Supplementation with a rich-polyphenols olive tree powder reduces circulating inflammatory markers, disease activity, and pain intensity in patients with rheumatoid arthritis: a 9-weeks randomized, double-blind, placebo-controlled clinical trial

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Abstract

Background- Notwithstanding the way that olive fruits polyphenols and olive leafs polyphenols have been known for their natural anti-inflammatory effect in the Mediterranean countries, there is little deep scientific study to confirm these benefits.

Objective- To assess the effect of rich-polyphenols olive tree powder (made of leafs, baby leafs, olive fruit and olive oil) on inflammatory process and pain intensity, a randomized, double-blinded, placebo-controlled trial was conducted on human subjects with rheumatoid arthritis (RA).

Methods- Seventy-nine RA patients were randomized to get either 1 g per day of olive tree powder or 1 g of placebo powder during 9 weeks. Laboratory analysis, questionnaires administration, pain intensity, disease activity score, and inflammatory biomarkers were determined at the baseline and at the end of the trial. Specialists have monitored eventual side effects and antagonistic impacts of taking the olive tree powder through the period of the study.

Results- Good compliance (over 95%) with the treatment was observed, without any side effect or study-intervention adverse. Significant decrease in disease activity score has shown at the end of intervention within the treated group, and between groups (P<0.0001). Compared with the placebo group, inflammatory biomarkers decreased significantly in treated participants (P<0.0001). Here are the changes noticed from baseline in treated group were -1.25 mg/L (CI, -1.75 to -0.75), -2.09 pg/mL (CI, -2.63 to -1.54), -0.82 pg/mL (CI, -1.14 to -0.49) and -1769 pg/mL (CI, -2254 to -1283) for hs-CRP, IL-6, TNF-α and PGE2 respectively. Additionally, it is important to note that pain

relief and global participants satisfaction increased significantly (*P*<0.0001) after 9 weeks of olive tree powder supplementation.

Conclusion- A net improvement in circulating inflammatory markers, disease activity, and pain intensity was observed in RA patients allocated to rich-polyphenols olive tree powder food supplement.

Keywords: Rheumatoid arthritis; olive tree powder; Inflammatory biomarkers; Pain intensity; Disease activity

Introduction

Rheumatoid arthritis (RA) is chronic autoimmune inflammatory disease responsible for joint destruction that contributes to functional impairment. RA remain the most common joint illness, occurring in 0.7-1% of worldwide population (Prado et al., 2018; Hresko et al., 2018). Its etiology is still not completely understood. However, several risk factors have been previously identified to be associated to RA development. Genetic factors can contribute by 50% to RA development beside other non-modifiable risk factors (presence of RA-related auto-antibodies, rheumatoid factor, and cyclic citrullinated peptide), while modifiable risk factors related to lifestyle (smoking, obesity, low fish intake, and poor dental health) participate by 41% (Nielen et al., 2004; Rantapaa-Dahlqvist et al., 2003).

Nowadays, Blockage of cytokines network –mediators of chronic inflammation– has taken a substantial proportion in the clinical management of inflammatory diseases like RA, which explain the progress of treatments from traditional nonsteroidal anti-inflammatory drugs to disease modifying anti-rheumatic drugs and biological drugs, since 1990s (Hresko et al., 2018). However, these drugs still had some unexpected adverse effects related to immune system impairment (Westra et al., 2014). Meanwhile, researchers have been also focusing on natural products as a source of alternative treatment that can stop the inflammatory flux associated to RA. In this sense, several *in vitro*, *in vivo*, and clinical studies have elucidated the effect of olive tree polyphenols –as principal components of Mediterranean Diet– on the inflammation process that characterizes RA (McKellar et al., 2007; Sköldstam et al., 2003). Thus, adherence to the Mediterranean diet decreased inflammatory activity, increased in physical function, and improved vitality in RA patients. Moreover, several *in vitro* studies have demonstrated the anti-inflammatory effect of hydroxytyrosol, tyrosol and

oleuropein (polyphenols coming olive leafs, baby leafs, olive fruits and olive oil) by acting directly on prostaglandin E_2 (PGE_2), leukotriene B_4 ($LTB_{4)}$, tumor necrosis factor-α (TNF- α), interleukin (IL)-6, IL-1 and high-sensitivity C-reactive protein (hs-CRP) (Camargo et al., 2014; Richard et al., 2011; Zhang et al., 2009). Her, we should also underline the similar anti-inflammatory effect of oleocanthal and the ibuprofen (drug from NSAIDs family) discovered by Beauchamp et al. (2005).

Although these promising results from *in vitro* and preclinical studies, more data from clinical trials are needed to prove the real anti-inflammatory effect of olive tree polyphenols in pathological cases like RA. Thus, the purpose of the present study was to verify whether supplementation with an olive tree powder rich in polyphenols could improve clinical and laboratory parameters of disease activity in Moroccan patients who have RA.

Methods

Study population

90 patients were recruited during July 2018 among those referred to rheumatology service of clinic ESSEHA in Casablanca, Morocco. In order to be enrolled to this study, patients have to be diagnosed with rheumatoid arthritis for more than one year according to the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) (Aletaha et al., 2010). The content of this study was explained in details to the voluntary participants. Patients that were not eligible were falling under the following categories: if they were pregnant, lactating, receiving contraceptive, smoking, if they were under the age of 20 years or over the age of 80 years, being diagnosed with metabolic syndrome as defined by the Adult Treatment Panel III, having inflammatory disorders, receiving NSAIDs and/or cytokine inhibitors, and having a white blood cell count $\leq 3.5\times10^9$/L, hemoglobin (Hb) level ≤8.5g/dl, platelet count $\leq 100\times10^9$/L, creatinine level ≥2.0 mg/dl and aspartate aminotransferase (AST) levels ≥2.5 times the upper normal limit. Subjects also agreed to avoid consumption of olive antioxidants and any other antioxidant supplements ≤3 weeks before and during the intervention. The history of allergy or intolerance to olive products was also considered during participants selection. Finally, a written consent was obtained from all voluntary participants before to be officially enrolled to this study.

Study design and intervention

The current study is a double-blind, randomized trial controlled by a placebo (Figure 1). Eligible participants were randomly assigned to be supplemented by the Olive Tree Powder (OTP) or the placebo using a computer-generated random-number sequence. Investigators, participants, and study personal were blinded to the type of supplement used by each group. After an overnight fasting (at least 12h), participants were invited by telephone to the clinic to undergo a screening visit including tender and swollen joints examination. The pre-examination has also included the adherence to the Mediterranean Diet by the modified questionnaire of Estruch et al (2006), the assessment of physical exercise by the International Physical Activity Questionnaire (Physical exercise was categorized as high, moderate, or low). Normal habitual diet of participants has been maintained during the study period whilst avoiding olive tree products intake (olive oil, olive table fruits) as well as nutrients with high n-3 PUFA contents (e.g. fish). The use of all herbs and products known to affect inflammation and immune function had also to be avoid throughout the study period. Dietary changes were monitored trough a 3-day dietary records at baseline, 4 and 9 weeks after treatment and placebo interventions. Study personnel provided necessary explanations about food intake estimations. Anthropometric and blood pressure data were measured and a sample of 8 ml fasting blood was obtained from each participant's antecubital vein. All examinations and measurements have been repeated at the end of the study. Participants and all study personnel (including investigators) had free and continuous access to clinic services for advices and consultations during this study.

Illegible participants have received 500 mg HPMC study capsules (the capsules for supplement and the capsules for the placebo were both the same, HPMC vegetal capsules of 500 mg). Participants received instructions about capsules taking (and storage) and were asked to administrate two capsules per day (equivalent to a dose of 1 $g.day^{-1}$) of either OTP or placebo (maltodextrin excipient) before each meal. Supplement and placebo intake was weekly controlled. Both OTP and placebo components were enclosed in a soft soluble HPMC vegetal capsules. OTP was obtained from olive leafs, olive baby leafs, olive fruits and olive oil using a purely physical extraction without the use of: solvents, purification process, or any chemicals. The temperatures implied in the process were cold (freeze drying) to preserve all the polyphenols potency. It is worth to note that this olive tree powder comes from olive trees planted in a specific rocky desert environment where temperatures attain 127 °F

in the summer, where it nearly never rains and where only little water in the dwells allow the tree survival. Also, the massive quantity of rocks do not allow olive tree roots to look for nutrients. These specific olive trees are under stress and produce abnormally high quantity of antioxidants (mainly hydroxytyrosol) to defend themselves and survive. For more details, please see www.olivie.ma.

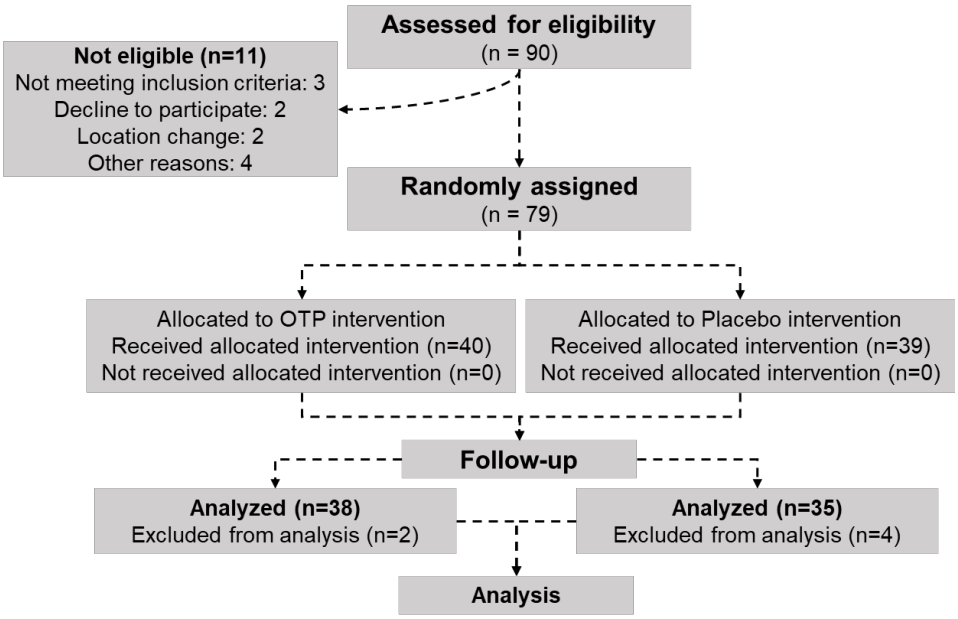

Figure 1. Study flow diagram.

Laboratory measurements and outcomes

Clinical indication of disease activity and laboratory parameters of study participants were measured at the baseline and at the end of study using internal methods of clinic ESSEHA and its associated laboratories. A calibrated scales and wall-mounted stadiometer with a precision of 0.1 cm, and a semi-automatic oscillometer (Boso Medicus smart Semi-automatic Blood Pressure Monitor, Germany) were used to measure anthropometric parameters and blood pressure. Blood samples were collected in EDTA and SST tubes. All erythrocytes, plasma, serum and urine samples were stored as 1 mL aliquots at -80°C until further analysis. Energy, nutrient intake and participants' diets assessment was carried out by Nutritionist 4.3 software (First Databank, Hearst Corp, San Bruno, CA). Serum quantification of PGE_2, LTB_4, TNF-α and cytokines IL-1 and IL-6 was performed using High-sensitivity enzyme-linked imminosorbent assay kits (DIA, Belgium), while the serum's hs-CRP level was determined by a turbidometric assay using a commercial kit at a wavelength of 500 nm.

Urinary hydroxytyrosol content, considered as marker of OTP intake, was measured by High Performance Liquid Chromatography (HPLC). First, hydroxytyrosol was extracted from acidified urine (hydrochloric acid, 0.6 N of final concentration) and then quantified by a Shimadzu chromatograph device equipped with a reverse phase C18 column (250 mm L. × 4.6 mm I.D., 5 µm) according to the protocol described by Visioli et al (2000).

A visual analog scale (VAS) has been considered to evaluate the pain intensity in this study, according to the protocol defined by DeLoach et al (1998). Participants have been instructed to draw a 100-mm line to describe their pain, 0 mm= no pain and 100 mm=most severe pain. Pain relief was assessed using a 5-point verbal rating scale (VRS) as, 0=no relief, 1=a little (perceptible) relief, 2=some (meaningful) relief, 3=a lot of relief, and 4 = complete relief. Disease Activity Score (DAS28) established by the EULAR (Wells et al., 2009), based on number of tender and swollen joint (TJC and SJC), serum hs-CRP concentration, and the result of Global Health (GH) assessed by the patient on a 10-cm VAS was calculated as follows:

$$DAS28\ (CRP) = [0.56\ \sqrt{TJC}] + [0.28\ \sqrt{SJC}] + [0.36\ Ln\ (CRP + 1)] + [0{,}014\ (GH)]$$

Possible adverse effects of OTP administration through the period of study (mouth symptoms, digestive disorders, fullness, allergic skin response, and other intervention-related symptoms) were assessed by doctors and study personnel. Finally, a 5-point categorical scale (0 = poor, 1 = fair, 2 = good, 3 = very good, and 4 = excellent) was used to assess the global satisfaction assessment in response to treatment (GAST) (including anxiety). This study was conducted according to the guidelines approved by Helsinki Declaration.

Statistical analysis

Data were statistically analyzed using GraphPad Prism version 6.00 (GraphPad Prism Inc, San Diego, California). For the baseline characteristics, continuous variables are expressed as mean values ± standard deviation (SD), and categorical variables are expressed as frequencies (percent). For inflammatory biomarkers, pain intensity, and pain relief mean values are expressed with 95% confidence intervals (CIs). Normal distribution of data was checked using the Kolmogorov-Smirnov test. The difference between baseline groups characteristic was performed by, the independent t test, the Mann-Whitney U test, and the $\chi 2$ test for normally continuous data, not normally

continuous data, and categorical data, respectively. the independent t test was also used to compare the mean changes from baseline to the end of the study (9 weeks) between OTP and placebo group. Results with two-sided P values of <0.05 were considered statistically significant.

Results

Study compliance and adverse effects

Out of the ninety eligible patients enrolled to this study, 11 were excluded before the intervention for several reasons (Figure 1). Moreover, six participants were dropped out of analysis (2 in OTP-group and 4 in placebo-group) because they were unable to follow the study protocol. No adverse reactions or side effects related to the intervention were observed and 95% compliance to the supplementation was reported. Results of Figure 2 illustrate the changes in urinary Hydroxytyrosol concentrations (used as biomarker of compliance) from initial values for both placebo and OTP groups. The content of hydroxytyrosol found in urine of OTP participant's group was significantly higher (*P*<0.0001) compared to that of placebo group. However, it is worth noting that literature data regarding olive phenols metabolism, and excretion are not in agreement (Covas et al., 2006; Visioli et al., 2003).

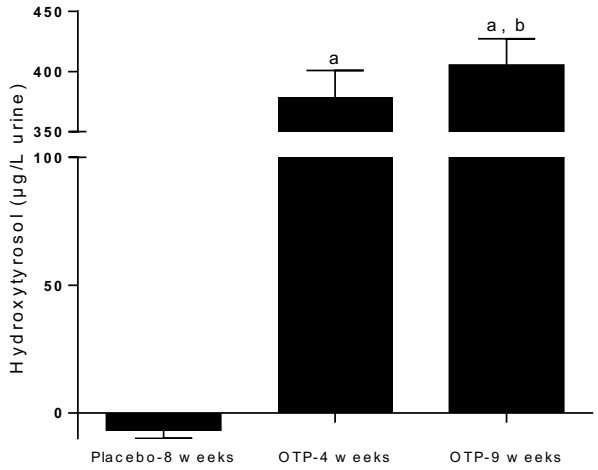

***Figure 2**. Change from baseline in urinary hydroxytyrosol excretion. Mean with 95% CI. [a]P<0.0001, between OTP-group (at 4 or 8 weeks); [b]P= 0.003, between OTP-group at 4 and 8 weeks.*

Baseline characteristics

The baseline characteristics of the 79th participants who were randomized into OTP and placebo groups are shown in table 2. No significant differences have been

revealed between the two study groups for all the baseline parameters. This include the adherence to the Mediterranean diet (*P*=0.296).

Table 2. Baseline characteristics of participants.

Parameter	OTP group (n=40)	Placebo group (n=39)	*P* value [a]
Age (years)	56.73 ± 1.61	54.31 ± 1.97	0.306
Female, n (%)	32 (80)	27 (69.23)	0.935
Weight (kg)	65.43 ± 3.06	63.54 ± 4.34	0.957
BMI (kg/m^2)	28.17 ± 1.662	27.83 ± 1.815	0.901
Disease duration (years)	7.77 ± 0.42	6.33 ± 0.56	0.356
Medical history of disease, n (%)	15 (37.50)	12 (30.76)	0.142
Family history of disease, n (%)	8 (20)	10 (25.64)	0.708
Exercise activity habits, n (%)	16 (40)	18 (45)	0.822
Alcohol drinking habits, n (%)	2 (5)	3 (7.69)	0.233
15-item Mediterranean diet score	2.04 ± 0.20	1.91 ± 0.15	0.307
DAS28	4.07 ± 0.67	4.40 ± 0.71	0.911
Pain VAS (0–100 mm)	77.11 ± 9.84	75.65 ± 9.12	0.710

Value are expressed as mean ± standard deviation or in percentage.
[a] *P* value (<0.05) by independent t-test or Mann-Whitney test.

Food, Energy, and Nutrient Intake

Results of table 3 show that there was no significant difference in diet intake at the baseline and after nine weeks of OTP and placebo supplement. Importantly, PUFAs intake was maintained constant (*P* value of 0.711 and 0.802 for OTP and placebo group). Actually, n-3 PUFAs intake may play a substantial role in the resolution of inflammation in RA (Park et al., 2013). Results of Table 3 indicate also a slight increase in participant's weight, which was no significant for both OTP (*P*=0.794) and placebo (*P*=0.906) groups. However, this was suitable for the current study, since the adipose tissue is also an active source of inflammatory cytokines (Lu et al., 2014). In general, all participants have met the daily recommended diet for this study, and the consumption of olive products and any other products known to have anti-inflammatory effects was avoided.

Inflammatory markers

Inflammation of synovial membrane is believed to be the main cause involved in RA outcomes. High concentration of circulating inflammatory markers, such as cytokines (IL-6, IL-1, TNF-α) and hs-CRP correlate with propensity to pain, warmth, redness associated to the join destruction. Changes from baseline in these markers are illustrated if the graphs of Figure 2. Significant decrease in the average hs-CRP of

participants allocated to OTP treatment compared to those in the placebo group (*P* value of 0.014 and <0.0001 after 4 and 9 weeks) has been observed. The mean changes from baseline in the hs-CRP levels were -0.56 (CI, -0,91 to -0.18) and -1.25 mg/L (CI, -1,75 to -0,74mg/L) after 4 and 9 weeks, respectively. Circulating interleukin-6, and TNF-α concentrations decreased only in the treated group, *P*<0.0001 for both parameters. The adjusted within treated group changes in IL-6 and TNF-α were -2.08 pg/mL (CI, -2.63 to -1.53) and -0.81 pg/mL (CI, -1.14 to -0.49). Compared to other parameters, changes in IL-1 were not so significative (*P* value of 0.413 and 0.084 at 4 and 9 weeks). However, this can be due to the significant decrease of plasma IL-6, leading to the stabilization of circulating IL-1 (Figure 2).

Table 3. Mean energy and nutrient intake at baseline and end of the study for tow study groups. Data are expressed as mean ± standard deviation.

Parameter	OTP group (n=45)	Placebo group (n=45)
Energy (cal)		
Baseline	1355.00 ± 209,54	1809.00 ± 130.3
9 weeks	1402.00 ± 205,11	1695.00 ± 318.4
P value [a]	0.603	0.445
Fat (g)		
Baseline	63.90 ± 11.40	78.32 ± 13.47
9 weeks	64.17 ± 11.95	75.06 ± 12.95
P value [a]	0.747	0.833
PUFAs (g)		
Baseline	11.33 ± 2.01	11.90 ± 1.22
9 weeks	12.77 ± 2.91	10.67 ± 1.43
P value [a]	0.711	0.802
MUFAs (g)		
Baseline	22.15 ± 1.87	23.22 ± 1.34
9 weeks	23.52 ± 2.23	22.54 ± 1.57
P value [a]	0.193	0.114
SFAs (g)		
Baseline	13.11 ± 1.52	12.93 ± 1.97
9 weeks	15.86 ± 1.62	15.57 ± 2.11
P value [a]	0.515	0.749
Weight (kg)		
Baseline	65.43 ± 3.06	63.54 ± 4.34
9 weeks	66.01 ± 4.77	63.97 ± 4.66
P value [a]	0.794	0.906

PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; SFAs, saturated fatty acids. [a] Paired Student *t* test (p<0.05).

Pain intensity and disease activity

RA has often been accompanied by high-intensity chronic pain. Table 4 summarizes the changes from baseline in pain intensity, pain relief as well as DAS28 in both OTP

and placebo groups. VAS (100-mm pain scale) values for the OPT-group (50.41 ± 8.86 mm) were significantly lower than those of the placebo group (76.03 ± 10.46 mm) at 9 weeks (*P*<0.0001). Similarly, pain relief score was significantly higher in the treated group compared to the placebo (P<0.0001), even after 4 weeks of intervention (Table 4). Here, we should underline that more than 30% of the OTP-group members have declared a high pain relief score (≥3), while the rest of the participants reported a meaningful pain relief (score ≥2) by the end of the study.

Table 4. Change from baseline in pain intensity, pain relief, DAS28. Data are expressed as mean and (95% CIs).

Parameter	OTP group (n=38)	Placebo group (n=35)	*P* value
Pain intensity	-26.70 (-30.39 to -20.90)	0.38 (-2.26 to 3.02)	<0.0001
Pain relief	2.14 (2.13 to 2.71)	-0.11 (-0,21 to -0,11)	<0.0001
DAS28	-1.84 (-2.43 to -2.03)	-0,13 (-0,21 to 0,09)	<0.0001
GART*	3.21 (2.93 to 3,48)	0.47 (0.28 to 0.65)	<0.0001

* Global satisfaction assessment in response to treatment. Only the average value measured at the end of the study.

Data for DAS28 show a significant decrease in disease activity reported by the treated participants compared to those allocated to the placebo (P<0.0001). Patients in OTP group with baseline active RA (DAS28 score ≥ 3.2) showed good therapeutic response by the end of the study (DAS28 score of 2.23 ± 0.54), which signal a RA remission (DAS28 score ≤ 2.6). Table 4 shows also the global satisfaction assessment in response to treatment, included the assessment of patient's anxiety. Very good satisfaction regarding the intervention (score of 3.21 of the 5-point categorical scale) was reported by participants received OTP, compared (*P*<0.0001) to those of placebo group. High degree of participant's satisfaction was correlated to a significant decrease in circulating inflammatory biomarkers level, pain intensity, and disease activity score, indicating the efficacy of the treatment with OTP.

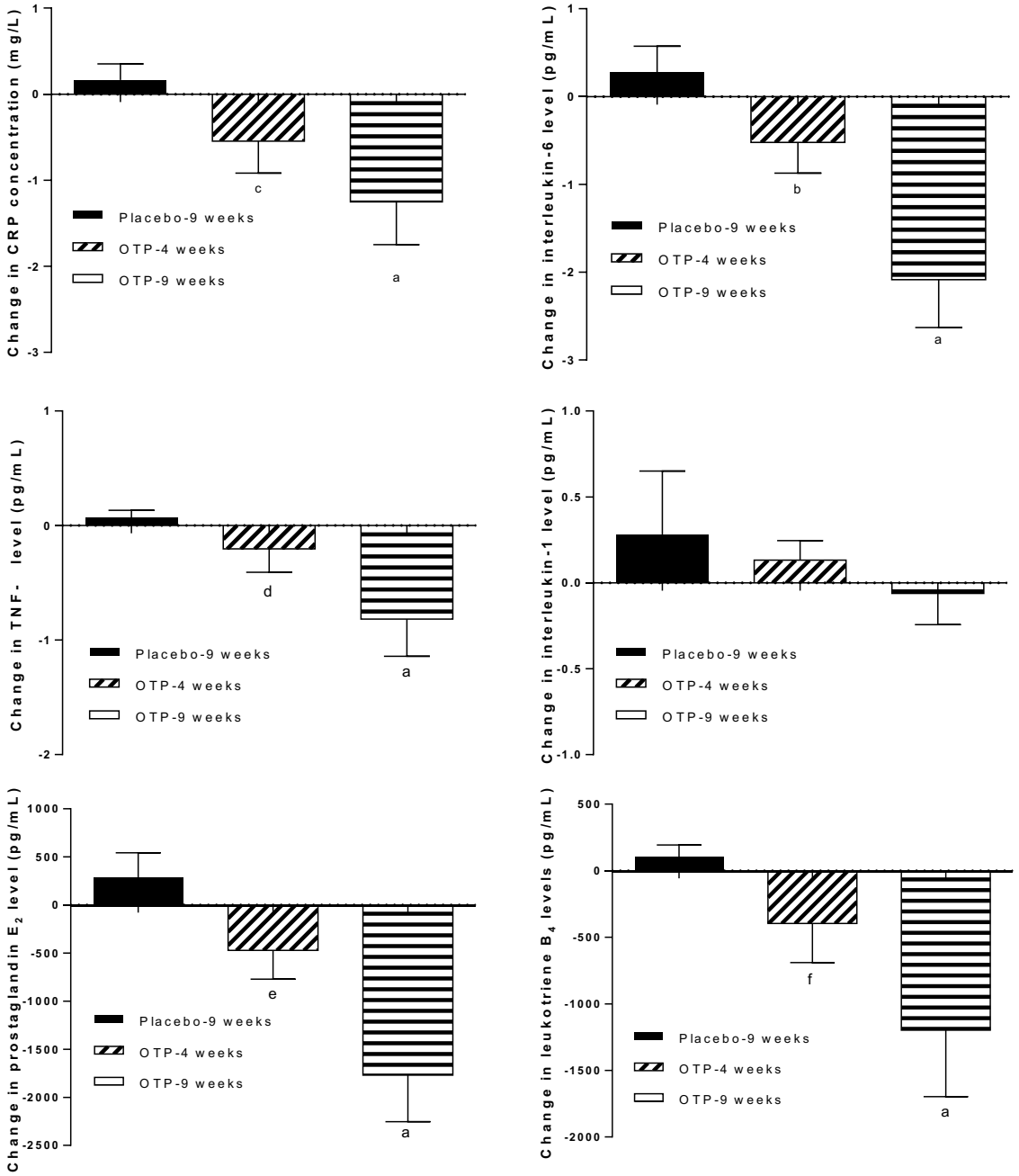

Figure 3. Change from baseline in circulating inflammatory biomarkers level in the tow study groups, hs-CRP, IL-6, TNF-α, IL-1, prostaglandin E2, and leukotriene B4. Error bars are 95% CIs. [a](P<0.0001), [b](P=0.014), [c](P=0.009), and [d](P=0.0247), e(P=0.0017), f(P= 0.0004).

Discussion

In the present study, rheumatoid arthritis patient (according to the ACR/ELUAR) were treated by a phenolic olive tree powder for 9 weeks, by receiving a daily dose of 1 g. No adverse reactions signs have been observed during the study as well as 3 weeks after (data not shown). We found that the olive tree polyphenols -carried in natural olive

tree powder- display a strong therapeutic effect against inflammation, disease activity, and joint pain associated to RA.

RA is characterized by a continuous and excessive influx of inflammatory cells into the synovial membrane. Chronic inflammation leads to cartilage damage and bone destruction (mediated by osteoclasts), which cause -finally- the loss of function. However, inflammatory reaction and osteoclasts differentiation are both mediated by a complex network of cytokines, mainly TNF-α, IL-6, IL-1, as well as other simple molecules such as eicosanoids (PGE_2 and LTB_4) (Smolen et Redluch, 2014; Boissier et al., 2012). RA treatment was evolved to the use advent of biologic treatments targeting specific immunologic pathways. Thus, it has been reported that the inhibition of TNF-α and IL-6 seems to be more efficient to predict inflammation compared to IL-1 (Smolen et Redlich, 2014). Current results demonstrate that supplementation by OTP (14% of total polyphenols and 10% of hydroxytyrosol) reduces the circulating TNF-α, IL-6, hs-CRP, PGE_2, and LTB_4 in patients with RA (Figure 2).

However, this result can be a direct consequence of OTP's polyphenols (particularly hydroxytyrosol), who may (i) act directly on DNA to reduce expression of inflammatory mediators or (ii) inhibit their biosynthesis pathways through a similar mechanism of glucocorticoids and/or NAIDs. In this sense, several studies have demonstrated the inhibitory effect that can be played by hydroxytyrosol on PGE2 levels through the repression of inducible cyclooxygenase (COX-2, key enzyme of PGE2 biosynthesis pathway from arachidonic acid) in isolated human monocytes (Rosignoli et al., 2013; Zhang et al., 2009a; Lu et al 2005) and murine macrophages (Richard et al., 2011). Moreover, hydroxytyrosol and oleuropein display a strong *in vivo* inhibitory effect against COX-2 and PGE_2 in mice with DSS-induced colitis (Sánchez-Fidalgo et al., 2011; Giner et al., 2011). Additionally, results from other cell culture models show that pure hydroxytyrosol ‑or carried in its natural matrix, like OTP and olive oil‑ can inhibit the synthesis of LTB_4, TNF-α, IL-6, IL-1 and hs-CRP (Camargo et al., 2014; Richard et al., 2011; Zhang et al., 2009a; Bitler et al., 2005; Maiuri et al., 2005). More importantly, results from clinical studies have highlighted such inhibitory effect against inflammatory markers in patients with stable coronary disease (Fitó et al., 2007; Estruch et al., 2006). Actually, results of the current study were consistent with previous *in vitro* and *in vivo* investigations (literature cited above), which explain the observed positive effect of rich-polyphenols powder from olive tree in patients with RA.

Besides, *in vitro* COX-2 targeting reduces PGE_2 level, which leads, in turn, to a decrease in circulating IL-6 in human macrophages and synovial fibroblasts (Inoue et al., 2002; Hinson et al., 1996). However, it has been known that IL-6 play an important role in the activation of inflammatory proteins like hs-CRP, which may explain the decrease in hs-CRP levels in treated participants. This was similar to the anti-inflammatory mechanism of NSAIDs drugs. Nevertheless, treatment with NSAIDs (celecoxib, rofecoxib, diclofenac) causes an increase in TNF-α contents in rheumatoid synovial membrane cultures as well as in blood (Rosignoli et al., 2013; Page et al., 2010), unlike our findings indicating a significant decrease of TNF-α levels. This mean that hydroxytyrosol and/or other OTP's polyphenols target other immunological pathway(s) leading to the decrease of IL-6 and TNF-α. Actually, Nuclear factor kappa β (NF-κβ) signaling and chronic inflammatory diseases has been in depth reviewed by Killeen et al (2014). These authors underlined the potential of hydroxytyrosol to drive new therapeutic opportunities by reducing NF-κβ activation and its nuclear translocation. NF-κβ triggers the expression of more than 150 genes including those encoding cytokines, TNF-α, IL-1, and IL-6 (Makarov, 2001). Richard et al (2011) found that the decrease of cytokines levels in murine macrophages after treatment with Hydroxytyrosol form aqueous olive extract was correlated to low expression of NF-κβp65. Hydroxytyrosol affects also NF-κβ activity in endothelial (Scoditti et al., 2012) and neural cells (St-Laurent-Thibault et al. 2011). Thus, one would think that OTP's polyphenols anti-inflammatory effect is, mainly, exerted by i) inhibiting COX-2 enzyme, and/or ii) reducing the expression of NF-κβ.

Otherwise, in RA, chronic inflammation induces proliferation of the synovium leading to formation of pannus and joint destruction, where neovascularization (angiogenesis) is major contributor (Semerano et al., 2011; Lee et al., 2001). A high correlation between RA progression and VEGF level (Vascular Endothelial Growth Factor, most important pro-angiogenic factor) has been observed in RA patients (Lee et al., 2001; Sone et al., 2001). Pro-angiogenic factors (VEGF and Angiopoietins-1) activation in the synovial membrane is a multi-targeted mechanism involving both cytokines (IL-1β and TNF-α) and COX-2 (Scoditti et al., 2012; Pettit et al., 2001). Our data (under publication process) elucidate that Hydroxytyrosol from the studied OTP modulates the angiogenic response of endothelial cells by repressing VEGF (isoforms A, B, and C), Ang-1 and Ang-2 gene expression, as it has been already stated (Scoditti et al., 2012; Fortes et al., 2012).

On the other hand, pain is a cardinal symptom in any illness state and especially the rheumatic diseases. In RA, pain intensity is strongly correlated to high PGE_2 level (Procházková et al., 2009; Scher et al., 2007; Kamei et al., 2004), explaining the effectiveness of NSAIDs as pain relief agents. Similar pain relief effect of Ibuprofen (most known NSAIDs) has been reported for oleocanthal (phenolic compound from olive oil) by Beauchamp et al (2005). The same applies to pain relief reported by participants received OTP. The decrease in circulating inflammatory markers, particularly in PGE_2 level, is likely the major responsible of pain intensity reduction observed in that group.

Conclusion

In summary, chronic inflammation and pain are the hallmark of RA. Our current findings demonstrate that the administration of rich-polyphenols extract from olive tree was associated to significant decrease of circulating inflammatory markers, pain intensity, and disease activity in RA patients. We suggest that the anti-inflammatory effect of olive polyphenols -as natural components of OTP- is linked to i) NF-κβ-dependent inhibition of cytokines (IL-6 and TNF-α), ii) like-NSAIDs inhibition of COX-2, and iii) VEGF and Ang-1 repression. Further research should focus on the anti-angiogenic activity of hydroxytyrosol in synovial membrane, as future target of new anti-inflammatory drugs based on hydroxytyrosol structure.

References

- Aletaha, D., Neogi, T., Silman, A., Funovits, J., Felson, D., Bingham III, C.O., et al. The 2010 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Rheumatoid Arthritis. Ann Rheum 2010; 69: 1580-1588.
- Beauchamp GK, Russell SJK, Diane M, Jianming L, Jana P, Qiang H, et al. Phytochemistry: Ibuprofen-like activity in extra-virgin olive oil. Nature 2005; 437: 45–46.
- Bitler CM, Viale TM, Damaj B, Crea R. Hydrolyzed Olive Vegetation Water in Mice Has Anti-Inflammatory Activity. J Nutr 2005; 135: 1475-1479.
- Boissier MC, Semerano L, Challal S, Saidenberg-Kermanac'h N, Falgarone G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction: a review. J Autoimmun 2012; 39: 222–8.
- Camargo A, Rangel-Zuñiga O, Haro C, Meza-Miranda E, Peña-Orihuela P, Meneses M, et al. Olive oil phenolic compounds decrease the postprandial

inflammatory response by reducing postprandial plasma lipopolysaccharide levels. Food Chem 2014; 162: 161-171.

- Covas MI, de la Torre K, Farre-Albaladejo M, Kaikkonen J, Fitò M, Lopez-Sabater C, et al. Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in human. Free Radic Biol Med 2006; 40: 608–16.
- DeLoach LJ, Higgins MS, Caplan AB, Stiff JL. The visual analog scale in the immediate postoperative period: intra subject variability and correlation with a numeric scale. Anesth Analg 1998; 86: 102–106.
- Estruch R. Anti-inflammatory effects of the Mediterranean diet: the experience of the PREDIMED study. Proc Nutr Soc 2010; 69: 333–40.
- Estruch, R., Martinez-Gonzalez, M.A., Corella, D., Salas-Salvadó J, Ruiz-Gutiérrez V, Covas MI, et al. Effects of a Mediterranean-Style Diet on Cardiovascular Risk Factors: A Randomized Trial. Ann Intern Med 2006; 145: 1-11.
- Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-2497.
- Fitó M, Cladellas M, Torre R, Martí J, Muñoz D, Schröder H, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr 2007; 62: 570–574.
- Fortes C, García-Vilas J, Quesada A, Medina M. Evaluation of the anti-angiogenic potential of hydroxytyrosol and tyrosol, two bio-active phenolic compounds of extra virgin olive oil, in endothelial cell cultures. Food Chem 2012; 134: 134–140.
- Giner E, Andújar I, Recio M, Ríos J, Cerdá-Nicolás J, Giner R. Oleuropein ameliorates acute colitis in mice. J Agric Food Chem 2011; 59: 12882–92.
- Hinson R, Williams J, Shacter E. Elevated interleukin 6 is induced by prostaglandin E2 in a murine model of inflammation: possible role of cyclooxygenase-2. Proc Natl Sci Acad 1996; 93: 4885–4890.
- Hresko A, Lin J and DH Solomon. Medical Care Costs Associated with Rheumatoid Arthritis in the US: A Systematic Literature Review and Meta-analysis. Arthritis Care Res. 2018; doi: 10.1002/acr.23512.

- Inoue H, Takamori M, Shimoyama Y, Ishibashi H, Yamamoto S, Koshihara Y. Regulation by PGE2 of the production of interleukin-6, macrophage colony stimulating factor, and vascular endothelial growth factor in human synovial fibroblasts. Br J Clin Pharmacol 2002; 136: 287-295.
- Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, et al. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. J Biol Chem 2004; 279: 33684-95.
- Killeen M, Linder M, Pontoniere P, Crea R. NF-κβ signaling and chronic inflammatory diseases: exploring the potential of natural products to drive new therapeutic opportunities. Drug Discov Today 2014; 19: 373-378.
- Lu B, Hiraki L, Sparks JA, Malspeis S, Chen CY, Awosogba JA, et al. Being overweight or obese and risk of developing rheumatoid arthritis among women: a prospective cohort study. Ann Rheum Dis 2014; 73: 205-459.
- Lu Y, Wahl LM. Oxidative stress augments the production of matrix metalloproteinase-1, cyclooxygenase-2, and prostaglandin E2 through enhancement of NF-kappa β activity in lipopolysaccharide-activated human primary monocytes. J Immunol 2005 ; 175: 5423–9.
- Maiuri M, Stefano D, Meglio P, Irace C, Savarese M, Sacchi R, et al. Hydroxytyrosol, a phenolic compound from virgin olive oil, prevents macrophage activation. Naunyn Schmiedebergs Arch Pharmacol 2005; 371: 457-465.
- Makarov S. NF-kappa β in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. Arthritis Res 2001; 3: 200–206.
- Mattey DL, Dawes PT, Clarke S, Fisher J, Brownfield A, Thomson W, et al. Relationship among the HLA-DRB1 shared epitope smoking and rheumatoid factor production in rheumatoid arthritis. Arthritis Rheum 2002; 47: 403-407.
- Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004; 50(2):380-6.
- Page TH, Turner JJ, Brown AC, Timms EM, Inglis JJ, Brennan FM, et al. Nonsteroidal anti-inflammatory drugs increase TNF production in rheumatoid

synovial membrane cultures and whole blood. J Immunol 2010; 185: 3694–3701.

- Park Y, Lee A, Shim S-C, Lee J, Choe J-Y, Ahn H, et al. Effect of n-3 polyunsaturated fatty acid supplementation in patients with rheumatoid arthritis: a 16-week randomized, double-blind, placebo-controlled, parallel-design multicenter study in Korea. J Nutr Biochem 2013; 24: 1367–72.
- Pettit AR, Ji H, von Stechow D, Müller R, Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001; 159 : 1689–1699.
- Prado M, Maura D. Iversen M, Yu Z, Miller Kroouze R, Triedman NA, Kalia SS, Lu B, Green RC, Karlson EW and Sparks JA. Effectiveness of a web-based personalized rheumatoid arthritis risk tool with or without a health educator for knowledge of RA risk factors. Arthritis Care Res. 2018; doi: 10.1002/acr.23510.
- Procházková M, Zanvit P, Doležal T, Prokešová L, Kršiak M. Increased gene expression and production of spinal cyclooxygenase 1 and 2 during experimental osteoarthritis pain. Physiol Res 2009; 58: 419-25.
- Rantapaa-Dahlqvist S, de Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. 2003;48(10):2741-9.
- Richard N, Arnold S, Hoeller U, Kilpert C, Wertz K, Schwager J. Hydroxytyrosol Is the Major Anti-Inflammatory Compound in Aqueous Olive Extracts and Impairs Cytokine and Chemokine Production in Macrophages. Planta Medica 2011; 77: 1890-1897.
- Rosignoli P, Fuccelli R, Fabiani R, Servili M, Morozzi G. Effect of olive oil phenols on the production of inflammatory mediators in freshly isolated human monocytes. J Nutr Biochem 2013; 24: 1513-1519.
- Salminen E, Heikkila S, Poussa T, Lagstrom H, Saario R, Salminen S, et al. Female patients tend to alter their diet following the diagnosis of rheumatoid arthritis and breast cancer. Prev Med 2002; 34: 529-35.
- Sánchez-Fidalgo S, Sánchez de Ibargüen L, Cárdeno A, Alarcón de la Lastra C. Influence of extra virgin olive oil diet enriched with hydroxytyrosol in a chronic DSS colitis model. Eur J Nutr 2011; 51: 497–506.

- Scher J, Pillinger M, Abramson S. Nitric oxide synthases and osteoarthritis. Curr Rheumatol Rep 2007; 9: 9-15.
- Scoditti E, Calabriso N, Massaro M, Pellegrino M, Storelli C, Martines G, et al. Mediterranean diet polyphenols reduce inflammatory angiogenesis through MMP-9 and COX-2 inhibition in human vascular endothelial cells: a potentially protective mechanism in atherosclerotic vascular disease and cancer. Arch Biochem Biophys 2012; 527: 81–89.
- Semerano L, Clavel G, Assier E, Denys A, Boissier MC. Blood vessels, a potential therapeutic target in rheumatoid arthritis? Jt Bone Spine 2011; 78: 118-123.
- Smolen J, Aletaha D, Redlich K. The pathogenesis of rheumatoid arthritis: new insights from old clinical data? Nat Rev Rheumatol 2012; 8: 235–43.
- Smolen JS, and Redlich K. Rheumatoid arthritis. In Rose N & Mackay I (Eds): The Autoimmune Diseases (Fifth Edition); 2014. p. 511-523.
- St-Laurent-Thibault C, Arseneault M, Longpré F, Ramassamy C. Tyrosol and hydroxytyrosol, two main components of olive oil, protect N2a cells against amyloid-b-induced toxicity. Involvement of the NF-κβ signaling. Curr. Alzheimer Res 2011; 8: 543–551.
- Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G, et al. Olive oil phenolics are dose-dependently absorbed in humans. FEBS Lett 2000; 468:159–160.
- Visioli F, Galli C, Grande S, Colonnelli K, Patelli C, Galli G, et al. Hydroxytyrosol excretion differs between rats and humans and depends on the vehicle of administration. J Nutr 2003; 133: 2612–2615.
- Wells G, Becker JC, Teng J, Dougados M, Schiff M, Smolen J, et al. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. Ann Rheum Dis 2009; 68: 954–60.
- Zhang X, Cao J, Jiang L, Zhong L. Suppressive effects of hydroxytyrosol on oxidative stress and nuclear Factor-kappa β activation in THP-1 cells. Biol Pharm Bull 2009a; 32: 578–582.

- Zhang X, Cao J, Zhong L. Hydroxytyrosol inhibits pro-inflammatory cytokines, iNOS, and COX-2 expression in human monocytic cells. Naunyn Schmiedebergs Arch Pharmacol 2009b; 379: 581–6.