Proof-of-concept “Stress & anxiety dampening effects of Lpc-37”

NH-03862 Sisu (PR)

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Proof-of-concept "Stress & anxiety dampening effects of Lpc-37"

Study Description: The purpose of this clinical trial is to determine whether a single strain of bacteria derived from the species *Lactobacillus paracasei* (Lpc-37) can modulate stress experienced by healthy male and female participants exposed to the Trier Social Stress Test (TSST). This randomized, double-blind, placebo-controlled, parallel-groups clinical trial is statistically powered to detect a significant modulation of the increase in heart rate (HR), as the primary outcome, in response to the TSST. Self-reporting inventories related to stress, anxiety, subjective feelings and sleep will be completed by participants to identify the potential impact of probiotic supplementation on other psychological and physiological outcomes. Designed as a proof-of-concept study, the results of this study will serve as an indication that the chosen study design is suitable to investigate stress-related effects of probiotics.

Objectives:

Primary Objective:

The primary objective of this trial is to evaluate the efficacy of *Lactobacillus paracasei* Lpc-37 (Lpc-37) on HR before, during and after the TSST.

Secondary Objectives:

The secondary objectives of this trial are to evaluate:

1. The effect of Lpc-37 on state anxiety as measured with the State-Trait-Anxiety-Inventory (STAI) before and after treatment.
2. The effect of Lpc-37 on state anxiety as measured with the STAI before and after the TSST.
3. The effect of Lpc-37 on the Cortisol Awakening Response (CAR) before and after treatment.
4. The effect of Lpc-37 on salivary cortisol before and after the TSST.
5. The effect of Lpc-37 on perceived stress as measured with the Perceived Stress Scale (PSS) before and after treatment.
6. The effect of Lpc-37 on depression, anxiety and stress as measured with the Depression-Anxiety-Stress Scale (DASS) before and after treatment.
7. The effect of Lpc-37 on anxiety as measured with the Beck Anxiety Inventory (BAI) before and after treatment.

8. The effect of Lpc-37 on perceived stress, anxiety, insecurity and exhaustion as measured by Visual Analog Scales (VAS) before and after treatment.
9. The effect of Lpc-37 on perceived stress, anxiety, insecurity and exhaustion as measured by VAS before, during and after the TSST.
10. The effect of Lpc-37 on sleep quality, health, wellbeing and mood as measured by a daily online diary over a period of 7 weeks.
11. The effect of Lpc-37 on systolic and diastolic blood pressure (BP) before and after treatment.
12. The effect of Lpc-37 on systolic and diastolic BP before and after the TSST.
13. The effect of Lpc-37 on salivary alpha-amylase (sAA) before and after the TSST.

Endpoints:

Primary Endpoint:

The primary endpoint is change in HR in response to the TSST. The efficacy is defined as a reduced increase in HR in response to the TSST in the verum group compared to the placebo group.

Secondary Endpoints:

Endpoints related to parameters assessed before (Visit 2) and after the intervention (Visit 3). The difference of:

- STAI-state scores (Form X1)
- PSS scores
- DASS depression scores
- DASS anxiety scores
- DASS stress scores
- BAI scores
- VAS stress perception scores
- VAS anxiety scores
- VAS insecurity scores
- VAS exhaustion scores
- Systolic BP
- Diastolic BP

Efficacy is defined as a larger reduction of the listed scores, as well as systolic and diastolic BP, after 5 weeks of daily Lpc-37 intake in the verum group compared to the placebo group.

Endpoints related to cortisol and the CAR assessed before (Visit 2) and after the intervention (Visit 3). The difference of:

- Area under the curve with respect to ground (AUC_G)
- Area under the curve with respect to increase (AUC_I)
- Cortisol at the time of awakening

- Cortisol at 8 pm

Efficacy is defined as a larger number of participants showing a normalization of the CAR after 5 weeks of daily Lpc-37 intake in the verum group compared to the placebo group.

Endpoints related to parameters assessed before and after the TSST. The difference of:

- STAI-state scores (Form X1)
- Systolic BP
- Diastolic BP

Efficacy is defined as a reduced increase of STAI-state (X1) scores, systolic and diastolic BP in the verum group compared to the placebo group.

Endpoints related to parameters assessed before, during and after TSST. The change of:

- VAS stress perception scores
- VAS anxiety scores
- VAS insecurity scores
- VAS exhaustion scores
- Salivary cortisol
- sAA

Efficacy is defined as a reduced increase of the VAS stress perception, anxiety, insecurity and exhaustion scores. For salivary cortisol and sAA, efficacy is defined as subgroup-specific normalization of the response, i.e. a higher response for chronically high stressed participants and a lower response for chronically low stressed participants in the verum group compared to the placebo group.

The changes over time from before (Visit 1) and after the intervention (Visit 3) as assessed with a daily online diary in:

- Sleep duration
- Sleep related recovery scores
- Sleep disruptions (binary)
- Reported number of sleep disruptions
- Perceived health status scores
- Mood scale scores
- Perceived productivity scores

Efficacy is defined as an increase of sleep duration, sleep related recovery scores, perceived health status scores, perceived productivity scores, mood scores and a decrease of number of sleep disruptions over the course of the intervention in the verum group compared to the placebo group.

Safety:

The safety objectives of this clinical trial are to evaluate if the vital signs (Body Mass Index (BMI), BP, HR, and the incidence and intensity of adverse events (AEs) are comparable between the verum group and the placebo group.

Study Population:

The study population included healthy male and female adults (18-45 years inclusive). A total of 120 healthy participants were recruited and randomized into one of two study groups (verum or placebo) after stratifying for chronic stress (below or above age-related median score of the Screening Scale of Chronic Stress subscale of the Trier Inventory for Chronic Stress (TICS) and sex (male and female).

For the stratification based on chronic stress, participants with a chronic stress score of ≤13 were stratified into the low chronic stress subgroups and participants with a chronic stress score of ≥14 were stratified into the high chronic stress subgroups.

Group 1: *Lactobacillus paracasei* Lpc-37 at 1.75 x 10^{10} colony forming units (CFU) per day

Group 2: Placebo

Participants took one capsule of their assigned investigational product every morning for 5 weeks.

Inclusion Criteria:

- Voluntary, written, informed consent to participate in the study
- Male or female aged between 18-45 years (inclusive)
- BMI between 18.5 – 29.9 kg/m^2
- Medical examination at baseline indicates they are healthy in the opinion of the Principal Investigator
- Ability of the participant (in the Principal Investigator's opinion) to comprehend the full nature and purpose of the study including possible risks and side effects
- Agreement to comply with the protocol and study restrictions
- Available for all study visits
- Females of child-bearing potential required to provide a negative urine pregnancy test and to use contraceptives
- Easy access to internet

Exclusion Criteria:

- Self-reported diagnosis of one or more DSM-IV axis 1 disorder(s), including but not limited to current major depression, anxiety disorder, bipolar spectrum disorder or schizophrenia
- Have a significant acute or chronic coexisting illness (cardiovascular, gastrointestinal (inflammatory bowel syndrome, inflammatory bowel disease), immunological, metabolic, neurodevelopmental or any condition which contraindicates, in the Principal Investigator's judgement, entry to the study
- Currently taking (from day of screening onwards) or have previously taken (last 4 weeks prior to screening) psychoactive medication (anxiolytics, sedatives, hypnotics, anti-psychotics, anti-depressants, anti-convulsants, centrally acting corticosteroids, opioid pain relievers)
- Currently taking (from day of screening onwards) medication or dietary supplements that the Principal Investigator believes would interfere with the objectives of the study, pose a safety risk or confound the interpretation of the study results (e.g. melatonin, omega-3 dietary supplements, non-steroidal anti-inflammatory drugs (NSAIDS), over-the-counter (OTC) sleep medication (not categorized as sedatives, hypnotics or anti-depressants), anti-coagulants, proton pump inhibitors, anti-histamines, pseudoephedrine, cortisone, beta-blockers)
- Recent (within last 4 weeks prior to screening) or ongoing antibiotic therapy during the intervention period
- Daily consumption of concentrated sources of probiotics and/or prebiotics within 2 weeks of screening and throughout the intervention period other than the provided study products (e.g., probiotic/prebiotic tablets, capsules, drops or powders)
- Pregnant or lactating female, or pregnancy planned during intervention period
- Not fluent in German
- Have self-reported dyslexia
- History of alcohol, drug, or medication abuse
- Self-declared illicit drug users (including cannabis and cocaine) for 3 weeks prior to screening and during the intervention period
- Contraindication to any substance in the investigational products
- Hypertension (systolic ≥ 140 mmHg, diastolic ≥ 90 mmHg)
- Known hyper- or hypothyroidism unless treated and under control (stable for more than 3 months)
- Persons having previously participated in the TSST
- Smoking > 5 cigarettes/day
- Employee of the sponsor or contract research organization
- Participation in another study with any investigational product within 60 days of screening and during the intervention period
- Principal Investigator believes that the participant may be uncooperative and/or noncompliant and should therefore not participate in the study

- Participant under administrative or legal supervision

Sample size:

The estimated sample size was computed for a repeated measurement ANOVA with

- 2 groups
- 7 measurements (HR during the TSST)
- a small effect size of f = 0.1
- α-error probability = 0.05
- power (1 – β-error probability) = 0.85

and resulted in a group size of 56 participants each. In order to account for protocol deviations, the estimated sample size was rounded up to a total sample size of 60 participants per study group.

Statistical analyses:

For all endpoints, analyses will be performed for the Intention-to-Treat (ITT) and Per Protocol (PP) population separately. Subgroup analyses will be performed for the different strata, *i.e.* female participants, male participants, high stressed participants and low stressed participants.

Efficacy analyses are planned as linear mixed model (lmm) analyses and repeated measures analyses of variance (RM ANOVA) or alternatively as t-tests, Friedman analyses of variance, Wilcoxon Signed-Rank test, Kruskal Wallis test, and Mann Whitney U tests in case of violation of model assumptions. Two-sided hypothesis testing (α=0.05) will be performed.

Mixed models will be built up gradually, first testing how many time polynomials should be included, then testing possible covariates, and lastly adding the effect of study group and time × group interaction terms (e.g. time1 × group, time2 × group). Models will be built including time and intercept as random factors. In case of convergence difficulties, time will be dropped from the random effects. Fixed effects include time, group, time × group interaction terms and covariates. Categorical variables are effect-coded, time will be centered. To assess main effects and interactions of variables with more than one coefficient (time, time x group interaction), Type II F-tests will be conducted using Satterthwaite's degrees of freedom method.

If the assumptions of a statistical analysis are violated in the efficacy analyses despite efforts of transformation, alternative tests will be used. If the assumptions for mixed models are violated, RM ANOVAs will be used. If the assumptions for RM ANOVAs are violated, one-way ANOVAs will be performed. If the assumptions for parametric testing are violated, non-parametric tests will be calculated. Time effects will then be evaluated by Wilcoxon Signed-Rank tests or Friedman ANOVAs, group effects by Mann

	Whitney U tests, and time × group interactions by Mann Whitney U tests on difference values between measurements (e.g. group difference in the difference value between HR sitting before the TSST minus HR standing before the TSST).
Phase:	2
Description of Sites/Facilities Enrolling Participants:	The sponsor contracted daacro GmbH & Co. KG (contract research organization) and their site located at the Science Park of Trier, Germany (Max-Planck-Straße 22, 54296 Trier, Germany) to perform the clinical trial. This was a single-site clinical trial.
Description of Study Intervention:	The investigational products are: **Group 1:** *Lactobacillus paracasei* Lpc-37 at 1.75 x 10^{10} CFU per day *Ingredients: Lactobacillus paracasei* Lpc-37, microcrystalline cellulose, magnesium stearate, silicon dioxide *Batch No.: 1103180371* **Group 2:** Placebo *Ingredients:* Microcrystalline cellulose, magnesium stearate, silicon dioxide *Batch No.: 1103180369* **Posology:** The study products will be provided in capsules. The study products will be consumed once daily for 5 weeks, 30 minutes before breakfast or their first meal of the day, with a glass of plain (non-sparkling) water.
Study Duration:	5 weeks date of First Participant First Visit: April 10, 2018 date of Last Participant Last Visit: October 9, 2018
Participant Duration:	The study visits are defined as; Screening Visit (Visit 1), Baseline Visit (Visit 2), and after 5 weeks of intervention with either verum or placebo, the Post-Treatment Visit (Visit 3). The investigation steps and duration of each study visit is outlined in Schema 1.2.

1.2 SCHEMA

Overall Study Design			
Study day	**No. of visit at study site**	**Investigation steps**	**Duration of visit**
	Visit 0	Telephone screening: Interested participants receive information via phone and a first check of inclusion/exclusion criteria is performed. Eligible participants are scheduled for a screening visit at the study site.	approx. 20 min.
1	Visit 1	Screening visit: At Visit 1, participants will be informed about the study procedure extensively. Eligibility criteria will be checked, and participants sign an informed consent. The TICS questionnaire will be filled in for stratification and participants receive a random number. Enrolled participants receive a training on how to handle the daily online diary entry and on how to collect saliva samples at home during the 2 days before Visit 2. A saliva collection kit will be handed out.	approx. 75 min.
2 - 15		Run-in: During the two-week run-in period participants are not allowed to consume products containing concentrated sources of probiotics/prebiotics. Participants fill in the daily online diary in the mornings. Saliva samples will be collected during the 2 days before Visit 2.	
16	Visit 2	Baseline visit: Participants return their saliva samples. Weight, HR and BP will be measured. Baseline questionnaires (STAI-Trait, STAI-State, VAS stress, anxiety, insecurity and exhaustion, BAI, DASS, PSS) will be assessed. The investigational product will be handed out and intake will be explained. A second saliva collection kit will be handed out for post-treatment assessment during 2 days prior to Visit 3.	approx. 45 min.
17-51		Intervention: Participants take 1 capsule of the investigational product and fill in the online diary daily in the morning. Saliva samples will be collected during the 2 days before Visit 3.	
51	Visit 3	After 5 weeks of intervention intake participants return to the study site. Again, saliva samples will be collected. Overall compliance with the study protocol will be checked and AEs will be assessed. Weight, HR and BP will be measured. Again, questionnaires (STAI-State, VAS stress, anxiety, insecurity and exhaustion, BAI,	approx. 125 min.

		DASS, PSS) will be assessed. The TSST will be conducted. 6 saliva samples for the assessment of cortisol and sAA will be collected before and after the TSST. A continuous HR measurement will take place before, during and after the TSST. BP will be assessed before and after the TSST. STAI state anxiety will be assessed before and after the TSST, while VAS stress, anxiety, insecurity and exhaustion will be assessed before, during and after the TSST. Participants receive their 400€ study compensation.	

Abbreviations: V, visit; TICS, Trier Inventory for Chronic Stress; HR, heart rate; BP, blood pressure; VAS, visual analog scale; BAI, beck anxiety inventory; DASS, depression-anxiety-stress scale; PSS, perceived stress scale; AE, adverse event; TSST, Trier Social Stress Test; sAA, salivary alpha amylase.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Visit	**V0**	**V1**	**Run-in**	**CAR pre**	**V2**	**Treatment**	**CAR post**	**V3 (TSST)**
Study day (treatment day)		***1***	***2-15***	***14-15***	***16***	***17-51***	***49-50***	***51***
Telephone screening	x							
Study inclusion, TICS		x						
Vital signs (BP, pulse, weight, height, BMI)		x			x			x
STAI-Trait					x			
General psychometric assessment (STAI-State, VAS stress, anxiety, insecurity and exhaustion, DASS, BAI, PSS)					x			x
Daily online diary			x		x	x		
Study treatment dispense					x			
Intake of investigational product						x		
Compliance check					x			x
TSST-protocol including questionnaires VAS, STAI-State, continuous HR, BP pre-/post- TSST, saliva samples (cortisol, AA)								x
CAR profile				x			x	

Abbreviations: TICS, Trier Inventory for Chronic Stress; BP, blood pressure; BMI, body mass index; VAS, visual analog scale; DASS, depression-anxiety-stress scale; BAI, beck anxiety inventory; PSS, perceived stress scale; TSST, Trier Social Stress Test; HR, heart rate; AA, alpha-amylase; CAR, cortisol awakening response