SUMMERY OF CLINICAL TRIAL REPORT

Title of the study

Efficacy of vitamin E and omega-3 unsaturated fatty acid in prevention of damage induced by oxidative stress in patients with schizophrenia

Study code

EudraCT number: 2009-018054-33

Sponsor number: 09-001

Investigators and study administrative structures

Sponsor
University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia

Principal Investigator
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Study centres

Clinical Study Centres	***Clinical investigators***
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Biochemical and Pharmacokinetic centre	***Investigators***
University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia	Assoc. Prof. Iztok Grabnar, M. Pharm., Ph. D. Assist. Prof. Tomaž Vovk, M. Pharm., Ph. D.
Molecular genetic centre	***Investigator***
University of Ljubljana, Medical Faculty, Institute of Biochemistry, Vrazov trg 2, 1000 Ljubljana, Slovenia	Prof. Vita Dolžan, M.D., Ph. D.

Time of Clinical part
08. 07. 2010 – 29. 06. 2011

Phase of clinical investigation
Phase 3

Study design
Double-blind, randomized, placebo-controlled study with four arms (placebo, vitamin E, omega 3 fatty acids, and vitamin E+ omega-3 fatty acids).

Diagnosis and main selection criteria
Patients diagnosed with schizophrenia chronically treated with depo haloperidol.

Investigated medical products
Apozema®, Vitamin E 600 I.U. Kapseln, soft gelatine capsules, Apomedica GmbH & co KG, Austria, control number 071411, Expired date 31. 12. 2011
Dr. Bohm Omega 3 Forte Kapseln, soft gelatine capsules, Apomedica Pharmazeutische Produkte GmbH, Austria, control number 093025, Expired date 31. 10. 2011

Placebo
500 mg of lactose in hard gelatine capsule, Lekarna Ljubljana, Galenic laboratory, Tbilisijska 87, 1000 Ljubljana, Slovenia

Patients were randomized to receive vitamin E (Vitamin E 600 IU/bid), Omega 3 (essential polyunsaturated fatty acids (EPUFAs), 132 mg eicosapentaenoic acid (EPA) and 88 mg docosahexaenoic acid (DHA)), all drugs or placebo.

Study aims
1. To evaluate effects of supplementation with EPUFAs and vitamin E on markers of oxidative stress and extrapyramidal symptoms in schizophrenia patients treated with haloperidol depot injection.
2. To evaluate association between oxidative stress markers and extrapyramidal symptoms with concentrations of haloperidol and neurotransmitters.
3. To evaluate association between positive and negative symptoms of schizophrenia with concentrations of neurotransmitters and markers of oxidative stress.
4. To evaluate influence of antioxidants enzymes and tumour necrosis factor-alpha genetic polymorphisms on oxidative stress markers and extrapyramidal symptoms.
5. To evaluate influence of CYP 2D6 and P-glycoprotein genetic polymorphism on metabolism of haloperidol.

Study design
Schizophrenia patients were on depo therapy with haloperidol. They were diagnosed based on a structured clinical interview for DSM-IV and randomly recruited from the outpatient clinic, as they were coming for their regular monthly visits. Patients were otherwise physically healthy, based on complete medical history and physical examination. The study design was presented to patients after which informed consent was obtained. 20 ml of venous blood was drawn and used for bioanalytical and molecular genetic studies. Psychopathology and extrapyramidal symptoms were assessed by experienced psychiatrists. Patients were randomized to four groups and after 4 months of adjuvant therapy 20 ml of blood was drawn for bioanalytical and molecular genetic studies. Moreover, psychopathology and extrapyramidal symptoms were assessed.

Group	Adjuvant therapy	
	Vitamin E	EPUFAs
A vitamin E (n=5)*	600 IU/bid	Placebo
B EPUFAs (n=9)*	Placebo	130/80 mg/tid
C vitamin e + EPUFAs (n=9)*	600 IU/bid	130/80 mg/tid
D control (n=11)*	Placebo	Placebo

Vitamin E – soft gelatine capsules (600 IU) two time per day
EPUFAs – soft gelatine capsules with 130 mg eicosapentaenoic acid in 80 mg docosahexaenoic acid three times per day
Placebo – hard gelatine capsules with 500 mg lactose, two times per day in case of placebo for Vitamin E group and three times per day in case of placebo group for EPUFAs group
* indicates number (n) of patients concluded the study

Inclusion criteria

Patients with schizophrenia according to DSM IV classification.
Patients treated with depo haloperidol.
Male and female patients over 18 years of age.
Healthy, other than primary disease, according to medical history, ECG and laboratory results.
Able to comprehend and be informed of the nature of the study. Capable of giving written informed consent.

Exclusion criteria

Disturbed blood coagulation.
Treatment with anticoagulants or vitamin K.
Diabetes mellitus.
Rheumatoid or psoriatic arthritis.
Pregnancy.

Ethical Conduct of the Study

The study protocol, informed consent form and written information about the study for the volunteers were reviewed and approved by National Ethic Committee on 15. 05. 2008, followed by approved second version of study protocol on 13. 01. 2009 and third version of the study protocol on 29. 01. 2011. The changes of study protocol were due to the change in the dose of EPA and DHA. In the first version of study protocol the dose of EPA and DHA was 170 mg and 120 mg bid, respectively. In the second version the dose of EPA and DHA was changed to 130 mg and 80 mb tid, respectively. Moreover, the placebo was changed from soft gelatine capsules with corn oil to hard gelatine capsules with lactose. In the third version of study protocol new study centres were included (Psychiatric Hospital of Begunje and Psychiatric Hospital Vojnik) to increase number of included patients since planed number of 20 patients per group was not reached.

The study was started after volunteers had given their approvals in writing. All clinical work was conducted in compliance with Good Clinical Practice (GCP) as referred in the ICH guidelines, with the directive 2001/20/EC of the European Parliament, with the principles enunciated in the Declaration of Helsinki and with the local regulatory requirements.

All subjects voluntarily accepted to participate in this study. The clinical investigator explained the nature, purpose and risk of the study. Study design was presented to every subject and clinical investigator was available to answer all volunteers' questions. The written informed consents were obtained from all the subjects before their inclusion to the study, in compliance with the recommendations of the Declaration of Helsinki.

Study methods

Psychopathology and extrapyramidal symptoms

Psychopathology was evaluated using Positive and Negative Symptoms Scale (PANSS) and global functioning with Global Assessment of Functioning (GAF) scale. Tardive dyskinesia symptoms were assessed with the Abnormal Involuntary Movement Scale (AIMS), akathisia with the Barnes Akathisia Rating Scale (BARS), and parkinsonism with the Simpson Angus Scale (SAS).

Evaluation of patient compliance

Patients were instructed to return their unused capsules at their regular monthly visits for assessment of adjuvant therapy compliance.

Molecular genetic study

Genetic polymorphisms for superoxide dismutase (MnSOD), glutathione peroxidase (GSGPx), catalase (CAT), tumour necrosis factor-alph (TNF), cytochrome P450 2D6 (CYP2D6) and P glycoprotein (MDR1) were determined using genomic DNA extracted from patient blood and real-time PCR genotyping.

Biochemical and Pharmacokinetic analysis

The following markers of oxidative stress were determined in blood or plasma samples:

- Antioxidant enzymes: glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase were determined using spectrophotometric methods
- Markers of nitric oxide production: nitrites and nitrates were determined using spectrophotometric method
- Non-enzymatic antioxidants: reduced and oxidized forms of glutathione were determined using high-pressure liquid chromatography method
- Markers of lipid peroxidation: malondialdehyde was determined using high-pressure liquid

chromatography method
- Markers of protein oxidative damage: protein carbonyls were determined using spectrophotometric method

Plasma concentration of dopamine, adrenaline, noradrenaline, and serotonin markers were determined using high-pressure liquid chromatography method.

The concentration of haloperidol and its main metabolites e.g. reduced haloperidol, haloperidol glucuronide and 4-(4-chlorophenyl)-4-hydroxy-piperidine were determined using liquid chromatography coupled to tandem mass spectrometry.

Statistical methods

Between-group differences in continuous variables were evaluated by one-way analysis of variance. Parametric Mann-Whitney test was used in cases where distribution was not normal. Between group differences in categorical data were assessed by Chi square test, while for ordinary data Kruskal–Wallis tests were used. Significance level was set at $p < 0.05$. Statistical analyses were carried out using SPSS version 20 (IBM SPSS, Chicago, IL, USA).

Study results

Patients

52 patients were included in the study. 34 patients finished the study. None of the patients were excluded because of side effects, but because of a personal decision.

Clinical results

1. Influence of supplementation with EPUFAs and vitamin E on markers of oxidative stress and extrapyramidal symptoms

Adjuvant therapy with EPUFAs has a positive effect on increasing the concentration of glutathione in the blood, while adjuvant therapy with vitamin E and the combination of vitamin E and EPUFAs shows positive trends in the increase in blood glutathione levels. The effect of adjuvant vitamin E therapy on the decrease in the concentration of the oxidised form of glutathione and motor retardation has been identified.
The effects of adjuvant therapy on assessments of the psychiatric scales PANSS, AIMS, SAS and BARS have not been observed.

2. Association between oxidative stress markers and extrapyramidal symptoms with concentrations of haloperidol and neurotransmitters

Our study showed that increased assessment of akathisia symptoms (BARS) is associated with lower activity of the antioxidative enzyme catalase. The assessment of symptoms of akathasi is also associated with the concentration of haloperidol and its reduced metabolite. Serotonin is associated with an increase in nitrite concentration. In addition, a relationship between serotonin concentrations with negative symptoms and GAF was confirmed.

3. Association between positive and negative symptoms of schizophrenia with concentrations of neurotransmitters and markers of oxidative stress

Association between increasing dopamine concentration and increasing assessment of positive symptoms on PANSS was determined. Moreover, association between adrenaline concentration and negative and global symptoms of PANSS and GAF was confirmed. No association was found between positive and negative symptoms and markers of oxidative stress.

4. Influence of antioxidants enzymes and tumour necrosis factor-alpha genetic polymorphisms on markers of oxidative stress and extrapyramidal symptoms

TNF-308G>A was associated with the increased risk of extrapyramidal symptoms in parkinsonism. Parkinsonism was more frequent in patients who were carriers of TNF A allele.

5. Influence of CYP 2D6 and P-glycoprotein genetic polymorphism on metabolism of haloperidol

Polymorphism of CYP2D6 and MDR1 are associated with plasma concentration of reduced haloperidol. Moreover, increased concentration of haloperidol and reduced haloperidol represent a risk factor for development of akathisia symptoms.

The final results of clinical trail were calculated on 22. 3. 2013.

Adverse events
No serious adverse events were reported during the clinical trial.

The following adverse events were observed:

Adverse event	*Patient number*
Vomiting	1 patient at inclusion and during the clinical trial, 1 patient during the clinical trial
Tremor	2 patients at inclusion to the clinical trial, 1 patient during the clinical trial
Constipation	1 patient at inclusion and during the clinical trial
Stomach pain	1
Bloating	1 patient at inclusion in the clinical trial
Nightmares	1 patient at inclusion in the clinical trial
Increased salivation	1
Sexual dreams	1
Nausea	1
Increased feeling of satiety, fear of weight gain	1
Puffiness of the legs and face	1 patient at inclusion in the clinical trial

Conclusions
Our study confirms the role of oxidative stress in patients with schizophrenia on long-term haloperidol treatment. Oxidative stress affects the pathophysiology of schizophrenia and the severity of extrapyramidal symptoms, but may also be associated with disturbances in neurotransmitter systems. A positive effect of EPUFAs adjuvant therapy on an increase in the concentration of the reduced glutathione was observed, and an uncharacteristic trend of increased glutathione concentration when treated with vitamin E or with a combination with vitamin E and EPUFAs. The effect of adjuvant vitamin E therapy on the decrease in the concentration of the oxidised form of glutathione and motor retardation has been observed. We can conclude that adjuvant therapy has potential positive effects on patients with schizophrenia on depo therapy with haloperidol, but further studies are needed to confirm this theory.

Archiving
All documentation of the clinical trial is kept in the University Psychiatric Clinic Ljubljana and the University of Ljubljana Faculty of Pharmacy.

Signature of Principal Investigator and Sponsor's Responsible

We, the undersigned, hereby certify that this study was performed according to the agreed protocol, in accordance with ethical principles stated in the Declaration of Helsinki, in compliance with Good Clinical Practice as referenced in the ICH Guidelines and in accordance with requirements of European Directive 2001/20/EC and other applicable regulations.

Signature of Principle Investigator

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22/11/2021

Date

Signature of Sponsor's Representative

Assist. Prof. Tomaž Vovk, M. Pharm., Ph. D.

22. 11. 2021

Date