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Short Communication

One year of omega 3 polyunsaturated fatty acid supplementation does not reduce circulating prothrombotic microvesicles in elderly subjects after suffering a myocardial infarction

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SUMMARY

Background & aims: Circulating microvesicles (cMV) are both effectors and biomarkers of cardiovascular disease (CVD), and the effects of omega 3 polyunsaturated fatty acids (n3 PUFA) in MV shedding are not yet well known. Therefore, we aimed to investigate the effects of long-term n3 PUFA supplementation on cMV release from cells of the vascular compartment in elderly subjects at very high risk of CVD.
Methods: We included 156 elderly patients 2–8 weeks after suffering an acute myocardial infarction from the OMEMI cohort. Subjects were randomly allocated to receive 930 mg EPA + 660 mg DHA (n3 PUFA intervention) or corn oil (56% linoleic acid, 32% oleic acid, 10% palmitic acid) used as placebo daily for two years. At inclusion and after one-year follow-up, prothrombotic [annexin V $(AV)^+$] cMV derived from blood and vascular cells were phenotyped by flow cytometry.
Results: No differences were observed in the levels of cMV between the randomized groups at inclusion in the study. After one-year follow-up, total AV^+, platelet-derived $CD61^+/AV^+$, and endothelial-derived $CD31^+/AV^+$ and $CD31^+/CD42b^-/AV^+$ cMV increased significantly in both groups. In the n3 PUFA supplemented group, platelet-derived $CD62P^+/AV^+$, $CD42b^+/AV^+$ and $CD31^+/CD42b^+/AV^+$; leukocyte-derived $CD62L^+/AV^+$, $CD45^+/AV^+$, and $CD11b^+/AV^+$, as well as endothelial derived $CD146^+/AV^+$, $CD62E^+/AV^+$, and $CD309^+/AV^+$ cMV also increased significantly. No significant differences were however, observed in the changes of cMV levels between groups.
Conclusion: In elderly Norwegians who have suffered a recent acute myocardial infarction and treated as per guidelines, long-term supplementation with 1.8 g/day n3 PUFA does not modulate prothrombotic MV release from blood and vascular cells.
Registration: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01841944.

1. Introduction

Circulating microvesicles (cMV) are a subset of large extracellular vesicles originated by cell membrane budding and released to the bloodstream by almost all cell types. Increased concentrations of cMV have been observed in plasma from subjects with cardiovascular risk factors, atherosclerosis, and both in primary and secondary fatal and non-fatal cardiovascular events [1].

Abbreviations: cMV, circulating microvesicles; n3, omega 3; PUFA, polyunsaturated fatty acids.

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Therefore, cMV are being considered a fingerprint of the onset and progression of cardiovascular disease (CVD). Some cMV expose phosphatidylserine during their biogenesis, thus conferring about 50- to 100-fold higher prothrombotic activity than platelets [2]. Whereas the mild effects of omega 3 polyunsaturated fatty acids (n3 PUFA) in platelet reactivity have been largely investigated, their involvement in secondary prevention of CVD, and more specifically though prothrombotic MV shedding, has been a matter of debate. In fact, the studies performed reported controversial or inconclusive results [3–7]. In many cases interventions were short-termed (weeks), or the doses of the n3 PUFA tested were too elevated to be sustained at the long term (around 5 g/day) [3], and only considered one or two specific MV phenotypes (of one or two cell origins). Moreover, all reported studies have been performed in middle-aged subjects. Considering that cMV exposing phosphatidylserine are prothrombotic, and the potential antithrombotic effects of n3 PUFA, we aimed to evaluate the effects of long-term n3 PUFA supplementation on prothrombotic cMV release from cells of the vascular compartment in elderly subjects with a recent acute myocardial infarction (AMI), and thereby at very high risk of CVD.

2. Materials and methods

We included the first 156 patients of the "OMega-3 fatty acids in Elderly patients with Myocardial Infarction" (OMEMI; NCT01841944, Clinical-Trials.gov) cohort [8,9]. The protocol was approved by the Regional Committee for Medical Research Ethics (ref. number: 2012/1422). The trial was conducted in compliance with the declaration of Helsinki and with the rules outlined in the guidelines for Good Clinical Practice. All participants provided written informed consent. Patients aged 70–82 years old were recruited at Oslo University Hospital Ullevål, Asker and Bærum Hospital, Vestre Viken and Akershus University Hospital Oslo, Norway, after a diagnosis of an AMI, and were included in the study 2–8 weeks after hospitalization. Patients were treated per guidelines with standard medication. Demographic data, clinical characteristics, cardiovascular history, classic cardiovascular risk factors, and current pharmacological treatment were obtained from all subjects using a standardized report form. At inclusion, patients were randomized in a 1:1 ratio to receive either 1.8 g n-3 PUFA (930 mg EPA + 660 mg DHA; Pikasol; Orkla Health, Oslo, Norway) or matching placebo (corn oil: 56% linoleic acid, 32% oleic acid, 10% palmitic acid) daily for 2 years. Subjects under n3 PUFA supplementation at inclusion were instructed to limit n3 supplements to one child spoon of cod liver oil daily [8].

Blood samples were obtained after overnight fasting and peripheral blood was withdrawn into 3.8% sodium citrate tubes for MV analysis, and into tubes for serum fatty acid quantification. Blood cells were removed by centrifuging whole blood 20 min at 2500×*g* at 4 °C. Plasma was carefully recollected, leaving about 0.2 cm of an undisturbed layer on top of the cells, and plasma and serum aliquots were immediately frozen and stored at −80 °C until processing for isolation and quantification of cMV and fatty acids, respectively. Additionally, biochemical and hematological parameters were quantified with standardized routine methods.

cMV analyses were performed between September and December 2015, and all researchers were blinded to the interventions. The randomization code was opened at the end of 2020. Phosphatidylserine exposure in the surface of the MV was characterized by annexin V (AV) binding. Therefore, prothrombotic (AV^+) cMV released by blood and vascular cells in plasma were quantified at baseline and at one-year follow-up by flow cytometry as previously described [10]. To phenotypically characterize the parental cells (blood and vascular cells) releasing the prothrombotic cMV into the bloodstream, cMV were also characterized with markers of cell lineage and activation. Cell biomarkers used for cMV phenotyping are listed in Supplementary Table 1. At baseline and at the end of the study follow-up (2 years), fatty acid profile of serum phospholipids were also measured as described in the data supplement of the reference [8].

Statistical analyses were performed using the SPSS Statistical Analysis System version 23.0 (IBM, NY). Descriptive statistics [mean ± SD or n (%)] were used to describe the baseline characteristics of the patients and the outcome variables. Changes in cMV after one-year follow-up were expressed as mean (95% confidence interval). Normality of variables was assessed with the Shapiro–Wilk test, and all variables with a skewed distribution were transformed to their natural logarithms for parametric analyses and are shown as antilogarithms to facilitate interpretation of the results. Differences in baseline concentrations of cMV between groups of intervention were analyzed with the Student's *t* test for unrelated samples. Changes in cMV after one-year follow-up within groups of intervention were analyzed with the Student's *t* test for paired samples. Differences in changes in plasma concentrations of cMV after one-year follow-up between groups of intervention were assessed by repeated measures ANOVA with the intervention group as the between subject factor. A two-tailed P-value of <0.05 was considered statistically significant.

3. Results

Detailed baseline characteristics of the 156 subjects included in this study can be found in Supplementary Table 2. Mean age was 74–75 years, approximately 75% of subjects were men and more than 95% were under dual antiplatelet therapy. No differences in baseline characteristics were observed between groups of intervention. After 2 years of intervention, the percentage of circulating EPA and DHA increased significantly at the expense of n6 PUFA in the intervention (n3 PUFA) group (Supplementary Table 3), indicating good compliance with the intervention. In addition, no differences were observed in the levels of cMV between the groups at inclusion in the study (Supplementary Table 4).

As depicted in Table 1, after one-year of intervention, total AV^+, platelet-derived $CD61^+/AV^+$, and endothelial-derived $CD31^+/AV^+$ and $CD31^+/CD42b^-/AV^+$ cMV, increased in both groups. In the n3 PUFA group, platelet-derived $CD62P^+/AV^+$, $CD42b^+/AV^+$, and $CD31^+/CD42b^+/AV^+$; leukocyte-derived $CD62L^+/AV^+$, $CD45^+/AV^+$, and $CD11b^+/AV^+$; as well as endothelial derived $CD146^+/AV^+$, $CD62E^+/AV^+$, and $CD309^+/AV^+$ cMV also increased significantly. However, no significant differences were observed in changes in any cMV phenotype between n3 PUFA and the placebo after one-year of intervention.

There were also no significant associations between changes in serum fatty acid concentrations and any cMV phenotype, also when exploring those with the most marked increase in EPA and/or DHA (data not shown).

4. Discussion

In our population of elderly Norwegians who have suffered an AMI within the last two months, daily supplementation with 1.8 g n3 PUFA for one year did not reduce MV shedding from cells of the blood and vascular compartment. In fact, after one year of n3 PUFA intervention, cMV from several phenotypes increased compared to baseline. Nevertheless, differences in these cMV concentrations did not achieve statistical significance compared to the non-significant increases of cMV in the placebo group. Overall, these results show no effect of n3 PUFA supplementation on MV

Table 1
Differences in the concentration of cMV at baseline and at one year of intervention in each study arm.

AV^+ cMV	n3 intervention (n = 80) mean (95% CI)	Placebo (n = 76) mean (95% CI)	*P*
Total	286.52 (136.51, 436.52)[a]	222.22 (10.30, 434.14)[a]	0.471
$CD142^+$	3.80 (−0.92, 8.52)	2.62 (−3.90, 9.13)	0.706
$CD61^+$	236.15 (73.88, 398.42)[a]	235.79 (20.23, 451.34)[a]	0.781
$CD142^+/CD61^+$	−0.28 (−1.63, 1.07)	0.80 (−0.61, 2.22)	0.900
$CD62P^+$	8.44 (1.88, 15.00)[a]	11.97 (−1.21, 25.15)	0.688
$CD62L^+$	9.60 (1.82, 17.37)[a]	29.72 (−16.17, 75.62)	0.626
$CD146^+$	1.05 (0.43, 1.67)[a]	0.67 (−0.32, 1.65)	0.232
$CD62E^+$	23.54 (2.29, 44.80)[a]	15.27 (−1.25, 31.79)	0.508
$CD146^+/CD62E^+$	0.39 (−0.02, 0.81)	0.19 (−0.15, 0.52)	0.763
$CD309^+$	17.61 (2.86, 32.35)[a]	16.34 (−8.44, 41.12)	0.192
$CD31^+$	162.00 (60.32, 263.67)[a]	138.70 (14.57, 262.84)[a]	0.996
$CD42b^+$	103.89 (26.61, 181.18)[a]	75.37 (−16.82, 167.57)	0.815
$CD31^+/CD42b^+$	88.92 (23.15, 154.68)[a]	67.24 (−8.15, 142.63)	0.977
$CD31^+/CD42b^-$	72.34 (23.65, 121.02)[a]	71.53 (3.99, 139.08)[a]	0.776
$CD235ab^+$	12.54 (−14.37, 39.44)	−9.34 (−32.98, 14.30)	0.114
$CD3^+/CD45^+$	1.81 (−3.95, 7.57)	0.89 (−3.42, 5.19)	0.386
$CD45^+$	15.24 (1.85, 28.63)[a]	7.82 (−3.54, 19.17)	0.995
$CD11b^+$	2.80 (0.11, 5.49)[a]	0.76 (−2.17, 3.69)	0.365
$CD14^+$	−0.59 (−3.02, 1.83)	0.90 (−1.04, 2.84)	0.804
$CD11b^+/CD14^+$	−0.84 (−1.87, 0.19)	0.26 (−0.47, 1.00)	0.457
$CD142^+/CD14^+$	−1.87 (−3.85, 0.11)	0.18 (−0.88, 1.24)	0.227

Results are expressed as mean (95% confidence interval) differences between before and after each intervention for each cMV phenotype (quantified as cMV/μL plasma). *P* from the comparison of the differences between interventions after 1 year (repeated measures ANOVA with the intervention group as the between subject factor).

[a] Significant changes after one year of intervention (Student's *t* test for paired samples).

release. This was despite satisfactory compliance to the study drug, as indicated by significant increases in serum levels of the relevant fatty acids. These results fit with the main results from the OMEMI trial [8], in which n3 PUFA supplementation (EPA + DHA) did not have a significant impact on secondary cardiovascular events. Importantly, these results endorse the potential role of cMV as biomarkers of progression and severity of CVD, and as biomarkers of the effect of diet on CVD.

The effects of n3 PUFA supplementation on MV release have been scarcely investigated. In a study performed in 46 subjects below 75 years, prothrombotic platelet-derived $CD61^+/AV^+$ and monocyte-derived $CD14^+/AV^+$ cMV concentrations decreased after the administration of 5.2 g/day of n3 PUFA for 12 weeks, compared to olive oil [3]. However, this study was performed in younger subjects after an AMI, with a short-term intervention with almost 3-fold the dose administered in our study. In 94 healthy middle-aged subjects, neither supplementation with EPA, nor with DHA for 4 weeks (1.2 g/day each intervention), did modulate $CD36^+$ cMV release [6]. However, in 84 moderate cardiovascular risk subjects aged 21–65 years, 1.5 g/day of n3 PUFA decreased the circulating levels of endothelial-derived $CD31^+/CD42b^-$ but not platelet-derived $CD31^+/CD42b^+$ MV [4]. Supplementation with 1.8 g/day of EPA only during 6 months, decreased the release of prothrombotic endothelial-derived $CD51^+/AV^+$ cMV in 126 diabetic and hyperlipidemic individuals, but not in non-diabetic hyperlipidemic subjects [5]. The disparities observed in the different studies, overall indicate that the dose of n3 PUFA supplementation might be crucial for the antithrombotic activity of the n3 PUFA, as for instance, 4 g of icosapent ethyl reduced the risk of ischemic events in the Reduce-IT study [11], whereas 1.8 g n3 PUFA did not in the OMEMI cohort [8]. Additionally, the proportion of EPA and DHA in the supplements, as well as the age and healthy status of the subjects, including use of medications such as antiplatelet agents, may significantly determine the effects of n3 PUFA on cell activation and cMV release.

This study is not exempt of limitations. Corn oil, used as placebo, is rich in n6 PUFA and may have potentially influenced the results. The population studied was mainly composed of males (75%), and consequently, sex-specific differences observed in other studies [7] could not be analyzed. In addition, our study population was Caucasian elderly subjects with a recent AMI treated as per guidelines, and were thus under double antiplatelet therapy. This guidelines-adhered best clinical practice may have rendered platelets inactivated and may have abolished any effect of n3 supplementation on prothrombotic MV shedding. The patients were also allowed to continue with one child spoon of cod liver oil if they used it before inclusion into the study, as previously discussed [8]. Our findings may therefore have limited relevance to other populations. Also, the limit of quantification of the cytometer used is around 0.2 μm, and therefore smaller MV that could be modulated by the interventions were not quantified.

5. Conclusion

In this elderly Norwegian population who recently had suffered an AMI, one-year supplementation with n3 PUFA did not have any effect on prothrombotic MV release to the bloodstream. Thus, our results do not support that supplementation with omega 3 PUFA reduce thrombotic risk through a MV-mediated mechanism in elderly subjects under dual antiplatelet therapy.

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Author contributions

Conceptualization, G.C.-B., L.B., and I.S.; data curation, K.L., V.B, and S.S.; formal analysis, G.C.-B., E.B-S, and V.B.; funding acquisition, H.A, and I.S.; investigation, G.C.-B., and V.B.; methodology, G.C.-B. and V.B.; resources, H.A., S.S., and I.S.; software, G.C.-B.; supervision, H.A.; writing—original draft, G.C.-B.; writing—review and editing, L.B., and I.S; final approval: all authors.

Conflict of Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.10.007.

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