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Original Article PHYSIOLOGY

Systemic peptide inhibitor of transendothelial migration administration attenuates microvascular leukocyte adhesion in an ovalbumin-induced mouse model of allergic inflammation

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ABSTRACT

OBJECTIVE: Allergic rhinitis (AR) is driven by type 2 immune polarization and excessive leukocyte trafficking. Current pharmacotherapies act symptomatically rather than at the level of transendothelial migration (TEM). Peptide inhibitor of TEM (PEPITEM) is a B lymphocyte-derived peptide that engages endothelial cadherin-15 to stimulate sphingosine-1-phosphate secretion and inhibit lymphocyte firm adhesion. Although effective in Type 1 autoimmune and metabolic inflammation, PEPITEM has not been examined in Type 2 allergic disease. To evaluate whether systemic PEPITEM administration attenuates systemic leukocyte trafficking in an ovalbumin (OVA)-induced murine AR model, using desloratadine as a clinically established comparator.

METHODS: Thirty-two C57Bl/6 mice (16 males, 16 females; 10–12 weeks; 20–25 g) were randomized to four groups (n=4 per sex per group): control, allergy, allergy+desloratadine (10 mg/kg oral gavage), and allergy+PEPITEM (200 µg/200 µL intraperitoneal). AR was induced by intraperitoneal OVA/alum sensitization (days 1–13) and intranasal 1% OVA challenge (days 22–25). Leukocyte rolling counts, stationary, and adherent leukocytes in mesenteric post-capillary venules were quantified by intravital microscopy (IVM). Peripheral leukocyte differentials were determined by hemogram. One-way analysis of variance was used as the primary analysis.

RESULTS: OVA sensitization and challenge increased peripheral lymphocytosis ($p=0.0446–0.0059$) and eosinophilia ($p=0.0019$) and significantly elevated stationary leukocytes and adhesion ($p=0.0003$) in mesenteric venules, while rolling was statistically unchanged. PEPITEM reduced firmly adherent leukocyte density ($p<0.05$ vs. allergy group) and normalized blood eosinophilia, with effects comparable to those observed in the desloratadine-treated group. Tissue oxidative and inflammatory marker analysis showed limited organ-specific changes; PEPITEM reduced myeloperoxidase activity in the liver ($p=0.0158$) and colon ($p=0.0288$), whereas malondialdehyde and glutathione levels were largely unchanged across tissues.

CONCLUSION: In this study, systemic PEPITEM administration reduces systemic leukocyte adhesion and several allergic phenotypes during OVA-driven Type 2 inflammation, with effects of similar magnitude to desloratadine.

Keywords: Allergic rhinitis; intravital microscopy; leukocyte trafficking; peptide inhibitor of transendothelial migration.

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Allergic rhinitis (AR) is one of the most prevalent chronic inflammatory diseases worldwide, affecting 15–25% of adults in Western populations [1] and 11.8–36.4% of the adult population in Turkiye [2]. It is defined by nasal congestion, rhinorrhea, sneezing, and pruritus in response to harmless environmental allergens and is associated with substantial impairment in quality of life, sleep disturbance, fatigue, and cognitive dysfunction [3]. AR is increasingly recognized as a systemic disease. Activated T lymphocytes, elevated circulating cytokines, and altered leukocyte trafficking produce distant organ effects beyond the nasal mucosa [3, 4]. Despite this burden, available pharmacological options – H1-antihistamines, intranasal glucocorticoids, leukotriene receptor antagonists – act on downstream mediators rather than the underlying immune dysregulation, and a substantial proportion of patients achieve only partial benefit [5].

The pathophysiology of AR is dominated by Type 2 immune polarization. Repeated mucosal allergen exposure causes dendritic cells to activate $CD4^+$ T helper 2 (Th2) lymphocytes, which drive immunoglobulin E (IgE) synthesis, mast cell sensitization, and eosinophil recruitment [6, 7]. Allergen cross-linking of IgE on sensitized mast cells releases histamine, tryptase, leukotrienes, and prostaglandins, which together activate endothelial cells, increase vascular permeability, and upregulate adhesion molecule expression to enhance leukocyte infiltration of the nasal mucosa [8]. $CD4^+$ T lymphocytes [9], eosinophils, and basophils accumulate at the inflamed site, and their abundance correlates with symptom severity [10]. Targeting leukocyte trafficking is therefore a mechanistically rational therapeutic strategy.

The leukocyte adhesion cascade encompasses up to seven discrete steps: Margination, tethering (capturing), rolling, activation and firm adhesion, intraluminal crawling, transmigration, and interstitial migration [11]. Rolling is mediated by selectin-ligand bonds; firm adhesion requires integrin activation (principally LFA-1/ICAM-1 and VLA-4/VCAM-1) elicited by chemokines on the activated endothelium [12]. Pharmacological disruption of firm adhesion and transmigration – without impeding the early rolling step – has shown efficacy in multiple inflammatory disease models and is emerging as a strategy for chronic inflammation [13, 14].

Peptide inhibitor of transendothelial migration (PEPITEM) is a 14-amino acid peptide (SVTEQGAELSNEER) proteolytically derived from the B lymphocyte-secreted 14-3-3ζδ protein [15]. PEPITEM binds endothelial cadherin-15, stimulating sphingosine-1-phosphate (S1P) synthesis and release, which in turn inhibits integrin-mediated lymphocyte firm adhesion and transendothelial migration without affecting selectin-mediated rolling [15]. In preclinical models of autoimmune disease – including systemic lupus erythematosus (SLE), rheumatoid arthritis, and Sjögren's syndrome – PEPITEM administration reduced T lymphocyte trafficking and tissue infiltration [15–17]. The PEPITEM/cadherin-15 axis suppresses T lymphocyte infiltration in murine SLE glomerulonephritis [16] and was recently shown to reduce leukocyte trafficking, macrophage accumulation in obese adipose tissue, and regulate the inflammatory response in peritonitis, psoriasis, and inflammatory arthritis [18, 19].

To our knowledge, all published PEPITEM studies have addressed Type 1 autoimmune or metabolic contexts. The peptide's behavior in Type 2 – driven allergic inflammation – where eosinophils and IgE-class B-cell responses dominate the cellular milieu and where leukocyte adhesion is amplified by mast cell mediators – has not been examined. We therefore designed this study to test the hypothesis that systemic PEPITEM administration attenuates systemic leukocyte trafficking in an OVA-induced murine AR model. Desloratadine, a second-generation H1-antihistamine of the class recommended as first-line pharmacotherapy for AR [5], served as the positive comparator. It was selected in preference to other antihistamines for three reasons specific to this design. First, among second-generation H1-antihistamines, its activity is best documented at the endothelial step that constitutes our primary endpoint: Desloratadine suppresses Nuclear factor kappa-B activation and downregulates ICAM-1 and P-selectin expression, together

Highlight key points

- Systemic administration of PEPITEM successfully prevents the rise of circulating eosinophils in an ovalbumin-induced murine model of allergic inflammation, achieving results similar to the antihistamine desloratadine.
- Treatment with PEPITEM significantly reduces the density of firmly adherent leukocytes in mesenteric post-capillary venules without impeding the early leukocyte rolling step.
- Beyond microvascular interactions, PEPITEM effectively lowers myeloperoxidase (MPO) activity in both the liver and colon, demonstrating a reduction in systemic, organ-specific inflammatory responses.
- This research provides the first preclinical evidence that PEPITEM can mitigate Type 2 allergic disease phenotypes, suggesting its potential as a novel therapeutic strategy targeting the leukocyte adhesion cascade.

with interleukin (IL)-6 and IL-8 release, in activated endothelial cells, and reduces inflammatory cell infiltration in OVA-induced AR in rodents [20, 21]. Second, it is non-sedating and essentially devoid of anticholinergic activity [20], thereby minimizing confounding of the microvascular hemodynamic variables measured under anesthesia, in contrast to first-generation agents. Third, a validated oral dosing regimen for desloratadine in a rodent ovalbumin (OVA) allergy model was already available, allowing an established dose to be used.

Sex was included as an independent experimental variable.

MATERIALS AND METHODS

Animals and Ethical Approval

Thirty-two C57Bl/6 mice (16 males, 16 females) aged 10–12 weeks and weighing 20–25 g were obtained from Koç University Animal Research Facility. Animals were housed in HEPA-filtered cages (≥70 cm^2/animal) at 22±2°C and 65–70% relative humidity under a 12-h light/dark cycle, with ad libitum access to standard pelleted chow and water. General health and stress indicators were monitored daily. All procedures were approved by the Marmara University Animal Experiments Local Ethics Committee (Protocol No: 09.2024mar). The experiments were performed in compliance with the Turkish law on the use of animals in experiments, regarding the ARRIVE guidelines, the guidelines of the New York Academy of Sciences, and in accordance with the Helsinki Declaration. Animals were randomly allocated to the four groups. All offline analyses (intravital microscopy [IVM] video review) were performed by observers blinded to group allocation.

Experimental Design and Groups

Mice were allocated, stratified by sex, to four groups (n=4 per sex per group; n=8 per group; total n=32). (1) Control – no intervention beyond vehicle injections, (2) Allergy – OVA sensitization and challenge, (3) Allergy+Desloratadine (Allergy+Des) – OVA model with oral desloratadine, used as the clinically established positive control [5, 20, 21], (4) Allergy+PEPITEM (Allergy+PEP) – OVA model with intraperitoneal PEPITEM.

Female mice were monitored for estrous cycle stage by vaginal cytology at 10× light microscopy. Sensitization and intranasal challenge for each female were timed to the same phase of the estrous cycle to minimize hormonal confounding of immune responses [22]. Sample size was estimated using the resource-equation approach [23, 24].

Induction of AR

The OVA-based AR model was adapted from established murine protocols [25]. OVA adsorbed to aluminum hydroxide was selected as the sensitizing allergen because it is the most extensively characterized and most reproducible allergen–adjuvant combination for inducing Type 2-polarised allergic airway and nasal inflammation in mice, reliably generating allergen-specific IgE, Th2 cytokine production and eosinophilia in a standardized acute sensitization-and-challenge format [26]. OVA is additionally a chemically defined, non-infectious single protein available at standardized purity, which minimizes the batch-to-batch variability in allergenic and endotoxin load associated with crude allergen extracts, because such variability can itself modify endothelial activation and leukocyte adhesion, the endpoints under study. Finally, the C57Bl/6 background was required for comparability with previous in vivo PEPITEM studies [15, 18, 19]. Because the murine allergic phenotype is strongly strain-dependent with respect to airway reactivity, inflammatory cell composition, and cytokine production [27], a robust adjuvanted OVA protocol was the appropriate means of achieving reliable Type 2 polarization in this background.

In the sensitization phase, mice in allergy groups received seven intraperitoneal injections, on days 1, 3, 5, 7, 9, 11, and 13, of 100 μg chicken OVA (OVA; Sigma-Aldrich, Grade V) in phosphate-buffered saline (PBS) adsorbed onto 2 mg aluminum hydroxide (Al(OH)3; alum), total volume 200 μL. Control mice received equivalent volumes of PBS. Following a 7-day rest period (days 14–21), in the challenge phase (days 22–25), sensitized mice received intranasal instillation of 1% OVA in PBS (10 μL per nostril; total 20 μL) on 4 consecutive days. Control mice received intranasal PBS.

Treatment Administration

Treatment was administered once daily on each of the 4 consecutive intranasal challenge days (days 22–25), 1 h before each intranasal OVA challenge, giving four administrations per animal. (1) PEPITEM group: PEPITEM 200 μg in 200 μL PBS intraperitoneally (i.p.) [15]; (2) Desloratadine group: desloratadine 10 mg/kg in 200 μL PBS by oral gavage (i.g.) [28]; (3) Control and allergy groups: equivalent volumes of PBS administered by the same routes.

The PEPITEM dose of 200 μg/animal corresponds to approximately 8–10 mg/kg in the 20–25 g mice used here and lies within the range of intraperitoneal doses previously shown to inhibit leukocyte trafficking in vivo, which include bolus i.p., doses of up to 300 μg/mouse in zymosan-induced peritonitis [29] and continuous subcutaneous delivery of approximately 0.8 mg/week in a diet-induced obesity model [18]. Repeated i.p. bolus dosing timed to the inflammatory stimulus follows the paradigm established in previous PEPITEM peritonitis studies [19, 29]. Administration 1 h before each challenge was intended to align peak peptide exposure with the acute challenge window and to match the exposure timing of the desloratadine comparator. A single dose level was used because this was a first-in-model proof-of-concept experiment.

Intravital Video Microscopy

On the 4th challenge day, IVM was performed. Mice were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (7.5 mg/kg, i.p.), with maintenance doses of ketamine (20 mg/kg) and xylazine (1.5 mg/kg, i.p.) administered as required. Anesthetic depth was monitored by limb-withdrawal reflex. Circulating leukocytes were fluorescently labeled by retro-orbital injection of rhodamine 6G (0.2 mg/kg in 0.1 mL saline, Sigma-Aldrich) and subsequently visualized within mesenteric postcapillary venules using IVM (Olympus BX53 Fluorescence Microscope and Olympus DP73 camera system). Rhodamine 6G labeling enabled visualization of circulating leukocytes but did not distinguish leukocyte subsets.

Following a midline laparotomy, the mesentery was gently exteriorized onto a temperature-controlled stage and transilluminated; tissue moisture was maintained by superfusion with Krebs–Ringer bicarbonate buffer (1 mL/min, pH 7.3–7.4, 37°C). Venules with 20–60 μm diameters were selected. For each mouse, at least three 100-μm segments or at least one 300-μm segment were recorded for ~120 s at 16 fps. For statistical analysis, values from all recorded venular segments within each animal were averaged. Leukocytes that adhered to the post-capillary venule for more than 2 s but left the venule before 30 s were considered "stationary." The number of "firmly adherent leukocytes" (leukocytes that adhered to the post-capillary venule for more than 30 s) per vessel segment was measured, averaged for time and each animal. Stationary and firmly adherent leukocytes were expressed as numbers/min/mm^2. Leukocyte rolling was quantified by drawing a reference line through the middle of each postcapillary venule and counting the number of leukocytes that crossed this line while rolling, expressed as cells/min [30, 31]. Leukocyte transmigration/extravasation was not directly quantified in this study. To calculate rolling velocity, 5–10 rolling leukocytes were selected from each venule. The distances traveled by these leukocytes in 5 s were measured. The rolling speed was calculated by dividing the measured distance by the time taken and reported as micrometers per second (μm/s). Venular flow was estimated from venular cross-sectional area and mean leukocyte flow velocity. Cross-sectional area was calculated as $\pi(d/2)2$, where d is venular diameter. Flow was calculated as cross-sectional area × mean leukocyte flow velocity. Values were converted from μm^3/s to μL/s [32].

After IVM, mice were sacrificed by cardiac puncture while under anesthesia.

Hematological Analysis

Whole blood obtained by cardiac puncture was immediately analyzed using an automated hematology analyzer (URIT-3300; URIT Medical Electronic, China), with manufacturer-supplied parameters validated for murine samples by reference QC.

A drop of whole blood was used to prepare thin smears for differential counts. Blood was stained with May–Grünwald (2 min) followed by Giemsa (15 min). For each smear, ≥100 leukocytes were classified to obtain neutrophils, lymphocytes, monocytes, and eosinophils proportions.

Measurement of Myeloperoxidase (MPO) Activity

MPO activity, a widely used enzymatic index of granulocyte (predominantly neutrophil) infiltration into tissue, was selected as a tissue-level correlate of the intravascular leukocyte–endothelial interactions quantified by IVM and was quantified by the H2O2-dependent oxidation of o-dianisidine dihydrochloride. Pieces of large intestine (colon), lung, and liver were homogenized at 9× tissue weight in potassium phosphate buffer containing hexadecyltrimethylammonium bromide (HETAB). Homogenates were centrifuged (12,000 rpm, 10 min, 4°C); supernatants were discarded and the pellets re-homogenized in 50 mmol/L K2HPO4 with 0.5% (w/v) HETAB and 10 mmol/L EDTA. MPO activity was read at 460 nm at 37°C and expressed as U/g tissue, where 1 U is the change in absorbance over 1.0 min^{-1}.

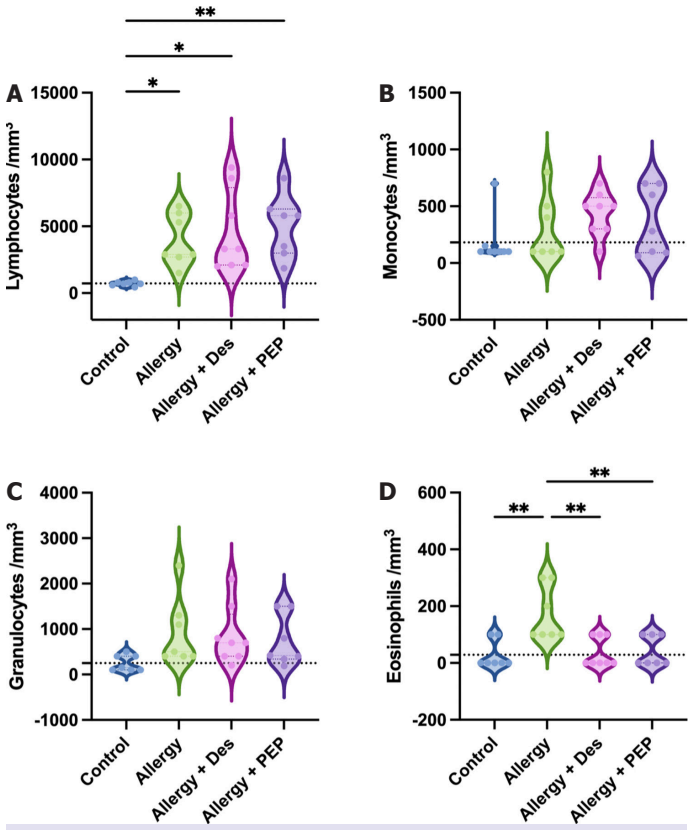

FIGURE 1. Effect of peptide inhibitor of transendothelial migration on peripheral blood leukocyte differentials. Violin plots showing **(A)** lymphocyte, **(B)** monocyte, **(C)** neutrophil (granulocyte), and **(D)** eosinophil counts (cells/mm³) (n=8/group). Groups: Control (blue), Allergy (green), Allergy+Des (pink), Allergy+PEP (purple).

*: P<0.05; **: P<0.01. One-way analysis of variance with *post hoc* Tukey's test.

Measurement of Malondialdehyde (MDA) and Reduced Glutathione (GSH)

The same tissues in which MPO activity was measured were homogenized in 10% trichloroacetic acid up to 10× tissue weight using an Ultra-Turrax, then centrifuged (3,000 rpm, 15 min, 4°C). Supernatants were clarified by a second spin (15,000 rpm, 8 min). MDA was measured at 535 nm and expressed as nmol/g tissue as an index of lipid peroxidation. GSH was quantified by the modified Ellman method at 412 nm and expressed as µmol/g tissue [33].

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. Normally distributed variables were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for single-factor comparisons. For sex-based data, two-way ANOVA (sex × treatment) followed by Tukey's post hoc test was used. Statistical significance was defined as $p<0.05$. All analyses were performed using GraphPad Prism 11 (GraphPad Software, USA).

RESULTS

Peripheral Blood Leukocyte Differentials

Peripheral blood leukocyte differentials showed distinct changes in lymphocyte and eosinophil populations after OVA sensitization and challenge. Circulating lymphocyte counts were elevated in all three allergy-receiving groups compared to control (one-way ANOVA, $p=0.0446–0.0059$; Fig. 1A). Lymphocytosis was not normalized by either treatment.

Eosinophil counts were elevated specifically in the untreated allergy group ($p=0.0019$ versus controls; Fig. 1D). Both desloratadine and PEPITEM significantly reduced eosinophil counts compared with untreated allergic mice, bringing values closer to the control range.

Monocyte and neutrophil counts did not differ significantly between groups (Fig. 1B, C). Sex-stratified analyses were broadly consistent with the pooled results, although the statistical significance of eosinophil count differences varied between sexes (Appendix 1).

Leukocyte Trafficking in Mesenteric Post-Capillary Venules

Representative IVM images and videos showed visibly increased leukocyte–endothelial interactions in the allergy group, with fewer vessel-associated leukocytes in the desloratadine- and PEPITEM-treated groups (Fig. 2D). Mean rolling leukocyte counts did not differ significantly between groups (Fig. 2A). Stationary leukocyte densities were significantly elevated in the untreated allergy group versus control while desloratadine significantly reduced this response ($p=0.0001$, $p=0.0103$; Fig. 2B). Stationary leukocyte counts tended to be lower in the PEPITEM-treated group than in the untreated allergy group, but the difference was not statistically significant. Total adherent leukocyte density was markedly higher in the allergy group than in the control ($p=0.0003$; Fig. 2C). Both desloratadine and PEPITEM significantly reduced adhesion compared with the untreated allergy group ($p=0.0310$ and 0.0008, respectively). Sex-stratified analysis showed similar overall patterns (Appendix 2).

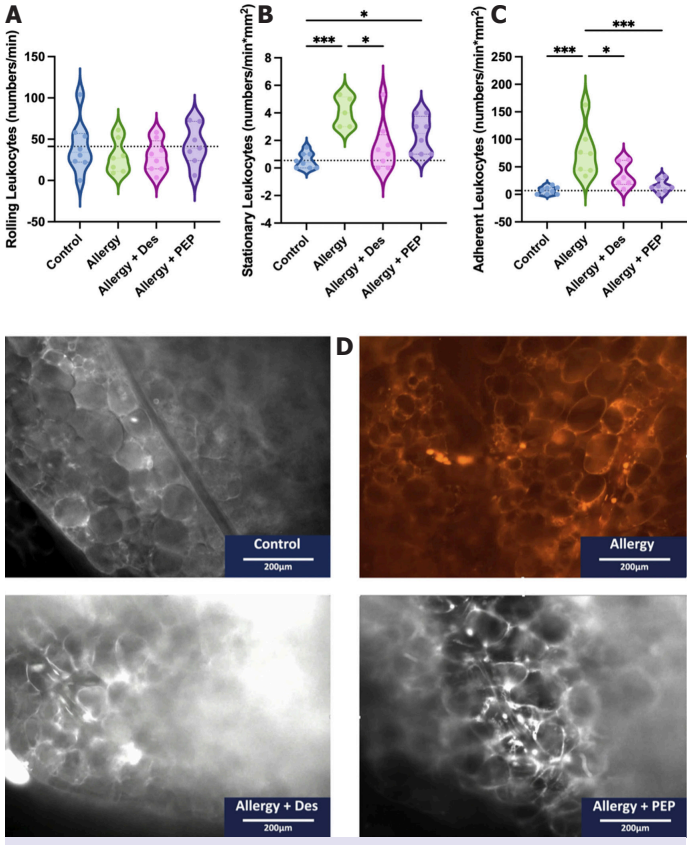

FIGURE 2. Effect of peptide inhibitor of transendothelial migration on leukocyte trafficking in mesenteric post-capillary venules. Violin plots showing **(A)** rolling counts, **(B)** stationary, and **(C)** adherent leukocyte densities; **(D)** representative intravital microscopy images of each group. Groups as in Figure 1.

*: P<0.05; ***: P<0.001. One-way analysis of variance with *post hoc* Tukey's test.

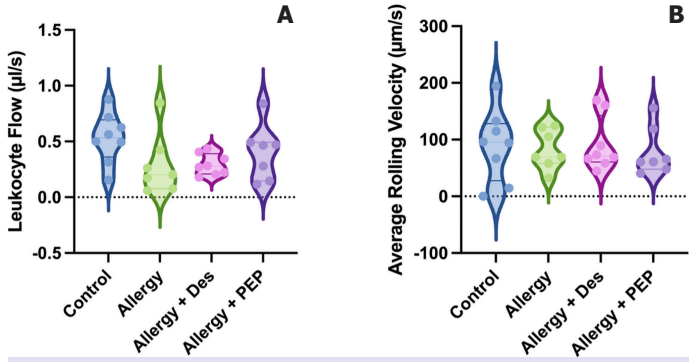

FIGURE 3. Measurements of leukocyte flow and leukocyte rolling velocity in mesenteric post-capillary venules. Violin plots showing **(A)** leukocyte flow, **(B)** average leukocyte rolling velocity. Groups as in Figure 1.

One-way analysis of variance with *post hoc* Tukey's test.

Leukocyte Flow and Rolling Velocity

Leukocyte flow and average rolling velocity were also evaluated to determine whether group differences in leukocyte adhesion could be attributed to changes in microvascular flow dynamics and changes in rolling pattern. Leukocyte flow did not differ significantly between groups (Fig. 3A). Average rolling velocity was likewise comparable across groups, with no statistically significant differences detected (Fig. 3B). Sex-stratified analysis is shown in Supplement (Appendix 3).

Tissue Levels of MDA, MPO Activity, GSH Content in Lung, Liver, and Colon

Tissue MPO activity, MDA levels, and GSH content were assessed in lung, liver, and colon samples. In the lung, allergy significantly increased MDA levels compared with controls (p=0.0184; Fig. 4D), whereas its increase was prevented in both desloratadine- and PEPITEM-treated groups. Lung MPO activity did not differ significantly among groups (Fig. 4A). In the liver, MPO activity was significantly lower in the PEPITEM-treated group than in the allergy group (p=0.0158; Fig. 4B), while MDA levels remained comparable across groups (Fig. 4E). In the colon, MPO activity was significantly reduced by both desloratadine and PEPITEM treatment (p=0.0358 and 0.0288, respectively; Fig. 4C); however, MDA levels showed no statistically significant differences between groups (Fig. 4F). No significant differences in GSH content were observed in any of the examined tissues (Fig. 4G–I). Overall, these findings indicate that OVA-induced allergic inflammation was associated with tissue-specific changes in oxidative and inflammatory markers.

DISCUSSION

In this study, systemic PEPITEM administration reduced peripheral eosinophilia and total leukocyte adhesion in mesenteric post-capillary venules during OVA-induced allergic inflammation, with a non-significant trend toward fewer stationary leukocytes. The magnitude of these reductions was of similar order to that achieved by desloratadine, a clinically established H1-antihistamine. Importantly, leukocyte rolling was not significantly altered, whereas firm adhesion was significantly reduced, a dissociation consistent with the cadherin-15/S1P mechanism described for PEPITEM in Type 1 autoimmune and metabolic inflammatory contexts [15–18].

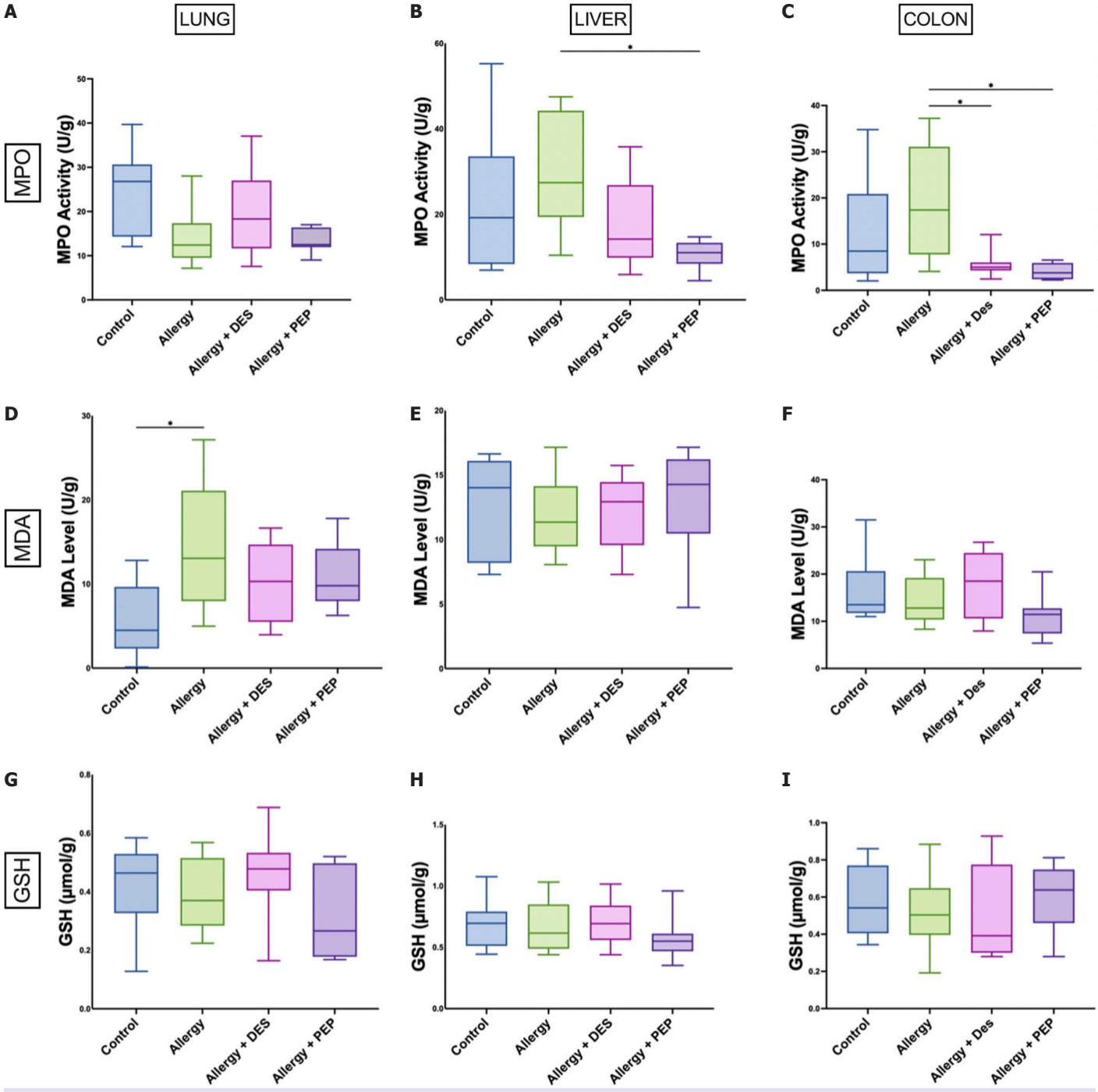

FIGURE 4. Tissue levels of malondialdehyde (MDA), myeloperoxidase (MPO) activity, and glutathione (GSH) content in lung, liver, and colon. Box-and-whisker plots show spectrophotometric assays in lung **(A, D, G)**, liver **(B, E, H)**, and colon **(C, F, I)** tissues. Groups as in Figure 1.

*: P<0.05. Center line indicating the median, boxes representing the interquartile range, and whiskers extending from the 5th to 95th percentiles. One-way analysis of variance with *post hoc* Tukey's test.

Both PEPITEM and desloratadine normalized peripheral blood eosinophil counts in the OVA model. Several mechanisms could produce this effect: (i) reduced eosinophil egress from circulation into tissue, which would be expected to elevate rather than reduce blood eosinophils unless tissue retention is dominant; (ii) reduced eosinophilopoiesis, plausibly through reduced IL-5 signaling; or (iii) accelerated peripheral clearance.

The present blood-only data cannot distinguish between these. We therefore restrict our interpretation to the observation that PEPITEM, like desloratadine, prevents the OVA-driven rise in circulating eosinophils. Mechanistic studies could incorporate IL-5 quantification and tissue eosinophil counts to dissect these possibilities.

Lymphocytosis persisted in allergy+PEPITEM and allergy+desloratadine animals. One possibility, drawn from the natalizumab (anti-VLA-4) literature, is that PEPITEM blocks lymphocyte exit from blood into inflamed tissue and thereby maintains, or elevates circulating counts despite reduced tissue infiltration [34]. However, the present design measured only blood lymphocytes; we did not quantify tissue (nasal, lung, lymph node) lymphocyte counts and therefore cannot confirm this redistribution hypothesis. An equally plausible alternative is that PEPITEM does not affect antigen-driven Th2 clonal expansion, leaving the circulating pool unchanged. Resolving these competing interpretations requires tissue lymphocyte phenotyping.

The dissociation we observe (rolling statistically unchanged, while adhesion reduced) is consistent with prior demonstrations that PEPITEM acts on the integrin-activation step of the leukocyte adhesion cascade [15]. The data are best described as consistent with, rather than confirmatory of, the canonical PEPITEM mechanism, and would benefit from corroboration in larger studies, ideally with cadherin-15 blockade or scrambled-peptide controls to address selectivity directly. Findings in IVM suggest that the observed differences in stationary and firmly adherent leukocytes were not accompanied by major changes in leukocyte flow or rolling velocity, which is also consistent with the reported absence of an effect of PEPITEM on selectin-mediated leukocyte rolling [15].

The IVM data report on mesenteric post-capillary venules, not nasal microvasculature. This choice was deliberate because intravital imaging of nasal mucosal microvessels in OVA-challenged mice is technically infeasible, while the mesenteric venule bed is a well-validated platform for quantifying systemic leukocyte trafficking responses [11, 35]. AR is increasingly recognized as a systemic disease, with circulating activated leukocytes and systemic vascular alterations [3, 4]. Nevertheless, mesenteric and nasal vascular beds differ in adhesion molecule expression patterns (mesenteric venules favor ICAM-1/LFA-1-driven neutrophil traffic; nasal mucosa eosinophil/Th2 recruitment is biased toward VCAM-1/VLA-4 and CCR3-mediated chemotaxis) [10, 36]. Accordingly, we frame the present IVM observations as evidence that systemic leukocyte adhesion is elevated by OVA challenge and reduced by PEPITEM and desloratadine, rather than as a direct measurement of nasal-mucosal leukocyte trafficking. Demonstrating equivalent effects in nasal/airway endothelium requires either airway IVM, leukocyte profiling, or nasal histology with leukocyte counts. Desloratadine acts acutely on H1 receptors expressed on sensory neurons and endothelial cells [37], while PEPITEM is hypothesized to act upstream by limiting leukocyte-derived mediator release at inflamed sites.

Tissue oxidative and inflammatory marker analysis was performed to assess whether OVA-induced allergic inflammation and treatment effects extended beyond circulating leukocyte responses and microvascular leukocyte–endothelial interactions. MPO, MDA, and GSH were selected as a deliberately focused panel rather than as a comprehensive inflammatory profile. MPO was chosen because it provides an enzymatic, tissue-level index of granulocyte accumulation and therefore extends the primary leukocyte-trafficking endpoint from the vascular lumen assessed by IVM into the tissue compartment, whereas MDA and GSH index the lipid peroxidation and thiol antioxidant reserve that accompany allergic tissue inflammation and that have previously been reported to change in OVA-driven AR and food allergy models [38–40]. All three can be assayed in the same tissue homogenate by validated spectrophotometric methods [33], which was a practical requirement because tissue and blood volumes were prioritized for the IVM and hematological endpoints that constituted the primary outcomes.

Allergic inflammation is closely associated with epithelial activation, leukocyte recruitment, and mediator release, all of which may promote local oxidative stress and tissue inflammatory enzyme activity. In the present study, the biochemical changes were tissue-specific rather than uniform across organs. MPO activity, a marker commonly associated with inflammatory activity of granulocytes, tended to increase in the colon and was reduced by both desloratadine and PEPITEM treatment. This could be a result of decreased leukocyte adhesion; however, transmigration counts are not assessed in this study. This finding is consistent with a previous OVA-induced AR study reporting increased colonic MPO activity, supporting the concept that AR may be accompanied by extra-nasal inflammatory alterations [38–40]. PEPITEM also reduced liver MPO activity, whereas

lung MPO activity was not significantly changed. In contrast, MDA levels showed only limited changes, and GSH content did not differ significantly among groups in lung, liver, or colon tissues. Previous experimental studies have reported increased MDA and/or reduced GSH in OVA-induced AR and food allergy models [39, 40], whereas these oxidative stress markers were largely unchanged in the present study. These differences may be related to the biological compartment analyzed, timing of tissue collection, animal strain, allergen exposure protocol, or the number of challenge exposures. Overall, our findings suggest that OVA-induced allergic inflammation was associated with selective tissue inflammatory changes. However, because tissue leukocyte subsets and local cytokine profiles were not assessed, these biochemical findings should be interpreted as supportive evidence of organ-specific inflammatory modulation rather than as direct mechanistic proof.

To our knowledge, this is the first study to evaluate PEPITEM in a type 2 allergic disease model. Previous investigations have focused primarily on Th1-dominant autoimmune disorders, including systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome, or on metabolic inflammation, whereas the present findings extend the potential anti-inflammatory activity of PEPITEM to allergic inflammation [15–18]. Nevertheless, several limitations should be acknowledged. Validation of the AR model was incomplete, as OVA-specific IgE and Immunoglobulin G (IgG)-1 levels, bronchoalveolar lavage eosinophil counts, and nasal histopathology were not assessed. In addition, mesenteric IVM provided a systemic assessment of leukocyte trafficking but did not directly evaluate cellular behavior within the nasal mucosa, and leukocyte subsets could not be distinguished because cell-type-specific fluorescent labeling was not performed. Furthermore, Type 2 cytokines (IL-4, IL-5, IL-13), interferons, immunoglobulin responses, and bronchoalveolar lavage composition were not measured. The primary endpoint of this study was leukocyte–endothelial interaction, and the canonical PEPITEM mechanism acts at the endothelial cadherin-15/S1P axis to block integrin-mediated firm adhesion downstream of chemokine signaling [15], so leukocyte adhesion rather than cytokine concentration constitutes the proximal readout of the hypothesized mechanism. The MPO, MDA and GSH panel was therefore intended to extend the trafficking readout into tissue and cannot substitute for direct cytokine profiling. Quantification of IL-4, IL-5, IL-13, interferon-gamma, eotaxin/CCL11 and OVA-specific IgE and IgG1 is a priority for mechanistic follow-up, particularly as PEPITEM has been shown to modulate cytokine and chemokine release in other inflammatory models [17, 19]. A further consideration is that, although OVA/alum is the best-characterized murine sensitization protocol, OVA is not a clinically relevant human aeroallergen and repeated mucosal exposure in the absence of adjuvant can induce tolerance. Confirmation in a model driven by a clinically relevant aeroallergen such as house dust mite is therefore an important next step [26]. Only one PEPITEM dose level was tested, so the dose–response relationship, the minimum effective dose, and the duration of effect after the final administration remain undefined, and no conclusion regarding relative potency versus desloratadine can be drawn. Importantly, the present study cannot determine whether PEPITEM directly modulates eosinophil trafficking or acts indirectly through suppression of Th2-associated cytokine signaling and subsequent reductions in IL-5- and eotaxin-mediated recruitment. Despite these limitations, the consistent reduction in leukocyte-endothelial interactions and eosinophilia observed across multiple analyses provides a rationale for further investigation. If confirmed in adequately powered studies incorporating comprehensive immunological, histological, and mechanistic endpoints, PEPITEM may represent a novel therapeutic strategy for AR that targets the endothelial cadherin-15/S1P regulatory axis rather than conventional histamine- or leukotriene-dependent pathways. While the current findings do not support direct clinical conclusions, they establish a foundation for future studies aimed at defining the therapeutic potential and mechanisms of PEPITEM in allergic disease.

Conclusion

This study provides initial evidence that systemic PEPITEM attenuates selected microvascular leukocyte–endothelial interactions associated with OVA-induced allergic inflammation. PEPITEM reduced peripheral eosinophilia and total adhesion in mesenteric post-capillary venules to a magnitude comparable to that of desloratadine, while leukocyte rolling and stationary leukocyte counts were statistically unchanged.

Ethics Committee Approval: This study was approved by The Marmara University Local Ethics Committee For Animal Experiments (09.2024mar – 12.02.2024).

Conflict of Interest: The authors declare that there is no conflict of interest.

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Appendix 1. Sex-stratified effects of PEPITEM on peripheral blood leukocyte differentials

Parameters	Groups	Female				Male			
		Mean	SEM	n	Tukey's test (p)	Mean	SEM	n	Tukey's test (p)
Lymphocytes (/mm^3)	Control	1300	70.71	4	–	1100	302.76	4	–
	Allergy	3450	1068.87	4	ns	4666.6	1003.88	4	ns
	Allergy+Des	2700	346.41	4	ns	6450	1672.07	4	0.0016 versus control
	Allergy+PEP	6900	862.1	4	0.0022 versus Control; 0.0242 versus Allergy+Des	3540	827.80	4	ns
Monocytes (/mm^3)	Control	225	25	4	–	200	40.82	4	–
	Allergy	200	100	4	–	375	170.17	4	–
	Allergy+Des	400	57.73	4	–	475	131.49	4	–
	Allergy+PEP	526.6667	126.6	4	–	237.5	154.4	4	–
Granulocytes (/mm^3)	Control	475	47.87	4	–	400	100	4	–
	Allergy	625	225	4	–	1333.333	560.75	4	–
	Allergy+Des	550	86.6	4	–	1150	413.31	4	–
	Allergy+PEP	906.6667	316.2	4	–	607.5	300.75	4	–
Eosinophils (/mm^3)	Control	25	25	4	–	25	25	4	–
	Allergy	166.6667	66.66	4	ns	175	47.87	4	0.0232 versus control
	Allergy+Des	50	28.86	4	ns	25	25	4	0.0232 versus allergy
	Allergy+PEP	66.66667	33.33	4	ns	25	25	4	0.0232 versus allergy

Sex-stratified data showing lymphocyte, monocyte, granulocyte, and eosinophil counts (cells/mm3). Groups: Control, Allergy, Allergy+Des, Allergy+PEP. Lymphocytes two-way analysis of variance (ANOVA): sex effect: $F(1, 22)=0.2929$, $p=0.5938$; treatment effect: $F(3, 22)=7.789$, $p=0.0010$. Monocytes two-way ANOVA: sex effect: $F(1, 25)=0.02424$ $p=0.8775$; treatment effect: $F(3, 25)=1.415$ $p=0.2617$. Granulocytes two-way ANOVA: Sex effect: $F(1, 22)=1.370$ $p=0.2543$; treatment effect: $F(3, 22)=1.385$ $p=0.2736$. Eosinophils two-way ANOVA: sex effect: $F(1, 22)=0.3427$ $p=0.5643$; treatment effect: $F(3, 22)=7.133$ $p=0.0016$. Two-way ANOVA with post hoc Tukey's test. PEPITEM: Peptide inhibitor of transendothelial migration.

Appendix 2. Sex-stratified effects of PEPITEM on leukocyte trafficking in mesenteric post-capillary venules

Parameters	Groups	Female				Male			
		Mean	SEM	n	Tukey's test (p)	Mean	SEM	n	Tukey's test (p)
Rolling (numbers/min)	Control	28.45	3.687	4	–	53.875	21.27	4	–
	Allergy	21	4.490	4	–	40.666	16.04	4	–
	Allergy+Des	18.375	6.132	4	–	40.20	9.866	4	–
	Allergy+PEP	50.166	22.08	4	–	36.75	5.170	4	–
Stationary leukocytes (numbers/min*mm^2)	Control	0.583	0.322	4	–	0.5	0.288	4	–
	Allergy	3.777	0.777	4	0.0192 versus Control	4.5	0.5	4	0.0091 versus Control
	Allergy+Des	0.875	0.381	4	0.0362 versus Allergy	2.25	1.165	4	ns
	Allergy+PEP	2.333	0.666	4	ns	2	0.707	4	ns
Adherent leukocytes (numbers/min*mm^2)	Control	3.23	1.928	4	–	10.20	3.656	4	–
	Allergy	78.40	29.39	4	0.0021 versus Control	74.09	15.70	4	0.0163 versus Control
	Allergy+Des	20.53	4.269	4	0.0191 versus Allergy	70.02	7.81	4	0.0259 versus Control
	Allergy+PEP	15.44	6.199	4	0.0181 versus Allergy	15.07	6.809	4	0.0283 versus Allergy; 0.0443 versus Allergy+Des

Sex-stratified data showing rolling counts, stationary, and adherent leukocyte densities. Groups as in Appendix 1. Rolling two-way analysis of variance (ANOVA): sex effect: F(1, 22)=2.373 p=0.1377; treatment effect: F(3, 22)=0.6811 p=0.5730. Stationary two-way ANOVA: sex effect: F(1, 20)=0.7062 p=0.4106; treatment effect: F(3, 20)=8.095 p=0.0010. Adhesion two-way ANOVA: Sex effect: F(1, 21)=1.865 p=0.1865; treatment effect: F(3, 21)=11.19 p=0.0001.Two-way ANOVA with post hoc Tukey's test. PEPITEM: Peptide inhibitor of transendothelial migration, SEM: Standard error of the mean.

Appendix 3. Sex-stratified measurements of leukocyte flow and leukocyte rolling velocity in mesenteric post-capillary venules

Parameters	Groups	Female				Male			
		Mean	SEM	n	Tukey's test (p)	Mean	SEM	n	Tukey's test (p)
Leukocyte flow (µl/s)	Control	0.3994	0.1038	4	–	0.662374	0.0849	4	–
	Allergy	0.3511	0.1852	4	–	0.212374	0.0261	4	–
	Allergy+Des	0.2753	0.0284	4	–	0.310782	0.0646	4	–
	Allergy+PEP	0.2871	0.1006	4	–	0.48702	0.1413	4	–
Average rolling velocity (µm/s)	Control	77.117	24.828	4	–	101.3724	39.926	4	–
	Allergy	70.336	18.786	4	–	99.70121	16.195	4	–
	Allergy+Des	68.334	9.2079	4	–	114.2602	29.375	4	–
	Allergy+PEP	77.3007	21.335	4	–	79.51546	25.937	4	–

Sex-stratified data showing leukocyte flow and average leukocyte rolling velocity. Groups as in Appendix 1. Leukocyte flow two-way analysis of variance (ANOVA): sex effect: F(1, 22)=1.323 p=0.2625; treatment effect: F(3, 22)=2.282 p=0.1073. Leukocyte rolling velocity two-way ANOVA: Sex effect: F(1, 22)=1.929 p=0.1788; treatment effect: F(3, 22)=0.09518 p=0.9619. Two-way ANOVA with post hoc Tukey's test. SEM: Standard error of the mean.