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Drug monitoring in systemic lupus erythematosus

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Abstract

Therapeutic drug monitoring (TDM) is not yet accepted by systemic lupus erythematosus (SLE) treatment guidelines. Studies in SLE, however, have proven benefit in three areas: identification of non-adherence or poor adherence; targets for clinical benefit; and ranges of toxicity. This review covers the data on three medications commonly used for SLE, drawing on studies from both the SLE and non-SLE literature.

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Systemic lupus erythematosus, Therapeutic drug monitoring, Hydroxychloroquine, Azathioprine, Mycophenolate.

Introduction

Therapeutic drug monitoring (TDM) is important when there are complicated pharmacokinetics and/or patient-related factors (i.e., genetic control of metabolism, adherence, obesity, renal impairment, liver disease, and gender or ethnicity) that affect the therapeutic or toxic effect. In non-rheumatology fields, such as neurology and gastroenterology, TDM is more widely accepted. To summarize:

> "The aim of therapeutic drug monitoring (TDM) is to optimize pharmacotherapy by maximizing therapeutic efficacy, while minimizing adverse events, in those instances where the blood concentration of the drug is a better predictor of the desired effect(s) than the dose" [1].

Hydroxychloroquine

Hydroxychloroquine remains the mainstay of initial and long-term treatment of systemic lupus erythematosus (SLE). In randomized clinical trials, about 70% of SLE patients have prescribed hydroxychloroquine. It has benefits on multiple organ manifestations. It reduces flares [2] and triples the complete renal response to mycophenolate [3]. One of its major benefits is prevention, in that it reduces organ damage [4] and reduces thrombosis [5,6]. It is the only drug proved in multiple studies to improve survival [7,8]. In fact, if it is stopped, the risk of mortality increases four-fold [9].

The benefit on reduction in flares was shown quite early in the clinical trial history of SLE. The Canadian hydroxychloroquine randomized withdrawal trial found the risk of flare was 2.5 times higher (95% CI 1.08–5.58) in those randomized to placebo versus those continuing on hydroxychloroquine. In addition, the time to flare was shorter ($p = 0.02$) in the placebo group. There was likely benefit on severe flares as well, although this did not reach statistical significance [2]. In nearly all SLE randomized clinical trials, a reduction in flares is an outcome. Incredibly, though, none of the trials have included the assessment of hydroxychloroquine whole blood levels in the study design to determine which patients were taking the prescribed medication.

The benefit on prevention of new organ involvement has been proven by several groups. In the Hopkins Lupus Cohort, SLE patients on hydroxychloroquine were less likely to develop new renal involvement. In the Systemic Lupus International Collaborating Clinics (SLICC) study, Hanly et al. found that using hydroxychloroquine reduced the later seizures [10].

Organ damage is a major predictor of mortality in SLE [11]. Organ damage begets more organ damage, and the higher the organ damage score, the greater the mortality risk [11]. The ability of hydroxychloroquine to prevent later organ damage was proven to occur early, by three years. The study adjusted for age and prednisone use (as prednisone is a major independent predictor of later organ damage). The odds ratio showed a two-thirds reduction (OR 0.34, 95% CI 0.13–0.87) [12].

One of the most important forms of organ damage in SLE is renal damage. A landmark study by Ward et al. found that the risk of end-stage kidney disease had not improved over many years and was much higher in African Americans than Caucasians [13]. Recently, deaths from lupus nephritis have been shown to have actually

increased in recent years (pre-COVID) [14]. Renal damage is an independent risk factor for later cardiovascular events [15]. In the Hopkins Lupus Cohort, hydroxychloroquine use prevented later renal damage in multivariable models adjusted for age, ethnicity, and prednisone use [15].

Cardiovascular events remain a major cause of morbidity and mortality in SLE [16] with risk increased over two-fold compared with Framingham controls [16]. Hydroxychloroquine reduces several cardiovascular risk factors, in particular hyperlipidemia [17] and risk of diabetes [18]. This may contribute to the reduction in mortality. Hydroxychloroquine and aspirin were shown by Kaplan−Meier analysis to significantly reduce cardiovascular events [19].

One of the major benefits of hydroxychloroquine is the reduction in thrombosis. This is likely mediated by multiple mechanisms of action, reviewed in Petri [20]. These include reduction in erythrocyte sludging, blood viscosity, platelet aggregation, binding of anti-beta 2 glycoprotein I, and protection of the annexin V shield from disruption by antiphospholipid antibodies. The protective effect has been shown in retrospective [21], prospective [8,17], and case−control study designs [22,23].

A potential cutaneous benefit is the inhibition of dermal fibroblasts [24]. Hydroxychloroquine is used almost universally for cutaneous lupus.

Hydroxychloroquine remains the only drug that has improved mortality in SLE in multiple studies. In a Hong Kong population, the hazard ratio (HR) was 0.59 (95% CI 0.37−0.93; p = 0.008) [25]. In the multicenter US cohort LUMINA, the odds ratio was 0.128 (95% CI 0.05−0.30) [26].

Given the myriad of hydroxychloroquine benefits, it is surprising that it was not until the last decade that the issue of TDM was raised. Non-adherence is an issue in multiple chronic diseases, leading former Surgeon General C. Everett Koop to state: "Drugs don't work in patients who don't take them!". Based on pharmacy refill data, 51% of patients were non-adherent to hydroxychloroquine at least 80% of the time [27]. Twenty-nine percent of adolescents had subtherapeutic hydroxychloroquine levels, correlating with refill rates [28]. Based on Medicaid refill data, four patterns of adherence were identified: persistent adherence (17%), persistent non-adherence (36%), and two dynamic patterns of partial adherence (47%). Adherence actually declined for most patients over the first year [29]. The entire field of hydroxychloroquine adherence changed with the advent of hydroxychloroquine whole blood monitoring.

Serum/plasma hydroxychloroquine levels are inferior to whole blood levels (which reflect the last month of hydroxychloroquine ingestion rather than being affected by very recent pill taking). Costedoat-Chalumeau and colleagues developed and validated hydroxychloroquine whole blood levels [30,31]. An initial study identified SLE patients hospitalized for severe flares who were not taking hydroxychloroquine (but reported that they did!). The old saying "Trust but verify" really applies well to adherence: basically, we cannot predict adherence; patients often tell us what they expect we want to hear, rather than their actual behavior. The hydroxychloroquine whole blood level assay technique has now been replicated [32] and is available in major commercial laboratories as well. For those without access to whole blood levels, a rough correction is to double the serum/plasma level [33].

The first issue is whether hydroxychloroquine whole blood levels are helpful in improving adherence. In a longitudinal study in the Hopkins Lupus Cohort, levels were sub-therapeutic (<500) in 44% at the first monitoring visit. Adherence improved at the first follow-up visit to 69%, 77% at the second follow-up visit, and plateaued at 80% by three or more visits [32]. In another study, hydroxychloroquine blood levels were lower in SLE patients with active disease. Lower baseline levels were predictive of disease flare [30]. In a third study, median whole blood hydroxychloroquine levels were higher in SLE patients with complete remission compared with partial remission and treatment failure [34].

The second issue was to determine whether hydroxychloroquine whole blood levels could identify a "therapeutic range" for optimal control of disease activity. A systematic review and meta-analysis suggested 750 ng/mL or higher [35]. Greater than 750 ng/mL was also the suggested goal in cutaneous lupus [36]. A median level of 910 ng/mL was found to be associated with remission in cutaneous lupus [34]. In the Hopkins Lupus Cohort, our therapeutic range is 1000−1500 ng/mL.

Although not all of the benefits of hydroxychloroquine in SLE have been studied, one of the most important benefits is the benefit on thrombosis. We showed that the risk of retinopathy was 11.46% 16−20 years after starting hydroxychloroquine [37]. There is an irrational fear of blindness with hydroxychloroquine. In one study of 31 patients on hydroxychloroquine with a diagnosis of blindness or toxic maculopathy, only three had hydroxychloroquine retinopathy, each without blindness or change in vision [38]. Instead of hydroxychloroquine, stroke, other macular diseases, hypertension or cataracts, were frequent causes. In the Hopkins Lupus Cohort, the mean hydroxychloroquine whole blood level was significantly higher in those without any thrombosis

or venous thrombosis (deep vein thrombosis or pulmonary embolus [39].

The benefit of hydroxychloroquine in mortality has not been studied at the level of whole blood levels, but instead, the surrogate of adherence has been used. Adherence to hydroxychloroquine improved long-term survival and decreased glucocorticoid and healthcare requirements [40]. Subgroup analyses showed a clear dose response of adherence with reduction in mortality.

The next issue is whether therapeutic blood monitoring of hydroxychloroquine can affect the risk of retinopathy. Currently, the goal has been to detect retinopathy early (subclinically) by using optical coherence tomography. This has led to retina screening guidelines of baseline testing, and then, starting at five years, yearly retina scans, adopted by ophthalmologists [41] and the American College of Rheumatology [42]. However, the Hopkins Lupus Cohort showed we could do better. In a prospective longitudinal cohort study, whole blood hydroxychloroquine levels in the highest tertile were predictive of later hydroxychloroquine retinopathy [37].

Genetic factors have been shown to play a role in both diabetic and hydroxychloroquine retinopathy. One study created a multilocus genetic risk score for diabetic retinopathy in a Han Chinese population from Taiwan [43]. An analysis of gene expression profiles identified novel gene markers for proliferative diabetic retinopathy [44]. Several studies have shown an association of variants in the ABCA4 gene, a gene related to Stargardt disease, with hydroxychloroquine retinopathy [45,46]. Genetic polymorphisms in cytochrome P450 affect the metabolism of hydroxychloroquine and could contribute to the disconnect between dosing and blood levels [47].

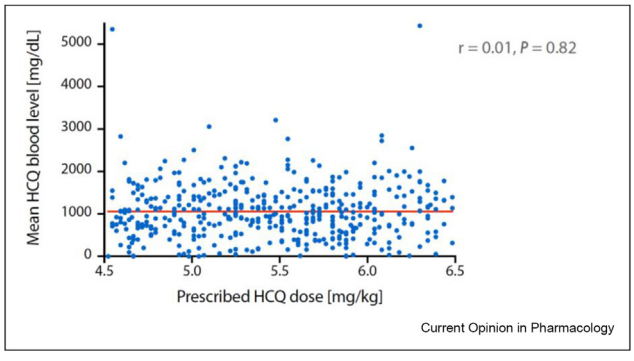
The American College of Rheumatology has not recognized the benefits of therapeutic blood monitoring for hydroxychloroquine. In a recent white paper, the fact that prospective longitudinal studies had ALREADY been done was omitted [42]. In the absence of therapeutic blood monitoring, fear of retinopathy has led to under dosing of many patients. A dose of 400 mg, for example, was given in 80% from 2007 to 2011 but only 40% in 2014 [48]. The guidelines say to dose at 5 mg/kg even though therapeutic blood monitoring studies have clearly shown this is scientifically incorrect. Data show there is no relationship between prescribed dose and whole blood level [39] (Figure 1).

Azathioprine

Azathioprine is an older oral immunosuppressant, still widely used in SLE as well as for autoimmune hepatitis, inflammatory bowel disease, and other diseases. It remains one of the very few oral immunosuppressants that can be used in pregnancy as the fetus lacks the enzyme that metabolizes it to an active form. Its metabolism can be understood by two pathways. First, the activation pathway converts azathioprine non-enzymatically by sulfhydryl compounds to mercaptopurine. Mercaptopurine is then converted to 6-thioguanine nucleotides. Second, there are competing pathways. Mercaptopurine is converted to methylmercaptopurine by thiopurine S-methyltransferase or to 6-thiouric acid by xanthine oxidase [49]. Genetic variability in thiopurine S-methyltransferase can affect metabolism in a major way. Allopurinol can lead to increased levels by its effect on xanthine oxidase (Figure 2).

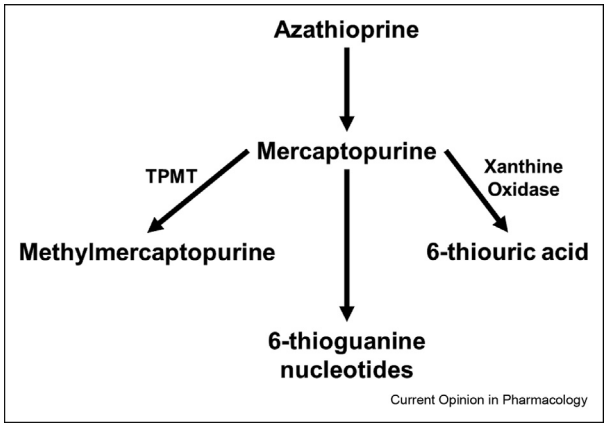
Thiopurine S-methyltransferase deficiency has a prevalence of 6 in 100, with three mutant alleles accounting for most cases. Homozygous mutant alleles (0.6%) lead to no activity, heterozygous mutant alleles (10%) to intermediate activity, and normal alleles (90%) to normal or high activity. African Caribbean and women are more

Figure 1



Prescribed hydroxychloroquine doses do not predict hydroxychloroquine levels. From Petri et al. [39].

Figure 2



Metabolism of azathioprine.

likely to have low thiopurine S-methyltransferase activity [49,50].

Reduced thiopurine S-methyltransferase activity can lead to more bone marrow suppression due to increased conversion of azathioprine to active 6-thioguanine nucleotides. In a trial of inflammatory bowel disease treated with azathioprine, those with intermediate activity were four times more likely to develop bone marrow suppression, 14.3% vs 3.5% [51]. In a rheumatoid arthritis trial, those with intermediate thiopurine S-methyltransferase activity had an increased risk of severe side effects with a RR of 3.1 (95% CI 1.6−6.2) [52].

Another common azathioprine side effect, gastrointestinal intolerance, is also more common in thiopurine S-methyltransferase heterozygotes, 37% vs 7% $p < 0.001$. Gastrointestinal intolerance occurred earlier, within six weeks of starting azathioprine, while bone marrow toxicity (29% in heterozygotes vs 0.5% in normal alleles $p < 0.01$) occurred 12 weeks or later, in the same study [53].

A further issue in therapeutic blood monitoring of azathioprine is that active metabolites are associated with both response and toxicity in inflammatory bowel disease and in SLE. In inflammatory bowel disease, 6-thioguanine $>$ 235 pmol/8 $\times$ 10^8 RBC was the efficacious range, $>$ 400 pmol/8 $\times$ 10^8 RBC was the bone marrow suppression range, and 6-methylmercaptopurine nucleotide $>$5700 pmol/8 $\times$ 10^8 RBC was the hepatotoxicity range [54−57]. In a study of 50 SLE patients given azathioprine, only 21 had a clinical response. In those with hepatotoxicity or leukopenia, the measurement of azathioprine metabolites allowed attribution to azathioprine or SLE [58].

Thiopurine S-methyltransferase is only part of the azathioprine story. Thiopurine S-methyltransferase variants explain only about one-tenth of leukopenias occurring on azathioprine [59,60]. Genome-wide association studies on azathioprine-associated leukopenia identified NUDT15 polymorphisms with OR 35.6 (95% CI 22.5−56.5). There is a 2.8−4% risk allele frequency [60−62]. This has led to new guidelines incorporating both thiopurine S-methyltransferase and NUDT15 screening [63]. Other genetic loci have been discovered which likely also contribute to azathioprine toxicity.

The benefits of thiopurine S-methyltransferase/NUDT15 screening are obvious. First, early identification of risk of bone marrow toxicity is essential, as relying on blood levels means bone marrow suppression and exhaustion of marrow stores of white blood cells has already occurred. Second, those with intermediate activity can be given lower azathioprine doses. Third, those with normal/high activity can be given higher azathioprine doses.

Mycophenolate mofetil

Data on therapeutic blood monitoring of mycophenolate mofetil come from both the transplant and rheumatology literature. In SLE, it is one of the primary therapies for both induction therapy [64] and maintenance therapy of lupus nephritis [65]. Mycophenolic acid is glucuronidated by uridine diphosphate-glucosyltransferases to the pharmacologically inactive 7-O-glucuronide metabolite mycophenolic acid glucuronide (MPAG). MPAG is excreted into bile via the multidrug resistance-associated protein 2 (MRP2). In a renal transplant study, a single nucleotide polymorphism, MRP2 c-24T, was associated with higher mycophenolic acid oral clearance and significantly more diarrhea [66].

In transplant patients, the mycophenolic acid area under the concentration−versus−time curve (AUC) varied ten-fold between patients treated with a daily dose of 2 g of mycophenolate mofetil per day [67]. This high variability is multifactorial with renal function, albumin concentration, co-medication, and pharmacogenetic factors playing a role [68]. In a study of 71 SLE patients, the mycophenolic acid-AUC variation was 10−100 mg h/L [69]. Sherwin et al. found a similar high variability in children with SLE [70]. Severe renal failure reduces mycophenolic acid exposure, thought to be the consequence of a higher non-protein bound fraction leading to faster clearance [71].

Given the great variability of mycophenolic acid-AUC, it is surprising that clinical trials of therapeutic blood monitoring of mycophenolate mofetil in renal transplant have been conflicting. The APOMYGRE study found a significant reduction in acute rejection in concentration-controlled patients [72]. In contrast, the FDCC study, with a similar design, did not find a benefit [73].

There is a robust literature in rheumatology on mycophenolic acid measurement. In SLE and ANCA-associated vasculitis, higher mycophenolic acid trough areas are protective against current flares. When mycophenolic acid was $<$3 mg/L, 29% had active disease versus 2% if the concentration was $\geq$3 mg/L. Remission persisted with mycophenolic acid pre-dose concentration $\geq$3.5 mg/L, with the pre-dose optimum concentration between 3.5 and 4.5 mg/L. To reach the ideal pre-dose mycophenolic acid concentration of 3 mg/L, the range of prescribed oral dose ranged from 1 g to 2.5−3 g mycophenolate mofetil per day [74].

A study of 18 Thai patients with lupus nephritis found the pre-dose mycophenolic acid concentration was higher in responders than non-responders, 3.1 $\pm$ 1.1 mg/

L vs 1.2 ± 0.9 mg/L, $p < 0.01$. In addition, the mean mycophenolic acid-AUC in responding patients was significantly higher (>4.5 mg h/L). However, therapeutic blood monitoring was only helpful for efficacy and did not associate with infection, gastrointestinal, or hematologic toxicity [75]. Although mycophenolic acid-AUC would be more difficult to use in clinical practice, a study in SLE also found it was associated with inactive vs active disease (26.8 ± 13.6 vs 46.5 ± 16.3 mg h/L, $p < 0.0001$). A mycophenolic acid-AUC threshold value of 35 mg h/L was associated with the lowest risk of active SLE [69]. Similarly, Djabarouti [76] found, for failures versus successes in MMF-treated SLE patients, the median mycophenolic acid-AUC was 37.7 vs 73.1 mg h/L ($p = 0.03$), and mycophenolic acid pre-dose concentrations were 1.5 vs 3.7 mg/L ($p = 0.08$). Using receiver operating characteristics curve analysis, mycophenolic acid pre-dose concentrations of 3 mg/L had 92% negative predictive value for flare. Thus, multiple studies point to a potential target level of pre-dose mycophenolic acid of 3.0 mg/L and an area under the concentration-versus-time curve between 35 and 45 mg h/L [69].

Summary

Therapeutic blood monitoring for three medications commonly used in SLE has demonstrated clinical benefit. Hydroxychloroquine whole blood levels identify adherence, can be used to achieve clinical efficacy including prevention of thrombosis, and also identify those at higher risk of retinopathy. Azathioprine guidelines now include the identification of two genetic risk factors, TPMT and NUDT15, for toxicity. Azathioprine metabolites may also be useful in achieving efficacy. For mycophenolate mofetil, the pre-dose mycophenolic acid concentration target can help to retain remission and prevent lupus nephritis flare. The cost of therapeutic blood monitoring for these drugs is acceptable given the clinical benefits of adherence, better efficacy, and less toxicity.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michelle Petri reports financial support was provided by National Institute of Arthritis and Musculoskeletal and Skin Diseases. Michelle Petri reports a relationship with AstraZeneca that includes consulting or advisory and funding grants. Michelle Petri reports a relationship with Alexion Pharmaceuticals Inc that includes consulting or advisory. Michelle Petri reports a relationship with Amgen Inc that includes consulting or advisory. Michelle Petri reports a relationship with Aurinia Pharmaceuticals Inc that includes consulting or advisory, funding grants, and speaking and lecture fees. Michelle Petri reports a relationship with AXDEV Group Inc that includes consulting or advisory. Michelle Petri reports a relationship with Boston Pharmaceuticals that includes consulting or advisory. Michelle Petri reports a relationship with Bristol Myers Squibb Co that includes consulting or advisory. Michelle Petri reports a relationship with Emergent BioSolutions Inc that includes advisory consulting. Michelle Petri reports a relationship with Eli Lilly and Company that includes consulting or advisory and funding grants. Michelle Petri reports a relationship with Exagen Inc that includes funding grants. Michelle Petri reports a relationship with Gilead Sciences Inc that includes consulting or advisory. Michelle Petri reports a relationship with GlaxoSmithKline USA that includes consulting or advisory and funding grants. Michelle Petri reports a relationship with IQVIA that includes consulting or advisory. Michelle Petri reports a relationship with Idorsia Pharmaceuticals Ltd that includes consulting or advisory. Michelle Petri reports a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory and funding grants. Michelle Petri reports a relationship with Kezar Life Sciences Inc that includes consulting or advisory. Michelle Petri reports a relationship with EMD Serono Inc that includes consulting or advisory. Michelle Petri reports a relationship with Momenta Pharmaceuticals that includes consulting or advisory. Michelle Petri reports a relationship with PPD Development that includes consulting or advisory. Michelle Petri reports a relationship with Sanofi-Aventis US LLC that includes consulting or advisory and speaking and lecture fees. Michelle Petri reports a relationship with Thermo Fisher Scientific Inc that includes funding grants. Michelle Petri reports a relationship with UCB that includes consulting or advisory.

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** of outstanding interest

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