A METHOD OF ENHANCING THE BIOAVAILABILITY OF A 2,4-DIENE ALKYLAMIDE FRACTION OF AN ECHINACEA FORMULATION

FIELD OF THE INVENTION

The present invention relates to a method of enhancing the bioavailability of a 2,4-diene alkylamide fraction of an Echinacea formulation for oral administration.

BACKGROUND OF THE INVENTION

Echinacea is a herb well known for its immune stimulatory activity. Echinacea formulations are widely available to the public and are commonly taken for the prevention and treatment of colds and flu.

Traditionally, herbal medicines were available in liquid form as tinctures, teas and the like. However, liquids are not favored by the public for reasons such as taste and convenience. Thus, herbal preparations are generally commercially available in tablet or capsule form. Many tablets are comprised simply of ground dried herb. A disadvantage of ground herbal products is that they contain large amounts of inert plant matter as compared to the active components. Thus relatively large numbers of relatively large tablets need to be consumed for a patient to obtain a therapeutic dose of active ingredients. This may meet with consumer resistance and lead to non-compliance. As such, formulations are generally classified simply by weight of the source herb, the actual content of purported active is not only unknown but can vary significantly from batch to batch.

Other preparations are available in the form of concentrated extracts which are obtained by hydroalcoholic extraction of the herb. An advantage of the concentrated extract is that the soluble constituents of the herb, including the actives, are separated from the inert plant material, thereby decreasing the total volume of material which must be ingested to obtain a therapeutic dose. Concentrated extracts are often standardized to a concentration of a marker compound. Herbal extracts are ideally standardized with respect to the level of an active ingredient such that the effects of batch to batch variations may at least partially be negated, whilst providing an indication as to potency of a particular dosage unit. Difficulties arise however, where the identity of the active compounds are unknown or uncertain. Many manufacturers of Echinacea formulations produce extracts standardized to echinacoside or

2-ene alkylamide and a synthetic 2,4-diene alkylamide. The synthetic alkylamides correspond to the major 2-ene and 2,4-diene alkylamides found in Echinacea. These alkylamides are illustrated in Figure 1 as compounds 6 and 3 respectively.

The results showed that all components tested except the 2-ene alkylamide significantly decreased lipopolysaccharide (LPS) stimulated NF-κB levels. Only cichoric, the Echinacea extract and the alkylamide mixture significantly decreased TNF-α production under LPS stimulated conditions in macrophages. Only the alkylamide mixture decreased LPS stimulated NO production. The mixture of alkylamides in the Echinacea ethanolic liquid extract did not respond in the same manner in the assays as the individual alkylamides investigated.

These results demonstrate that the alkylamides, cichoric acid and Echinacea are an effective modulator of macrophage immune responses *in vitro*. However, it is believed that it is unlikely that cichoric acid would have any observable effect *in vivo* in view of it's low permeability across Caco-2 monolayers.

The present invention relates to the surprising and unexpected discovery by the inventors that the 2-ene alkylamides are metabolized by the liver at much lower rates that the 2,4 diene alkylamides and still further that the 2-ene alkylamides can actually inhibit 2,4-diene liver metabolism.

DESCRIPTION OF THE INVENTION

According to ~~a first broad form of~~ the invention there is provided ~~an Echinacea formulation which includes an alkylamide fraction comprising a 2-~~ ene alkylamide fraction and a 2,4-diene alkylamide fraction and the weight ratio of the 2-ene alkylamide fraction to the 2,4-diene alkylamide fraction is between 1:10 to 1:2, wherein the 2-ene alkylamide fraction is at least partially sourced from *E. angustifolia* root and the 2,4-diene fraction is at least partially ~~sourced from *E. purpurea* root.~~

The ratio of the 2-ene alkylamide fraction to the 2,4-diene alkylamide fraction is preferably between ~~about 1:9 to about 1:1. Especially preferred is a ratio between about~~ 1:8 to ~~about~~ 1:5.

The formulation may be in any form suitable for oral administration. Such forms are well known in the art and include liquids, tablets, capsules, powders and the like. Typically, the formulation is in the form of a solid unit dose.

The formulation includes an extract of *E. angustifolia* root as a source of the 2-ene alkylamide fraction. Typically, the formulation includes a dried hydroalcoholic extract of *E. angustifolia* root, although ground herb could also be used. The extract may be obtained by any suitable method which will at least partially extract the 2-ene alkylamides contained therein. The *E. angustifolia* extract may be in the form of an alkylamide enriched extract.

E. angustifolia root contains a number of 2-ene alkylamides, of which (2E)-N-isobutylundeca-2-ene-8, 10-diynamide is generally the most common. Thus a typical formulation ~~of the invention~~ may include a 2-ene alkylamide fraction containing (2E)-N-isobutylundeca-2-ene-8, 10-diynamide and at least one or more further 2-ene alkylamides. It is preferred that (2E)-N-isobutylundeca-2-ene-8,10-diynamide is the major 2-ene alkylamide in the 2-ene alkylamide fraction of the formulation ~~of the invention~~. Typically, the 2-ene alkylamide fraction includes at least 30wt% (2E)-N-isobutylundeca-2-ene-8,10-diynamide. It will be appreciated that (2E)-N-isobutylundeca-2-ene-8,10-diynamide may also be the sole or essentially the sole 2-ene alkylamide in the 2-ene alkylamide fraction.

Other 2-ene alkylamides which may be present in an *E. angustifolia* extract include, but are not limited to, (2E)-N-(2-methylbutyl)dodeca-2-ene-8,10-diynamide, (2E,7Z) –N-isobutyltetradeca-2,7-diene-10,12-diyamide, (2E,9Z)-N-isobutylpentadeca-2,9-12,14-diynamide and (2E,9Z)-N-(2-methylbutyl)pentadeca-2,9-diene-12,14-diynamide.

The 2,4-diene alkylamides may be found in the aerial parts and roots of both *E. purpurea* and *E. angustifolia.* Preferably both plants are used as a source of the 2,4-diene alkylamides. Although the 2,4-diene alkylamides are found in root and aerial parts of these species, it is preferred that the alkylamides are sourced from the root of each plant. These plants typically contain a number of 2,4-dienes, of which (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide is generally the most common. Thus a typical

formulation ~~of the invention~~ may include a 2,4-diene alkylamide fraction containing (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide and at least one or more further 2,4-diene alkylamide. It is preferred that (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide is the major 2,4-diene alkylamide in the 2,4-diene alkylamide fraction of the formulation ~~of the invention.~~ Typically, the 2,4-diene alkylamide fraction includes at least 30wt% (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide.

Other 2,4-diene alkylamides which may be present in the 2,4-diene alkylamide fraction include, but are not limited to, are (2E,4Z)-N-isobutylundeca-2,4-diene-8,10-diynamide, (2E,4Z)-N-isobutyldodeca-2,4-diene-8,10-diynamide, (2E,4Z,8Z)-N-isobutyldodeca-2,4,10-triene-8-ynamide, (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide, (2E,4E,8Z)-N-isobutyldodea-2,4,8-trienamide, (2E,4E)-N-isobutyldodeca-2,4-dienamide and (2E,4Z)-N-(2-methylbutyl)dodeca-2,4-diene-8,10-diynamide.

Typically, a formulation ~~of the present invention~~ will include both (2E)-N-isobutylundeca-2-ene-8, 10-diynamide and (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide. Typically, these alkylamides are in a weight ratio of between ~~about 1:10 to about 1:1, preferably between about~~ 1:6 to ~~about~~ 1:2, most preferably between about 1:4.5 to about 1:2.5.

The relative amounts of 2-ene and 2,4-diene alkylamides in *E. angustifolia* root may vary depending upon the age of the plant, flower developmental stages and growing, harvesting and storage conditions. Further, extraction parameters such as solvent, temperature and length of extraction period will also affect the total and relative alkylamide levels in any extract. Accordingly, the amounts of *E. angustifolia* extract required to be included with a formulation containing a mixture of *E. angustifolia* and *E. purpurea* may vary on a batch to batch basis. Thus in order to manufacture the formulation herein described it is required ~~of the present invention it is desirable~~ to measure the relative amounts of the 2-ene alkylamides and 2,4-diene alkylamides in the respective Echinacea extracts prior to admixture such that the relative amounts of *E. angustifolia* and *E. purpurea* may be adjusted to provide the desired ratio of alkylamides. The respective extracts may be analyzed for either the total 2-ene or 2,4-diene alkylamide content or alternatively the content of the

generally most common alkylamides (2E)-N-isobutylundeca-2-ene-8,10-diynamide and (2E,4E,8Z,10Z)-N isobutyldodeca-2,4,8,10-tetraenamide.

Alternatively the *E. angustifolia* extract and/or combined formulation is standardized to 2-ene alkylamide and/or (2E)-N-isobutylundeca-2-ene-8,10-diynamide content.

According to a further form of the invention, there is provided an Echinacea formulation which includes an extract of *E. angustifolia* comprising a 2,4- diene alkylamide fraction and a standardized amount of a 2-ene alkylamide fraction, wherein the weight ratio of the 2-ene alkylamide fraction to the 2,4-diene alkylamide fraction is from about 1:15 to about 2:1, the formulation comprising E. *angustifolia* root or an extract thereof and *E. purpurea* or an extract thereof.

The formulation may alternatively be standardized with respect to a specific 2-ene alkylamide. In the latter case, it is preferred that the formulation is standardized to (2E)-N-isobutylundeca-2-ene-8,10-diynamide. Typical standardized amounts of the whole 2-ene alkylamide fraction range from about 0.002 to about 2w/w%, typically between about 0.02 to about 1w/w%, preferably between about 0.04 to about 0.1w/w%. Typical standardized amounts of (2E)-N-isobutylundeca-2-ene-8,10-diynamide range from about 0.001 to about 1w/w%, typically between about 0.01 to about 0.5w/w%, preferably between about 0.02 to about 0.05w/w%.

The total level of alkylamides in the formulations ~~of the invention~~ is typically between about 0.01 to 10w/w%, preferably between about 0.2 to about 3w/w%.

It will be appreciated that the formulation may also include other herbs, or parts or extracts thereof and pharmaceutically acceptable, recipients, diluents, carriers and/or adjuvant.

The formulation may also include means for enhancing the solubility of the alkylamides. Such means are known in the art and include microencapsulation.

DESCRIPTION OF THE FIGURES

Figure 1 shows structures of isobutylamides and methylbutylamides.

(1) (2E,4Z)-N-isobutyldodeca-2,4-diene-8,10-diynamide mw = 243;

(2) (2E,4Z,10Z)-N-isobutyldodeca-2,4,10-triene-8-ynamide mw = 245;

(3) (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide mw = 247;

(4) (2E,4E,8Z)-N-isobutyldodeca-2,4,8-trienamide mw = 249;

(5) (2E,4Z)-N-(2-methylbutyl)dodeca-2,4-diene-8,10-diynamide mw = 257;

(6) (2E)-N-isobutylundeca-2-ene-8,10-diynamide mw = 231;

(7) (2E)-N-(2-methylbutyl)dodeca-2-ene-8,10-diynamide mw = 259;

(8) (2E,9Z)-N-isobutylpentadeca-2,9-diene-12,14-diynamide mw = 285

(9) (2E,4E)-N-(sec-butyl)hexa-2,4-dienamide mw = 167 ;

(10) (2E)-N-(sec-butyl)hex-2-enamide mw = 169 ;

(11) ~~(2E)-N-(sec-butyl-hex-2-enamide mw = 247~~; (2E)-N-isobutyldodeca-2-ene-8,10-diynamide mw = 245

(12) (2E-4E)-N-isobutyldodeca-2,4-dienamide mw = 251;

Figure 2 shows representative structures of possible isobutylamide metabolites for (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide mw = 247 (3). (a) Parent compound; (b) an example of the epoxidation of a double bond; (c) an example of hydroxylation at a saturated C; (d) dealkylated product resulting from hydroxylation at the methylene next to the amide N; (e) an example of a diol formed from further transformation of the initial epoxide metabolite as seen in (b);

Figure 3 shows the time-dependent metabolism of synthetic

or total alkylamides in the fasted versus the fed subjects.

Discussion

The present study investigated the disposition and pharmacokinetics of phytochemicals from a dry ethanolic preparation of Echinacea ingested in tablet form. Alkylamides were detected in plasma at the first sampling time (20 minutes after dosing) from both fed and fasted subjects and remained at measurable concentrations for the 12 hours of the study.

Bioavailability is essentially defined as the fraction of a given dose of compound that reaches the systemic circulation as intact compound. As is the case for prescription pharmaceuticals, it is necessary to know the amounts of the potentially active constituents (in this case the various alkylamides) present in the Echinacea products ingested. Many previous clinical trials investigating the efficacy of Echinacea preparations have not disclosed these phytochemical profiles making direct comparison of one study to another impossible. The phytochemical profile of any herb is dependent on many factors including growing conditions and post-harvest treatment Differences in either or both of these can contribute to marked differences in the phytochemical profiles of different batches from the same manufacturer, let alone from different sources. The alkylamide content of the tablets given in Table 2 are therefore only relevant for the particular batch of Echinacea used in this study.

It may therefore be appreciated that for therapeutic use it is important to provide an Echinacea formulation having a level of 2-ene sufficient to inhibit metabolism of the 2,4-enes present ~~/and/or to standardize formulations according to 2-ene content/~~.

The fast appearance of alkylamides in plasma (20 minutes) is in agreement with the ease of uptake seen with their rapid permeation across Caco-2 monolayers [1]. It is also in agreement with the tablet disintegration time of 13 minutes. The presence of food appears to make no difference to the speed of alkylamide uptake into the plasma as plasma concentrations in the fasted state were within the range found for subjects who ingested

Echinacea after a standard high fat breakfast. The alkylamides exhibit classic single dose pharmacokinetic profiles, both individually and as a summed total group. Maximum plasma concentrations for the individual alkylamides differ as expected based on the varying amounts of each present in the starting material.

Thus it may be seen that protecting the akylamide fraction from liver metabolism may enhance the bioavailability of solid Echinacea formulations in particular.

It may therefore be appreciated that for therapeutic use it is important to provide an Echinacea formulation having a level of 2-ene sufficient to inhibit metabolism of the 2,4-enes present ~~and/or to standardize formulations according to 2-ene content~~.

In the specification the term "comprising" shall be understood to have a broad meaning similar to the term "including" and will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. This definition also applies to variations on the term "comprising" such as "comprise" and "comprises".

CLAIMS

1. A method of enhancing the bioavailability of a 2,4-diene alkylamide fraction of an Echinacea formulation which includes an alkylamide fraction comprising a 2-ene alkylamide fraction which is at least partially sourced from *E. angustifolia* root, and a 2,4-diene alkylamide fraction which is at least partially sourced from *E. purpurea* root, the method comprising:

(i) determining the relative amounts of 2-ene alkylamides and 2,4-diene alkylamides in an extract of *Echinacea angustifolia*;
(ii) determining the relative amounts of 2-ene alkylamides and 2,4-diene alkylamides in an extract of *Echinacea purpurea*; and
(iii) combining the extract of *Echinacea angustifolia* and the extract of *Echinacea purpurea* to provide said formulation in which the weight ratio of the 2-ene alkylamide fraction to the 2,4-diene alkylamide fraction is between 1:10 to 1:2.

2. The method of claim 1, wherein the weight ratio of the 2-ene alkylamide to the 2,4-diene alkylamide fraction of said Echinacea formulation is between 1:8 to 1:5.

3. The method according to claim 1 or 2, wherein the 2-ene alkylamide fraction comprises (2E)-N-isobutylundeca-2-ene-8,10-diynamide.

4. The method according to any one of claims 1 to 3, wherein the 2-ene alkylamide fraction comprises at least 30 wt % (2E)-N-isobutylundeca-2-ene-8,10-diynamide.

5. The method according to any one of claims 1 to 4, wherein the 2,4-diene alkylamide fraction comprises (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide.

6. The method according to any one of claims 1 to 5, wherein the 2,4-diene alkylamide fraction comprises at least 30 wt% (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide.

7. The method according to any one of claims 1 to 6, wherein the Echinacea formulation produced comprises (2E)-N-isobutylundeca-2-ene-8,10-diynamide and (2E,4E,8Z,10Z)-N-isobutyldodeca-2,4,8,10-tetraenamide in a weight ratio of between ~~1:10 to 1:1.9.~~

~~8. The method of claim 7 wherein the weight ratio is between~~ 1:6 to 1:2.

~~9~~8. The method according to any one of claims 1 to ~~8~~7, wherein said Echinacea formulation is a solid unit dosage form.

~~10~~9. The method according to any one of claims 1 to ~~8~~7, wherein said Echinacea formulation is in a liquid form.

~~11. The method according to claim 1 substantially as hereinbefore described with reference to any one of the Examples.~~