

APITHERAPY IN IMMUNE MEDIATED DISORDERS

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WHAT IS APITHERAPY

- APITHERAPY, or “bee therapy” (*from the Latin apis which means bee*) is the medical use of products made by honeybees.
- Products of the Honeybee include :
 - Bee venom,
 - Honey,
 - Pollen,
 - Royal jelly,
 - Propolis,
 - Beeswax.

- It is important to note that Apitherapy is **not only the use of the venom for healing**, often called **BEE STING THERAPY**, but the use of all the hive products, and usually a combination of them.
- These products are also sometimes mixed with **other ingredients, specifically different essential oils**, dependent on the condition being treated.

HISTORY OF APITHERAPY

- The exact place and pattern of origin of apitherapy is **not clear**.
- History of apitherapy can be traced back to **ancient Egypt, Greece, and China** (*Hegazi, 1998*)
- Even **Hippocrates**, the great Greek physician renowned as the "father of medicine," used bee venom to treat joint pain and arthritis. Ancient Greeks athletes used honey to boost an energy. (*Broffman, 1999*).

- The modern systematic study of apitherapy was initiated through the efforts of the Austrian physician **PHILLIP TERC**.
- He published the results of intentional bee sting and bee in his article "Report about a Peculiar Connection Between the Beestings and Rheumatism" in 1888.

- The holly Quran 1400 years ago mentioned that the bee products contain cure to people.

Wherein is healing for people

Al Nahl :69

Active components of apitherapy

1-Peptide constituents

PEPTIDE 401

- **Mast cell degranulating (MCD) peptide—MCD peptide**, also known as peptide 401, a bee venom polypeptide with 22 amino acids and constituting 2–3% of dry bee venom.
- It was originally named due to its biological action of causing release of histamine from mast cells (*Banks et al., 1990*).

APAMIN

- Another important bee venom neurotoxic polypeptide of 18 amino acids comprising 2–3% of dry bee venom.
- It possesses a selective inhibitory action on *calcium-dependent potassium channels* that are involved in regulation of the **after-hyperpolarization period** and **frequency of action potential generation** in the central nervous system (CNS) (*Hugues et al., 1982*).

- **Afterhyperpolarization**, describes the phase of a neuron's action potential where the cell's membrane potential falls below the normal resting potential.
- This is also commonly referred to as an action potential's undershoot phase (*M. Shah, and D. G. Haylett, 2000*).

MELLITIN

- A strongly basic 26 amino-acid polypeptide which constitutes 40–60% of the whole dry honeybee venom.
- It has various biological, pharmacological and toxicological actions including **strong surface activity on cell lipid membranes, hemolyzing activity, antibacterial** and **antifungal activities** (*Lariviere and Melzack, 1996*).

- The cytotoxic effect through the activation of PLA2 by melittin is believed to be an important mechanism of anti-cancer activity of BV.
- Several cancer cells, including renal, lung, liver, prostate, bladder, and mammary cancer cells as well as leukemia cells, can be targets of melittin (*Moon et al., 2006*).

- The induction of apoptotic cell death through several cancer cell death mechanisms, including the activation of caspase and matrix metalloproteinases (MMP), is important for the melittin-induced anti-cancer effects (*Holle et al., 2003*).
- The binding of the cell lytic peptide (melittin) to the hormone receptors as well as gene therapy carrying melittin can be useful as a novel targeted treatment for some types of cancer, such as prostate and breast cancers (*Li et al., 2004*).

- Recently, **Melittin** has also been demonstrated to cause neural plastic changes along pain-signaling pathways by activation and sensitization of nociceptor cells via phosphorylation of mitogen-activated protein kinases (MAPK) (*Hao et al., 2008; Yu et al., 2009*).
- The effect of mellitin was studied in animal models with amyotrophic lateral sclerosis (ALS) it was found that administering melittin decreased microglial activity and the expression of the pro-inflammatory factor TNF- α (*Yang EJ., et al 2010*).

ADOLAPIN

- **ADOLAPIN**, a basic polypeptide with 103 amino acids residues and comprising 1% of dry bee venom, it has been shown to have **anti-nociceptive** “decreasing pain sensation” **anti-inflammatory** and **antipyretic effects** (*Koburova et al., 1984,1985*).
- Adolapin can **inhibit prostaglandin synthesis via inhibition of cyclooxygenase activity** (*Shkenderov and Koburova, 1982*).

ENZYMES

PHOSPHOLIPASE A2

- **PLA2**, which constitutes 10–12% of dry bee venom, has inflammatory and nociceptive effects (*Landucci et al., 2000*).
- PLA2 is a membrane-associated phospholipid converting enzyme that is **important in the production of arachidonic acid**, which is further metabolized to prostaglandins by cyclooxygenase and to leukotrienes by lipoxygenase (*Landucci et al., 2000*).

- **PLA2** exhibits complex interactions with melittin that can result in potentiation of secretory PLA2 effects or in inhibition depending on the peptide/phospholipid ratio (*Koumanov et al., 2003*).
- **PLA2** has effects in a range of cells related to **nociception** including astrocytes and neurons and possibly microglial cells , it is also involved in **nerve regeneration** (*Sun et al., 2004a*).

HYALURONIDASE

- **HYALURONIDASE** constitutes 1.5–2% of dry bee venom (*Lariviere and Melzack, 1996*).
- Hyaluronidases break down hyaluronic acid in tissues such as in synovial bursa of rheumatoid arthritis patients (*Barker et al., 1964*).
- Hyaluronidase in bee venom shares this property with endogenous hyaluronidase (*Barker et al., 1963*).

IMMUNE EFFECTS OF APITHERPAY

- In most of the diseases which are considered to benefit from propolis, cellular immune reaction is activated, **neopterin** levels in body fluids are increased and enhanced **tryptophan** degradation is observed.
- Increased amounts of **neopterin** are produced by human monocytes/macrophages upon stimulation with the cytokine interferon- γ (**Murr C., et al 2002**).

- Caffeic acid phenethyl ester (**CAPE**) is a biologically active component of propolis, a resinous material obtained from bee hives (*Girgin et al., 2009*).
- **CAPE** has several positive effects, including **anti-inflammatory, anti-oxidation, anti-cancer, anti-bacterial, anti-viral, anti-fungal,** and **immunomodulatory effects** (*Jung et al., 2008*).

- ***Song et al., (2008)*** evaluated the anti-inflammatory effect of CAPE on cultured human middle ear epithelial cells (HMEECs).
- They suggested that the anti-inflammatory effect of caffeic acid phenethyl ester (CAPE) is due to its **inhibition of tumor necrosis factor (TNF)-alpha expression** and **interleukin (IL)-8 production** (***Song et al., 2008***) .

- *Márquez et al., (2004)* evaluated the immunosuppressive activity of **CAPE** in human T-cells, discovering that this phenolic compound is a potent inhibitor of early and late events in T-cell receptor-mediated T-cell activation.
- They found that **CAPE** specifically inhibited both interleukin (IL)-2 gene transcription and IL-2 synthesis in stimulated T-cells.

- ***Kohno et al., (2004)*** examined the anti-inflammatory actions of ***Royal Jelly (RJ)*** at a cytokine level. When supernatants of RJ suspensions were added to a culture of mouse peritoneal macrophages stimulated with lipopolysaccharide and IFN-gamma.
- the production of proinflammatory cytokines, such as **TNF-alpha, IL-6, and IL-1**, *was efficiently inhibited in a dose-dependent manner without having cytotoxic effects on macrophages.*

POLLEN

- At the mucosal surfaces, pollen grains do not only release allergens but also proinflammatory and immunomodulatory lipids, termed **pollen-associated lipid mediators**.
- Among these, the E1-phytoprostanes (PPE1) were identified to modulate dendritic cell (DC) function: PPE1 inhibit the DC's capacity to **produce IL-12** and enhance DC mediated **TH2 polarization of naive T cells** (*Gilles et al., 2009*).

ULCERATIVE COLITIS

WHAT IS ULCERATIVE COLITIS?

- ***Ulcerative colitis (UC)*** is one of the 2 major types of inflammatory bowel disease (IBD), along with Crohn disease.
- ***Unlike Crohn disease (CD)***, which can affect any part of the gastrointestinal (GI) tract, UC characteristically involves only the large bowel.

Signs and symptoms

Patients with UC predominantly complain of the following:

- Rectal bleeding.
- Frequent stools.
- Mucous discharge from the rectum.
- Tenesmus (occasionally).
- Lower abdominal pain .

In some cases, UC has a fulminate course marked by the following:

- Severe diarrhea and cramps
- Fever
- Leukocytosis
- Abdominal distention

UC is associated with various extracolonic manifestations, as follows:

- Uveitis
- Pyoderma gangrenosum
- Pleuritis
- Erythema nodosum
- Ankylosing spondylitis
- Spondyloarthropathies

EPIDEMIOLOGY

- In North America, incidence rates range from **2.2 to 19.2 cases per 100,000** person-years for ulcerative colitis and **3.1 to 20.2 cases per 100,000** person-years for Crohn disease (*Molodecky NA et al., 2012*).
- The incidence and prevalence of Crohn disease and ulcerative colitis appear to be **lower in Asia and the Middle East** (*Ng SC,. Gastroenterology 2013*).

DIAGNOSIS

Laboratory studies are useful principally in excluding other diagnoses and assessing the patient's nutritional status. They may include the following:

- Complete blood count (**CBC**).
- Comprehensive metabolic panel.
- Inflammation markers (eg, erythrocyte sedimentation rate [**ESR**], C-reactive protein [**CRP**]).
- Stool assays.
- Serologic markers (eg, antineutrophil cytoplasmic antibodies [**ANCA**], anti-*Saccharomyces cerevisiae* antibodies [**ASCA**]).

Diagnosis is best made with endoscopy and biopsy, on which the following are characteristic:

- *Abnormal erythematous mucosa*, with or without ulceration, extending from the rectum to a part or all of the colon
- *Uniform inflammation*, without intervening areas of normal mucosa (skip lesions tend to characterize Crohn disease)
- *Contact bleeding* may also be observed, with mucus identified in the lumen of the bowel



HISTOLOGY

- In untreated disease, UC usually exhibits a histological pattern of **CHRONIC ACTIVE COLITIS**, which refers to the presence of active inflammation accompanied by features of chronic mucosal injury.
- **Activity** is defined as the presence of neutrophil-mediated epithelial injury, which may take the form of neutrophils infiltrating crypt epithelium (**cryptitis**), collections of neutrophils within crypt lumens (**crypt abscesses**), or by infiltration of surface epithelium with or without mucosal ulceration (*Gupta RB., et al 2007*).

- **Chronicity** is defined by crypt architectural distortion, basal lymphoplasmacytosis, or cell metaplasia.
- Architectural distortion is represented by shortening of the crypts (**Gupta RB., et al 2007**).

ETIOLOGY

- The exact etiology of ulcerative colitis is unknown, but certain factors have been found to be associated with the disease, and some hypotheses have been presented.
- Genetic factors , immune conditions, environmental factors and NSAIDs use may be associated with the development and affect the course of ulcerative colitis (***Jantchou P., et al 2010***).

Genetics

- The current hypothesis is that genetically susceptible individuals have *abnormalities of humoral and cell-mediated immunity* **and/or** *generalized enhanced reactivity against commensal intestinal bacteria* and that this deregulated mucosal immune response predisposes to colonic inflammation (*Xavier RJ, et al 2007*).

Immune reactions

- Immune reactions that compromise the integrity of the intestinal epithelial barrier may contribute to ulcerative colitis.
- Serum and mucosal autoantibodies against intestinal epithelial cells may be involved. The presence of **antineutrophil cytoplasmic antibodies (ANCA)** and **anti-*Saccharomyces cerevisiae* antibodies (ASCA)** is a well-known feature of inflammatory bowel disease (***Dubinsky MC, et al 2001***).

Environmental factors

- Environmental factors also play a role. For example, **sulfate-reducing bacteria**, which produce sulfides, are found in large numbers in patients with ulcerative colitis, and sulfide production is higher in patients with ulcerative colitis than in other people (*Almeida MG, et al 2008*).

NSAID use

- Nonsteroidal anti-inflammatory drug (NSAID) use is higher in patients with ulcerative colitis than in control subjects, (*Felder Jb et al 2000*) .

PATH PHYSIOLOGY

- Subsets of **T cells** accumulate in the lamina propria of the diseased colonic segment.
- These T cells are cytotoxic to colonic epithelium, with increased production of **immunoglobulin G (IgG) and immunoglobulin E (IgE)** (*Himmel ME, et al 2008*).

- Also this is linked to excessive immune responses to intestinal microbiota which are triggered by **increased activity of effector T cells and/or decreased activity of regulatory T cells**, changes in the composition of intestinal microflora, and/or damaged epithelial barrier (***N. A. Molodecky and G. G. Kaplan, 2010***).

- **Elevated expression of TNF** was detected in IBD patients more than 20 years ago (*D. Owczarek., et al 2012*).
- A recent report showed that elevated concentration of TNF was present in blood serum of IBD patients while other groups found increased levels of TNF protein both in serum and in the intestinal lamina propria of UC patients (*R. Matsuda., et al 2009*)

MANAGEMENT

Medical treatment of mild UC includes the following:

- Mild disease confined to the rectum: Topical mesalazine via suppository or budesonide rectal foam.
- Left-side colonic disease: Mesalazine suppository and oral aminosalicylate (oral mesalazine is preferred to oral sulfasalazine).
- Systemic steroids, when disease does not quickly respond to aminosalicylates.
- Oral budesonide.
- After remission, long-term maintenance therapy (eg, once-daily mesalazine).

Medical treatment of acute, severe UC may include the following:

- Hospitalization.
- Intravenous high-dose corticosteroids.
- Alternative induction medications: Cyclosporine, tacrolimus, infliximab, adalimumab, golimumab.

- **INFLIXIMAB:** *REMICADE* an antibody administered intravenously , it works by blocking the effects of tumor necrosis factor alpha (TNF alpha).

“**Dosing in UC:** 5 mg/kg IV at 0, 2, and 6 weeks, then every 8 weeks”.

- **ADALIMUMAB:** *HUMIRA* other form of injectable anti TNF used in autoimmune disorders.

“ Dosing in UC

Induction: 160 mg SC either as 4 injections of 40 mg on day 1 or as 2 injections of 40 mg daily on 2 consecutive days, then 80 mg SC 2 weeks later (day 15).

Maintenance :(beginning Week 4 Day 29): 40 mg SC q2wk.”

- **Adverse effects of biological therapy:**

- ❖ Antinuclear antibodies (50%).

- ❖ Infection (36%).

- ❖ Nausea (21%).

- ❖ Infusion reaction and Headache (18%).

- ❖ Antibodies to double-stranded DNA (17%).

- ❖ Elevated alanine transaminase (ALT; rarely >3 times upper limit of normal)

- **Increased risk for:**

- ❖ Active tuberculosis.

- ❖ Invasive fungal infections.

- ❖ Infections caused by other opportunistic pathogens, including bacteria (eg, Legionella, Listeria).

- ❖ **Malignancy:** Lymphoma and other malignancies.

Indications for urgent surgery include the following:

- Toxic megacolon refractory to medical management.
- Fulminant attack refractory to medical management.
- Uncontrolled colonic bleeding.

Indications for elective surgery include the following:

- Long-term steroid dependence.
- Dysplasia or adenocarcinoma found on screening biopsy.
- Disease present 7-10 years.

Surgical options include the following :

- Total colectomy (panproctocolectomy) and ileostomy.
- Ileoanal pouch reconstruction or ileorectal anastomosis.
- In an emergency, subtotal colectomy with end-ileostomy (***Shen B. 2009***).

DISEASE COURSE

- Approximately **67 %** of patients have at least one relapse 10 years following the diagnosis (***Scand J. 2009***).
- The risk of relapse depends on the age at initial diagnosis (***Ha CY., et al 2010***).
- A disease flare within two years of the diagnosis, the presence of fever or weight loss at diagnosis, and active disease in the preceding year increase the risk of subsequent relapse (***Scand J. 2009***).

- Extension of colonic disease is seen in up to **20 %** of patients within five years (*Allison J Clin. 2008*).
- Approximately **20 - 30 %** of patients with ulcerative colitis will require colectomy for acute complications or for medically intractable disease (*Scand J. 2009*).

- Patients with ulcerative colitis are at increased risk for colorectal cancer (CRC) (***Lutgens MW. 2013***).
- ***EXTENSION:*** The risk of CRC appears to be highest in patients with *pancolitis*, while those with *proctitis* and *proctosigmoiditis* are probably not an increased risk of CRC, regardless of the duration of disease.
- ***TIMING:*** The CRC risk begins to increase 8 to 10 years following the onset of symptoms in patients with pancolitis (***Gyde SN. 1988***).

CLINICAL TRIAL

- A clinical trial was performed **2009** by inducing UC by administering trinitrobenzene sulfonic acid in experimental rats.
- The rats were then treated, in groups of six, with a **single enema of manuka honey or sulfasalazine medication** (as a positive control) or a **combination of manuka honey and sulfasalazine**, or **not treated** (as a negative control).

- Visual examination of the colon showed that manuka honey on its own significantly decreased ulcerative colitis compared with no treatment and treatment with sulfasalazine (to about one sixth of that with the no-treatment control, being twice as effective as sulfasalazine).
- Histopathology showed that there was severe inflammation (evidenced by infiltration of inflammatory cells) with no treatment, but only mild inflammation with the honey treatment, this being less than with the sulfasalazine treatment.
(Medhi B,.et al 2009)

CONCLUSION

- The immune disorders associated with development of UC , the disease course and progression , the need of various methods of treatment with multiple relapses and major side effects encourage for more safe and effective methods of treatment.
- The role of bee products as natural defense against the precipitating factors of the disease as **tumor necrosing factor** and **T cells** represents a safe and effective way in the management of UC patients.

- The role of CAPE as inhibitor of stimulation of T lymphocytes and its action as natural inhibitor of the tumor necrosing factor (***Song et al., 2008***) .
- Also the role of royal jelly as inhibitor of tumor necrosing factor without any cytotoxic effects ***Kohno et al., (2004)*** rendering the usage of apitherapy as a safe, effective and promising modality in the treatment of UC .

RECOMMENDATIONS

- Large multicenter study should be done to investigate the long term effect and ability of the apitherapy products to induce and maintain remission in UC patients.
- Comparing the effects and results and adverse effects with the traditional medical therapy

- Different routes of application should be studied including topical application in the form of enemas.
- Bee sting therapy also should be studied in cases of UC to detect the possible benefit to these patients

- Clinical , laboratory and histological studies should be performed before and after the course of apitherapy .
- Any side effects or abnormal results should be considered to detect about the safety and effectiveness of apitherapy in UC patients.

THANK YOU