promiscuous kinase inhibitor that has EGFR ligand properties and avapritinib is a more selective inhibitor of D816V-mutated c-KIT.[8]

From 2015 to 2022, current therapy for ISM was largely supportive using H1 and H2 histamine receptor blockers, antileukotrienes, mast cell stabilizers, omalizumab, and glucocorticoids, but patients continued to experience symptoms with a reduced quality of life.[9] Our approach to this patient's care using KIT inhibitors to control recurrent anaphylaxis in a patient with ISM is validated by the recent US Food and Drug Administration approval of avapritinib for this patient population.[10] The use of KIT inhibitors for this patient permitted the successful use of osimertinib resulting in progression-free survival.

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Melatonin reduces exo-endocytosis in mast cells, making it a useful therapeutic tool in inflammatory diseases

Mast cells were previously known for their role in the type I hypersensitivity responses of the Gell and Coombs 1963 classification, where they are activated by immune complexes, FcRI–IgE allergens, on their cell surface. However, the function of mast cells is much broader than hypersensitivity, being considered tissue sentinels of the immune system.[1] Many other stimuli, such as IgG, TLR, neuropeptides, cytokines, or chemicals such as compound 48/80 or PMACI [PMA (phorbol 12-myristate 13-acetate) plus CI (calcium ionophore A23187)] can trigger the selective release of vasoactive mediators and stimulate proliferation, differentiation, and migration of these cells. In addition, some of the stimuli have a synergistic effect with IgE stimulation. Activation of the mast cells results in the following 3 types of biological responses: (1) secretion of the preformed contents of the granule by exocytosis (degranulation), (2) synthesis and secretion of lipid mediators, and (3) synthesis and secretion of cytokines. Stimulation of mast cells with PMACI leads to increased intracellular Ca^{2+} and degranulation and synthesis of new compounds released outside through exocytosis. Mast cell degranulation involves compound exocytosis which, in addition to vesicle fusion at the plasma membrane, generates vesicle fusion in a multivesicular or sequential manner to allow the formation of degranulation channels.[2] During the secretion process, granules translocate across microtubules from the cell interior to the cell periphery, and depolymerization of the cortical actin assembly is necessary for exocytosis to occur, allowing vesicles to pass through and fuse with the mastocyte plasma membrane.[3] In pathologies with intense inflammation, such as irritable bowel disease or multiple sclerosis, it has been found that one of the main participants in this inflammation are mast cells and the secretion of the contents of their granules. The work of Kim et al[4] revealed that the co-treatment of mast cells with melatonin nanoparticles at 1 μM concentration and the C48/80 stimulus inhibited beta-hexosaminidase, a preformed component of mast cell granules, by 25%, facilitating the anti-inflammatory effect of melatonin.

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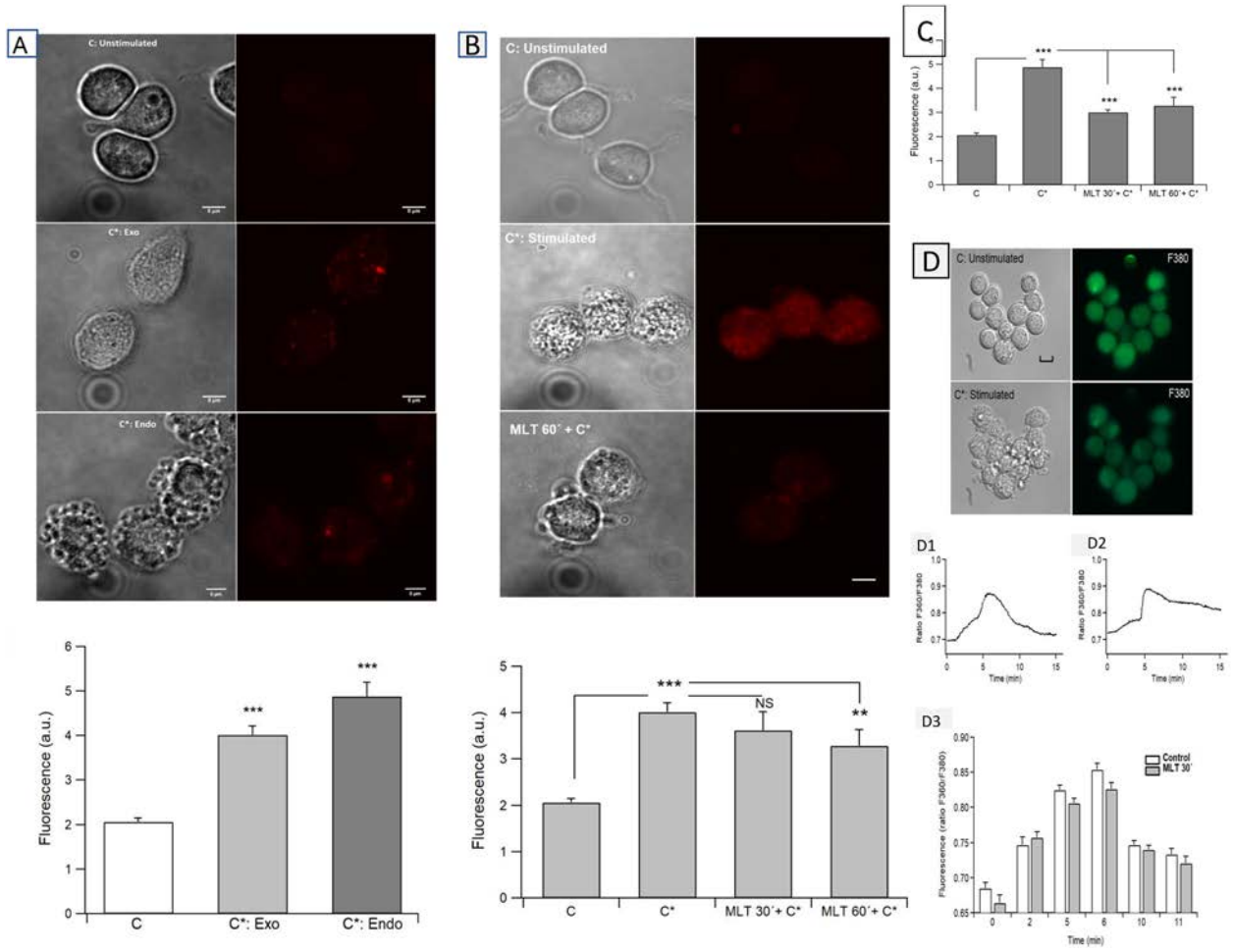

Figure 1. (A) Effects of PMACI stimulus on RBL-2H3 cells. Confocal microscopy of RBL-2H3 mast cells stimulated with PMACI produces an increase in exocytosis-endocytosis as evidenced by increased red (FM1-43) fluorescence in the stimulated cells. Each bar represents the mean ± SEM of 5 experiments, on different days, where n ≥ 30 cells were analyzed. ***$P < .001$ indicates statistical significance when compared with unstimulated RBL-2H3 cells. (B) Melatonin reduced exocytosis in PMACI-stimulated RBL-2H3 cells. Pretreatment with melatonin 1 mM for 30 and 60 minutes reduced exocytosis generated by the PMACI chemical stimulus in RBL-2H3 cells, with statistically significant values at 60 minutes (**$P < .01$). Scale bar: 5 μm. (C) Melatonin reduced endocytosis in PMACI-stimulated RBL-2H3 cells. Pretreatment with melatonin 1 mM for 30 and 60 minutes reduced endocytosis generated by the PMACI chemical stimulus in RBL-2H3 cells, with statistically significant values at both 30 and 60 minutes (***$P < .001$). (D) Calcium signals of RBL-2H3 cells pretreated with melatonin. Bright field and F380 nm fluorescence images of unstimulated and stimulated RBL-2H3 mast cells. The cells after receiving the PMACI stimulus have a decrease in green fluorescence, indicative of an increase in intracytoplasmic calcium. Calcium signal recordings (ratio F360/F380) of (D1) a PMACI-stimulated cell and (D2) a PMACI-stimulated cell pretreated with 1 mM melatonin for 30 minutes. (D3) Comparison of the increase in fluorescence between control cells and cells pretreated with melatonin after stimulating with PMACI at different recording times. Scale bar: 10 μm. No statistically significant differences were found. C, unstimulated RBL-2H3 cells or control cells; C*, stimulated RBL-2H3 cells with PMACI; C*exo, exocytosis in stimulated RBL-2H3 cells; C*endo, endocytosis in stimulated RBL-2H3 cells; MLT30´+ C*, cells stimulated and preincubated with melatonin for 30 minutes; MLT60´+ C*, cells stimulated and preincubated with melatonin for 60 minutes.

In previous work, our research group has found that mast cells possess sufficient machinery to synthesize and release melatonin. Furthermore, they express the melatonin-specific receptors MT1 and MT2 in their peripheral membrane, and this expression occurs constitutively, increasing after stimulus.[5] All these findings have led us to believe that melatonin could play a relevant role in modulating mast cell functions, especially at the level of inflammation. For this reason, we have carried out measurements of exo-endocytosis and intracytosolic calcium concentration in the RBL-2H3 mast cell line stimulated with PMACI and pretreated or not with melatonin at pharmacologic concentration (1 mM), for 30 and 60 minutes.

Initial experiments with PMACI revealed that chemical stimulation resulted in increased exo-endocytosis in RBL-2H3 cells (Fig 1A) and increased intracytosolic calcium compared with unstimulated RBL-2H3 cells. This was evidenced by a reduction in green fluorescent emission, as can be found in Figure 1D. However, pretreatment of these same RBL-2H3 cells with 1 mM melatonin for 30 and 60 minutes (Fig 1B), before stimulation with PMACI, resulted in a decrease in exocytosis of 18% and 38%, respectively, with statistically significant values at 60 minutes, and the mechanism of action was independent of calcium (Fig 1 D1, D2, and D3). Reduced mast cell activation by pretreatment with melatonin occurred in a concentration- and time-dependent manner. Thus, melatonin for 60 minutes reduced exocytosis with statistically significant values, but this was not the case when 30 minutes of pretreatment was used, although its column illustrated a downward trend (Fig 1B). Melatonin pretreatment also reduced endocytosis and did so with statistically significant values at both 30 and 60 minutes preincubation (Fig 1C). Endocytosis is a membrane retrieval process in which the cell prevents the depletion and losses that exocytosis entails.[6] Therefore, we might think that if melatonin reduced endocytosis, which is a recovery process, by 38%, it would harm the cells by not allowing them to recover properly and achieve equilibrium or homeostasis. The reduction in exocytosis may be closely related to less endocytosis, because in mast cells 60% of exo-endocytosis occurs through reversible fusion processes (kiss-and-run and cavicapture).[7] These are rapid mechanisms for membrane recovery and new vesicle formation. Thus, the time-dependent effect of melatonin could indicate a progressive

depletion of ready vesicles for exocytosis. Another plausible explanation is that if melatonin reduces exocytosis or granule loss, the cell will have lost fewer extracellular membranes and will need less recovery processing. In previous work, our research group has revealed that chemical stimulation of the mast cell with PMACI leads to excessive mast cell activation, increased cell stress, and entry into apoptosis.[7] Melatonin, owing to its antioxidant action, could act by reducing oxidative stress or blocking some of the mast cell activation pathways, such as the nuclear factor NF-KB, thus reducing the synthesis and release of proinflammatory cytokines such as IL-6 or TNF-alpha, which are components of the content of mast cell granules, and exerting a cytoprotective role.[8,9]

Therefore, blocking the NF-KB factor in mast cells may be one of the mechanisms of action of melatonin, although it is not the only one. Based on how melatonin acts on other cells containing granules that undergo exo-endocytosis, such as neurons or epithelial cells, its intervention was at the cytoskeleton level by modulating it,[10] preventing its assembly and reducing their exo-endocytosis, and even acting on adhesion molecules.[11] In the case of mast cells, melatonin could act at the microtubule level, calcium independent,[2] reinforcing them and preventing exocytosis of the granules. Our findings establish that melatonin restores mast cell integrity by decreasing exo-endocytosis after PMACI stimulation and protects against an excessive inflammatory response. The ability of melatonin to restore mast cell membranes and prevent their degranulation makes it a useful tool for combating diseases involving acute or chronic inflammatory processes, in which mast cells play a key role. The mechanism of action by which it achieves these effects is independent of calcium.

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Recall urticaria with infusion of agalsidase beta

Check for updates

Fabry disease is an X-linked inherited neurologic disorder that results when the enzyme alpha-galactosidase A cannot break down globotriaosylceramide (Gb3). The accumulation of Gb3 within the cells, especially lysosomes, results in pathogenic effects including neurologic, dermatologic, cardiac, and kidney manifestations. Replacement enzyme therapy for patients with Fabry disease, especially when started before signs of cardiac or kidney disease, has reduced the morbidity and mortality of this condition.[1]

A 20-year-old woman with Fabry disease had been receiving treatment with agalsidase beta (Fabrazyme) every 2 weeks for the past 3 years. She reported a localized infusion site reaction characterized by erythema and swelling which began 1 year ago. She experienced symptoms with each infusion in the arm at the same site the reaction first occurred regardless of the arm she received the infusion (Fig 1). She could only tolerate the infusions for 30 minutes because of a burning sensation that occurred despite premedication with diphenhydramine and acetaminophen. As a result of inadequate treatment of Fabry disease, she had begun experiencing tingling in her hands.

Recall urticaria to agalsidase beta was suspected. Skin prick and intracutaneous testing to agalsidase beginning at 1:10,000 and increasing the concentration by 1 log up to a 1:10 dilution (eg, 1:1000, 1:100, and 1:10 weight/volume dilutions) were performed to rule out a hypersensitivity immunoglobulin (Ig)E–mediated reaction, which were

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