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Lack of interaction between meloxicam and warfarin in healthy volunteers

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Abstract *Objective*: The effect of multiple oral doses of meloxicam 15 mg on the pharmacodynamics and pharmacokinetics of warfarin was investigated in healthy male volunteers. Warfarin was administered in an individualized dose to achieve a stable reduction in prothrombin times calculated as International Normalized Ratio (INR) values. Then INR- and a drug concentration-time profile was determined. For the interaction phase, meloxicam was added for 7 days and then INR measurements and the warfarin drug profiles were repeated for comparison. Overall, warfarin treatment lasted for 30 days.
Results: Warfarin and meloxicam were well tolerated by healthy volunteers in this study. Thirteen healthy volunteers with stable INR values entered the interaction phase. Prothrombin times, expressed as mean INR values, were not significantly altered by concomitant meloxicam treatment, being 1.20 for warfarin alone and 1.27 for warfarin with meloxicam cotreatment. *R*- and *S*-warfarin pharmacokinetics were similar for both treatments. Geometric mean (% gCV) AUC_{SS} values for the more potent *S*-enantiomer were 5.07 $mg \cdot h \cdot l^{-1}$ (27.5%) for warfarin alone and 5.64 $mg \cdot h \cdot l^{-1}$ (28.1%) during the interaction phase. Respective AUC_{SS} values for *R*-warfarin were 7.31 $mg \cdot h \cdot l^{-1}$ (43.8%) and 7.58 $mg \cdot h \cdot l^{-1}$ (39.1%).
Conclusion: The concomitant administration of the new non-steroidal anti-inflammatory drug (NSAID) meloxicam affected neither the pharmacodynamics nor the pharmacokinetics of a titrated warfarin dose. A combination of both drugs should nevertheless be avoided and, if necessary, INR monitoring is considered mandatory.

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Introduction

Meloxicam is a new non-steroidal anti-inflammatory drug (NSAID) for the treatment of osteoarthritis and rheumatoid arthritis. Meloxicam is a potent inhibitor of cyclooxygenase (COX) [1] with a selectivity for the inducible COX-2 isoenzyme in several models [2–4]. This may explain the good effectiveness and superior safety profile of this drug [5]. Warfarin is an orally active anticoagulant of the coumarin class which is widely used in the treatment and prophylaxis of various thromboembolic diseases such as deep venous thrombosis [6], transient ischaemic attacks [7], reoccurrence of myocardial infarction and other thromboembolic disorders [8, 9]. Warfarin is prone to several kinds of interactions, which may be clinically important, since an interaction leading to enhanced action might result in bleeding. Conversely, any interaction which hampers the anticoagulant effect might worsen the underlying disease for which the coumarin was prescribed. Warfarin and other coumarins, such as phenprocoumon or acenocoumarol, were shown to interact with phenylbutazone [10, 11], ibuprofen [12] or flurbiprofen [13]. Some of these interactions were of clinical importance and thus it was necessary to investigate whether meloxicam causes a pharmacodynamic or pharmacokinetic interaction with the coumarin anticoagulants. Warfarin was chosen as it is the best-studied anticoagulant in this respect [14]. The similarity between the individual coumarins may be enough, in terms of metabolism and pharmacodynamic action [14], to allow the extrapolation of results to the other coumarins.

Coumarins may interact via interference with their mechanism of action or their pharmacokinetic behaviour. Most NSAIDs are highly bound to plasma albumin [15], which also binds warfarin [16], and thus an interaction can occur by displacement of warfarin from

albumin. In some cases this leads to an enhanced effect of warfarin [17]. The clinical importance of this type of interaction may be less than is usually assumed [18], since the more free drug available for action, the more free coumarin is also available for metabolism. This means that the drug is metabolized faster, which may result in an only temporary increase in free drug. This is less likely to be of clinical importance, as the onset of action of warfarin is delayed because of a pool of clotting factors which will have been produced earlier. Meloxicam is more than 99% plasma albumin bound and thus a protein-binding interaction with warfarin would be theoretically possible.

A more important type of interaction may occur due to enzyme induction, which was described for barbiturates [19]. This type of interaction seems not to occur with the NSAIDs developed more recently and no signs of enzyme induction were seen with meloxicam during the preclinical development. Most often an interaction may occur by inhibition of metabolism, if two drugs are metabolized by the same enzyme. *S*-Warfarin [20] as well as some [21, 22], if not all, NSAIDs, are metabolized by the isoenzyme CYP 2C9. Meloxicam is also predominantly metabolized this isoenzyme (J. Schmid, personal communication). The R-enantiomer of warfarin, which is approximately 5 times less active than the S-enantiomer, is predominantly metabolized by the 3A4 isoenzyme [23]. CYP 3A4 is much more abundant in human liver (up to one-third of all cytochrome P450 [24]) than the isoenzyme 2C9. Thus clinically important interactions on the basis of this isoenzyme are less likely. However, potent inhibitors of the isoenzyme 3A4 such as quinidine do interact clinically with warfarin [25].

Any drug which is tightly bound to albumin and is predominantly metabolized by CYP 2C9 may interact with warfarin. Thus the possibility of an interaction between meloxicam and warfarin was investigated.

Materials, methods and subjects studied

Study design, clinical procedures and subjects

The study was of a two-period, sequential-treatment design, with the administration of individual warfarin doses to achieve a stable enhanced INR value on day 17 after the first warfarin dose. An initial fixed-dose regimen of 10 mg on day 1, 5 mg on day 2 and 4 mg on days 3 to 5 was followed by a dose titration according to the daily measured INR values. An INR value between 1.2 and 1.8 was intended. From day 14, an individual fixed dose of warfarin was maintained. After 3 more days, to demonstrate a stabilization of the INR value, a drug concentration-time profile was determined by sampling blood on day 17 predose and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after the warfarin dose. Beginning on day 18, all volunteers also received meloxicam 15 mg once daily as capsules. To monitor the achievement of steady state for meloxicam, additional blood samples were taken in the morning to quantify meloxicam predose plasma concentrations. Steady state for meloxicam was achieved within 4–5 days. On day 24 all measurements done on day 17 were repeated and the administration of meloxicam was terminated. The fixed warfarin dose was continued until day 30 and the INR values monitored during the meloxicam washout until day 31. A post-treatment examination was performed on day 39.

Thirteen healthy male volunteers with a mean age of 27 years (range 19–42), a mean body weight of 80 kg (range 70–99) and a mean body height of 179 cm (range 170–185) completed both periods of this study. The study received Ethics Committee approval and written informed consent was obtained prior to the trial. No concomitant medications were allowed during the study and smoking was prohibited. All subjects received the drugs after a standardized continental breakfast with 200 ml tap water.

Blood was collected into heparinized tubes, and the plasma separated by centrifugation and stored frozen at −20 °C until analysis.

Pharmacodynamic measurements

Prothrombin time (PT) was determined from citrated plasma samples using an automated nephelometric coagulation laboratory analyser, ACL (Instrumentation Laboratory Co., Lexington, MA, USA), and a high-sensitivity calcium thromboplastin, a lyophilized extract from rabbit brain with the addition of an optimal concentration of calcium ions (IL Test produced by Instrumentation Laboratory Co., Lexington) as reagents.

Each thromboplastin batch was calibrated against the International Sensitivity Index (ISI) of a reference standard. International normalized ratio (INR) was derived from [PT ratio sample/reference]^{ISI} [9].

Protein binding

Freshly prepared human ethylenediaminetetraacetate (EDTA) plasma was spiked with ^{14}C-labelled warfarin (Amersham, Braunschweig, Germany and subsequently with various concentrations of meloxicam. Then the mixture was subjected to ultrafiltration (Amicon ultrafiltration vials) and radioactivity of the filtrate measured by liquid scintillation counting.

Assay procedures

Warfarin was quantified in plasma by an enantioselective high-performance liquid chromatography (HPLC) method using ultraviolet detection based on a previously used method [26]. Assay precision was within 10.9% and deviation from theoretical values (accuracy) was less than ±10.7%. The limit of quantification was 12.8 $ng \cdot ml^{-1}$ for both enantiomers.

Meloxicam was quantified in plasma by a specific HPLC method using column switching and ultraviolet detection. Plasma (100 µl) was combined with internal standard and applied to enrichment columns filled with Perisorb RP2 by a stream of water. Proteins and other plasma constituents were removed by washing the enrichment columns for 4 min at 1.5 $ml \cdot min^{-1}$ with water. Chromatographic separation was achieved on a analytical reversed-phase column (ODS Hypersil 5 µm, 50 °C) after column switching with eluent (1.5 $ml \cdot min^{-1}$). The eluent consisted of a mixture of methanol (24.66 ml), tetrahydrofurane (253 ml) and 0.067 $mol \cdot l^{-1}$ potassium phosphate buffer pH 7.2 (1.60 l) and contained 7.2 g cetyltrimethylammonium bromide. Ultraviolet detection at 365 nm was used for quantitation of the eluting analytes. The intrastudy validation resulted in an assay precision within 6.5% and an assay accuracy with ±2.5% in quality control samples. The limit of quantification was 0.05 $\mu g \cdot ml^{-1}$.

Sample Size Estimation and Data Analysis

A sample size of 12 volunteers was found necessary to demonstrate, by a paired *t*-test, whether the difference in INR values exceeded 25% between test (warfarin alone) and reference (warfarin with meloxicam) treatment. This was found valid for an intraindividual coefficient of variation of 20%. An INR elevation of 25% was considered to be of at least borderline clinical importance. It is known that some subjects do not achieve stable INR values within

a certain time. For this reason 16 volunteers were enrolled in the stabilization period to ensure 12 evaluable subjects with stable INR values in the subsequent interaction phase. A P-value of 0.05 was considered to be significant.

R- and *S*-Warfarin plasma concentration-time data were analysed by established non-compartmental procedures [27]. The drug predose concentration in steady state ($C_{pre,ss}$), the maximum drug plasma concentrations in steady state ($C_{max,ss}$) and the time to reach $C_{max,ss}$ ($t_{max,ss}$) were determined directly from the data. The area under the plasma concentration-time curve during one dosing interval in steady state ($AUC_{0-24h} = AUC_{ss}$) was determined by the logarithmic trapezoidal method.

Lack of interaction for pharmacokinetic parameters was tested by applying bioequivalence criteria [28] to *R*- and *S*-warfarin $C_{max,ss}$ and AUC_{SS} data using a multiplicative model. The equivalence range was 80–125%.

Results

Tolerability

Both warfarin and meloxicam were well tolerated. There were no adverse events associated with the administration of either drug. In particular, no bleeding events occurred.

Pharmacodynamics

Arithmetic mean with (standard deviation) prothrombin times, calculated as INR values, are shown in Fig. 1. The first 5 days with a fixed-dose regimen generated INR values outside the range desired for this trial and the warfarin dose was consequently lowered in the titration phase, which lasted until day 14. Sufficiently stable mean INR values were achieved by day 10 of the study and a mean value of 1.20 (0.14) was measured on day 17, when the plasma concentration time profiles were generated for *R*- and *S*-warfarin. The individual warfarin dose was then kept constant. The subsequent addition of meloxicam did not change INR value as shown Fig. 1. On day 24, when steady-state conditions for meloxicam were reached, a mean INR value of 1.27 (0.26) was found. The values on days 17 and 24 were statistically not different ($P = 0.13$, paired t-test). Ninety per cent confidence intervals ranged from 99.4% to 112%. On day 31, after washout of meloxicam and 24 h after the last warfarin dose, the mean INR value was 1.20 (0.10) $n = 12$).

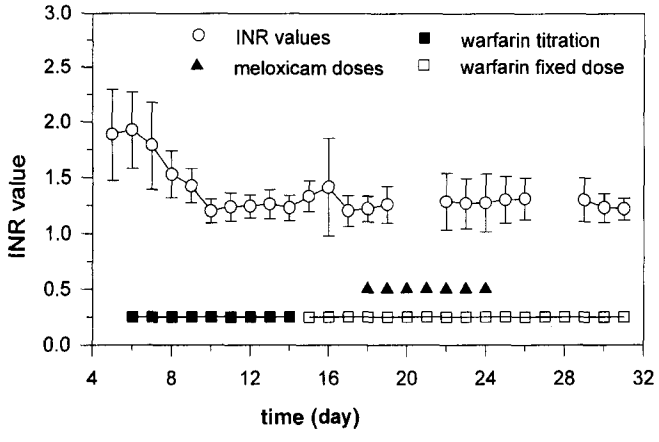

Fig. 1 Arithmetic mean INR values (with SD) without (day 17) and with (day 24) concomitantly administered meloxicam in 13 healthy male volunteers. A high INR value on day 16 (2.76) in one volunteer, preceded by (1.31) and followed by a lower INR value, caused the deviating behaviour on this day

Pharmacokinetics

The geometric man *R*- and *S*-warfarin plasma concentrations determined on days 17 (without meloxicam) and 24 (with meloxicam) are shown in Fig. 2, protein binding data in Table 1, mean pharmacokinetic parameters in Table 2 and statistical analyses are summarized in Table 3.

The in vitro measured protein binding of radiolabelled racemic warfarin was not affected by meloxicam (Table 1). Warfarin and meloxicam concentrations were selected to cover the complete therapeutic range of both substances.

R-Warfarin concentrations were higher than *S*-warfarin concentrations (Fig. 2), which is in line with the published higher clearance for *S*-warfarin [29]. Maximum concentrations were achieved 3 h after drug in-

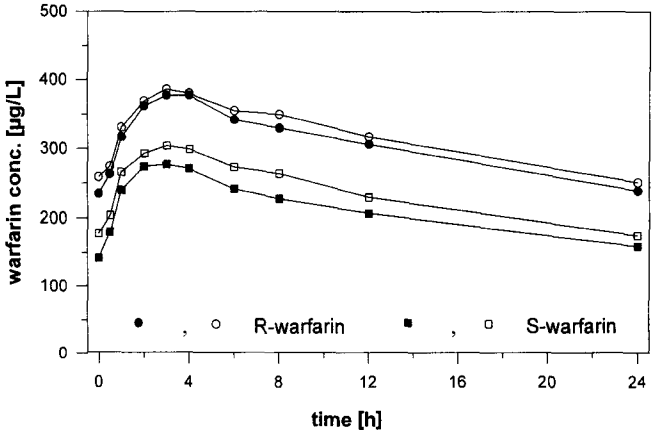

Fig. 2 Geometric mean *R*- and *S*-warfarin plasma concentrations without (day 17) and with (day 24) concomitant meloxicam administration in 13 healthy male volunteers. *Filled symbols* without meloxicam, *open symbols* with meloxicam

Table 1 Protein binding of racemic warfarin in human plasma in the presence of meloxicam. In vitro measurements, plasma from other healthy volunteers. Arithmetic means (SD)

	Warfarin concentration (10^{-6} mol·l^{-1})	(10^{-5} mol·l^{-1})
Control	99.32 (0.03)	99.32 (0.02)
+ 0.3 mg·l^{-1} meloxicam	99.29 (0.02)	99.32 (0.01)
+ 3.0 mg·l^{-1} meloxicam	99.30 (0.02)	99.30 (0.01)
+ 10.0 mg·l^{-1} meloxicam	99.29 (0.02)	99.31 (0.01)

Table 2 Pharmacokinetic parameters for *R*- and *S*-warfarin with and without meloxicam coadministration in 13 healthy male volunteers

	R-Warfarin				*S*-warfarin			
	With meloxicam gMean	(gCV)	Without gMean	(gCV)	With meloxicam gMean	(gCV)	Without gMean	(gCV)
$C_{max,ss}$ ($\mu g \cdot l^{-1}$)	413	(42.1)	416	(42.4)	336	(25.5)	309	(24.2)
$t_{max,ss}$ (h)[a]	3	(0.5–8)	3	(0.5–4)	3	(0.5–8)	3	(0.5–4)
$C_{pre,ss}$ ($\mu g \cdot l^{-1}$)	259	(39.1)	235	(40.8)	178	(27.4)	142	(30.6)
AUC_{ss} ($mg \cdot h \cdot l^{-1}$)	7.58	(39.1)	7.31	(43.8)	5.64	(28.1)	5.07	(27.5)

[a]Median and range

take, with no apparent influence by the additional intake of meloxicam. *R*-Warfarin concentrations were similar for both periods. There was no evidence that meloxicam changed *R*-warfarin pharmacokinetics. *S*-Warfarin showed a trend to slightly higher (+11%) plasma concentrations (AUC_{ss}) with intake of meloxicam (Table 2), which is not unexpected, since meloxicam and *S*-warfarin are metabolized by the same isoenzyme. Bioequivalence criteria were still fulfilled, which is illustrated by the shortest 90% confidence intervals (90% CI) of 98.6–120% for $C_{max,ss}$ (point estimate 109%) and 107–116% for AUC_{ss} (point estimate 111%) (Table 3).

Geometric mean meloxicam predose concentrations were 0.817 $mg \cdot l^{-1}$ (52.5% gCV) on day 24, and the time course of concentrations on the previous days demonstrated that steady state was properly achieved on day 24 (data not shown).

Discussion

The current study indicates a lack of interaction between racemic warfarin and concomitantly administered meloxicam, on the basis of both protein binding and metabolic interaction. The lack of a clinically important pharmacodynamic interaction is clear by the observation that INR values did not change significantly. Lack of a pharmacokinetic interaction was evident because the 90% CI for *S*-warfarin were within the bioequivalence range 80–125%. Vice versa, the observed mean meloxicam predose concentration was similar to values obtained in earlier trials with healthy volunteers [30], indicating that warfarin seems to have no effect on meloxicam pharmacokinetics. Similar results were obtained with tenoxicam [31]. Lornoxicam, on the other hand, has shown a statistically significant 20% increase in INR values [32].

It may be argued that the actual warfarin dose given to the volunteers was quite low as reflected by small INR values. This low dose was chosen to keep the risk of bleeding for the healthy volunteers as low as possible. However, the interaction of oral anticoagulants with drugs inhibiting their metabolism is dose dependent. Thus an interaction might best be seen using a small warfarin dose and a high dose of the interacting drug, in this case the NSAID meloxicam: The recommended dose of meloxicam is 7.5 mg, which may be increased to 15 mg, if necessary. In other words, concerns regarding safety of the healthy volunteers were compatible with the intention to detect even a small pharmacodynamic or pharmacokinetic interaction. In therapeutic practice, where much higher warfarin doses are used, but the same or even smaller meloxicam doses are applied, a smaller rather than a greater interaction would therefore be expected.

A combination of meloxicam with an oral anticoagulant can by no means be advocated due to several conditions, which are outside the scope of this trial. NSAIDs may produce gastrointestinal bleeding and ulcers, which can be worsened by oral anticoagulants. Furthermore, there is good evidence for inhibition of platelet function by NSAIDs and this may further complicate the titration of the dose of a therapeutically necessary anticoagulant. Finally, there might be a few patients, e.g. with abnormal low CYP 2C9 expression, in whom an interaction can occur. Such rare cases cannot be covered by a clinical interaction trial with 13 volunteers and this possibility should be borne in mind. Thus a combination of an NSAID with an oral anticoagulant remains an exception, which has to be carefully justified on a case-by-case basis. INR values should be monitored in such patients receiving both meloxicam and oral anticoagulant to prevent bleedings.

Table 3 90% Confidence intervals for warfarin $C_{max,ss}$ and AUC_{ss} with and without concomitant meloxicam

	R-Warfarin		*S*-Warfarin	
	Point estimate	90% CI	Point estimate	90% CI
$C_{max,ss}$	99.3	89.9–110	109	98.6–120
AUC_{ss}	104	99.1–108	111	107–116

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