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# Vitamin K deficiency amplifies anticoagulation response to ximelagatran: possible implications for direct thrombin inhibitors and their clinical safety

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**Abstract** Dietary vitamin K is known to influence the anticoagulation response to warfarin. It is possible that dietary vitamin K availability also influences the pharmacological activity of other oral anticoagulants, which target the vitamin-K dependent clotting proteins in the coagulation cascade. This study examined whether vitamin K insufficiency affected anticoagulation response to the direct thrombin inhibitor, ximelagatran. Anticoagulation response to ximelagatran and warfarin in rats on a normal diet was compared to those on a vitamin K deficient diet. Ximelagatran and warfarin increased prothrombin time (PT) by 1.4- and 1.3-fold, activated partial thromboplastin time (APTT) by 1.8- and 1.4-fold and ecarin clotting time (ECT) by 6.8- and 1.2-fold, respectively, in rats on normal diet. Vitamin K deficient diet alone caused modest increases in PT, APTT and ECT. The anticoagulant activity of both ximelagatran and warfarin was significantly greater in rats on vitamin K deficient diet (6.1- and 12.3-fold for PT, 2.6- and 5.1-fold for APTT and 2.9- and 1.6-fold for ECT, respectively) compared to those on normal diet. Factor II activity was reduced by both ximelagatran (58%) and warfarin (44%) in rats on normal diet. However, factor II activity was virtually abolished (<0.1%) by both drugs in rats on vitamin K deficient diet. The results suggest that oral anticoagulant drugs, whose primary site of action is not within the vitamin K cycle, may also exhibit variability in clinical response due to dietary variation as the established coumarin drugs such as warfarin.

**Keywords** Anticoagulants · Diet · Direct thrombin inhibitors · Vitamin K · Warfarin

## Introduction

Few drugs have stood the test of time for as long as the coumarin anticoagulants. Warfarin, the most commonly used derivative, has been in clinical use for several decades, despite well-known difficulties in dose optimisation and a safety record, which would normally have caused it to be deleted from most drug formularies, if a suitable alternative existed. Its clinical usage has continued because of its proven and well-established effectiveness in the treatment and prophylaxis of thromboembolic disease together with the failure of more novel drugs to meet modern requirements for efficacy and safety.

Warfarin has a narrow therapeutic range and the anticoagulation response to it is notoriously unpredictable, both between and within patients. These enigmatic properties require laborious anticoagulant monitoring to prevent adverse effects and to ensure an adequate therapeutic response. Patients often fail to stabilise on one particular dose and overdosing can lead to life-threatening haemorrhage and under-dosing to therapeutic failure, both with potentially life-threatening consequences.

Various reasons for the inter-patient differences in response have been suggested, including patient age, liver size, genotype and vitamin K status [1–3], although the whole picture is incomplete. It has been suggested that alterations in dietary vitamin K may also explain some of the intra-patient variability [4–6]. Vitamin K, in its hydroquinone form, is an essential co-factor for the carboxylation of the glutamic acid residues of the amino-terminals of the coagulation proteins II, VII, IX and X, leading to their

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activation. Warfarin causes anticoagulation by inhibiting the reductase enzymes, which generate vitamin K hydroquinone from vitamin K epoxide in the liver, thus preventing activation of the clotting proteins [7]. The availability of vitamin K thus plays a role in determining the effectiveness of warfarin, and dietary modification by either lowering or increasing vitamin K intake can increase or decrease patients' sensitivities to the drug [5, 8–10].

The variability in anticoagulation response to warfarin, its implications on patient safety and the costs associated with the frequency with which anticoagulation in patients has to be monitored, has stimulated the quest for new strategies and novel classes of drugs, which may produce more consistent effects, less toxicity and require less monitoring. The direct thrombin inhibitors belong to one class of drug, which have been developed with this in mind [11]. These agents exert their effect by inhibition of thrombin (factor IIa) activity [11] and thrombin generation [12], thus preventing the conversion of fibrinogen to fibrin.

The possibility exists that the availability of vitamin K in the diet may affect not only the anticoagulant effect of the coumarins, but also the pharmacological response to newer therapeutic agents, which affect other parts of the coagulation cascade. To test this hypothesis, studies are reported here to determine the effect of a diet, deficient in vitamin K, on the anticoagulant activity of warfarin and the direct thrombin inhibitor, ximelagatran, in rats.

## Materials and methods

The study protocol was approved by the Newcastle University Ethics Committee. A Home Office project licence was obtained for the study.

### Animals and drugs

Ximelagatran (Astra Production Chemicals) was prepared as an aqueous solution, acidified to a pH less than 3.0. Warfarin (Nycomed Pharma AS, Oslo) was prepared as an aqueous solution.

**Study 1** Male Wistar rats (200–300 g) were housed in special cages to prevent coprophagy in groups of five. They were fed water and food ad libitum. Half of the animals ( $n=45$ ) were fed a normal diet and the other half received a vitamin K deficient diet (Special Diets Services, Witham, Essex, UK) for 14 days. Fifteen rats from each diet cohort acted as controls. Of the remainder, 15 rats were given a solution of ximelagatran (20  $\mu\text{mol/kg}$ ) twice daily and a similar number were administered warfarin (0.125  $\mu\text{g/kg}$ ) once daily, by gavage, starting on day 8, for 7 days. The treatment duration enabled plasma-steady state

concentrations to be achieved for both melagatran and warfarin. In the morning of day 8 of the drug regimens, animals were anaesthetised and blood collected by cardiac puncture. The blood samples were stored in either citrate tubes before measurement of ecarin clotting time (ECT), activated partial thromboplastin time (APTT) and prothrombin time (PT), and factors II, V and VIII activity or heparin tubes for the determination of plasma vitamin K and warfarin concentrations. For the rats receiving ximelagatran blood sample was taken 1 h after the morning dose of ximelagatran on day 7 of drug regimen. This was to ensure that there were measurable plasma concentrations of melagatran (pharmacologically active metabolite of ximelagatran), which would otherwise not have been detectable if the sample was taken in the morning of day 8 (trough concentration) because of the relatively short half-life of melagatran. Plasma melagatran concentration at 1 h post-dose also corresponds to the drug's  $C_{\text{max}}$  value (AstraZeneca data on file). For the ximelagatran-treated rats, the above-mentioned haematological parameters were therefore also determined 1 h after the morning dose of ximelagatran on day 7.

**Study 2** This was conducted to investigate whether vitamin K deficiency had an effect on melagatran pharmacokinetics following the earlier demonstration in study 1 that vitamin K deficiency in rats significantly increased plasma melagatran concentration 1 h after ximelagatran dosing. Rats were divided into two groups of 96, with one group receiving a normal diet and the other a vitamin K deficient diet for 2 weeks under the same conditions as for study 1. After 1 week on the diets both groups were given ximelagatran (20  $\mu\text{mol/kg}$ ) twice daily by gavage for 7 days. An additional six rats in each diet group acted as controls and received no drug. On days 1 and 7 of the drug regimen, blood samples (approximately 4 ml) were collected by abdominal aorta puncture under anaesthesia at 0 (control rats), 0.25, 0.5, 1, 2.5, 5, 8 and 24 h post-dose for measurements of plasma melagatran concentrations.

For both studies, blood samples were centrifuged at  $2,500\times g$  immediately after collection and the resultant plasma stored at  $-80^{\circ}\text{C}$  before analysis.

### Haematological measurements

Various tests have been found to be useful for measuring the anticoagulant effect of ximelagatran in patients and volunteers such as the PT, APTT and the ECT (13), but with varying sensitivities. PT is a measure of the activity of the vitamin K dependent factors II, VII, IX and X and also of factor V, which is not vitamin K dependent. Although its use to measure the anticoagulant effect of warfarin in patients in a concentration-dependent manner is well established, it has been reported to have low sensitivity to

therapeutic plasma melagatran concentrations in patients [13]. APTT is prolonged when any of the factors VIII, IX and X in the intrinsic system are inhibited such as by DTIs and warfarin. ECT is prolonged when thrombin is inhibited and can provide a measure of the effect of ximelagatran [13] but not warfarin.

#### Activated partial thromboplastin time (APTT)

Activated partial thromboplastin time was determined using a KC10A coagulometer. Plasma (25 µl) was incubated for 3 min with 25 µl reagent (PTT-Automate, Diagnostika Stago, Asnières, France). Coagulation was started with 25 µl of 25 µmol/l calcium chloride and the time to coagulation was measured.

#### Ecarin clotting time (ECT) test

Ecarin (Triolab, Mölndal, Sweden) was dissolved to a working solution of 4 Units/ml; 50 µl of the solution was then added to 50 µl of plasma and the ECT determined using a KC10 coagulometer (Amelung, Lemago, Germany).

#### Prothrombin Time (PT)

Prothrombin time was measured by adding 25 µl of citrated rat plasma to the coagulometer (KC10A micro). After a 1-min incubation period at 37°C, 50 µl of Thromborel® S (Dade Behring, Marburg, Germany) dissolved in 10 ml water was added and the time to coagulation measured.

#### Factor II and V assay

Factor II and V assays were performed using the Coag-A Mate MTX II (Organon Teknika, Basingstoke, UK). Plasma (5 µl) was diluted 1:10 with imidazole buffer (45 µl) and added to a cuvette. Fifty microlitres of Human Factor Deficient Plasma (Organon Teknika) was added to the cuvette, and incubated for 25 s at 37°C. Coagulation was activated by adding Simplastin Excel S thromboplastin. The absorbance was followed for 150 s and the point of coagulation determined. The percent activity of Factor II or V was determined by comparing the coagulation times with a reference curve constructed from the coagulation times obtained from the clotting times of a series of dilutions of Human Verify Reference Plasma (Organon Teknika).

#### Factor VIII assay

Factor VIII assay was performed using the Coag-A Mate MTX II. Plasma (5 µl) was diluted 1:10 with imidazole buffer (45 µl) and added to a cuvette. Fifty microliters of

Human Factor VIII Deficient Plasma (Organon Teknika) was added to the cuvette and 50 µl of Platelin LS Reagent (Organon Teknika) was added and incubated for 220 s at 37°C. Coagulation was indicted by addition of 50 µl of pre-warmed Platelin LS calcium chloride 0.025 mol/l. The well was optically inspected for 150 s and the time point of coagulation determined by transforming the clotting time through a reference curve. The reference curve was constructed from the clotting time of a series of dilutions of Human Verify Reference Plasma (Organon Teknika).

#### Plasma melagatran concentrations

Melagatran plasma concentrations were determined by a liquid chromatographic-mass spectrometric method [14]. The relative standard deviation of the assay at melagatran concentrations of 10 and 25 nmol/l was 1–5%.

#### Plasma warfarin concentrations

Warfarin plasma concentrations were determined by high-performance liquid chromatography according to an established method [15]. The coefficient of variation of the assay at warfarin concentration of 280 ng/ml was 3%.

#### Plasma vitamin K concentrations

Plasma vitamin K concentrations were determined by high-performance liquid chromatography using post-column chemical reduction with zinc and fluorimetric detection, according to an established method [16]. The coefficient of variation of the assay at vitamin K concentration of 1,440 pg/ml was 12%.

#### Pharmacokinetic analysis

Pharmacokinetic analysis was carried out using the programme WinNonlin®, version 4.0.1 (Pharsight Corporation, CA, USA) using standard non-compartmental methods. Plasma concentrations that were below the limit of quantification were treated as zero for all pharmacokinetic calculations. The maximum observed plasma melagatran concentration ( $C_{\max}$ ) and time to maximum observed plasma melagatran concentration ( $t_{\max}$ ) were determined by visual inspection of the data. Terminal elimination half-life ( $t_{1/2}$ ) was calculated as  $0.693/\lambda_z$ , where  $\lambda_z$  is the rate constant associated with the terminal part of the log concentration versus time curve and estimated from linear regression using at least three data points. Area under the plasma melagatran concentration versus time curve was determined using the linear trapezoidal rule. The area under the curve from time 0 to the time of the last quantifiable concentration (AUC 0- $\tau$ )

extrapolated to infinity (AUC 0- $\infty$ ) was calculated as a measure of melagatran exposure following the first dose of ximelagatran on day 1 and the final dose of ximelagatran on day 7.

### Statistical analysis

The Minitab statistical software (version 14, Coventry, England) was used for the statistical analysis of data. Student's *t* test was used to compare plasma concentrations of vitamin K, warfarin and melagatran concentrations. Mann–Whitney test was used to compare the haematological parameters measured. Kruskal–Wallis test was used for multiple comparisons of data. Data are presented as mean  $\pm$  SD or mean  $\pm$  SEM as appropriate. Correlations between melagatran concentrations and haematological parameters were evaluated using Pearson's correlation and regression analysis. A *P* value of  $<0.05$  for comparisons was considered to be statistically significant.

## Results

### Plasma vitamin K

Plasma vitamin K concentrations were significantly lower in control rats, which were fed the vitamin K deficient diet, compared to those fed the normal diet ( $P<0.005$ ). Predictably, plasma concentrations of the vitamin were significantly lower in animals given warfarin in both the normal ( $P<0.001$ ) and vitamin K deficient ( $P<0.001$ ) groups, compared either to controls ( $P<0.001$ ) or those treated with ximelagatran ( $P<0.001$ ) (Fig. 1).

### Coagulation factors

As expected, factor II activity in control rats, which were given no drugs, was significantly lower in the group receiving the vitamin K deficient diet, compared to those on the normal diet ( $P<0.001$ ). Factor II activity was reduced in all animals receiving either ximelagatran ( $P<0.001$ ) or warfarin ( $P<0.001$ ) and was reduced to below detectable levels in those receiving either drug on the vitamin K deficient diet (Fig. 2a).

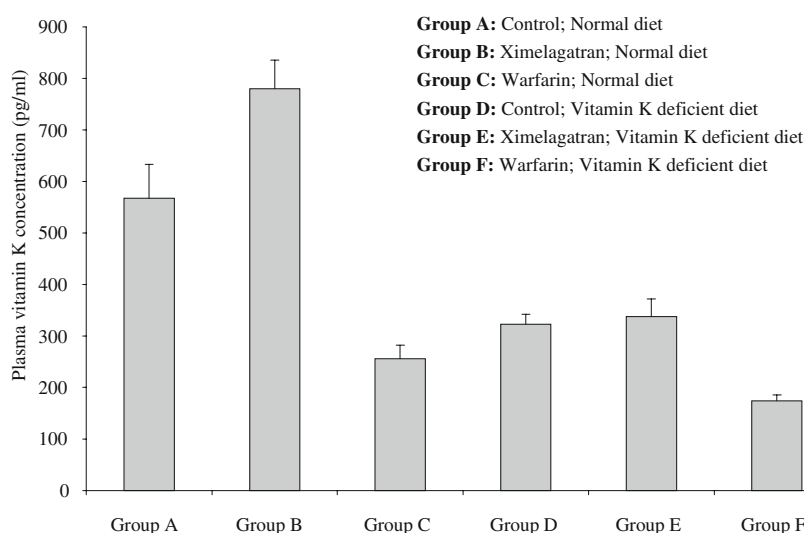
Factor V activity was not significantly different in the control groups on either diet. Similarly, factor V activity was not significantly altered in rats that received warfarin on either diet. However, rats on the normal diet that were given ximelagatran had significantly lower activity than controls ( $P<0.005$ ), and this was even lower in rats on the vitamin deficient diet that were given ximelagatran ( $P<0.001$ ; Fig. 2b).

Factor VIII activity was lower in control rats fed the vitamin K deficient diet compared to the control rats on the normal diet. In rats on normal diet factor VIII activity was decreased by over fourfold following ximelagatran ( $P<0.001$ ) and by nearly twofold following warfarin ( $P=0.01$ ) administration. In rats on vitamin K deficient diet factor VIII activity was decreased by threefold following ximelagatran ( $P<0.001$ ) administration, but there was no significant alteration in the activity following warfarin administration (Fig. 2c).

### Coagulation tests

An enhanced anticoagulant effect, evident in the three different coagulation tests, was observed when rats fed the vitamin K deficient diet were treated with either warfarin or

**Fig. 1** Mean (SEM) plasma vitamin K concentration in rats on normal diet and in rats on vitamin K deficient diet after 7 days dosing with warfarin or ximelagatran

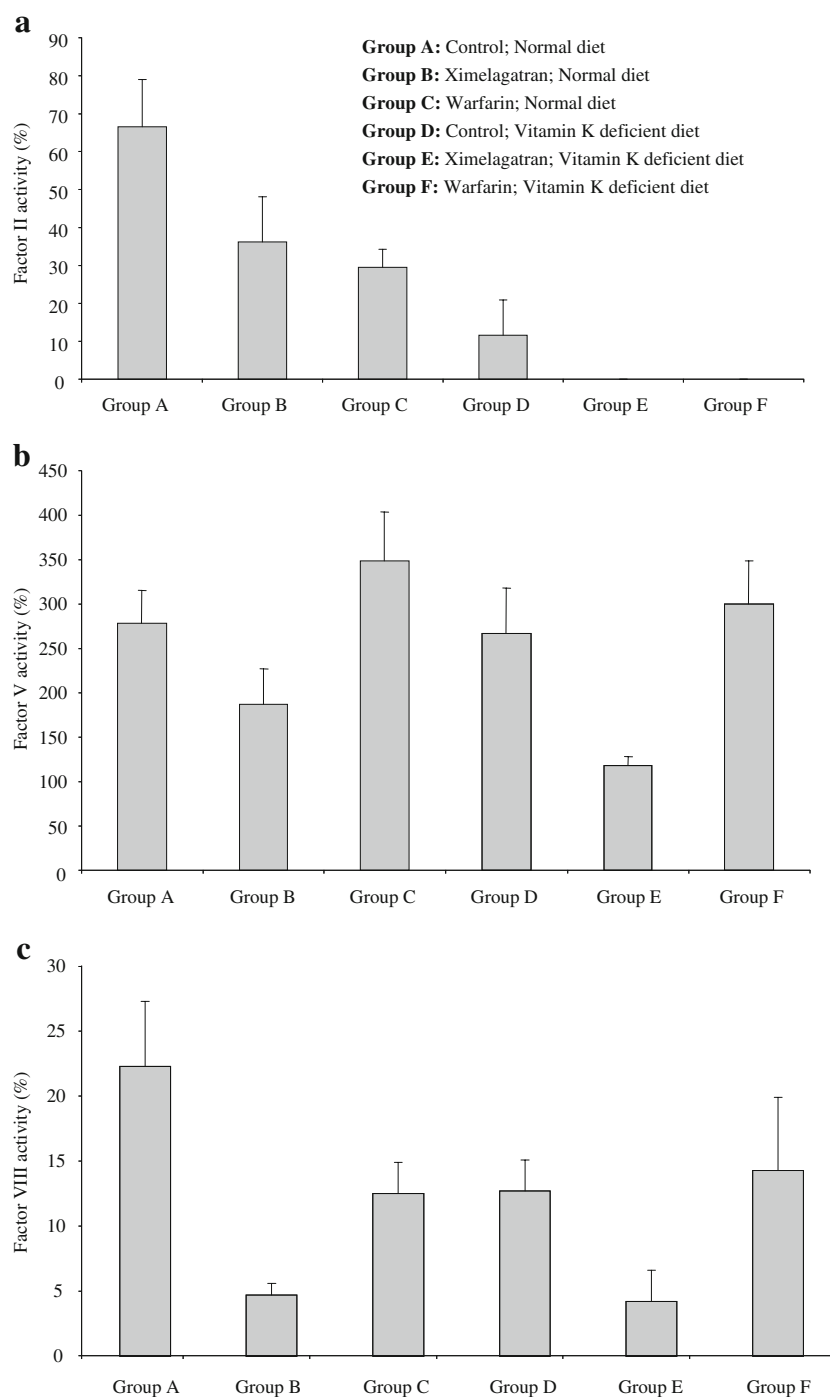


ximelagatran compared with rats on the normal diet receiving either drug.

There was a small increase in PT in rats treated with warfarin and ximelagatran on the normal diet, compared to controls. The mean PT was increased sixfold following ximelagatran ( $P<0.001$ ) and 12-fold ( $P<0.01$ ) after warfarin administration in rats on the vitamin K deficient diet compared to those fed the normal diet (Table 1).

Activated partial thromboplastin time (APTT) was significantly increased in rats on the normal diet receiving either ximelagatran or warfarin, compared to their respective controls. The mean APTT increased further by approximately threefold and by fivefold following administration of ximelagatran ( $P<0.001$ ) and warfarin ( $p<0.001$ ), respectively, in the vitamin K deficient groups (Table 1).

**Fig. 2** Mean (SEM) activity for factor II (a), Factor V (b) and Factor VIII (c) in rats on normal diet and in rats on vitamin K deficient diet after 7 days dosing with warfarin or ximelagatran





**Table 1** Coagulation assays after 7 days treatment with drugs (mean  $\pm$  SD)

	Control (no drugs)	Warfarin	Ximelagatran
PT normal diet (s)	9.37 $\pm$ 1.04	12.08** $\pm$ 1.30	12.76* $\pm$ 1.68
PT vitamin K deficient	15.24 $\pm$ 4.13	148.49** $\pm$ 65.29	77.36** $\pm$ 36.34
APTT normal diet	21.16 $\pm$ 5.86	28.05** $\pm$ 6.16	32.99** $\pm$ 7.70
APTT vitamin K deficient	30.73 $\pm$ 7.26	145.40** $\pm$ 36.41	85.38** $\pm$ 17.53
ECT normal diet	25.61 $\pm$ 0.94	31.15** $\pm$ 3.09	124.71** $\pm$ 33.67
ECT vitamin K deficient	35.68 $\pm$ 4.04	50.25** $\pm$ 3.80	364.66** $\pm$ 167.61

All values marked with asterisks are significantly different from the controls.

\* $P<0.01$ ; \*\* $P<0.001$ .

There was a moderate but significant increase in mean ECT in rats that were treated with warfarin on vitamin K deficient diet ( $P<0.001$ ). The rats on normal diet given ximelagatran showed a fivefold increase in ECT compared with controls ( $P<0.001$ ) and this was increased to more than tenfold in the vitamin K deficient animals ( $P<0.001$ ) (Table 1).

#### Plasma melagatran concentrations

Ximelagatran is a pro-drug that is rapidly absorbed after oral administration and converted to its pharmacologically active metabolite, melagatran [17, 18]. In study 1, plasma melagatran concentrations at 1 h post-dose were higher in rats on the vitamin K deficient diet compared to the normal diet [ $0.92\pm0.29$   $\mu\text{mol/l}$  vs  $0.51\pm0.23$   $\mu\text{mol/l}$  (mean $\pm$ SD);  $P<0.001$ ]. In study 2, which evaluated the impact of vitamin K deficiency on melagatran pharmacokinetics, maximum plasma melagatran concentration ( $C_{\text{max}}$ ) after the first oral dose of ximelagatran on day 1 was significantly higher ( $P<0.05$ ) in rats fed the vitamin K deficient diet compared to those fed the normal diet. Whilst maximum plasma melagatran concentration was slightly raised after twice daily dosing of drug for 7 days in rats on vitamin K deficient diet compared to those on normal diet, the differences were not significantly different (Fig. 3 and

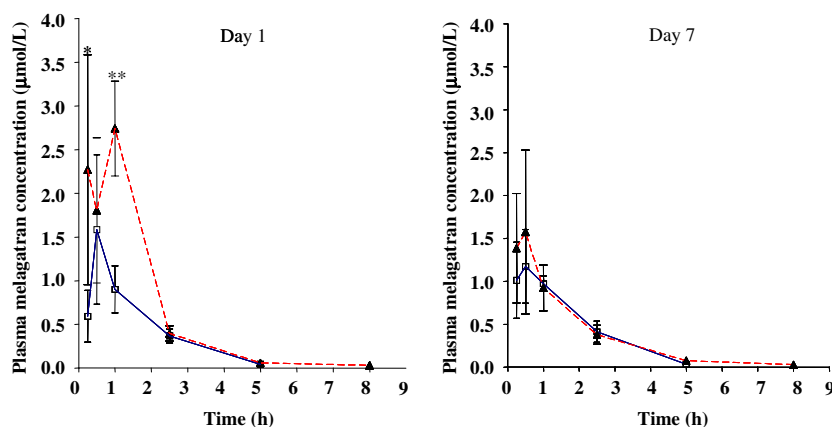
Table 2). The areas under the plasma melagatran concentration versus time curves,  $\text{AUC}_{0-\text{tau}}$  and  $\text{AUC}_{0-\infty}$ , were both nearly twofold greater in rats fed the vitamin K deficient diet compared to those fed the normal diet on day 1 but were similar between the two groups after dosing for 7 days (Table 2).

#### Relationships between PT, APTT, ECT and plasma melagatran concentrations

In study 1 the coagulation time/plasma melagatran concentration ratios on day 7 for PT ( $105\pm103$  vs  $28\pm12$  s/ $\mu\text{mol/l}$ ;  $P=0.01$ ), APTT ( $95\pm17$  vs  $71\pm30$  s/ $\mu\text{mol/l}$ ;  $P=0.02$ ) and ECT ( $444\pm213$  vs  $252\pm44$  s/ $\mu\text{mol/l}$ ;  $p<0.01$ ) were significantly greater in rats on vitamin K deficient diet compared to rats on normal diet.

In study 2, plasma melagatran concentrations highly significantly correlated with PT, ECT and APTT after dosing with ximelagatran on days 1 and 7 in both groups of rats (Table 3). The slopes of the regression lines for PT and APTT versus plasma melagatran concentration were significantly steeper (i.e. PT and APTT values were increased by a greater amount with increasing plasma melagatran concentrations) in rats on vitamin K deficient diet compared to those on normal diet. However, such a trend was not observed with ECT (Table 3).

**Fig. 3** Plasma melagatran concentrations in rats on normal ( $\square$ ) and vitamin K deficient ( $\blacktriangle$ ) diet on days 1 and 7 post-dose (single asterisk  $p=0.02$ ; two asterisks  $p<0.001$ )



**Table 2** Melagatran pharmacokinetics following ximelagatran administration (20 µg/kg) in rats on either normal or vitamin K deficient diet after the first dose on day 1 and after 7 days twice daily dosing

	Normal diet		Vitamin K deficient diet	
	Day 1	Day 7	Day 1	Day 7
$C_{\max}$ (µmol/l)	1.59±0.85	1.17±0.43	2.74±0.54*	1.57±0.95
Mean±SD (range)	(0.025 – 2.38)	(0.63 – 1.87)	(2.21 – 3.66)	(0.70 – 3.02)
$C_p$ 1 h (µmol/l) <sup>a</sup>	0.90±0.27	0.97±0.09	2.74±0.54**	0.92±0.27
Mean±SD (range)	(0.698 – 1.38)	(0.863 – 1.06)	(2.21 – 3.60)	(0.585 – 1.31)
$AUC_{(0-\tau)}$ (µmol/l)	2.30	2.28	4.20	2.54
$AUC_{(0-\infty)}$ (µmol/l)	2.30	2.32	4.26	2.65
$T_{1/2\beta}$ (h)	0.9	0.8	0.7	1.1
$t_{\max}$ (h)	0.5	0.5	1	0.5

$C_{\max}$  and  $C_p$  1 h values in the table are based on plasma melagatran concentrations from six different rats. All the other pharmacokinetic analyses are based upon plasma melagatran concentrations determined from pooled data in six rats at each sampling time point.

\* $P=0.02$ ; \*\* $P<0.001$

<sup>a</sup> Plasma melagatran concentration at 1 h after ximelagatran administration.

### Plasma warfarin concentrations

Plasma (mean ± SD) warfarin concentrations in rats on normal diet were not significantly different from those on vitamin K deficient diet (352 ng/ml±153 ng/ml vs 322±136).

### Adverse events

Six rats, administered warfarin, on the vitamin K deficient diet died with internal haemorrhages prior to completion of the experiment. One animal in the vitamin K deficient group, which received ximelagatran in study 1, also died with multiple haemorrhages before the completion of the experiment.

### Discussion

The novel direct thrombin inhibitors are purported to have a much better safety profile than the existing coumarins for

the treatment and prophylaxis of thromboembolic disorders. It is well established that alterations in the dietary intake of vitamin K in patients on coumarin therapy can lead to altered anticoagulation response. However, the impact of changes in the dietary intake of vitamin K on the anticoagulation response to direct thrombin inhibitors has not previously been explored. This is because of the perception that the mode of action of direct thrombin inhibitors, which exert their pharmacological activity by inhibition of thrombin activity, is different from that of coumarins that inhibit the recycling of vitamin K, necessary for the activation of vitamin K dependent clotting proteins, including thrombin. However, since the activation of thrombin is inextricably linked with the vitamin K cycle in the coagulation cascade, there is the possibility that the pharmacological activity of direct thrombin inhibitors could be influenced by alterations in vitamin K availability.

The enhanced pharmacological activity of warfarin in vitamin K deficient rats observed in this study confirms the well-established impact of vitamin K deficiency on anti-coagulation response to warfarin in man [4–6, 8–10]. This study also demonstrates that overt vitamin K deficiency enhances the anticoagulant activity of the direct thrombin inhibitor, ximelagatran, in rats.

Plasma vitamin K concentrations were lowered by about twofold after warfarin administration in rats on both the normal and vitamin K deficient diets. This reflects the inhibitory action of warfarin on the vitamin K epoxide reductase, which prevents the conversion of vitamin K from vitamin K epoxide in the vitamin K cycle. However, ximelagatran administration did not affect plasma vitamin K concentrations as it does not influence the reductase activity.

The effects of ximelagatran on all the coagulation tests were enhanced in vitamin K deficient animals as were its

**Table 3** Correlations between plasma melagatran concentration and PT, ECT and APTT

	Day 1		Day 7	
	$r^*$	Slope (SE)*	$r^*$	Slope (SE)*
PT normal diet (s)	0.84	6.5 (0.7)	0.93	3.6 (0.2)
PT vitamin K deficient	0.93	14.5 (0.9)	0.89	11.7 (1.0)
APTT normal diet	0.54	20.7 (5.05)	0.77	25.9 (5.28)
APTT vitamin K deficient	0.86	40.0 (2.48)	0.71	34.5 (5.46)
ECT normal diet	0.98	133.7 (4.3)	0.98	141.3 (4.5)
ECT Vitamin K deficient	0.98	119.6 (3.7)	0.95	123.6 (6.7)

$r$  Pearson's correlation coefficient.

\* $P<0.001$  for all values.



effects on the activity of factors II, V and VIII. Factor VIII is activated by thrombin in the coagulation cascade and hence inhibition of the latter by ximelagatran would prevent factor VIII activation. Vitamin K deficiency by itself caused a 50% reduction in factor VIII activity. This can also be explained by a reduction in thrombin activation caused by vitamin K deficiency.

Whilst inexplicably, melagatran  $C_{\max}$  and AUC values were significantly greater in the rats fed the vitamin K deficient diet, compared to those fed the normal diet after the first dose of ximelagatran, the corresponding values were similar between the two groups of rats after 7 days of treatment with the drug. It appears unlikely therefore that vitamin K deficiency caused any permanent changes in melagatran bioavailability and therefore the changes seen in the anticoagulant effects of ximelagatran can reasonably be explained by alterations in its pharmacodynamics, likely caused by reduced thrombin generation. The significantly greater PT, APTT and ECT to plasma melagatran concentration ratio in rats on vitamin K deficient diet compared to those on normal diet further demonstrated that the enhanced anticoagulant activity of ximelagatran is caused in the main by vitamin K deficiency rather than by any increase in drug exposure.

Predictably, warfarin did not reduce the activity of factor V, since this factor is not vitamin K dependent, but the drug did have an unexplained effect on factor VIII in rats on the normal diet. This effect was not seen in the vitamin K deficient animals.

Although it is possible that reference curves for the determination of clotting protein activity using human plasma may not parallel that generated from rat plasma, this should not, in the main, make any significant differences in the relative changes observed in clotting protein activity between rats on normal diet and those on the vitamin K deficient diet.

The present study results show that overt vitamin K deficiency in rats enhanced the anticoagulant response to ximelagatran. However, it is not clear at this stage as to whether subclinical vitamin K deficiency causes any exaggerated response to ximelagatran in the anticoagulated patient population.

Although clinical trials showed that ximelagatran, the first oral direct thrombin inhibitor to be developed, is an effective anticoagulant for many clinical indications [19–22], it was withdrawn from the market by the manufacturer in February 2006 [23] because of fears of liver toxicity. There are, however, other direct thrombin inhibitors that are under development and will appear in its wake. If ximelagatran is representative of oral direct thrombin inhibitor class of drugs, then it is reasonable to assume that vitamin K deficiency influences all the drugs in this class as well as possibly other novel oral anticoagulants that target the coagulation cascade, such as factor Xa inhibitors.

Should vitamin K deficiency be demonstrated to affect the pharmacological activity of direct thrombin inhibitors in man, then the anticoagulant response to such drugs may be less predictable than originally thought. Consequently, certain patient groups with erratic poor diets such as the elderly [24, 25] might be at a greater risk of becoming over-anticoagulated and unstable. It may therefore be prudent to further examine the influence of vitamin K deficiency on anticoagulant response for other novel oral anticoagulants currently under development.

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