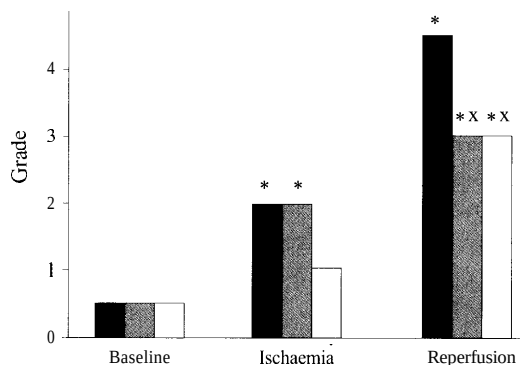


Fig. 2. Grading of mucosal injury after 120 min complete intestinal ischaemia and 120 min reperfusion. Effects of Compound 48/80 (gray bars) and Cremophor-El pretreatments (open bars) on post-occlusive morphological changes. *: $P < 0.05$, Dunnett test against baseline values. X: $P < 0.01$ in Wilcoxon test against identical sample of control group.

Mucosal Damage

The control samples exhibited a grade of injury of 0.5 in each group. In the nontreated group, the 120 min ischaemia induced moderate tissue damage and resulted in a grade 2.1 injury. Reperfusion initiated an increase in mucosal injury and resulted in severe mucosal damage of grade 4.5 by the end of the 120-min reperfusion (Fig. 2).

Semiquantitative evaluation of samples from Groups 2 and 3 revealed a statistically significant ($P < 0.05$) mucosal injury during the 120 min ischaemia. The mucosal damage was significantly exacerbated after the 30 min reperfusion, and there was also a tendency toward a more intense injury following the 120 min reperfusion. However, in these groups, pretreatment with Compound 48/80 or Cremophor-El before the ischaemia-reperfusion challenge statistically significantly protected the ileal

mucosa, and the degree of tissue damage was reduced during 30 min and 120 min reperfusion ($P < 0.05$). These groups each exhibited a grade of injury of 3, a value statistically significantly different from the corresponding one for the nontreated control group. The decreasing villus MMC number correlated significantly with the median grade of injury ($r = 0.8$, $P < 0.05$).

Discussion

Intestinal ischaemia leads rapidly to an impairment of the barrier function of the mucosa, allowing luminal constituents to gain access to the mesenteric lymphatics and portal circulation. Although no single process has been identified as the critical event in ischaemia-reperfusion-induced mucosal damage, it is recognized that granulocyte infiltration and leukocyte-endothelial cell adherence are both major factors in the mediation of mucosal destruction. MCs are located in close proximity to submucosal collecting venules, which are primary targets of leukocyte-endothelial interactions during reperfusion. It has been shown that MC-stabilization protocols reduce leukocyte recruitment, a potential source of oxygen free radicals in the afflicted tissues.¹⁵ It has also been demonstrated that granulocyte recruitment in the postischaemic small intestine may be closely related to the activation of MCs,^{15,16} and recent data indicate that MC may be involved in neutrophil sequestration even after remote ischaemia.¹⁷ If the MMC can act as sentinel cells, providing a chemotactic signal for the neutrophil leukocytes to eliminate the insult, any alteration in the secretory mechanism of MC-derived mediators could have important consequences in local tissue responses.

In our experiments, Compound 48/80 or Cremophor-El pretreatment decreased the number of MMC and clearly ameliorated the reperfusion-induced tissue injury. This suggests that MC degranulation contributes to the tissue destruction during ischaemia-reperfusion. The MMCs are located at high density in the intestinal mucosa, with a close relationship to the microvasculature of the villus. MC granules may be released either through a selective, noncytotoxic exocytosis or through a nonselective (cytotoxic) alteration of the plasma membrane after activation by various stimuli. In our model, intestinal ischaemia was accompanied by the release of histamine upon reperfusion. Histamine liberation appears to be secondary to the generation of oxygen-derived free radicals in the previously ischaemic tissues, as shown by studies in our laboratory.^{3,18} We may therefore assume that MMC discharge their granular contents into the extracellular space following the oxidative stress accompanying reintroduction of the blood flow. In a previous report, we demonstrated the release of type II rat MC protease (RMCP II) into the extracellular environment after intestinal ischaemia in the rat.⁵ RMCP II is a pro-

tease product of MCs found exclusively in the MMC.⁶ We assume that MC degranulation may play a role in the degradation of the proteoglycans of the connective tissue,¹⁹ since type IV collagen, the structural framework of epithelial and endothelial basement membranes, is one of the few known substrates of protease.²⁰

Our data indicate that MMC degranulation results in an enhanced mucosal HDC activity. The synthesis and release of non-MC histamine are largely controlled by the enzyme HDC, and the activity of HDC is an accurate index of mucosal histamine formation.^{21,22} It is recognized that the histamine produced in the gastrointestinal tract is rapidly cleared and degraded by histaminases.¹ Similarly, it has been demonstrated that the regranulation of degranulated MC is a long process.²³ Thus, it may be assumed that MMC degranulation is followed by a compensatory increase in non-MC histamine formation in the intestinal mucosa.

In MMC-depleted animals the ischaemia-reperfusion-induced histamine output was significantly reduced, whereas the mucosal HDC activity was elevated. The inverse changes in mucosal HDC activity and plasma histamine content may indicate a balance between the rates of non-MC histamine synthesis and MMC degranulation. It has been demonstrated that HDC may be involved in mucosal growth and contributes significantly to the intestinal repair that is observed after the small intestine has been injured.²⁴ Accordingly, it is tempting to speculate that the increased mucosal protection in Compound 48/80 and Cremophor-El-pretreated animals may be related to the stimulation of HDC activity prior to ischaemia.

The present results show that reperfusion of the ischaemic intestine decreased pHi, evoked a rise in plasma histamine level, and induced severe structural alterations. Tonometry allows indirect monitoring of the mucosal metabolism, microcirculation and tissue ischaemia.¹³ Arterial occlusion induced a steep decline in pHi, which was followed by a slow increase toward the normal range during reperfusion. Intramucosal pHi changes caused by arterial occlusion were not influenced by previous MMC degranulation. These observations may indicate a metabolically reversible tissue injury and may suggest that MC-mediator-associated vascular reactions do not play a significant role in the blood flow-related acute metabolic changes in the mucosa.

Previously, we demonstrated that MC-derived histamine is involved in the mechanism of the postischaemic vasodilator flow response via the activation of H₂ receptors.²⁵ In our present study, the applied MC degranulator pretreatments ameliorated the degree of postischaemic bowel injury, but did not affect the functional parameter. Several possible mechanisms can account for these results. Although it is recognized that the postocclusive liberation of MC mediators, histamine or

proteases may play a central role in the immediate haemodynamic, permeability and pHi changes, several additional, potentially destructive humoral and cellular pathways may also induce tissue damage. The release of peptide mediators, PAF or peptidoleukotrienes, and the activation of other chemotactic systems may also explain the leukocyte invasion and subsequent leukocyte-mediated tissue damage. Another explanation may be provided by the observation that the current MC depleter pretreatment regimens were only partially effective in reducing the MMC numbers, the MC degranulation and the postocclusive liberation of histamine. This suggests that the residual responses to ischaemia in the Compound 48/80 and Cremophor-El-pretreated animals may be mediated by a MMC population unaffected by the pretreatment regimens. With ischaemia, MMC degranulation occurs and contributes to the injury. The 120 min arterial occlusion induced a significant fall in the number of MMC in the ileal villi, irrespective of the MMC numbers during baseline conditions. This suggests that the MMCs in the ileal villi are involved in response to ischaemia after arterial occlusion. It may be assumed that the proficiency of MMC-degranulator compounds in inhibiting ischaemia-reperfusion-induced morphological and haemodynamic changes is related to their capacity to deplete the mediator content of MMC. This explanation seems plausible, but complete characterization of this process requires an in-depth investigation. However, the results of our previous investigations and the present study lead us to propose that MMCs play an important role in the initiation of tissue damage after intestinal ischaemia. The depletion of MC-derived mediators prior to ischaemia decreases the degree of tissue injury, most probably in consequence of stimulation of the mucosal HDC activity.

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