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Effect of ciprofloxacin on the hypoprothrombinemic activity of warfarin in rats

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Abstract

The interaction between the fluoroquinolone ciprofloxacin and the oral anticoagulant warfarin was investigated in male Wistar rats in two phases. In phase I, the optimum oral dose of warfarin was determined to be 0.1 mg/kg in rats. This dose prolonged the prothrombin time (PT) 1.5-2 times the control values. On phase II, for a period of 5 consecutive days, three groups of animals received daily oral doses of one of the following treatments: warfarin sodium 0.1 mg/kg (group I), ciprofloxacin 20 mg/kg (group II), or a combination of ciprofloxacin and warfarin (group III). PT was determined for all animals before and after each treatment. Ciprofloxacin serum concentrations were measured using an HPLC assay. The mean PT before treatment was 12.3 ± 2.8 seconds. One hour after the last treatment in the three groups, the PT of the combination group was significantly higher than that of both of the warfarin and the ciprofloxacin groups (32.7 ± 10 vs. 18.9 ± 5.2 and 14.4 ± 0.9 seconds, respectively). Whereas, 3 days after the last treatment, the PTs were found to be 15.8 ± 1.1, 18.6 ± 0.7 and 14.6 ± 2 seconds for the combination, warfarin and ciprofloxacin groups, respectively. Interestingly, animals in group three seemed to recover more rapidly and fully on day 8. The results indicate that this interaction is serious and could be fatal if not monitored, however it is reversible if ciprofloxacin is discontinued.

Keywords: Ciprofloxacin; Prothrombin time; Warfarin; Metabolism; Inhibition; Interaction

1. Introduction

The oral anticoagulant warfarin is widely used to treat and prevent thromboembolic events. It is beneficial in patients with chronic atrial fibrillation and those who have experienced a myocardial infarction (Smith et al., 1990). Ciprofloxacin is an oral, broad spectrum fluoroquinolone antibiotic. It is shown to exhibit minimal side effects and is frequently used to treat various infections. However, several initial reports indicated a possible drug interaction between fluoroquinolones and warfarin, that result in clinically significant changes in hypoprothrombinemic response (Hoffbrand, 1974; Leor et al., 1987; Leor and Matetzki, 1988; Linville and Matanin, 1989; Mott et al., 1989; Kamada, 1990). Some of these cases have been reported to the Food and Drug Administra-

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tion which have prompted the FDA to recommend revisions in product labeling. Some of these initial case reports were summarized in one review (Jolson et al., 1991). The report indicated that patients with steady state prothrombin time who were on warfarin therapy for a long time experienced a significant increase (at least 50%) in prothrombin time (PT) following initiation of fluoroquinolone therapy. Although the patients were on other concomitant drug therapy, none were considered a contributory factor. None of the reported cases determined the underlying mechanism responsible for the interaction. However, single-dose-controlled studies of ciprofloxacin, enoxacin and norfloxacin in man failed to show this interaction (Toon et al., 1987; Rocci et al., 1990; Rindone et al., 1991). In addition, randomized multiple dose studies of patients on long-term anticoagulation receiving therapeutic course of ciprofloxacin for 7-10 days also did not detect any significant effect of ciprofloxacin on the mean PT ratios (Rindone et al., 1991; Bianco et al., 1992). This lack of interaction in the above studies does not rule out that the interaction may occur in occasional patients or a subgroup of patients with a higher dose of ciprofloxacin or at greater levels of anticoagulation.

In the current study, an attempt to stimulate this interaction in an animal model was undertaken. A multiple dose study was carried out in male Wistar rats to determine the feasibility of investigating the mechanism of this serious and possible fatal interaction.

2. Materials and methods

2.1. Materials

Ciprofloxacin HCl, quinine hemisulphate and warfarin sodium were purchased from Sigma Chemical Co. (St Louis, MO, USA). All reagents and chemicals were HPLC or analytical grade and used as received.

2.2. Animals

Thirty six male Wistar rats (weighing 300–400 gm) were used in phase I and II of this study. Animals were kept in a controlled environment with a constant humidity and temperature (25.5 ± 1°C). Drinking water and dry food pellets were available ad libitum.

3. Methods

3.1. Dosing and sampling

3.1.1. Phase I

This part of the study was carried out to determine the optimum daily dose of warfarin that would result in a PT of 1.5-2 fold of the control value. Three groups of rats (six in each group) were placed in separate cages. Blood samples were collected from the orbital venous plexus and the PT was determined before warfarin treatment. For 5 consecutive days, each group received multiple daily oral dose of 0.1, 0.2 or 0.6 mg/kg of warfarin sodium. Two hours after the last treatment, blood samples were collected and the PT was determined. The animals were observed daily for any signs of bleeding or other adverse effects.

3.1.2. Phase II

The results obtained from phase I indicated that a daily dose of 0.1 mg/kg was suitable to produce a prothrombin time range of 1.5–2 of control values. In another three groups, each group consisted of six rats, animals received daily oral doses of one of the following treatments for 5 consecutive days: warfarin sodium 0.1 mg/kg (group I), ciprofloxacin suspension 50 mg/kg (group II), or a combination of 50 mg/kg ciprofloxacin followed by 0.1 mg/kg warfarin solution 1 h later (group III). On day 5, blood samples were collected from all three groups at 2 h after the last dose of respective treatments. Blood samples were collected into two tubes to be used for PT measurements and serum analysis for ciprofloxacin concentrations for group II and III. After allowing the animals to recover for 3 days, blood samples were withdrawn for another PT

evaluation. Animals were under close observations for any signs of stress or bleeding.

3.2. Determination of prothrombin time

PT was measured by the manual tilt tube technique described by Quick (Quick, 1966). Briefly, the blood samples were collected in 3.8% sodium citrate in a ratio of 9:1. The samples were kept on ice until analyzed. Blood was then centrifuged for at least 10 minutes at 3000 rpm in a refrigerated centrifuge. The plasma was then separated and kept at 4°C until tested. 0.1 ml thromboplastin (Manchester reagent, stored at 2–8°C) was added to 0.1 ml plasma, and the mixture was incubated at 37°C for 5 min. An aliquot of 0.1 ml of prewarmed calcium chloride (0.025 mole/L) was added to the mixture and the stopwatch was started. A normal control was run along with the test samples. The tube were tilted gently and continually until a solid clot formed or fibrin threads appeared. The speed and angle of tilting was standardized to 3 tilts through 90° every 5 s to control glass activation and minimize cooling. The clotting time was recorded in seconds. Each test was performed in duplicates.

3.3. HPLC assay of Ciprofloxacin

Serum concentrations of ciprofloxacin was determined by a specific HPLC assay (Jim et al., 1992). Briefly, serum samples (0.2 ml) were precipitated with 400 μl of acetonitrile after the addition of 50 μl of quinine solution as the internal standard (IS) and the mixture was vortexed for 30 s. Following centrifugation, the supernatant was evaporated at 45°C under a stream of nitrogen to about 200 μl. An aliquot was thinjected directly into the loop injector. The drug and the IS were eluted from a 10 μm μ-Bondapack C-18 cartridge with a mobile phase consisting of 20% acetonitrile in 0.1 M sodium dihydrogen phosphate. The pH was adjusted to 3.9 with phosphoric acid. The flow rate was 2.5 ml/min. The effluent was monitored on a fluorescence detector using an excitation and emission wavelength of 280 and 455 nm, respectively.

3.4. Statistical analysis

A one-way analysis of variance using SAS computer program was conducted to compare the prothrombin time of the three different treatments on day 5 and after the recovery on day 8. Group comparisons was done using the LSD multiple comparison procedure. The PT of the ciprofloxacin group was compared to that of the control values (before treatments) using the paired t-test. A P value of < 0.05 was considered significant.

4. Results and discussion

Phase I study was conducted to determine the optimum dose of warfarin that can be used throughout the interaction study. The mean control PT for all rats before treatments was 12.3 $\pm$ 2.8 seconds. Following oral administration of 0.1, 0.2, and 0.6 mg/kg for 5 consecutive days, the mean prothrombin time determined on the last day were: 20.1 $\pm$ 7.5, 31.4 $\pm$ 18.5 and 66.8 $\pm$ 64.8 seconds, respectively (Fig. 1). The lowest given dose (0.1 mg/kg) was chosen for the interaction study (Phase II) since it resulted in a PT of 1.63 of the control value. Moreover, the rats in this group did not show any signs of stress or sickness. On the contrary, the rats in the other two groups showed some signs of bleeding around the nose, weakness and stress. Furthermore, all the rats in the high dose group (0.6 mg/kg) died on day 8 after dosing. However, only two rats died in the medium dose group (0.2 mg/kg).

The interaction between ciprofloxacin and warfarin was conducted in a another group of 18 rats (Phase II). Serum samples from group II and III were analyzed for ciprofloxacin concentration using HPLC assay to assure good absorption of ciprofloxacin. All rats in the above two groups showed a detectable serum concentrations of ciprofloxacin (mean $\pm$ S.D. 1.25 $\pm$ 0.28 μg/ml on day 5, that is decreased to < 0.1 μg/ml 3 days after the drug was stopped). The concentration of ciprofloxacin indicated that the drug was well absorbed and available at the therapeutic maximum concentrations (1–3 μg/ml) (Nightingdale, 1993) throughout the study.

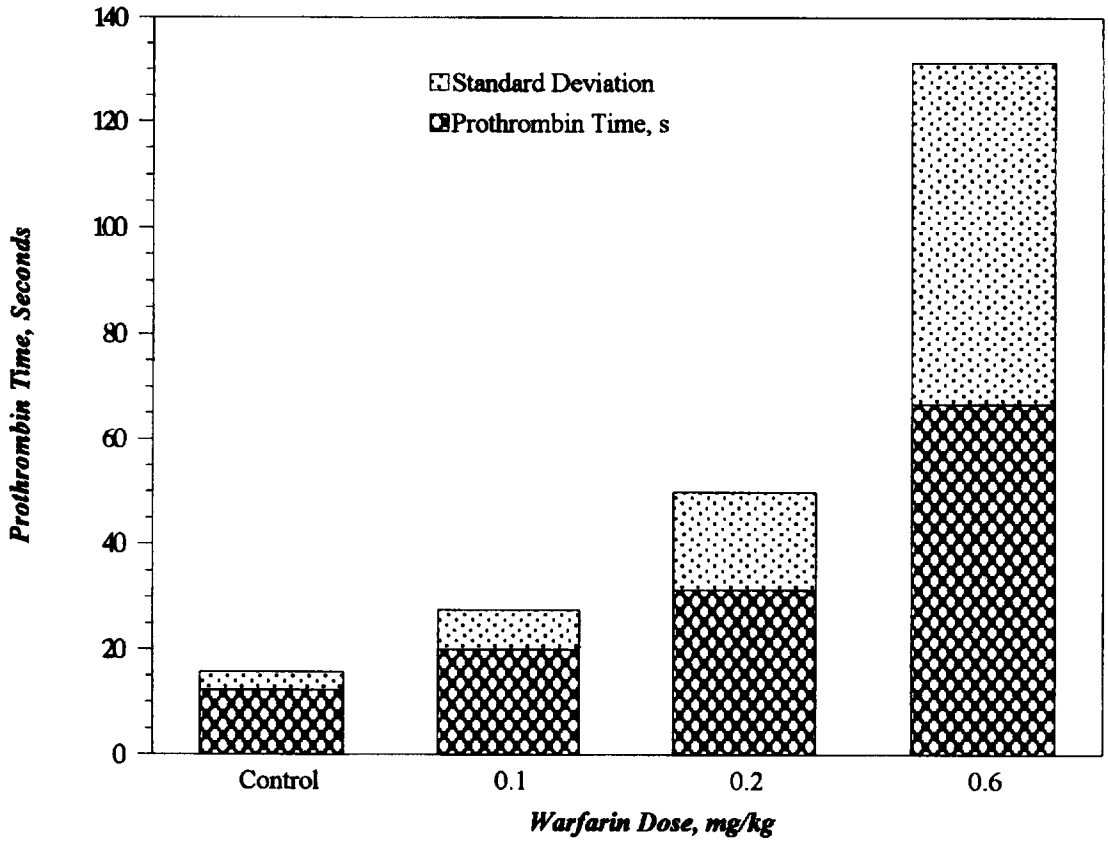

Fig. 1. Mean prothrombin time (± S.D.) in rats (n = 6) determined after daily oral administration of different doses of warfarin sodium for 5 days

The mean control PT of all rats in phase II was 12.8 ± 3.1 seconds. The PT determined in this phase on the last day of treatment were 18.9 ± 5.2, 14.42 ± 0.9 and 32.7 ± 10 seconds for I, II, and III, respectively, as shown in Fig. 2. The PT of the three groups were compared using one way analysis of variance (ANOVA). A statistical significant difference was found between the three groups ($P < 0.05$). In the combination group, the PT was increased significantly to 32.7 as compared to 18.9 seconds for warfarin alone. The PT measured for ciprofloxacin group was not significantly different from that of the control group (14.4 vs. 12.8 seconds). These results indicate that a pronounced interaction exists between ciprofloxacin and warfarin and also that the use of ciprofloxacin alone has no effect on prothrombin time.

Three days following cessation of treatment, PT for the three groups were 18.6 ± 0.72, 14.6 ± 2.0 and 15.8 ± 1.1 seconds for groups I, II, and III, respectively, as presented in Figure 2. A statistical significant difference existed between the PT of the three groups after recovery ($P < 0.01$). As indicated, the combination group rapidly recovered on day 8 to normal PT value from 32.7 to 15.8 seconds. However, the two other groups did not show any noticeable change in PT from day 5 to day 8. These data suggest that for the combination group, warfarin was probably cleared much faster when ciprofloxacin was discontinued probably due to the removal of ciprofloxacin that was inhibiting warfarin metabolism.

In all case reports submitted to the FDA as individual cases, the proposed mechanisms of action were the inhibition of warfarin metabolism

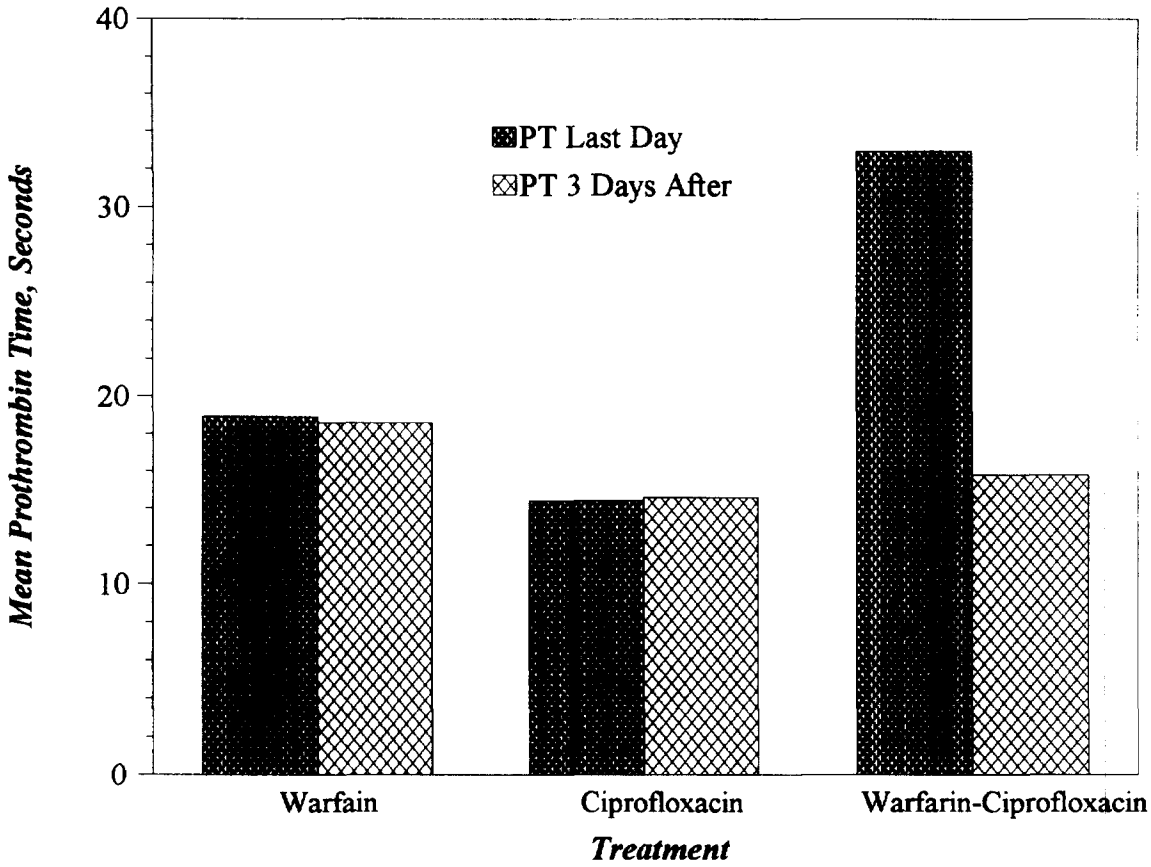

Fig. 2. Mean prothrombin time in rats (n = 6), measured 2 h (on day 5) and 3 days (on day 8) after the last oral daily doses of warfarin sodium, ciprofloxacin, and the combination.

by the anti-infective agent, displacement of warfarin's binding to protein and/or the effect of the fluoroquinolones on inhibition of vitamin K-producing bowel flora (Jolson et al., 1991). However, the actual mechanism was not investigated. The FDA recommendations to physicians were to be aware of this interaction and to closely monitor the PT in patients concurrently receiving warfarin and a fluoroquinolone. The significant increase in PT, observed following the coadministration of both drugs in the current study, indicated that this interaction can occur under normal conditions of dosing and in healthy animals and in a reasonable short period of time. Although warfarin pharmacokinetics were not investigated in this study, the interaction seems to be of a pharmacokinetics nature. According to the above results, this interaction could be explained by the metabolic inhibition of warfarin by ciprofloxacin. Warfarin is a low extraction ratio drug in which its clearance is mainly affected by its intrinsic clearance and its free fraction in plasma. The inhibition of warfarin's intrinsic clearance by the fluoroquinolone will result in significant reduction of warfarin clearance. This in turn will increase the steady state concentration of warfarin in plasma, increase its pharmacological effect and prolong the PT. The mechanism of ciprofloxacin inhibition to warfarin metabolism is supported by a significant reduction of theophylline clearance when ciprofloxacin was co-administered (Rybak et al., 1987; Thomson et al., 1987; Bem and Mann, 1988; Wijnands et al., 1986). On the other hand, discontinuation of ciprofloxacin in the com-

bination group, led to rapid recovery of PT from 32 to 15.6 seconds. This fast recovery might be due to the fact that the metabolic inhibitor, ciprofoxacin, was discontinued that resulted in a faster elimination of warfarin.

This animal model will allow us to do further investigation of the speculated mechanisms underlying this interaction.

References

Bem, J.L. and Mann, R.D., Danger of interaction between ciprofloxacin and theophylline. *Br. Med. J.*, 296 (1988) 1131

Bianco, T.M., Bussey, H.I., Farnett, L.E., Linn, W.D., Roush, M.K. and Wong, Y.W.J., Potential Warfarin-Ciprofloxacin interaction in patients receiving long-term anticoagulation. *Pharmacotherapy*, 12 (1992) 435–439.

Hoffbrand, B.I., Interaction of nalidixic acid and warfarin (letter). *Br. Med. J.*, 2 (1974) 666.

Jim, L.K., El-Sayed, N. and Al-Khamis, K.I., A simple high-performance liquid chromatographic assay for ciprofloxacin in human serum. *J. Clin. Pharm. Ther.*, 17 (1992) 111–115.

Jolson, H.M., Tanner, A., Green, L. and Grasela, T.H., Adverse reaction reporting of interaction between warfarin and fluoroquinolones. *Arch. Intern. Med.*, 151 (1991) 1003–1004.

Kamada, A.K., Possible Interaction between ciprofloxacin and warfarin. *Drug Intell. Clin. Pharm.*, 24 (1990) 27–28.

Leor, J., Levartowsky, D. and Sharon, C., Interaction between nalidixic acid and warfarin (letter). *Ann. Intern. Med.*, 107 (1987) 601.

Leor, J. and Matetzki, S., Ofloxacin and Warfarin (letter). *Ann. Intern. Med.*, 109 (1988) 761.

Linville, T. and Matanin, D., Norfloxacin and Warfarin (letter). *Ann. Intern. Med.*, 110 (1989) 751–752.

Mott, F.E., Murphy, S. and Hunt, V., Ciprofloxacin and Warfarin (letter). *Ann. Intern. Med.*, 111 (1989) 452–453.

Nightingdale, C., Pharmacokinetic Considerations in Quinolone Therapy. *Pharmacotherapy*, 13 (1993) 34S–38S.

Quick, A.J. *Hemorrhagic diseases and thrombosis*, 2nd. ed., Lea and Febiger, Philadelphia, 1966.

Rindone, J.P., Keuy, C.L., Jones, W.N. and Garewal, H.S., Hypoprothrombinemic effect of warfarin not influenced by Ciprofloxacin. *Clin. Pharm.*, 10 (1991) 136–138.

Rocci, M.L., Vlasses, P.H., Distlerath, L.M., Gregg, M.H., Wheeler, S.C., Zing, W. and Bjornsson, T.D., Norfloxacin does not alter warfarin's disposition or anticoagulant effect. *J. Clin. Pharmacol.*, 30 (1990) 728–732.

Rybak, M.J., Bowles, S.K., Chandrasekar, P.H. and Edwards, D.J., Increased theophylline concentrations secondary to ciprofloxacin. *DICP*, 21 (1987) 879–881.

Smith, P., Arnesen, H. and Holme, I., The effect of warfarin on mortality and reinfarction after myocardial infarction. *N. Engl. J. Med.*, 323 (1990) 147–152.

Thomson, A.H., Thomson, G.D., Hepburn, M. and Whiting, B., A clinically significant interaction between ciprofloxacin and theophylline. *Eur. J. Clin. Pharmacol.*, 33 (1987) 435–436.

Toon, S., Hopkins K.J., Garstang, F.M., Aarons, L., Sedman, A. and Rowland, M., Enoxacin-Warfarin interaction: Pharmacokinetic and stereochemical aspects. *Clin. Pharmacol. Ther.*, 42 (1987) 33–41.

Wijnands, W.J.A., Vree, T.B. and Van Herwaarden, C.L.A., The influence of quinolone derivatives on theophylline clearance. *Br. J. Clin. Pharmacol.*, 22 (1986) 677–683.