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Tobian AAR, Canaday DH, Harding CV. (2004b) Bacterial heat shock proteins enhance class II MHC antigen processing and presentation of chaperoned peptides to CD4+ T cells. The Journal of Immunology, 173: 5130 - 5137.


References


Stress Protein Study Case Record Form (Control)

Initials .....................
Date of Enrolment .....................
Time of blood collection .....................

Factors influencing hsp expression

Sex
DOB
Smoking ..................... per day
Alcohol ..................... per week
Supplements
  Antioxidants yes/no (details) .....................
  Vitamins yes/no (details) .....................
  Others .....................
Stress Protein Study Case Record Form

Place ASC sticker here

HSP Study Number ......................
Treatment On/Off (circle)
Date of Enrolment ......................
Time of blood collection .................
VL ... Date ........ CD4 ... Date ........

Factors influencing hsp expression

Sex MALES ONLY
Age ......................
Smoking .............. per day
Alcohol .............. per week
Supplements Antioxidants yes/no (details) ......................
Vitamins yes/no (details) ......................
Others ......................
Duration of HIV ......................
Duration VL < 50 ......................
Information Sheet

Biochemical changes, particularly in the synthesis of stress proteins, associated with intake of standard amounts of Echinacea

Human Research Ethics Committee approval number:

Proposed Schedule:

Friday May 9th 2003, first blood sample & commence Echinacea
Friday May 23rd 2003, second blood sample & end of trial
(blood samples will be taken by trained personnel at Pathology, Armidale & New England Hospital, between 8 – 10 am on the designated days, transport can be arranged to & from the University/other)

Echinacea, also known as Rudbeckia, purple coneflower & red sunflower, is a member of the daisy (Compositae) family. It has been used for centuries as an herbal medicine as a treatment for colds, coughs and respiratory infections. However, its actual mode of action is unclear despite numerous relatively recent clinical trials, many of which have been conducted in Germany. More information is needed before a definitive statement can be made regarding the mode of action and efficacy as an herbal medicine.

It is widely accepted that stimulation of the human immune system is responsible for the perceived benefits of Echinacea as an herbal medicine. The small scale, pilot study, proposed in the present study is to examine the effect of a well defined and widely used Echinacea preparation upon the synthesis of heat shock or stress proteins and other serum parameters. The stress proteins are a highly evolutionary conserved set of proteins associated with the human immune system. The present proposal will thus be a novel approach towards our understanding of how Echinacea works in healthy individuals to support the immune system.

Participants must be 18 years of age or older. Participants currently taking other medications or who have never previously taken Echinacea will be excluded. There are no known side effects due to Echinacea root extracts, however, contact dermatitis may occur rarely in susceptible individuals. In this respect, individuals who are allergic to the daisy (Compositae) family should not participate in this study. No cases of overdose in humans have been reported.

Participants are free to withdraw at anytime from the experimental protocol.

The funding source for the research will be provided by Mediherb Australia, a provider of herbal medicines based in Warwick, QLD. Mediherb will also provide the Echinacea tablets, containing:
Echinacea purpurea dry root 675 mg, containing alkylamides 2.65 mg
Echinacea augustifolia dry root 600 mg, containing alkylamides 2.50 mg
Excipients for the tablets are:
- cellulose, calcium hydrogen phosphate, silica, sodium starch glycollate,
- hypromellose & magnesium stearate.

The Echinacea tablets are the well-defined preparations from Mediherb and widely used by the public as recommended by complementary therapy practitioners. The product is listed on the Australian Register of Therapeutic Goods AUST L75124.

Participants will be required to take two Echinacea tablets per day for 2 weeks.

In the present studies, participants will be required to provide a blood sample (approx. 20 ml) taken prior to commencement of the trial and another blood sample two weeks after intake of Echinacea. Trained personnel at the Pathology Laboratory, Armidale & New England Hospital, will take blood samples. Transport can be provided for participants. Total blood counts and liver function tests will be conducted and participants will be informed of any abnormal results by Armidale Pathology, contact:

Mr Neil Horton, Operations Manager, Pathology New England, Armidale & New England Hospital, Rusden Street, Armidale ph (02) 67 764840

Blood samples will be centrifuged to separate out the lymphocytes and stress protein synthesis measured by standard methods at the University of New England, contact:

Professor Ken Watson, Human Biology, School of Biological, Biomedical & Molecular Sciences, Armidale ph (02) 67 733125, fax (02) 67 733267, E-mail kwatson2@pobox.une.edu.au

All samples (blood, lymphocytes, plasma) will be destroyed after testing is complete.

Participants should be aware that the information obtained from these studies may be presented at scientific meetings and submitted for publication in peer-reviewed journals (participants may request a copy of any relevant publication arising from the research). The information will be retained for future reference (up to 5 years) given the requirement for Universities & other institutions to retain experimental data relating to publications for a reasonable period of time. Moreover, the data may be subject to reinterpretation as the scientific knowledge base changes. However, the data will be shredded after 5 years.

Should you have any concerns regarding the manner in which the research is conducted, please contact the Human Research Ethics Committee at the following address:

The Secretary, Human Research Ethics Committee
University of New England
Armidale NSW 2351
Ph: (02) 67 733449
Fax: (02) 67 733543
E-mail: fprater@pobox.une.edu.au
Information Sheet

Biochemical changes, particularly in the synthesis of stress proteins, associated with intake of standard amounts of Echinacea

Human Research Ethics Committee approval number:
Commencement date: April 16th 2003 valid to: August 1st 2003

Echinacea, also known as Rudbeckia, purple coneflower & red sunflower, is a member of the daisy (Compositae) family. It has been used for centuries as an herbal medicine as a treatment for colds, coughs and respiratory infections. However, its actual mode of action is unclear despite numerous relatively recent clinical trials, many of which have been conducted in Germany. More information is needed before a definitive statement can be made regarding the mode of action and efficacy as an herbal medicine.

It is widely accepted that stimulation of the human immune system is responsible for the perceived benefits of Echinacea as an herbal medicine. The small scale, pilot study, proposed in the present study is to examine the effect of a well defined and widely used Echinacea preparation upon the synthesis of heat shock or stress proteins and other serum parameters. The stress proteins are a highly evolutionary conserved set of proteins associated with the human immune system. The present proposal will thus be a novel approach towards our understanding of how Echinacea works in healthy individuals to support the immune system.

Participants must be 18 years of age or older. Participants currently taking other medications will be excluded. There are no known side effects due to Echinacea root extracts, however, contact dermatitis may occur rarely in susceptible individuals. In this respect, individuals who are allergic to the daisy (Compositae) family should not participate in this study. No cases of overdose in humans have been reported.

Participants are free to withdraw at anytime from the experimental protocol.

The funding source for the research will be provided by Mediherb Australia, a provider of herbal medicines based in Warwick, QLD. Mediherb will also provide the Echinacea tablets, containing:
Project Title: Modulation of Stress Proteins by Hormones and Cytokines

Human Research Ethics Committee approval number: 04/026

Proposed dates: Tuesday 5 October – Tuesday 30 November

Dear Participant,

Thank you for taking the time to participate in this study to determine if stress proteins are influenced by hormones and cytokines. Stress proteins are highly conserved proteins associated with the human immune system. Research has demonstrated that males and females have different levels of stress protein expression. Animal studies have suggested that these differences may be due, in part, to the actions of hormones and cytokines. It is unclear, however, whether this is the case in humans, and if so, what the mechanisms of interaction are.

Participants must be women between 18 and 50 years of age who are healthy and cycling regularly. The funding source for the research will be University research grants and academic pursuits funds held by Professor Ken Watson and Dr Jim McFarlane, School of Biological, Biomedical and Molecular Sciences, University of New England.

Participants are free to withdraw from the experimental protocol at any time.

In the present studies, participants will be required to provide a fasting blood sample (approximately 10 ml) on between four and six occasions during one monthly cycle. Blood will be taken by certified personnel at the Pathology Laboratory, Armidale & New England Hospital, Armidale. Transport can be provided for participants.

A portion of the blood sample will be used for whole blood flow cytometric analysis of heat shock protein 70 (hsp70) synthesis in leukocytes. The remaining blood will be centrifuged to separate out the plasma which will also be examined for hsp70 levels at the University of New England, contact:

Professor Ken Watson, Human Biology, School of Biological, Biomedical and Molecular Sciences, Armidale ph (02) 67733125, email kwatson2@pobox.une.edu.au

Plasma will also be examined for a number of other parameters including: hormone and cytokine levels, contact:

Dr Jim McFarlane, Human Biology, School of Biological, Biomedical and Molecular Sciences, Armidale ph (02) 67733201, email jmcfarla@pobox.une.edu.au

All samples (whole blood, leukocytes and plasma) will be destroyed after testing is complete.
Participants should be aware that the information obtained from these studies may be presented at scientific meetings and submitted for publication in peer-reviewed journals (participants may request a copy of any relevant publication arising from the research). The information will be retained for future reference (up to 5 years) given the requirements for Universities and other institutions to retain experimental data relating to publications for a reasonable period of time. Moreover, the data may be subject to reinterpretation as the scientific knowledge base changes. However, the data will be shredded after the 5 year period expires.

Should we find any results that are outside the normal ranges expected you will be notified and advised to discuss the matter with your medical practitioner.

This project has been approved by the Human Research Ethics Committee of the University of New England (Approval No. 04/026).

Should you have any complaints concerning the manner in which this research is conducted, please contact the Research Ethics Officer at the following address:

Research Services
University of New England
Armidale, NSW 2351.
Telephone: (02) 6773 3449 Facsimile (02) 6773 3543
Email: Ethics@metz.une.edu.au
Consent Form

Project Title: Modulation of Stress Proteins by Hormones and Cytokines

Human Research Ethics Committee approval number: 04/026

This project is summarized in the accompanying information document. The extent of individual involvement is the donation of approximately 10 ml of blood, a procedure that takes less than 5 minutes. A blood sample will be collected on a number of occasions (between four and six) over a period of approximately one month.

Results will be coded to remove names and identifiers and only the chief investigator will have access to names. Records will be kept in a locked cabinet.

I have read the information above and any questions have been answered to my satisfaction. I agree to participate in the study and the research data collected for the study may be published, provided my name is not used. I am aware that I may withdraw from the study at any time.

I will be notified if anything abnormal is found in the blood tests I agree that data can be forwarded to my GP:

Name: ........................................................................
Address: .................................................................Phone: ............

To the best of my knowledge I have no medical condition which would prevent me from participating in this research.

Participant...............................................................Date.............

Investigator...............................................................Date.............
Questionnaire

Project Title: Modulation of Stress Proteins by Hormones and Cytokines

Human Research Ethics Committee approval number: 04/026

Name:__________________________________________________________

Contact details:__________________________________________________________________________

Date of birth:__________________________________________

Are you currently taking oral contraceptives? (circle one) YES NO

If so, please provide details:____________________________________________________________________

Do you regularly take any other medications? (circle one) YES NO

Details:____________________________________________________________________________________

Are you taking any vitamins or herbal supplements? (circle one) YES NO

Details:____________________________________________________________________________________

What was the date of the first day of your last period...
Please mark on the calendar below when you are menstruating, any medications that you take and any days that you are unwell.

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ALTERED EXPRESSION OF HEAT SHOCK PROTEINS (HSP70, HSP90, HSP110) IN LYMPHOCYTES FROM HIV-POSITIVE INDIVIDUALS

Agnew L L¹, Kelly M², Ffrench R A³, Gold J², Watson K¹

1. School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale, NSW 2351, Australia. 2. Albion Street Centre, Sydney, NSW 2010, Australia. 3. Department of Immunology & Infectious Diseases, Sydney Children’s Hospital, Sydney, NSW 2031, and School of Women’s and Children’s Health, University of New South Wales, Sydney, NSW 2031, Australia.

Heat shock proteins (hsp) are a group of highly conserved proteins that represent between 2% and 15% of total cellular protein and are expressed by every living organism. The main functions of hsp’s are to regulate apoptosis and to act as intracellular molecular chaperones that facilitate protein folding and assembly. Some hsp’s are highly immunogenic and elicit humoral, cytotoxic T-lymphocyte (CTL) and natural killer (NK) cell responses against viruses, tumors and infectious diseases.

Twenty male patients with HIV disease and fifteen age-matched controls were recruited. Lymphocytes were isolated and incubated at either 37°C for 1 h or heat shocked at 42.5°C for 1 h. Lymphocytes were then allowed to recover at 37°C for 3 h and hsp expression was measured using both western immunoblots and 1D-SDS-PAGE (β-actin used as internal control). Parameters of oxidative stress including lipid peroxidation, protein oxidation and antioxidant status were measured in plasma and kinetics of haemolysis induced by free radical challenge [peroxyl radicals generated by 2,2'-azobis (2-amidinopropane) dihydrochloride] were measured in erythrocytes.

After a mild heat shock (from 37°C to 42.5°C for 1 hr) lymphocytes displayed an augmented synthesis of hsp 110, hsp 90 and hsp 70, relative to actin, in all individuals regardless of HIV-status. This adaptive response is thought to complement the constitutive antioxidant defense system found in mammalian cells. There were apparent differences in levels of expression of newly synthesized hsp’s between the HIV-positive and HIV-negative groups. Within this cohort these differences were not correlated with CD4+ count, viral load, dietary supplement use, smoking or the use of HAART. However, further studies including larger numbers in each of these groups within the HIV-positive cohort are warranted. We have also demonstrated increased plasma protein carbonyl formation (p <0.05) and decreased plasma antioxidant status (p < 0.05), both measures of oxidative stress, in the HIV infected cohort.

The significance of altered hsp expression remains to be determined. However, given the recent reports on the role of these proteins in cross-presentation of antigens, α-defensin internalization and pro-inflammatory cytokine production, further investigation is merited.
ALBERTO EXPRESSION OF HEAT SHOCK PROTEINS (HSP70, HSP90, HSP110) IN LYMPHOCYTES FROM HIV-POSITIVE INDIVIDUALS

Linda L Agnew1, Mark Kelly2, Rosemary A Frenche3, Julian Gold2, Kenneth Watson1

1. School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale, NSW 2351, Australia. 2. Alfred Street Centre, Sydney, NSW 2010, Australia. 3. Department of Immunology & Infectious Diseases, Sydney Children's Hospital, Sydney, NSW 2031 and School of Women's & Children's Health, University of New South Wales, Sydney, NSW 2031, Australia.

BACKGROUND
Heat shock proteins (hsp) are a group of highly conserved proteins that represent between 2% and 15% of total cellular protein and are expressed by every living organism. The main functions of hsp are to regulate apoptosis and to act as intracellular molecular chaperones that facilitate protein folding and assembly. Some hsp are highly immunogenic and elicit humoral, cytotoxic T-lymphocyte (CTL) and natural killer (NK) cell responses against viruses, tumors and infectious diseases. Previous studies by this group have reported altered leukocyte expression of hsp70 in HIV-positive individuals, compared with age-matched, uninfected controls (1).

AIMS
The aims of this study were to examine de novo synthesis of Hsp70, Hsp90 and Hsp110 in lymphocytes from HIV-positive individuals compared with uninfected control subjects and to investigate whether these parameters were related to CD4+ expression, viral load or oxidative stress status.

METHOD
Twenty male patients with HIV disease and fifteen age-matched controls were recruited. Lymphocytes were isolated by Ficoll-Paque density centrifugation and incubated at either 37°C for 1 h or heat shocked at 42.5°C for 1 h. Lymphocytes were then allowed to recover at 37°C for 3 h and hsp expression was measured using both western immunobots and 1D-SDS-PAGE (β-actin used as an internal control). Parameters of oxidative stress including lipid peroxidation, protein oxidation and antioxidant status were measured in plasma and kinetics of hsp expression induced by free radical challenge (peroxyl radicals generated by 50 mM 2,2'-azobis (2-aminompropane) dihydrochloride) were measured in erythrocytes.

RESULTS AND DISCUSSION
After a mild heat shock (from 37°C to 42.5°C for 1 hr) lymphocytes displayed an augmented synthesis of hsp 110, hsp 90 and hsp 70, relative to β-actin, in all individuals regardless of HIV-status. This adaptive response is thought to complement the constitutive antioxidant defense system found in mammalian cells (2). There were apparent differences in levels of expression of newly synthesized hsp110 and hsp 90 between the HIV-positive and HIV-negative groups as measured by autoradiography. Significant differences were also apparent in absolute amounts of hsp 70 as measured by western immunoblot (data not shown). Specifically, constitutive expression of hsp 70 was down regulated in HIV-positive individuals (p<0.009), within the HIV-positive cohort differences in hsp expression were not correlated with CD4+ count, viral load, dietary supplement use, smoking or the use of HAART. However, further studies including larger numbers in each of these groups within this cohort are warranted. We have also demonstrated increased plasma protein carbonyl formation (p<0.05) and decreased plasma antioxidant status (p<0.05), both measures of oxidative stress, in the HIV-injected cohort (data not shown). It is possible that increased oxidative stress parameters may alter hsp expression as it has previously been demonstrated that dietary supplements of mixed antioxidants modulate hsp synthesis (3).

CONCLUSION
The significance of altered hsp expression in HIV-positive individuals remains to be determined. However, given the recent reports on the role of these proteins in cross-presentation of antigens, α-defensin internalization and pro-inflammatory cytokine production, further investigation is merited.

REFERENCES

ACKNOWLEDGEMENTS: AIDS Trust of Australia - UNS Research Grant
HIV-POSITIVE INDIVIDUALS HAVE ALTERED HSP70 EXPRESSION IN A NUMBER OF LEUKOCYTE SUBTYPES.

Linda L Agnew¹, John Zaunders², Jega Sarangapany³, Anthony Kelleher², Julian Gold³, Kenneth Watson¹.

¹School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale, NSW, 2351, Australia. ²Centre for Immunology, St Vincent's Hospital, Sydney, NSW, 2010, Australia. ³Albion Street Centre, Sydney, NSW, 2010, Australia.

It has been reported that heat shock protein 70 (hsp70) is highly immunogenic, plays critical roles in antigen presentation, promotes pro-inflammatory cascades and induces immune responses. These immunogenic properties may be a result of a number of mechanisms that include, and may not be limited to, heat shock proteins acting as classic antigens, their ability to redistribute to cell surface membranes following infection or transformation, the adjuvant properties of heat shock proteins, and their ability to be integrally involved in antigen presentation.

We have previously reported that leukocyte hsp70 expression is altered in patients with HIV disease. The significance of altered hsp expression remains to be determined, however given the recent reports on the role of these proteins in cross-presentation of antigens, α-defensin internalization and pro-inflammatory cytokine production, further investigation is merited.

Flow cytometric methods have demonstrated that hsp70 is constitutively expressed in human leukocytes but that the level of expression varies considerably between different cell types. Specifically, monocytes express significantly more hsp70 than any other leukocyte subtype. The aim of the present study, therefore, was to compare these expression patterns in HIV-positive and HIV-negative cohorts. The results of this study demonstrated significant differences (p<0.05) in hsp70 expression at both basal and heat shock levels, in a number of leukocyte sub-populations, between HIV-positive (n = 42) and HIV-negative (n = 11) individuals. These differences were apparent in cytotoxic T cells (CD8+), helper T cells (CD4+) and monocytes (CD14+). There were no apparent differences in natural killer cell (CD56+) expression of hsp70. These results may provide insight into the mechanisms of altered leukocyte hsp70 expression in patients with HIV disease.
HIV-positive individuals display altered hsp70 expression in a number of leukocyte subtypes

Linda L Agnew¹, John Zaunders², Jega Serangapani³, Anthony Kelleher¹, Julian Gold³, Kenneth Watson¹

¹ School of Biological, Biomedical & Molecular Sciences, University of New England, Armidale, NSW 2351, Australia. ² Centre for Immunology, St Vincent’s Hospital, Sydney, NSW 2010, Australia. ³ Albion Street Centre, Sydney, NSW 2010, Australia.

Introduction

It is widely accepted that hsp70 is highly immunogenic, plays critical roles in antigen presentation, promotes pro-inflammatory cascades and induces immune responses. These immunogenic properties may be a result of a number of mechanisms that include, and may not be limited to, heat shock proteins acting as classical antigens, their ability to redistribute to cell surface membranes following infection or transformation, the adjuvant properties of heat shock proteins, and their ability to be integrally involved in antigen presentation.

We have previously reported that leukocyte hsp70 expression is altered in patients with HIV disease (1). The significance of altered hsp expression remains to be determined, however, given the recent reports on the role of these proteins in cross-presentation of antigens, o-defensin internalization and pro-inflammatory cytokine production, further investigation is merited.

Methods

Forty-two HIV-positive males (mean age 42 years) and eleven HIV-negative control subjects (mean age 41 years) participated in this study. All procedures were approved by the South Eastern Sydney Area Health Service and the University of New England human research ethics committee.

For heat shock, samples of heparinized whole blood were pre-equilibrated in a 37°C water bath then placed in a 42.5°C water bath for 1 h, followed by recovery in a 37°C water bath. Control blood samples were maintained in the 37°C water bath for the entire period. Blood samples were incubated with the appropriate extracellular marker antibodies. Erythrocytes were lysed then leukocytes were permeabilized. Samples were then incubated with either (anti-hsp70-FITC) or control anti-IgG-FITC. Cells were washed then fixed with 0.5% paraformaldehyde. Tubes were stored at 4°C until flow cytometric analysis was performed on a BD-LSR 2 with BD-FACSDiva software. This analysis was completed within 24 h of staining.

Results

Expression of hsp70 in leukocyte subtypes

The role of hsp70 in HIV disease pathogenesis is now beginning to be elucidated. A recent report that expression of the hsp receptor, CD91, is increased in monocytes from patients with long term non-progressive HIV disease (2) is of particular importance given that key components of the soluble factor, termed CAP, that suppresses HIV-replication, and which is secreted from stimulated CD8 T lymphocytes in high amounts from such individuals have been identified as α-defensins (3). The latter in turn have been demonstrated to be associated with CD91 which mediates internalisation of α-defensins (4). Furthermore, hsp70 selectively incorporated into the HIV virion during the assembly process (5).

The current study demonstrated that patterns of expression of hsp70 vary among leukocyte subtypes. It also showed that HIV-positive individuals express significantly different amounts of hsp70 in a variety of leukocyte cell types despite there being no difference in the numbers of cells expressing the protein. These results may provide insight into the mechanisms of altered hsp70 expression in patients with HIV disease.

Discussion

CD4+ CD8+ CD14+ monocytes CD56+ natural killer cells

CD4+ CD14+ CD16+ CD14+ CD16+ EXPRESSION OF HSP70 MEAN FLUORESCENCE INTENSITY (MFI)

- Positive
- Negative
- Fold Increase

CD4+ lymphocytes CD8+ lymphocytes CD14+ monocytes CD56+ natural killer cells

- Positive
- Negative
- Fold Increase

Summary

- No significant difference between HIV-positive & HIV-negative individuals in numbers of cells expressing hsp70 in any cell type examined except monocytes (heat shock)
- HIV-positive individuals displayed significantly higher relative amounts of hsp70 in:
  - CD4+ helper T lymphocytes (control & heat shock)
  - CD8+ cytotoxic T lymphocytes (control & heat shock)
  - CD56+ natural killer cells (heat shock)
  - CD14+ monocytes (fold increase between control & heat shock)

References


Acknowledgements

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EFFECT OF ECHINACEA ON HUMAN IMMUNE RESPONSES IN VIVO

Lehmann R\textsuperscript{1}, Agnew LL\textsuperscript{2}, Matthias A\textsuperscript{1}, Guffogg SP\textsuperscript{2}, Bone K\textsuperscript{3}, Watson K\textsuperscript{2}.

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The immunomodulatory effects of oral dosing with an Echinacea preparation were investigated in a small scale clinical trial (n = 11). Expression of leukocyte heat shock protein 70 (hsp70), serum chemistry, haematological values and plasma alkylamide concentrations were evaluated in eleven healthy individuals (26 to 61 years of age) at baseline (day 1) and on day 15 after consuming two commercially blended Echinacea tablets (containing both \textit{Echinacea angustifolia} and \textit{Echinacea purpurea} root) per day for fourteen days.

Plasma alkylamide levels were determined one hour after ingestion of one Echinacea tablet and concentrations were found to be 12 ± 2 ng equiv/ml plasma. Echinacea supplementation significantly enhanced the fold increase in leukocyte hsp70 expression after a mild heat shock from 2.2 ± 0.4 to 3.3 ± 0.7 (p = 0.03). Serum chemistry and haematological values for subjects after Echinacea supplementation did not vary significantly from baseline levels with the exception of white cell counts which increased from 6.6 ± 0.4 to 7.2 ± 0.3 x 10\textsuperscript{9} (p = 0.04). Differential white cell counts displayed modest increases after Echinacea supplementation although only the lymphocyte sub-population approached significance (p = 0.06).

The enhanced hsp70 stress response found is indicative of an improved immune response given that increases in hsp70 expression following cellular stress may play a critical role in antigen presentation, lymphocyte effector function and cytokine induction. This pilot study therefore suggests that supplementation with Echinacea may invoke an immune response through altered expression of hsp70 and increased white cell counts.
Effect of Echinacea on Human Immune Responses In Vivo

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**INTRODUCTION**

Although Echinacea has been used for many years as an immunomodulator [1], its mode of action is still unclear. As such, this study investigated the potential immunomodulatory effects of an Echinacea preparation by measuring leucocyte heat shock protein 70 (hsp70) expression as a biomarker of the immune response. Hsps are among the most highly conserved proteins in nature and are found in all organisms. They are expressed constitutively as well as induced in response to mild, generally non-lethal stress such as inflammation and microbial infections. There is increasing evidence that hsps play key roles as prominent antigens in the humoral and cellular immune responses mediated by antibodies and T cells respectively [2,3]. The involvement of altered hsp expression in a number of disease states has emphasised the important role of these proteins in the modulation of the immune response [4].

The present study was a pilot scale clinical trial involving eleven subjects (five male, six female), orally dosed with the tablet form of an ethanolic extract of two species of *Echinacea* – *purpurea* and *angustifolia*. The aim of the study was to investigate the potential immunomodulatory effects of *Echinacea* in healthy subjects by measuring leucocyte hsp70 expression as a biomarker of the immune response. Haematology alterations in response to chronic dosing with Echinacea were also examined.

**Results**

![Figure 1: Representative Western immunoblots for hsp70 expression in human leucocytes.](image)

Control (37°C), Heat shock (42.5°C), \( \text{pre} = \) before Echinacea intake, \( \text{post} = \) after 2 weeks Echinacea supplementation.

<table>
<thead>
<tr>
<th></th>
<th>Before Echinacea</th>
<th>After Echinacea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell counts ((10^6/\mu L))</td>
<td>4.84 ± 0.15</td>
<td>4.81 ± 0.16</td>
</tr>
<tr>
<td>Haemoglobin ((g/L))</td>
<td>146 ± 4</td>
<td>146 ± 4</td>
</tr>
<tr>
<td>White cell counts ((10^3/\mu L))</td>
<td>6.6 ± 0.4</td>
<td>7.2 ± 0.3</td>
</tr>
<tr>
<td>Neutrophils ((10^3/\mu L))</td>
<td>3.6 ± 0.3</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>Lymphocytes ((10^3/\mu L))</td>
<td>2.2 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Monocytes ((10^3/\mu L))</td>
<td>0.4 ± 0.0</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>Eosinophils ((10^3/\mu L))</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>Basophils ((10^3/\mu L))</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Platelets ((10^3/\mu L))</td>
<td>276 ± 20</td>
<td>272 ± 15</td>
</tr>
</tbody>
</table>

**Table 1: Comparison of blood haematology before and after two weeks supplementation with Echinacea.**

All values are mean ± SE (n = 11). *p = 0.043

Heat shock increased hsp70 expression levels in leucocytes. Although neither basal nor heat shock hsp70 levels were different, there was a significantly greater fold increase in hsp70 after Echinacea supplementation. Total white cell counts increased after Echinacea supplementation.

- Differential cell counts displayed only non-significant increases after Echinacea supplementation.

Plasma alkylamide levels were 12 ± 2 ng/mL plasma one hour after ingestion of one Echinacea tablet.

**Summary**

Echinacea may induce an immune response through:
- Increased white cell counts.
- Increased expression of leucocyte hsp70 in response to heat shock.

These are indicative of an improved immune response.

**Acknowledgments:** This work was supported in part by a University of New England research grant, by AusIndustry through a Biotechnology Innovation Fund Grant (No. BIF02651) and MediHerb Pty. Ltd.

**References:**


**Methods**

Echinacea Premium Tablets containing 675 mg of *E. purpurea* root extract and 600 mg of *E. angustifolia* root extract prepared by ethanol extraction were obtained from MediHerb, Warwick, Australia. Eleven individuals with BMI 19-30 participated in the study. Participants then consumed 2 Echinacea tablets per day for 14 days. A further blood sample was collected on day 15. Leucocytes were isolated and incubated at either 37°C or 42.5°C for 1 hour. They were then allowed to recover for 3 hours at 37°C prior to protein extraction. Hsp70 expression was measured using western immunoblots and densitometric analysis. Blood was also assessed using standard haematological analyses. Plasma alkylamide levels were determined by LC-MS [5].

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