Mast Cell Anergy: absence of symptoms after accidental re-exposure to amoxicillin/clavulanic acid 3 days after anaphylaxis

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Abstract

Empty Mast Cell Syndrome, also named Post Anaphylaxis Mast Cell Anergy (PAMA), is a temporary state of loss of mast cell responsiveness after a severe immediate hypersensitivity reaction. We describe a case of PAMA after accidental re-exposure to amoxicillin in a patient who developed severe anaphylaxis to this drug three days earlier in the operating room.

1. Introduction

Post-anaphylactic mast cell anergy defines a state of mast cell unresponsiveness following massive activation. It is manifested by a loss of cutaneous reactivity to skin tests, and has been described in several series[1, 2] and clinical cases[3, 4, 5, 6] in the context of anaphylaxis to hymenoptera venoms and perioperative anaphylaxis. Current recommendations by allergology societies indicate diagnostic skin testing between 4 and 8 weeks[7, 8] after a severe hypersensitivity reaction to avoid false negative results. The mechanisms involved in this phenomenon are incompletely understood and implicate mast cells mediators depletion.

We report a case of mast cell anergy which enabled a patient to tolerate accidental reintroduction of amoxicillin, despite having presented with intraoperative anaphylactic shock to amoxicillin 3 days earlier.

2. Case report

A 77-year-old patient was admitted for a left ureteroileoplasty. During anesthetic induction, he presented hypotension after mechanical ventilation treated with epinephrine from which he recovered, and when receiving prophylactic antibiotic therapy with 2 grams of amoxicillin/clavulanic acid (AC) intravenously he developed diffuse urticaria with bronchospasm, followed by cardiac arrest within 2 minutes. Cardiopulmonary resuscitation was successful, and the patient required 6 mg of intravenous epinephrine, external electric shock, 11 puffs of salbutamol and 100 mg of hydrocortisone. Serum biomarkers were obtained and elevated tryptase and histamine levels confirmed mast cells and possibly basophils activation and degranulation during anaphylaxis (**Table I**). The surgical procedure was not performed, and the patient was transferred to the intensive care unit for recovery and monitoring. Allergy to amoxicillin or clavulanic acid was suspected. On day 3 after the event, he developed pneumonia and 1 g of oral amoxicillin/clavulanic acid was administered without symptoms. As the prescribing error was quickly identified, no additional doses were administered, and the antibiotic therapy was replaced by levofloxacin. The patient was informed of the prescribing error.

The allergology consultation carried out four weeks later revealed that the patient had been taking amoxicillin (3 grams/day for 4 days), which had been well tolerated one month before the event, due to urinary tract infection. Skin prick tests (SPTs) and intradermal skin tests (IDTs) for amoxicillin, amoxicillin/clavulanic acid, cefazolin, piperacillin-tazobactam and cefotaxime; SPT for latex and basophil activation tests (BATs)

(expression of CD63/CD203c by flow cytometry) for amoxicillin, amoxicillin/clavulanic acid, cefazolin, piperacillin-tazobactam and cefotaxime were performed. The results confirmed an IgE-mediated allergy to amoxicillin (**Table II**): IDTs and BATs were positive for amoxicillin and amoxicillin/clavulanic acid and negative to all other drugs. A challenge with 3000 mg of piperacillin-tazobactam was negative.

Amoxicillin and clavulanic acid were considered responsible for the per-operative anaphylactic shock, and the diagnosis of post-anaphylactic mast cell anergy was proposed to explain the absence of clinical reaction to the reintroduction of 1 gram of amoxicillin/clavulanic acid on day 3 after anaphylaxis.

3. Discussion

We report a case of perioperative grade 4 anaphylaxis to amoxicillin/clavulanic acid with re-exposure to the molecule 72 hours later without reaction. The patient had become sensitized to amoxicillin during a treatment received one month before the event. Positive skin tests and BATs carried out one month after the anaphylaxis confirmed the sensitization. The absence of an allergic reaction when 1 gram of amoxicillin/clavulanic acid was reintroduced orally 3 days after anaphylaxis can be explained by a transient mast cell and potentially basophil anergy and natural desensitization. A case of post-anaphylactic re-exposure to amoxicillin/clavulanic acid has been reported in the literature in a patient who took a 7-day course of oral AC without reactions, 4 weeks after anaphylactic shock induced by 1.2 grams of intravenous AC.[9] The antigenic epitopes of beta lactams are thought to be related to the R1 chain and while cefazolin, piperacillin and cefotaxime can be used due to negative skin test, in this patient cefadroxil, cefprozil and cefatrizine (with shared R1 chain)[10] along with clavulanic acid should be avoided. The use of penicillin and ampicillin will require further testing. Tryptase was found 25 fold higher than baseline and histamine over 10 times higher within 60 min of the event, providing insight into the mechanism of the reaction and the extent of mast cells and basophils activation.

Several studies have evaluated mast cell anergy using skin tests.[11]

In 1997, Goldberg and Cofino-Cohen[1] performed SPTs, IDTs and specific IgE on patients with a history of anaphylaxis to hymenoptera venom, at 1 week and then 4-6 weeks after the incident. Of the 38 patients tested, 9 had negative skin tests at the first visit but positive specific IgE. The skin tests of these 9 patients became all positive at the second visit. In 2013, Lafuente et al[2] conducted a similar study (without specific IgE testing) on 25 patients with perioperative anaphylaxis in whom the offending allergen was identified. Ten of these patients had initial skin tests (performed between 0 and 4 days after the incident) that were negative and then positive at the second visit (4 to 8 weeks later). Several factors such as the nature of the allergen, the severity of the index reaction and the patient genetic factors are likely to influence mast cells mediators depletion and skin test reactivity .

Several mechanisms are thought to be involved in post-anaphylactic mast cell anergy (**Figure 1**): (i) mast cell re-granulation after massive release of mast cell mediators; (ii) the activation of a signalosome inhibiting mast cell activation ; (iii) changes in FcεRI (high-affinity IgE receptor) availability and signalling following internalization of the allergen/IgE/FcεRI complexes after anaphylaxis; (iv) external factors such as the introduction of β-adrenergic therapies blocking mast cell activation, as in the patient's case.

After activation, mast cells do not undergo apoptosis and need time for the biosynthesis of mediators, to regranulation and to become functional again. Mast cell "recovery" times can be variable: from 24-48h for IL-6 and IL-13 gene expression and release of B-hexosaminidase;[12, 13] to a few days for endocytosis and granule recycling capacity.[14] Hammel et al[15] observed a significant decrease in the size of rat mast cells after strong IgE-dependent stimulation, followed by a gradual recovery of the mast cells size and their granules over 4 to 5 weeks. Cytoskeletal changes also appear to be involved in mast cell anergy. Seagrave et al showed that over-stimulated mast cells retained degranulation capacity in an environment containing inhibitors of actin polymerization,[16] supporting the hypothesis that cytoskeletal rearrangements and post-stimulation actin polymerization would play an inhibitory role on hypothetical second degranulation.

The internalization of allergen/IgE/FcεRI complexes following anaphylaxis leads to FcεRI membrane de-

pletion, which can last several days, explaining mast cell anergy despite the presence of specific IgE and allergens.[17]

Repeated IgE mast cell activation with low dose allergens induces profound quantitative and qualitative changes in FcεRI-dependent signaling (known as FcεRI desensitization), resulting in a state of unresponsiveness whose mechanisms have been analyzed in the induction of rapid drug tolerance (acute drug desensitization) and involve the activation of inhibitory phosphatases such as SHIP-1 (src homology 2-containing inositol phosphatase).[18] The suppression of mast cell responses secondary to exposure to increasing concentrations of allergens is due to the activation of inhibitory signaling pathways.[19] An inhibitory signalosome with SHIP-1,[20] Lyn (src kinase family),[21]protein kinase C- δ (PKC- δ)[22] and Cbl family proteins such as Cbl-c[23] (figure 1) has been proposed. As allergen concentrations increase, the proteins making up this signalosome are recruited. Mice or cells deficient in these various enzymes or inhibitory proteins do not develop inhibition of mast cell activation at supra-optimal allergen doses.[20, 21, 22, 23, 24] The description of the change from positive to negative skin test in a patient allergic to carboplatin after desensitization provides evidence that mast cell inhibitory mechanisms do not need massive release of mediators.[25]

Studies have shown that β-adrenergic receptor agonists inhibit IgE-dependent histamine release by human mast cells,[26]and that exposing healthy volunteers to salmeterol and terbutaline attenuates the cutaneous mast cell response for up to 24 hours post-exposure.[27] However, it is not known whether these inhibitory effects follow a dose-response curve, nor whether they are durable with molecules such as adrenaline, the first-line treatment for anaphylaxis and used in large quantities in our patient's case. Systemic corticoids, on the other hand, do not appear to be involved in inhibiting the mast cell response and the release of mediators.[28, 29]

Post-anaphylactic mast cell anergy affects mast cells in all tissues, which explains the negativity of skin tests and the absence of clinical hypersensitivity reactions on re-exposure to the allergen in the days and weeks following systemic mast cell activation. The duration of this anergy has been defined as 4 to 8 weeks, based on studies of skin test positivity after anaphylaxis. However, practitioners should be aware that this duration may be longer in some patients, as suggested by the recently published clinical case in which skin tests remained negative 8 weeks after the initial accident.[9] The work-up carried out 4 to 8 weeks after anaphylactic shock should be repeated if negative. Clinicians may be able to explore this window of opportunity allowing drug allergic patients to tolerate the culprit drug if given within a short time after anaphylaxis while skin test is still negative.

4. Conclusion

Post-anaphylactic mast cell anergy is described here in a patient allergic to amoxicillin/clavulanic acid, leading to natural desensitization at the time of accidental reintroduction of the drug within 3 days of the initial event. Research is needed to further understand this window of opportunity in patients in need of first line therapy.

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Table I. Biomarkers during and after anaphylaxis

	Standards	T= h0^{+}	T= h1	T= h2	T= d5
Tryptase (µg/L)	<11	81	132	79.9	5.5
Histamine (nmol/L)	<10	>98	>98	ND	ND

h= hour ; d= day ; µg/L= microgram(s) per liter; nmol/L= nanomole(s) per liter ; IgE= immunoglobulin E ; ND: not done

+: blood sample taken 20 minutes after the onset of the shock.

	SPT	IDT 10-3	IDT 10-2	IDT 10-1	B.A.T^{++}	Specific IgE
Amoxicillin	-	-	+ **6mm/20mm^{+}**	ND	15%	Negative (<0.1 kU/L)
Amoxicillin-clavulanic acid	-	-	+ **6mm/20mm^{+}**	ND	15%	NA
Cefazolin	-	ND	-	-	3%	NA
Piperacillin-tazobactam	-	ND	-	-	3%	NA
Cefotaxime	-	-	-	-	4%	NA
Latex	-	NA	NA	NA	NA	Negative (<0.1 kU/L)

Table II. Allergological work-up carried out 4 weeks after the incident

NA= not applicable ; ND= not done ; SPT= skin prick-test ; IDT= intradermal skin test ; B.A.T= basophil activation test

+= size of the wheal in millimetres / size of the flare in millimetres

++: BAT positive control: 93% ; BAT negative control : 3%. On the basis of the concentrations tested, the result is positive for amoxicillin and amoxicillin/clavulanic acid and negative for the other molecules tested.

Figure legend

Figure 1. *Pathophysiology of IgE-mediated mast cell activation and of post-anaphylaxis mast cell anergy. Adapted from Mohamed et al.*[11]

IgE-dependent mast cell activation (blue labeling). Upon antigen cross-linking of the IgE/FcεRI complex, β and g chains of the receptor aggregate and their immunoreceptor tyrosine-based activation motifs (ITAM) are phosphorylated by Lyn, which also activates Syk. This leads to a cascade involving numerous intermediates such as PI3K, PLC- g and PKC. The influx of extracellular calcium (Ca^{2+}) leads to mast cell degranulation, internalization of the Ag/IgE/FceRI complexes and cytoskeletal movements. ITAM phosphorylation is also involved in the activation of MAPK, resulting in eicosanoid synthesis and cytokine gene transcription.

Post-anaphylactic mast cell anergy (red labeling) . Massive degranulation can lead to depletion of mast cell mediators, requiring a period for synthesis and regranulation. It is possible that Lyn can activate inhibitory molecules such as SHIP-1, a phosphatase inhibiting the mast cell response via the PI3K pathway. The internalization of antigen-IgE-FcεRI complexes can further reduce the number of FcεRIs available at the cell surface, impairing mast cell activation; while β-adrenergic receptor agonists can block mast cell degranulation via an inhibitory action on PKC. There is no proof that anergy is not a universal phenomenon.

Ag= Antigen; Fc εRI= high-affinity receptor for IgE ; ITAM= immunoreceptor tyrosine-based activation motif; Lyn= member of the Src family of protein tyrosine kinases ; MAPK= mitogen-activated protein kinase ; P= phosphorylation; PI3K= phosphoinositide 3-kinase ; PKC= protein kinase C ; PLC-g = phospholipase C-g ; SHIP 1= Src homology 2-containing inositol polyphosphate-5'-phosphatase 1 ; Syk= spleen-associated tyrosine kinase.