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Seasonal variation in blood drug concentrations and a potential relationship to vitamin D

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Abstract

The most important enzyme in hepatic drug metabolism is cytochrome P450 (CYP) 3A4. Published *in vitro*-data indicate that vitamin D may up-regulate the expression of the *CYP3A4* gene. Individual vitamin D-levels are highly dependent on sunlight exposure and show great seasonal variability in northern countries. The aim of the present study was to investigate whether plasma concentrations of CYP3A4 drug substrates exhibit seasonal changes compatible with a stimulatory effect of vitamin D on drug metabolism. Three immunosuppressants (tacrolimus, sirolimus, and cyclosporine) were analysed, as these CYP3A4 drug substrates are subject to long-term use and repeated concentration determinations. In addition, mycophenolic acid was included in the analysis as a control drug independent of CYP3A4 metabolism. Concentration-to-dose ratios were extracted from the Karolinska Therapeutic Drug Monitoring database, and compared between the three-month-periods of lowest and highest vitamin D levels. Sirolimus and tacrolimus levels showed seasonal variability highly consistent with changes in vitamin D; i.e. significantly lower drug concentrations in July-September than in January-March. As expected, no significant difference was evident for mycophenolic acid but this was also the case with cyclosporine, possibly due to cross-reactivity of CYP3A4-mediated metabolites with the immunoassay used for quantification. In conclusion, there is cyclic variation in blood levels of important immunosuppressants over the year that correlates with UV-light dependent changes in vitamin D levels. Even though a causal relationship remains to be established, it is suggested that individual differences in vitamin D may contribute to variability in drug metabolism and disposition.

Introduction

CYP3A4 is the most important drug metabolising cytochrome P450 enzyme with regards to hepatic and intestinal expression, as well as the number of identified drug substrates (Evans and Relling, 1999; Daly, 2006). The activity of this enzyme is known to show a significant variability not only between different individuals but also within the same individual at different time points. The reason for this is unknown, but a recent study on the upstream regulatory region of the *CYP3A4* gene suggests an extraordinary potential for interactions with exogenous and endogenous ligands of different nuclear receptors (Qiu et al., 2010).

In vitro-studies have indicated that vitamin D may induce the expression of CYP3A4 by a vitamin D-receptor mediated increase in gene transcription (Schmiedlin-Ren et al., 1997; Fan et al., 2009), and this would result in increased metabolism of CYP3A4 drug substrates. Indeed, these molecular findings suggest a potential influence of vitamin D on the turnover of many drugs, but the relevance for drug exposure in patients is yet completely unexplored. Vitamin D is actually not a true vitamin since it can be formed *in vivo* from endogenous 7-dehydrocholesterol. This synthesis takes place in the skin and is dependent on sunlight (UV-B) exposure. Since the amount of sunlight varies widely between summer and winter especially in countries closer to the arctic circles, plasma levels of vitamin D exhibit cyclic changes over the year (Landin-Wilhelmsen et al., 1995; Virtanen et al., 2010). Compared to the endogenous synthesis of vitamin D, the dietary intake of vitamin D is quantitatively less important and does not abolish the seasonal variation in serum vitamin D levels (Lund and Sorensen, 1979; Virtanen et al., 2010).

The aim of the present study was to test the hypothesis that plasma concentrations of typical CYP3A4 drug substrates exhibit seasonal variations compatible with enzyme induction by vitamin D. Focus on the immunosuppressants tacrolimus, sirolimus, and cyclosporine was motivated by their CYP3A4-dependent metabolism, and that most patients on these drugs are subject to long-term use and repeated concentration determinations.

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Methods

Drug concentrations

Study data was extracted from a large routine therapeutic drug monitoring (TDM) service database within Clinical Pharmacology, Karolinska University Hospital Laboratory. This laboratory is quality-assured by Swedish authorities (SWEDAC) and participates in external quality control programs. All routine blood concentration measurements of tacrolimus, sirolimus, cyclosporine (based on commercial immunoassays) or serum concentrations of mycophenolic acid (based on chromatography) performed between January 1, 2000 and May 31, 2010, were screened. Samples meeting the following criteria were included in the analysis; sample drawn at the end of a normal dosage interval (trough value), specified dosage of the analysed immunosuppressant, and patients being at least 18 years old. Mycophenolic acid was included as a negative control, since its elimination is known to be independent of CYP3A4 (Fulton and Markham, 1996).

For each sample, the dose-corrected drug concentration (C/D ratio) was calculated by dividing the serum concentration of the immunosuppressant by the daily dose. In the main analysis, the C/D ratio of each drug was compared between the three months associated with the lowest endogenous levels of vitamin D (January, February and March) and the three months with the highest levels (July, August and September) (Virtanen et al., 2010). To verify the robustness of the results, these comparisons were performed using both unpaired and paired analysis methods. In the unpaired analysis, all samples drawn during any of the two time intervals were included and the dose-corrected plasma concentrations were compared using the Mann-Whitney U test. If a patient contributed with more than one sample in January-March or in July-September, these C/D ratios were substituted with an individual median value for the three-month-period. The paired analysis included only ratios from patients contributing with samples in both of the two time periods. In this analysis, the Wilcoxon signed ranks test was used to calculate the median within-patient change in dose-corrected drug concentration between January-March and July-September.

When analyses indicated a significant seasonal change in exposure to a drug, the temporal pattern was further investigated by calculating median C/D ratios for each of the twelve calendar months, presented graphically as deviations from the year-average. To avoid correlated samples and over-weighting of patients subjected to repeated sampling, multiple values from the same calendar month were replaced with a median value, as described above.

In all analyses, p-values <0.05 (two-sided) were considered statistically significant. Data extraction was performed using Crystal Reports XI, Business Objects Software Ltd, and statistical analyses were performed using StatsDirect statistical software version 2.7.2 (StatsDirect, Sale, Cheshire, UK).

UV-light data and vitamin D levels

Data on UV-light exposure in the Stockholm area of Sweden was obtained from The Swedish Meteorological and Hydrological Institute (SMHI) and average monthly UV-light exposure was calculated using data from the years 2000-2008.

Published data on serum vitamin D levels in a Finnish cohort of 1136 participants (aged 53-73) was obtained by correspondence with Dr Virtanen and co-workers (Virtanen et al., 2010). These data were used to identify the time period associated with the highest levels of vitamin D (July-September) and the period with the lowest levels (January-March). This was also, to a great extent, in accordance with data reported from other European countries at similar latitudes; more specifically in Scandinavian countries and the United Kingdom (Lund and Sorensen, 1979; Finch et al., 1992; Landin-Wilhelmsen et al., 1995) .

Ethical approval

The study was approved by the Regional Ethics Committee at Karolinska Institutet, Stockholm (#2010/1735-31/2).

Results

A total of 39 316 tacrolimus concentrations (from 1671 patients) meeting the inclusion criteria were identified in the database. The corresponding numbers for sirolimus, cyclosporine and mycophenolic acid were 3239, 24 414, and 1305, from 344, 1555 and 530 patients, respectively.

The comparison of drug exposures between the three months with the lowest vitamin D levels and the three months with the highest levels is presented in table 1. Both tacrolimus and sirolimus exhibited significantly lower dose-corrected concentrations in July-September compared to January-March. For tacrolimus, the reduction was statistically significant in both paired and unpaired analyses, with a similar magnitude of 5-7%. For sirolimus, the reduction was more pronounced, 17% in the unpaired comparison and 8% in the paired. However, the smaller number of included samples rendered the analysis a lower statistical power, and the change in sirolimus C/D ratio reached statistical significance only in the unpaired comparison. The large fraction of patients who actually exhibited a reduction in drug concentration between winter and summer was virtually identical for tacrolimus (62%) and sirolimus (63%). There was no evidence of a change in cyclosporine or mycophenolic acid concentration between the two time periods.

The two drugs showing a significant seasonal change in blood concentration were further analysed by calculation of C/D ratios separately for each month (figure 1C-D). Both tacrolimus and sirolimus showed a very similar pattern with above-average dose-corrected concentrations during the period December-April and predominantly below-average concentrations during May-September. The changes in drug concentration closely mirrored those of vitamin D in plasma (Figure 1B) and although the temporal pattern was very similar for both drugs, the amplitude was several-fold larger for sirolimus. Figure 1A demonstrates the monthly UV-light exposure in Sweden with a peak during the summer period. Maximum vitamin D levels (Figure 1B) are reached somewhat later due to an accumulation of vitamin D in the late summer. The half-life of vitamin D is reported to be approximately 1 month (Heaney et al., 2008) resulting in a delayed decline in vitamin D-levels during the early autumn.

Discussion

The data presented in this investigation support the hypothesis that seasonal changes in UV exposure and vitamin D has an impact on CYP3A4-dependent drug metabolism. Accordingly, the bioavailability of sirolimus and tacrolimus is reduced during the summer. The findings indicate a novel mechanism behind inter- and intraindividual differences in drug disposition that needs to be challenged by other data sets and study designs. However, the current approach to utilize accumulated data in a large TDM-database indeed offered enough statistical power to detect even subtle differences between different months during the year (Fig 1).

In contrast to the seasonal variation of tacrolimus and sirolimus, the concentrations of two other immunosuppressants, cyclosporine and mycophenolic acid, did not show any significant seasonal change from winter to summer. While being used essentially by the same patient category as the other three drugs, mycophenolic acid is metabolised primarily by glucuronidation and not by CYP3A4 (Fulton and Markham, 1996). Hence, it was included to serve as a negative control.

One possible explanation for the diverging effect of vitamin D on measured cyclosporine, tacrolimus and sirolimus concentrations is that the immunoassays used for quantification may differ with regard to analyte specificity. Commercially available cyclosporine assays have been shown to cross-react with major cyclosporine metabolites and co-detection of CYP3A4-mediated metabolites could effectively obscure the association between enzyme induction and measured drug concentrations (Stettin et al., 2006). To examine whether different contributions of drug metabolites in the three analyses could explain the seemingly heterogeneous effects of sun exposure, we utilised validation data collected at the laboratory as the cyclosporine, tacrolimus and sirolimus immunoassays were replaced by highly specific LCMS/MS methods in June 2010. In a series of cyclosporine samples analysed with both the immunoassay and the LCMS/MS method, the former resulted in concentrations 35% higher than the latter indicating a substantial cross-reactivity with cyclosporine metabolites. For tacrolimus, the corresponding value was +20% while there was no indication of cross-reactivity with sirolimus metabolites (unpublished data). These differences in assay specificity are in good

correspondence with the differential influence of sun exposure and indicate that the cyclosporine and tacrolimus results may underestimate the true effect of CYP3A4 induction. To evaluate the impact of the assay performance, we repeated the unpaired main analysis in cyclosporine samples analysed with the newly introduced LCMS/MS method and found a seasonal effect very similar to that of sirolimus in the original analysis, with 17.6% lower C/D ratios in the summer compared to the winter (p=0.028). Although this clearly supports the notion that co-detection of cyclosporine metabolites could have diluted the results obtained by the immunologic method, the re-analysis was based on a small number of samples (n=272) and larger materials are required to allow firm conclusions.

Another factor that could have contributed to the lack of effect on cyclosporine concentrations might simply relate to the route of drug administration, and that cyclosporine more often is administered intravenously compared to tacrolimus and sirolimus (Swedis on line, 2010). A predominant effect of vitamin D on first pass elimination in the intestinal wall and in the liver would then be lost, and this could have influenced the results. Indeed, a *post hoc* reanalysis of only those cyclosporine samples in which oral administration could be verified, indicated a trend towards the seasonal shifts noticed for tacrolimus and sirolimus, albeit not statistically significant (data not shown).

In addition to differences in assay performance and drug administration routes, the individual pharmacokinetic properties of cyclosporine, sirolimus and tacrolimus may have contributed to the heterogeneous results. Although all three drugs are primarily metabolised by CYP3A (Lampen et al., 1998; Iwasaki, 2007; Staatz et al., 2010), they are also substrates of the drug efflux pump P-glycoprotein (P-gp), which limits oral bioavailability by pumping absorbed drug molecules back to the intestinal lumen (Lo and Burckart, 1999). Similar to CYP3A4, P-gp is induced by vitamin D, but at the vitamin D concentration investigated ($1,25(OH)_2$vitamin D_3 100 nmol/L) the level of P-gp induction is much lower than that of CYP3A4 (Fan et al., 2009). If the relative influence of CYP3A4 and P-gp on the overall bioavailability varies between cyclosporine, sirolimus and tacrolimus this could affect their sensitivity to vitamin D-associated enzyme induction. Although the relative contribution of CYP3A4 and P-gp has not been well-characterised for these drugs, their dissimilar bio-

availabilities (cyclosporine 30-60%, sirolimus 14%, tacrolimus 20-25%) indicate potentially important differences with regard to first-pass metabolism and/or drug efflux (AstellasPharma, 2010; Novartis, 2010; Wyeth, 2011). Furthermore, at therapeutic doses cyclosporine is in itself an inducer of CYP3A4 and an inhibitor of P-gp gene expression and this could theoretically have limited the potential for further induction of its first-pass metabolism by vitamin D (Lo and Burckart, 1999; Staatz et al., 2010).

Compared to tacrolimus and sirolimus, the blood concentrations achived at therapeutic doses of cyclosporine are approximately ten times higher (100 nmol/L vs 10 nmol/L for the former drugs), a difference that could have implications for the molecular interactions with drug metabolising enzymes and drug transporters. Although the blood drug concentrations are well below the associated K_m for CYP3A4 (4-7 μmol/L for cyclosporine, tacrolimus, and sirolimus) (Lampen et al., 1995; Lampen et al., 1996; Lampen et al., 1998) and P-gp (8 μmol/L for cyclosporine) (Saeki et al., 1993), the intracellular concentrations in enterocytes and hepatocytes may be higher resulting in a transition to non-linear kinetics for cyclosporine.

It should be noted, that cyclosporine, tacrolimus and sirolimus are not only metabolised by CYP3A4 but also by CYP3A5. Although CYP3A5 is induced by vitamin D, the induction is less pronounced than that of CYP3A4 (Schmiedlin-Ren et al., 1997) and the CYP3A5-mediated metabolism might be devoid of a pronounced seasonal variation related to UV light exposure. However, the majority of the Swedish, primarily Caucasian population do not express the CYP3A5 gene and this pathway is expected to be of little importance for the first-pass metabolism and elimination of immunosuppressants in the included patients (Mirghani et al., 2006). On the other hand, patients of African descent, a group at high risk of vitamin D deficiency during periods of low sunlight exposure, commonly express functional CYP3A5 and this additional pathway could theoretically curb the influence of UV light exposure on the turnover of immunosuppressants (Mirghani et al., 2006).

In vitro, CYP3A4 induction has been demonstrated at 25-(OH)-vitamin D_3 concentrations from 250 nmol/L and reaches a maximum at 5 µmol/L (Schmiedlin-Ren et al., 1997). In human blood, the concentration of 25-(OH)-vitamin D_3 is generally lower, with population mean values ranging from 65-100 nmol/L in samples collected during the summer (Virtanen et al., ; Lund and Sorensen, 1979; Burgaz et al., 2009; Christensen et al., 2010) . However, it is generally difficult to draw reliable conclusions regarding concentration-effect relationships from in vitro studies and the apparent concentration discrepancy does not exclude the possibility of enzyme induction *in vivo*. For example, the blood concentration may differ substantially from that in hepatocytes and enterocytes where the induction processes take place. In addition, in vitro experiments have addressed the inducing properties of 25-(OH)-vitamin D_3 and 1,25-$(OH)_2$-vitamin D_3 (a more potent inducer of CYP3A4) separately while the induction *in vivo* reflects the combined effect of the two forms of vitamin D.

An obvious limitation of this study is that seasonal vitamin D data originated from a geographically related but still different population than the patients in the TDM database. Hence, confirmation of a direct relationship between vitamin D levels in plasma and drug exposure requires further studies in which intraindividual comparisons are made between vitamin D and drug levels or other biomarkers of drug metabolic capacity. In addition, the possible effect of exogenous vitamin D intake on drug metabolism deserves further attention.

The clinical significance of the observed seasonal change in C/D ratios of tacrolimus and sirolimus is unclear at this stage. The relative effect, despite being statistically significant, is limited and these immunosuppressants are also subject to routine monitoring by concentration determinations. However, a seasonal change in drug exposure might be of greater importance in other conditions where TDM is rarely used, such as cardiovascular disease or diabetes. This notion gains some support from a small study by Negero and co-workers, in which increased blood pressure was noted during co-administration of activated vitamin D and the calcium channel blocker nifedipine (Negoro et al., 2007). However, drug levels were not measured and the mechanism of the proposed interaction could not be established.

In summary, we propose a mechanism behind inter- and intraindividual variability in drug disposition that would explain the observed changes over time in blood concentrations of important immunosuppressants. Seasonal differences in UV-light and the resulting impact on individual vitamin D-levels may lead to seasonal changes in the activity of enzymes and transporters of major relevance for drug disposition. Clearly, this hypothesis needs to be tested by additional studies based on different methodological approaches.

Authorship contributions:

All four authors contributed to study design, data analysis and writing of the manuscript.

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Footnote:

The authors have no conflict of interest to declare.

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Legends to figure

Figure 1

A: Monthly UV-radiation in Stockholm, Sweden.

B: Monthly serum levels of vitamin D (25OH vitamin D) in a Finnish cohort (n=1136). Raw-data kindly obtained from Dr Virtanen and co-workers (Virtanen et al., 2010).

C: Monthly sirolimus concentration-to-dose ratios in patients monitored at Karolinska (n=344 patients).

D: Monthly tacrolimus concentration-to-dose-ratios in patients monitored at Karolinska (n=1671 patients).

All values are presented as deviations from the yearly average. Lines represent moving average of three adjacent months.

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Table 1

Change in dose-corrected concentration (C/D ratio) of four immunosuppressants between winter (January-March) and summer (July-September). N refers to the total number of values included in the analysis (i.e. two per patient in the paired comparison).

	Unpaired test			Paired test		
	n	Change in C/D ratio	P-value	n	Change in C/D ratio	P-value
Tacrolimus	2439	-4.9% (-8.8; -0.9)	0.02	1898	-6.9% (-9.0; -4.9)	<0.0001
Sirolimus	366	-17.2% (-25.3; -4.0)	0.007	184	-8.1% (-16.5; 0.3)	0.06
Cyclosporine	2020	2.0% (-2.8; 4.4)	0.67	1418	0.7% (-1.5; 2.9)	0.52
Mycophenolic acid	397	1.2% (-12.4; 19.3)	0.71	106	-6.1% (-27.4; 14.5)	0.55

Figure 1 Lindh et al.