Beneficial effects of a nano formulation of pomegranate seed oil, GranaGard®, on the cognitive function of Multiple Sclerosis patients

Panayiota Petrou MD[1], Ariel Ginzberg PhD[1], Orli Benyamin MSc[2], Ruth Gabizon PhD[2] & Dimitrios Karussis MD, PhD[1]

[1]Multiple sclerosis Center and cell therapies Unit, Laboratory of Neuroimmunology,

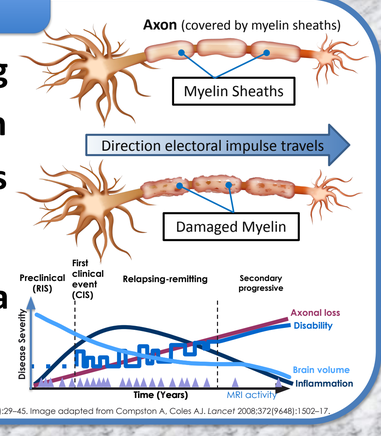
[2]The Agnes-Ginges Center for Neurogenetics, Neurology department

Hadassah University Hospital, Jerusalem

Introduction

Multiple sclerosis(MS) is a chronic demyelinating disease of the central nervous system associated with inflammation and neuro-degeneration that exhibits significant oxidative damage.

Though often neglected, cognitive impairment is a common feature of MS that affects 40-70% of patients.



Bar-Or A. *Semin Neurol* 2008;28(1):29–45. Image adapted from Compston A, Coles AJ. *Lancet* 2008;372(9648):1502–17.

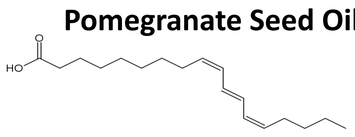
Rationale of the study

Multiple sclerosis treatments successfully reduce the systemic inflammatory component but fail to affect the degenerative component of the disease. The aim of our study is to examine the effect of PSO in MS patients as an add-on therapy. PSO is an inexpensive food additive, has no risks and can be easily used as daily oral treatment.

Background

Pomegranate seed oil (PSO) comprises, Punicic Acid (PA), a poly-saturated fatty acid, which is considered as one of the strongest antioxidants in nature. To increase its bioavailability and activity, PSO was prepared in oil-in-water nano-emulsions. This approach allows the distribution to organs and especially reach and pass the blood brain barrier

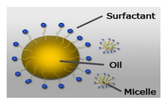
Punicic acid from Pomegranate Seed Oil



Punicic acid – among the strongest natural antioxidants

- Polysaturated fatty acid with 3 conjugated double bonds
- Can cross the BBB
- 70-80% punicic acid in PSO

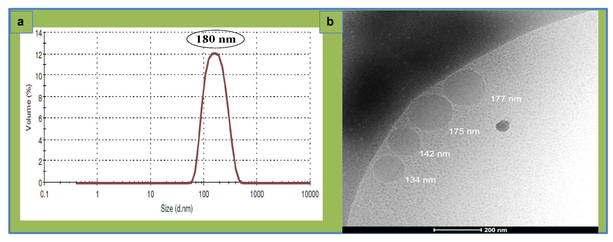
Transforming PSO into bioavaliable compound



Natural oil drops → Absorption through liver

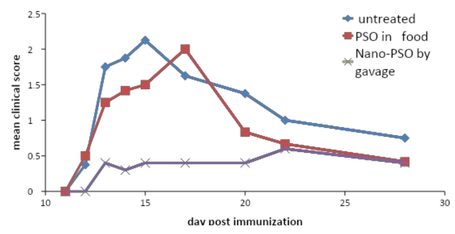
Small micelles → Highly available

Artificially formed stable and small micelles may avoid the first passage of PSO through the liver, then enter the thoracic duct and absorbed, depending on size and composition, by lymphatic organs or other cells



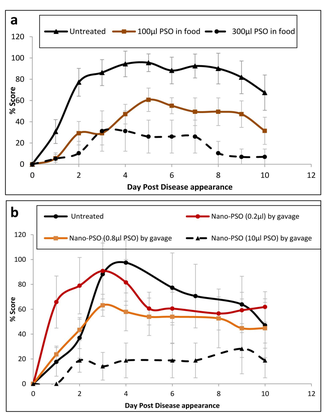
Droplet size analysis

a: DLS results for Nano-PSO; b. Cryo TEMimage of Nano-PSO.



PSO in food in low concentration <u>are not</u> effective

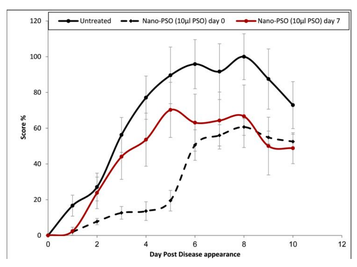
In previous studies, PSO was administered to mice afflicted with the animal model of Multiple Sclerosis. The treatment reduced demyelination and oxidation of lipids in the brains of the animals and improved their clinical disease course



Nano-PSO as an α-EAE agent

a: designated EAE induced groups were fed either with normal mouse chow (untreated group; n=10) or with chow enriched with PSO at the concentration in which 3 g (daily intake) comprise the levels designated in the figure insert; 100µl(n=10) PSO or 300µl(n=8). P<0.05 for all PSO treated groups versus the untreated group.

b: designated EAE induced groups were either left untreated or treated (by gavage) with 150µl solution comprising; 0.2, 0.8(n=6), or 10 µl(n=7) PSO in the form of Nano-PSO . P<0.05 for 0.8 and 10 µl PSO treated group versus the untreated group.



P<0.05 untreated VS day 0 until day 5

Nano-PSO in the prevention and treatment of EAE

Mice induced for EAE were administrated Nano-PSO. While one group of induced mice was left untreated (n=8), a second group was treated with Nano-PSO from day one of the induction (n=6) and a third group from day seven of the induction (n=7). Mice were scored daily for EAE signs for 2 additional weeks. P<0.05 for both Nano-PSO treatments.

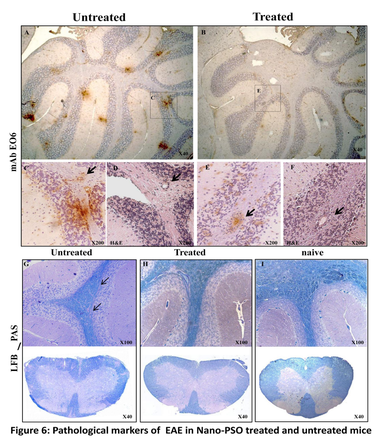


Figure 6: Pathological markers of EAE in Nano-PSO treated and untreated mice

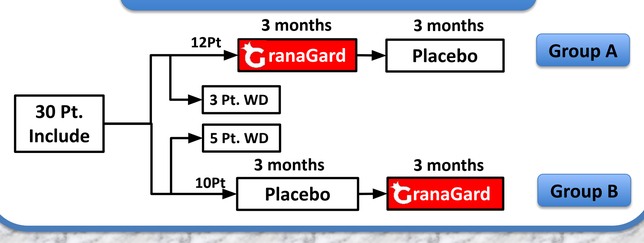
Pathological markers of EAE in Nano-PSO treated and untreated mice.

Nano-PSO treated and untreated mice were sacrificed 3 weeks after induction and their formalin fixed, paraffin embedded brain sections as well as those of age matched naive mice stained by mAb EO6 (A, B, C, E), H&E (D, F) and LFB/PAS (G-I). C& E represents an enlargement of the squares in A&B; D&F are serial section of C&E respectively. Arrows in C-F indicate blood vessel infiltration. Arrows in G represent demyelinated areas.

Methods

We included 30 MS patients, of which 15 were given GranaGard for the first three months, then placebo for three months and 15 received placebo for the first three months, and GranaGard for the following three months. All patients received GranaGard for additional six months. GranaGard®, was added to the designated MS treatment. Patients follow up included: Short quality of life and fatigue questionnaires (SF-12, MFIS-5), Expanded Disability Status Scale (EDSS), Multiple Sclerosis Functional Composite (MSFC) and cognitive tests: Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS), Symbol Digit Modalities Test (SDMT), California Verbal Learning Test (CVLT-II), Brief Visuospatial Memory Test (BVMT-R).

Study Design



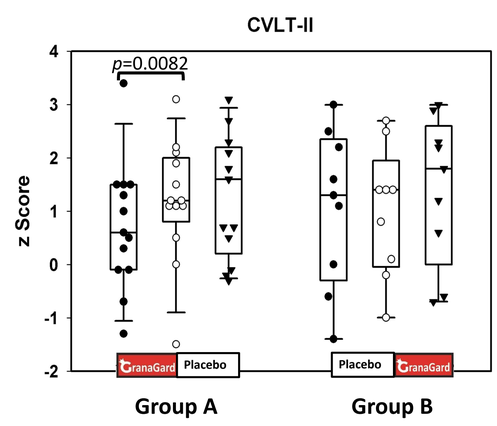
Demographics

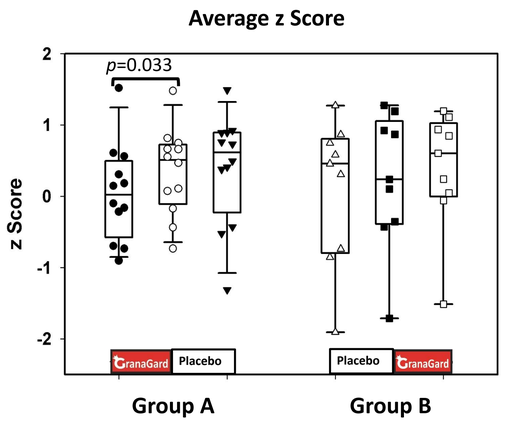
	Group A	Group B	p Value
n	12	10	
F/M	7/5	7/3	0.57
Age (y)			
mean±SD	47.7±14.0	50.1±8.9	0.62
Onset disease (y)			
mean±SD	14.8±12.0	19.6±11.5	0.18
EDSS			
mean±SD	4.5±1.9	5.0±1.2	0.81

	Group A	Group B
Type MS		
RRMS	9	7
PPMS	2	1
SPMS	1	2
DMT		
OCREVUS	4	5
TYSABRI	2	2
TECFIDERA	2	1
GILENYA	1	0
MABTHERA	1	0
AUBAGIO	1	0
PLEGRIDY	1	0
BETAFERON	0	1

Results

There was a significant beneficial effect of GranaGard®, on the verbal testing in the relevant periods of treatment. This was reflected in an increase in z score from 0.891 to 1.415 in CVLT-II (p=0.00825, paired T test) in the period that patients received GranaGard®. At that time there was no significant change in the placebo group. For the patients receiving GranaGard in the initial 3 months, the value of z score remained high (z=1.415) at the following three months, while they received placebo, suggesting a long - term effect.





Conclusions

This is the first study in which GranaGard, a brain targeted nano-formulation of PSO, was tested in humans.
Our preliminary results suggest that while no side effects were observed, GranaGard administration to MS patients under diverse treatments may improve/maintain the cognitive status of these patients.