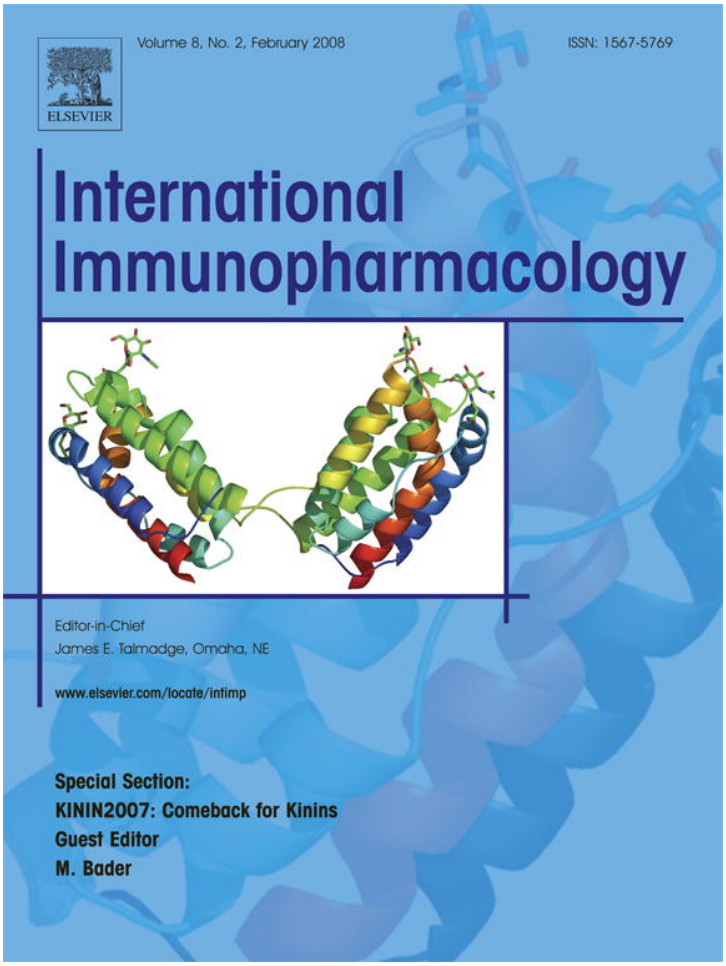

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Small bowel injury associated to allergy is triggered by platelet-activating factor, mast cells, neutrophils and protected by nitric oxide

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Abstract

Allergy to components of the diet is followed by gut inflammation which in children, sometimes progress to mucosal lesions and anaphylaxis. In newborns suffering of cow's milk allergy, bloody stools, rectal bleeding and ulcerations are found. The rat systemic anaphylaxis is a suitable model to study the intestinal lesions associated to allergy. In the present study we used this model to investigate some mechanisms involved. We found that 15 min after antigen challenge of sensitized rats, hemorrhagic lesions develop in the small intestine. The lesions were more severe in jejunum and ileum compared to duodenum. Pretreatment of the rats with a platelet-activating factor-receptor antagonist (WEB-2170) reduced the lesions whereas inhibition of endogenous nitric oxide by L-NAME, greatly increased the hemorrhagic lesions and mortality. Both, lesions and mortality were reversed by L-arginine. The hemorrhagic lesions were also significantly reduced by the mast cell stabilizers, disodium cromoglycate and ketotifen as well as by neutrophils depletion (with anti-PMN antibodies) or inhibition of selectin binding (by treatment with fucoidan). Thus, the intestinal hemorrhagic lesions in this model are dependent on platelet-activating factor, mast cell granule-derived mediators and neutrophils. Endogenous nitric oxide and supplementation with L-arginine has a protective role, reducing the lesions and preventing mortality. These results contributed to elucidate mechanisms involved in intestinal lesions which could be of relevance to human small bowel injury associated to allergy.

1. Introduction

Anaphylaxis is an allergic condition where cardiovascular and respiratory collapse may cause death. Gastrointestinal symptoms, as cramps and diarrhea, are found in humans affected by allergic diseases, mainly those induced by diet components. In children, food allergy frequently progress to

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anaphylaxis [1]. Rubin [2] described the presence of bloody stools in newborns suffering from cow's milk allergy, a common condition in the first year of life. Infantile allergic proctocolitis is another feature of food allergy induced by cow's milk, soy or even breast milk, where rectal bleeding is found and biopsies show ulcerations and signs of inflammation [3,4].

Although the mechanisms involved in the cardiovascular and respiratory symptoms of anaphylaxis are well known, those responsible for the intestinal inflammation and mucosal lesions are still incompletely understood. The rat systemic anaphylaxis is a suitable experimental model to study intestinal lesions associated to allergy, because in this animal species, the small intestine is the shock organ, and marked hemorrhagic lesions are observed minutes after systemic antigen challenge of sensitized animals [5].

Platelet-activating factor (PAF) is a potential mediator of these lesions since it is produced during anaphylaxis and causes alterations in endothelial cells cytoskeleton and permeability, increases neutrophils adherence and transendothelial migration and induces priming of inflammatory cells among other effects in inflammation [6]. There is one report showing PAF involvement in hemorrhagic lesions consequent to anaphylaxis and this was observed in rats passively immunized with monoclonal IgE antibodies [7]. Some effects of PAF are dependent on nitric oxide (NO) production, among them the increased vascular permeability, in organs of gastrointestinal system of rats [8]. NO has been shown to participate in several conditions that lead to gut injury; as a general rule, small concentrations produced under basal conditions seems to be necessary to keep the gut mucosa intact, whereas release of large amounts of nitric oxide, by stimuli that induce the expression of inducible nitric oxide synthase, may cause injury [9]. Mast cell granule-associated mediators play a central role in allergic inflammation and are responsible for the early vascular events. However, their contribution to intestinal mucosa lesions associated to allergy is not known. Neutrophils are potentially able to cause tissue injury. For neutrophils to cause tissue injury a multistep paradigm was proposed, in which several mediators participate in neutrophils priming and triggering, where neutrophils adherence to the endothelium is a prerequisite for subsequent tissue injury [10]. In inflammation, the first step towards leukocyte contact to endothelium is mediated through reversible selectin carbohydrate interactions [11]. The involvement of neutrophils in the cascade of events leading to gut mucosal injury caused by allergic reactions, however, remains to be determined.

In the present study we investigated the contribution of PAF, endogenous NO, mast cells granule-derived mediators, neutrophils and selectins to the small bowel injury, represented by hemorrhagic lesion, induced by a sub-lethal anaphylactic reaction in rats. It was found that the segments of the small bowel (duodenum, jejunum and ileum) presented marked hemorrhagic lesions which were dependent on PAF, mast cell granule-associated mediators and neutrophils. Selectins blockade did also inhibit hemorrhagic lesions. While PAF was injurious, endogenous NO seems to exert a protective role since inhibition of its synthesis markedly increased the lesions and turned a non-lethal into a lethal allergic reaction. Moreover, the lesions and mortality were reversed by supplementation with the substrate for NO synthesis, L-arginine.

2. Materials and methods

2.1. Animals

Male Wistar rats 2 months old (160–180 g) obtained from our own animal facilities, were used in this study. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA), and approved by the Ethics Committee for Animal Research, from the Biomedical Sciences Institute, USP.

2.2. Experimental model

Rats were sensitized with an intraperitoneal (i.p.) injection of ovalbumin (10 g/rat) in aluminum hydroxide ("Alumen", Aldrox-Wyeth; 10 μg/rat). Fourteen days after the sensitization, the animals were challenged with an intravenous (i.v.) dose of ovalbumin (1 mg/kg) in sterile isotonic saline and 15 min later they were killed and the small intestine removed. The survival of the animal was monitored up to 24 h after immunological challenge.

2.3. Measurement of hemoglobin content in small bowel segments

Increased hemoglobin content, taken as index of hemorrhagic lesion, was assessed by a colorimetric assay based on the conversion of hemoglobin to cyanometahemoglobin using Drabkin's solution [12]. Fifteen minutes after immunological challenge the rats were anaesthetized with ether and exsanguinated, a portion about 2 cm of each small bowel segment (duodenum, jejunum and ileum) was removed. The mucosa of each segment was extensively washed (more than 20 ml/segment) until no stool could be observed. The tissues were then weighed and incubated with Drabkin solution (8 ml/g tissue). After 24 h tissues were removed and the solution centrifuged for 10 min at 5000 ×*g*. The concentration of hemoglobin in the supernatant solution was measured spectrophotometrically in an ELISA plate reader, at 540 nm. The optical density obtained was compared to a rat hemoglobin standard curve, and the values of hemoglobin in the tissues were expressed as mg of hemoglobin/g wet weight of tissue. Since a colorimetrical method for hemoglobin was employed as index of hemorrhagic lesion, histological preparations of intestine sections (stained with hematoxylin/eosin) were performed to confirm the presence of intestinal hemorrhagic lesion. Basal hemoglobin intestinal content was determined in sensitized rats that received saline i.v. instead of the antigen.

2.4. Pharmacological modulation on platelet-activating factor and nitric oxide

To study the participation of PAF and NO in the hemorrhagic lesions, groups of animals were treated with a PAF-receptor antagonist, WEB-2170 (5 mg/kg), an inhibitor of NO synthesis, Nω-nitro-L-arginine methyl ester (L-NAME 10 mg/kg), or L-arginine (300 mg/kg), the physiological substrate for NO production. For control purposes groups of animals were treated with the inactive enantiomers D-NAME (10 mg/kg) and D-arginine (300 mg/kg). The role of endogenous NO was also studied (with the use of L-NAME and L-arginine as described above) on the rate of mortality of rats over a period of 24 h after the antigen challenge. All drugs were dissolved in sterile isotonic saline and given i.v. 30 min before the challenge.

2.5. Mast cell inhibition, neutrophils depletion and selectin blockade

Rats were treated i.v., 30 min before antigen challenge, with the mast cell stabilizers, disodium cromoglycate (30 mg/kg) or ketotifen

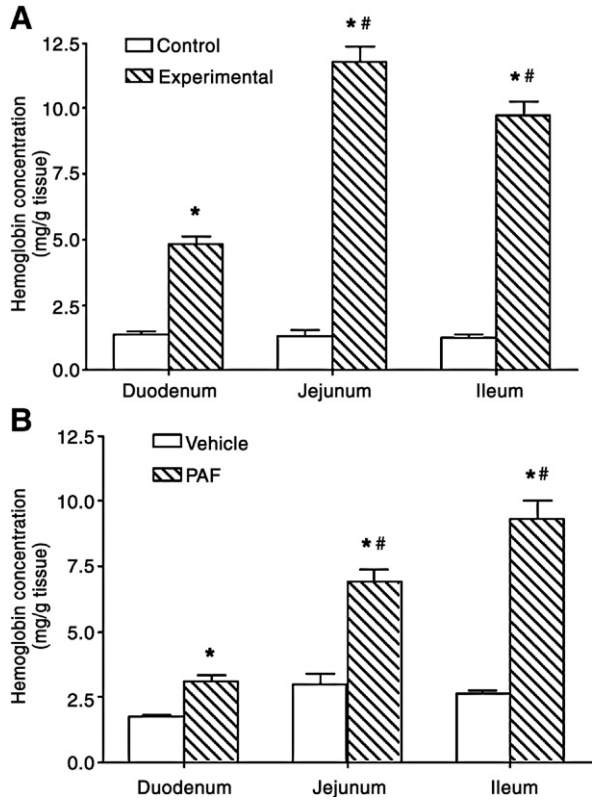

Figure 1 Intestinal hemorrhagic lesions induced by immunological challenge (A) or platelet-activating factor injection (B). Hemoglobin concentration was measured 15 min after the stimuli (immunological challenge or platelet-activating factor). In A the selected tissues from 12 immunized and challenged rats (experimental group) were compared to tissues from 12 non-immunized rats that received the antigen i.v. (control group). The values of this control were not significantly different from those obtained in non-manipulated rats. In B, tissue hemoglobin in 5 rats injected i.v. with platelet-activating factor (3 μg/kg, $n=7$) were compared to 5 rats from the control group which received the platelet-activating factor vehicle (0.25% bovine serum albumin in saline). Data represent the mean±SEM of hemoglobin concentration (mg/g of wet tissue).*$P<0.05$ comparing control and experimental groups; #$P<0.05$ comparing data from tissues of the same group.

(5 mg/kg). Depletion of neutrophils was achieved by intravenous administration of 100 μl of rabbit anti-rat neutrophil hyper immune serum, 6 h before the challenge. Immediately before the immunological challenge a 20 μl aliquot of blood was taken and total and differential (smears stained with Giemsa dye) cell counts performed to confirm the neutrophil depletion. The treatment depleted more than 90% of neutrophils. Control rats were treated with the same amount of serum from non-immunized rabbits. To inhibit neutrophils adherence to the endothelium, rats were treated with the selectin antagonist, fucoidan (30 mg/kg). Fucoidan was diluted in sterile saline and injected i.v. 30 min before the immunological challenge. In another set of experiments, compound 48/80 (0.5, 1.0 mg/kg) diluted in sterile isotonic saline, was injected i.v. and intestinal hemoglobin concentration was measured 15 min later.

2.6. Statistical analysis

Results are shown as means (±SEM) and were analysed with a two-way analysis of variance, followed by Tukey's multiple comparisons. Values of $P<0.05$ were taken as showing a significant difference between means. Data were compared against their own controls. No significant difference was observed between the experimental non-treated group with the experimental groups treated with the drug diluent or with the irrelevant antibodies.

2.7. Materials

The following compounds were purchased from Sigma Chemical Co., St. Louis, USA—ovalbumin (Grade III), rat hemoglobin, disodium cromoglycate, ketotifen, L- and D-NAME, L- and D-arginine, fucoidan and compound 48/80. Aluminium hydroxide (Aldrox®) was obtained from Wyeth, Whitehall Ltd., Brazil. Rabbit anti-rat neutrophil hyper immune serum was purchased from Accurate Chemical & Scientific Corp., Westbury, N.Y., U.S.A. The PAF (1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphorylcholine) was purchased from Cayman Chem. Co. Ann Arbor, USA. The WEB-2170 was supplied by Boehring Ingelheim, Germany.

3. Results

3.1. Hemorrhagic lesions in small bowel segments

Hemorrhage in small bowel segments (duodenum, jejunum and ileum) of sensitized rats challenged intravenously with the antigen (ovalbumin) was evaluated by measuring tissue hemoglobin content as described in Material and Methods. As shown in Fig. 1A, the hemoglobin concentration in small bowel segments of control rats (sensitized animals that received saline i.v.) was low and not different among them, but the concentration was markedly increased after antigen challenge, in all segments. Although an expressive hemorrhage was detected in the three segments, differential sensitivity to immunological challenge

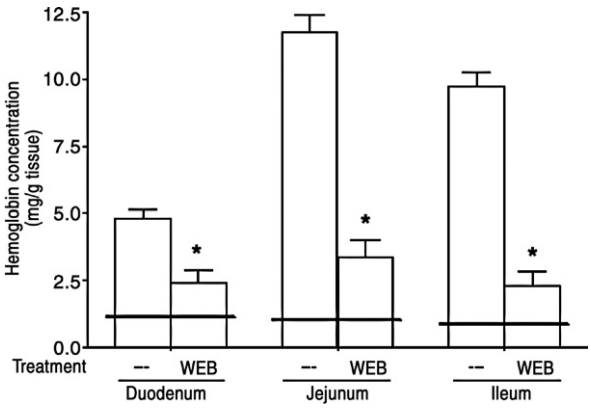

Figure 2 Effect of a platelet-activating factor-antagonist on intestinal hemorrhagic lesions induced by immunological challenge. Fifteen minutes after immunological challenge hemoglobin concentration was measured in the segments of small bowel of the control group treated i.v. with the vehicle of WEB2170 and compared to the experimental group treated i.v. with the platelet-activating factor-antagonist WEB-2170 (5 mg/kg), 30 min before challenge. *$P<0.05$ comparing WEB-2170-treated group with the treated group; (non-treated group $n=6$, WEB-2170 group $n=7$). Data represent the mean (±SEM) of hemoglobin concentration (mg/g of wet tissue). Lines through bars represent the mean of basal concentration of hemoglobin present in tissues from non-immunized rats that received the antigen i.v.

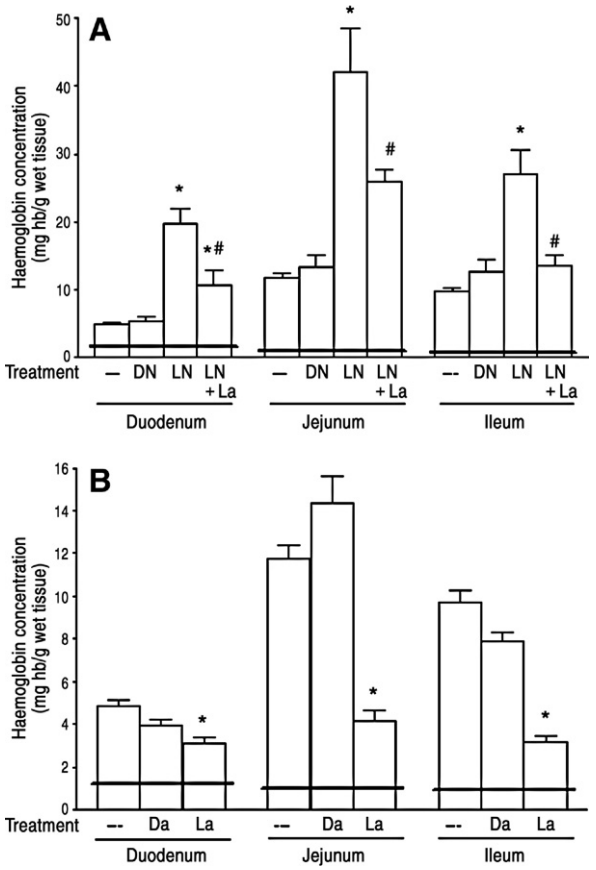

Figure 3 Role of endogenous nitric oxide on intestinal hemorrhagic lesions induced by immunological challenge. Endogenous nitric oxide production was inhibited treating animals with L-NAME (10 mg/kg). L-arginine, substrate for nitric oxide synthesis, was given at 300 mg/kg. Each of them was injected i.v., 30 min before challenge. Hemoglobin concentration was measured 15 min after immunological challenge. In A is shown the effect of L-NAME (LN, n = 9), D-NAME (DN, $n = 6$) or a combination of L-NAME and L-arginine (LN + La, $n = 7$) on the hemorrhagic lesions. In B is shown the effect of L-arginine (La, $n = 7$) and D-arginine (Da, $n = 6$) on the hemorrhagic lesions. Data represent the mean ± SEM of hemoglobin concentration (mg/g of wet tissue). $^{*}P < 0.05$ comparing treated with non-treated groups; $^{\#}P < 0.05$ comparing the LN + La with LN group. Lines through bars represent the mean of basal concentration of hemoglobin present in tissues from non-immunized rats that received the antigen i.v.

was observed. The increase in hemoglobin content varied among the segments: 900, 750 and 350% increase over control levels in jejunum, ileum and duodenum, respectively. These values were statistically different when compared among them. Histological analysis of the gut segments confirmed the occurrence of hemorrhage in all segments (data not shown). For comparative purpose we injected a group of rats with platelet-activating factor and measured the hemorrhagic lesions. Lesions were not observed when 1 μg/kg of platelet-activating factor was injected i.v. (not shown); with 3 μg/kg the localization and intensity of the gut lesions were comparable to those obtained with immunological stimulation (Fig. 1B) and with 10 μg/kg the hemorrhagic lesions were much more intense than those caused by antigen challenge (data not shown).

3.2. Effect of a platelet-activating factor-receptor antagonist on the hemorrhagic lesions

Sensitized rats were pretreated with the platelet-activating factor-receptor antagonist WEB-2170 (5 mg/kg) given i.v. 30 min before the antigen challenge. Fig. 2 shows that the antagonist of platelet-activating factor-receptor was able to significantly reduce the hemorrhagic lesions. The range of inhibition varied from 50% in duodenum to near 75% in jejunum and ileum. WEB-2170 treatment of control rats did not modify the hemoglobin concentration in any tissue segment (data not shown).

3.3. Modulation of the hemorrhagic lesions and lethality by nitric oxide

The contribution of endogenous NO to the hemorrhagic lesions was assessed by pretreatment of the animals with an inhibitor of NO synthesis (L-NAME 10 mg/kg) or by giving the substrate for NO synthesis, L-arginine (300 mg/kg). A group of rats was given L-arginine and L-NAME together. As control for L-NAME, its enantiomer D-NAME was used. Whereas D-NAME had no effect on the intensity of hemorrhagic lesions (Fig. 3, graph A), L-NAME strongly increased the intensity of the lesions in the three segments of small bowel analyzed. L-arginine given together with L-NAME partially reversed the effect of L-NAME in the duodenum and jejunum and totally reversed it in the ileum. Rats that received only L-arginine (Fig. 3, graph B) presented reduced levels of hemoglobin, particularly in jejunum and ileum, but its content was still above the basal levels. Treatment with D-arginine had no effect on hemoglobin levels.

When the survival of the animals was monitored, we found that 24 h after the immunological challenge only one rat from the saline treated group died (Fig. 4). In contrast, L-NAME pretreatment had a severe effect on the outcome of allergic reaction—80% of the animals died in the first hour following antigen challenge, and one after 18 h. The lethality induced by L-NAME was reversed by L-arginine administered together with L-NAME.

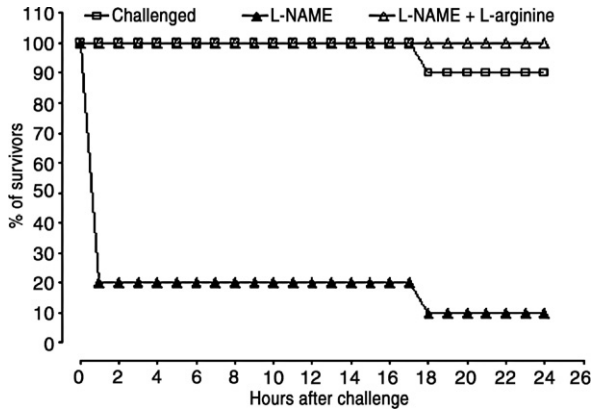

Figure 4 L-NAME treatment transformed a non-lethal in a lethal anaphylaxis. After 24 h of the immunological challenge only one rat from the saline treated group died whereas in the group treated with L-NAME (10 mg/kg, i.v.), 90% of the animals died during this period. The lethality induced by L-NAME was reversed by L-arginine (300 mg/kg, i.v.) administered together with L-NAME ($n = 10$ per group).

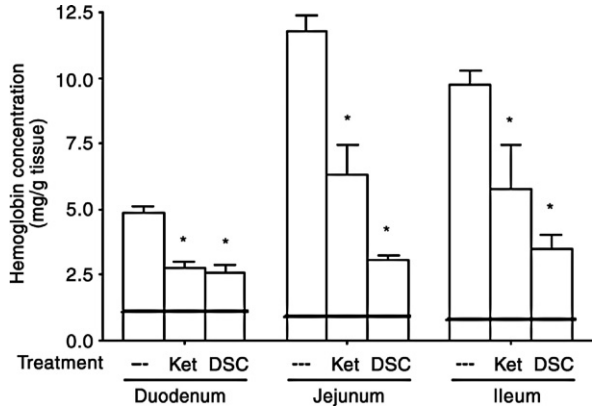

Figure 5 Involvement of mast cells on intestinal hemorrhagic lesions induced by immunological challenge. Hemoglobin concentration was measured 15 min after the immunological challenge of immunized rats. Ketotifen (Ket, 5 mg/kg, $n=6$) or disodium cromoglycate (DSC, 30 mg/kg, $n=6$) were given i.p. 30 min before antigen challenge. Control group ($n=5$) received the vehicle of the drugs (saline), 30 min before antigen challenge Lines through bars represent the mean of basal concentration of hemoglobin present in tissues from non-immunized rats that received the antigen i.v. Data represent the mean ± SEM of hemoglobin concentration (mg/g of wet tissue) *$P<0.05$ comparing the treated with the control group.

Treatment of control rats (immunized and not challenged) with L-NAME, L-arginine or both did not affect the concentration of hemoglobin in intestinal segments (data not shown).

3.4. Involvement of mast cells, neutrophils and selectins in the hemorrhagic lesions

The involvement of mast cell granule-derived mediators in the intestinal hemorrhagic lesions was studied by the administration of two mast cell "stabilizer" drugs, disodium cromoglycate (DSC) and ketotifen, given before antigen challenge. Both drugs significantly reduced the hemoglobin concentration in all segments of the small intestine (Fig. 5). These treatments did not affect the concentration of hemoglobin in intestinal segments of control rats (immunized and saline challenged).

Table 1 Intestinal hemorrhagic lesions produced by administration of compound 48/80

	Hemoglobin concentration (mg of hb/g wet tissue)		
	Duodenum	Jejunum	Ileum
Control	1.4±0,1	1.5±0.2	1.2±0.2
48/80 (0.5 mg/kg)	1.5±0.1	4.4±0.3*	3.3±0.3*
48/80 (1 mg/kg)	2.5±0.2*	4.6±0.3*	2.9±0.3*

This table shows hemoglobin concentration in intestinal segments 15 min after injection with compound 48/80 (0.5 or 1 mg/kg, i.v.). The control group received an i.v. injection of isotonic saline. Values shown are the mean (±SEM), results from 5 animals in each group. *Significantly different from control group, $P<0.05$.

Moreover, injection of the compound 48/80, a well recognized connective tissue mast cell degranulating agent, induced hemorrhagic lesions in all intestinal segments (Table 1). The hemoglobin values were lower with 48/80 than with antigen challenge. A higher dose of 48/80 (2 mg/kg) killed all animals in less than 5 min. To investigate the role of neutrophils, the hemorrhagic lesions were evaluated in animals depleted of circulating neutrophils. Anti-rat neutrophils serum was given i.v. in a dose that reduced by more than 90% the number of circulating neutrophils, measured 6 h after treatment with the antiserum. At this time, the sensitized animals were challenged with the antigen and hemoglobin content measured in intestine segments. Data presented in Fig. 6A show that hemorrhagic lesion were significantly less intense in jejunum and ileum of the

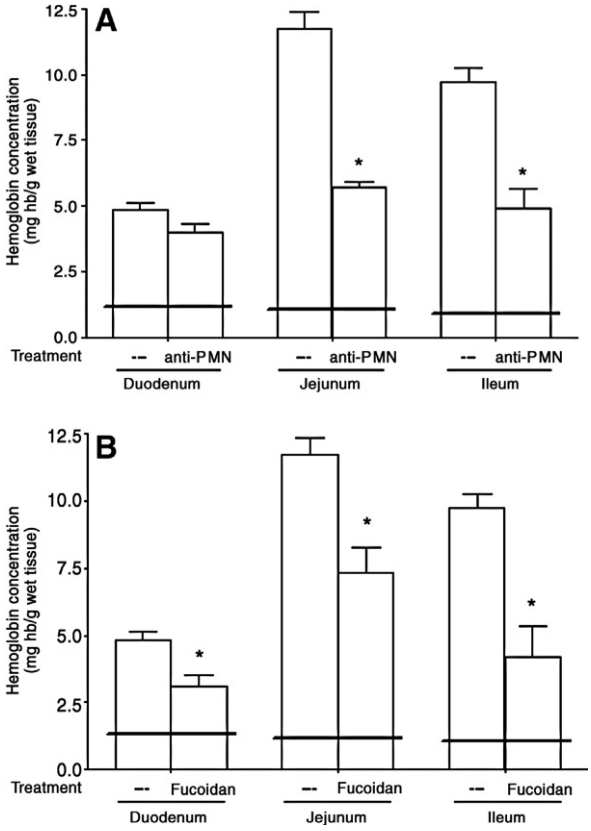

Figure 6 Participation of neutrophils (A) and selectins (B) on intestinal hemorrhagic lesions induced by immunological challenge. Hemoglobin concentration was measured 15 min after the immunological challenge of immunized rats. In A, rats were treated with anti-rat neutrophils serum i.v. 6 h before immunological challenge (anti-PMN, $n=7$). Control group ($n=5$) was treated with a non-related antiserum. In B the rats were treated with fucoidan (30 mg/kg, i.v., $n=7$), 30 min before the immunological challenge. Non-treated group received NaCl 0.9% before antigen challenge. Data represent the mean ± SEM of hemoglobin concentration (mg/g of wet tissue). Lines through bars represent the mean of basal concentration of hemoglobin present in tissues from non-immunized rats that received the antigen i.v. *$P<0.05$ comparing the treated with the respective control groups.

neutrophils-depleted group, whereas the hemoglobin content in duodenum was not modified by neutrophils depletion. The control group (treated with a rabbit antiserum of unknown specificity) did not differ significantly from the not treated group. Since selectins are required for the initial contact between neutrophils and endothelium, we therefore assessed the effect of pretreatment with a selectin antagonist (fucoidan 30 mg/kg). Fig. 6B shows that fucoidan pretreatment, reduced the hemorrhagic lesion in the three intestinal segments analyzed. This dose of fucoidan reduced the neutrophils infiltration (76%) into the peritoneal cavity of sensitized rats challenged i.p. with ovalbumin. In control rats (immunized and not challenged) fucoidan treatment did not affect the hemoglobin concentration in small intestine (data not shown).

4. Discussion

We described here the involvement of endogenous PAF, NO, mast cell granule-derived mediators and neutrophils, in the hemorrhagic lesions that develop in the small intestine of sensitized rats following systemic antigen challenge. First we observed that all three segments of the small intestine (duodenum, jejunum and ileum) presented hemorrhagic lesions soon after antigen challenge and that the intensity of the hemorrhage varied among the segments analyzed. Endogenous platelet-activating factor seems to contribute to these lesions while endogenous nitric oxide has a protective role, not only in the development of the lesions but also in animals' survival to the anaphylactic reaction. Finally, we found that both, mast cells and neutrophils contribute to the development of the hemorrhagic lesions.

In our experiments hemorrhage occurred in all segments of the small intestine being more intense in jejunum and ileum than in the duodenum corroborating previous finding by Levine and Saltzman [13]. However in their study the lesions were more intense in jejunum than in duodenum and ileum. Differences in the type of adjuvant and in the protocol of immunization may account for these differences.

Treatment with the PAF-receptor antagonist (WEB-2170) was able to inhibit from 50 up to 75% the hemoglobin content in small intestine segments indicating that PAF contributes to the onset of the hemorrhagic lesions. Pellón et al. [7] obtained similar results in a slightly different model, where rats were passively immunized with monoclonal IgE antibodies whereas we used a model of active anaphylaxis. Moreover, we extended the observations made by Pellón et al. [7], showing that all segments of small intestine are affected by anaphylaxis and modulated by PAF. These results implicate PAF as mediator of hemorrhagic lesion in anaphylaxis. Functional PAF-receptor is constitutively expressed in intestinal epithelium of rats and humans, where it seems to play a role in the induction and regulation of gut inflammation [14,15]. In our study, intravenous injection of PAF induced a pattern of hemorrhagic lesions in small intestine segments similar to that observed in anaphylaxis; less intense in duodenum than in jejunum and ileum. This differential response of intestinal segments to PAF and immunological challenge suggests that the number or sensitivity of PAF receptors varies along the small intestine. Regarding which is the cell type responsible for the production of the PAF that is contributing to the hemorrhagic lesions in this model, mast cell is a natural candidate since it is a well known source of PAF [16,17]. However, we can not exclude other cell types since, at least in mice, PAF is crucial for fatal IgE-induced anaphylaxis even in mast cell-deficient animals [18]. Strait et al. [19] have shown that IgE and IgG may trigger distinct pathways of anaphylaxis, being the IgE pathway more dependent on histamine released by mast cells (but also with PAF participation) and IgG pathway strongly dependent on PAF released by cross-linking of FcγRIII on macrophages. Since our immunization protocol induces both IgE and IgG isotypes, it is possible that the PAF involved in the intestinal lesions comes from macrophages as well.

We showed here that in the hemorrhagic lesions caused by anaphylaxis, NO plays a protective role; L-NAME given before challenge enhanced the hemorrhage, in contrast to L-arginine treatment which inhibited the lesions. It has been previously reported that NO-generating compounds were able to inhibit intestinal hemorrhage induced by anaphylaxis [20], however, the lesions were not affected by an inhibitor of nitric oxide production (L-NMA). One possible explanation for this conflicting result could be the type of antibody isotypes involved. In the case of Tavares et al. [20] anaphylaxis was triggered exclusively by IgE antibodies (passive transfer of monoclonal IgE anti DNP antibodies) whereas in our case, the protocol of immunization employed, induces also IgG2a besides IgE [21].

In the experimental model used in this study, nitric oxide was probably produced by constitutive NO-synthases (endothelial or neuronal isoforms) since the time elapsed between challenge and evaluation of intestinal hemorrhage (15 min) is not sufficient for the expression of the inducible form of NO-synthase (iNOS). Also, under basal conditions, iNOS mRNA was not detected in small intestine in rats [22,23], although it is expressed in the ileum of mice [24]. Moreover, NO produced by constitutive NO-synthases seems to have a protective role in tissue damage in LPS-induced inflammation [25]. Additionally, Vallance et al. [26] found that constitutive NOS knock out mice were more susceptible to trinitrobenzensulfonic acid-induced colitis. The protective effect of endogenous NO in our model was so important that L-NAME, a non-selective inhibitor of NO-synthases [27], turned a non-lethal allergic reaction into a lethal one. The potentiating effect of L-NAME on hemorrhage and mortality was reversed by administration of a high dose of L-arginine. Also, the administration of L-arginine alone was very efficient to inhibit the hemorrhage, particularly in jejunum and ileum. This effect of L-arginine has been observed in other models of intestinal injury such as that induced by trinitrobenzenesulphonic acid [28] and indomethacin [29,30]. Jiménez et al. [31] showed a protective role for L-arginine in ibuprofen-induced rat gastric mucosal damage.

Our results indicate that the mast cell granule-associated mediators also contribute to the hemorrhagic lesions caused by anaphylaxis since the mast cell "stabilizers" (DSC and ketotifen) inhibited the intestinal lesions and a well known mast cell degranulating agent, compound 48/80 [32] reproduced the lesions. Ketotifen was shown to inhibit the activation of both mast cell types, the connective tissue and the mucosal. Similarly effect was attributed to DSC, which acts probably by phosphorilation of a 78-kDa protein related to moesin [33,34]. The mast cell degranulating agent,

compound 48/80 injected intravenously induced intestinal hemorrhagic lesions which showed a pattern similar to that caused by anaphylaxis. The observation that compound 48/80 caused less intestinal hemorrhage than the systemic anaphylaxis can be explained by the fact that compound 48/80 activates only one type of mast cells, the connective tissue one [35]. Since in the intestine both connective tissue and mucosal mast cells are found, the weaker response generated by 48/80 would be expected.

Although the central role of mast cells in anaphylaxis is well established, some symptoms of anaphylaxis can occur independently of the presence of these cells, as it was found in studies that used mast cell-deficient rats [36,37]. In our study one of the symptoms of systemic anaphylaxis, the intestinal hemorrhagic lesions, seems to be dependent on mast cells as discussed above. However, we do not know if the mast cells from the intestinal mucosa are the ones contributing to the lesions or whether mast cells from other anatomic sites are activated and the mediators released act systemically to injure the intestinal mucosa.

Our results implicated the neutrophils as effectors of intestinal hemorrhage; reduction of neutrophil number (by treatment with anti-neutrophil antibodies) or inhibition of adherence to endothelium by treatment with a selectin antagonist (fucoidan) prevented the development of the haemorrhagic lesions. This important role of neutrophils in intestinal lesions induced by anaphylaxis is in accordance with results obtained by Kimura et al. [38], showing that the mortality associated with anaphylactic shock in mast cell-deficient mice was severely reduced by anti-neutrophil serum. A prerequisite for neutrophils to cause endothelial injury is its activation. A multi step paradigm involving Fc gamma receptors and mediators produced by activated endothelium such as platelet-activating factor, nitric oxide and endothelins has been proposed [10]. In active immunization, as we did in our study, the rats produce IgE and IgG antibody isotypes, and thus, Fc gama receptors-activation of neutrophils can occur. Neutrophils also present a particular type of IgE receptor called Mac-2/εbp [39], and occupation of this receptor was shown to activate neutrophils [40].

In conclusion we showed the participation of endogenous platelet-activating factor and nitric oxide, mast cells, neutrophils and selectins in a web of very rapid events culminating in hemorrhagic lesions in small bowel induced by anaphylaxis. They also show an important protective role of nitric oxide, preventing gut lesions and mortality and suggest that L-arginine supplementation could be a useful strategy to treat bowel diseases of allergic etiology, at least the acute phase, as observed in this study. In chronic inflammatory conditions, with high expression of iNOS and changes in the inflammatory milieu, the effect of L-arginine could de different. The role of cNOS and iNOS in intestinal injury is complex and L-arginine supplementation should be considered with caution.

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