LETTER TO THE EDITORS

Increased systemic exposure of once-daily tacrolimus in renal transplant recipients on marine omega-3 fatty acid supplementation

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Dear Editors,

High daily doses of marine omega-3 fatty acids (FAs) from fish and seafood may have beneficial cardiovascular effects in renal transplant recipients (RTRs) [1,2]. A recent randomized clinical trial demonstrated that daily supplementation with high-dose marine omega-3 FAs lowered plasma triglyceride and C-reactive protein levels [1]. In some parts of the world, high intake of marine omega-3 FAs is recommended for the general population and it is likely that RTRs use omega-3 FA supplements without consulting a transplant physician [2]. Previous reports indicate increased systemic exposures of cyclosporine and sirolimus in patients receiving high-dose marine omega-3 FA supplements [3,4], which could be related to reduced drug metabolism supported by the *in vitro* experimental observation of an inhibitory effect of omega-3 FAs on cytochrome P450 (CYP) 3A enzymes [5] expressed in the intestine and liver. The present study aimed to examine the effect of marine omega-3 FAs on the pharmacokinetics of tacrolimus.

A total of 15 RTRs included in the ORENTRA-trial (ClinicalTrial.gov identifier NCT01744067) treated with once-daily tacrolimus were recruited for this prospective pharmacokinetic sub-study at one-year post-transplant [1]. The study design of the ORENTRA-trial and the immunosuppressive protocol has been described previously [1]. The present sub-study was approved according to ethical research laws in Norway and performed in accordance with the Declaration of Helsinki. All patients provided sub-study specific written informed consent. After a wash-out period of 4 weeks from the last study visit in the ORENTRA-trial, an 8-h pharmacokinetic investigation of tacrolimus was performed. Following this investigation, all patients were administered 2.6 g marine omega-3 FAs (Omacor®, Pronova Biopharma) daily for 4 weeks, after which the 8-h pharmacokinetic investigation was repeated. Prior to these investigations, the association between the tacrolimus area under the concentration-time curve (AUC) from 0 to 24 h and the abbreviated AUC from 0 to 8 h was assessed. The association was strong (Spearman´s $\rho = 0.97$, $P < 0.001$) and ratified the use of AUC_{0-8} to investigate the potential interaction. Blood samples were collected in the morning prior to the administration and then again at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 h after drug intake. Patients abstained from food and drugs from the evening before the investigations. Breakfast and administration of marine omega-3 FAs and other concomitant medications were allowed after 2 h. Blood samples were drawn in EDTA vacutainers (4 ml Vacuette® K_2EDTA) and stored at −80 °C until analysis. Whole blood concentrations of tacrolimus were determined at Oslo University Hospital using liquid chromatography–tandem mass spectrometry as previously reported [6]. Trough concentrations (C_0), peak concentration (C_{max}) and time to C_{max} (T_{max}) are the actual observed values. The AUC was calculated at steady state in accordance with the log-trapezoidal rule. Natural logarithmic transformation was used prior to statistical analysis for all individual pharmacokinetic parameters, except T_{max}. Geometric mean ratios with 90% confidence interval (CI) for AUC_{0-8} and C_{max} of

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tacrolimus with and without daily marine omega-3 FA administration were calculated in accordance with the European Medicines Agency guidelines for bioequivalence studies of narrow therapeutic index drugs [7,8]. Paired sample t-test and Wilcoxon signed-rank test were used to compare means of variables, for parametric and nonparametric data analyses, respectively. The estimated glomerular filtration rate (eGFR) was assessed using the Modification of Diet in Renal Disease.

As one patient withdrew from the study and two patients had their tacrolimus dose changed during the study period, the final number of patients available for analysis was reduced to 12 patients (mean age of 56 ± 14 years, two women and 10 men). Eleven patients received treatment with mycophenolate mofetil, and three patients expressed functional CYP3A5 enzymes. Concomitant medications remained unchanged and no adverse events were reported. There were no changes in the eGFR ($P = 0.052$) during the study period. Mean whole blood concentrations versus time profiles of tacrolimus before and after 28 days of high-dose marine omega-3 FA supplementation are shown in Fig. 1. Daily administration of marine omega-3 FAs induced a 25 ± 30% increase in $AUC_{0–8}$ ($P = 0.02$) and a 22 ± 25% increase in C_{max} ($P = 0.01$). The bioequivalence criteria of a 90% CI within 90%-111% and 80–125% for AUC and C_{max}, respectively, were not fulfilled. Mean with and without marine omega-3 FA supplementation $AUC_{0–8}$ and C_{max} geometric ratios were 1.22 (90% CI: 1.09–1.35, $P = 0.02$) and 1.20 (90% CI: 1.10–1.31, $P = 0.01$), respectively. Tacrolimus trough concentrations numerically increased from 5.5 ± 1.2 to 6.3 ± 1.8 μg/l after marine omega-3 FAs supplementation ($P = 0.19$).

The main finding in the present study was that the systemic exposure of once-daily tacrolimus was increased on average by 25% after daily high-dose marine omega-3 FA supplementation. The mechanism for the interaction between tacrolimus and omega-3 FAs could not be fully elucidated using the present study design. The findings in this study are in line with previous observations of increased exposure of cyclosporine and sirolimus in RTRs receiving omega-3 FA supplements [3,4]. Previous experimental studies that investigated the effects of FAs on CYP3A activity both *in vitro* and *in vivo* have reported that FAs inhibit the activity of intestinal CYP3A enzymes [5,9]. Since tacrolimus, cyclosporine and sirolimus are mainly metabolized by CYP3A, inhibition of these enzymes in the intestine and liver leads to an increased bioavailability of these immunosuppressive drugs. As clearance appeared to be unchanged in our study

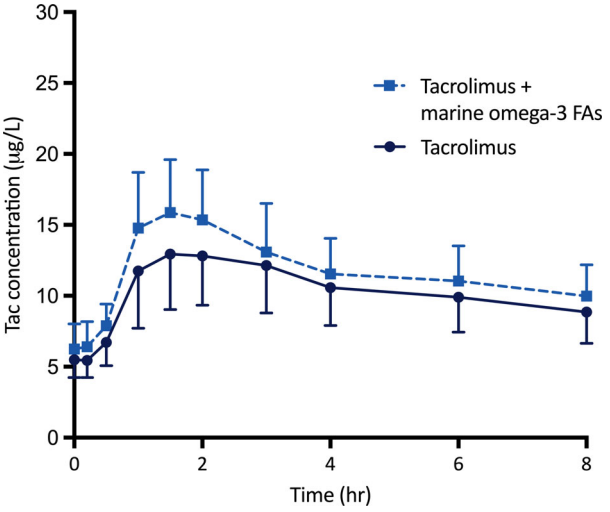

Figure 1 Mean (SD) concentration–time profiles of tacrolimus ($n = 12$) before and after high-dose marine omega-3 fatty acids supplementation for 4 weeks. Patients were treated with individualized doses of once-daily tacrolimus (Advagraf®, target C_0 of 3–7 μg/l) for at least 7 days before the first pharmacokinetic investigation. Doses were kept unchanged between the two sub-study pharmacokinetic investigations.

(similar curve profiles in the elimination phase), it is plausible that the increased systemic tacrolimus exposure may have been caused by an increased bioavailability, either due to the inhibition of intestinal CYP3A enzymes or as an effect of greater drug absorption. Intravenous administration of tacrolimus could help us to better understand the actual underlying mechanism and to differentiate potential differences in the inhibitory effect on intestinal and hepatic CYP3A enzymes. Physicians should be aware that this clinically relevant increase in the systemic exposure of tacrolimus may not necessarily induce a similar increase in trough concentrations [10], which make it difficult to monitor tacrolimus levels following the initiation and discontinuation of marine omega-3 FA supplementation. Patients who are using or considering using marine omega-3 FA supplements should be informed of this interaction.

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Conflict of interest

The authors have no conflict of interest to disclose.

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