Effects of N-methyl-thiotetrazole cephalosporin on haemostasis in patients with reduced serum vitamin K₁ concentrations

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SUMMARY Two patients with low random serum vitamin K₁ concentrations but with normal prothrombin times and normal biological assays of the vitamin K dependent coagulation proteins were treated with an N-methyl-thiotetrazole cephalosporin (cefotetan) postoperatively. Four to six days later both patients developed a prolonged prothrombin time and a noticeable and specific lowering of the clotting activities of factors II, VII, IX and X, though the serum vitamin K₁ concentrations remained unchanged. Crossed immunoelectrophoresis of prothrombin showed the appearance of a second peak corresponding to descarboxyprothrombin (PIVKA II). These abnormalities corrected after vitamin K administration. These data are consistent with the hypothesis that cephalosporins with an N-methyl-thiotetrazole side chain inhibit the hepatic utilisation of vitamin K but that this only causes hypoprothrombinaemia when liver reserves of vitamin K are low.

Cephalosporin antibiotics have been known for some years to be associated with reduced synthesis of the vitamin K dependent clotting factors II, VII, IX and X and occasionally to be responsible for clinical bleeding episodes.¹ Clinical reports have usually implicated cephalosporins with N-methyl-thiotetrazole (NMTT) side chains such as latamoxef,^{2,3} cefamandole,⁴ and cefoperazone.⁵ These events occurred most in patients with renal failure, those who are severely malnourished with chronic gastrointestinal disease, or those who had been receiving prolonged parenteral nutrition.⁶ It has been suggested that NMTT cephalosporins may cause vitamin K deficiency by suppressing the vitamin K producing micro-organisms of the colonic microflora.^{7,8} Contradicting this hypothesis is the lack of evidence that menaquinones (vitamin K₂) can be absorbed from the colon and the fact that other antibiotics such as tetracyclines, which also strongly suppress the bowel flora, are not known to be associated with vitamin K deficiency. Another hypothesis is that the NMTT side chain directly inhibits the vitamin K dependent γ -carboxylation of clotting factors in the liver cell.^{9,10}

Cefotetan (ICI) is a new compound in the cephamycin subdivision of the cephalosporins with an

NMTT side chain.¹¹ We investigated the effects of this new cephalosporin on vitamin K₁ metabolism and hepatic synthesis of the vitamin K dependent coagulation factors after parenteral nutrition given over several days to two patients with a normal prothrombin time and normal factor II, VII, IX and X assays before treatment. Both patients, however, had subnormal serum vitamin K₁ concentrations before treatment was started.

Patients

CASE 1

An 81 year old woman presented with a two month history of generalised abdominal pain and vomiting over the previous week. On admission (day 1), a diagnosis of a strangulated left femoral hernia was made and operative repair was carried out that day. Prophylactic antibiotic cover was started with intravenous cefotetan (2 g) given every 12 hours; treatment was continued for four days. She gave a history of recent weight loss, but routine liver function and renal function tests were within normal limits. On day four, after six doses of cefotetan, treatment was stopped and 10 mg of vitamin K₁ was given intramuscularly later that day when the prothrombin time was found to be prolonged.

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CASE 2

A 74 year old man presented with a 24 hour history of generalised abdominal pain that later localised to the right iliac fossa. On admission (day 1), peritonitis was diagnosed, and at operation that day a perforated gangrenous appendix was removed. Prophylactic antibiotic cover was started preoperatively with intravenous cefotetan (2 g) given every 12 hours and continued as treatment because free pus was found at operation. Liver function and renal function tests were within normal limits on admission. On day 6, after ten doses of cefotetan, treatment was stopped and 10 mg of vitamin K₁ was then given intramuscularly because he was noted to have a prolonged prothrombin time.

Methods

Blood was collected by clean venepuncture into plain glass tubes for serum, or 0.106M trisodium citrate (9:1) for plasma.

Serum concentrations of Vitamin K₁ were measured by high performance liquid chromatography with dual electrode electrochemical detection, as described previously,¹² except that quantification was made by reference to an internal standard (menaquinone-6) added at the extraction stage. The inter-run coefficient of variation for 37 replicate analyses of a plasma pool (mean concentration 1100 pg/ml) carried out over six months was 11.5%.

Prothrombin time was measured manually using the human brain Manchester comparative reagent. Factor V and IX coagulant assays were performed by one stage methods using congenitally deficient substrate plasma.¹³ One stage factor VII assay was carried out with an artificially depleted substrate plasma.

Factor X was assayed using Russell's viper venom and "Diagen" factor X deficient plasma (Diagnostic Reagents Ltd). Prothrombin was measured by a two stage clotting assay¹³ or by a chromogenic substrate assay using Ecarin (Pentapharm Ltd) and S2238 (Kabi Vitrum Ltd).¹⁴ Factors II and IX antigen values were measured by immunoelectrophoresis,¹⁵ using suitable antisera (Dako Ltd, Diagnostica Stago Ltd). All factor II, VII, IX and X assays were standardised against a commercial reference plasma (Immuno Ltd) with a normal range of 50–200 U/dl. Crossed immunoelectrophoresis of prothrombin was performed,¹⁶ using calcium lactate buffer to separate descarboxyprothrombin (PIVKA-II) from γ -carboxylated prothrombin.

Results

Tables 1 and 2 show the results of assays for plasma coagulation factors and serum vitamin K₁. Before treatment with antibiotics both patients had normal prothrombin times (13.7 and 13.7 seconds, normal range 12–14 seconds) and normal activities in the biological assays for the vitamin K dependent clotting factors II, VII, IX and X, but serum concentration of vitamin K₁ (54 and 78 pg/ml) were substantially reduced compared with those in the range (170–680 pg/ml) in 45 normal fasting adults using the same electrochemical method. After four and six days of treatment with cefotetan the serum vitamin K₁ concentrations remained unchanged (52 and 73 pg/ml), but both patients developed a severe hypoprothrombinaemia, evidenced both by an increased prothrombin time (34.7 and 20.5 seconds) and clotting assays that showed a specific lowering of the four vitamin K dependent factors. Hypo-

Table 1 Coagulation results (case 1) after six 12 hourly doses of cefotetan and then six and 24 hours after 10 mg vitamin K₁ given intramuscularly

Day	Vitamin K ₁ (pg/ml)	Prothrombin time (seconds)	V:C (U/dl)	VII:C (U/dl)	IX:C (U/dl)	IX:Ag (U/dl)	X:C (U/dl)	II:C (U/dl)	II Ag (U/dl)	Ecarin II (U/dl)
1	54	13.7	52	59	180	76	77	87	78	77
4	52	34.7	47	3	2	46	36	30	59	72
+ 6 hours	1700	38.2	54	39	38	75	32	21	108	77
+ 24 hours	36 000	14.4	90	68	160	51	32	33	99	72

Table 2 Coagulation results (case 2) after ten 12 hourly doses of cefotetan and then 12 hours after 10 mg vitamin K₁ given intramuscularly

Day	Vitamin K ₁ (pg/ml)	Prothrombin time (seconds)	V:C (U/dl)	VII:C (U/dl)	IX:C (U/dl)	IX:Ag (U/dl)	X:C (U/dl)	II:C (U/dl)	II Ag (U/dl)	Ecarin II (U/dl)
1	78	13.7	50	65	150	88	90	50	108	63
6	73	20.5	72	37	38	80	18	29	135	77
+ 12 hours	27 900	14.2	61	47	126	90	59	26	132	70

Figure Crossed immunoelectrophoresis of prothrombin (case 1) Top gel: day 1 before treatment with Cefotetan. Middle gel: day 4 after six doses of Cefotetan. Bottom gel: 24 hours after 10 mg vitamin K₁.

prothrombinaemia was most severe in the patient having the lower serum vitamin K₁, a serum concentration that was less than one third the lower limit of the normal range. In this patient the reduction in clotting factors was most noticeable for factors VII and IX (half lives four to six hours and 24 hours, respectively) than for factors X and II, which have longer circulating half lives (48 hours and 60 hours, respectively). Over the same period the clotting activity of factor V, which is also synthesised in the hepatocyte but is not a vitamin K dependent protein, did not change appreciably in either patient. Factor II activity measured by the Ecarin method and factors II and IX antigenic activity, however, did not change in either patient. The Ecarin assay measured both descarboxyprothrombin (PIVKA-II) and the biologically active fully γ -carboxylated prothrombin: the similar values before and after treatment with cefotetan indicate that the fall in biological activity of factor II is due to an impaired vitamin K dependent γ -carboxylation rather than an impaired hepatic synthesis of the core protein. This was confirmed by crossed immunoelectrophoresis, which showed the characteristic faster migrating peak of non-carboxylated species of prothrombin after treatment with cefotetan and a single peak of normal prothrombin before treatment (case 1) (figure). Similar findings were found in case 2 by crossed immunoelectrophoresis (results not shown).

The effect of 10 mg of vitamin K₁ given by intramuscular injection was most noticeable, producing after 12 to 24 hours a substantial normalisation of the prolonged prothrombin time and increased circulating concentration of biologically active vitamin K dependent factors.

Discussion

Vitamin K deficiency is usually identified by the presence of a prolonged prothrombin time that corrects after vitamin K administration. In vitamin K deficiency, or in the presence of vitamin K antagonists (such as the coumarin anticoagulant warfarin), the postribosomal γ -carboxylation of certain glutamic acid residues of the vitamin K dependent clotting factors (factors VII, IX, X and II, proteins C and S) is inhibited.¹⁷ Non γ -carboxylated vitamin K dependent proteins are unable to bind calcium ions and are inactive in the blood coagulation cascade.¹⁸ Patients with severe vitamin K deficiency have abnormal inactive non γ -carboxylated proteins in their blood. These abnormal proteins have been called PIVKAs or descarboxyproteins.

A feature of the investigations in these two patients was the measurement of their plasma concentrations of vitamin K₁ (phylloquinone). Until recently, such

measurements have been hampered by the analytical problems associated with the detection of the low circulating concentrations in blood: consequently, very little is known about plasma concentrations of vitamin K in health and disease and nothing about those concentrations that may relate to an overt or marginal deficiency of vitamin K. Using a recently developed assay, based on high performance liquid chromatography with electrochemical detection¹² but refined for use with an internal standard, the normal range of plasma vitamin K₁ in 45 healthy fasting adults was 170–680 pg/ml (median 372, mean 412 pg/ml). The plasma values of vitamin K₁ in the patients (54 and 52 pg/ml in case 1, 78 and 73 pg/ml in case 2) were notable in several respects. Firstly, they were well below the normal range; secondly, they did not change during the period of the study and; thirdly, although low, the plasma vitamin K₁ was sufficiently high to maintain normal plasma concentrations of the vitamin K dependent clotting factors until they were treated with cefotetan. The relation between plasma concentrations of vitamin K₁ and tissue reserves remains to be established, but it seems reasonable to assume that, as with other fat soluble vitamins, low plasma concentrations reflect low body stores. It is likely, therefore, that both these patients had low vitamin K reserves in the liver, which is the site of synthesis of the vitamin K dependent clotting factors. Both patients had a chronically poor nutritional state, and in the week or so before hospital admission, their dietary intake had further diminished due to generalised abdominal pain and vomiting.

It has previously been suggested that cephalosporins containing the NMTT side chain such as latamoxef and cefamandole cause prolongation of the prothrombin time by inhibiting the action of the vitamin K 2,3-epoxide reductase enzyme.¹⁹ This would seem to occur after in vivo degradation of the antibiotic and release of the NMTT side chain.^{9,10} This has been confirmed using in vitro rat liver systems showing partial inhibition of the epoxide reductase enzyme similar to the action of the oral anticoagulant warfarin.²⁰ Previous studies with cefotetan in well nourished subjects with normal serum vitamin K₁ concentrations, however, have shown no prolongation in the prothrombin time or decreased synthesis of the vitamin K dependent coagulation factors.²¹ In vivo degradation of the NMTT side chain occurs less readily in cefotetan than other NMTT cephalosporins such as latamoxef.²² Thus only in clinical situations in which there is chronic vitamin K deficiency such as elderly patients with a poor dietary intake, patients receiving prolonged parenteral feeding without vitamin K supplements,²³ or patients with chronic gastrointestinal malabsorption

states²⁴ is cefotetan likely to inhibit the vitamin K oxidation reduction cycle and cause decreased γ -carboxylation of the K dependent proteins. Obviously in patients receiving warfarin and antibiotics with a cephalosporin containing the NMTT side chain, there is a risk of a synergistic reaction with a sudden decreased γ -carboxylation of the vitamin K dependent factors and excessive prolongation of the prothrombin time.

Although both these patients developed a prolonged prothrombin time, neither had any clinical bleeding episode or spontaneous bruising. Other NMTT containing cephalosporins, particularly latamoxef²³ have often been associated with a clinical bleeding diathesis. These antibiotics, as well as inhibiting the vitamin K epoxide-reductase activity; also appreciably inhibit platelet function and prolong the bleeding time at standard therapeutic doses.²⁵ Cefotetan does not inhibit platelet function or prolong the bleeding time²¹ and is thus much less likely to cause bleeding events.

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