Effect of tenidap sodium on the pharmacodynamics and plasma protein binding of warfarin in healthy volunteers

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1 An open-label, randomised study was performed to assess the effect of tenidap sodium on the pharmacodynamics and plasma protein binding of warfarin.
2 Fourteen healthy male volunteers received either a single oral dose of 120 mg tenidap sodium or matching placebo capsules from days 11 to 36. A single oral dose of 0.75 mg kg\(^{-1}\) warfarin was administered on days 1 and 32.
3 The mean prothrombin AUC(0,120h) value between baseline and day 32 increased from 1692.4 ± 234.5 s h to 1769.3 ± 218.0 s h in the group given tenidap, and decreased from 1747.6 ± 289.4 s h to 1708.1 ± 236.8 s h in the placebo group.
4 Tenidap caused a slight delay in the normalisation of prothrombin times following the second dose of warfarin on day 32 compared with the first dose on day 1. This was significant at 36, 48, 72 and 96 h but not at 120 h after administration of warfarin.
5 The mean percentage of unbound warfarin in the tenidap group (0.08% ± 0.09) was significantly different (\(P = 0.047\)) from that in the placebo group (~0.03% ± 0.10) but this was not considered to be clinically meaningful.
6 These data indicate that prothrombin times should be monitored during concomitant administration of tenidap and warfarin.

Keywords tenidap sodium warfarin pharmacodynamics plasma protein binding

Introduction

Tenidap sodium is a novel, cytokine-modulating anti-rheumatic agent currently undergoing clinical investigation in the USA and Europe for the treatment of rheumatoid arthritis and osteoarthritis. Tenidap inhibits cyclo-oxygenase activity [1] and has significant effects on acute phase proteins; for example, tenidap has been shown to lower the plasma concentration of C-reactive protein in patients with rheumatoid arthritis [2, 3]. In vitro, tenidap has also been shown to inhibit production of the cytokines tumour necrosis factor, interleukin-1 and interleukin-6 [4], which mediate release of acute phase proteins from the liver [5].

Warfarin is a coumarin anticoagulant widely used in the treatment and prophylaxis of a variety of thromboembolic disorders. It is used in the prophylaxis of atrial fibrillation [6], thromboembolic complications with prosthetic heart valves [7], transient ischaemic attacks [8] and prophylaxis and treatment of deep venous thrombosis [9].

Non-steroidal anti-inflammatory drugs (NSAIDs) also inhibit cyclo-oxygenase; these include salicylates [10], ibuprofen [11–13], phenylbutazone [14] and flurbiprofen [15, 16], which have all been shown to interact with warfarin, and these interactions may be of clinical importance [14]. Interactions may occur through a number of different mechanisms, including displacement of warfarin (which is highly plasma protein bound) from plasma protein binding sites, and stereoselective inhibition of the metabolism of the S-isomer of warfarin [14, 17]. The result is a prolonged prothrombin time without a proportional increase in the concentration of warfarin.

More than 99% of tenidap is tightly bound to plasma protein [18]; therefore, the effect of its administration to patients taking other highly bound drugs, such as warfarin, merits investigation. The potential clinical importance of an interaction between tenidap and warfarin makes this study and assessment of the
effect of tenidap on prothrombin time as well as plasma protein binding in warfarin-treated subjects both necessary and important.

Methods

Subjects

Healthy male volunteers who provided written, informed consent were enrolled in an open-label, randomised, parallel-group study. At their initial screening visit all subjects underwent a medical history, full physical examination, urine drug screen, urinalysis, a 12-lead resting electrocardiogram, and standard laboratory tests for haematological, renal and hepatic functions, which were required to be within 10% of normal limits for the subject to be eligible for entry. Subjects were included only if they had not used any prescription or over-the-counter medications in the previous 2 weeks, if they had a negative urine drug screen and negative ethanol breath test, and were not vegetarians.

Protocol

Subjects received a single oral dose of 0.75 mg kg⁻¹ warfarin (Du Pont Pharmaceuticals) on day 1 and either a once daily oral dose of 120 mg tenidap sodium (Pfizer Central Research) (eight subjects) or matching placebo (six subjects) between days 11 and 36 inclusive. On day 32, 2 h after the dose of tenidap or placebo, they received a second dose of 0.75 mg kg⁻¹ warfarin.

The protocol was approved by the Ohio State University Human Subjects Biomedical Sciences Review Committee. Subjects were confined to the Research Unit for at least 72 h after each dose of warfarin.

Prothrombin times

Blood samples (3 ml) were obtained for determination of prothrombin times immediately before and at 12, 24, 36, 48, 60, 72, 96 and 120 h after each dose of warfarin. Prothrombin times were determined by a standardised assay with thromboplastin and photo-optical detection using a Coag-A-Mate X2 (General Diagnostics) [19].

Determination of plasma tenidap concentrations

Blood samples sufficient to give 3 ml plasma were collected prior to dosing with warfarin on day 1 and prior to dosing with tenidap or placebo on days 32 and 33. Plasma was prepared and stored frozen at −15° C until being used for assay.

In preparation for assay, acetonitrile (0.1 ml) containing CP-66,993 as an internal standard was added to 50 µl of plasma. The samples were vortexed and centrifuged at 4° C, and 50 µl of the supernatant was added to vials containing 50 µl of 0.025 M Tris-phosphate buffer (pH 7.4). Part of this sample (20 µl) was analysed by h.p.l.c. (Waters 481) using a 200 × 4.6-mm Novapak C-18 column packed with 5 µm particles, preceded by a 50 × 3.9 mm guard column consisting of 40 µm glass beads. The mobile phase comprised 0.025 M Tris-phosphate buffer (pH 6.5) and methanol in a ratio of 55:45 (v/v) with a flow rate of 1 ml min⁻¹. Absorbance was monitored at 365 nm [20]. Standard curves, constructed over a concentration range of 0.5–25.0 µg ml⁻¹, were run after every 30 samples. Mean correlation coefficients were 0.997 ± 0.002, and the mean slope was 0.379 ± 0.130.

Determination of plasma protein binding of warfarin

Blood samples sufficient to provide 5 ml plasma were collected immediately before the administration of warfarin on days 1 and 32. Plasma was prepared and stored at −15° C until assayed for plasma protein binding of warfarin by equilibrium dialysis. [¹⁴C]-Warfarin (46 µCi mmol⁻¹; Amersham) was added to a 2.0 ml aliquot of plasma to a final concentration of 2.0 µg ml⁻¹ and 0.3 µCi ml⁻¹ and was dialysed against 0.8 ml 0.13 M sodium phosphate buffer (pH 7.4) for 18 h at room temperature. Concentrations of warfarin in plasma and dialysate were measured by liquid scintillation counting using Ecolite scintillation fluid and a Packard scintillation counter.

Statistical evaluation

The area under the prothrombin time-time curve during the 120 h after administration of warfarin (AUC(0,120h)) was calculated by the linear trapezoidal method. Comparison of the difference in AUC(0,120h) between the first and second doses of warfarin was undertaken using a paired, two-tailed, Student's t-test. Mean differences in prothrombin time between the first and second doses of warfarin were compared at each time point (0–120 h). From scintillation counting data the percentage of unbound warfarin was calculated:

\[
\% \text{ unbound warfarin} = 100 - \left( \frac{\text{dpm ml}^{-1} \text{ plasma} - \text{dpm ml}^{-1} \text{ buffer}}{\text{dpm ml}^{-1} \text{ plasma}} \right)
\]

The mean change in plasma protein binding of warfarin was also calculated using a paired, two-tailed Student's t-test.

Results

A total of 15 volunteers were enrolled into the study and their baseline demographic details are shown in Table 1. Of these, 12 subjects completed the study and were included in the pharmacodynamic analysis. The three subjects who were withdrawn were all from the tenidap group: two were excluded for non-compliance (one before receiving tenidap), and the third subject was withdrawn on day 34 (2 days after the second dose of warfarin) because of probable treatment-related adverse events (moderate nausea, vomiting and abdominal discomfort). One subject in the placebo group continued in the study despite developing skin bruising and myalgia approximately 1 week after the first dose of warfarin.
Table 1 Demographic characteristics of healthy male volunteers entered into a study to determine the effect of 120 mg day⁻¹ tenidap vs matching placebo on prothrombin time during concomitant treatment with 0.75 mg kg⁻¹ warfarin

<table>
<thead>
<tr>
<th>Warfarin + tenidap</th>
<th>Warfarin + placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>(all male)</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22–45</td>
</tr>
<tr>
<td>Mean</td>
<td>33.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>64.6–84.1</td>
</tr>
<tr>
<td>Mean</td>
<td>73.5</td>
</tr>
<tr>
<td>Ethnic origin</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>8</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
</tr>
</tbody>
</table>

Plasma concentrations of tenidap

Concentrations of tenidap in plasma were in the range of those observed previously in normal volunteers receiving daily doses of 120 mg tenidap (trough levels ranged from 3.4 to 7.0 μg ml⁻¹) and indicated compliance with the study regimen [20].

Prothrombin times

All prothrombin times were within the normal range (9.5–12.5 s) before administration of each dose of warfarin.

Normalisation of mean prothrombin AUC(0,120h) values following the first and second doses of warfarin are shown in Table 2. The mean difference in AUC(0,120h) between the first and second doses of warfarin in the tenidap group was significantly different from that in the placebo group (P = 0.007). The mean change in prothrombin time between the first and second doses of warfarin was 4.5% in the tenidap group compared with −2.3% in the placebo group.

The differences in prothrombin times at 36, 48, 72 and 96 h between the second and first doses of warfarin were significantly different for the tenidap and placebo groups (P ≤ 0.05) (Table 2). The change in mean prothrombin times after the second and first dose of warfarin for the tenidap and placebo groups was not significantly different at 120 h.

Plasma protein binding of warfarin

The mean percentages of unbound warfarin determined from plasma obtained before the first and second doses of warfarin (2 h after the dose of tenidap on day 32) for subjects in the tenidap and placebo groups are shown in Table 3. The mean change of 0.08% (range −0.06% to 0.18%) between day 32 and day 1 for subjects in the tenidap group was significantly different (P = 0.047) from the mean change of −0.03% (−0.15% to 0.10%) for subjects in the placebo group.

Discussion

Concomitant administration of warfarin and 120 mg day⁻¹ of tenidap, compared with warfarin and placebo, resulted in a small but statistically significant increase in the prothrombin time AUC(0,120h). This is likely, however, to be of little or no clinical significance. A slight delay in normalisation of prothrombin times in the tenidap group compared with placebo followed the second dose of warfarin. The daily administration of 120 mg tenidap had a small, but probably clinically insignificant, effect on the plasma protein binding of warfarin.

Table 3 Mean percentage of unbound warfarin in plasma following administration of either 120 mg day⁻¹ tenidap or matching placebo in warfarin-treated healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Tenidap + warfarin</th>
<th>Placebo + warfarin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.00 ± 0.09</td>
<td>1.04 ± 0.07</td>
</tr>
<tr>
<td>Day 32</td>
<td>1.08 ± 0.10</td>
<td>1.01 ± 0.09</td>
</tr>
<tr>
<td>Day 32-Day 1</td>
<td>0.08 ± 0.09*</td>
<td>−0.04 ± 0.10</td>
</tr>
</tbody>
</table>

*P = 0.047 vs placebo.

Table 2 Mean prothrombin time (s) and AUC(0,120h) following administration of either 120 mg day⁻¹ tenidap or matching placebo to warfarin-treated healthy volunteers

<table>
<thead>
<tr>
<th>Time after warfarin dose (h)</th>
<th>Tenidap + warfarin (n = 5)</th>
<th>Placebo + warfarin (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 Day 32</td>
<td>Day 32 Day 32</td>
</tr>
<tr>
<td>0</td>
<td>10.60 10.94 0.34</td>
<td>10.75 10.92 0.17</td>
</tr>
<tr>
<td>12</td>
<td>11.38 11.54 0.16</td>
<td>11.92 12.25 0.33</td>
</tr>
<tr>
<td>24</td>
<td>15.30 15.74 0.44</td>
<td>15.73 15.78 0.05</td>
</tr>
<tr>
<td>36</td>
<td>17.94 19.00 1.06</td>
<td>18.92 17.96 −0.70</td>
</tr>
<tr>
<td>48</td>
<td>17.60 18.60 1.00</td>
<td>18.77 17.18 −1.59</td>
</tr>
<tr>
<td>60</td>
<td>15.84 16.64 0.80</td>
<td>16.63 15.82 −0.81</td>
</tr>
<tr>
<td>72</td>
<td>14.58 15.02 0.44</td>
<td>15.05 14.20 −0.85</td>
</tr>
<tr>
<td>96</td>
<td>12.30 12.98 0.68</td>
<td>12.97 12.67 −0.03</td>
</tr>
<tr>
<td>120</td>
<td>11.20 11.96 0.76</td>
<td>11.82 11.76 −0.04</td>
</tr>
</tbody>
</table>

AUC(0,120h) (s h) = 1692.4 ± 234.5

warfarin. In the tenidap group the percentage of unbound warfarin in plasma prior to the first dose compared with prior to the second dose of warfarin increased by 0.08%, as opposed to a decrease of 0.03% in the placebo group.

Warfarin is metabolised by the cytochrome P450-III A isozyme [21]. The lack of a clinically significant interaction between tenidap and warfarin that is indicated by the present study suggests that tenidap neither induces nor inhibits the P450III A isozyme. Similarly tenidap is unlikely to interact with other drugs which are metabolised by P450III A.

The effect of tenidap on plasma protein binding, although statistically significant, was small. Other anti-inflammatory agents, such as the NSAIDs mefenamic acid, phenylbutazone and salicylates, have also been shown to displace warfarin from plasma protein binding sites [22]. However, clinically significant interactions between different NSAIDs occur by mechanisms other than displacement of plasma protein binding, and the likelihood of clinical interactions cannot be predicted on the basis of plasma protein binding [22].

Most NSAIDs potentiate oral anticoagulants via a number of mechanisms [14]. Schulman & Henriksson [23] reported that in 20 patients (the majority elderly) stabilised on warfarin, addition of ibuprofen (600 mg three times daily) significantly prolonged bleeding times after 1 week ($P < 0.05$). In four patients, bleeding times were prolonged above the normal range of 140–570 s, and one of these patients experienced a doubling of bleeding time. However, analysis of the results from this study was complicated by the many concomitant drugs some patients received.

Drug interactions with antirheumatic medications are a significant problem because of the numerous medications utilised in the geriatric population. Elderly patients may be at further risk from decreased metabolic activity and reduced renal function. Awareness of potential drug interactions is essential, and appropriate monitoring should be undertaken to minimise clinical toxicity.

The findings of this study suggest that concurrent treatment of warfarin and tenidap was safe and that the pharmacodynamics and plasma protein binding of warfarin were not altered to a clinically meaningful extent by administration of tenidap. The statistically significant increase in free warfarin in tenidap-treated subjects is also unlikely to be clinically relevant, and there appears to be no evidence to discourage the advisability of co-administration of warfarin and tenidap. However, since many cyclo-oxygenase inhibitors alter platelet function and haemostasis, additional monitoring of prothrombin time is recommended when tenidap is added to the regime of patients stabilised on warfarin.

References

18 Gardner MJ, Wilner KD, McMahon GF, Fouda HG. The pharmacokinetics of tenidap following single and multiple 120 mg doses to healthy, male volunteers. Clin Pharmac Ther 1993; 53: 211.
