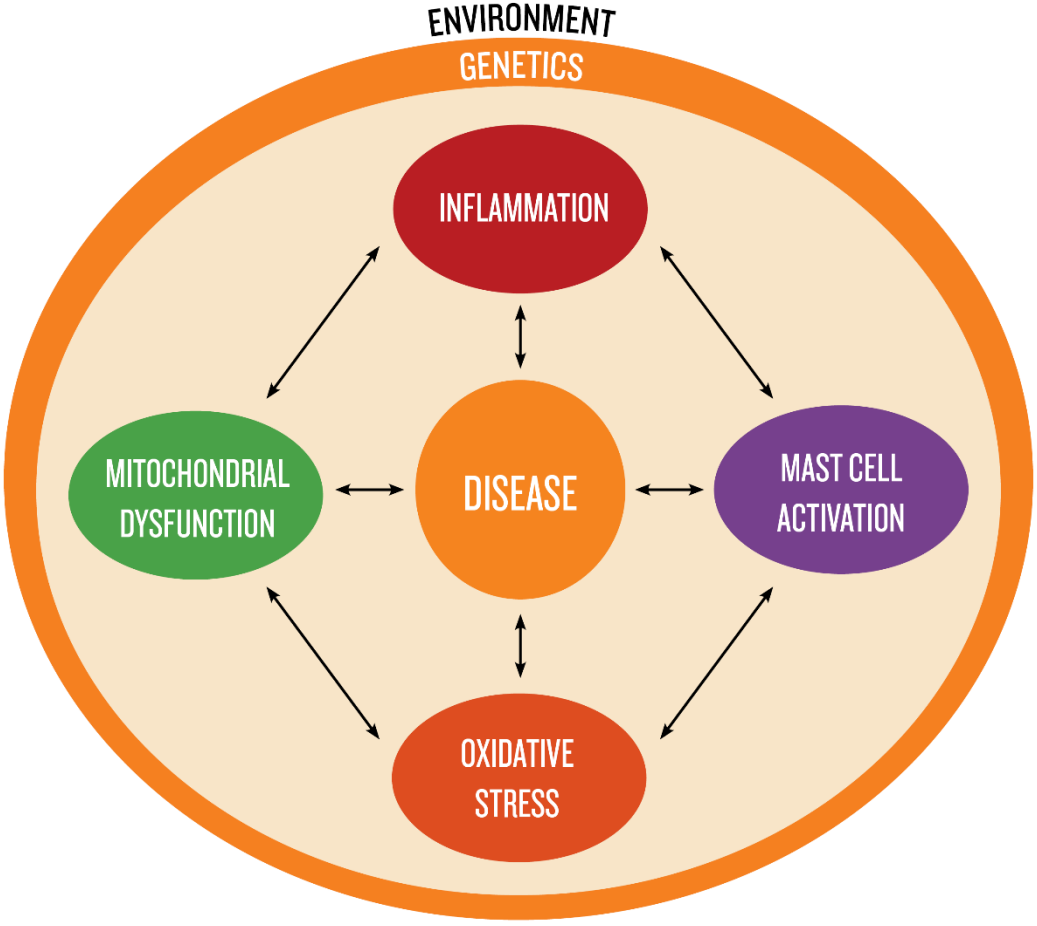


Understanding the Role of Mast Cell Activation in Chronic Disease

DEBBY HAMILTON, MD, MPH

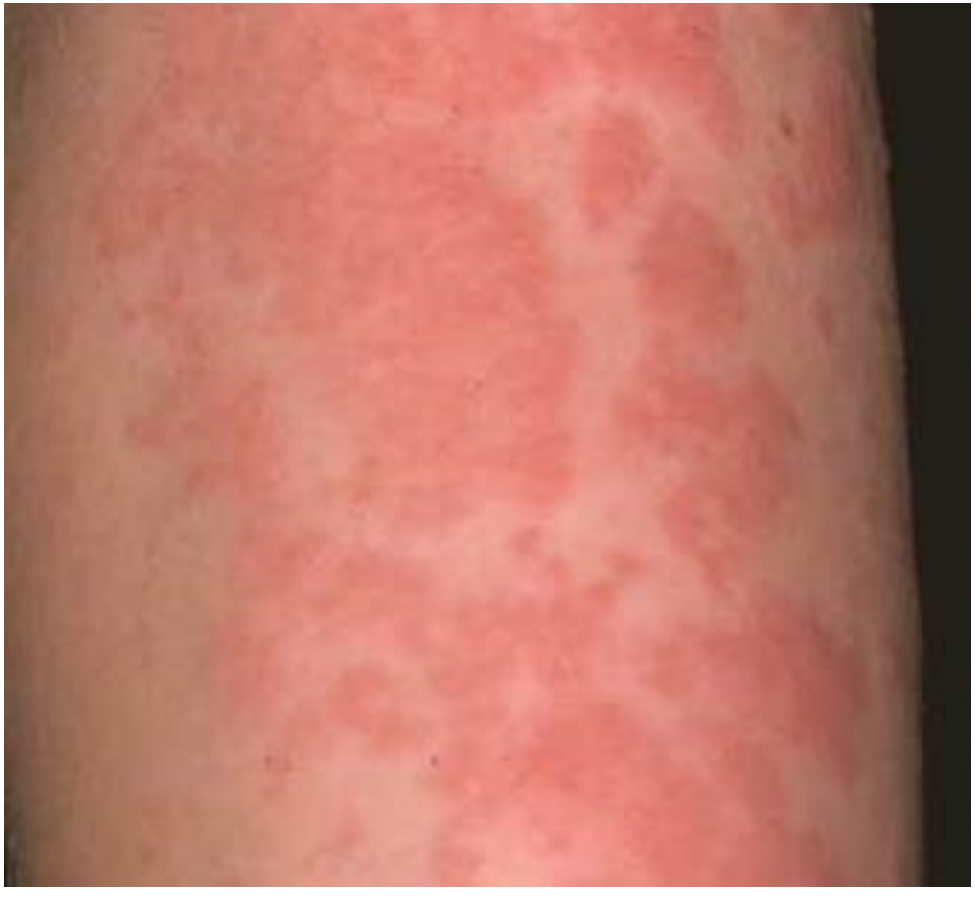
RESEARCHED NUTRITIONALS

Objectives

- Understand the symptoms associated with activated mast cells and how to diagnose mast cell issues
- Discuss mast cells location and function in the immune system
- Learn about abnormal mast cell activation from infections, inflammation, and toxins
- Describe the role that mast cells play in the underlying pathology of chronic disease including digestive issues, infections, and degenerative diseases such as autism
- Learn about natural herbal treatments for inhibiting mast cell activation and blocking the histamine receptor
- Discuss a comprehensive treatment approach to chronic disease that targets mast cell activation, inflammation, oxidative stress, mitochondrial dysfunction, and glutathione depletion

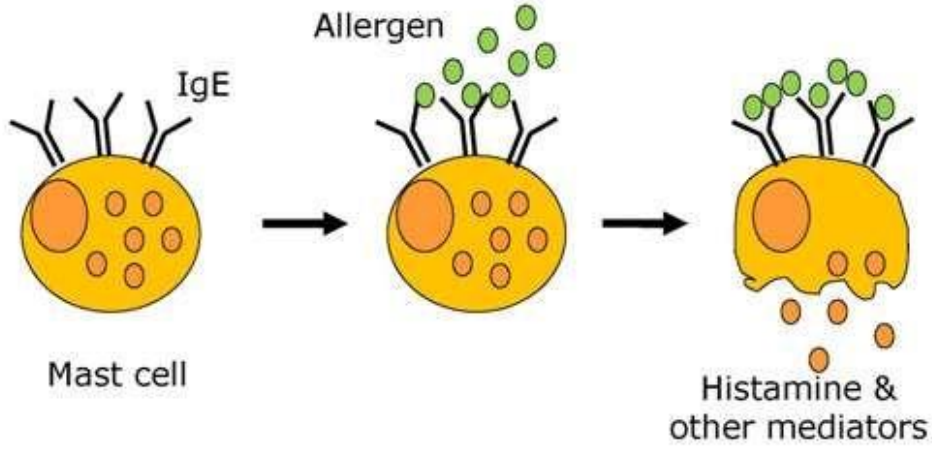
Symptoms Associated with Elevated Histamine from Activated Mast Cells

- Allergies
- Asthma
- Eczema
- Hives
- Anaphylaxis
- Histamine Intolerance
- Mast Cell Activation Syndrome(MCAS)
- Mastocytosis



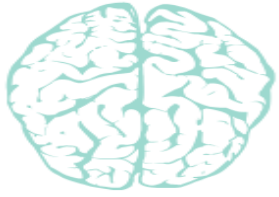
Histamine Release from Mast Cells

- Causes:
  - Blood vessel dilation
  - Bronchoconstriction
  - Increased heartbeat
  - Release of adrenaline
  - Increased capillary permeability
  - Increased gastric acid secretion
  - Pruritis



Histamine Intolerance

Histamine Intolerance Symptoms



- Headaches
- Migraines
- Vertigo or dizziness
- Insomnia
- Anxiety
- Circadian rhythm problems

- Flushes
- Hives, rashes, eczema
- Itchy skin
- Excessive sudden sweating
- Swelling (lids, water retention)

- Difficulty breathing
- Chronic coughing
- Asthma
- Throat clearing
- Post nasal drip
- Sore throat

- Racing heart
- Palpitation
- Arrhythmia
- Low blood pressure
- Blood clots

- Period cramps and dysregulation
- Missed periods
- Endometriosis
- Estrogen dominance

- Muscle and joint pain
- Fibromyalgia
- Muscle twitches

- Nausea/Vomiting
- Diarrhea
- Loose stool
- Stomach pain
- Bloating
- Acid reflux

Mast Cell Disorders

- Mastocytosis: Increase in number of mast cells
- Mast Cell Activation Syndrome: abnormal increase in activation of existing mast cells

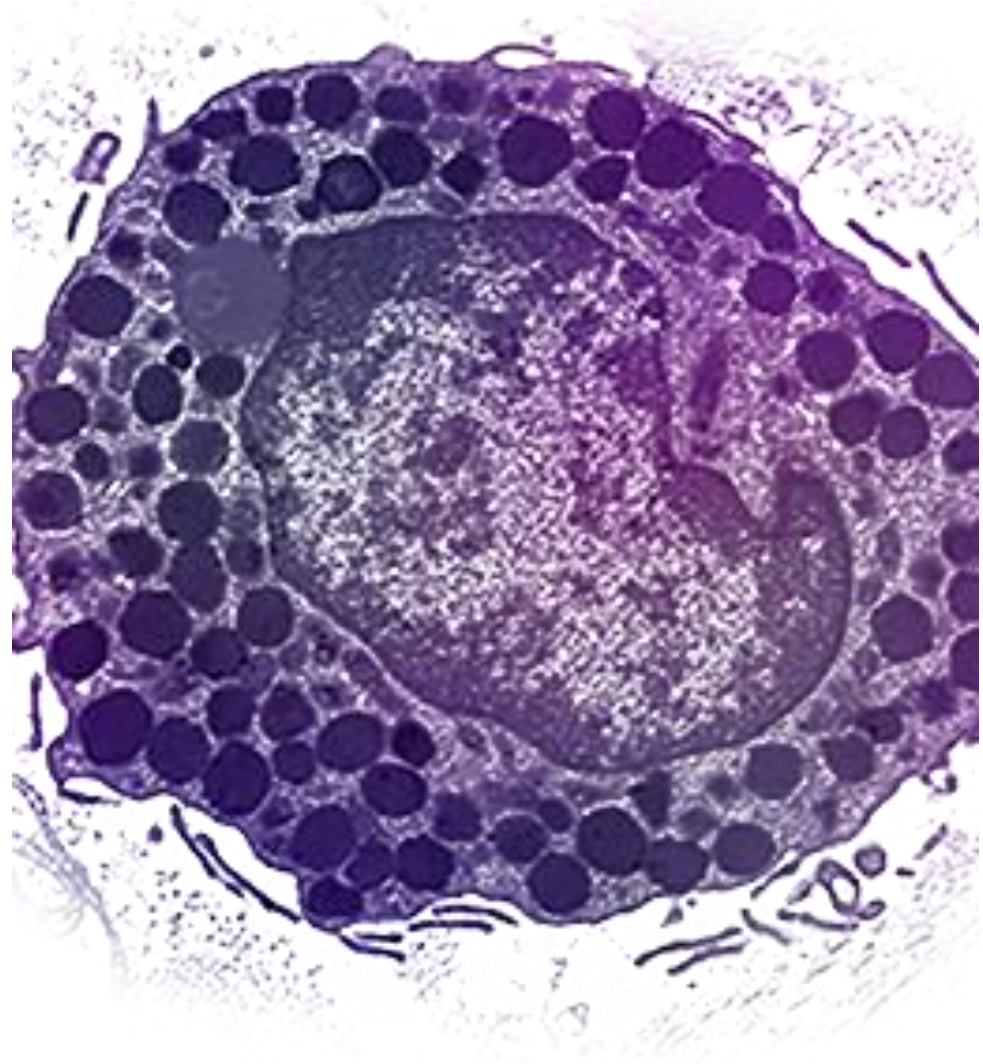


Table **Symptoms and Findings in MCAD[a]**

System	Potential Manifestations of MCAD
Constitutional	Fatigue, subjective or objective hyperthermia and/or hypothermia, sweats, flushing, plethora or pallor, increased or decreased appetite, weight gain or loss, pruritus, chemical/physical sensitivities (often odd), poor healing
Dermatologic/ integument	Rashes/lesions of many sorts (eg, classic urticaria pigmentosa, telangiectasias, xerosis, striae, warts, tags, folliculitis, ulcers, dyshidrotic eczema, migratory but sometimes focally persistent patchy macular erythema), migratory pruritus (sometimes aquagenic), angioedema, dermatographism, alopecia, onychodystrophy
Ophthalmologic	Irritated eyes, episodic difficulty focusing, lid tremor/tic (blepharospasm)
Otologic/osmic	Infectious or sterile otitis externa and/or media, hearing loss and/or tinnitus, dysosmia, coryza, congestion, epistaxis.
Oral/ oropharyngeal	Pain or irritation (sometimes "burning"), leukoplakia, ulcers, angioedema, dysgeusia, dental or periodontal inflammation/decay
Lymphatic	Adenopathy (usually subpathologic and spontaneously waxing/waning in size, sometimes migratory), adenitis, splenitis
Pulmonary	Airway inflammation at any or all levels, cough, dyspnea (usually mild, episodic, and accompanied by normal pulmonary function tests), wheezing, obstructive sleep apnea, pulmonary hypertension
Cardiovascular	Presyncope or syncope, hypertension and/or hypotension, palpitations, migratory edema, chest pain (usually nonanginal), atherosclerosis, odd heart failure (eg, takotsubo), allergic angina (Kounis syndrome), vascular anomalies
Gastrointestinal	Dyspepsia, reflux, nausea, vomiting (sometimes cyclical), diarrhea and/or constipation (often alternating), angioedema, dysphagia (often proximal), bloating/gas, migratory abdominal pain from luminal or solid organ inflammation, malabsorption, ascites
Genitourinary	Migratory luminal and solid organ inflammation, chronic kidney disease, endometriosis, chronic back/flank/ abdominal pain, infertility, decreased libido; miscarriages may signal an MCAS-rooted antiphospholipid antibody syndrome
Musculoskeletal	Migratory bone/joint/muscle pain, joint laxity/hypermobility, osteopenia and/or osteosclerosis
Neurologic	Headache, sensory, and/or motor neuropathies, seizure disorders, pseudoseizures, dysautonomia
Psychiatric	Mood disturbances, anxiety/panic, psychoses, cognitive dysfunction (most commonly memory and word-finding difficulties), sleep disruption
Endocrinologic/ metabolic	Abnormal electrolytes and liver function tests, hypothyroidism, hyperthyroidism, dyslipidemia, impaired glucose control, hyperferritinemia, nutritional deficiencies, delayed puberty, dysmenorrhea
Hematologic/ coagulopathic	Polycythemia or anemia (macrocytic, normocytic, or microcytic), leukocytosis or leukopenia, monocytosis/ eosinophilia/basophilia, thrombocytosis or thrombocytopenia, arterial and/or venous thromboembolic disease, otherwise inexplicable "easy" bruising/bleeding; usually no histologic or molecular evidence of MC aberrancy in the marrow in MCAS
Immunologic	Hypersensitivity reactions, increased risk for malignancy and autoimmunity, impaired healing, increased susceptibility to infection, increased or decreased levels of immunoglobulin of any isotype, monoclonal gammopathy of undetermined significance

[a]Most symptoms are chronic and low-grade; some are persistent, but many are either episodic or waxing/waning. More comprehensive lists and discussions are available. MC = mast cell; MCAD = mast cell activation disease; MCAS = mast cell activation syndrome. Data from: Afrin L. Presentation, diagnosis, and management of mast cell activation syndrome. In: Murray D. Mast cells: phenotypic features, biological functions, and role in immunity. 2013.[9]

Table 1. Mast cell mediators considered to contribute to mast cell activation syndrome clinical symptoms

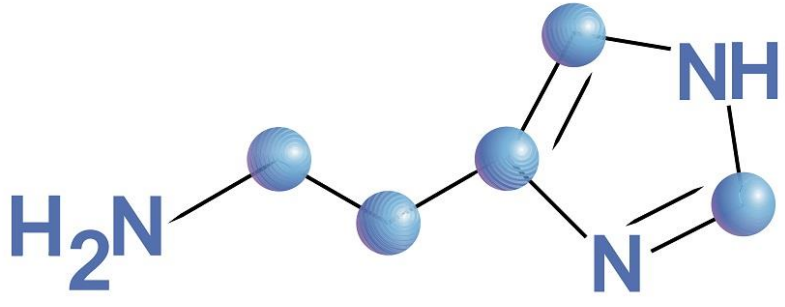
Mediator	Symptoms/signs
Histamine	Headache, hypotension, pruritus, urticaria with or without angioedema, diarrhea, anaphylaxis
Tryptase	Endothelial activation with consecutive inflammatory reactions, bleeding diathesis
Prostaglandin D_2	Flushing, mucus secretion, bronchoconstriction, vascular instability, headache, "mixed organic brain syndrome" (poor concentration, memory loss), nausea, abdominal pain
Platelet-activating factor	Abdominal cramping, pulmonar edema, urticaria, bronchoconstriction, hypotension, arrythmia, anaphylaxis
Cytokines (IL-1, IL-6, TNF-) and chemokines	Constitutional symptoms (fatigue), inflammation, osteoporosis
Leukotriene C_4 and leukotriene D_4	Mucus secretion, bronchoconstriction, edema formation, vascular instability
Chemokines	Acute inflammation and leukocyte recruitment, leukocyte migration
Renin	Cardiac arrhythmias, myocardial infarction

Mast Cell Activation Syndromes.

Bonamichi-Santos R. Current Treatment Options in Allergy. 2016. 3(4):384-400.

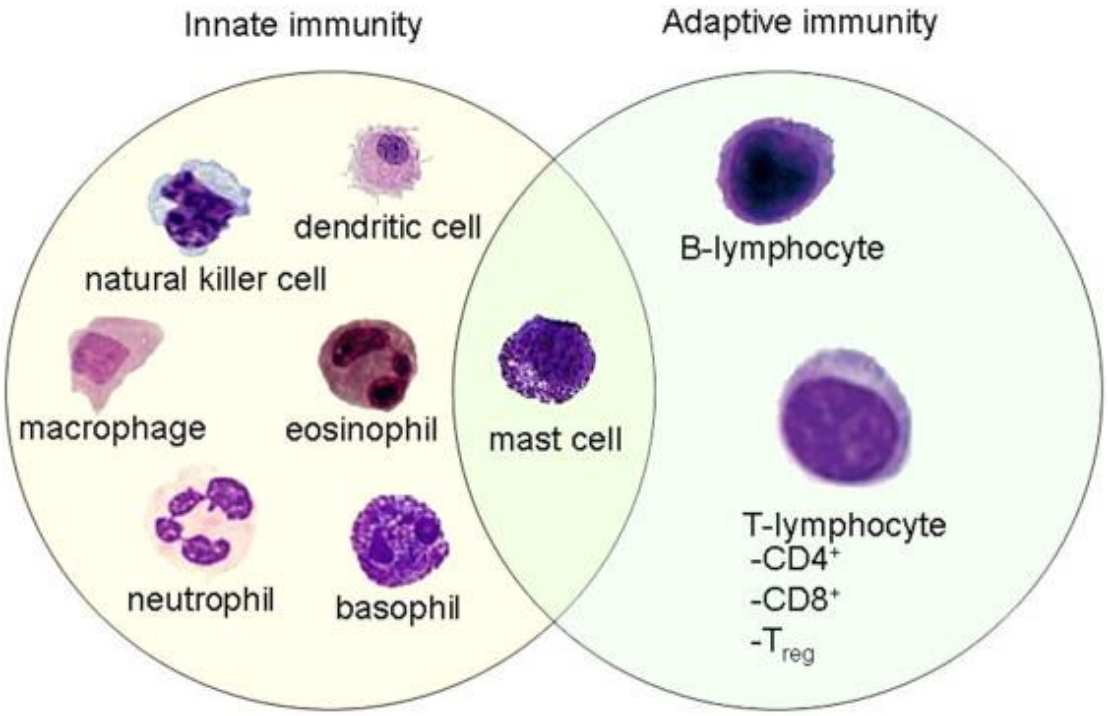
Diagnosis:
Lab tests sensitive to heat
Often negative
Clinical Diagnosis

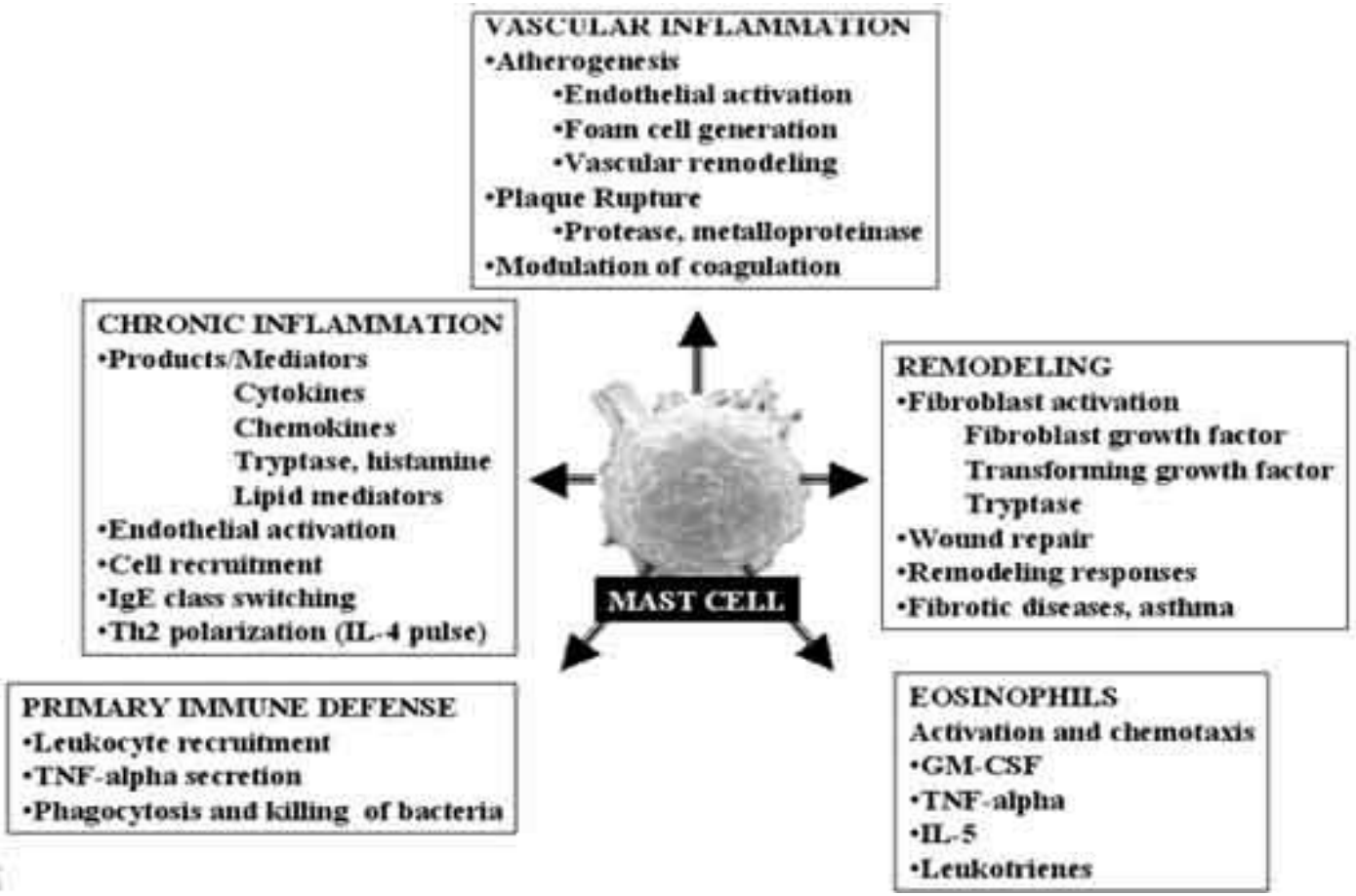
- Histamine: plasma
  - Normal range: 28-51 ug/l.
- N-Methylhistamine: 24-hour urine
  - Normal range: less than 200 mcg/
- Prostaglandin D2: plasma
  - Off of NSAIDS or aspirin for test
- Prostaglandin D2 (PGD2): 24-hour urine
  - Off of NSAIDS and aspirin for test
- Chromogranin A
  - Off proton pump inhibitors and H2 blockers for test
- Tryptase



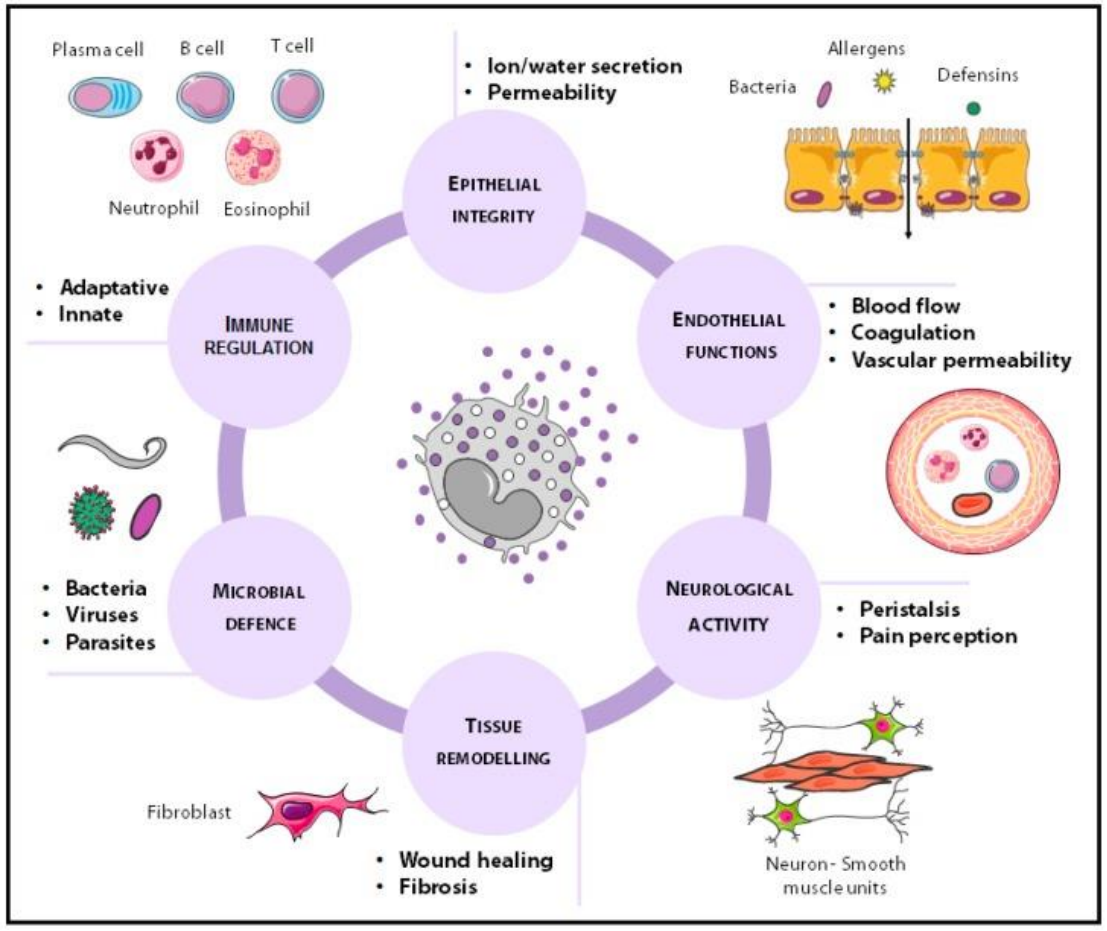
Histamine

Mast Cells role in the Immune System





The Human Mast Cell: Functions in Physiology and Disease.
Krishnaswamy G. et al. Frontiers in Bioscience 6. 2001. 1109-1127



Intestinal Mucosal Mast Cells: Key Modulators of Barrier Function and Homeostasis. Albert-Bayo M. et al. *Cells*. 2019;8(2):135.

Receptor	Location	Effects
H4	Mast, eosinophil, T, dendritic cells	Immune response regulation
H3	Central Nervous System	Neurotransmitter control
H2	Gastric parietal cells	GI gastric acid secretion
H1	Smooth muscle, Endothelial cells	Acute allergic response

Histamine Receptors

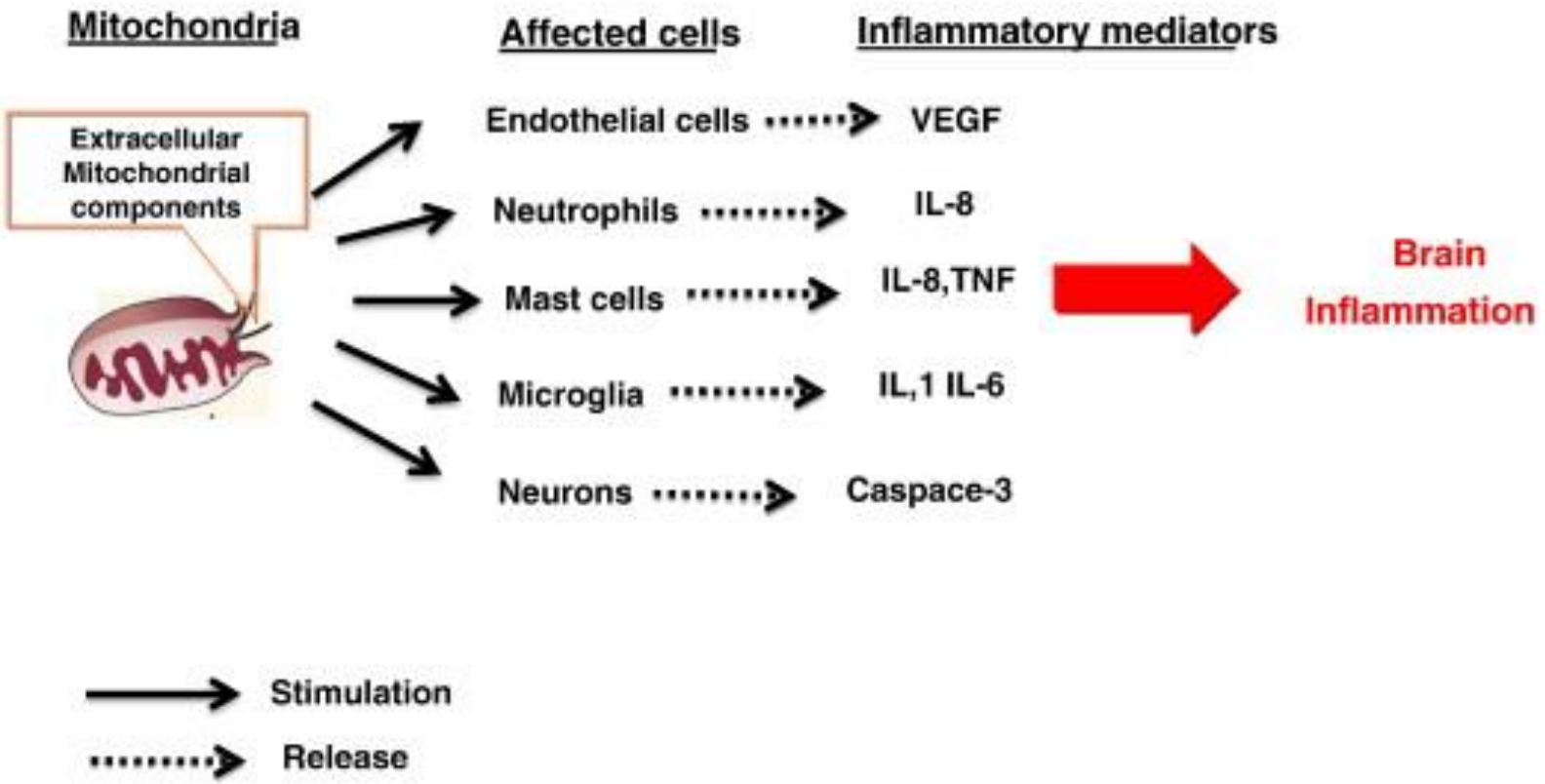
Diseases associated with Mast Cell Activation

- Irritable bowel syndrome
- CIRS
- Dysbiosis: gastrointestinal
- Candida overgrowth
- Obesity
- Diabetes
- Asthma and allergies
- Autism
- Autoimmune diseases (such as lupus, rheumatoid arthritis, and Hashimoto's)
- Celiac disease
- Skin conditions such as eczema and psoriasis
- Food intolerances and allergies
- Gastroesophageal reflux (GERD)
- Infertility and endometriosis
- Chemical and medication sensitivities
- Postural orthostatic hypotension (POTS)
- Mold
- Migraines
- Depression
- Fibromyalgia
- Tinnitus
- Multiple Sclerosis
- Cancer
- Tick-born infections: Lyme
- Ehlers-Danlos Syndrome

Interrelationship between Mast Cell Activation and inflammation, oxidative stress, and mitochondrial dysfunction

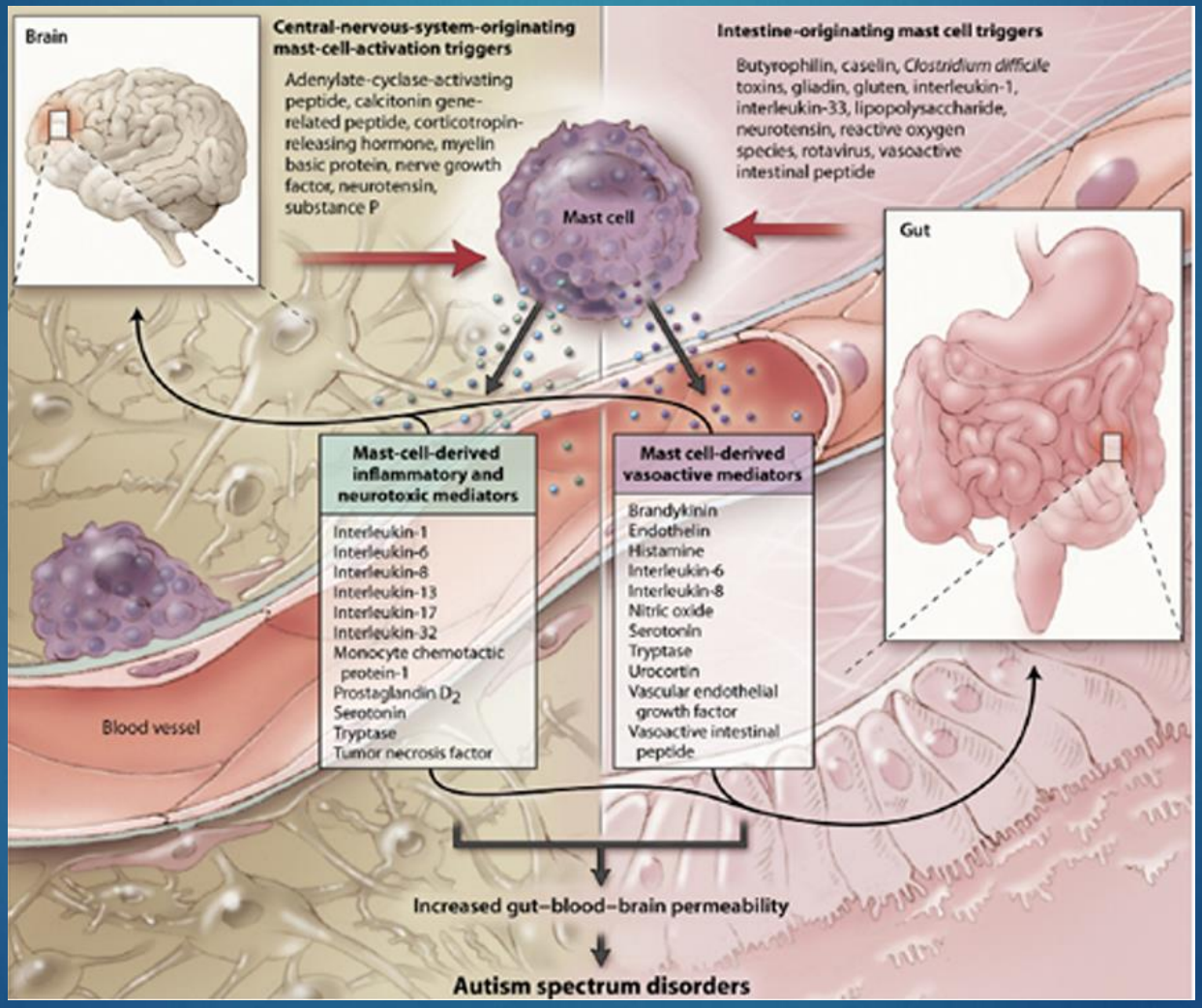
- Inflammation
- Oxidative Stress (Reactive Oxygen Species)
- Extracellular mitochondrial components from damaged mitochondria
- Glutathione depletion (can't clear free radicals)

- All Trigger Mast cell activation



Mast Cells Involved in Neuroinflammation

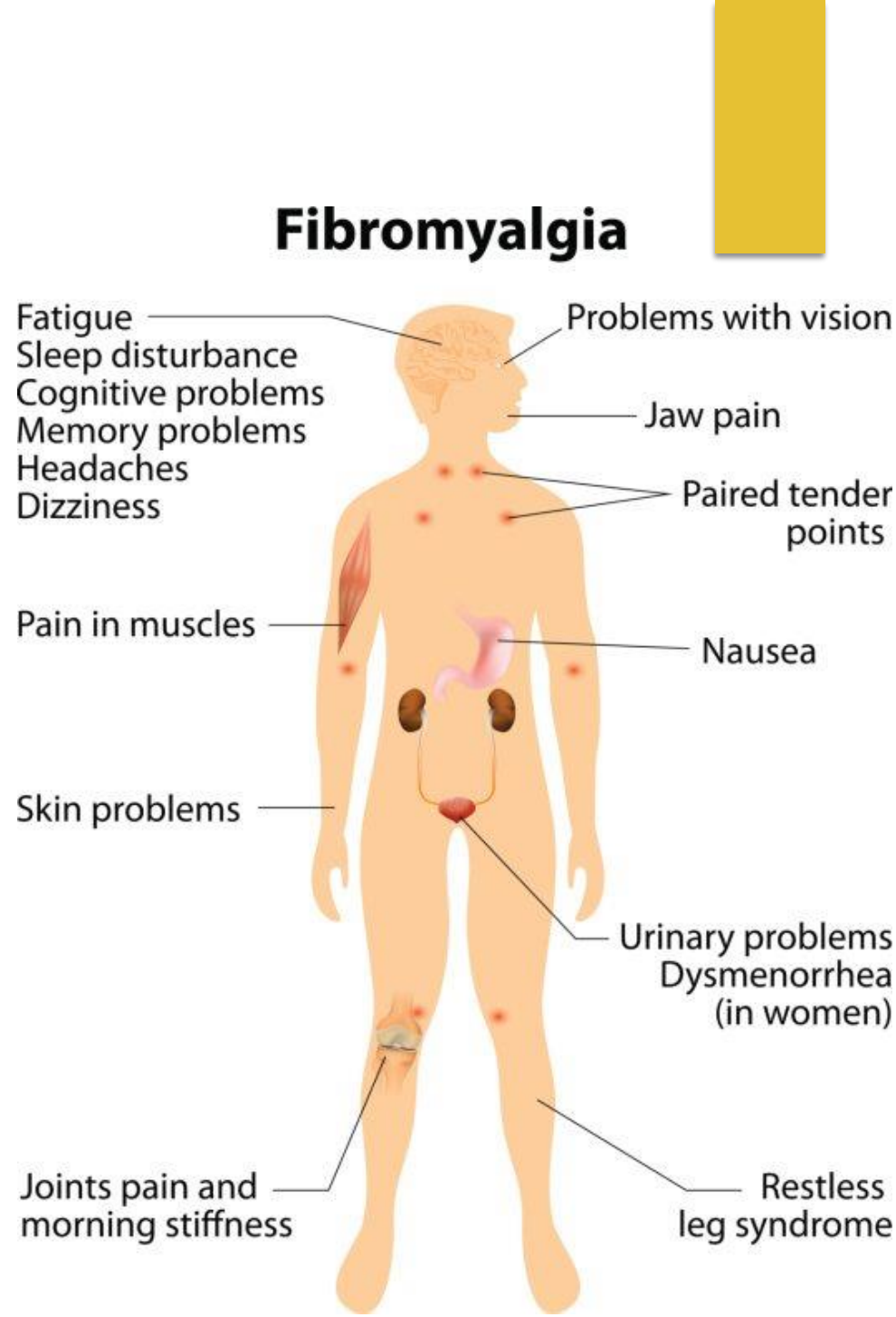
Autism: An emerging neuroimmune disorder in search of therapy. Theoharides TC. Et al. Expert Opinion in Pharmacother. 2009. 10(13)P:2127-2143



Oxidativestress, mitochondrial dysfunction and, inflammation common events in skin of patients with Fibromyalgia. Sanchez-Dominguez B. Mitochondrion. 2015 Mar;21:69-75.

- Recent studies have shown some evidence demonstrating that oxidative stress, mitochondrial dysfunction and inflammation may have a role in the pathophysiology of fibromyalgia.

- Overlap of symptoms between MCAS and Fibromyalgia



- Increase of Mast Cell Activation Syndrome seen in patients with Ehlers-Danlos Syndrome

American Journal of Medical Genetics Part C (Seminars in Medical Genetics)

RESEARCH REVIEW

Mast Cell Disorders in Ehlers–Danlos Syndrome

SURANJITH L. SENEVIRATNE, ANNE MAITLAND ,* AND LAWRENCE AFRIN

Well known for their role in allergic disorders, mast cells (MCs) play a key role in homeostatic mechanisms and surveillance, recognizing and responding to different pathogens, and tissue injury, with an array of chemical mediators. After being recruited to connective tissues, resident MCs progenitors undergo further differentiation, under the influence of signals from surrounding microenvironment. It is the differential tissue homing and local maturation factors which result in a diverse population of resident MC phenotypes. An abundance of MC reside in connective tissue that borders with the external world (the skin as well as gastrointestinal, respiratory, and urogenital tracts). Situated near nerve fibers, lymphatics, and blood vessels, as well as coupled with their ability to secrete potent mediators, MCs can modulate the function of local and distant structures (e.g., other immune cell populations, fibroblasts, angiogenesis), and MC dysregulation has been implicated in immediate and delayed hypersensitivity syndromes, neuropathies, and connective tissue disorders (CTDs). This report reviews basic biology of mast cells and mast cell activation as well as recent research efforts, which implicate a role of MC dysregulation beyond atopic disorders and in a cluster of Ehlers–Danlos Syndromes, non-IGE mediated hypersensitivity disorders, and dysautonomia. © 2017 Wiley Periodicals, Inc.

How to cite this article: Seneviratne SL, Maitland A, Afrin L. 2017. Mast cell disorders in Ehlers–Danlos syndrome. Am J Med Genet Part C Semin Med Genet 175C:226–236.

INTRODUCTION: MAST CELLS AND THEIR PROPERTIES

In the late 19th century, Paul Ehrlich named a granule-dense cell, "mast-zellen," situated near blood vessels in the mucosa and connective tissue. He theorized these cells were providing nourishment to the local tissue environment. Using commercial dyes such as dahlia, toluidine blue, methylene blue, and neutral red, he noted metachromatically staining mature mast cells (MCs) in the connective tissue of several organs.

MCs develop from multipotent hemopoietic progenitors in the bone marrow [Moon et al., 2010]. Stem cell factor (KIT ligand) binds to homodimeric KIT (a transmembrane tyrosine kinase receptor) and influences MC differentiation, growth, survival, migration, and effector functions. MCs acquire a tissue specific phenotype depending on signals they receive from the local tissue environment. Several factors such as interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-9 (IL-9), and transforming growth factor β1 (TGFβ1) have been shown to influence the number and mediator content of MCs [Galli et al., 2011].

Under non-pathological states, mature differentiated MCs are found exclusively within tissues, compared to other innate immune cells, such as basophils, neutrophils, and eosinophils. Within tissues, MCs congregate around nerves, blood vessels, and lymphatic vessels. Based on their location (connective tissue or mucosal) and content of their granules, two types of MCs have been described. MCs residing in connective tissue, skin, and the peritoneal cavity contain tryptase (MC_T) in their granules and express interleukin-5 (IL-5) and interleukin-6 (IL-6). MCs homing to the gut and respiratory mucosa contain tryptase and chymase (MC_{TC}), and express IL-4 [Sigal, 2011]. When fully differentiated, MCs exhibit a wide range of biological properties including phagocytosis, antigen presentation, cytokine and chemokine production, and the immediate release of vasoactive substances. They have a role in local tissue homeostasis (tissue repair, angiogenesis) and co-ordination of immune responses to a myriad of pathogens, recognized through evolutionarily conserved surface receptors like toll-like receptors, complement receptors, and receptors for adenosine phosphate, oestrogen, and immunoglobulins), physical stimuli (pressure, temperature), and toxins. As yet, no animal model or disease state has

Suranjith L. Seneviratne, Institute of Immunity and Transplantation, Royal Free Hospital and University College London, United Kingdom; Faculty of Medicine, Department of Surgery, University of Colombo, Colombo, Sri Lanka.
Anne Maitland, Division of Clinical Immunology, Department of Medicine, Mount Sinai Hospital, New York, New York.
Lawrence Afrin, Department of Medicine, University of Minnesota, Minneapolis, Minnesota.
Disclosure of potential conflict of interest: Anne Maitland receives honoraria for participation in the speakers' bureau for Genentech.
*Correspondence to: Anne Maitland, MD, PhD, Division of Clinical Immunology, Department of Medicine, Mount Sinai Hospital, Box 1089, 1 Gustave L. Levy Place, New York, NY 10028. E-mail: anne.maitland@mssm.edu
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Hindawi

Review Article

The Role of Mast Cells in Irritable Bowel Syndrome

Kang Nyeong Lee and Oh Young Lee

Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Republic of Korea

Correspondence should be addressed to Kang Nyeong Lee; leekn@hanyang.ac.kr

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Academic Editor: Magdy El-Salhy

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders, but its treatment is unsatisfactory as its pathophysiology is multifactorial. The putative factors of IBS pathophysiology are visceral hypersensitivity and intestinal dysmotility, also including psychological factors, dysregulated gut-brain axis, intestinal microbiota alterations, impaired intestinal permeability, and mucosal immune alterations. Recently, mucosal immune alterations have received much attention with the role of mast cells in IBS. Mast cells are abundant in the intestines and function as intestinal gatekeepers at the interface between the luminal environment in the intestine and the internal milieu under the intestinal epithelium. As a gatekeeper at the interface, mast cells communicate with the adjacent cells such as epithelial, neuronal, and other immune cells throughout the mediators released when they themselves are activated. Many studies have suggested that mast cells play a role in the pathophysiology of IBS. This review will focus on studies of the role of mast cell in IBS and the limitations of studies and will also consider future directions.

1. Introduction

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal (GI) disorders with a worldwide prevalence of 5–20% [1, 2]. IBS diagnosis is based on symptoms such as recurrent abdominal pain related to defecation and accompanied by a change in the frequency or form of stool [3]. However, neither diagnostic nor therapeutic approaches are satisfactory because IBS is a multifactorial disorder and its manifestation differs from patient to patient. It has traditionally been thought to result from two abnormalities: visceral hypersensitivity and intestinal dysmotility. However, recent intensive studies have revealed that low-grade inflammation of the intestines [4], as well as alterations of gut barrier function, epithelial permeability, mucosal immunity, and gut-brain axis [5–8], is also involved.

It has been suggested that intestinal mast cells are intimately involved in these pathophysiologic changes [9, 10]. Mast cells can activate adjacent cells by releasing mediators and can also be activated themselves via IgE-mediated or non-IgE-mediated pathways. They are thus closely associated both anatomically and functionally with intestinal components such as intrinsic and extrinsic nerves of the GI tract, intestinal smooth muscles, and secretory glands [11–15]. Furthermore, symptoms of IBS are often provoked by the ingestion of food or psychological stress, which is one of the factors to activate intestinal mast cells [12].

This connection between mast cells and IBS pathophysiology and symptomatology has been supported by numerous studies. In this review, we describe the results of those studies and their limitations and consider potential future developments.

2. Mast Cells in the Regulation of GI Physiology and Pathophysiology

The many roles of mast cells depend on their ability to secrete mediators after being activated by a variety of stimuli [13]. Mast cells can be activated via either IgE-dependent or IgE-independent pathways [16, 17]. First, IgE-dependent pathways are activated, as in allergic reactions, by binding of allergen to IgEs bound to high affinity Fc epsilon receptor (FcRI) and their subsequent cross linking [16]. Second, IgE-independent pathways are activated by various receptors on mast cells to other agents, including cytokines, neurotransmitters, anaphylatoxins such as venom, and physical

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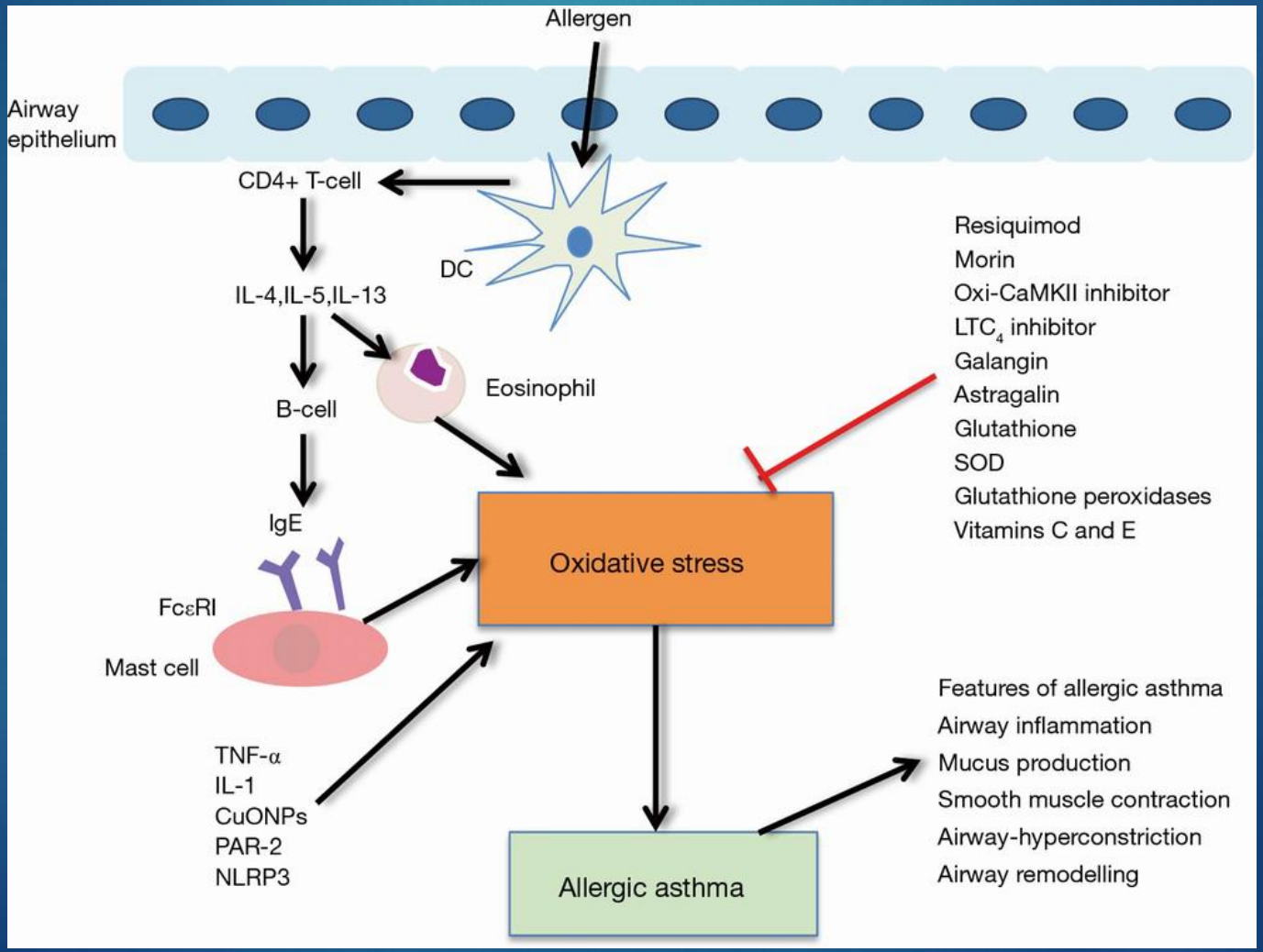
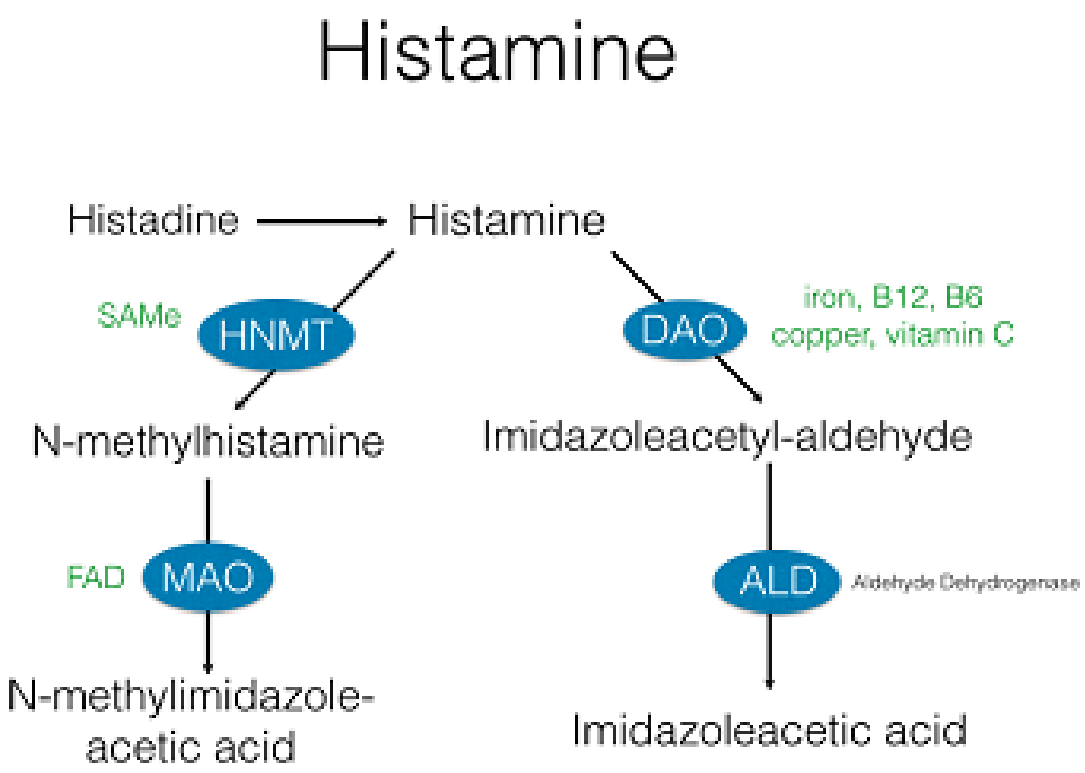
Correlation between mast cells and gastrointestinal disorders: Role in IBS

Asthma:
oxidative stress, mast cell activation, inflammation: leads to "leaky lungs" increased epithelial permeability





Histamine Intolerance Treatment

Breakdown on Histamine dependent on genetic SNP's of HNMT and DAO

DAO: Diamine Oxidase Enzyme

DAO Inhibitors

- Alcohol
- NSAID's
- Immune modulators: Enbrel, Plaquenil
- Anti-histamines: Benadryl, Zyrtec, Zantac

Decrease DAO

- SIBO, leaky gut, dysbiosis from histamine producing probiotics
- Deficiencies in cofactors: Copper, vitamin C, B6
- Genetic polymorphisms: DAO enzyme

Foods to Avoid if You Are Histamine Intolerant

Histamine-Rich Foods

Fermented Alcoholic Beverages,
Fermented Foods: Sauerkraut, Vinegar, Soy Sauce, Kefir, Yogurt, Kombucha, etc.
Vinegar-containing Foods: Pickles, Mayonnaise, Olives
Cured Meats: Bacon, Salami, Pepperoni, Luncheon Meats and Hot Dogs
Soured Foods: Sour Cream, Sour Milk, Buttermilk, Soured Bread, etc.
Dried Fruit: Apricots, Prunes, Dates, Figs, Raisins
Most Citrus Fruits
Aged Cheese Including Goat Cheese
Nuts: Walnuts, Cashews, and Peanuts
Vegetables: Avocados, Eggplant, Spinach, and Tomatoes
Smoked Fish and Certain Species of Fish: Mackerel, Mahi-Mahi, Tuna, Anchovies, Sardines

Histamine-Releasing Foods

Alcohol
Avocados
Bananas
Chocolate
Cow's Milk
Nuts
Papaya
Pineapple
Shellfish
Strawberries
Tomatoes
Wheat Germ
Many Artificial Preservatives and Dyes

DAO enzyme Supplements

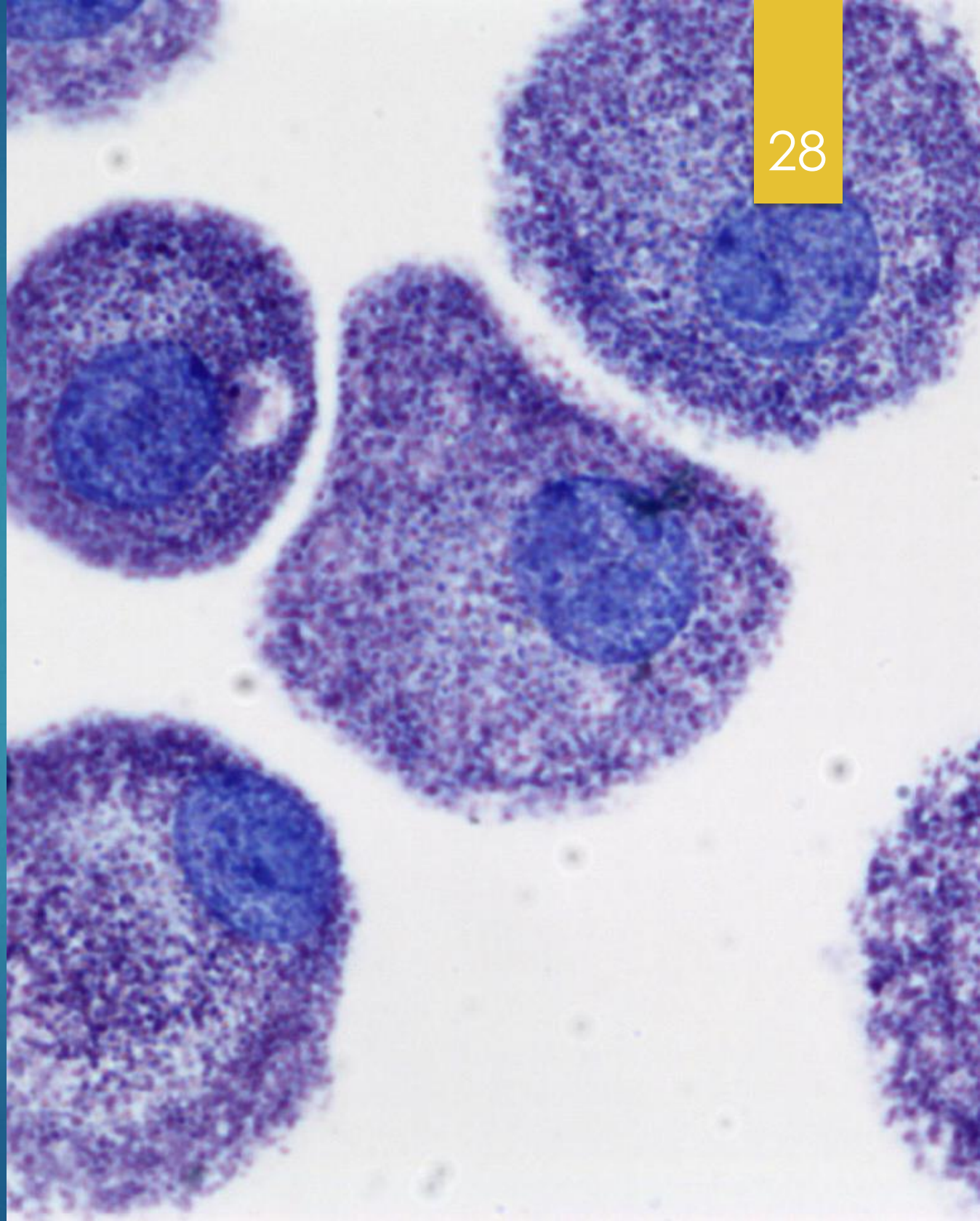
- Break down histamine in the digestive tract
- Good for histamine intolerance but not mast cell activation issues
- Will not change the amount of histamine released
- Break down histamine from foods contributing to overall histamine level

  - DO NOT inhibit release of histamine from mast cells
  - DO NOT block IgE binding on mast cell receptor

Histamine Degrading Probiotics

- Bacteroides fragilis
- Bifidobacterium lactis
- Bifidobacterium infantis
- Bifidobacterium longum
- Lactobacillus casei
- Lactobacillus gasseri
- Lactobacillus rhamnosus
- Lactobacillus plantarum
- Lactobacillus plantarum
- Lactobacillus salivarius

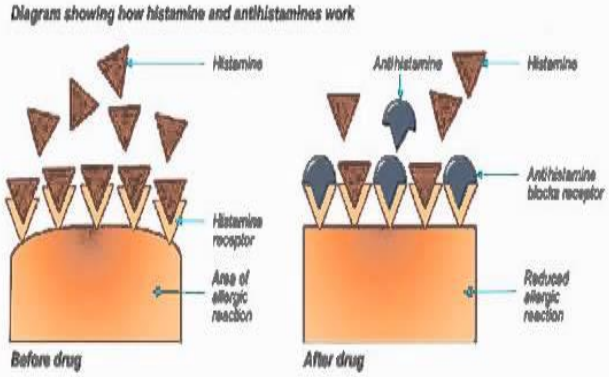
Mast Cell Disorders Treatment



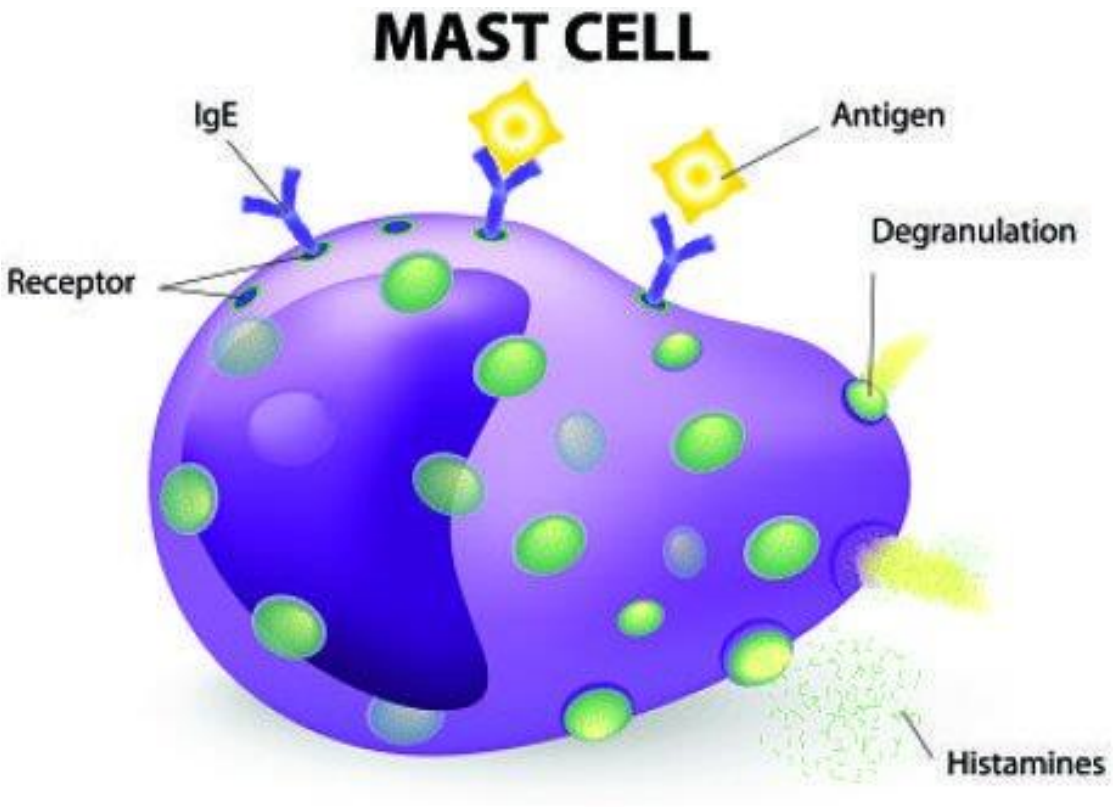
Anti-Histamines

- H1 receptor: Blocked
  - Benadryl, Claritin
  - Natural H1: Stinging nettle,
- H2 receptor: blocked
  - Zantac, Pepcid
  - Natural H2: Peppermint, Ginger

Antihistamines



- Compete for and block receptor sites
- Results in less severe allergic reactions

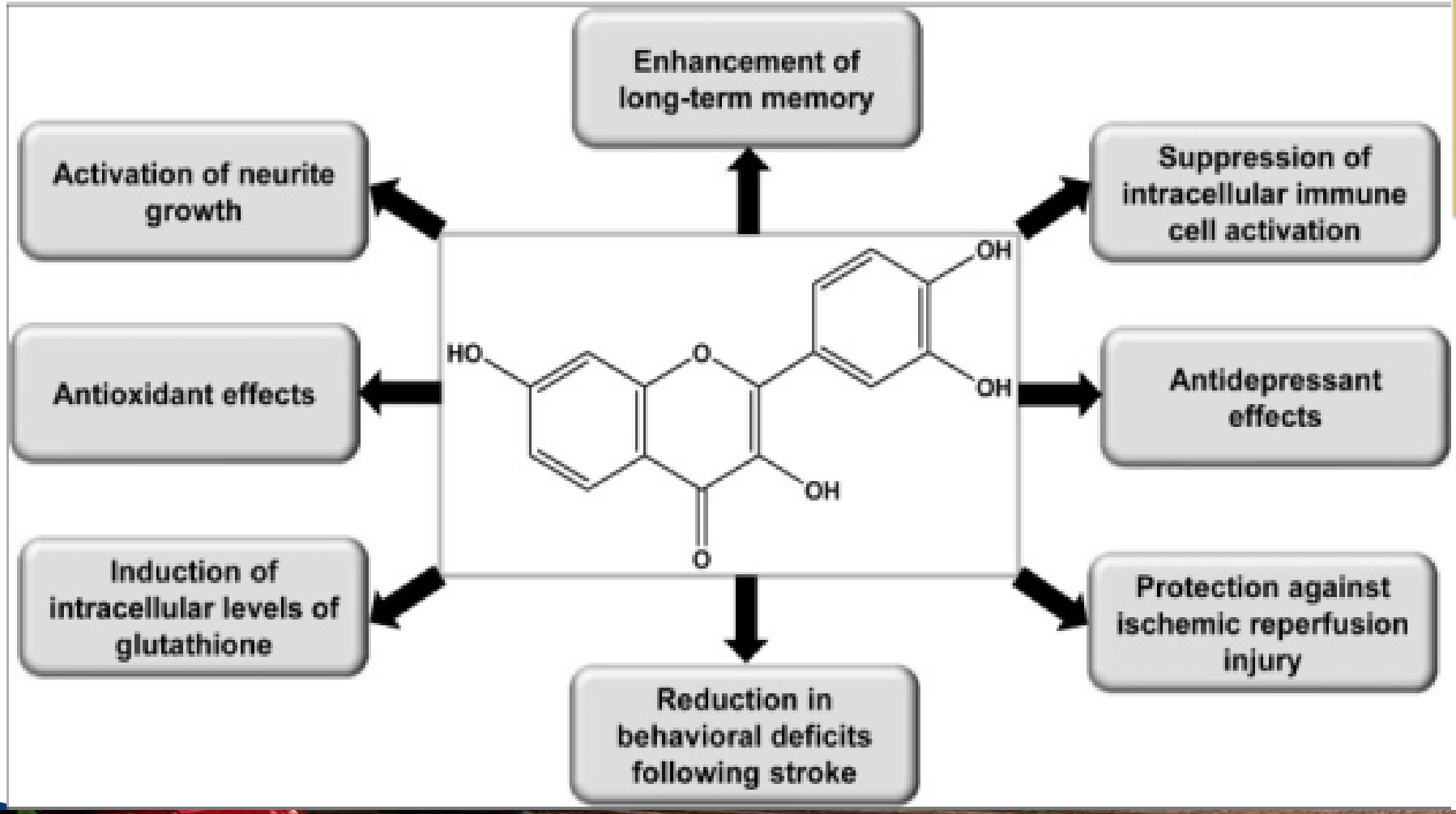


Mast Cell Release Inhibitors

Flavonoids

- Group of polyphenolic compounds found in fruits, flowers, seeds, and vegetables
- Flavonoids are naturally occurring molecules with
  - antioxidant
  - cytoprotective
  - anti-inflammatory actions
- Inhibit IgE mediated histamine release
- Decrease production of proinflammatory cytokines
- Down-regulate mast cell activation

- Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. Park HH. Et al. *Arch Pharm Res*. 2008 Oct;31(10):1303-11


Enhancement of long-term memory
Activation of neurite growth
Suppression of intracellular immune cell activation
Antioxidant effects
Antidepressant effects
Induction of intracellular levels of glutathione
Protection against ischemic reperfusion injury
Reduction in behavioral deficits following stroke
HO
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WHAT ARE THE BENEFITS OF FISETIN?

The hydroxyflavone, Fisetin, suppresses mast cell activation induced by interaction with activated T cell membranes. Nagai K. et al. *British Journal of Pharmacology*. 2009

- Conclusions and implications:
  - Fisetin suppressed activation of HMC-1 cells by activated T cell membranes by interfering with cell-to-cell interaction and inhibiting the activity of NF-κB and MAPKs and thereby suppressing gene expression. Fisetin may protect against the progression of inflammatory diseases by limiting interactions between mast cells and activated T cells.

Asthma and Fisetin research

- Bronchodilation of lungs comparable to medicine

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EUROPEAN PHARMACEUTICAL JOURNAL

The effect of short-term and long-term application of fisetin on experimentally induced airway hyperreactivity

Original Paper

Kazimierová I.,[1,✉] Pappová L.,[2] Šutovská M.,[2] Fraňová S.,[1,2]

[1]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Biomedical Center Martin, Slovak Republic
[2]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Department of Pharmacology, Biomedical Centre Martin, Slovak Republic

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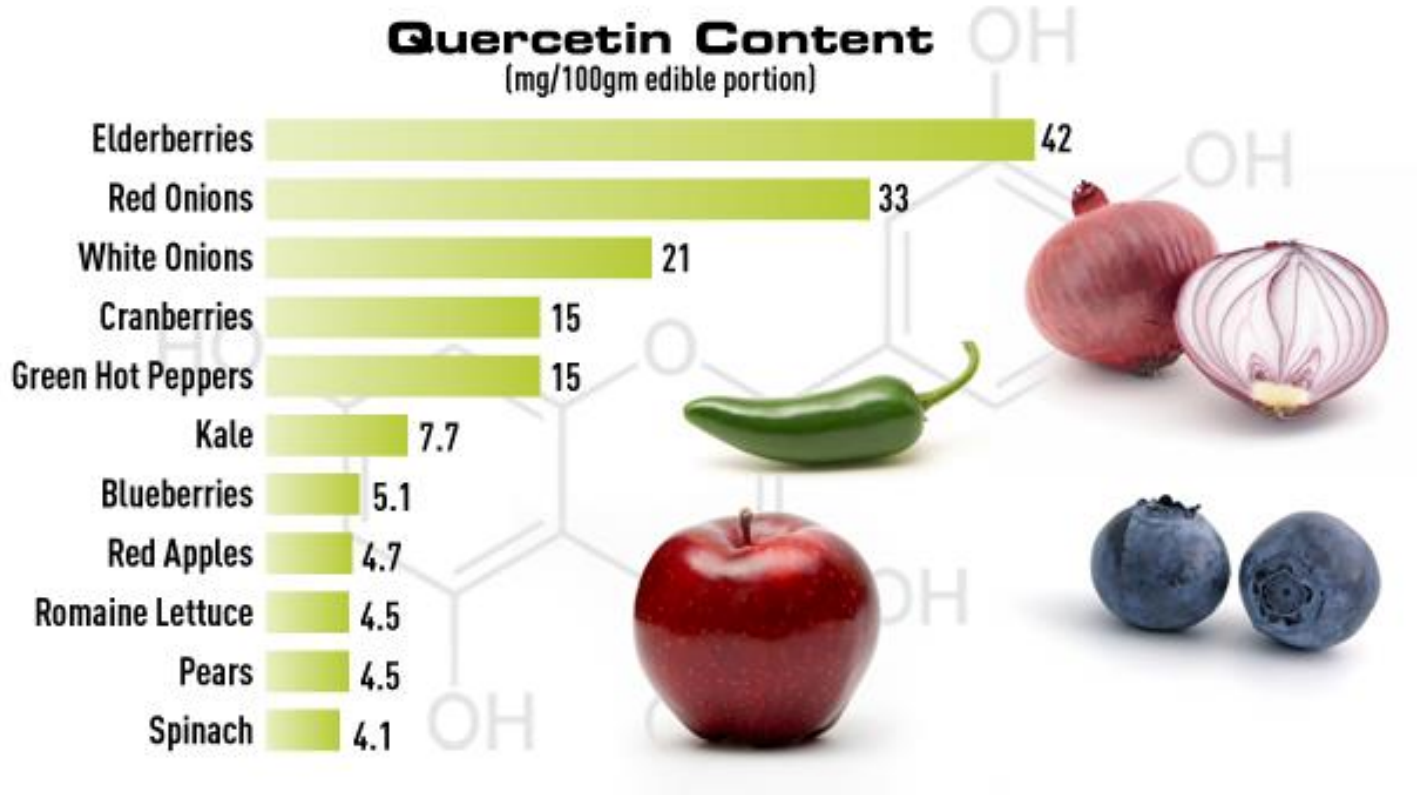
Abstract **Background:** Fisetin, a derivate from the flavonol group may possess a variety of pharmacological effects. The aim of the presented study was to evaluate the bronchodilatory effect of fisetin after the acute or the chronic administration to guinea pigs with allergic airway inflammation.
Methods: Experimental animals were sensitized and challenged by ovalbumin. Fisetin was administered in dose 5mg/kg/ p.o., either once after the end of 21-days sensitization or daily during the 21-days sensitization. By using the whole-body plethysmograph, we monitored the specific airway resistance, a parameter of airway hyperreactivity *in vivo*. The changes of the specific airway resistance were evaluated after the short-term inhalation of the bronchoconstriction mediator -histamine (10^{-6} mol.l^{-1}).
Results: Our results showed that the short-term as well as the long-term administration of fisetin caused decrease of the specific airway resistance values. The bronchodilatory effect of fisetin was comparable to the long-acting $beta_2$ sympathomimetic – salmeterol after the long-term administration. The measurements of the bronchodilatory activity after single administration have revealed more prolonged effect of fisetin comparing to the short-acting $beta_2$ sympathomimetic – salbutamol, as this remained even after the 5 hours, when salbutamol was already ineffective.
Conclusion: In conclusion, flavonol – fisetin has shown bronchodilatory potential. In the light of this fact, fisetin may represent potential substance that can be effective in both prevention as well as control of airway inflammation symptoms.

Keywords *Airway hyperreactivity – fisetin – airway inflammation*

INTRODUCTION

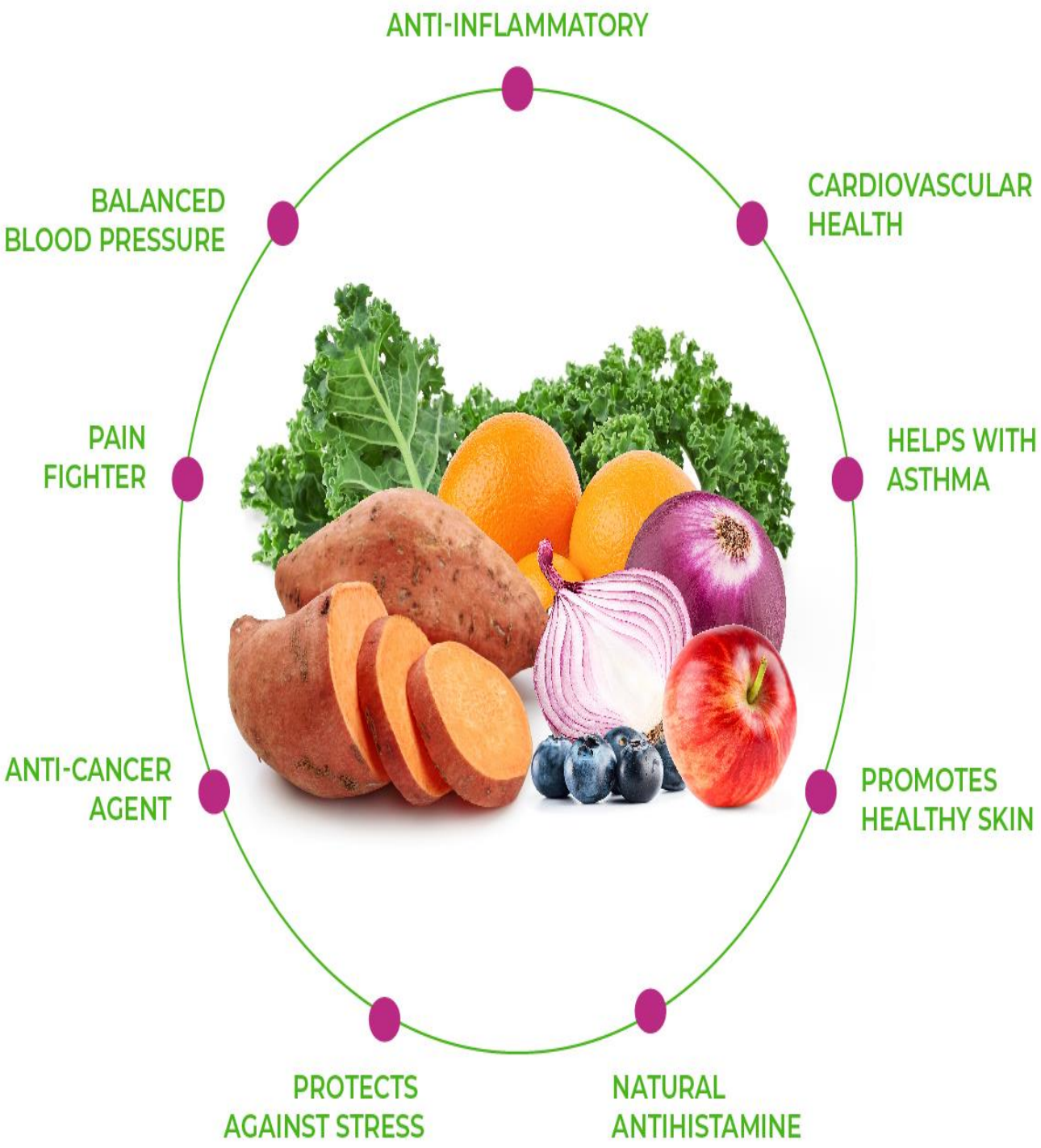
The airway hyperreactivity is one of the main attribute of allergic asthma. It is defined as an exaggerated contraction response of the airways to different kind of endogenous and exogenous stimuli (Bossé, 2012). It is believed, that airway inflammation plays a central role in the development of airway hyperreactivity. Airway infiltration by inflammatory cells can impair the integrity of the bronchial epithelium. Due to this change, the release of bronchodilating substances is decreased and the formation of bronchoconstriction active kinins is augmented. Moreover, inflammatory cells such as eosinophils and mast cell release the mediators with the capacity to cause bronchial smooth muscle contraction and exudation of plasma, resulting in the thickening of airway wall and the modification of mechanical properties of airway smooth muscle (O´Byrne & Inman, 2003; Fredberg, 2004).
There are evidences, that flavonoids possess bronchodilatory effect and inhibit the synthesis of pro-inflammatory cytokines and the release of chemical mediators in allergic asthma (Tanaka & Takahashi, 2013). In the light of this fact, the aim of the presented study was to estimate the effect of short-term and long-term application of flavonol derivate fisetin on airway hyperreactivity. The bronchodilatory activity of fisetin was evaluated on experimental model of allergic airway inflammation in guinea pigs. This model simulates the development of airway hyperreactivity (Franova et al., 2013). The ability of fisetin to relax airway smooth muscle was

* *E-mail: Ivana.Kazimierova@jfmed.uniba.sk*



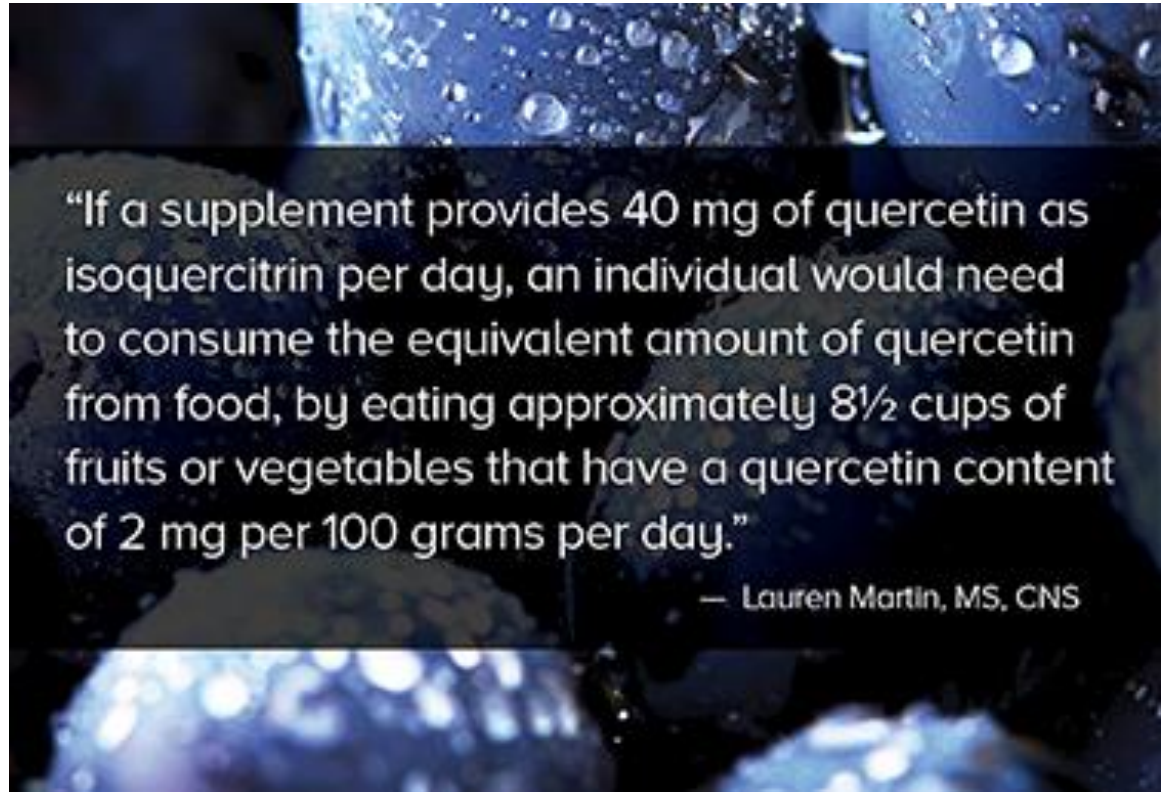
Quercetin: Flavonoid

Quercetin Health Benefits



Quercetin

- Concern with absorption



European Journal of Drug Metabolism and Pharmacokinetics
https://doi.org/10.1007/s13318-018-0517-3

ORIGINAL RESEARCH ARTICLE

Improved Oral Absorption of Quercetin from Quercetin Phytosome®, a New Delivery System Based on Food Grade Lecithin

Antonella Riva[1] · Massimo Ronchi[1] · Giovanna Petrangolini[1] · Stefania Bosisio[1] · Pietro Allegrini[1]

Abstract

Background and Objectives The importance of quercetin and flavonoids in the diet and as food supplements is well known, and literature studies support their potential use to treat several human diseases. Many beneficial properties have been described for quercetin, so much effort has been directed into overcoming the major drawbacks of this natural compound—its poor solubility and low oral absorption. The aims of this study were to compare a new food-grade lecithin-based formulation of quercetin, Quercetin Phytosome®, to unformulated quercetin in terms of solubility in simulated gastrointestinal fluids and oral absorption in a randomized crossover pharmacokinetic study of healthy volunteers.

Methods The solubility of the new formulation was determined by in vitro incubation in simulated gastrointestinal fluids, and quercetin was detected by ultra performance liquid chromatography. A single-dose, randomized, six-sequence/three-period crossover clinical trial (3×3×3 crossover design) with a balanced carryover effect was conducted in healthy volunteers under fasting conditions. Twelve healthy volunteers of both sexes with an age range of 18–50 years were recruited; one dose of quercetin and two different doses of Quercetin Phytosome were administered orally as film-coated tablets. Pharmacokinetic samples were collected at twelve time points (from 0 h to 24 h) after administration, and quercetin levels were measured by HPLC/MS/MS. Data were analyzed using the Phoenix WinNonlin (v.6.4) software package, and the most significant pharmacokinetic parameters were calculated. Statistical analysis involved performing a two-way ANOVA with repeated measures followed by post hoc analysis (Tukey's test).

Results Significant improvements in both in vitro solubility and oral absorption (in terms of both exposure and maximum concentration achieved) by healthy volunteers in a human clinical study were obtained with the Quercetin Phytosome formulation as compared to unformulated quercetin.

Conclusions A more soluble formulation of quercetin based on lecithin, Quercetin Phytosome, has recently been developed, and was found to facilitate the attainment of very high plasma levels of quercetin—up to 20 times more than usually obtained following a dose of quercetin—when the novel formulation was administered orally in human volunteers, and it did not have any notable side effects. These results suggest that Quercetin Phytosome allows the oral administration of quercetin in a safe and bioavailable manner, thus facilitating the effective utilization of this natural compound to treat various human diseases.

Key Points

It is well known from the literature that flavonoids, especially quercetin, are very important biological molecules, strongly suggesting their potential use to treat several human diseases

A new food-grade lecithin-based formulation of quercetin, Quercetin Phytosome, was developed and validated in healthy volunteers.

Quercetin Phytosome overcomes the low bioavailability hurdle of quercetin and should help to fulfill the great health benefit potential of this flavonoid in the diet and as food supplements

✉ Antonella Riva
antonella.riva@indena.com

[1] Research and Development Department, Indena SpA, 20139 Milan, Italy

Published online: 16 October 2018

Quercefit™

20 times the absorption of regular quercetin

Review paper DOI: https://doi.org/10.5114/ceji.2018.81347

Impact of polyphenols on mast cells with special emphasis on the effect of quercetin and luteolin

YASDANI SHAIK[1], ALESSANDRO CARAFFA[2], GIANPAOLO RONCONI[3], GIANFRANCO LESSIANI[4], PIO CONTI[5]

[1]Department of Medicine, Boston University School of Medicine, Boston, USA
[2]Department of Pharmacology, University of Perugia, Perugia, Italy
[3]Clinica dei Pazienti del Territorio, Policlinico Gemelli, Roma, Italy
[4]Internal Medicine, Villa Serena Hospital, Cittŕ Sant'Angelo, Italy
[5]Universitŕ G. d'Annunzio, Chieti-Pescara, Italy

Abstract

Polyphenols are ubiquitous in food and have long been recognized to possess antioxidant, anti-inflammatory and anticancer activities. Mast cells (MCs) are implicated in the pathogenesis of inflammatory diseases, allergy, autoimmunity and cancer. MCs derive from hematopoietic progenitor cells, reside virtually in all vascularized tissue and are activated by crosslinking of FceRI-bound IgE (at very high affinity: 1 × 10^{10} M^{-1}) with multivalent antigen. MCs in cytoplasmic granules release preformed chemical mediators, and also they can release lipid mediators and cytokines/chemokines without degranulation.

Luteolin, 3',4',5,7-tetrahydroxyflavone, is a flavonoid contained in many kinds of plants including vegetables and fruits. This anti-oxidant product inhibits interleukin (IL)-6, IL-8 and vascular endothelial growth factor (VEGF) production from tumor necrosis factor (TNF)-triggered keratinocytes, and is a candidate for use in alternative therapies in the treatment of inflammatory skin disorders.

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a ubiquitous flavonoid which exhibits anti-cancer, anti-oxidative and anti-inflammatory properties and causes a reduction in the availability of nitrite that influences vascular function. Quercetin exerts physiological functions though the interaction with phosphatidylinositol-3-phosphate kinase (PI3K), mitogen-activated protein kinase (MAPK), extracellular signal regulated kinase (ERK), kinase (MEK) 1, and others, and has a negative effect on FceRI cross-linking and other activating receptors on mast cells. In this article we report for the first time the interrelationship between mast cells and polyphenols.

Key words: *polyphenols, mast cells, immunity, inflammation.*

(Centr Eur J Immunol 2018; 43 (4): 1-6)

At the beginning of the last century, Rusznyak and Szent-Gyorgyi reported that citrus fruits contain diverse substances other than vitamin C, which can prevent capillary fragility. These substances were the polyphenols (flavonoids, phenolic acids, lignans, coumarins) or flavonoids contained in large quantities in fruit, vegetables, cereals, and beverages, and which may have a positive effect under conditions of stress and other neurological dysfunctions [1]. Constituents of grapes, such as quercetin, resveratrol, kaempferol, catechin, epicatechin and anthocyanins, constitute more than 70% of the grape polyphenols. Natural compounds have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticancer activities.

Flavonoids, previously called vitamin P or vitamin C2, are numerous; in fact, to date, about 800 different flavonoids have been isolated. Flavonoids are polyphenolic secondary metabolites classified into anthocyanins, flavonols, flavones, flavan-3-ols, flavanones, isoflavones, and chalcones; they are ubiquitous in food and potentiate the anti-scorbutic activity shown in a number of animal studies. Flavonoids cannot be considered vitamins, since they produce pharmacologic effects rather than nutritional ones. It is well known that polyphenols such as coumarin, curcumin, catechin, resveratrol, anthocyanidin, tannin, rutin, isoflavone, quercetin, etc. exert antioxidant properties, and these are found in red wine, chocolate, tea, pomegranate and fruit juices, where they are the greatest contributors of flavonoids in the human diet along with vegetables [2].

Correspondence: Prof. Pio Conti, Universitŕ G. d'Annunzio, viale Unitŕ d'Italia, 66100, Chieti, Italy, e-mail: pconti@unich.it
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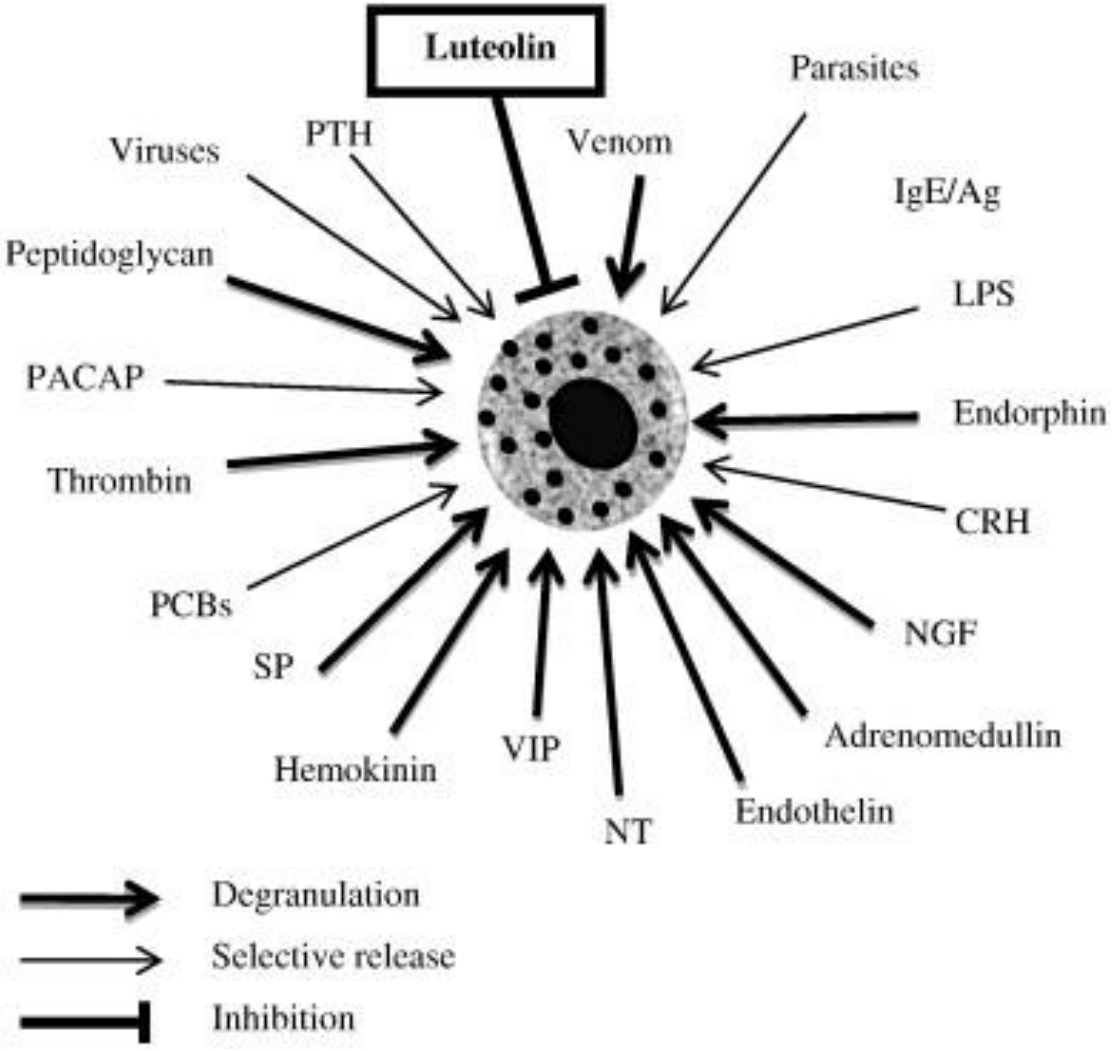
Polyphenols Effect on Mast Cells

Luteolin



Theoharides TC. Et al. Mast Cells and Inflammation. Biochimica et Biophysica Acta. 2012. 1822:21-33.

A flavanone derivative from the Asian medicinal herb (Perilla frutescens) potently suppresses IgE-mediated immediate hypersensitivity reactions. Kamei R. et al. 2017 Jan 29;483(1):674-679.

1

Perilla frutescens is a dietary leafy herb consumed as a traditional Japanese condiment

2

Suppresses IgE-mediated type I hypersensitivity reactions.

3

Significantly inhibits IgE-mediated histamine release

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.

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http://dx.doi.org/10.1155/2015/324265

Research Article

Ethanol Extract of *Perilla frutescens* Suppresses Allergen-Specific Th2 Responses and Alleviates Airway Inflammation and Hyperreactivity in Ovalbumin-Sensitized Murine Model of Asthma

Miaw-Ling Chen,[1] Chi-Heng Wu,[2] Li-Shiuan Hung,[2] and Bi-Fong Lin[2]

[1]*Department of Nutrition and Health Sciences, College of Health Sciences, Chang Jung Christian University, No. 1, Changda Road, Guiren District, Tainan City 71101, Taiwan*
[2]*Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Sec. 4, No. 1 Roosevelt Road, Taipei 10617, Taiwan*

Correspondence should be addressed to Miaw-Ling Chen; miaw-ling@mail.cjcu.edu.tw and Bi-Fong Lin; bifong@ntu.edu.tw

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This study was to investigate the effects of different fractions of *Perilla frutescens* (Pf) leaves extracted by water or ethanol on asthma. BALB/c mice sensitized intraperitoneally and challenged with ovalbumin (OVA) were divided into six groups. Each group of mice was tube-feeding with 0 (control), 80 μg (PfWL), or 320 μg (PfWH) water extracts or 80 μg (PfEL) or 320 μg (PfEH) ethanol extracts of perilla leaves daily for 3 weeks. A negative control group (PBS) was neither sensitized nor treated with Pf. The effects of perilla leave extracts on allergic immune response were evaluated. The results showed that OVA-specific IL-5 and IL-13 secretions from OVA-stimulated splenocytes were significantly suppressed in the ethanol extract groups PfEL and PfEH. Serum level of anti-OVA IgE tended to be lower in the PfEH group. The inflammatory mediators, such as eotaxin and histamine, and total cells, particularly eosinophils in bronchoalveolar lavage fluid (BALF), were also decreased in the PfEL and the PfEH groups. Therefore, the PfEL and the PfEH groups had significantly lower methacholine-induced hyperresponsiveness (AHR). In conclusion, ethanol extracts, rather than water extract, of perilla leaves could significantly suppress Th2 responses and airway inflammation in allergic murine model of asthma.

1. Introduction

Allergic asthma is a chronic disease that clinically augments bronchial hyperresponsiveness and inflammation. The asthmatic inflammation is clearly associated with the high level of type 2 T cell (Th2) cytokines that induced immunoglobulin (Ig) E production and eosinophilic infiltration [1]. The Th2 cytokines IL-4, IL-5, and IL-13 are the major cytokines for development of atopic diseases, such as asthma, rhinitis, and dermatitis [2]. IL-4 promotes the immunoglobulin class switch from IgM to IgE [3]. IL-5 induces eosinophilia activity and infiltration, which is the critical response in the allergic asthma [4]. IL-13 acts to induce airway hyperresponsiveness (AHR) that contributes to atopic disease [5]. The suppression of Th2 responses is a feasible attempt to attenuate the symptoms of allergic asthma. Therefore, biologic targeted therapies have been developed to target the specific molecular pathways to treat asthma, especially those targets at IL-4, IL-5, IL-13, and IgE [6]. However, due to complex clinical symptoms and multiple mechanisms involved, the outcome of these trials has not been satisfied. Epidemiological studies indicate that patients often turn to complementary and alternative therapies, including dietary supplements [7]. Studies also showed that dietary oil, adlay, and medicinal herbs, such as Ganoderma and Andrographis, decreased Th2 cytokines productions and thus alleviated allergic responses in a murine model of asthma [8–12], suggesting the potential application of traditional herbal medicine for immunomodulation.

42

Perilla

Decreases TH2 immune response

Role in asthma treatment

The Health Benefits of Butterbur (Petasites)

Treatment For Migraine

Relieves migraines by reducing the spasm of the muscle linings of blood vessels in the brain

Treatment For Allergies

Butterbur can reduce histamine activity, so it is also effective for allergic conditions like asthma and rhinitis

Treatment For Inflammation

Inhibits the lipoxygenase pathway and production of leukotrienes so it can also be effective for inflammation

Anti type I allergic property of Japanese butterbur extract and its mast cell degranulation inhibitory ingredients.

Shimoda H. et al. J Agric Food Chem. 2006 Apr 19;54(8):2915-20.

inhibitory activity on mast cell degranulation

fukinolic acid, a principal polyphenol constituent, showed potent inhibitory activity.

suppress the type I allergic reaction.

Nettle extract (Urtica dioica) affects key receptors and enzymes associated with allergic rhinitis. Roschek B. et al. 2009 Jul;23(7):920-6.

- A nettle (Urtica dioica) extract shows in vitro inhibition of several key inflammatory events that cause the symptoms of seasonal allergies.
- antagonist against the Histamine-1 (H(1)) receptor
- inhibition of mast cell tryptase
- inhibits prostaglandin formation

HistaQuel™

Two-Fold Mechanism of Action

- Block histamine and other mediator release from Mast cells
- Block histamine receptors
- Quercefit: 10x absorption of regular quercetin

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.

HistaQuel™ Ingredient Blends

Ingredient Blend	Constituents	Mechanisms of Action*
Mast Cell Secure™ (Flavonoids)	• Fisetin • Luteolin • Perilla Frutescens • Quercefit™ (10x the absorption of regular quercetin)	• Targeted flavonoid blend supports a decrease in mast cell release of histamine • Moderates interactions between mast cells and activated T cells. • Decreases Th2 release of allergy promoting cytokines IL-4 and IL-5
HistaCheck™	• Stinging Nettle leaf • Butterbur extract	• Decreases ability of histamine to bind to its receptors by competitive inhibition • Supports management of a healthy immune response to allergens

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HistaQuel™

Mast Cell Secure™ Blend Ingredient Research-Summary

- **Flavonoids (general)**
  - Inhibit IgE mediated histamine release[1]
  - Decrease production of proinflammatory cytokines[1]
  - Down-regulate mast cell activation[1]
- **Fisetin**
  - Significantly inhibited infiltration of inflammatory cells including eosinophils, mast cells and CD4(+) T and CD8(+) T cells[2]
  - Total serum immunoglobulin E (IgE) levels were markedly reduced by Fisetin[2]
  - Reduced the production of interferon-gamma and interleukin-4 by activated CD4(+) T cells in a dose-dependent manner[2]
  - limits interactions between mast cells and activated T cells[3]
- **Quercefit™**
  - 20x the absorption of regular Quercetine[4]
- **Perilla Frutescens**
  - Suppresses IgE-mediated type I hypersensitivity reactions[7]
  - Significantly inhibits IgE-mediated histamine release[7]

HistaQuel™

HistaCheck™ Blend Ingredient Research Summary

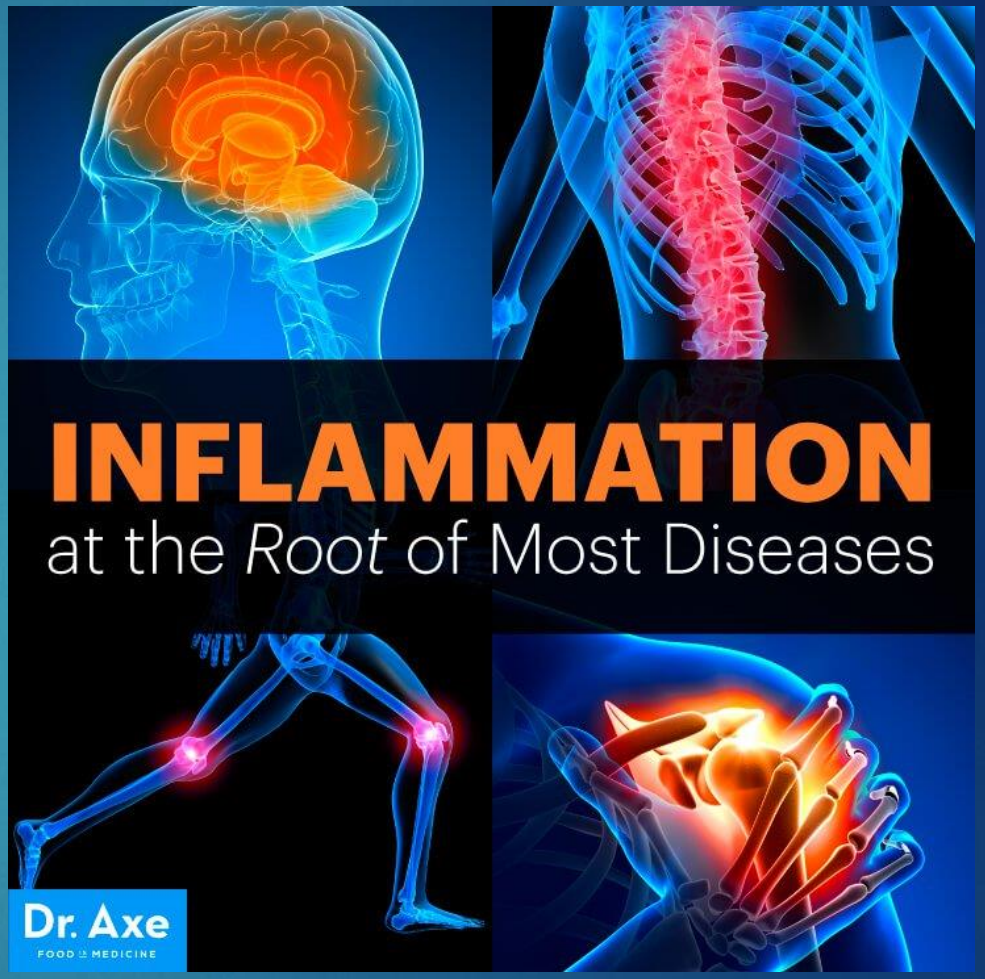
- **Nettle Extract**
  - Shows in vitro inhibition of several key inflammatory events that cause the symptoms of seasonal allergies[5]
  - Antagonist against the Histamine-1 (H(1)) receptor[5]
  - Inhibition of mast cell tryptase[5]
  - Inhibits prostaglandin formation[5]
- **Butterbur Extract**
  - Showed inhibitory activity on mast cell degranulation[6]
  - Suppresses the type I allergic reaction[6]

Treating Chronic Disease through Treating Chronic Pathology

- Repair and support mitochondria
  - Membrane repair
  - Support nutrients in ATP production from breakdown of macronutrients to Krebs cycle to oxidative phosphorylation
- Decrease causes of oxidative stress and inflammation
- Increase anti-oxidants to counteract oxidative stress including activating Nrf2
- Decrease inflammation through down regulating inflammatory mediators and improving microbiome
- Decrease mast cell activation by inhibiting release and blocking histamine receptors

Support for Inflammation and Abnormal Cytokines

- Glutathione
- Molecular Hydrogen (H_2 : Hydrogen gas)
- Vitamin E: mixed tocopherols and tocotrienols
- Curcumin
- Tea: EGCG
- Resveratrol
- N-acetyl-cysteine
- Probiotics



CytoQuel®
Key Ingredients

- Black Tea extract (EGCG)
  - High EGCG content
  - Promotes healthy levels of IL-23, which controls IL-17 levels
  - Supports healthy levels of NF-k Beta, TNF-a, IL-1 beta, IL-6, IL-8 (Herxheimer reactions increase TNF-a, IL-6 & IL-8)
- N-Acetyl Cysteine
  - Promotes healthy NF-k Beta, IL-6, IL-8 levels
- CurcuWIN™ brand Curcumin
  - Promotes healthy inflammation response
  - 46x more absorbable vs standard curcumin
- Tocotrienols (Vitamin E) from Annatto (not rice or palm derived)
  - Pure delta and gamma tocotrienols --- no tocopherol
  - Promotes healthy inhibition levels of NF-k Beta &nitric oxide
  - Protects against oxidative stress
  - Promotes healthy cardiovascular function
  - Protects from neurodegeneration
- Resveratrol
  - Promotes healthy inhibition levels of TNF-a, IL-1 beta & NF-k Beta
  - Activates Sirt-1 & Nrf2

CytoQuel®

Peer-reviewed Clinical Research*

- Participants with chronic pain for at least 6 months where given CytoQuel® for 8 weeks.
- Pain scoring and activities of daily living questioners were given throughout the study
- Study results…
  - 65% decrease in primary pain within the last 24 hours*
  - 44% improvement in quality of sleep*
  - Normalization of the ankle brachial index*

Conclusion: Consuming CytoQuel® helped manage pain, increased comfort during daily activities, and improved vascular function… *

Journal of Pain Research

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Pain reduction and improved vascular health associated with daily consumption of an anti-inflammatory dietary supplement blend

Debby E Hamilton, MD[1]
Gitte S Jensen, PhD[2]

[1]Researched Nutritionals, Los Olivos, CA, USA; [2]NIS Labs, Klamath Falls 1437, OR, USA

Purpose: The objective for this clinical pilot study was to evaluate changes to chronic pain, vascular health, and inflammatory markers when consuming a dietary supplement blend (DSB, CytoQuel®), containing curcumin, resveratrol, tocotrienols, N-Acetylcysteine, and epigallocatechin gallate.

Materials and methods: An open-label study design was used where 21 study participants were evaluated at baseline and at 2 and 8 weeks after consuming DSB. Participants were randomized to consume 3 capsules once daily versus 2 capsules twice daily. Pain and activities of daily living questionnaires were used to gather subjective data on pain levels and interference with daily living. Blood pressure was measured in both arms and ankles, and the ankle-brachial index (ABI) calculated. Blood samples were used to evaluate markers associated with inflammation and cardiovascular health.

Results: Highly significant reduction of chronic pain was seen after 8 weeks ($p<0.01$), both at rest and when physically active. Faster improvement was seen when consuming 3 capsules once daily, compared to 2 capsules twice daily. The pain reduction resulted in improved sleep quality ($p<0.1$), and improved social functioning ($p<0.01$), and less need for support from others ($p<0.05$). Normalization of mildly elevated ABI at study start was seen after 2 weeks. Plasma fibrinogen and von Willebrand Factor and serum matrix metalloproteinase-9 (MMP-9) showed reduction after 2 weeks (not significant), whereas a reduction in serum interleukin-1 receptor antagonist-a (IL-1ra) was statistically significant after 2 weeks ($p<0.05$). Correlation between pain reduction and changes to MMP-9 after 8 weeks was highly significant ($P<0.01$), whereas correlation between pain reduction and changes to IL-1ra reached significance at 2 weeks for the group consuming 3 caps once daily ($p<0.04$).

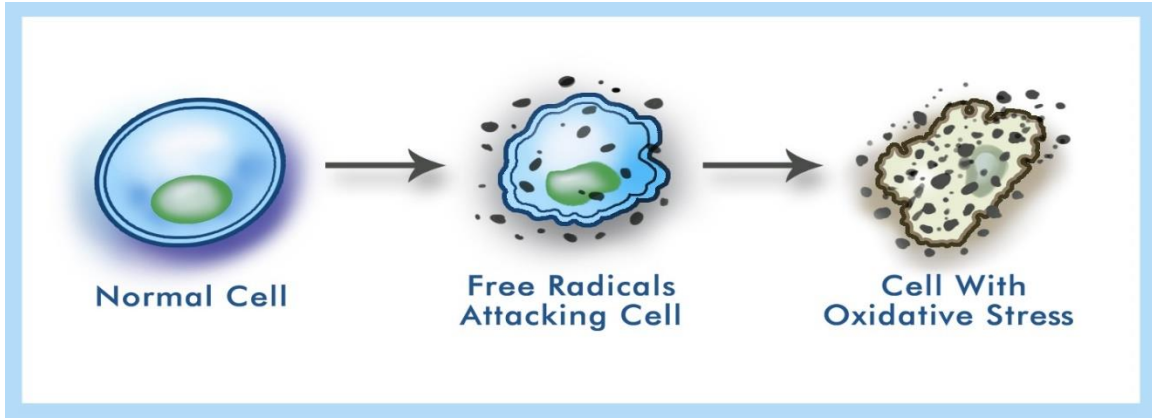
Conclusion: Consuming DSB helped manage pain, increased comfort during daily activities, and improved vascular function. This was associated with selective effects on specific blood biomarkers associated with inflammation and vascular health.

Keywords: ankle-brachial index, cardiovascular disease, fibrinogen, interleukin-1 receptor antagonist, matrix metalloproteinase-9, von Willebrand factor

the [illegible] products are not intended to diagnose, treat, cure, or prevent any disease.

Support for Oxidative Stress

- Anti-oxidants to combat oxidative stress
  - Glutathione
  - Vitamin C
  - Alpha-lipoic Acid
  - Molecular Hydrogen (H_2 : Hydrogen gas)
  - Vitamin E: mixed tocopherols and tocotrienols
  - Curcumin
  - Tea: EGCG
  - Resveratrol
  - N-acetyl-cysteine



Tri-Fortify®

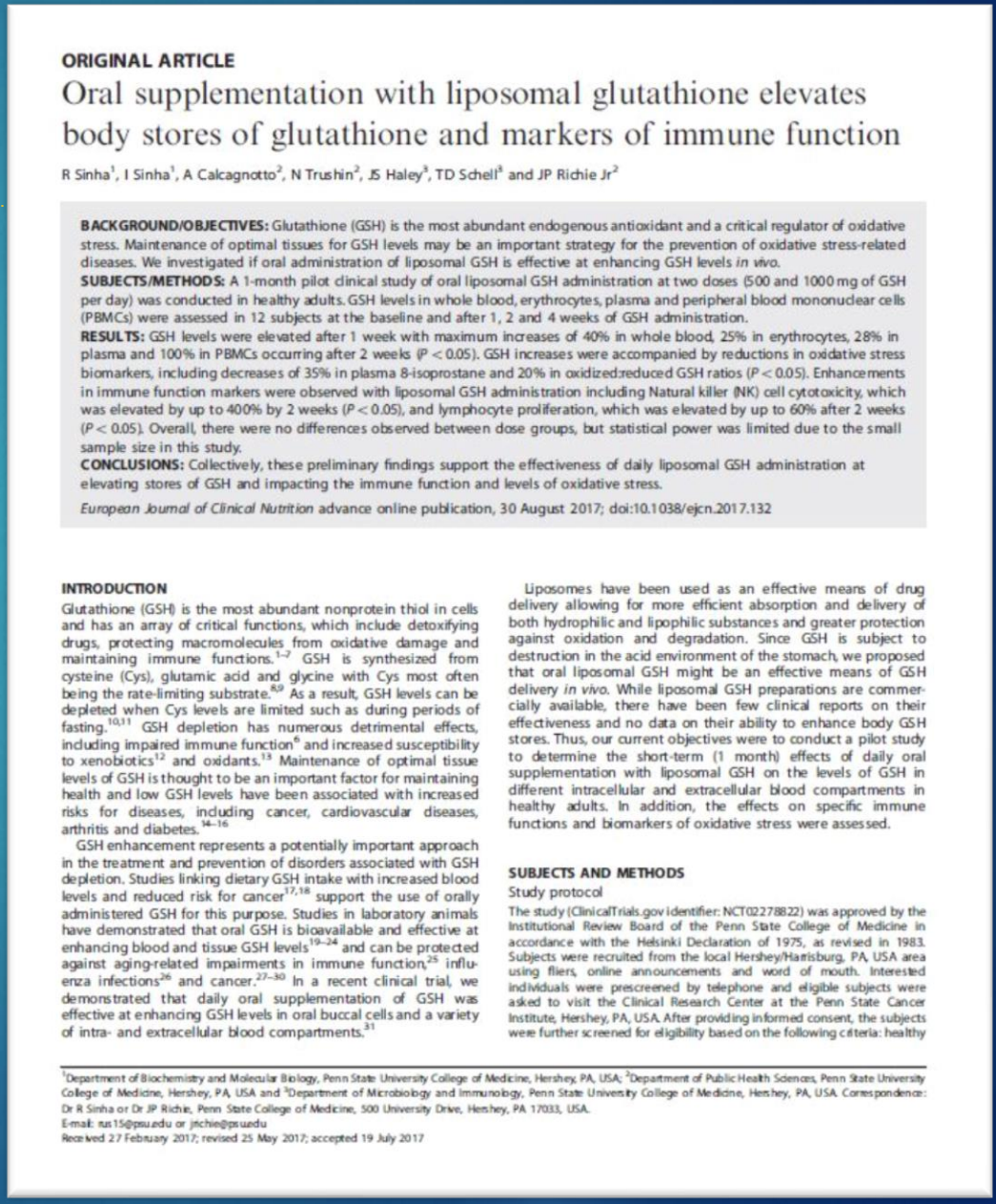
Published, Peer-Reviewed Research

CLINICALLY RESEARCHED

Research Summary

In just 2 weeks…

- **28%** increase in red blood cell glutathione levels
- **400%** increase in natural killer cell activity
- **25%** reduction in lipid peroxidation (oxidative stress marker)
- Research conducted at Penn State University
- Published in European Journal of Clinical Nutrition..2017



ORIGINAL ARTICLE

Oral supplementation with liposomal glutathione elevates body stores of glutathione and markers of immune function

R Sinha[1], I Sinha[1], A Calcagnotto[2], N Trushin[2], JS Haley[3], TD Schell[3] and JP Richie Jr[2]

BACKGROUND/OBJECTIVES: Glutathione (GSH) is the most abundant endogenous antioxidant and a critical regulator of oxidative stress. Maintenance of optimal tissues for GSH levels may be an important strategy for the prevention of oxidative stress-related diseases. We investigated if oral administration of liposomal GSH is effective at enhancing GSH levels *in vivo*.
SUBJECTS/METHODS: A 1-month pilot clinical study of oral liposomal GSH administration at two doses (500 and 1000 mg of GSH per day) was conducted in healthy adults. GSH levels in whole blood, erythrocytes, plasma and peripheral blood mononuclear cells (PBMCs) were assessed in 12 subjects at the baseline and after 1, 2 and 4 weeks of GSH administration.
RESULTS: GSH levels were elevated after 1 week with maximum increases of 40% in whole blood, 25% in erythrocytes, 28% in plasma and 100% in PBMCs occurring after 2 weeks ($P < 0.05$). GSH increases were accompanied by reductions in oxidative stress biomarkers, including decreases of 35% in plasma 8-isoprostane and 20% in oxidized:reduced GSH ratios ($P < 0.05$). Enhancements in immune function markers were observed with liposomal GSH administration including Natural killer (NK) cell cytotoxicity, which was elevated by up to 400% by 2 weeks ($P < 0.05$), and lymphocyte proliferation, which was elevated by up to 60% after 2 weeks ($P < 0.05$). Overall, there were no differences observed between dose groups, but statistical power was limited due to the small sample size in this study.
CONCLUSIONS: Collectively, these preliminary findings support the effectiveness of daily liposomal GSH administration at elevating stores of GSH and impacting the immune function and levels of oxidative stress.

European Journal of Clinical Nutrition advance online publication, 30 August 2017; doi:10.1038/ejcn.2017.132

INTRODUCTION

Glutathione (GSH) is the most abundant nonprotein thiol in cells and has an array of critical functions, which include detoxifying drugs, protecting macromolecules from oxidative damage and maintaining immune functions.[1–7] GSH is synthesized from cysteine (Cys), glutamic acid and glycine with Cys most often being the rate-limiting substrate.[8,9] As a result, GSH levels can be depleted when Cys levels are limited such as during periods of fasting.[10,11] GSH depletion has numerous detrimental effects, including impaired immune function[6] and increased susceptibility to xenobiotics[12] and oxidants.[13] Maintenance of optimal tissue levels of GSH is thought to be an important factor for maintaining health and low GSH levels have been associated with increased risks for diseases, including cancer, cardiovascular diseases, arthritis and diabetes.[14–16]

GSH enhancement represents a potentially important approach in the treatment and prevention of disorders associated with GSH depletion. Studies linking dietary GSH intake with increased blood levels and reduced risk for cancer[17,18] support the use of orally administered GSH for this purpose. Studies in laboratory animals have demonstrated that oral GSH is bioavailable and effective at enhancing blood and tissue GSH levels[19–24] and can be protected against aging-related impairments in immune function,[25] influenza infections[26] and cancer.[27–30] In a recent clinical trial, we demonstrated that daily oral supplementation of GSH was effective at enhancing GSH levels in oral buccal cells and a variety of intra- and extracellular blood compartments.[31]

Liposomes have been used as an effective means of drug delivery allowing for more efficient absorption and delivery of both hydrophilic and lipophilic substances and greater protection against oxidation and degradation. Since GSH is subject to destruction in the acid environment of the stomach, we proposed that oral liposomal GSH might be an effective means of GSH delivery *in vivo*. While liposomal GSH preparations are commercially available, there have been few clinical reports on their effectiveness and no data on their ability to enhance body GSH stores. Thus, our current objectives were to conduct a pilot study to determine the short-term (1 month) effects of daily oral supplementation with liposomal GSH on the levels of GSH in different intracellular and extracellular blood compartments in healthy adults. In addition, the effects on specific immune functions and biomarkers of oxidative stress were assessed.

SUBJECTS AND METHODS

Study protocol

The study (ClinicalTrials.gov identifier: NCT02278822) was approved by the Institutional Review Board of the Penn State College of Medicine in accordance with the Helsinki Declaration of 1975, as revised in 1983. Subjects were recruited from the local Hershey/Harrisburg, PA, USA area using fliers, online announcements and word of mouth. Interested individuals were prescreened by telephone and eligible subjects were asked to visit the Clinical Research Center at the Penn State Cancer Institute, Hershey, PA, USA. After providing informed consent, the subjects were further screened for eligibility based on the following criteria: healthy

[1]Department of Biochemistry and Molecular Biology, Penn State University College of Medicine, Hershey, PA, USA; [2]Department of Public Health Sciences, Penn State University College of Medicine, Hershey, PA, USA and [3]Department of Microbiology and Immunology, Penn State University College of Medicine, Hershey, PA, USA. Correspondence: Dr R Sinha or Dr JP Richie, Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA.
E-mail: rus15@psu.edu or jrichie@psu.edu
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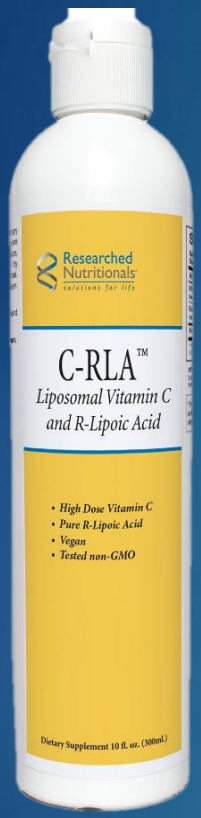
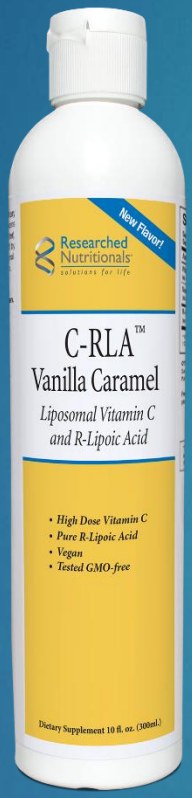
C-RLA™

Liposomal High Dose Vitamin C and R-Lipoic Acid





Original Flavor

Supplement Facts

Serving Size: 2 teaspoons (10 mL)
Servings Per Bottle: 30

Amount Per Serving		%Daily Value**
Vitamin C (as sodium ascorbate)	1500 mg	1667%
Sodium (as sodium ascorbate)	197 mg	9%
R-Lipoic Acid	70 mg	†

** Percent Daily Values are based on a 2,000 calorie diet.
† Daily Value not established.

Other Ingredients: Purified water, non-GMO sunflower phospholipids, glycerin, natural flavors, potassium sorbate.

Free of: Milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, soy, corn and gluten.

Vanilla Caramel

Supplement Facts

Serving Size: 2 teaspoons (10 mL)
Servings Per Bottle: 30

Amount Per Serving		%Daily Value**
Calories	5	
Carbohydrates	6 g	2%
Total Sugars 6g		
Vitamin C (as Sodium Ascorbate)	1500 mg	1667%
Sodium (as Sodium Ascorbate)	197 mg	9%
R-Lipoic Acid (as Sodium R-Lipoic Acid)	70 mg	†

** Percent Daily Values are based on a 2,000 calorie diet.
† Daily Value not established.

Other Ingredients: Allulose, deionized water, sunflower lecithin, vanilla extract, grapefruit seed extract, stevia extract.

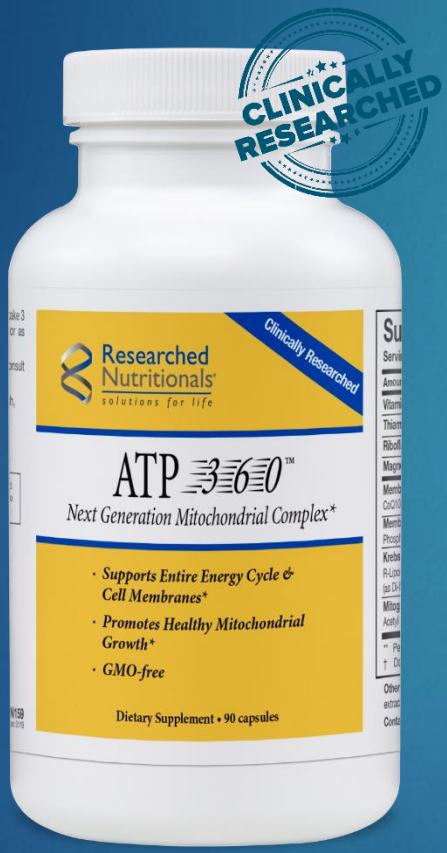
Free of: Milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, soy and gluten.

Suggested Use: Take 2 teaspoons daily. May be taken straight or mixed in water.

*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.

GMO Free – Vegan – Soy Free

ATP 360™
Mitochondrial Dysfunction Support



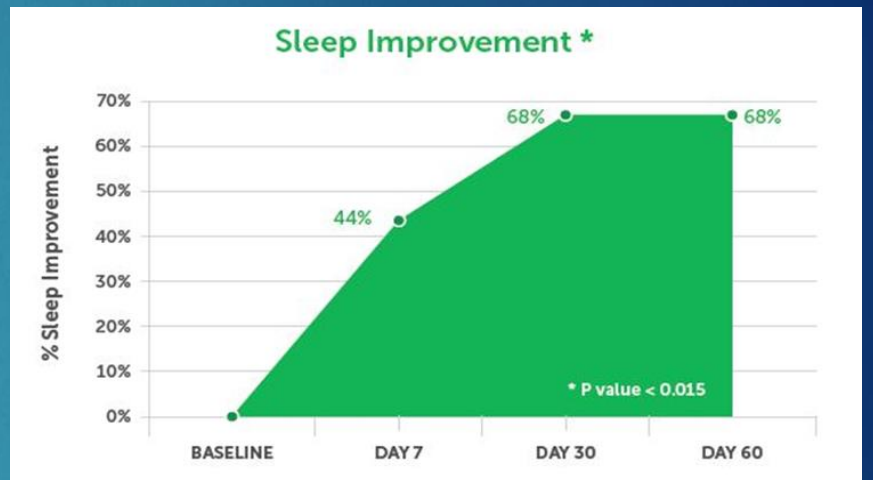
- **Phospholipid blend**
  - Repairs damaged mitochondrial membranes
- **PQQ**
  - Increases mitochondrial mass
- **Pure Tocotrienols and R-Lipoic Acid**
  - Reduces oxidative stress which damage membranes
- **NADH & CoQ10**
  - Supports Kreb's cycle

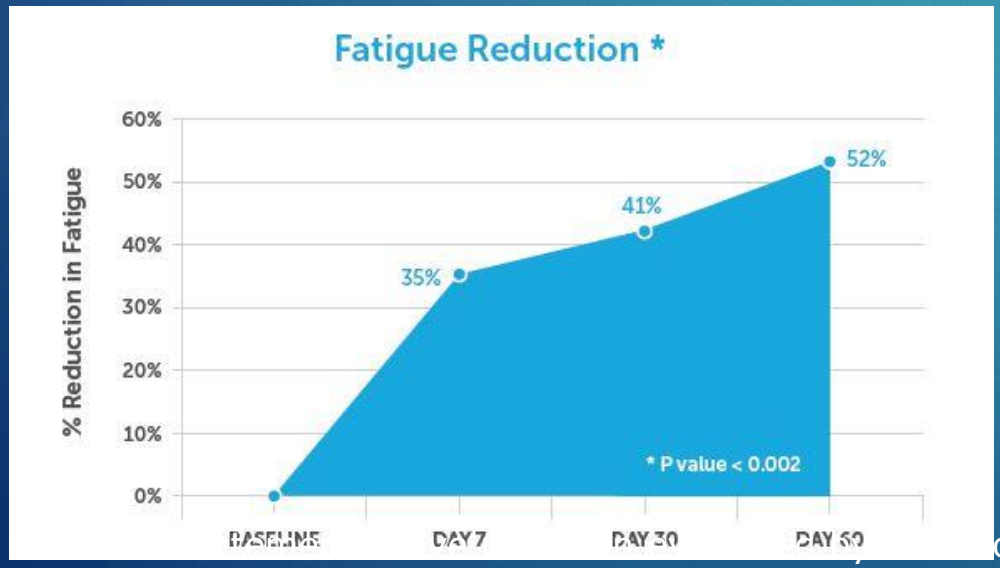
*These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.

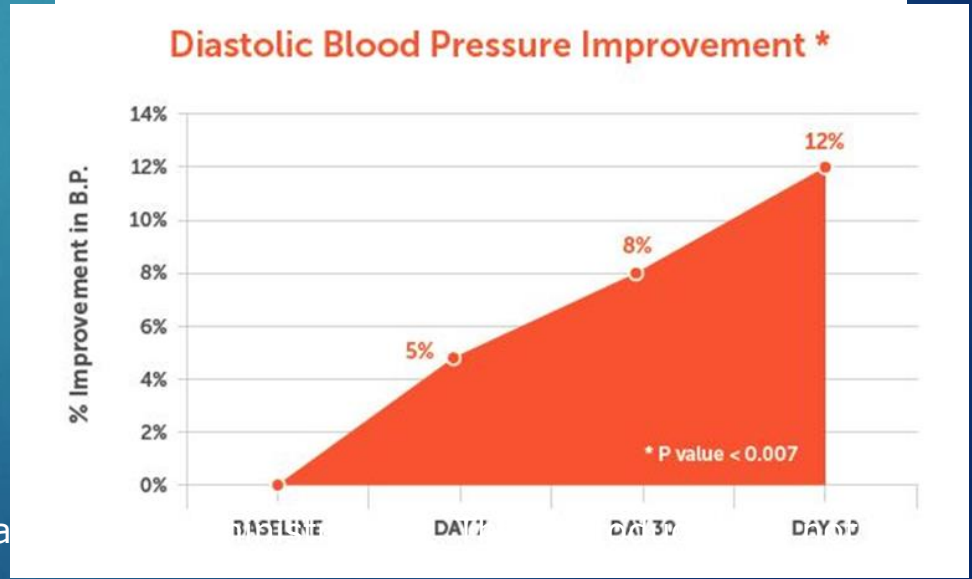
ATP 360™
Clinical Pilot Study

8-week study with 24 patients complaining of long-term fatigue demonstrated…

- **52%** Reduction in Fatigue
- **68%** Improved Sleep
- **12%** Reduction in Diastolic Blood Pressure







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intended to diagnose, treat, cure, or prevent any disease.

Summary of Mast Cell Activation Syndrome

- Symptoms involve all body systems
- Component of most chronic diseases
- Clinical diagnosis- positive lab tests helpful but not necessary
- Treatment
  - Diet: Low histamine diet and DAO enzymes can be helpful for food histamine levels and break down of food histamines
  - Inhibit mast cell activation
  - Block histamine receptors
  - Anti-inflammatories
  - Anti-oxidants
  - Mitochondrial support

Treating Chronic Disease through Treating Chronic Pathology

- **Mast Cell Activation:**
  - **HistaQuel™** **d**ecrease mast cell activation by inhibiting release and blocking histamine receptors
- **Inflammation:**
  - **CytoQuel®** - support healthy cytokine activity and manage oxidative stress and inflammation
  - **CoreBiotic™**: dysbiosis triggering inflammation and mast cell activation
- **Mitochondrial Dysfunction:**
  - **ATP360™** - repair mitochondrial membranes and support mitochondrial function
- **Oxidative Stress:** neutralize free radicals
  - **Tri-Fortify® Liposomal Glutathione**
  - **C-RLA™**