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Alteration of Coagulation and Fibrinolysis Systems After Multidrug Anticancer Therapy for Lung Cancer

E. C. Gabazza, O. Taguchi, T. Yamakami, M. Machishi, H. Ibata, S. Suzuki and T. Shima

Recently, an increased frequency of thromboembolic events has been reported after the administration of anticancer drugs. The precise mechanism by which these vascular phenomena occur is unknown. The current work aims at evaluating the alterations of the coagulation and the fibrinolysis systems during the administration of antineoplastic agents by means of newly developed markers of haemostasis. This investigation comprised 25 lung cancer patients treated with multidrug combination chemotherapy. D-dimer, plasmin-α_2-antiplasmin complex, fibrin degradation products, fibrinogen, antithrombin III, thrombin–antithrombin III complex, prothrombin time and activated partial thromboplastin time were measured from samples taken before and on days 2, 5, 7, 14 and 21 after the administration of antineoplastic drugs. A significant reduction in plasma concentration of fibrinolytic activity markers, DD and PAP, was observed on days 5 and 7, and on days 2, 5, 7 and 14, respectively, following the administration of chemotherapeutic drugs. Statistically significant shortening of PT and APTT on days 2, 5, 7 and 14, as well as significant elevation of the thrombin generation marker TAT were observed on days 5 and 7 after chemotherapy. These results show that relatively higher levels of coagulation activation and a lower fibrinolytic activity occur during cytotoxic drug therapy compared with basal values. Small variations of haemostatic values and a short follow-up period may explain why no thrombotic events were observed during this study. Although further studies must be done to clarify these findings, the results of this investigation suggest that an imbalance of the coagulation-fibrinolysis system might be a contributing factor in the pathogenesis of thrombotic complications during chemotherapy.

Key words: blood coagulation, fibrinolysis, chemotherapy, thrombosis
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INTRODUCTION

IT HAS been recently appreciated that antineoplastic drug therapy may predispose patients to the development of thromboembolic disease. Venous thrombosis, pulmonary embolism, thrombotic microangiopathy, cerebrovascular accidents and myocardial infarction have been described with increasing frequency in chemotherapy-treated cancer patients [1–5]. Although the lack of untreated control populations in these retrospective studies makes it difficult to differentiate whether those thrombotic complications occurred due to toxicity of the drug or were just secondary effects of the cancer itself, a recently published prospective controlled study gave unequivocal evidence that chemotherapy independently contributes to the risk of thrombosis [6].

The pathogenesis whereby such drug-induced adverse reactions occur has not as yet been elucidated. Vascular endothelial cell injury, disturbance of the clotting system and/or autonomic dysfunction have been proposed as potential mechanisms of these vascular phenomena [7]. Dysfunction of the coagulation inhibitory system or low fibrinolytic activity have been implicated, in isolated reports, as potential mechanisms of this hypercoagulation response [8, 9]. However, studies on clotting system alterations during chemotherapy provided conventional haemostatic parameters data only in the immediate post-therapy period [10]. Assessment of newly developed haemostatic tests as well as their sequential postchemotherapy changes are lacking.

The current investigation aims to evaluate the alterations of the coagulation-fibrinolysis system and the serial changes of various haemostatic parameters in cancer patients treated with a combination of antineoplastic agents. To achieve our objective, newly developed markers of *in vivo* ongoing thrombin formation (thrombin–antithrombin III complex) and of plasminogen–plasmin system activity (D-dimer, plasmin-α_2-antiplasmin complex) were measured serially in blood samples taken from lung cancer patients treated with multidrug anticancer therapy. In addition, the effect of cytotoxic drug administration on these haemostatic parameters, according to the histological type of the tumour, the clinical response to chemotherapy and after a second cycle of cytotoxic drug administration were also evaluated.

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Table 1. Patients' characteristics

	Cases
Total number of patients	25
Sex	
Male	21
Female	4
Histological classification	
Adenocarcinoma	9
Squamous cell carcinoma	4
Small cell carcinoma	12
Stage	
I	4
II	1
III	7
IV	13
Chemotherapy	
Induction	17
Adjuvant	2
Neoadjuvant	6

MATERIALS AND METHODS

This investigation comprised a total of 32 consecutive lung cancer patients hospitalised in our institution. 7 cases were excluded from this study because of liver metastasis or hepatic dysfunction. Table 1 shows the characteristics of the 25 eligible patients. There were 21 men and 4 women, age varied from 20 to 75 years (mean age 59.8). Histological grouping was carried out according to the World Health Organization classification. There were 13 cases of non-small cell lung cancer(SCLC) and 12 of SCLC.

None of the 25 cases presented laboratory abnormalities suggestive of intravascular coagulation, and no patients were receiving any drug that could alter the clotting system before the beginning of the study. D-dimer (DD), plasmin-α_2-antiplasmin complex (PAP), fibrin degradation products (FDP), fibrinogen (FBG), antithrombin III (AT-III), thrombin–antithrombin III complex (TAT), prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured in blood samples taken immediately before starting chemotherapy on day 1 and, thereafter, on days 2, 5, 7, 14 and 21 after the first cycle of chemotherapy. Measurements of the aforementioned haemostatic parameters were also carried out in midtherapy (day 5) of a second cycle of anticancer therapy.

Chemotherapeutic agents combination and schedule were as follows: (a) non-SCLC: cisplatin 80 mg/m^2 day 1, mitomycin 8 mg/m^2 day 1, vindesine 3 mg/m^2 days 1, 8 and 15; (b) SCLC: cisplatin 80 mg/m^2 day 1, etoposide 100 mg/m^2 days 1, 3 and 5.

Blood samples to test AT-III, PT, APTT and FBG were drawn into a syringe containing 3.8% trisodium citrate and measured using automated techniques (Coagu-master, Sankyo Co, Japan. Cobas Fara, Roche, Switzerland) [11]. FDP was collected with aprotinin and determined by a method previously described [12]. Heparin and aprotinin were used as anticoagulants for measuring DD, and trisodium citrate for PAP and TAT. Fragments of DD were measured using a monoclonal antibody and an enzyme-linked immunoassay developed by Elms and colleagues [13]. Samples of TAT were assayed by a solid phase enzyme immunoassay kit (Enzygnost-TAT) from Behring Werke, A.G. (Germany) in accordance with the manufacturers' instructions, and following a technique previously described [14]. Blood concentrations of PAP were assayed by ELISA (commercially radioimmunoassay kit provided by Teijin Ltd, Tokyo, Japan) using antiplasminogen antibody and peroxidase-conjugated anti-α_2-plasmin inhibitor monoclonal antibody [15].

All the samples were taken after having obtained formal and written consent of the patients, and the research was carried out in accordance with stipulations of the Helsinki Declarations. It must be noted that samples taken at the same intervals in untreated controls were not available for comparison.

STATISTICAL METHODS

Data on DD, PAP, FDP, FBG, AT-III, TAT, PT and APTT are reported as means ± standard deviation, unless otherwise specified. To test whether the six matched groups' haemostatic parameter concentrations on day 0 (baseline), 2, 5, 7, 14 and 21 come from the same population, the Friedman two-way analysis of variance by rank was applied. Multiple comparison of values obtained on day 2, 5, 7, 14 and 21 against the baseline values was employed when the ANOVA showed significant difference among the groups. A similar procedure was used to evaluate haemostatic changes during different cycles of chemotherapy. The relationship among pairs of haemostatic parameters was analysed using the Pearson product–moment correlation in normally distributed variables, or the Spearman correlation when variables showed non-Gaussian distribution. $P < 0.05$ was considered as a significant difference [16].

RESULTS

Indexes of the coagulation pathway, TAT, APTT and PT, correlated significantly with markers of fibrinolytic activity, PAP, FDP, FBG and DD (data not shown). Figure 1 shows sequential changes of the fibrinolytic system in all lung cancer patients before the initiation of chemotherapy and on days 2, 5, 7, 14 and 21 after starting the cytotoxic therapy. Compared with baseline values, significant reductions in plasma concentrations of fibrinolytic activity markers DD and PAP were observed on days 2, 5, 7 and on days 2, 5, 7, 14, respectively, following the administration of chemotherapeutic drugs. FDP and FBG blood concentrations also showed the same trend, with a significant

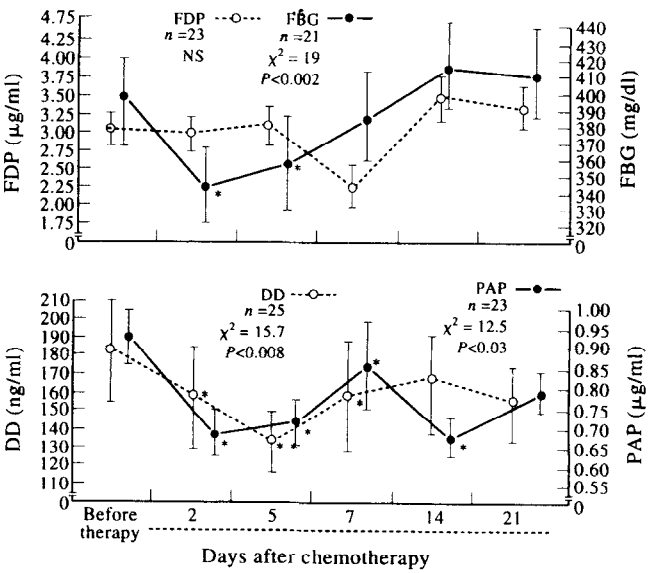

Figure 1. Time-course of plasma values of D-dimer (DD), plasmin-α_2-antiplasmin complex (PAP), fibrin degradation product (FDP) and fibrinogen (FBG) during chemotherapy for lung cancer (cycles: mean values; bars: S.E., * $P < 0.05$; ** $P < 0.01$).

decrease of the latter on days 2 and 5 (Figure 1). Concerning the coagulation system, there was a progressive increase of thrombin generation marker TAT and of functional AT-III after chemotherapy, and compared to baseline values, the elevation was statistically significant on days 5 and 7 (Figure 2). In addition, significant shortening of PT and APTT values was observed on days 5, 7, 14 and on days 2, 5, 7, respectively (Figure 2).

Sequential changes of clotting tests were also analysed in both SCLC and non-SCLC (Table 2). Significant alterations in PAP, FBG and TAT were observed in the SCLC group, but not in that of non-SCLC. DD plasma concentration changed more significantly in non-SCLC. Although PT and APTT values varied significantly in both groups, their changes were more striking in SCLC. Patients were also divided into responder and non-responder groups, according to clinical response to chemotherapy. Serial coagulation changes were not different between responder and non-responder patients (data not shown). To evaluate the effect of successive treatment with cytotoxic drug on the clotting system, values of haemostatic variables prior to initiation of therapy and in midtherapy (day 5) of a first and a second cycle of chemotherapy were measured. This latter study group comprised 14 patients that included adenocarcinoma (5 cases), squamous cell carcinoma (2 cases) and small cell carcinoma (7 cases). The results are depicted in Table 3. Compared to basal values, significant increments of TAT, shortening of PT and APTT, as well as low values of fibrinolytic markers (DD, FBG, PAP) were observed during the first and the second cycle of anticancer drug therapy.

DISCUSSION

For more than a century it has been recognised that malignant disease is frequently associated with a variety of coagulopathies and thromboembolic disorders [17]. Although the mechanism of these cancer-related disturbances of haemostasis is not completely clear, previous works demonstrated that clotting activation in malignancy is characterised by a continuous balance among procoagulant, anticoagulant and fibrinolytic processes [18]. This fact was also illustrated in the present study by the significant correlation observed between coagulation and fibrinolytic markers before the initiation of chemotherapy. However, metastatic disease with liver dysfunction, surgical manipulations, radiation or hormonal therapies and some drugs may rupture this clotting system homeostasis and so increase the susceptibility for the occurrence of localised intravascular coagulation or the classic consumption coagulopathy syndrome [19].

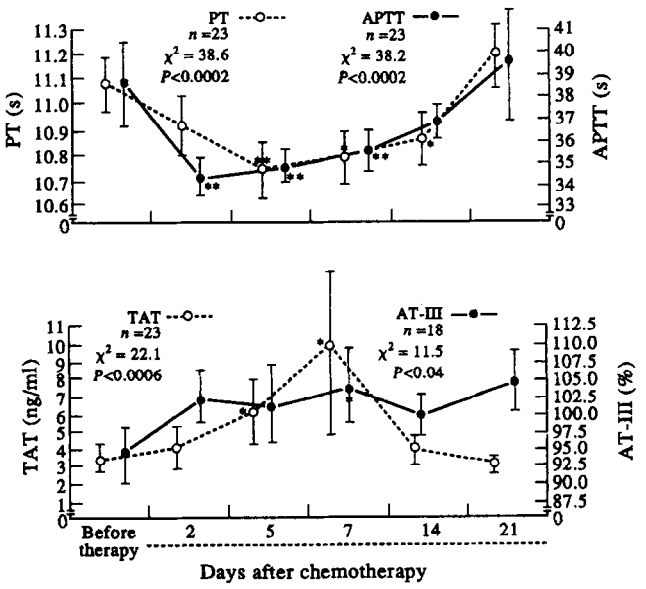

Figure 2. Time-course of thrombin–antithrombin III complex (TAT), functional antithrombin III (AT-III), prothrombin time (PT) and activated partial thromboplastin time (APTT) values during chemotherapy for lung cancer (cycles: mean values; bars: S. E.; *$P < 0.05$; ** $P < 0.01$).

Regarding the influence of cytotoxic drugs on haemostasis, there are convincing data in the literature indicating that the antineoplastic agents themselves may enhance the risk of developing thrombotic events in cancer patients [4, 6]. The cytotoxic drugs used in our chemotherapeutic protocol have also been incriminated as trigger factors of a wide variety of vascular events. Cisplatin-based chemotherapy has been associated with the occurrence of myocardial ischemic attacks, cerebrovascular accidents, Raynaud's phenomenon and veno-occlusive disorders [2, 5, 20, 21]. Clinical and pathological findings of thrombotic microangiopathy have been described in patients receiving mitomycin-containing regimens [22]. Etoposide has been reported as a potential causative factor in patients suffering from acute myocardial infarction [23]. The pathogenesis of these chemotherapy-related vascular phenomena has not yet been elucidated. Perturbation of the activity of some coagulation factors or an autonomic dysfunction with increased α-adrenergic tone have been proposed as possible mechanisms [7]. Recent investigations have suggested that drug-induced endovascular damage may also be involved in the pathogenesis of chemotherapy-related coagulopathies [24].

In the current investigation, the alterations of the coagulation and fibrinolytic pathways during chemotherapy were evaluated by measuring newly developed markers of the clotting system. These parameters have been demonstrated to be very sensitive as indicators of alterations of the clotting system, even in early clinical stages of malignancy [25]. Significant increases in TAT, AT-III and shortening of PT and APTT show, at least temporarily, a tendency toward hypercoagulability during anticancer therapy. Subclinical intravascular coagulation with compensatory hepatic production might explain this AT-III elevation. Although the decreased plasma levels of DD, PAP and FBG during chemotherapy may be accounted for by drug-induced normalisation of the fibrinolytic system, the increased activation of the coagulation system suggests that a transient imbalance of the coagulatin-fibrinolytic system is the most probable mechanism of the reduced fibrinolytic activity during administration of systemic cytotoxic drug. This transient disruption of the coagulation-fibrinolysis system might be another contributing factor for the previously reported triggering of thrombotic complications by anticancer drug therapy.

Administration of various cycles of cytotoxic drugs is the common approach for the treatment of patients with malignancy. On these grounds, the cumulative effect of a second cycle of chemotherapy was also assessed. Although all parameters tended to return to baseline values after completion of the first cycle of chemotherapy, they showed higher degree of coagulation and fibrinolytic alterations during a second cycle of anticancer therapy compared with that observed during the first cycle. However, the small number of patients and the heterogeneous components (different histological types) of our study's group did not allow us to assure the certainty of these findings.

The mechanism by which these alterations of the haemostatic system occur is still controversial. A number of factors has been

Table 2. Sequential changes of haemostatic markers after chemotherapy in each histological group

		Basal values	Number of days after chemotherapy 2	5	7	14	21	χ^2	*P* value‡
SCLC									
DD (NV:53 ± 28 ng/ml)	(*n* = 12)	176.4 ± 144.5	147.7 ± 108.3	135.1 ± 86.5	147.4 ± 123.8	168.5 ± 148.2	143.4 ± 87.2	9.1	<0.11
PAP (NV:0.4 ± 0.1 μg/ml)	(*n* = 12)	0.9 ± 0.3	0.6 ± 0.2*	0.7 ± 0.2	0.9 ± 0.7	0.6 ± 0.2*	0.6 ± 0.3*	11.4	<0.05
FDP (NV:< 10 μg/ml)	(*n* = 10)	2.8 ± 1.5	2.9 ± 1.7	2.8 ± 1.6	3.2 ± 1.6	3.3 ± 2.0	3.1 ± 1.9	2.5	<0.8
FBG (NV:200–400 mg/dl)	(*n* = 10)	414.4 ± 80.3	332.2 ± 73.8*	328.9 ± 111.1†	365.2 ± 89.2	409.1 ± 105.9	422.1 ± 133.6	19.9	<0.002
TAT (NV:1.2 ± 0.4 ng/ml)	(*n* = 11)	3.7 ± 2.3	3.5 ± 2.5	6.4 ± 7.3	16.6 ± 27.4	4.0 ± 2.6	3.1 ± 1.5	16.4	<0.006
PT (NV:10-13 s)	(*n* = 12)	11.0 ± 0.5	10.9 ± 0.4	10.7 ± 0.3*	10.7 ± 0.4	10.8 ± 0.3	11.2 ± 0.3	26.9	<0.0002
APTT (NV:29–42 s)	(*n* = 12)	40.1 ± 9.5	35.0 ± 3.5†	34.7 ± 3.4†	35.7 ± 3.2*	36.7 ± 3.3	41.2 ± 14.9	25.7	<0.0002
AT-III (NV:70–130%)	(*n* = 7)	93.5 ± 11.6	95.4 ± 15.2	97.5 ± 18.5	102.7 ± 17.5	94.5 ± 11.0	102.0 ± 19.8	8.7	<0.13
Non-SCLC									
DD (NV:53 ± 28 ng/ml)	(*n* = 13)	329.6 ± 510.9	170.0 ± 135.1	130.6 ± 68.5*	172.7 ± 150.9	171.6 ± 111.9	169.6 ± 88.1	10.9	<0.05
PAP (NV:0.4 ± 0.1 μg/ml)	(*n* = 11)	0.9 ± 0.4	0.7 ± 0.4	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.3	0.8 ± 0.3	3.0	<0.7
FDP (NV:< 10 μg/ml)	(*n* = 13)	3.2 ± 1.6	3.0 ± 1.2	3.4 ± 1.8	3.2 ± 1.5	3.6 ± 1.5	3.5 ± 1.5	7.6	<0.2
FBG (NV:200–400 mg/dl)	(*n* = 11)	387.3 ± 113.7	355.0 ± 118.1	385.4 ± 118.7	406.0 ± 143.2	419.8 ± 148.7	402.0 ± 134.7	5.6	<0.4
TAT (NV:1.2 ± 0.4 ng/ml)	(*n* = 12)	3.1 ± 1.2	4.7 ± 4.1	5.6 ± 3.7	3.8 ± 1.7	3.7 ± 1.6	2.9 ± 0.5	9.5	<0.09
PT (NV:10–13 s)	(*n* = 11)	11.0 ± 0.3	10.8 ± 0.5	10.7 ± 0.5†	10.8 ± 0.4	10.9 ± 0.4	11.2 ± 0.5	14.9	<0.02
APTT (NV:29–42 s)	(*n* = 11)	37.1 ± 3.2	33.7 ± 3.7†	35.0 ± 3.2*	35.5 ± 3.6	36.9 ± 3.2	37.6 ± 4.0	13.7	<0.02
AT-III (NV:70–130%)	(*n* = 11)	94.0 ± 12.6	106.1 ± 15.3	102.0 ± 22.4	104.7 ± 18.8	101.7 ± 12.8	105.5 ± 13.6	6.0	<0.4

TAT, Thrombin–antithrombin III complex; PT, prothrombin time; APTT, activated partial thromboplastin time; AT-III, antithrombin III; DD, D-dimer; PAP, plasmin-α_2-antiplasmin complex; FDP, fibrin degradation product; FBG, fibrinogen; SCLC, small cell lung cancer; non-SCLC, non-small cell lung cancer; NV, normal values. *$P < 0.05$ (compared to basal values); †,$P < 0.01$ (compared to basal values); ‡ *P* values were obtained comparing all groups.

Table 3. Clotting system during different cycles of chemotherapy

Variables		Basal values	First cycle‡ (5th day)	Second cycle‡ (5th day)
DD	(ng/ml, n = 10)	167.8 ± 117.8	107.2 ± 48.2*	92.4 ± 44.4*
PAP	(μg/ml, n = 14)	0.9 ± 0.4	0.7 ± 0.3*	0.7 ± 0.2†
FDP	(μg/ml, n = 14)	3.1 ± 1.7	2.8 ± 1.6	3.0 ± 1.7
FBG	(mg/dl, n = 11)	415.2 ± 85.7	339.3 ± 90.9†	337.4 ± 65.9†
TAT	(ng/ml, n = 11)	2.9 ± 1.2	3.8 ± 1.3*	5.4 ± 3.6*
PT	(second, n = 11)	11.0 ± 3	10.6 ± 0.4†	10.7 ± 0.2*
APTT	(second, n = 11)	39.7 ± 9.2	34.8 ± 3.1†	35.3 ± 1.0†
AT-III	(%, n = 12)	110.1 ± 15.4	94.9 ± 12.3	108.4 ± 15.5

See footnote to Table 2 for abbreviations. *$P < 0.05$ compared to basal values. †$P < 0.01$ compared to basal values. ‡Values are from the 5th day of chemotherapy. Values are expressed as mean ± S.D.

suggested. Drug-induced systemic endovascular damage and activation of some clotting factors are the most probable explanation for these abnormalities. In this respect, reduced endothelial cell release of inhibitors of the coagulation system, protein C and S, in association with an increased endothelial secretion of abnormal von Willebrand factor into the circulation, have been reported during chemotherapy [9, 22]. Further, some drugs have been described to have the property of provoking platelet activation [26]. The abnormal fibrinolytic response probably results from decreased functional levels of tissue plasminogen activator alone or coupled with higher systemic concentration of plasminogen activator inhibitor during cytotoxic drug therapy [10]. The levels of these two substances, which are secreted products of the endothelial cell, have also been found to be altered in other hypofibrinolytic states [27].

Moreover, cumulative administration of some cytotoxic drugs has been demonstrated to result in a direct toxic effect on small vessels [24, 28]. Bleomycin has been shown to provoke vacuolisation, detachment and necrosis of endothelial cells [29]. The occurrence of these pathological alterations of the endothelium could explain the alterations of the coagulation-fibrinolysis system observed in this study. Haemostatic changes showed similar patterns in both SCLC and non-SCLC, though they were more significantly expressed in the former histological group. This difference could be explained by the effect of the etoposide component of our SCLC chemotherapy protocol. Evaluation of haemostatic tests according to response to chemotherapy did not disclose major differences between responder and non-responder groups. These findings may indicate that destructive or necrotic changes of the tumours themselves are not preponderant factors in chemotherapy-inducing clotting abnormalities.

Intravascular coagulation disorders that complicate the clinical course of patients with malignant disease present a major problem in therapeutic management. They are notoriously resistant to anticoagulant therapy, and this worsens their life expectancy [30]. Therefore, it is necessary to recognise that cytotoxic drugs can act as potential triggers of haemostatic abnormalities and, consequently, carry out careful clinical and laboratory evaluations during their administration. The small variation of the haemostatic values, as well as the short follow-up period (6 weeks), might explain why no thrombotic events were observed during this investigation.

In brief, these results show that relatively higher levels of coagulation activation and a lower fibrinolytic activity occur transiently during cytotoxic drug therapy compared with basal values. Although further studies must be carried out to clarify these findings, this investigation suggests that an imbalance of the coagulation-fibrinolysis system might be a contributing factor in the pathogenesis of thrombotic complications during chemotherapy.

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Pharmacokinetics of Folinic Acid and 5-Methyltetrahydrofolic Metabolite After Repeated Oral Administration of Calcium Folinate Following Methotrexate Treatment

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The pharmacokinetic profiles of folinic acid (FA) and its active metabolite, 5-methyltetrahydrofolic acid, were studied after oral administration of decreasing doses of calcium folinate during 37 courses of high and intermediate dose methotrexate treatment in 25 lymphoma patients. FA was administered at a dose of 6 × 50 mg in 15 courses, 6 × 25 mg in seven courses, 6 × 15 mg in 10 courses and 6 × 7.5 mg in 5 courses. FA, 5-methyltetrahydrofolic acid, methotrexate and 7OH-methotrexate were assayed simultaneously by high performance liquid chromatography. When FA was administered at doses between 50 and 15 mg, maximum concentrations of both the drug and its metabolite were always obtained after 1 to 2 h and remained stable. The same was true for the equilibrium concentration of the two products at doses over 15 mg. These findings suggest saturation of absorption and metabolism of folinic acid at doses over 15 mg.

Key words: folinic acid, 5-methyltetrahydrofolate, metabolite, pharmacokinetic, methotrexate, rescue, repeated dosing

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INTRODUCTION

THE CLINICAL use of high dose (HD) methotrexate (MTX) treatment is feasible in association with the subsequent administration of 5-formyl tetrahydrofolate (THF), commonly known as folinic acid (FA) [1, 2]. Rescue has been successfully achieved by both the oral and intravenous routes [3, 4]. However, many questions concerning the optimal dose, mode and frequency of administration of FA remain unresolved.

Commercially available FA is a racemic mixture of L-CHO-THF and D-CHO-THF. Absorption of L-CHO-THF is 4 times greater than D-CHO-THF: 80 versus 20% [5]. L-CHO-THF is rapidly converted to 5-methyl tetrahydrofolic acid [6], which