FIGURA 1. *Panax ginseng*.
Foto: Bernat Vanaclocha.

PL05 Molecular bases of the immunomodulatory activity of medicinal plant extracts

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Resumen

El análisis histórico de las culturas antiguas muestra una riqueza en el uso de fitomedicamentos como agentes curativos y para favorecer la vitalidad y la salud en general. Algunos de estos productos se utilizan bajo la premisa de que pueden modular el sistema inmunológico de los pacientes. Sin embargo, un buen número de ellos y de sus constituyentes químicos han sido poco investigados desde la perspectiva de los mecanismos de acción moleculares. Entre las plantas a las que se les atribuyen propiedades inmunomoduladoras, dos de las más interesantes y mejor estudiadas son la equinácea (*Echinacea purpurea*) y el ginseng (*Panax ginseng*). En el caso de la equinácea se describen los diversos mecanismos involucrados en su efecto inmunoestimulate y se destaca el papel de las alquilamidas como potentes moduladores y ligandos potenciales de los receptores CB2. Se describe el efecto adaptógeno del ginseng y su relación con la síntesis de óxido nítrico y con los receptores de la inmunidad innata denominados TLRs.

Palabras clave: Inmunomoduladores, *Panax ginseng*, *Echinacea* sp.

Abstract

Historical analysis of ancient cultures is rich in the use of phyto-medicines as curative agents of disease, and modulators of overall health and vitality. Some of these natural products are now used under the presumption that they may modify the immune system. However, a number of botanicals consumed directly or as constituents in various products currently on the market have not been subjected to thorough scientific investigation as far as their molecular mechanisms of action are concerned. Regarding the immune system, *Echinacea purpurea* and *Panax ginseng*, are two of the most interesting and well studied herbal drugs with attributed immunomodulating properties. In the case of *echinacea* the immuno-stimulant effect is brought about several mechanisms, emphasizing the role of alkylamides as potent modulators and potential ligands for CB2 receptors. The adaptogenic effect of ginseng is described and linked to enhanced nitric oxide synthesis and to receptors of innate immunity named Toll-like Receptors (TLRs).

Keywords: Immunomodulators, *Panax ginseng*, *Echinacea* sp.

Introduction

The use of herbals or botanicals in all parts of the world has been gaining wide popularity. In its recent survey report [1], the National Center for Complementary and Alternative Medicine of the United States (NCCAM) stated that about 19% of adult Americans are using some form of natural products. In a 1998 study published in the Journal of the American Medical Association, 42% of Americans were using alternative medicine, representing an 8% increase from 34% in 1990. Currently, the use of complementary or alternative medicine (CAM), inclusive of vitamins, dietary supplements, herbal therapies and alternative medicine professional services, represents an industry ranking second in expenses being paid out-of-pocket, with a 45.2% increase in expenditures between 1990 and 1997 estimated at US $21.2 billion in 1997 [2]. Dietary supplements are used by more than one-half of the adult population in the United States [3]. Around the globe, individuals in a wide array of age groups, lifestyles and states of health or infirmity are using CAM with expectations of beneficial health improvements, boosted immunity, enhanced sports performance and overall perception of well-being. Far from being "new age," these expectations may well be rooted in a long history of "natural therapies" passed down from person to person as well as in documented use over hundreds of years. Indeed, historical analysis of ancient cultures is rich in the use of phytomedicines as curative agents of disease, and modulators of overall health and vitality.

A number of botanicals consumed directly or as constituents in various products currently on the market have not been subjected to through scientific investigation as far as their molecular mechanisms of action are concerned. The "proof" of their beneficial effects mostly comes from claims and anecdotal reports originating from their traditional uses. With the advances in our knowledge of the biochemical and molecular basis of inter-individual differences in drug/xenobiotic transport and metabolizing ability, it is becoming increasingly important that systematic studies on the molecular basis of action of these herbals/botanicals are undertaken. Such studies might yield information that was not realized from traditional use, yet these effects may have far-reaching consequences. Regarding the immune system, there is a continuing interest in the development of natural compounds which act on immunological function. Two of the most interesting and well studied herbal drugs with attributed immunomodulating properties are the products developed from *Echinacea* sp. and *Panax sp.*

Echinacea sp. (echinacea)

First used by Native Americans, the echinacea or "purple coneflower" (*Echinacea purpurea* and *E. angustifolia*) has become one of the most popular phytomedicines and herbal supplements in North America and Europe. More recent studies reported that the herb exerts many immunological functions both, *in vivo* and *in vitro*. It has the ability to activate human phagocytic function [4] and protects mice against systemic infections [5]. There is also evidence that some phytochemicals have the capacity to annihilate tumours and reduce viral infections [6-8]. Several clinical trials have been carried out with echinacea preparations and it appears that certain preparations shorten the duration and severity of colds and other upper respiratory tract infections, when given as soon as the symptoms become evident. Despite these benefits, the therapeutic potential of echinacea is controversial [9, 10] and many published clinical trials have produced negative results [11, 12]. Previous *in vitro* investigations with different echinacea extracts have reported stimulatory effects on macrophages (M/s), activation of natural killer cells (NK-cells) as well as non-specific induction of pro-inflammatory cytokines in monocytes and M/s [9]. Since the role of NK-cells is essential in clearing several types of viral infections and the findings that echinacea may play a role in protection against viral infections, it is plausible that echinacea activates NK-cells and NK-cells participate in the anti-viral effect. Studies with echinacea have been reported on its effect on NK-cell function both *in vitro* and *in vivo*. See *et al*. [8] have demonstrated that *E. purpurea* enhanced NK cytotoxic activity *in vitro* by human peripheral blood lymphocytes in both, normal individuals and in patients with chronic fatigue syndrome or acquired immune deficiency syndrome. Currier and Miller [13] studied aged mice fed with diet with *E. purpurea* and demonstrated that there was a significant activation of NK-cell cytotoxic activity and an increase in NK-cell numbers.

The mechanisms by which echinacea regulates NK cytotoxic function were recently studied in order to understand its biological effects [14]. This study examined *in vitro* the effects of soluble extracts of *E. purpurea* on natural killer (NK) cells present in human peripheral blood mononuclear cells (PBMC). Treatment of PBMC with echinacea overnight resulted in the activation of CD69 expression and in the CD16+ and CD16 + CD56+ NK subsets. However, the frequency of CD16+ cells was decreased as well as the mean fluorescence intensity was down regulated. NK cytotoxicity was augmented 100% at the concentration of 0.1 Ag/ml of echinacea in a short time (4-h) assay. Examination at the single cell level revealed augmentation of the frequency of CD56+

NK–target conjugates and a plateau was reached after 30–60 min of incubation. Likewise, the frequency of CD56+ killer cells in the conjugates was also significantly increased by echinacea. There was recruitment of non-conjugated CD56+ cells into CD16+ NK–target conjugates and activation of the NK–target non-killer conjugates into killer cells.

These findings demonstrate that echinacea extracts are potent activators of NK cytotoxicity; augment the frequency of NK target conjugates and activate the programming for lysis of NK cells.

In other study, a standardized tincture of echinacea induced *de novo* synthesis of a tumour necrosis factor (TNF-α mRNA in primary human monocytes/macrophages, but not the TNF-α protein [15]. Moreover, LPS-stimulated TNFα protein was potently inhibited in the early phase but prolonged in the late phase. The study of the main constituents of the extract showed that the alkylamides dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides, trienoic and dienoic acid derivatives are responsible for this effect. The up-regulation of TNF-α mRNA was found to be mediated by CB2 receptors, increased cAMP, p38/MAPK and JNK signalling, as well as NF-jB and ATF-2/CREB-1 activation. This study is the first report of a possible molecular mechanism of action of Echinacea, highlighting the role of alkylamides as potent immunomodulators and potential ligands for CB2 receptors. It is interesting to note that although echinacea alkylamides induce TNF-α mRNA, which is not translated, they inhibit

LPS-stimulated TNF-α protein expression too. This dual modulation on the non-specific immune response may also explain previous reports on the anti-inflammatory action of echinacea preparations. Since TNF-α is a strong endogenous signal with multiple auto-regulatory mechanisms in different cell types, and a broad spectrum of physiological roles, the finding that alkylamides modulate this factor via CB2 receptors might open up new avenues in e*chinacea* research.

Panax sp. (ginseng)

Panax ginseng (ginseng) has been reported to have immunomodulatory properties, stimulates the immune system eliciting a specific immune response. Various studies *in vitro* and *in vivo* have shown that ginseng extract modulates different parameters of the immune response. These findings have been confirmed in clinical studies [16, 17]. The results showed that ginseng is able to improve the immune response *in vivo* in humans and can protect against influenza and common flu. Moreover ginseng is able to reduce the bacterial count in the bronchial system of patients undergoing an acute attack of chronic bronchitis. A systematic evaluation of immune system components revealed stimulation of basal natural killer (NK) cell activity following sub chronic exposure to ginseng, which helped stimulate recovery of NK function in cyclophosphamide-immunosuppressed mice [18]. A recent study demonstrated an enhanced *in vitro* interleukin-12 (IL-12) production in Ginseng modulated human peripheral blood mononuclear cell (PBMC) which could induce a stronger Th1 response, resulting in better protection against infection with a variety of pathogens [19].

Recently it has been suggested that the immunomodulating activities of ginseng may be linked to enhanced nitric oxide (NO) synthesis in peritoneal murine macrophages, producing reactive nitrogen intermediates [20]. Ginseng acts on stimulation of inducible nitric oxide synthetase (iNOS) expression *via* necrosis-factor (NF-kB), providing a valuable contribution to understand the molecular basis of action of *P. ginseng* [21].

Furthermore, some reports have shown that ginseng and its components improve the lesions evoked by stress [22, 23]. *P. ginseng*, the first clinically used adaptogen, has been extensively investigated experimentally and clinically for its stress attenuating activity. Ginseng saponins have been proposed as possible candidates in the therapeutic modulation of stress-induced disorders for their inhibitory effect on the level of stress-induced plasma interleukin-6 (IL-6) in mice. The immunomodulatory effects of ginseng in physical stress have recently been shown to be related to immunity and to receptors of innate immunity named Toll-like Receptors (TLRs). This work [24] facilitates the understanding of the mechanism of action of ginseng during physical stress and how the extract modulates the innate immune cell response. Ginseng, administered orally, modulated the expression of TLR4 mRNA, as well as the release of lipopolysaccharide (LPS)-stimulated IL-1β and TNF-α, both in control and in stressed mice. Moreover ginseng increased the expression of TLR4 and cytokines release with a different pattern to the exercise alone group. Ginseng was able to gradually modulate the immune response to stressors, making the response more specific and thus increasing the magnitude of the response against bacterial or pathogenic challenge. The activity of ginsenosides as functional ligands of glucocorticoid receptors may be involved in this process. Ginsenosides, or other components of ginseng extract, could act as partial agonists of TLR4, protecting the host from the massive release of inflammatory cytokines activated by the endogenous ligands.

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