Clinical Importance of Drug-Drug Interaction Between Warfarin and Prednisolone and Its Potential Mechanism in Relation to the Niemann-Pick C1-Like 1-Mediated Pathway

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Background: Warfarin is an anticoagulant drug used to prevent thromboembolic disorders, but its pharmacological effect is affected by co-administered drugs. Therefore, careful management of warfarin-related drug-drug interactions (DDIs) is necessary for its safety and effectiveness. Recently, intestinal vitamin K1 absorption through the Niemann-Pick C1-like 1 (NPC1L1)-mediated pathway was found to affect the pharmacological effect of warfarin. This study aimed to identify high-frequency warfarin-related DDIs in a clinical setting and elucidate their mechanism(s) in terms of changes in NPC1L1 expression and/or activity.

Methods and Results: Prednisolone was the most frequently suspected drug in retrospective surveys of medical records of patients who experienced warfarin-related DDIs. Prednisolone significantly increased the international normalized ratio of prothrombin time (PT-INR) values in warfarin-treated patients. To demonstrate the involvement of NPC1L1 in warfarin-prednisolone DDI, we conducted an in vitro vitamin K1 uptake assay using NPC1L1-overexpressing cells and found that prednisolone inhibited NPC1L1-mediated vitamin K1 uptake. Additionally, we found that prednisolone downregulates NPC1L1 in a glucocorticoid receptor α-dependent manner.

Conclusions: Co-administration of warfarin and prednisolone frequently enhanced the anticoagulant effect of warfarin in a clinical setting. Prednisolone-mediated suppression of NPC1L1 expression and activity could be the mechanism of DDI between warfarin and prednisolone. To manage warfarin therapy, the potential of concomitant drugs to change its anticoagulant effect through NPC1L1-related mechanisms merits consideration.

Key Words: Drug-drug interactions; Niemann-Pick C1-like 1; Prednisolone; Vitamin K; Warfarin

Warfarin is a commonly used anticoagulant drug characterized by its narrow therapeutic index, which requires careful clinical management to maintain the balance between under- and over-anticoagulation. To date, a number of drugs have been reported to affect responsiveness to warfarin and their concomitant use with warfarin causes harmful events in patients, such as severe bleeding or thrombosis formation. Therefore, the warfarin package insert gives warnings about drug-drug interactions (DDIs) between warfarin and a wide variety of drugs.

The anticoagulant effect of warfarin is regulated by hepatic concentrations of warfarin and vitamin K, a micro-nutrient facilitating blood coagulation by activating clotting factors such as prothrombin and factors II, VII, IX, and X in the liver. Warfarin exerts its anticoagulant effect by inhibiting the vitamin K-dependent activation of blood clotting factors. It is known that warfarin (particularly, pharmacologically active S-isomers of warfarin) is primarily metabolized by CYP2C9, and therefore, co-administration of warfarin with CYP2C9 inhibitors (e.g., fluconazole or capetibatine) or inducers (e.g., phenobarbital or carbamazepine) alters the hepatic concentration of warfarin, resulting in changes in the anticoagulant effect in warfarin-taking patients.

Besides CYP2C9-mediated DDIs, we recently revealed a novel interaction mechanism between warfarin and ezetimibe caused by ezetimibe-mediated vitamin K1 malabsorption, which resulted in enhancement of warfarin-mediated anticoagulation. Ezetimibe is a cholesterol absorption inhibitor clinically used for dyslipidemia. The target molecule of ezetimibe is the Niemann-Pick C1-like 1 (NPC1L1) protein, a cholesterol importer expressed in the intestine. Our previous study revealed that NPC1L1 plays a central role in the intestinal absorption of not only cholesterol, but also vitamin K1. In addition, we demonstrated that ezetimibe inhibits vitamin K1 absorption, as well as cholesterol absorption. These findings, together...
with the fact that vitamin K1 intake affects the anticoagulant effect of warfarin and that warfarin-ezetimibe interaction is observed with high frequency in clinical settings, suggest that NPC1L1-mediated vitamin K1 absorption is an important regulatory factor for warfarin therapy. However, unlike CYP2C9-mediated DDIs, there is little information about the influence of drugs other than ezetimibe on NPC1L1-mediated vitamin K1 transport activity and/or NPC1L1 expression.

In the present study, we examined high-frequency warfarin-related DDIs that are difficult to explain by CYP2C9-mediated mechanisms in the clinical setting and to elucidate the interaction mechanism in terms of changes in NPC1L1 expression and/or activity. Our retrospective medical records research indicated that co-administration of prednisolone with warfarin unintentionally potentiates the pharmacological effect of warfarin at a very high frequency (>90% of patients) in clinical settings. In addition, as a possible mechanism of warfarin-prednisolone interaction, we demonstrated in vitro that prednisolone can inhibit NPC1L1-mediated vitamin K1 uptake and downregulate mRNA expression of NPC1L1 via glucocorticoid receptor α (GRα)-mediated mechanisms. These findings suggest that, in order to manage warfarin therapy adequately, it is necessary to consider the potential of concomitant drugs to change the anticoagulant effect of warfarin through NPC1L1-related mechanisms.

Methods

Comprehensive Analysis of DDIs Involving Warfarin
To find out which drugs unintentionally enhance the anticoagulant effect of warfarin in the clinical setting, the medical records of patients admitted to the University of Tokyo Hospital were analyzed retrospectively. The research period was from September 2014 to April 2015. Patients who experienced warfarin DDIs were selected using 3 criteria: (I) international normalized ratio of prothrombin time (PT-INR) value ≥3.0 during warfarin treatment, (II) warfarin administration ceased after the date of PT-INR >3.0, and (III) warfarin dose not increased (unchanged or decreased) within 5 days before the date of PT-INR >3.0. We based the cutoff point (PT-INR >3.0) according to the recommended therapeutic range of PT-INR values (1.6–3.0) in several Japanese guidelines for anticoagulant therapy.

After screening, 49 patients were selected (Figure 1) and the drugs administered concomitantly with warfarin (oral drugs only, except for on-demand use) within the 5 days before the date of PT-INR >3.0 were investigated.

Analysis of PT-INR Values Before and After Prednisolone Administration
The effects of prednisolone administration on clinical laboratory values were examined using the medical records of patients admitted to the University of Tokyo Hospital during September 2014 to August 2015. Patients were selected using 2 criteria: (I) diagnosed with a disease requiring anticoagulant therapy (e.g., cerebral infarct, pulmonary embolism, thromboembolism or atrial fibrillation), and (II) prednisolone administration (>25 mg/day) was started during the research period. The total number of selected patients was 194 (Figure 2). Of them, 45 were treated with warfarin and 149 were treated with other anticoagulant drugs. From the warfarin-treated group, we excluded patients who changed warfarin dose within 5 days before prednisolone administration. Finally, patients whose PT-INR values were monitored before (within 30 days before) and after starting prednisolone administration were selected: 18 patients in the warfarin-treated group and 87 patients in the non-warfarin-treated group (Figure 2).

The data were calculated as the average of 3 data sets of PT-INR values obtained before/after prednisolone administration. When 3 independent data sets within the same warfarin dose period could not be obtained, the average of 2 or 1 data sets was used.

The institutional review board of the Graduate School of Medicine and Faculty of Medicine at the University of Tokyo approved the study protocols described and waived the need for a written informed consent from each patient.
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Figure 2. Flowchart of selection of patients for the analysis of PT-INR values before and after prednisolone administration. AF, atrial fibrillation; CI, cerebral infarct; PE, pulmonary embolism; PSL, prednisolone; PT-INR, international normalized ratio of prothrombin time; TE, thromboembolism.

Micellar Preparation
Micellar preparation was conducted as described in our previous studies.8,14-16 Cholesterol (Wako, Osaka, Japan) diluted in ethanol (1 μmol/L), phosphatidylcholine (Sigma-Aldrich, MO, USA) diluted in methanol (50 μmol/L), sodium taurocholate (Sigma-Aldrich) diluted in 96% ethanol (2 mmol/L) and Vitamin K1 (Nacalai Tesque, Kyoto, Japan) diluted in ethanol (20 μmol/L) were mixed with [or without (for mock control)] either prednisolone diluted in methanol (100 μmol/L) or ezetimibe (KEMPROTEC Limited, Cumbria, UK) diluted in methanol (40 μmol/L) and then evaporated through mild heating under N2 gas. Transport buffer (118 mmol/L NaCl, 23.8 mmol/L NaHCO3, 4.83 mmol/L KCl, 0.96 mmol/L KH2PO4, 1.2 mmol/L MgSO4, 12.5 mmol/L HEPES, 5 mmol/L glucose, and 1.53 mmol/L CaCl2 adjusted to pH 7.4) was then added to prepare the medium for uptake experiments. The micellar solution was thoroughly vortexed and stirred at 37°C for a few hours.

Vitamin K1 Uptake Assay
The in vitro vitamin K1 uptake assay was conducted using human NPC1L1-overexpressing Caco-2 cells and control Caco-2 cells. These cells were engineered in our previous studies by stable transfection with a vector carrying human NPC1L1 cDNA with hemagglutinin (HA) tag (YPYDVPDYA) sequences at the 3′-end for NPC1L1-overexpressing cells or with an empty vector [pcDNA3.1(+)] for control Caco-2 cells.8,14-16 All cells were cultured in Eagle’s minimum essential medium (Nacalai Tesque) with 10% fetal bovine serum (Biological Industries, CT, USA), penicillin and streptomycin (100 U/mL) (Nacalai Tesque), 1% nonessential amino acids (Thermo Fisher Scientific, MA, USA), and G418 sulfate (500 μg/mL) (Nacalai Tesque) at 37°C in an atmosphere supplemented with 5% CO2.

For the uptake assay, cells were seeded on 12-well plates at a density of 1.2 × 10⁵ cells per well and cultured for 14 days to allow them to differentiate. During this period, the medium was replaced every 2 or 3 days. After 14 days, cells were washed twice with the transport buffer and preincubated with the same buffer for 30 min. After preincubation, mixed micelles were added, and cells were incubated for 3 h. Cells were then washed with ice-cold transport buffer and disrupted with 0.2 N NaOH overnight. Vitamin K1 in the cell lysate was measured with the UPLC system (see later) to determine cellular uptake. For normalization, the protein concentration of each well was determined with the BCA Protein Assay Kit (Thermo Fisher Scientific).

Quantification of Vitamin K1 Using UPLC
The UPLC system consisted of the ACQUITY UPLC sample manager and binary solvent manager (Waters, MA, USA). Separations were performed with a VanGuard BEH C18 (1.7 μm) as the precolumn (Waters) and an ACQUITY UPLC BEH C18 (1.7 μm, 2.1×100 mm) Column (Waters) as the main column. Vitamin K1 and menaquinone 7 (as internal standard) were detected with the XEVO TQ-XS tandem quadrupole mass spectrometer (Waters). The sample temperature was kept at 4°C, and the column temperature was kept at 50°C. The mobile phase was a mixture of MilliQ water (solvent A) and liquid chromatography-grade methanol (Nacalai Tesque) (solvent B). The UPLC conditions are shown in Supplementary Table 1 and the mass spectrometer conditions are shown in Supplementary Table 2. Data analyses were performed using MassLynx NT software version 4.1 (Waters).
Vector Construction of Reporter-Linked Human NPC1L1 Promoters

pGL3-Basic vectors containing a part of the promoter region of human NPC1L1 were obtained as described previously. The resulting plasmids were referred to as p1315/Luc containing the region from nucleotides -1,315 to +20 of human NPC1L1 promoter, p1110/Luc containing the region from -1,110 to +20, p821/Luc containing the region from -821 to +20, p455/Luc containing the region from -455 to +20, and p264/Luc containing the region from -264 to +20.

Construction of Expression Vector for Human GRα

The cDNA of human GRα (GRα/NR3C1) (accession No. NM_000176) was amplified with the Not I site attached at both the 5’-end and the 3’-end by PCR from the total RNA of HepG2 cells. After insertion of the amplified human GRα cDNA into pGEM-T Easy Vector, the inserted fragments were digested with Not I and were ligated into pcDNA3.1(+) vector plasmid.

Luciferase Assay

HepG2 cells were plated on day 0 at a density of 1.5×10⁵ cells/well on 24-well plates and grown in phenol red-free DMEM with 10% charcoal-adsorbed fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine. On day 1, cells were transfected with 500 ng/well of pGL3-Basic vectors, with or without human NPC1L1 promoter, using Lipofectamine 2000 (Thermo Fisher Scientific) at a DNA/lipid ratio of 1:2.5. In some experiments, co-transfections were performed with GRα (250 ng/well) in pcDNA3.1(+) vector. All wells were also co-transfected with 50 ng/well of pRL-TK vector (Promega, WI, USA) to correct the transfection efficiency. On day 2, 10 µmol/L prednisolone was added to the culture medium and the incubation was continued for an additional 24 h. After incubation, luciferase activities were quantified by Luminescer MCA (Atto, Tokyo, Japan) using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol.

Statistical Analysis

Student’s t-test and a chi-square test were used for characteristic comparison between the warfarin- and non-warfarin treated groups. A paired t-test was used for analysis of changes in PT-INR values. ANOVA followed by
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To elucidate the effect of prednisolone on anticoagulant therapy, we analyzed the PT-INR values before and after starting prednisolone administration of the patients selected according to a flowchart (Figure 2). Clinical characteristics (sex ratio, age, diagnosed disease requiring anticoagulant therapy, prednisolone dose, and warfarin dose) of the selected patients are shown in Supplementary Table 3. We confirmed that the sex ratio and mean age of patients were not statistically different between the warfarin-treated group (n=18) and non-warfarin-treated group (n=87). The warfarin-treated group included 1 of 6 prednisolone cases found in the preceding analysis. We then analyzed the PT-INR values in each patient group and found that those in the warfarin-treated group increased significantly after prednisolone administration (mean value of PT-INR before: 1.83, after: 2.61, fold change: 1.41) (Figure 4A, B). Indeed, 17 of 18 patients in the warfarin-treated group showed an increase in PT-INR values (fold change >1.0) (Figure 4B). Such increases were observed regardless of the type of disease requiring anticoagulant therapy (e.g. atrial fibrillation, cerebral infarct, thromboembolism, pulmonary embolism) (data not shown). By contrast, the PT-INR values of the non-warfarin-treated group slightly decreased after prednisolone administration (mean value of PT-INR before: 0.99, after: 0.95, fold change: 0.97) (Figure 4C, D). These results indicated that co-administration of prednisolone with warfarin may enhance the anticoagulant effect of warfarin in the clinical setting.

Bonferroni’s test was used for Vitamin K1 uptake assay, luciferase assays and for comparisons of NPC1L1 mRNA levels. P<0.05 was considered significant.

Results

Warfarin-Related DDIs

Firstly, we investigated all drugs used concomitantly with warfarin in 49 patients exhibiting a warfarin-related DDI (Figure 1). In these selected patients, the average daily dose of warfarin was 2.75±0.19 mg and the mean PT-INR value before the occurrence of warfarin-related DDI was 1.94±0.10. A typical DDI case is shown in Figure 3. In this case, the PT-INR value reached 3.62 on October 18, although the warfarin dose was unchanged for more than 5 days before the date, resulting in discontinuation of warfarin administration on October 24. Within the 5 days before October 18, the daily dose of febuxostat was increased from 20 mg to 40 mg and administration of 50 mg prednisolone was newly started. Thus, in this case, febuxostat and prednisolone were listed as drugs possibly interacting with warfarin (Figure 3). By comprehensive investigation of 49 patients, we found 42 drugs co-administered with warfarin, of which the dosages were increased or administration was started within the 5 days before the date of PT-INR >3.0 (Table). Of these concomitant drugs, prednisolone was found most frequently (6/49 cases) (Table). Because the prednisolone doses in these cases were relatively high (25–60 mg/day), we selected patients who received a prednisolone dosage ≥25 mg/day for subsequent investigation.

Prednisolone Administration and PT-INR of Warfarin-Treated Patients

To elucidate the effect of prednisolone on anticoagulant therapy, we analyzed the PT-INR values before and after starting prednisolone administration of the patients selected according to a flowchart (Figure 2). Clinical characteristics (sex ratio, age, diagnosed disease requiring anticoagulant therapy, prednisolone dose, and warfarin dose) of the selected patients are shown in Supplementary Table 3. We confirmed that the sex ratio and mean age of patients were not statistically different between the warfarin-treated group (n=18) and non-warfarin-treated group (n=87). The warfarin-treated group included 1 of 6 prednisolone cases found in the preceding analysis. We then analyzed the PT-INR values in each patient group and found that those in the warfarin-treated group increased significantly after prednisolone administration (mean value of PT-INR before: 1.83, after: 2.61, fold change: 1.41) (Figure 4A, B). Indeed, 17 of 18 patients in the warfarin-treated group showed an increase in PT-INR values (fold change >1.0) (Figure 4B). Such increases were observed regardless of the type of disease requiring anticoagulant therapy (e.g. atrial fibrillation, cerebral infarct, thromboembolism, pulmonary embolism) (data not shown). By contrast, the PT-INR values of the non-warfarin-treated group slightly decreased after prednisolone administration (mean value of PT-INR before: 0.99, after: 0.95, fold change: 0.97) (Figure 4C, D). These results indicated that co-administration of prednisolone with warfarin may enhance the anticoagulant effect of warfarin in the clinical setting.
Prednisolone and NPC1L1-Mediated Vitamin K₁ Uptake

Our medical records study suggested that the anticoagulant effect of warfarin was enhanced by prednisolone treatment, although the mechanism of this DDI was unclear. Recently, we revealed that NPC1L1, a cholesterol uptake transporter expressed on the luminal membrane in the intestine, functions as a modulator of warfarin therapy by regulating intestinal absorption of dietary vitamin K₁ in addition to cholesterol. Based on the fact that prednisolone is a steroid compound and that some steroid compounds, including cholesterol and plant sterols, have been reported to affect the expression and/or transport activity of NPC1L1, we hypothesized that prednisolone may affect NPC1L1-mediated vitamin K₁ uptake and/or NPC1L1 expression. To test this possibility, we conducted a series of in vitro experiments.

We first examined the effect of prednisolone on NPC1L1-mediated vitamin K₁ uptake by using human NPC1L1-overexpressing Caco-2 cells (human NPC1L1 cells) and control Caco-2 cells slightly expressing endogenous NPC1L1 (Control cells). Consistent with our previous study, we observed that the vitamin K₁ uptake activity of human NPC1L1 cells was significantly higher than that of Control cells and that this vitamin K₁ uptake activity was effectively inhibited by ezetimibe. In addition, we found that prednisolone also inhibited NPC1L1-mediated vitamin K₁ uptake, although the inhibitory effect of prednisolone was not as high as that of ezetimibe (Figure 5). These in vitro results suggested that prednisolone potentially can inhibit NPC1L1-mediated vitamin K₁ uptake.

Prednisolone and Transcription of Human NPC1L1 Gene Via GRα-Mediated Mechanisms

Next, we elucidated the effect of prednisolone on NPC1L1 expression. Because steroid compounds are known to regulate the transcription of multiple genes, together with nuclear factors such as glucocorticoid receptors, we focused on the effects of prednisolone on the transcriptional regulation of NPC1L1.

To clarify the effect of prednisolone on NPC1L1 transcription, we performed reporter gene assays. As shown in Figure 6, luciferase activity of the NPC1L1 promoter was...
decreased by prednisolone treatment in combination with transfection of human GRα, a nuclear factor for which prednisolone has been reported to be a ligand23 (Figure 6A), whereas luciferase activity of pGL3-Basic (not containing human NPC1L1 promoter) was not decreased by the same treatment (Figure 6B). These results indicated that prednisolone negatively regulated transcription of the human NPC1L1 gene via GRα-mediated mechanism(s).

To determine the NPC1L1 promoter region responsible for GRα-mediated downregulation, we performed luciferase assays using deletion mutants of human NPC1L1 promoter. Administration of prednisolone with GRα also decreased the luciferase activity of the p1110/Luc construct and that of the p821/Luc construct, but the extent of the reduction was less than that of the p1315/Luc construct. In contrast, p455/Luc and p264/Luc constructs exhibited no response (Figure 6C). These results suggested that the regulatory regions responsible for the GRα-prednisolone signaling were located between −1,315 and −1,110 and between −821 and −455 upstream of the human NPC1L1 gene.

**Figure 6.** Luciferase assays using human NPC1L1 promoter constructs. (A) Luciferase activity of human NPC1L1 promoter (p1315/Luc) by human GRα and prednisolone (10μmol/L). (B) Luciferase activity of pGL3-Basic (not containing human NPC1L1 promoter). (C) Luciferase activity of deletion mutants of human NPC1L1 promoter. (i) p1110/Luc, (ii) p821/Luc, (iii) p455/Luc, (iv) p264/Luc. The fold activation values were calculated by dividing the luciferase activity of each promoter by that of control cells. Data are mean±SD (n=3). **P<0.01, significantly different as determined by ANOVA followed by Bonferroni’s test. GRα, glucocorticoid receptor α; N.S., no significant difference.

**Figure 7.** Effect of human GRα and prednisolone (10μmol/L) on the mRNA expression of NPC1L1 in HepG2 cells. The fold changes were calculated as the relative expression levels compared with that of Control cells. Data are mean±SD (n=3). *P<0.05, significantly different as determined by ANOVA followed by Bonferroni’s test. GRα, glucocorticoid receptor α.

Prednisolone and Endogenous Expression of NPC1L1 in GRα-Dependent Manner

To demonstrate whether prednisolone downregulated
endogenous NPC1L1 expression, HepG2 cells, in which NPC1L1 is expressed endogenously, were treated with prednisolone with or without transfection of a GRα-expressing plasmid. As shown in Figure 7, prednisolone treatment significantly reduced the expression of NPC1L1 mRNA in GRα-transfected cells (71.3±4.7% compared with Control cells). Meanwhile, the mRNA level of NPC1L1 was hardly affected by prednisolone in GRα-non-transfected cells. These results indicate that prednisolone can decrease the endogenous expression of NPC1L1 mRNA in HepG2 cells in GRα-dependent manner.

Taken together, our in vitro results suggested that prednisolone can inhibit NPC1L1-mediated vitamin K1 uptake, and in addition, downregulate the expression of NPC1L1 via GRα-mediated mechanisms. From these findings, together with our clinical observation that patients exhibiting warfarin–prednisolone DDI (Figure 4A,B) did not receive ezetimibe therapy, it is possible that prednisolone may reduce NPC1L1-mediated intestinal absorption of vitamin K1, thus enhancing the anticoagulant effect of warfarin.

Discussion

Our retrospective review of the medical records of 49 patients being treated with warfarin revealed a variety of drugs that could unintentionally enhance the anticoagulant effect of warfarin (Table). For instance, a number of previous reports3,24–28 suggest that fluconazole and sulfamethoxazole/trimethoprim, widely known as CYP2C9 inhibitors,29 can cause DDIs with warfarin. However, the number of cases in our study was limited (Table), probably because of the careful attention of medical personnel to DDIs between warfarin and CYP2C9 inhibitors (Table). By contrast, although the warfarin package insert warns that prednisolone may alter the intensity of warfarin anticoagulant activity, we found many suspected cases of warfarin–prednisolone DDI (Table). This finding indicated that prednisolone was not well recognized by medical personnel as a causative drug for warfarin DDIs, resulting in interruption of warfarin treatment. Moreover, febuxostat, about which little is known regarding effects on warfarin therapy, was also listed as a causative drug for warfarin-related DDIs in our study (Figure 3 and Table). Further studies to clarify the frequency, degree, and mechanism of these unreported DDIs are necessary and important to improve patient outcomes.

Based on our findings that relatively high doses (25–60 mg/day) of prednisolone were administered in all cases where prednisolone was suspected as a causative drug for warfarin-related DDIs (Table), and that most warfarin-treated patients experienced unintentional increases in PT-INR values after starting co-administration of prednisolone (≥25 mg/day) (Figure 4A,B), PT-INR values should be carefully monitored especially when co-administration of high-dose prednisolone is started in warfarin-treated patients. Meanwhile, the PT-INR values of patients who were not treated with warfarin were slightly decreased after prednisolone administration (Figure 4C,D), probably because of activation of blood coagulation by the steroid.30 These observations suggested that an increase in PT-INR values in patients treated with warfarin could not be attributed to a direct effect of prednisolone on blood coagulation. Our results, together with previous reports about interactions of warfarin with prednisone,31–33 methylprednisolone,34,35 and dexamethasone,36 suggest that we should pay attention to DDIs between warfarin and clinical agents classified as corticosteroid compounds.

The mechanism of DDIs between warfarin and prednisolone has not been reported. CYP2C9 is generally known to mediate warfarin DDIs, but to the best of our knowledge, there is no report demonstrating that prednisolone affects the expression and/or activity of CYP2C9. However, we have demonstrated that prednisolone can inhibit the vitamin K1 uptake activity of NPC1L1 and, in addition, downregulate NPC1L1 expression via GRα-mediated mechanisms (Figures 6,7). Considering our previous report demonstrating that ezetimibe enhanced the anticoagulant effect of warfarin by decreasing hepatic vitamin K1 levels through inhibition of NPC1L1-mediated intestinal vitamin K1 absorption, our results raise the possibility that DDIs between warfarin and prednisolone are associated with changes in NPC1L1-mediated vitamin K1 absorption by prednisolone treatment. To support this assumption, future clinical studies investigating changes in serum vitamin K1 concentration after starting prednisolone treatment would be helpful. In addition, in cases where NPC1L1 expression is downregulated by prednisolone in humans, it is possible that, even after cessation of prednisolone treatment, the effect of the prednisolone-warfarin DDI continues until NPC1L1 expression is restored.

Previous reports have indicated that glucocorticoids, including prednisolone, can increase serum cholesterol levels in humans.36,37 Indeed, serum total cholesterol levels in the patients we analyzed were significantly increased after starting prednisolone administration (data not shown). One plausible mechanisms of such a glucocorticoid-induced hyperlipidemia is that the activity of hydroxymethylglutaryl-coenzyme A reductase, a rate-limiting enzyme for de novo cholesterol synthesis, is increased by glucocorticoids.40 Given the stimulation of cholesterol synthesis by glucocorticoids, and the fact that NPC1L1 is involved in intestinal cholesterol absorption, our finding that NPC1L1 expression and function were suppressed by glucocorticoids (prednisolone) seems to be reasonable from the viewpoint of maintaining cholesterol homeostasis.

As well as ezetimibe and prednisolone, other drugs that have the potential to affect NPC1L1 expression/function may cause warfarin DDI. For instance, drugs with similar chemical structures to prednisolone and/or high affinity to GRα may be able to interact with warfarin through NPC1L1-related mechanisms. In fact, dexamethasone, a synthetic glucocorticoid with GRα agonist activity, was suspected of warfarin DDI in our study (Table), and in a previous report.38 In addition, because it has been reported that an ezetimibe-warfarin interaction more frequently appeared in patients taking statins, probably because of an increase in NPC1L1 expression by statin therapy,39 positive regulation of NPC1L1 expression by statins should be considered.40–43 In this regard, if prednisolone-mediated NPC1L1 inhibition accounts for the mechanism of warfarin-prednisolone interaction, statin treatment may potentiate the effect of the interaction as well as that of warfarin–ezetimibe: this is an important future research topic. Altogether, it is necessary to consider the influence of drugs on NPC1L1 expression and/or function for appropriate management of anticoagulant therapy with warfarin.
Our results indicated 2 possible regulatory regions responsible for GRα-prednisolone signaling upstream of the human NPC1L1 gene. It is as yet unknown whether GRα binds to the regulatory regions directly or indirectly. Further research is needed to clarify the mechanism (key proteins) of transcriptional regulation of NPC1L1 via the GRα-prednisolone signaling.

In conclusion, our study revealed that co-administration of warfarin and prednisolone frequently enhanced the anti-coagulant effect of warfarin in the clinical setting. Moreover, functional inhibition and mRNA downregulation of NPC1L1 by prednisolone are proposed as possible mechanisms of the warfarin-prednisolone DDI. In order to manage anti-coagulant therapy with warfarin more appropriately, health professionals should be careful with the administration of concomitant drugs that could affect NPC1L1.

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Conflicts of Interest (Within the Past 12 Months)

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References


**Supplementary Files**

Please find supplementary file(s);