The Role of Fatty Acids in Vaccine Efficacy: A Randomized Controlled Clinical Trial

Study Protocol

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1. Background

Rabies is an acute zoonotic infectious disease caused by central nervous system invasion by rabies virus. At present, there is still a lack of effective treatment, and once clinical symptoms appear, it is almost 100% fatal. Human rabies is a 100% vaccine-preventable disease, yet it continues to kill. Rabies vaccination within 24 hours of exposure may reduce the incidence of rabies. The efficacy of rabies vaccination is crucial to avert deaths. Pre-exposure prophylaxis is also effective, and the current recommended pre-exposure vaccination program in China is: 0 days, 7 days, 21 days.

The health of the human body is inseparable from various nutrients. There are studies suggesting that arachidonic acid (ARA) has an immunomodulatory effect, has potential ability to promote efficacy of certain vaccination, but there is limited evidence for its effect on rabies vaccines. Therefore, this study will explore the effects of nutritional supplements on the effectiveness of rabies vaccination.

2. Study design

This study intends to use a randomized controlled trial design to vaccinate all participants against rabies and perform ARA interventions during vaccination.

3. Study objectives:

The objectives of this study are:

1) to test the ARA intervention on promoting rabies vaccination efficacy;

2) to evaluate the safety of using ARA intervention;

3) to explore the potential mechanism

4. Methods

4.1 Participants

Inclusion criteria

1）18-45 years old;

2）BMI 18.5-24.9 kg/m^2;

3）Have not received rabies vaccination.

Exclusion criteria

1) Those who have severe disorders of abnormal lipid metabolism;

2) Those who have used lipid-lowering drugs, weight loss drugs, and insulin drugs in the past three months;

3) Those who have received other vaccines in the past three months;

4) Those who have used probiotics or prebiotics in the past three months;

5) Those who have used steroids and immunosuppressants, other hormonal drugs in the past year;

6) Those with immunodeficiency diseases;

7) Those with a history of severe vaccine allergies;

8) Those who have disorders of liver and kidney metabolism;

9) Those who have had fever, cold, severe diarrhea and other diseases in the past month.

10) Smokers in the last year.

Drop out

1) Use of other nutritional supplements during the intervention;

2) Those who do not consume nutritional supplements as prescribed by the doctor

3) Those who develop a major illness;

4) Those who experience severe vaccine allergy;

5) Voluntary withdrawal.

4.2 Sample size

According to the mean and standard deviation of the two groups of neutralizing

antibody, 1323±1093.9 in the experimental group and 164.8±123.4 in the control group; using the standard deviation combined formula s = $\sqrt{\frac{s_1^2(n_1-1)+s_2^2(n_2-1)}{n_1+n_2-2}}$, the combined standard deviation was calculated as 778.4.

With reference to the above results, it was determined that the expected difference between the outcome indicators of the experimental and control groups in this study was 1158.2, and the standard deviation was 778.4. Taking the test level α = 0.05 and the degree of certainty 1-β = 0.9, the minimum sample size for each group was calculated as 10 people according to the following formula, and setting the expected loss of follow-up rate at 20%, 13 people needed to be recruited in each group.

$$N = 2 \times \left(\frac{z_{1-\frac{\alpha}{2}} + z_{1-\beta}}{\delta} \right)^2 \times s^2$$

4.3 Randomizing

Using the random number method, the study subjects were randomized into four groups, performed on R version 4.1.3, and after 3 days of a low-volume diet of a certain nutrient, nutritional supplement intervention was performed 3 days before the first dose of vaccine to the second dose of vaccination, in which the first group will receive nutritional supplements of 500mg/day from days 3 of the study; The second group will receive nutritional supplements 500mg/day, starting on days 6 of the study; The third group will receive a placebo and days 3 of the study.

Grouping concealment

After randomly grouping the study subjects, the groupings will be replaced with numbers 1, 2, and 3 to mask the specific grouping information.

Using the envelope method, the number of each study subject's grouping was placed in an envelope according to the grouping, and the name and ID were written on the outside of the envelope and distributed to each study subject.

Masking:

The three-blind design was adopted, and the study implementers, research subjects, data monitoring and statistical analysts were blinded.

Supplements used in interventions:

Placebo group: six ×500 mg sunflower oil capsules each day.

Day 3 ARA group: 512.4mg/day ARA via 6 × 500mg ARA capsules.

Day 6 ARA group: 512.4mg/day ARA via 6 × 500mg ARA capsules.

4.4 Study content

Baseline Investigation

1) Baseline data collection: age, gender, education level, monthly income, etc;

2) Collection of dietary intakes: 3-day, 24-hour dietary review method;

3) Lifestyle information: physical activity, sleep, anxiety, depression;

Follow-up indicators:

1) Primary indicators：

① Rabies virus antibody titers: to examine the specific antibody after intervention. Will be tested on day 6, 13, 16, 19 and 26.

② Neutralizing antibody: to test the ability of binding to rabies virus

Will be tested on day 6, 13, 16, 19 and 26.

2) Secondary indicators

① Serum blood fatty acid profiles: to reflect the change of fatty acid profiles after intervention. Will be tested on day 6, 13, 16 and 19.

② Cytokines: to test the potential blood cytokines changes triggered by ARA supplementation. Will be tested on day 6, 13, 16, 19 and 26

③ Feces microbiota: the microbiota in feces samples will be examined with metagenome to explore the potential gut-immune response caused by intervention. Will be tested on Day 3, 6，13, 16，19 and 26 of the study.

④ Blood metabolites: To examine the metabolites changes in blood after intervention.

Will be tested on Day 6，13 and 16 .

2) Safety indicators

①Blood glucose and lipids: tested on days 3 and 13 of the test;

②Blood count: tested on day 3 and 26 of the trial;

③Anxiety: to evaluate the mood changes after intervention with GAD-7. tested on day 13 of the trial.

④Adverse reaction records: allergy, gastrointestinal discomfort during the trial.

Biological sample collection (6 times in total):

Test Day 3: venous fasting blood collection of 30 ml and feces of 10 g;

Trial Day 6 (vaccination Day 0): 5 ml of blood collected intravenously; 10 g of feces;

Trial Day 13 (vaccination Day 7): 5 ml of blood collected intravenously on an empty stomach; 10 g of feces;

Trial Day 16 (vaccination Day 10): 5 ml of blood collected intravenously; 10 g of feces;

Trial Day 19 (vaccination Day 13): 30 ml of blood collected intravenously; 10 g of feces;

Trial Day 26 (vaccination Day 20): 5 ml of blood collected intravenously; 10 g of feces.

Preservation of serum, plasma and whole blood: frozen at -80°C after sampling.