Original article

Mast cell activation by fibrinogen-related homologous c-terminal peptides (haptides) modulates systemic blood pressure

Maamoun Basheer, MSc,[a] Herzl Schwalb, PhD,[b] Maoz Nesher, MSc,[c] Dan Gilon, MD,[d] Irit Shefler, PhD,[f] Yoseph A. Mekori, MD,[f] Oz M. Shapira, MD,[b,e] and Raphael Gorodetsky, PhD[a] *Jerusalem and Kfar Saba, Israel*

Background: Haptides are a family of short peptides homologous to C-termini sequences of fibrinogen chains β and γ (haptides Cβ and preCγ, respectively) which were previously shown to penetrate and bind cells.
Objectives: This work investigates the systemic effect of the haptides with possible clinical implications.
Methods: Intra-arterial monitoring in rats recorded the haptides' effects on systemic blood pressure. In parallel, their effect was also tested *in vitro* on isolated rat peritoneal mast cells and on human mast cells.
Results: Intra-arterial monitoring in rats showed that intravenous administration of low haptides concentrations (35-560 μg/kg rat) caused a shocklike behavior with transient decrease in the systolic and diastolic blood pressure by up to 55% ($P < .05$) in a dose-dependent manner and a minor increase in their heart rate. Randomly scrambled sequences of the haptides had no such effect, suggesting a specific interaction with receptors. Intravenous administration of blockers to histamine receptors H1 and H2 before haptides administration attenuated this effect. Furthermore, *in vitro* incubation of human LAD2 mast cell line or isolated rat peritoneal mast cells with the haptides caused degranulation of the mast cells. We found that the haptides Cβ and preCγ activated mast cells causing histamine release, resulting in a steep decrease in blood pressure, comparable to anaphylactic shock.
Conclusion: In treating vascular occlusive diseases, massive fibrinolysis is induced, and haptide-containing sequences are released. We suggest that treatment with histamine receptor blockers or with mast cell stabilizing agents in such pathological conditions may overcome this effect. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: *Haptides, fibrin, mast cells, hypotension, histamine, serotonin*

Fibrinogen molecules and fibrin gels were found to be highly cell-binding.[1] Part of the mediation of cell binding was recently attributed to small conserved family of short homologous (~20mer) sequences on the c-termini of β chain and just preceding the c-termini of the γ chain (Cβ and preCγ, respectively).[2] Another partially homologous sequence is present on the extended α-chain αE, which is seen only in small fraction of fibrinogen molecules in adult mammals because of posttranslational modification.[2-4] We termed this family of homologous peptides *haptides* because of their haptotactic (cell-binding) properties. Haptides also showed high penetration properties into cultured cells,[2,5] probably mediated by their structure and their relatively high content of both basic and cyclic or hydrophobic amino acids. It is of major interest that all the haptides in a wide range of concentrations tested had no apparent cytotoxic effect *in vitro* on different cell types, including normal fibroblasts and endothelial cells.[2] When bound to collagen matrix implants in rats, haptides increased cell binding with no adverse effects.[6] Haptides' effect was also tested *ex vivo* on isolated perfused beating hearts, in which high levels of haptides caused significant vasoconstriction of the coronaries, resulting in impaired heart functions.[7] Nevertheless, the systemic effect of the haptides has not been investigated previously.

The possible effect of haptides administered into the circulation is highly relevant to clinical practice. Fibrinolysis is a prominent treatment for salvaging ischemic organs caused by obstructive thrombotic events, resulting in a release of peptides containing haptidic sequences.[8,9] The effectiveness in reducing morbidity in acute myocardial infarction is dependent on early thrombolytic therapy.[10-12] Streptokinase is widely used to initiate this cascade by inducing the activation of the fibrinolytic enzyme plasmin, which degrades fibrin clots at specific sites, yielding identifiable soluble fragments that are released to the circulation.[13-16] Elevation of the level of large D-dimers, as well as small circulating fragments, was observed in numerous pathological conditions[14-17] and could serve as a marker of many vascular disorders.[18]

Low-molecular-weight fibrinogen degradation products derived from plasmin digestion exhibit a variety of biological activities. The fibrin degradation products mediate platelet activation,[19,20] fibroblast proliferation,[21] endothelial cells spreading, proliferation and capillary tube formation,[20] and promotion of angiogenesis.[22,23]

The current study examines whether haptides can contribute to the systemic side effects that are observed after massive fibrinolysis. Our findings in the current report suggest that haptides exhibit hemodynamic effects with significant induction of

From [a]the Laboratory of Biotechnology and Radiobiology, Sharett Institute of Oncology, [b]the Cardiothoracic and Thoracic Research Center, Department of Cardiothoracic Surgery, and the Departments of [c]Physiology, [d]Cardiology, and [e]Cardiothoracic Surgery, Hadassah-Hebrew University Medical Center, Jerusalem; and [f]the Laboratory of Allergy and Clinical Immunology, Meir Medical Center, Kfar Saba.

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Reprint requests: Raphael Gorodetsky, PhD, Head, Biotechnology and Radiobiology Laboratory, Sharett Institute of Oncology Hadassah-Hebrew University Medical Center, POB 12000, Jerusalem 91120, Israel. E-mail: rafi@hadassah.org.il.

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Abbreviations used
BP: Blood pressure
Cβ: Haptides, homologous to sequences on the C-termini of fibrinogen beta chains
DBP: Diastolic blood pressure
β-hex: β-Hexosaminidase
HR: Heart rate
LAD2: Human mast cell line
MAP: Mean arterial pressure
preCγ: Haptides, homologous to sequences on the C-termini of gamma chains
SBP: Systolic blood pressure

hypotension. Furthermore, we propose that the mechanism of such effect may be associated with histamine and serotonin secretion as a result of systemic activation of mast cells.

METHODS

Peptides synthesis

The synthetic haptides Cβ (KGSWYSMRKMSMKIRPFFPQQ) and preCγ (KTRWYSMKKTTMKIIPFNRL) correspond to addresses 471-491 of the C-terminal of fibrinogen precursor of the β chain and 399-418 of the precursor of the γ chain, respectively. The control peptide Cα (SEADHEGTHSTKRGHAKSRP) corresponds to a sequence close to the C-terminal of fibrinogen α chain. The arbitrary scrambled sequences of Cβ and preCγ were ISSWYKFPMQGQKRPRMSFKM and FIKLMNPWSTRYIKMRTKTK, respectively. The peptides were synthesized by GeneScript (Piscataway, NJ). The experiments used peptides that were >90% pure as determined by HPLC and mass spectrometry.

Fibrinogen

Human fibrinogen as a component of a tissue sealant was obtained from Omrix (Rehovot, Israel). Compound 48/80, ketanserin tartrate salt, fexofenadine, famotidine, pyrilamine, cimetidine, cromolyn A, and *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide were supplied by Sigma (Rehovot, Israel).

Animals

Male Sprague-Dawley rats (275-300 g; Harlan Laboratories, Rehovot, Israel) were grown in specific pathogen-free conditions. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (http://www.nap.edu/catalog/5140.html). The experiments were approved by the Institutional Animal Care and Utilization Committee.

Blood pressure monitoring

Male Sprague-Dawley rats (250-300 g; Harlan Laboratories) were kept in specific pathogen-free conditions in accordance with the Guide for the Care and Use of Laboratory Animals (NIH pub no. 86-23, revised 1996). Experiments were approved by the Institutional Animal Care and Utilization Committee (Application MD-103.05-1).

The rats were anesthetized with urethane (1 g/kg, intraperitoneal) and placed on a heated pad. Rectal temperature was adjusted to 37.5 to 38°C by a rectal probe. After tracheotomy and insertion of a PE260 catheter, the right femoral artery and vein were cannulated with a PE50 heparinized tube. Blood pressure (BP) was recorded from the right femoral artery using an SP 844 pressure transducer (MEMSCAP, Skoppum, Norway) coupled to an amplifier (Laboratory Linc V; Coulbourn Instruments, Allentown, Pa). Heart rate (HR) was measured in parallel. Mean arterial blood pressure (MAP) was calculated as the sum of two thirds diastolic blood pressure and one third systolic pressure (mmHg).

After 20 minutes of equilibration, peptides dissolved in saline were administered by a bolus injection through the femoral vein cannula in a final volume of 100 μL, and the hemodynamic parameters were measured. After 15 minutes, a higher dose of the peptide was injected, and the hemodynamic parameters were measured for additional 15 minutes. The maximal effect of the haptides was recorded after 3 minutes.

In the histaminergic blockade experiment, rats were intravenously administrated with the histamine antagonists cimetidine (15 mg/kg) and pyrilamine (1 mg/kg), famotidine (2 mg/kg), or fexofenadine (2 mg/kg) 10 minutes before haptide injection. Cromolyn A (intravenous, 1 mg/kg/min) was injected for 5 minutes before haptide injection. Administration of the saline and antagonist H1 and H2 alone had no significant effect on the BP.

Isolation and purification of rat peritoneal mast cells

Rat peritoneal mast cells were obtained from Sprague-Dawley rats by a peritoneal lavage, and the cells were purified as previously described.[24] Briefly, a suspension of washed peritoneal cells was layered over a cushion of 30% Ficoll 400 (Pharmacia Biotech, Uppsala, Sweden) in buffered saline supplemented with 0.1% BSA and centrifuged at 1500*g* for 15 minutes. The purity of mast cells recovered from the bottom of the tube was 90% as assessed by toluidine blue staining.

Purified mast cells (5×10^5 cells/mL in a final volume of 0.5 mL) were incubated in Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 20 mM HEPES, pH 7.4, 1 mM $CaCl_2$, 5.6 mM glucose, 1 mg/mL BSA) and were stimulated by the activating agent for 20 minutes. Reactions were terminated by placing the tubes on ice, followed by a brief centrifugation by microfuge (12,000 rpm for 20 seconds) at 4°C. Aliquots of 50 μL were collected from the supernatant for determination of histamine levels.

The amount of histamine released was determined by using the *O*-phthaldialdehyde fluorometric method, as previously described.[24,25] The results are presented as final concentration of histamine above the baseline values.

Histamine released after haptides administration

Blood samples were collected after intravenous administration of haptides (preCγ or Cβ) or the control peptide Cα at a concentration of 280 μg/kg animal. The blood samples were centrifuged for 10 minutes at 3000 rpm. Aliquots of 50 μL plasma were used for the determination of histamine, as previously described.[24,25] Results are presented as net concentration of histamine released. The baseline histamine and negligible readings of haptides alone in the system were deducted from the relevant readings.

Human-derived LAD2 mast cells line

The LAD2 human mast cell line was maintained and used as previously described.[26] The LAD2 human mast cells (10^6/mL) were incubated with the Cβ and preCγ haptides. A dose response for both haptides activity was performed for 2 minutes incubation for doses of 0.1 to 100 μg/mL. Incubation of LAD2 mast cells was also performed for 2 time intervals (2 and 30 minutes) with the peptides Cβ or preCγ as well as with the control Cα at concentration of 50 or 100 μg/mL. Mast cell degranulation was measured by β-hexosaminidase (β-hex) release as previously described.[27,28] Briefly, 20 μL sample supernatants were mixed with 50 μL substrate solution (8 mmol/L *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 48 mmol/L citric acid and 56 mmol/L Na_2HPO_4, pH 4.5), and incubated for 90 minutes at 37°C. The reaction was stopped by addition of 150 μL ice-cold glycine (0.2 mol/L, pH 10.7), and the optical density measurement was immediately performed by using a spectrophotometer (OD 405 nm). Results were expressed as percentage of total β-hex activity present in the cells as previously described.[26,29] Total activity was the one measured in cell lysates plus supernatant. Haptides alone had no effect on the readings. For IgE-mediated activation, cells were sensitized overnight with 100 ng/mL human myeloma IgE-biotin (Calbiochem, Merck KGaA, Darmstadt, Germany), then stimulated with 100 ng/mL streptavidin (Jackson ImmunoResearch Laboratories, West Grove, Pa) as previously described.[30]

Statistical analysis

All values are expressed as means ± SEs. One-way ANOVA and a 2-tailed Student *t* test were used for comparing between relevant groups. Statistical differences of $P < .05$ were considered significant.

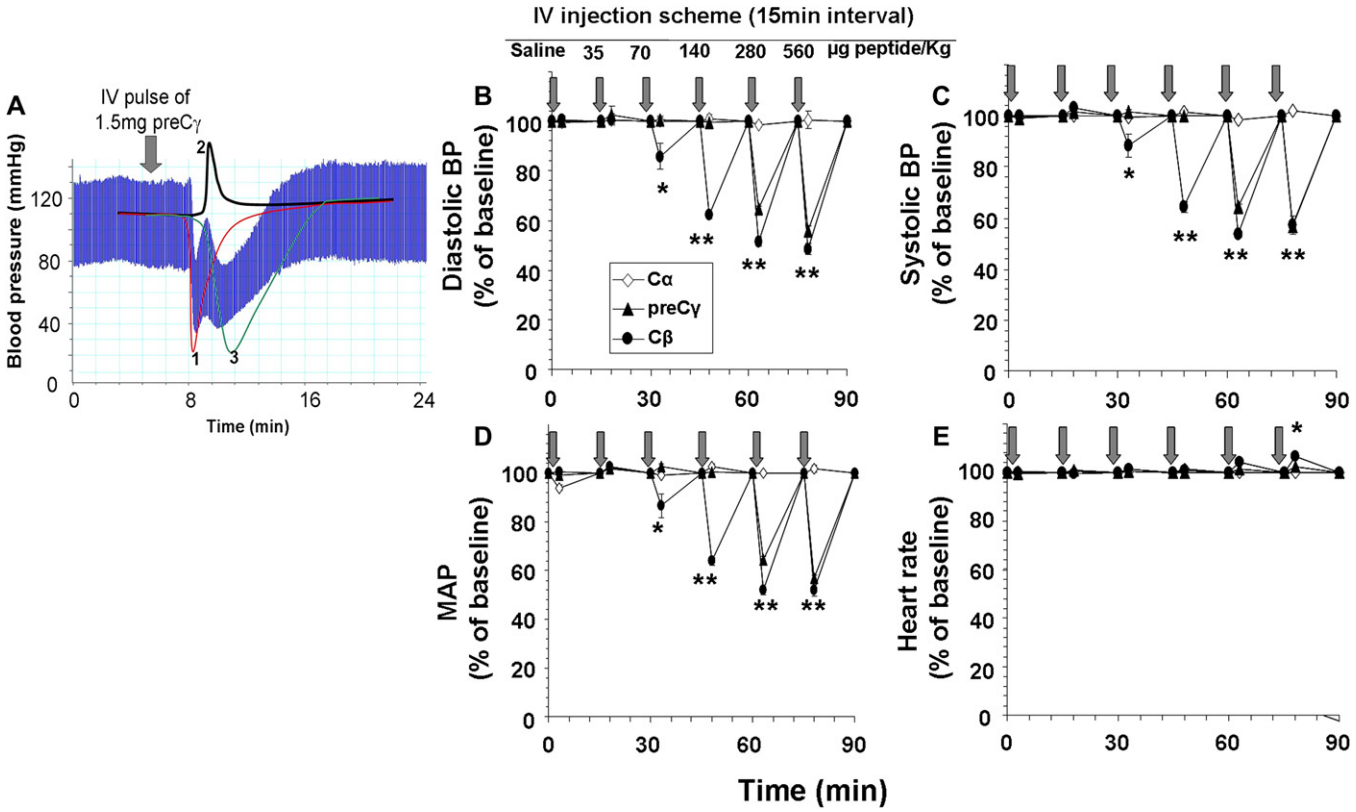


FIG 1. Effect of escalating doses of intravenous *(IV)* administration of haptides on blood-pressure and heart rate in the rat. **A,** An arterial pressure records at baseline and 6 minutes after intravenous injection of 1.5 mg/kg Cβ showing a triphasic response effect on blood pressure (lines 1-3). Haptides effect (35-560 µg/kg) on diastolic, systolic, and mean arterial BP as well as heart rate were tested (n = 6) (**B-E,** respectively). Results were compared with the control peptide Cα (*$P < .05$; **$P < .01$).

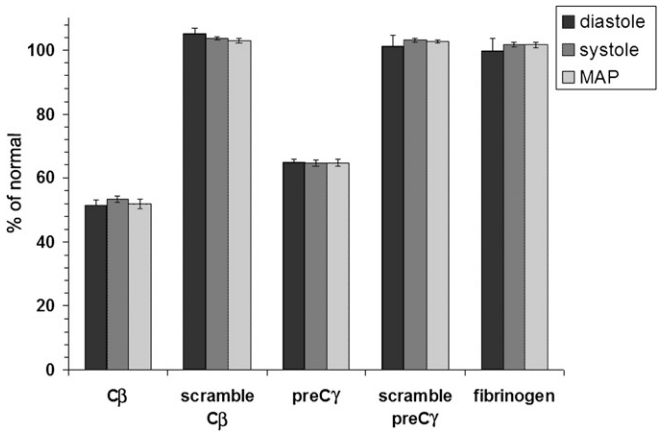


FIG 2. Effect of haptides versus scrambled haptides sequences on DBP, SBP, and MAP.Rats were injected with 280 µg/kg of the haptides Cβ, preCγ or their scrambled versions as well as with whole human fibrinogen (n = 6). The results (mean ± SE) are presented as percentages of the baseline values. The effects are compared with the baseline values ($P < .01$).

RESULTS

Effect of haptides on the rat hemodynamic parameters

Intravenous injection of a 1.5 mg/kg bolus of both fibrin-related Cβ and preCγ haptides in nonsedated rats caused immobilization of the animals with a shocklike behavior. In sedated rats, intravenous administration through the femoral vein resulted in significant reduction of blood pressure as monitored by intra-arterial monitor (Fig 1). The maximal effect was observed within 3 minutes after pulse administration of the haptides. Full recovery was recorded within 15 minutes. High-dose bolus of haptides resulted in triphasic effect on arterial blood pressure, made up of an initial intense but brief vasodepressor response followed by a moderate vasopressor response. The third phase was a longer-lasting vasodepressor response that persisted until the full recovery in about 15 minutes (Fig 1, *A*).

The administration of haptides (preCγ and Cβ) within the concentration range of 35 to 560 µg/kg produced a dose-dependent decrease of the systolic BP (SBP), diastolic BP (DBP), and MAP (Fig 1, *B-D*). Maximal MAP decrease of as much as 50% ($P < .01$) was recorded at a higher dose of 280 µg/kg (Fig 1, *D*). In addition, the HR minimally increased in correlation with doses of haptides given, reaching a significant elevation at 560 µg/mL ($P < .05$; Fig 1, *E*). However, the control peptide Cα at the same concentration did not affect both BP and HR (Fig 1, *B-E*).

Effect of scrambled haptides sequences and whole fibrinogen on rat blood pressure

To study the specific effect of haptides sequences that modulate BP, scrambled sequences of haptides were tested. In parallel, the effect of the whole intact fibrinogen molecule was also studied. Administration of 280 µg/kg scrambled sequences of the haptides, preCγ and Cβ, or fibrinogen did not affect the

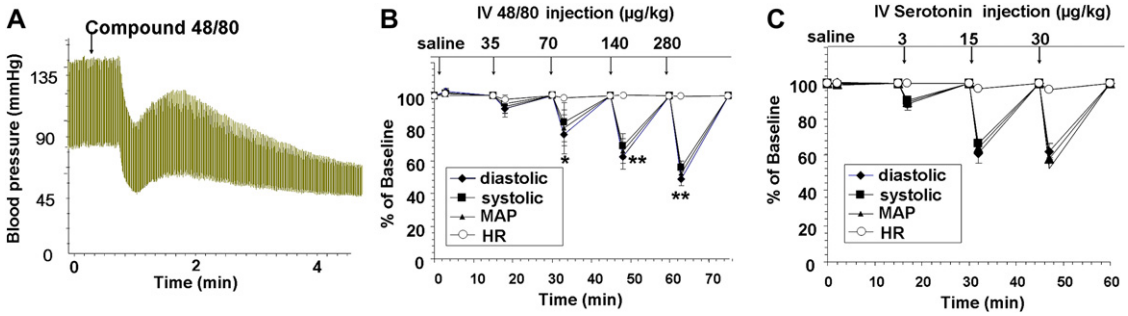


FIG 3. Compound 48/80–mast cell activator and serotonin decreases rat BP in the same manner as haptides. **A,** Trace record of arterial pressure at baseline and following intravenous *(IV)* injection in rat of 2 mg/kg compound 48/80. Hemodynamic parameters in response to escalating doses of compound 48/80 and serotonin are summarized in **B and C,** respectively (n = 6). Results presented as percentages of the baseline (mean ± SE). The effects are compared with the baseline values (**P* < .05; ***P* < .01).

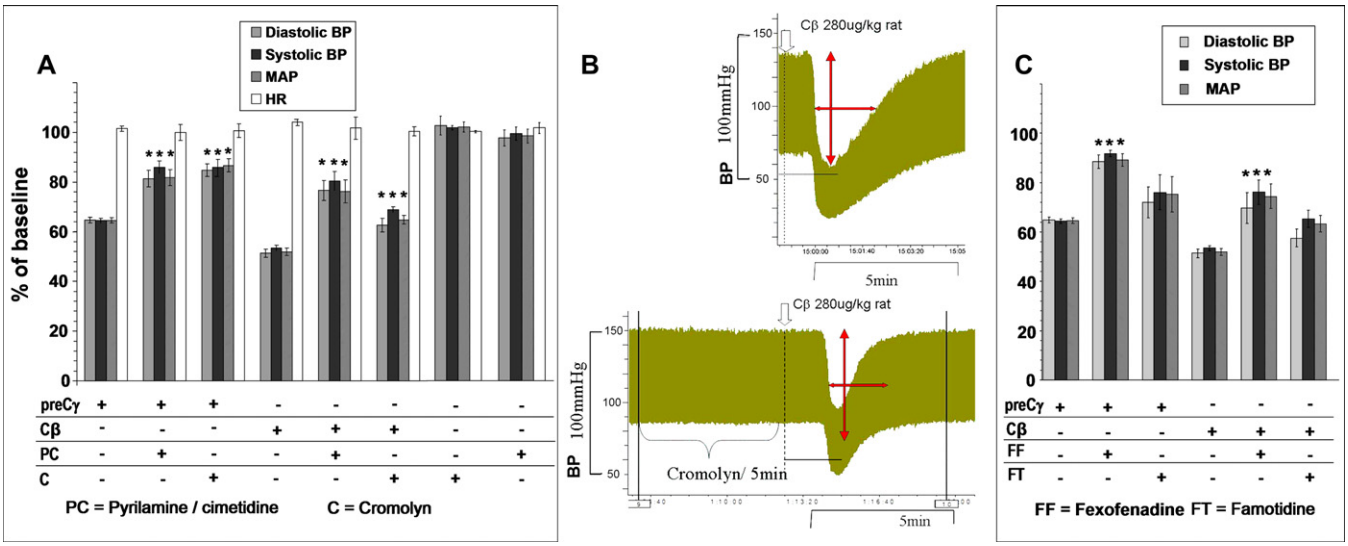


FIG 4. Histaminergic blockade attenuates haptides' effect on rat blood pressure. **A,** Effect of haptides (280 µg/kg) on hemodynamic parameters in rats pretreated with cromolyn or with pyrilamine and cimetidine. **B,** A chart of blood pressure drop caused by intravenous haptide administration and pretreatment with cromolyn. **C,** Effects of haptides on BP in rats pretreated with fexofenadine or famotidine. The results (mean ± SE) are presented as percentages of the baseline (n = 6; **P* < .05).

SBP, DBP, MAP, or HR (Fig 2). In contrast, haptides preCγ and Cβ at a concentration of 280 µg/kg decreased SBP, DBP, and MAP by 50% of baseline blood pressure values ($P < .01$).

Effect of the mast cell secretagogue compound 48/80 and serotonin on BP

The reduction of BP by haptides is similar to the effect observed after repeated injections of the mast cell secretagogue compound 48/80 (Fig 3, *A*). This suggests that haptides-mediated hypotension could be generated by mast cell activation and histamine release.

Intravenous bolus injection of compound 48/80 (35-280 µg/kg) in rats resulted in a dose-dependent decrease in SBP, DBP, and MAP (Fig 3, *B*). Serotonin injection in a range of 3 to 30 µg/kg also resulted in a dose-dependent decrease in the BP (Fig 3, *C*).

Histaminergic blockade attenuates haptide hypotension effect

To verify that haptides-induced hypotension is associated with mast cell activation, the rats were pretreated with histamine receptor blockers or with mast cell stabilizers. Pretreatment of the rats with cimetidine, a histamine receptor type 2 antagonist, and pyrilamine, a histamine receptor type 1 antagonist (Fig 4, *A*), resulted in a significant attenuation of haptides-induced decrease of the BP ($P < .05$). Infusion of the mast cell stabilizing agent cromolyn A 5 minutes before haptide injection hindered the haptide-mediated hypotension effect (Fig 4, *A*). The effect was most pronounced for preCγ ($P < .05$). Cromolyn alone did not affect any of the parameters tested. An example of a chart of the recorded BP drop caused by intravenous administration of haptide and modulation with pretreatment with cromolyn is presented in Fig 4, *B*. The *red arrows* indicate the amplitude and time interval of the response with no pretreatment with cromolyn *(upper chart)*.

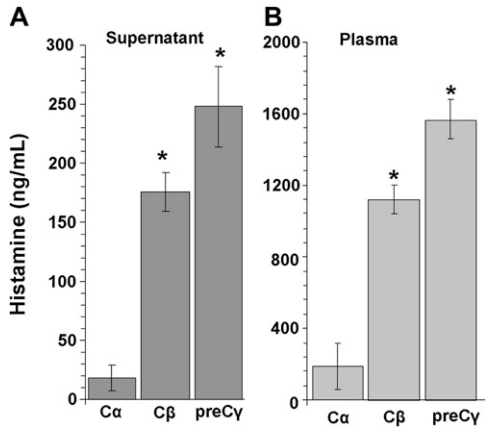


FIG 5. Haptides activate rat mast cells. Effect of haptides on histamine release in isolated rat peritoneal mast cells. The mast cells (2×10^5 cells/mL) were preincubated for 30 minutes with 100 μg/mL haptides **(A)**. Plasma histamine level in rats pretreated with 280 μg/kg preCγ, Cβ, or the control (Cα; n = 5; **B**). Results are presented as means ± SEs. The effects are compared with the control peptide Cα values (*$P < .01$).

The same arrows are superimposed on the response recorded with pretreatment with cromolyn *(lower chart)* for better manifestation of the reduction of the haptide effect. The newly introduced antihistamine agent fexofenadine showed a significant inhibition ($P < .05$) of haptides-induced decrease of BP (Fig 4, *C*). These results clearly demonstrate that mast cell stabilizers and histamine antagonists significantly attenuated the haptides-induced hypotensive response. This further indicates that the systemic effect of the haptides, as reflected by a drop of BP, seems to be associated with regulation of histamine secretion by mast cells.

Haptides activate rat peritoneal mast cells

The effect of haptides on the activation of isolated rat peritoneal mast cells was tested. Thus, peritoneal mast cells were incubated in the absence or presence of haptides (100 μg/mL), and histamine content was measured in the supernatant. Trypan blue exclusion assay showed that the haptides had no effect on cell viability, which was found to be greater than 98% in all experiments.

Incubation of rat peritoneal mast cells with haptides, preCγ, and Cβ for 30 minutes resulted in a significant histamine release (Fig 5, *A*) relative to the group that was treated with the Cα inactive control peptide ($P < .05$). Furthermore, intravenous haptides administration induced a significant increase in plasma histamine level (Fig 5, *B*) relative to the control group ($P < .05$).

Haptides activate cultured human mast cells

Haptides' effect on human mast cell (LAD2) degranulation was tested by β-hex release. A dose response of the haptides' effect after 2 minutes of exposure is shown in Fig 6, *A*. The effect was tested after incubation for 2 or 30 minutes in the presence of 2 doses of the haptides (50 and 100 μg/mL), as seen in Fig 6, *B and C*. In both concentrations, the haptides induced a highly significant β-hex release (Fig 6, *B and C*; $P < .05$) not dependent on the time of incubation. In the same conditions and experimental system, activation of the LAD2 human mast cells for 2 and 30 minutes via the FcεRI (biotinylated IgE plus streptavidin) resulted in only 5% and 15% β-hex release, respectively (data not shown). The effect of the haptides on β-hex release was also evident within the very short time of 2 minutes after the addition of the haptides, which coincides with the time scale of the systemic effect by *in vivo* administration. The control unrelated peptide Cα had no such effect (Fig 6, *B and C*).

Effect of serotonin receptor antagonist on the vasopressor phase mediated by haptides

Both the injection of serotonin and high-dose haptides induced a triphasic effect on arterial BP made up of an initial intense but brief vasodepressor response followed by a moderate vasopressor response (Fig 1, *A*). Finally, a longer-lasting vasodepressor response was recorded. The vasopressor phase varied quantitatively depending on the species and the experimental conditions.[31-34] The vasopressor response to haptide, preCγ, and Cβ, was blocked by ketanserin, a serotonin receptor type 2 (5-HT2) antagonist (Fig 7, *A-D*).

DISCUSSION

We have previously identified and characterized the haptides, a family of cell-binding and penetrating fibrin-related peptides.[2,5] The importance of the current study is in demonstrating that the haptides have major direct effects on systemic BP. Systemic intravenous administration of relatively low doses (35-560 μg/kg) of haptides Cβ and preCγ induced significant hypotension (Fig 1). The maximal effect was observed within 3 minutes after the bolus injection of the haptides. Higher doses of haptides (1.5 mg/rat) resulted in triphasic effect on the arterial BP, made up of an initial intense brief vasodepressor response followed by a moderate vasopressor response. Later, a longer-lasting vasodepressor response that returned to baseline within ~15 minutes was recorded (Fig 1,*A*). These findings have specific importance in cases in which massive fibrinolysis occurs or is induced as part of the treatment of occlusive arterial diseases, where vast amounts of fragments of fibrin with haptides sequences are released into the circulation.[8,9]

We demonstrated that the effect of haptides on BP dropping is a result of the activation of mast cells, followed by histamine and serotonin release, as observed during anaphylaxis.[31] This is further supported by the direct evidence whereby intravenous haptides injection indeed caused a significant histamine release (Fig 5, *A*). A similar effect on BP was also observed, with similar timing, after the injections of compound 48/80 (Fig 3), a known mast cell secretagogue, or serotonin, an active mediator and histamine analog in rats that is stored in mast cell granules.[32-34] In parallel, the effect of haptides on blood pressure was suppressed by histamine receptor type 1 and 2 antagonists or cromolyn A, a mast cell–stabilizing agent (Fig 4). Fexofenadine, a newly introduced agent, significantly suppressed the effect of haptides on blood pressure more than famotidine. Although famotidine is a histamine H2-receptor antagonist that inhibits H-pump and is used in the treatment of peptic ulcer disease and gastroesophageal reflux,[35] it is only marginally involved in regulating blood pressure compared with histamine H1 receptors that are blocked more efficiently by fexofenadine.[36] Therefore, it is not surprising that famotidine did not mask the effect of haptides as much as recorded with fexofenadine.

The time interval of haptides-induced *in vivo* maximal effect was short, within ~3 minutes after their administration. To

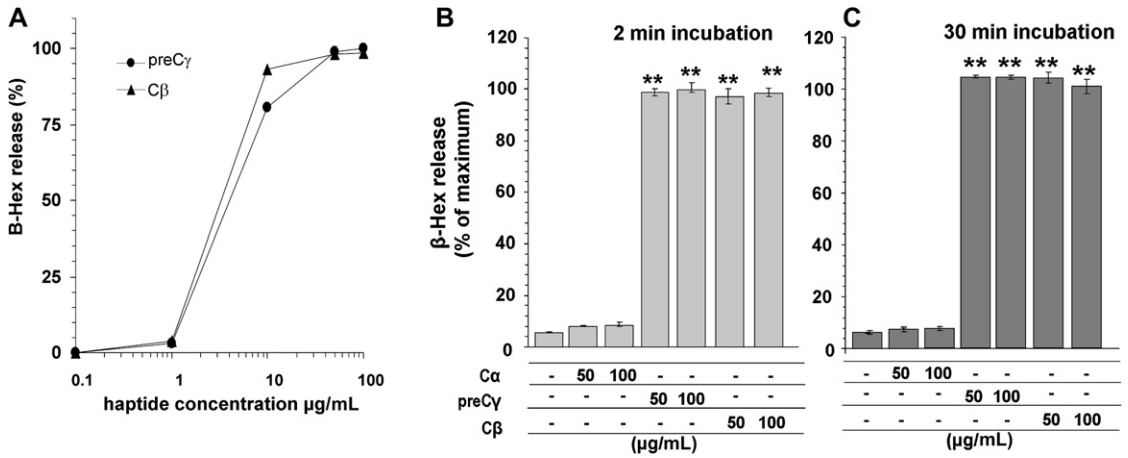


FIG 6. Effect of haptides on degranulation of cultured LAD2 cells. Haptides effects on LAD2 cells activation as monitored by β-hex secretion. **A,** Dose response of 2-minute incubation with haptides. The cells were preincubated for 2 minutes **(B)** or 30 minutes **(C)** with 50 or 100 μg/mL with haptides preCγ, Cβ, or the control Cα (n = 4). Values are percentages of total β-hex release. The haptides effects are presented relative to Cα (**$P < .01$).

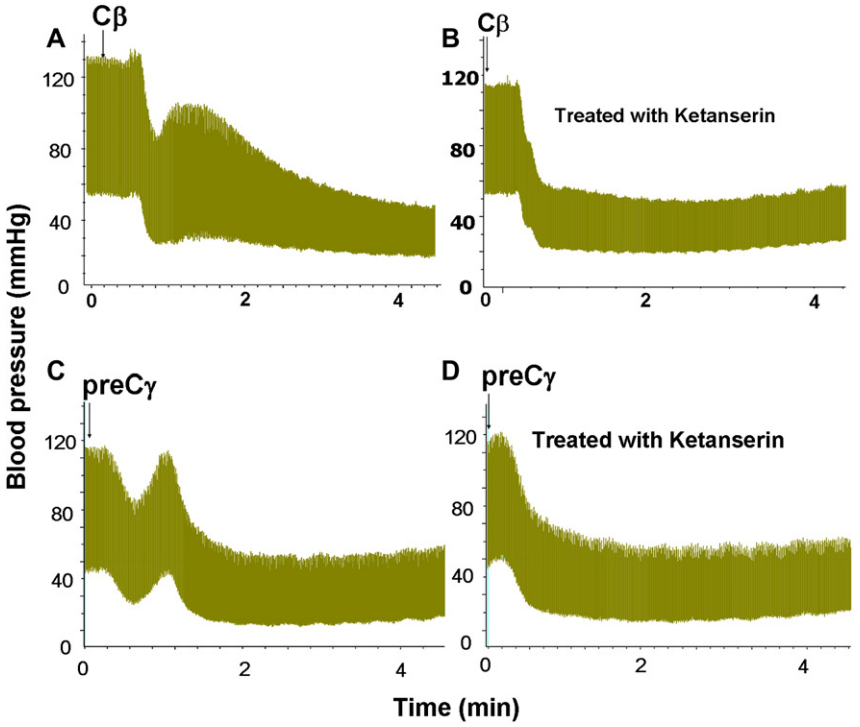


FIG 7. Effect of intravenous administration of ketanserin, 5-HT2 receptor antagonists, on BP variability in rats. Trace records of arterial BP at baseline and 6 minutes after injection into the femoral veins of 2 mg/kg haptides Cβ **(A)** or preCγ **(C)**. Tracings of arterial BP records where both haptides were injected 10 minutes after a single dose of intravenous ketanserin (1 mg/kg) administration are presented in **B and D,** respectively.

investigate whether this timing is sufficient for an induced effect caused by mast cells activation, we tested the timing for a direct effect of haptides on cultured mast cells. Both haptides preCγ and Cβ induced significant degranulation of the LAD2 cells (Fig 6) within a short time of 2 minutes. This indeed conforms to the timing of the onset of the effect observed in the whole animal (Fig 1).

The vasopressor response to serotonin can be blocked by 5-HT2 receptor antagonists such as ketanserin.[37,38] Accordingly, the vasopressor effects on arteries and veins from rats and humans are generally mediated by 5-HT2 receptors.[33,37-39] We found that the vasopressor response to haptides was blocked by 5-HT2 receptor antagonist ketanserin (Fig 7), indicating that haptides affect on cardiovascular function is associated with activation of the serotonin receptors.

It is expected that in treating certain pathological conditions such as occlusive blood vessels disease, massive degradation of fibrin and fibrinogen is induced. In such conditions, peptides

containing haptides sequences are released into the circulation. Each fibrinogen molecule contains 4 haptidic sequences.[8,9] In cases with massive fibrinolysis, fibrin degradation products with haptide containing sequences are equivalent to haptides levels of ~1 to 5 μg/mL.[40,41] In our rat model, the effective levels of haptides in the circulation that influenced BP were in the range of 0.5 to 7 μg/mL. In the *in vitro* system with LAD2 cells (Fig 6, *A*), the potent concentrations tested (<10 μg/mL) were equivalent to those used *in vivo* (~0.5-7 μg/mL blood or 70-560 μg/kg animal). Therefore, the potent levels used in the experimental rat model seem to be equivalent to the physiological situation in cases associated with massive fibrinolysis.

It was previously demonstrated that low-molecular-weight fibrinogen degradation products caused dose-dependent secretion of collagenase and histamine from mast cells.[42] Our study identifies the haptides as potential fibrin-related sequences that could trigger such effects after fibrinolysis.

The damage to blood vessels caused by clot formation induced endothelial-mediated mast cell attraction. The activated mast cells were previously shown to release β-tryptase, a specific enzyme of mast cells.[43] Tryptase, a very potent proteolytic enzyme, was shown further to enhance full degradation of fibrin, as well as fibrinogen, to produce fibrinopeptides of smaller size containing haptides sequences.[44] This positive feedback cycle may increase the haptides release, which may further activate the mast cells that in turn secrete more tryptase.

Therapeutic use of plasminogen activators remains controversial because of the sometimes inefficient restoration of blood vessel tonus, coupled with the risk of hypotension as a side-effect. Streptokinase-based treatment was found to be associated with a risk of developing hypotension.[12,45] The mechanism responsible for this streptokinase effect remains unknown.[18,46,47] We suggest that streptokinase-induced fibrinolysis through plasmin activation results in the release of smaller fragments containing the C-termini of fibrin with haptide sequences,[8,9] which, as shown here, may trigger hypotension.

It is of interest whether the specific haptides effect may be mediated by a receptor. The control peptide Cα, which is not related to the haptides, did not affect the BP (Fig 1). Even arbitrary scrambled sequences of the haptides that have similar mean hydrophobicity and positive charge had no such effect (Fig 2). Furthermore, we found that haptides caused major mast cell activation in a system without IgE and added complement (Fig 6), whereas IgE and the specific antigen caused merely 15% activation at 30 minutes.[29] These findings suggest that the haptides effect is probably not equivalent to classical IgE mediated activation. Their major effect may be mediated through a specific receptor on the target cells that has not been identified yet. Nevertheless, a direct effect, such as the one recorded by basic secretagogues that were previously proposed as effectors of anaphylaxis, still cannot be ruled out.[29,48]

Integrin αIIβb3 is highly expressed in mouse, rat, and human mast cells. In the presence of stem cell factor and FcεRI, it was reported to bind to fibrinogen and activate the mast cells.[49] We found that the haptides alone, but not the whole fibrinogen, with no addition of other factors, induced mast cells activation. Therefore, other candidate targets may also be considered.

To conclude, haptides were shown to induce release of histamine and serotonin from mast cells, which can explain their mediation of hypotension. This response can be abrogated by histamine receptor antagonists and other anti-inflammatory drugs. These findings may be relevant to the possible mode of action of circulating fibrinogen fragments containing haptides as a result of induced fibrinolysis in the management of vascular occlusive diseases.

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Clinical implications: Haptides, short peptides homologous to sequences on the C-termini of fibrinogen, induce hypotension by mast cell activation; thus, they are presented as possible key mediators of hypotension after streptokinase-induced fibrinolysis.

REFERENCES

1. Gorodetsky R, Vexler A, An J, Mou X, Marx G. Haptotactic and growth stimulatory effects of fibrin(ogen) and thrombin on cultured fibroblasts. J Lab Clin Med 1998;131:269-80.
2. Gorodetsky R, Vexler A, Shamir M, An J, Levdansky L, Shimeliovich I, et al. New cell attachment peptide sequences from conserved epitopes in the carboxy termini of fibrinogen. Exp Cell Res 2003;287:116-29.
3. Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost 2005;3:1894-904.
4. Fu Y, Cao Y, Hertzberg KM, Grieninger G. Fibrinogen alpha genes: conservation of bipartite transcripts and carboxy-terminal-extended alpha subunits in vertebrates. Genomics 1995;30:71-6.
5. Gorodetsky R, Levdansky L, Vexler A, Shimeliovich I, Kassis I, Ben-Moshe M, et al. Liposome transduction into cells enhanced by haptotactic peptides (haptides) homologous to fibrinogen C-termini. J Control Release 2004;95:477-88.
6. Marx G, Hotovely-Salomon A, Levdansky L, Gaberman E, Snir G, Sievner Z, et al. Haptide-coated collagen sponge as a bioactive matrix for tissue regeneration. J Biomed Mater Res B Appl Biomater 2008;84:571-83.
7. Basheer M, Schwalb H, Gilon D, Doviner V, Sherman Y, Shapira OM, et al. Short homologous peptides based on C-terminal sequences of fibrinogen beta- and gamma-chains (haptides) affect cardiovascular function by eNOS inhibition. J Vasc Res 2010;47:507-518.
8. Walker JB, Nesheim ME. The molecular weights, mass distribution, chain composition, and structure of soluble fibrin degradation products released from a fibrin clot perfused with plasmin. J Biol Chem 1999;274:5201-12.
9. Lucas MA, Fretto LJ, McKee PA. The relationship of fibrinogen structure to plasminogen activation and plasmin activity during fibrinolysis. Ann N Y Acad Sci 1983;408:71-91.
10. Nee PA. Thrombolysis after acute myocardial infarction. J Accid Emerg Med 1997; 14:2-9.
11. Glogar D, Yang P, Steurer G. Management of acute myocardial infarction: evaluating the past, practicing in the present, elaborating the future. Am Heart J 1996; 132(2 pt 2 suppl):465-70; discussion 496-502.
12. Menon V, Harrington RA, Hochman JS, Cannon CP, Goodman SD, Wilcox RG, et al. Thrombolysis and adjunctive therapy in acute myocardial infarction: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004; 126(3 suppl):549S-75S.
13. Marder VJ, Shulman NR, Carroll WR. High molecular weight derivatives of human fibrinogen produced by plasmin, I: physicochemical and immunological characterization. J Biol Chem 1969;244:2111-9.
14. Wells PS, Anderson DR, Rodger M, Forgie M, Kearon C, Dreyer J, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. N Engl J Med 2003;349:1227-35.
15. Baglin T. Disseminated intravascular coagulation: diagnosis and treatment. BMJ 1996;312:683-7.
16. Gaffney PJ, Brasher M, Lord K, Strachan CJ, Wilkinson AR, Kakkar VV, et al. Fibrin subunits in venous and arterial thromboembolism. Cardiovasc Res 1976;10: 421-6.
17. Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM. C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are

inflammation and fibrin turnover linked in pathogenesis? Arterioscler Thromb Vasc Biol 2001;21:603-10.
18. Michiels JJ, Palareti G, de Moerloose P. Fibrin D-dimer testing for venous and arterial thrombotic disease. Semin Vasc Med 2005;5:311-4.
19. McRedmond JP, Harriott P, Walker B, Fitzgerald DJ. Streptokinase-induced platelet activation involves antistreptokinase antibodies and cleavage of protease-activated receptor-1. Blood 2000;95:1301-8.
20. Chalupowicz DG, Chowdhury ZA, Bach TL, Barsigian C, Martinez J. Fibrin II induces endothelial cell capillary tube formation. J Cell Biol 1995;130:207-15.
21. Sporn LA, Bunce LA, Francis CW. Cell proliferation on fibrin: modulation by fibrinopeptide cleavage. Blood 1995;86:1802-10.
22. Ribes JA, Ni F, Wagner DD, Francis CW. Mediation of fibrin-induced release of von Willebrand factor from cultured endothelial cells by the fibrin beta chain. J Clin Invest 1989;84:435-42.
23. Francis CW, Bunce LA, Sporn LA. Endothelial cell responses to fibrin mediated by FPB cleavage and the amino terminus of the beta chain. Blood Cells 1993;19:291-306.
24. Aridor M, Traub LM, Sagi-Eisenberg R. Exocytosis in mast cells by basic secretagogues: evidence for direct activation of GTP-binding proteins. J Cell Biol 1990; 111:909-17.
25. Shore PA, Burkhalter A, Cohn VH Jr. A method for the fluorometric assay of histamine in tissues. J Pharmacol Exp Ther 1959;127:182-6.
26. Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, et al. Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcepsilonRI or FcgammaRI. Leuk Res 2003;27:677-82.
27. Shalita-Chesner M, Koren R, Mekori YA, Baram D, Rotem C, Liberman UA, et al. 1,25-Dihydroxyvitamin D3 enhances degranulation of mast cells. Mol Cell Endocrinol 1998;142:49-55.
28. Bhattacharyya SP, Drucker I, Reshef T, Kirshenbaum AS, Metcalfe DD, Mekori YA. Activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact. J Leukoc Biol 1998;63:337-41.
29. Kulka M, Sheen CH, Tancowny BP, Grammer LC, Schleimer RP. Neuropeptides activate human mast cell degranulation and chemokine production. Immunology 2008;123:398-410.
30. Salamon P, Shoham NG, Puxeddu I, Paitan Y, Levi-Schaffer F, Mekori YA. Human mast cells release oncostatin M on contact with activated T cells: possible biologic relevance. J Allergy Clin Immunol 2008;121:448-55, e5.
31. Page IH, Mc CJ. Modification of vascular response to serotonin by drugs. Am J Physiol 1953;174:436-44.
32. Saxena PR, Villalon CM. Cardiovascular effects of serotonin agonists and antagonists. J Cardiovasc Pharmacol 1990;15(suppl 7):S17-34.
33. Villalon CM, Heiligers JP, Centurion D, De Vries P, Saxena PR. Characterization of putative 5-HT7 receptors mediating tachycardia in the cat. Br J Pharmacol 1997; 121:1187-95.
34. Villalon CM, Centurion D, Lujan-Estrada M, Terron JA, Sanchez-Lopez A. Mediation of 5-HT-induced external carotid vasodilatation in GR 127935-pretreated vagosympathectomized dogs by the putative 5-HT7 receptor. Br J Pharmacol 1997;120:1319-27.
35. Pounder R. Histamine H2-receptor antagonists. Baillieres Clin Gastroenterol 1988; 2:593-608.
36. Devillier P, Roche N, Faisy C. Clinical pharmacokinetics and pharmacodynamics of desloratadine, fexofenadine and levocetirizine: a comparative review. Clin Pharmacokinet 2008;47:217-30.
37. Martin GR. Vascular receptors for 5-hydroxytryptamine: distribution, function and classification. Pharmacol Ther 1994;62:283-324.
38. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 1994;46:157-203.
39. Villalon CM, Centurion D. Cardiovascular responses produced by 5-hydroxytriptamine: a pharmacological update on the receptors/mechanisms involved and therapeutic implications. Naunyn Schmiedebergs Arch Pharmacol 2007;376:45-63.
40. Altiay G, Ciftci A, Demir M, Kocak Z, Sut N, Tabakoglu E, et al. High plasma D-dimer level is associated with decreased survival in patients with lung cancer. Clin Oncol (R Coll Radiol) 2007;19:494-8.
41. Tick LW, Nijkeuter M, Kramer MH, Hovens MM, Buller HR, Leebeek FW, et al. High D-dimer levels increase the likelihood of pulmonary embolism. J Intern Med 2008;264:195-200.
42. Wojtecka-Lukasik E, Maslinski S. Fibronectin and fibrinogen degradation products stimulate PMN-leukocyte and mast cell degranulation. J Physiol Pharmacol 1992; 43:173-81.
43. Bankl HC, Valent P. Mast cells, thrombosis, and fibrinolysis: the emerging concept. Thromb Res 2002;105:359-65.
44. Schwartz LB, Bradford TR, Littman BH, Wintroub BU. The fibrinogenolytic activity of purified tryptase from human lung mast cells. J Immunol 1985;135: 2762-7.
45. Lew AS, Laramee P, Cercek B, Shah PK, Ganz W. The hypotensive effect of intravenous streptokinase in patients with acute myocardial infarction. Circulation 1985;72:1321-6.
46. Pachai A, Erlendsson AK, Brandslund I. Streptokinase, complement activation and hypotension. APMIS 1997;105:650-4.
47. Gemmill JD, Hogg KJ, Douglas JT, Dunn FG, Lowe GD, Rae AP, et al. The incidence and mechanism of hypotension following thrombolytic therapy for acute myocardial infarction with streptokinase-containing agents—lack of relationship to pretreatment streptokinase resistance. Eur Heart J 1993;14:819-25.
48. Simons FE. Anaphylaxis. J Allergy Clin Immunol 2008;121(suppl 2):S402-7.
49. Oki T, Kitaura J, Eto K, Lu Y, Maeda-Yamamoto M, Inagaki N, et al. Integrin alphaIIbbeta3 induces the adhesion and activation of mast cells through interaction with fibrinogen. J Immunol 2006;176:52-60.