

A Research Report on the Biological Activity of Resprin

Prepared for NuCentury Herbs

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Background: Derangements and dysfunctions of the immune system give rise to reactive airway diseases, and are responsible for the clinical syndromes commonly known as asthma, chronic obstructive pulmonary disease, and allergic rhinitis (1). In addition, asthma is referred to as an ‘atopy’(2). Sly and Holt for instance state ‘*Asthma is essentially a developmental disease, in which the normal growth and development of the respiratory and immune systems are affected by environmental exposures acting on underlying genetic predispositions*’(3).

Commonly encountered atopies include atopic dermatitis, eczema, allergic rhinitis, allergic conjunctivitis and allergic asthma (1, 2). These diseases have the common characteristic of excessive and disproportionate immune system responses to environmental stimuli. On exposure to antigenic stimulus/i, for example pollen, a patient suffering from one of these diseases suffers an excessive local and systemic immune response. In the case of asthma, the immune dysfunctions can result in the death of the patient.

The immune system is composed of a variety of specialized cellular types that operate in a coordinated fashion in an enormously complicated and incompletely understood network, the purpose of which is to respond effectively to various physiological challenges.

To choose one simple example, dendritic cells function as sentinels responsible for detecting microbiological invaders such as bacteria and viruses. On encountering an invader, the dendritic cells respond in such a way as to alert other immune system elements to the presence of the invader. This alarm function is effected both by direct interaction of the activated dendritic cell with other immune system cells and by the release of a broad range of soluble mediators. These soluble mediators are a form of longer range biochemical communication that sends signals to other immune cell types, causing them to respond in ways appropriate to the nature of the invader. This type of cell to cell communication is known as ‘paracrine signaling’.

The normal function of the immune system is to react to the presence of an invader by first producing and releasing pro-inflammatory signaling molecules. These molecules activate other immune system cells causing some cell types to migrate to the site of infection where they work to kill the invader. Soluble immune mediators include proteins and small molecules. Histamine and nitric oxide are examples of small molecules, and the interleukins are examples of one type of proteinaceous mediator. The list of protein immune mediators is large and constantly expanding. Some types include the cytokines, chemokines, growth factors and adipokines. Hereinafter we will refer to these protein signaling molecules as ‘cytokines’ for simplicity.

After the invader is neutralized, the inflammatory response **must** be turned off. This anti-inflammatory immune response limits the degree and duration of inflammation and the damage to tissue that results.

Inflammation is a vital part of the immune response, but it is very much a double edged sword. Excessive inflammation and hyperactive inflammatory response is the primary cause of immune dysfunctions such as asthma. The immune cells of patients suffering from immune system dysfunctions such as asthma release aberrant types and quantities of cytokines and small molecule signals.

Scientific Literature: There is an exceptionally well-developed body of scientific literature discussing the inflammatory nature of the lung disease commonly known as asthma.

Appendices 1 and 2 provide the interested reader with a comprehensive listing of the indexed extant medical literature on these subjects. The term MESH in the titles is an abbreviation for 'MEDical Search'. The collected references represent the entirety of the currently available scientific literature through Pub Med as of February 25, 2011. The authors have selected a set of representative publications for the purposes of this discussion. These articles and discussion should not be considered to represent the entirety of scientific knowledge in these areas, but are chosen to illustrate certain points. However, the chosen articles are consistent with and are supported by the current scientific thinking in the subject area.

The various causes of asthma are as yet relatively poorly understood. Asthma attacks are often provoked by exposure to inflammatory environmental stimuli (*See Appendices 3 and 4*). The knowledge of the pathologies of the physiological biochemistry of the immune systems of patients, however, is extensive and detailed. A number of key cytokines, such as interleukins 1, 6 and 8 have been shown to be dysregulated in asthmatics (3) and treatments designed to alter levels of these mediators with the goal of altering the disease course are evaluated by monitoring cytokine responses (4). The central role of cytokine dynamics in asthma has stimulated much interest in drugs designed to control these responses (5-8).

Thus anti-inflammatory drugs find considerable clinical use in the treatment of asthma (1, 7-11, 15-17). The medical community has for years relied on methylxanthines and steroids for asthma therapy. Many currently used asthma medications have significant anti-inflammatory activity. The reader is referred the attached references and to Appendices 5 through 8 for a comprehensive listing of the extant literature in these areas.

Recently a number of natural products having anti-inflammatory properties have been used to treat asthma. Extracts of *Glycyrrhiza*, *Ganoderma*, *Clerodendrum* (several of which are included in the NuCentury Herbs product Resprin) and many other herbal preparations have been studied in the laboratory and subjected to clinical trials (10-14).

Glycyrrhiza and its extracts have a long history of human use (18-20). There are a number of pharmacologically active constituents present in this plant, the most intensively investigated of which is glycyrrhizin. Glycyrrhizin possesses potent anti-inflammatory properties and it and related compounds contained in *Glycyrrhiza* are

responsible for many of the beneficial health effects associated with consumption of licorice root. *Glycyrrhiza* extracts have an established record of safety in human consumption when used wisely (20). *Glycyrrhiza* is a key ingredient present in the Resprin formulation.

Ganoderma extracts also have an extensive history of human use (21). *Ganoderma*, like *Glycyrrhiza*, contains a variety of active components including the acidic triterpenes known as ganoderic acids, which possess potent anti-inflammatory activity (22). *Ganoderma* has also been shown to be safe for human consumption (23). While this material is not included in Resprin it is discussed here for the purpose of illustrating the breadth and depth of traditional Chinese medicine and its use in the treatment of inflammatory diseases.

Clerodendron, an ingredient included in Resprin, also has been shown to have anti-inflammatory activity in a number of models and has been tested in FDA Phase 1 Clinical trial for the treatment of childhood asthma (14, 24, 25).

Platycodon, another major ingredient in Resprin, has also been shown to have activity in the treatment of asthma (26). Various extracts of *Platycodon* have been shown to have anti-inflammatory effects (27).

Peppermint (*Mentha* Spp.) extracts have also been used for the treatment of asthma as reviewed in (28). Peppermint has also shown activity in experimental studies of allergic asthma (29).

This listing should not be construed to represent the entirety of the extant scientific literature covering the various ingredients of Resprin, it is a sampling only for the purposes of illustration.

Traditional Chinese Medicine (TCM) has been a particularly fertile area in the production of herbal medications having activity in asthma (15, 16). A search of herbal TCM on PubMed (3/7/11) yielded over seven thousand article citations. A recent paper (30) discusses the presence of nearly nine thousand different kinds of reported herbal TCM with several tens of thousands citations for the most commonly used five hundred plant species.

TCM has been investigated for treatment of cancer (31), asthma (32), heart disease (33), and many other diseases. Modern medicine and the pharmaceutical industry have recognized TCM as a rich resource for new therapies and lead compounds (34). The scientific literature on TCM is so large as to defy easy comprehension in this discussion. The reader is referred to *Appendices 9 through 12* for a comprehensive listing of the extant literature in these areas. (All Appendices are being sent electronically.)

Studies: In our experimental work we have tested the Resprin preparation on human tissues *in vitro* (See Presentation—sent in Notebook Binder at Tab C.) These tissue

specimens were prepared from samples taken from the respiratory tracts of patients suffering from asthma and compared to tissues derived from normal patients (MatTek Corporation). A more detailed experimental description can be found in the body of the Presentation (Tab C).

Briefly, the tissues were exposed to a variety of noxious agents intended to irritate the tissues and provoke the release of inflammatory cytokines. The irritated tissues were also exposed to a variety of drugs currently used for the treatment of asthma, as well as a water extract of the Resprin herbal preparation. The quantities of cytokines released were measured by multiplex immunoassay using the Luminex analytical platform. The results clearly demonstrated that Resprin is capable of suppressing inflammatory cytokine released from irritated human tissues, and that it produces a pattern of cytokine responses similar, though not identical, to the commonly used asthma drug fluticasone (as marketed in the drugs Flovent and Advair).

Figure 2 of the attachment (Presentation, Tab C; Fig. 2 sent electronically) presents graphically the direction and significance level of the cytokine modulation patterns induced by the various drugs in tissues exposed to the irritant urethane.

Blue squares in this figure indicate the direction of change relative to control. For instance, in the second and third rows of the figure are compared the baseline levels of a range of markers comparing asthma patient tissues to those of a normal subject denoted 'N vs. AST TBE12 and N vs. AST TBE18'. The color of the entry indicates the direction of the change of the particular cytokine under consideration; red = up-regulation, blue = down-regulation.

For instance, in the column marked 'Resistin' the N vs. AST TBE12 box is dark blue, indicating that the normal tissue showed a lower expression of resistin than did the AST TBE12 tissue, and that the difference was statistically significant. The light blue box shown for the comparison of N vs. AST TBE18 signifies that the normal tissue expression of resistin was lower than that of AST TBE18, but that the difference measured was not statistically significant. This convention is carried through all of the differences presented in this figure. Red boxes indicate higher expression, with bright red representing statistically significant differences, and pink or light red boxes representing differences that were not statistically significant.

In the sets of rows wherein various concentrations of Resprin were applied to urethane exposed tissues it is clear that the marker interleukin 6 (IL-6) is consistently downregulated by the treatment relative to the control. Comparing these results to those observed for the tissues treated with Fluticasone one can clearly see that it too down-regulates IL-6. IL-6 is a key immunological signaling molecule and is directly associated with other inflammatory modulators such as IL-1 β , IL-8 and NF κ -B (4). Control of the production and release of these species is an area of intense research (3, 7, 8).

These observations are consistent with the results presented in Edwards *et al.* where the authors state '**GCs** (*n.b.*, glucocorticoids, including Fluticasone) **suppress IL-1 β and RV**

(n.b. rhinovirus) -induced IL-6 via a unique mechanism involving an nGRE element proximal to the TATA box.'(17).

The results presented in Figure 1 (electronically attached) also illustrate the relative efficacy of Resprin in regulating another key pro-inflammatory signal widely acknowledged to be important in the pathophysiology of asthma. Administration of adenosine triphosphate (ATP) to asthmatic patients resulted in worsening of asthma symptoms while it had no observable effects in normal subjects (20). For instance, the authors state '*Our results indicate that the inhalation of ATP has more potent effects than AMP on perception of dyspnea, induction of cough, and bronchoconstriction.*'

Thus, ATP acts as an agent that aggravates the symptoms of asthma in asthmatics, and Figure 1 shows that both Fluticasone and Resprin decrease ATP release and therefore both can be expected to alleviate ATP induced asthma symptoms and reactions.

We concluded on the basis of these results that Resprin possesses significant anti-inflammatory activity, and that the similarity in the pattern of evoked cytokine responses shows that Resprin holds significant promise for the treatment of asthma and other inflammatory airway diseases.

Conclusion: Asthma and other chronic airway diseases have in common the presence of excessive and aberrant inflammation. The morbidity and mortality associated with these diseases is a direct consequence of inflammatory crisis in asthma sufferers. The weight of scientific evidence supports the use of anti-inflammatory drugs in the treatment of these diseases. Many drugs and many natural products have well documented anti-inflammatory activity and have demonstrated salutary effects in the treatment of asthma. The medical literature and our experimental data show that Resprin and many of its components are of use in the treatment of asthma and inflammation more generally. The data demonstrating the ability of Resprin water extracts to suppress inflammatory cytokine and mediator release from cultured human tissues are evidence of its pharmacological activities. Resprin, by merit of its anti-inflammatory activity, thus is a promising treatment for reactive airway diseases such as asthma. Similarly, both the experiments and the scientific literature above suggest that (or indicate that) the herbal product Resprin helps to promote proper immune function and normal inflammatory response in healthy adults.

Attachments: Figures 1 and 2

NOTE: Hard paper copies of these Reference articles are included in a Notebook of supporting documents sent via FedEx to the National Advertising Division, for March 8, 2011 delivery.

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