15. Naguib FNM, El Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985, **45**, 5405–5412.
16. Kovach JS, Beart RW Jr, Svingen P, *et al.* Concentration of 5-fluorouracil (FU) and metabolites in human colon carcinomas and normal tissues after i.v. FU. *Proc Am Soc Clin Oncol* 1986, **5**, 177.
17. Aiba K, Allegra CJ, Park JG, Drake JC, Chabner BA. An investigation of the determinants of 5-fluorouracil (FU) resistance in human colon carcinoma cell lines. *Proc Am Assoc Cancer Res* 1989, **30**, 2383.
18. Lévi F, Blazsek I, Ferlé-Vidovic A. Circadian and seasonal rhythms in murine bone marrow colony-forming cells affect tolerance for the anticancer agent 4'-0-tetrahydropyranyladriamycin (THP). *Exp Hematol* 1988, **16**, 696–701.
19. Smaaland R, Laerum OD, Lote K, Sletvold O, Sothern RB, Bjerknes R. DNA synthesis in human bone marrow is circadian stage dependent. *Blood* 1991, **77**, 2603–2611.
20. Smaaland R, Laerum OD, Sothern RB, Sletvold O, Bjerknes R, Lote K. Colony-forming unit-granulocyte-macrophage and DNA synthesis of human bone marrow on circadian stage-dependent and show covariation. *Blood* 1992, **79**, 2281–2287.
21. Lévi F, Misset JL, Brienza S, *et al.* A chronopharmacologic phase II clinical trial with 5-FU, folinic acid and oxaliplatin using an ambulatory multichannel programmable pump. *Cancer* 1992, **69**, 893–900.
22. Longo DL, Duffey PL, De Vita VT, *et al.* The calculation of actual or received dose intensity: a comparison of published methods. *J Clin Oncol* 1991, **9**, 2042–2051.
23. Soussan A. Chronothérapie des cancers colorectaux par perfusion veineuse continue sur 5 jours de 5-fluorouracile (5-FUra) administré par pompe programmable. *Mémoire en vue du Diplôme d'Etudes et de Recherches en Biologie Humaine*, Université Paris XI, 1988.
24. Shah A, MacDonald W, Goldie J, Gudanskas G, Brisebois B. 5-FU infusion in advanced colorectal cancer : a comparison of three dose schedules. *Cancer Treat Rep* 1985, **69**, 739–742.
25. Hryniuk W, Figueredo A, Goodyear M. Applications of dose-intensity to problems in chemotherapy of breast and colorectal cancer. *Semin Oncol* 1987, **14**, 3–11.
26. Bailleul F, Lévi F, Metzger G, Regensberg C, Reinberg A, Mathé G. Chronotherapy of advanced breast cancer with continuous doxorubicin infusion via an implantable programmable device. *Proc AACR* 1987, **28**, 771.
27. Von Roemeling R, Hrushesky W. Circadian patterning of continuous floxouridine infusion reduces toxicity and allows higher dose intensity in patients with widespread cancer. *J Clin Oncol* 1989, **7**, 1710–1719.
28. Caussanel JP, Lévi F, Brienza S, *et al.* Phase I trial of 5-day continuous venous infusion of oxaliplatinum at circadian-modulated vs constant rate. *J Natl Cancer Inst* 1990, **82**, 1046–1050.
29. El Kouni M, Naguib F. Circadian rhythm of hepatic pyrimidine metabolizing enzymes and plasma uridine concentration in mice. *Proc 5th Int Conf Chronopharmacol.* Florida, Amelia Island, 1992, IV–2.
30. Zhang R, Lu E, Liu T, *et al.* Circadian rhythm of rat spleen cytoplasmic thymidine kinase: possible relevance to 5-fluorodeoxyuridine chemotherapy. *Proc 5th Int Conf Chronopharmacol.* Florida, Amelia Island, 1992, IV–3.
31. Diasio R, Zhang R, Lu Z, *et al.* Circadian variation of fluoropyrimidine catabolic enzymes:importance to fluorodeoxyuridine (FdURD) chemotherapy. *Proc 5th Intern Conf Chronopharmacol.* Florida, Amelia Island, 1992, IV–1.

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A Toxic Interaction Between Mitomycin C and Tamoxifen Causing the Haemolytic Uraemic Syndrome

A. Montes, T.J. Powles, M.E.R. O'Brien, S.E. Ashley, J. Luckit and J. Treleaven

A comparison of patients receiving combination chemotherapy with mitomycin C, mitozantrone and methotrexate (3M) with and without tamoxifen for treatment of primary breast cancer indicates an increased risk of anaemia ($P < 0.0001$) and thrombocytopenia ($P < 0.001$), but not leucopenia for patients receiving tamoxifen with their chemotherapy compared to those receiving the chemotherapy alone. Furthermore, 9 out of 94 patients receiving tamoxifen with 3M developed progressive anaemia, thrombocytopenia and abnormal renal function as early features of microangiopathic haemolytic anaemia, progressing on to various degrees of the haemolytic uraemic syndrome (HUS). This is only rarely seen with patients receiving mitomycin C alone at higher doses than used in the 3M combination and in the presence of active metastatic disease. This syndrome can be fatal and 1 of our 9 patients died. These observations indicate that there may be an interaction between tamoxifen and mitomycin C, causing an increased incidence of anaemia, thrombocytopenia and an increased risk of HUS. The combination of these two drugs should be avoided or carefully monitored.
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INTRODUCTION

THE HAEMOLYTIC uraemic syndrome (HUS) is an acquired syndrome consisting of intravascular haemolysis, thrombocytopenia (TCP) and acute renal failure with hypertension, neurological symptoms, pulmonary oedema and intolerance to blood transfusions. It was originally described by Gasser and his co-workers in 1955 as a fatal illness affecting five small children [1]. It seems probable that the major cause of the syndrome is localised microangiopathy mainly in the glomerular capillaries, this being primarily produced by an abnormal interaction between platelets and the vascular endothelium, probably mediated by abnormalities by prostacyclin production which

normally limits platelet adherence to the vascular endothelium [2]. A similar syndrome can occur in women postpartum [3] or following treatment with oral contraceptives [4]. It also occurs in patients with metastatic cancer who have received mitomycin C in doses greater than 40 mg/m^2 [5, 6] and occasionally in patients receiving tamoxifen [7] or medroxyprogesterone acetate [8], with a mortality from the syndrome as high as 50% [6, 7].

Microangiopathic haemolytic anaemia (MAHA) was the term proposed by Brain *et al.* to describe the blood film picture in patients with the HUS, the main feature being evidence of red cell fragmentation and TCP caused by an interaction between red cells and diseased blood vessels. MAHA may develop in patients suffering from disseminated mucin-secreting adenocarcinomas where contact between red cells and tumour emboli may be involved in the pathogenesis. These patients generally do not develop renal failure characteristic of the HUS [9].

The chemotherapy combination of mitomycin C, mitozantrone and methotrexate (3M) has low subjective toxicity and has been used extensively in the treatment of metastatic breast cancer [10]. Since 1990, we have used this combination in a randomised trial for the presurgical treatment of primary breast cancer. In order to achieve maximum response, we added tamoxifen 20 mg/day to the chemotherapy combination.

In December 1991, a 33-year-old female with metastatic breast cancer who had previously received 3M chemotherapy, including five injections of 7 mg/m^2 of mitomycin C, relapsed and, therefore, started tamoxifen 3 months after the last mitomycin C injection. Twenty days after starting tamoxifen she developed rapidly fatal acute renal failure. A postmortem examination showed renal changes similar to those described in the HUS associated with mitomycin C [6]. We had not seen this problem previously in patients on tamoxifen nor in over 600 patients treated with 3M alone, which raised the possibility of an unexpected drug interaction.

We, therefore, compared 94 patients who received 3M chemotherapy with tamoxifen to 45 patients who received 3M alone for the treatment of primary breast cancer.

PATIENTS AND METHODS

Between February 1990 and December 1991, 127 patients with primary operable breast cancer were included in a randomised trial of neoadjuvant vs. adjuvant chemoendocrine therapy for treatment of primary breast cancer using our standard 3M regimen [mitomycin C 7 mg/m^2 intravenously (i.v.) every 42 days for four courses, mitozantrone 7 mg/m^2 and methotrexate 35 mg/m^2 i.v. (maximum dose 50 mg) every 21 days for eight courses] with tamoxifen (20 mg orally od). At the time of this analysis 94 of these patients have been followed up for at least 3 months postchemotherapy. At the same time, a separate group of 45 patients with locally advanced primary breast cancer were treated with our standard 3M chemotherapy without tamoxifen and have similarly been screened for the presence of the syndrome. All patients had clinical records, including sequential haematology and biochemistry, available for analysis and had a blood film available for review.

The initial screen for the syndrome in both groups was done by identifying those patients with the triad of anaemia (haemoglobin < 10 g/dl), TCP (platelets < 100 × 10^9/l) and renal dysfunction (creatinine > 120 μmol/l). These patients' records were then retrospectively analysed for other characteristics of mitomycin C-induced HUS including hypertension, neurological abnormalities and abnormal reactions to blood transfusions. In 5 patients, clotting screen (prothrombin time, partial thromboplastin time, fibrinogen) and in 4 patients bone marrow was available for review.

The stage, histology, treatment of the primary disease, age and menopausal status of the patients, together with the mitomycin C dose, time to onset of the syndrome and the outcome, were included in the analysis.

Similarly, the cumulative haematological toxicity of 3M with tamoxifen was compared with 3M alone using the Mann–Whitney test for detecting significant differences.

RESULTS

The patients' characteristics for 94 patients who received 3M chemotherapy with tamoxifen (3MT) compared to 45 patients who received 3M alone as adjuvant or neoadjuvant therapy, are shown in Table 1. The patients who received 3MT were somewhat older (not significant, NS) and less likely to be premenopausal ($P < 0.01$), had significantly smaller primary

Table 1. *Characteristics of* 94 *patients with primary breast cancer receiving mitomycin C, mitozantrone and methotrexate chemotherapy* (3M) *with tamoxifen* (3MT) *compared to* 45 *patients who received only* 3M

	3MT (n = 94)	3M (n = 45)	P value
Age			
Median	56	47	NS
Range	31–71	33–69	
T Stage			
1	11	8	
2	75	16	
3	7	7	< 0.005
4	1	14	
N Stage			
0	78	28	
1	16	15	< 0.05
2		2	
Menopausal status			
Pre	24	23	< 0.01
Peri	5	4	
Post	52	16	
Surgical	13	2	
Surgery			
None		9	NS
Biopsy	0	4	
WE	79	18	
Mastectomy	15	14	
Radiotherapy			
Adjuvant	79	24	< 0.001
Chemotherapy			
Adjuvant	44	22	NS
Neoadjuvant	50	23	
Mitomycin C dose			
Mean total dose	42.7	39.4	
95% confidence interval	41.1–44.4	35.9–42.9	NS
S.D.	8.3	12.0	

NS, not significant; S.D., standard deviation.

Correspondence to T.J. Powles.
A. Montes, T.J. Powles, M.E.R. O'Brien and S.E. Ashley are at the Department of Medicine; and J. Luckit and J. Treleaven are at the Haematology Department, The Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, U.K.
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tumours ($P < 0.005$) and axillary lymph node involvement ($P < 0.05$) and were more likely to have had local radiotherapy than those who received 3M without tamoxifen ($P < 0.001$). There was no obvious difference in the mean total dose of mitomycin C (42.7 vs. 39.4 mg). However, the triad of progressive anaemia, TCP and renal dysfunction was found in 9/94 (9.6%) patients who received 3M with tamoxifen compared to none of the 45 patients who received 3M alone.

These 9 patients were marginally older (mean 63 years) than the 85 unaffected 3MT patients (mean 57 years, NS) and significantly older than the 45 3M patients (mean 47 years, $P < 0.05$). These 9 patients have been tabulated in Table 2 according to the severity of anaemia, TCP and abnormal renal function, indicating an association with other features of the syndrome such as hypertension, evidence of haemolysis and increased requirement for blood transfusion. Clotting screen was normal in 5 patients who were tested.

All 9 patients had some degree of hypertension, which was present in 4 patients prior to starting mitomycin C. Red blood cell fragments were identified in 6 patients. 8 patients required blood transfusion for progressive anaemia, 3 of whom had an adverse reaction including headache (3 patients) and pulmonary oedema (1 patient). 1 patient developed transient quadrantanopsia.

With regard to prognosis, 1 patient has died from renal failure and postmortem examination showed histological evidence of HUS in the kidney, the other 8 patients are alive with no evidence of active breast cancer, although 3 have mild HUS and 3 have residual renal dysfunction.

The mean total dose of mitomycin C was 26.5 mg/m^2 (range 23.4 to 29.3 mg/m^2), with no obvious correlation between the dose and the severity of the HUS. The mean time from the start of 3M to the first identification of HUS was 10 months (range 7–12).

The cumulative haematological toxicity in patients receiving 3M with or without tamoxifen is summarised in Fig. 1 which shows a progressively greater fall in the mean haemoglobin ($P < 0.0001$) and platelet counts ($P < 0.001$) but not white count for patients receiving 3M with tamoxifen compared to 3M alone.

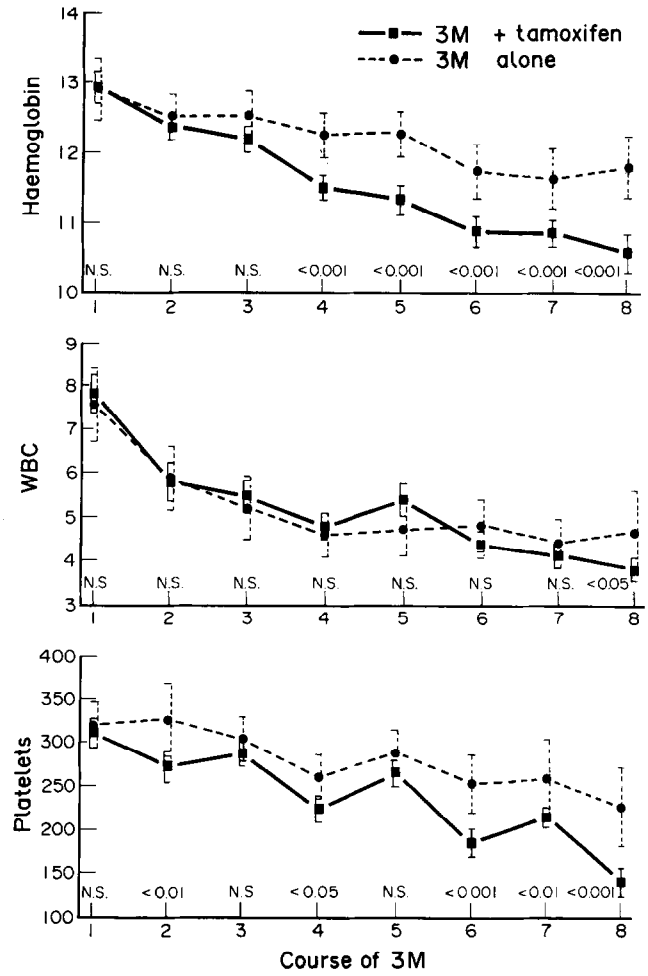

Fig. 1. Haematological toxicity. Mean value with 95% confidence interval.

Table 2. Clinical and investigative characteristics of 9 patients with the triad of anaemia, thrombocytopenia and abnormal renal function

	Patient								
	1	2	3	4	5	6	7	8	9
Anaemia (WHO grade)	4	4	3	2	3	2	2	1	1
Thrombocytopenia (WHO grade)	3	3	2	2	1	2	1	2	2
Renal toxicity (WHO grade)	4	2	2	1	1	1	1	1	1
Hypertension	Mod	Mod	Mod	Mild*	Mild	Mod	Mild*	Mild*	Mild*
RBC fragments	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Clotting screen	Norm	Norm	Norm	ND	Norm	Norm	ND	ND	ND
Blood transfusion (units)	15	15	5	9	2	4	5	0	1
Adverse reaction	+	+	+	0	0	0	0	0	0
Neurological abnormalities	0	+	+	0	0	0	0	0	0
Bone marrow	Norm	Norm	Norm	—	—	—	—	—	Norm
Mitomycin C dose (mg/m^2)	29.3	25.5	27.0	26.6	28.1	23.4	27.7	25.2	25.8
Persistent abnormal renal function			+	+				+	
HUS status at present		+			+	+			
Died of HUS	+								

*Hypertensive prechemotherapy. Norm = normal, Mod = moderate, ND = not done.

Table 3. Haematological toxicity (WHO grade) 3 months after completion of treatment with 3M only (45 patients, 46 treatments) or with continued tamoxifen (3MT) (94 patients)

	Anaemia ($P < 0.001$)		Leucopenia ($P < 0.01$)		Thrombocytopenia ($P < 0.001$)	
	3M ($n = 46$)	3M + TAM ($n = 94$)	3M ($n = 46$)	3M + TAM ($n = 94$)	3M ($n = 46$)	3M + TAM ($n = 94$)
WHO grade						
0	31 (67.4%)	27 (28.7%)	19 (41.3%)	27 (28.7%)	39 (84.8%)	52 (55.3%)
1	13 (28.3%)	46 (48.9%)	18 (39.1%)	26 (27.7%)	3 (6.5%)	12 (12.8%)
2	2 (4.3%)	14 (14.9%)	9 (19.6%)	23 (24.5%)	2 (4.3%)	16 (17.0%)
3	0	6 (6.4%)	0	13 (13.8%)	2 (4.3%)	12 (12.8%)
4	0	1 (1.1%)	0	5 (5.3%)	0	2 (2.1%)
> 1	2 (4.3%)	21 (22.3%)	9 (19.6%)	41 (43.6%)	4 (8.7%)	30 (31.9%)
Any	15 (32.6%)	67 (71.3%)	27 (58.7%)	67 (71.3%)	7 (15.2%)	42 (44.7%)

TAM, tamoxifen.

Assessment of the blood counts 3 months after completion of 3M chemotherapy indicates a continued myelotoxic effect of 3M which is greater for those patients who also received tamoxifen (Table 3). Anaemia persisted in 33% and TCP in 15% of patients having received 3M compared to 71% ($P < 0.0001$) and 45% ($P < 0.001$) of patients having received 3M and tamoxifen. There was also a similar but smaller decrease in the incidence of leucopenia in patients having received 3M (59%) compared to 3M with tamoxifen (71%) ($P < 0.01$).

DISCUSSION

These retrospective, non-randomised data indicate that the addition of tamoxifen to 3M combination chemotherapy may increase the risk of developing MAHA and HUS, causing the appearance of progressive anaemia, TCP and renal dysfunction with one treatment-related death. This high incidence at the low total dose of mitomycin C may in part be a result of our screen, which may account for the relative mildness of the syndrome which in some cases went unnoticed [5, 6]. The absence of features of this syndrome in patients receiving 3M alone indicates a possible drug interaction between tamoxifen and one of the cytotoxic drugs, presumably mitomycin C. Furthermore, the progressively greater fall in haemoglobin and platelet count but not white cell count with tamoxifen would seem to indicate a more specific effect such as MAHA rather than a non-specific enhancement of bone marrow toxicity.

A possible explanation for this interaction may involve a combination of subclinical endothelial damage induced by mitomycin C and a thrombotic effect on platelets caused by tamoxifen. This would allow a possible precipitation of the syndrome by tamoxifen after previous exposure to mitomycin C which may have occurred in our initial metastatic patient. All 9 of our study patients had no evidence of metastases at the time of development of the syndrome, which would indicate that the pathogenesis relates to the treatment rather than any tumour activity.

In conclusion, we have identified a possible serious interaction between tamoxifen and mitomycin C which may cause haemolytic anaemia, TCP and renal dysfunction progressing on to the potentially fatal HUS. We recommend that patients on or having recently received mitomycin C and tamoxifen should be screened for hypertension, renal dysfunction, haemolytic anaemia and TCP. We advise that tamoxifen should not be used in combination with or shortly after treatment with mitomycin C unless carefully monitored. In our primary medical treatment programme we have stopped using mitomycin C.

1. Gasser Von C, Gautier E, Steck A, Siebenmann RE, Oechslin R. Hämolytisch-urämische Syndrome: Bilaterale Nierenrindennekrosen bei akuten erworbenen hämolytischen Anämien. *Sonderabdruck aus der Schweizerischen Medizinischen Wochenschrift* 1955, **38/39**, 905.
2. Webster J, Rees AJ, Lewis PJ, Hensby CN. Prostacyclin deficiency in haemolytic-uraemic syndrome. *Br Med J* 1980, 271–272.
3. Weiner CP. Thrombotic microangiopathy in pregnancy and the postpartum period. *Semin Hematol* 1987, **10**, 119–129.
4. Brown CB, Clarkson AR, Robson JS, Cameron JS, Thomson D, Ogg CS. Haemolytic uraemic syndrome in women taking oral contraceptives. *Lancet* 1973, **I**, 1479.
5. Lesesne JB, Rothschild N, Erickson B, *et al.* Cancer-associated hemolytic-uremic syndrome: analysis of 85 cases from a national registry. *J Clin Oncol* 1989, **7**, 781–789.
6. Cantrell JE Jr, Phillips TM, Schein PS. Carcinoma-associated hemolytic-uremic syndrome: a complication of mitomycin C chemotherapy. *J Clin Oncol* 1985, **3**, 723–734.
7. Rabadi SJ, Khandekar JD, Miller HJ. Mitomycin-induced hemolytic uremic syndrome: case presentation and review of literature. *Cancer Treat Rep* 1982, **66**, 1244–1247.
8. Rumpf KW, Rieger J, Lankisch PG, von Heyden HW, Nagel GA, Scheler F. Mitomycin induced haemolysis and renal failure. *Lancet* 1980, 1037–1038.
9. Brain MC, Azzopardi JG, Baker LRI, Pineo GF, Roberts PD, Dacie JV. Microangiopathic heamolytic anaemia and mucin-forming adenocarcinoma. *Br J Haem* 1970, **18**, 183–193.
10. Powles TJ, Jones AL, Judson IR, Hardy JR, Ashley SE. A randomised trial comparing combination chemotherapy using mitomycin C, mitozantrone and methotrexate (3M) with vincristine, anthracycline and cyclophosphamide (VAC) in advanced breast cancer. *Br J Cancer* 1991, **64**, 406–410.