LifeWave X49 Patch Proof of Concept Explores Changes in the AHK-Cu Peptide in Blood

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# Abstract

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# Purpose

This study focused on the impact of LifeWave X49 patch usage on AHK-Cu levels in men and women.

## Materials

BD Vacutainer Safety Loc Blood Collection set with Pre-attached holder sized 21GX0.75 or 23GX0.75, lavender top tubes, Kendro Sorvall Biofuge centrifuge 7500-5184+, Cryo tubes, American Biotech Supply medical freezer, 2" thick polystyrene containers, thermal box liners and double walled boxes.

# Method

Participants were recruited using flyers and would call into the main study phone number. At this point they were assessed for inclusion and exclusion criterion, and scheduled for consenting. All consented participants wore the LifeWave X49 patch for one hour prior to sample data taking at each data point except baseline and for a minimum of 8-12 hours per day during the study period. The GV 14 point was used for simplicity so that participants could easily find patch placement point. If appropriate, participant were consented, and then baseline data was taken. Blood sample was taken and placed in lavender top tubes. Each blood sample was then placed in the cryo tubes and flash frozen using a medical -22C degree freezer. Samples were then placed in 2" thick polystyrene containers, wrapped in thermal box liners and placed in double walled boxes for overnight shipping. Samples were sent to HK Labs, a division of Axis Pharm. In addition to wearing the patch and having blood samples taken, each participant was asked to keep a food log and were given listings of common foods which include Leucine.

# Results

50% of the time participants increased AHK-Cu over a period of 7 days. When vegetarians were removed from the sample, a clear pattern developed in the mean change.

# Conclusion

While there was no statistically significant change in the concentration on AHK-Cu over 7 days, 50% of the time participants did show increase in AHK-Cu. Once vegetarians were removed, a clear pattern developed which implies promotion of positive benefits to the body. Further study will need to be done with larger sample sizes over longer periods of time to determine if there is a consistency of results over repeated trials.

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## Introduction

This study explores the impact of wearing the LifeWave X49 patch over the period of one week on levels of L-alanine-L-histidyl-L-lysine-Cu2+ (AHK-Cu) tripeptide levels in the blood as a result. AHK-Cu has been shown to have several systemic effects on the body, including enhancing the expression of growth hormones, blood vessel growth, and new collagen, elastin, and glycosaminoglycan components of tissue to increase tissue repair (Patt, 2009, McCormack et al, 2001). "The response of human dermal fibroblasts to AHK-Cu has been investigated using cultured cells. The results show that AHK-Cu increases the growth and viability of dermal fibroblasts while stimulating the production of collagen" (Patt, 2009). An increase in AHK-Cu production in the body could have a significant impact on both resiliency and recovery rates in individuals, even with the small amounts of damage done through normal living (Nunez et al, 1994).

## Background

The LifeWave X49 patch uses light to stimulate the substance in the patch to produce the photobiomodulation of the body to increase production of AHK-Cu. Based on previously done research on this peptide, it was felt that a possible change in the amount of the tripeptide AHK-Cu could show a change in tissue remodeling and possible stem cell stimulation (DeHaven, 2014). The tri-peptide has been demonstrated to improve tissue remodeling and research has identified that the peptide is used as part of the natural skin, hair and muscle repair process (Han et al, 2004, Maquart et al, 1993). It is also possible that AHK-Cu is part of stem cell stimulation

within the body, given its many similarities with GHK-Cu (Pickart et al., 2014, Rhee et al.,

2006). Unfortunately at this point the mechanism of action is still unknown.

# The Tripeptide (AHK-Cu)



Figure 1. Structure of AHK-Cu

# Non-transdermal Patch

All LifeWave X49 patches are sealed so that none of the substances in the patch actually penetrate the skin. This allows for consistent patch promotion of the natural light flow throughout the time the patch is worn. Patches are designed to reflect wavelengths of light in the infrared, near infrared, and visible light bands. The specific wavelengths were chosen to trigger the body to increase production of AHK-Cu. The patches use the same adhesives as band-aids, which limits the level of irritation which might be developed through consistent daily use of the patch.

## Phototherapy

Phototherapy is the use of various wavelengths of light, both in and out of the visual light spectrum, to produce specific health effects and changes within the physical body. It has been used in various forms for over 100 years. In that time there has been little evidence of negative side effects. This suggests that this is a relatively untapped option for healing with relatively few risks.

## **Patch placement**

The LifeWave patches are placed on meridian points to create clear placement and ease of use. Current information now maps the meridian system to parts of the lymphatic system and that acupuncture points are (at least in part) strategic conductors of electromagnetic signals (Feinstein, 2010). This study used a point on the back known as GV14, Du-14, or Tao Dao, at the place where the cervical spine and thoracic spine meet. It is easy to find, reach and use.

## Purpose

To determine the impact of usage of the LifeWave X49 patch on AHK-Cu levels in men and women.

#### Procedure

Once human research studies ethics board approval was received (NFFEH 06-22-20-3) recruitment was begun. Flyers advertising for interested research participants were posted at various local sites. Participants would call into the main study phone number and were assessed for inclusion and exclusion criterion. If appropriate they were scheduled for consenting. At the time of arrival at the study site, each participant was consented. Blood samples to measure AHK-Cu concentrations were taken at baseline, 24 hours and at 7 days of wearing the patch. A sample of convenience of 10 subjects made up of both men and women aged 40-81 were selected to participate in this study.

Individual participants were then taken into the exam room and a blood sample was taken using BD Vacutainer Safety Loc Blood Collection set with Pre-attached holder sized 21GX0.75 or 23GX0.75 and placed in lavender top tubes. Each blood sample was then placed in the centrifuge, spun for 10 minutes to separate the plasma, which was then placed in the cryo tubes, and then flash frozen using a medical -22C degree freezer. Samples were then placed in 2" thick polystyrene containers, wrapped in thermal box liners and placed in double walled boxes for overnight shipping. Samples were sent to HK Labs, a division of Axis Pharm's laboratory in San Diego, CA under the direction of Dr. David Yue. Patches were worn for 8-12 hours per day beginning after baseline data taking. Participants were asked to wear the patch for an hour minimum prior to data being taken at the rest of the data points.

In addition to wearing the patches and having blood samples taken, each participant was asked to keep a food log. Participants were given listings of common foods which include leucine. Lysine, one of the building blocks of this tri-peptide, is used by the body to maintain leucine economy in the body in situations where there is insufficient intake. (Motil, 1981) This is likely to be an issue both within research and in wider use, since leucine is a substance which many individuals in modern society do not get in sufficient quantities. Participants were asked to keep the food log each day and eat a minimum of 4 oz of foods with Leucine in them. Food logs were evaluated at the conclusion of data taking to confirm that the minimum amount of Leucine containing foods were ingested each day.

## **Analysis of Blood Samples**

The blood samples were processed according to the original thesis on AHK-Cu. One lavender top tubes was drawn per participant and then placed in the Kendro Sorvall Biofuge centrifuge 7500-5184+ HERAEUS 7591 with a 4000 RPM rotor. Samples were spun for 10 minutes at 1300rcf at room temperature to separate the plasma. The plasma was separated, placed in cryo tubes and then flash frozen using a medical -22C degree freezer. Once frozen, the cryo tubes were then placed in 2" thick polystyrene containers, which were wrapped in thermal box liners and placed in double walled boxes. These boxes were shipped overnight to HT-Labs, a division of AxisPharm in San Diego, CA for analysis. The filtrate was concentrated by speed-vac and reconstituted with de-ionized water to 50ul and analyzed with AB Sciex API4000 Qtrap. The data was analyzed with Analyst software 1.6.2.

## **Statistical Analysis**

Absolute changes in AHK-Cu levels from baseline to the 24 hours and day 7 assessments were summarized in terms of means, standard deviations, medians and ranges. Changes from baseline to the 24 hours and day 7 assessments were evaluated using a nonparametric Wilcoxon signed rank test. All reported P-values are two-sided and P<0.05 was used to define statistical significance. Statistical analyses were conducted using R software (version 3.5.1; http://www.r-project.org/).

## Results

A sample of convenience of individuals consisted of 10 individuals. There were four men and six women in the study which had a mean age of 64.2, with an age range of 41 - 73. Significant results of the LifeWave X49 patch testing are as follows:

Table 1: Study X49 - Evaluation of Changes in	Outcomes from Day 1 to Day 2, Day 1 to
Day 7, and Day 2 to Day 7 within Groups	

Outcome	Time	Mean Change	SD	p-value*
AHK-Cu Concentration (ng/ml)	Day 1 to Day 2	-154.92	380.06	0.3750
	Day 1 to Day 7	-150.91	401.91	0.4922
	Day 2 to Day 7	4.01	90.25	1.0000
Total AHK-Cu in Sample (ng)	Day 1 to Day 2	-464.76	1140.17	0.3750
	Day 1 to Day 7	-452.73	1205.74	0.4922
	Day 2 to Day 7	12.03	270.76	1.0000

\*:p-value for evaluating changes from Day 1 to Day 2, Day 1 to Day 7, and Day 2 to Day 7

While there was no statistically significant change in the concentration on AHK-Cu in the samples over the period of one week, once the two vegetarians were removed from the sample a clear pattern developed of increase in the mean change. This may be because in general

vegetarians are low in leucine and lycine which is used by this peptide is then substituted for leucine to keep the body functioning. This data confirms the importance of sufficient leucine ingestion for vegetarians so that they may maintain optimal health.

Table 2: Study X49 - Evaluation of Changes in Outcomes from Day 1 to Day 2, Day 1	to
Day 7, and Day 2 to Day 7 within Groups (without vegitarians.)	

Outcome	Time	Mean Change	SD	p-value*
AHK-Cu Concentration (ng/ml)	Day 1 to Day 2	-38.71	117.41	1.0000
	Day 1 to Day 7	-31.71	138.42	0.8438
	Day 2 to Day 7	7	100.15	0.9453
Total AHK-Cu in Sample (ng)	Day 1 to Day 2	-116.14	352.23	1.0000
	Day 1 to Day 7	-95.13	415.27	0.8438
	Day 2 to Day 7	21.01	300.45	0.9453

\*:p-value for evaluating changes from Day 1 to Day 2, Day 1 to Day 7, and Day 2 to Day 7

During the evaluation period, 50% of the data samples showed an increase over baseline. However, the total change was not at the p < 0.05 for statistical significance. Given the use of this peptide in the development of hair, skin, collagen, cartilage and muscle tissue it is possible that humans are regularly short in lycine.

Food logs were kept through out the study and participants were confirmed to be eating at least 4 oz of foods which included Leucine as part of their diet.

## Discussion

It is important to recognize that this was both a sample of convenience with a small sample size and was done as a proof of concept study. There are only a hand full of studies on the AHK-CU tri-peptide world wide. In the national library of medicine we only found four in humans, so the material developed in this study while not statistically significant is very significant in other ways. First, it is clear that the LifeWave X49 patch did produce a change in AHK-Cu production. This suggests that changes in patch placement to increase effects, or changes in the life style of participants (for example: increase in the amount of exercise or participation in hormone replacement therapies) may demonstrate more complete information.

Second, in Table 1 you will see that there is no clear differentiation between each day and no clear pattern appears. However, when we remove the two vegetarians from the study, confirming data from other studies that many vegetarians have a chronic shortage of leucine in particular, which causes a critical shortage in lycine, a building block in this tri-peptide, we see a clear pattern appear in the mean change of amount of AHK-CU in the period of one week in Table 2. It would be valuable to ask participants to move to 8oz of leucine foods each day to see the effects of that change in future studies.

Third, and perhaps more important, is that there was a clear effect as shown by the mean change. While the change did not meet the statistical significance recognized by science, there is a clear upward trend in the amount of AHK-CU being found in the blood samples. Given the wide age range of study participants and the small sample, this is a very important finding. So the patch has a clear effect. It is the strength of the effect which remains a question. What would be important to take as a next step would be to determine the correct period of time to have the study run as the data implies a level of pattering and periodicity which should be explored.

Of equal interest is the balancing of the body systems. The question of what amount of any particular amino acid production for the optimal functioning of the body is necessary is an ongoing dialogue between many metabolism experts. This study contributes to the overall data with the clear increase in the mean change over the course of the week.

# Conclusion

This study goal was to measure the changes of AHK-Cu concentration in the blood as a result of wearing the LifeWave X49 patch for 1 week. While there was not a statistically significant change in the concentration on AHK-Cu in the samples over the period of one week, once the two vegetarians were removed from the sample a clear pattern in mean change developed which is appropriate for exploration. With 50% of samples taken over baseline showing and increase, the pattern implies promotion of positive benefits to the body. Further study will need to be done with larger sample sizes, over longer periods of time to determine if there is a consistency of results over repeated trials.

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