Ramipril and metoprolol intake aggravate human and murine anaphylaxis: Evidence for direct mast cell priming

Maria Nassiri, MS,[a] Magda Babina, PhD,[a] Sabine Dölle, PhD,[a] Günter Edenharter, PhD,[b] Franziska Ruëff, MD,[c] and Margitta Worm, MD[a] *Berlin and Munich, Germany*

Background: Cofactors contribute to the elicitation of anaphylaxis. β-Blockers and angiotensin-converting enzyme (ACE) inhibitors are widely used cardiovascular drugs. We specially designed a mouse model to further analyze the cofactor potential of these drugs.

Objective: We sought to test the hypothesis that β-blockers and ACE inhibitors alter the risk for severe anaphylaxis and to pinpoint the associated mechanism.

Methods: The risk factor potency of cardiovascular drugs on the severity of anaphylaxis in patients from German-speaking countries was analyzed. *In vivo* interaction of the cardiovascular drugs metoprolol (β-blocker) and ramipril (ACE inhibitor) with the anaphylactic response was determined. Mast cell (MC) mediators (histamine, serotonin, leukotriene C_4, prostaglandin D_2, and mouse mast cell protease 1) were quantified in serum. Bone marrow–derived cultured MCs served to identify whether the therapeutics targeted MCs directly.

Results: Our anaphylaxis database indicated a higher risk of severe anaphylaxis after monotherapy with β-blockers or ACE inhibitors, which was more pronounced when both drugs were combined. This was confirmed in our mouse model. While single therapeutics had either no significant (ramipril) or a modestly aggravating (metoprolol) effect, their combined administration exacerbated anaphylactic symptoms potently and simultaneously enhanced MC mediators, hinting at MCs as direct targets. In fact, FcεRI-mediated MC histamine release was synergistically increased by metoprolol/ramipril or metoprolol/bradykinin (the latter increased after ACE inhibitor intake), whereas the substances had no significant effect on their own. MC priming was particularly pronounced when FcεRI aggregation was in the suboptimal range, reflecting common clinical settings.

Conclusion: β-Blockers and ACE inhibitors synergistically aggravate anaphylaxis at least partly by decreasing the threshold of MC activation. (J Allergy Clin Immunol 2015;135:491-9.)

Key words: *Anaphylaxis, β-blocker, angiotensin-converting enzyme inhibitor, cofactor, mast cells, cardiovascular medication*

Abbreviations used

ACE:	Angiotensin-converting enzyme
BMcMCs:	Bone marrow–cultured mast cell
cAMP:	Cyclic AMP
LT:	Leukotriene
MC:	Mast cell
mMCP-1:	Mouse mast cell protease 1
PGD_2:	Prostaglandin D_2
PK:	Protein kinase
PSA:	Passive systemic anaphylaxis

Anaphylaxis is a severe IgE-mediated and mast cell (MC)–dependent hypersensitivity reaction with a potentially fatal outcome. Together with cutaneous and gastrointestinal symptoms, the respiratory and cardiovascular systems are frequently involved.[1-3] The most prevalent elicitors of anaphylaxis are food, drugs, and venom,[4-6] but reactions do not always occur on contact with the offending allergen, even in the same patient.

In fact, about 30% of reactions are elicited under specific circumstances only, and these observations have led to the concept of so-called augmenting factors or cofactors, which need to combine with the triggering allergen for anaphylaxis to ensue.[7-10] Drugs are an important group of relevant cofactors.[5] They can interact at several levels and through different mechanisms with the anaphylactic response. The best understood examples are cases in which the drug itself acts as the allergen (eg, β-lactams), so that MC degranulation is triggered like a classical type I allergic reaction.[11] Another group of therapeutics (eg, opioids) activate MCs directly in an IgE-independent manner.[12,13] The third and largest group comprises a heterogeneous collection (eg, nonsteroidal anti-inflammatory drugs or most contrast media), which generally do not degranulate MCs directly[14,15] but which can nevertheless affect anaphylactic responses; their mechanisms of action are poorly defined thus far. β-Blockers and angiotensin-converting enzyme (ACE) inhibitors are commonly used in the management of cardiovascular diseases[16,17] and supposedly belong to the latter category. Their use has increased over the last decades,[18,19] but the intersection of these drugs with anaphylaxis is not well understood. The question of whether single cardiovascular drugs serve as potentiating factors in anaphylaxis is likewise still a matter of debate.[20-23] Cardiovascular medication is commonly prescribed as a combination of drugs from different categories because single substances are often insufficient to control blood pressure appropriately.[24,25] The possibility that such cardiovascular drugs

From [a]Allergy Center Charité, CCM, Department of Dermatology and Allergology, Charité-Universitätsmedizin Berlin; [b]Edenharter Research, Berlin; and [c]the Department of Dermatology and Allergology, Ludwig-Maximilians-Universität, Munich.

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Corresponding author: Margitta Worm, MD, Allergy Center Charité, CCM, Department of Dermatology and Allergology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany. E-mail: Margitta.worm@charite.de.

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might aggravate anaphylactic responses when used alone or in combination has not been studied in detail thus far. Here we first assess the potential role of β-blockers and ACE inhibitors in the context of anaphylaxis based on data extracted from our extensive registry for German-speaking countries,[8] showing that both therapeutics display modest yet discernible anaphylaxis-promoting effects on their own and more pronounced effects if combined. After establishing a mouse model to investigate the role of potential cofactors on anaphylactic responses, we confirm the above effects from epidemiologic data in the mouse and furthermore show that the β-blocker metoprolol and the ACE inhibitor ramipril have modest anaphylaxis-promoting activities as single substances but display clearly potentiating effects when administered in combination. In search of the underlying mechanism, we identify MCs as direct targets of the drugs, which are primed for enhanced responses to FcεRI aggregation. Effects preferentially occur when the degree of FcεRI cross-linking is in the suboptimal range, presumably reflecting the scenario most commonly encountered in clinical settings.

METHODS

Anaphylaxis registry, data extraction, and analysis

The anaphylaxis registry contains data from patients with severe allergic reactions entered by allergists using a standardized online questionnaire, as described previously.[3] We analyzed data sets from 4783 patients collected in 2006-2014 and defined 4 groups according to their anaphylaxis severity grades. Severity grading was based on the clinical reaction patterns assessed before first aid treatment was given. The groups were stratified for the variables of use of antihypertensive medication either as monotherapy or in combination or patients without antihypertensive medication.

Mice

Female BALB/c wild-type mice (10-12 weeks old) were obtained from Charles River (Sulzfeld, Germany). Animals were kept under specific pathogen-free conditions in a temperature-controlled environment with free access to standard chow and water. All experiments were approved by the local State Office of Health and Social Affairs.

Passive cutaneous anaphylaxis

Mice received ramipril (2.5 mg/kg; kindly provided by Sanofi-Aventis, Frankfurt, Germany) through drinking water and metoprolol (30 mg/kg dissolved in water; Sigma-Aldrich, Hamburg, Germany) by means of oral gavage, either alone or in combination, for 14 days. Sham-treated mice received water instead. The average amount of daily fluid intake was monitored for 2 weeks before the experiment and during the experiment every day to ensure the mice received the correct dose of ramipril. On day 14, mouse anti-trinitropehnyl-IgE (anti-TNP-IgE; BD Bioscience PharMingen, San Jose, Calif) was injected subcutaneously (3 ng) into the right ear and PBS alone into the left ear. Twenty-four hours later, mice were challenged with 7 μg of TNP-BSA (Biocat, Heidelberg, Germany) together with 1% Evans Blue administered intravenously. After 30 minutes, mice were killed, and vascular permeability was visualized based on blue staining of the ear. Skin samples were taken, and Evans blue was extracted with dimethylformamide. The amount of dye was quantified based on OD_{600} measurement.

Passive systemic anaphylaxis

Mice were passively sensitized with anti–TNP-IgE administered intravenously (7 μg), followed by an intravenous injection of TNP-BSA 24 hours later at the dosages shown in the figure legends. Changes in rectal temperature were assessed for 70 minutes with a digital thermometer (Physitemp Instruments, Clifton, NJ).

After preoptimization of the passive systemic anaphylaxis (PSA) model, mice received oral ramipril and metoprolol alone or in combination for 14 days. Sham-treated mice received water instead. Mice were then sensitized with mouse anti–TNP-IgE, followed 24 hours later by challenge with the optimized dose of TNP-BSA (7 μg). Anaphylaxis was determined as above, and plasma samples were collected.

Quantification of MC mediators in serum

Levels of histamine (LDN, Nordhorn, Germany), serotonin (IBL, Hamburg, Germany), leukotriene (LT) C_4, prostaglandin D_2 (PGD_2; Cayman Chemical, Ann Arbor, Mich) and mouse mast cell protease 1 (mMCP-1; eBioscience, Frankfurt, Germany) were measured by using ELISA, according to the manufacturers' instructions.

MCs

Bone marrow specimens were obtained from female BALB/c mice and cultured in IMDM medium (PAA, Cölbe, Germany) in the presence of mouse recombinant IL-3 (ImmunoTools, Friesoythe, Germany) for 4 to 6 weeks at 37°C and 5% CO_2 in a humidified atmosphere to obtain bone marrow–cultured mast cells (BMcMCs), which were identified as MCs by the expression of c-Kit and FcεRI. BMcMCs were sensitized with anti–TNP-IgE (1 μg/mL) overnight, washed with PAG-CM buffer (PIPES albumin glucose buffer, pH 7.5, containing 3 mmol/L $CaCl_2$ and 1.5 mmol/L $MgCl_2$), and preincubated with different concentrations of ramiprilat (active form of ramipril, kindly provided by Sanofi-Aventis), metoprolol, or bradykinin (Sigma-Aldrich) alone or in several combinations for 20 minutes at 37°C to investigate the effect of cardiovascular medication on histamine release. Drug concentrations were based on clinical plasma concentrations achievable during antihypertensive medication.[26-28] Cells were activated by the addition of 2 concentrations of anti-IgE (0.4 or 4 μg/mL; BD PharMingen, San Jose, Calif) or kept in PAG-CM only (spontaneous release) for 25 minutes at 37°C. Calcium ionophore A23187 (2 μmol/L; Calbiochem, San Diego, Calif) was used as a positive control to assess the degree of MC release. Total histamine content of the cells was determined after cell lysis with 1% perchloric acid (complete). Histamine release was assessed in cell supernatants by using an autoanalyzer (Borgwald Technik, Hamburg, Germany).[29,30] Net histamine release was calculated as follows:

$$\text{Net histamine release } (\%) = [(\text{Stimulated release} - \text{Spontaneous release})/\text{Complete}] \times 100.$$

Statistical analysis

Logistic regression to compute crude and adjusted odds ratios was performed with STATA software, version 12 (StataCorp, College Station, Tex). All other statistical analyses were performed with PRISM 5.0 software (GraphPad Software, La Jolla, Calif) by using nonparametric 1-way ANOVA, followed by the Bonferroni test. Data are shown as means ± SEMs. A P value of less than .05 was considered statistically significant.

RESULTS

Increased risk for severe anaphylaxis in patients taking ACE inhibitors and β-blockers

It is still controversial whether monotherapies with either β-blockers or ACE inhibitors aggravate anaphylaxis. Therefore we analyzed the effect of β-blocker and ACE inhibitor intake on the severity of anaphylaxis using clinical data from the anaphylaxis registry. A summary of anaphylactic cases collected in 2006-2014 is presented in Table I. We performed a risk factor analysis, which included 4 groups of anaphylactic patients, classified into severity grades according to Ring and Messmer.[31] Our results show a slight increase in odds ratios for patients who used the combined drugs (Fig 1, *A*) if severity grades I/II and III/IV were used as dependent variables. This effect was more pronounced when patients with the most severe reactions were analyzed in comparison with patients with less severe