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Activity and Unexpected Lung Toxicity of the Sequential Administration of Two Alkylating Agents—Dacarbazine and Fotemustine—in Patients with Melanoma

Béatrice Gerard, Steinar Aamdal, Siow-Ming Lee, Serge Leyvraz, Catherine Lucas, Maurizio D'Incalci and Jean-Pierre Bizzari

We report the results and discuss the toxicity of clinical trials based on a single concept: the decrease in O⁶alkyl DNA alkyltransferase (O⁶AT) resistance mechanism when a chloroethylating agent is used sequentially after a methylating agent. This decrease in O⁶AT being dose dependent, several increasing doses of dacarbazine (DTIC) have been tested (400 mg/m² to 1000 mg/m² every 4 weeks, 3–4 h before fotemustine (100 mg/m² intravenously every 4 weeks). These results (mean overall response rate 27%) compared with reference regimes, demonstrate that DTIC is able to increase the alkylating power of fotemustine: same range of response rate with only half of the two drug doses compared to an alternated combination, high activity rate especially in lung metastases (10/42 complete responses + 13/42 partial responses), different pattern for haematotoxicity, and occurrence of a new side-effect: acute lung toxicity as adult respiratory distress syndrome (ARDS). This lung toxicity was totally unexpected since several hundreds of patients had been so far treated with fotemustine as single agent or in other combinations with DTIC without any case of acute or delayed lung toxicity. Prophylactic administration of corticoids was not effective and monitoring of the respiratory function was of no predictive value. Due to the additional depleting effects of DTIC on at least two main defence mechanisms—the O⁶AT system and cytosolic and/or nuclear glutathione—we suppose that the sequence is able to increase the alkylating power of fotemustine to an excessive extent and/or that the detoxication capacity of the cell against DTIC and/or fotemustine metabolites is overwhelmed. Other depletors of the O⁶AT activity which do not generate metabolites that compete for the same detoxication pathway as the chloroethylnitrosurea (CENU) metabolites should be tested.
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INTRODUCTION

WE HAVE RECENTLY reported the results of the first phase II clinical trial based on the application of the abundant preclinical knowledge about resistance to nitrosoureas in relation to DNA repair [1]. In a further series of clinical trials, the same sequence of two alkylating agents has been used at different dosages in a total of 107 patients with disseminated melanoma.

The sequence is based on *in vivo* and *in vitro* demonstrations of the role of a DNA repair system termed O⁶ alkyl DNA alkyltransferase (O⁶AT) in the sensitivity of tumour cells to

treatment with chloroethylnitrosoureas (CENU). For CENU, the main mechanism of action is the formation of DNA interstrand cross-links starting with carbonium ion attacks at the O^6 position of the guanine and subsequent binding to the opposite cytidine 6–12 h later [2–6]. The number of crosslinks (i.e. alkylating power) will, therefore, depend on the ability of a cell to remove in this interval the chloroethyl group from the guanine before the cross-link (i.e. lethal lesion) is established [7]. This removal is due to the O^6AT system, a 'suicide' enzyme that needs > 12 h to be regenerated [8]. Cells encoding the O^6AT system have been termed Mer^+. It is considered that about 75% of human cells are Mer^+ [2]. Cellular content in O^6AT is, therefore, crucial for the cytotoxicity of CENU and other cytotoxics interacting at the O^6 position of the guanine, like methyltriazenes [2–9]. It is possible to transiently saturate the enzyme, more specifically with methylating agents [10]. The saturating effect is dose dependent [10, 11]. The interval and the sequence are of particular importance [11]. Among the clinically useful agents, 5-(3,3 dimethyl-1 triazeno)-imidazole-4-carboxamide or dacarbazine (DTIC) seems to be the most potent for inhibiting O^6AT activity [12].

Fotemustine, an alpha aminophosphonate chloroethyl nitrosourea with promising *in vitro* activity and higher cytotoxicity in Mer− cells, has proven to be a useful agent in the treatment of melanoma [13]. Its limiting toxicity is thrombopenia. The only other relevant toxicity is mild transient and reversible hepatotoxicity.

After *in vitro* confirmation of the role of temozolomide—the prodrug of the active metabolite of DTIC—in increasing the sensitivity of a L_{1210}/1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) resistant cell line expressing the O^6AT activity to further treatment with fotemustine [14], it was decided to administer DTIC at increasing dosages from 400 to 1000 mg/m²/ month 3–4 h before fotemustine 100 mg/m². This interval proved optimal for obtaining 23 to 100% O^6AT inhibition in human blood lymphocytes of 13 patients receiving 500 or 800 mg/m² DTIC with a median decrease of 70% [15]. This interval was extrapolated for tumour tissues but for ethical reasons no tissue biopsy could be obtained. The early onset of haematotoxicity, compared to the delayed nadir observed with CENU containing regimes, permitted repetition of the treatment every 4 weeks.

MATERIALS AND METHODS

Patient characteristics are listed in Table 1.

The therapeutical schedules were all based on the same sequence: DTIC intravenous administration over 10 min followed 3–4 h later by fotemustine intravenously over 15–30 min as outpatient treatment. The dose of fotemustine was 100 mg/m² in all trials. The treatment was planned every 4 weeks depending on haematological recovery. The dose of DTIC in regime A was 400 mg/m² for the first administration and then 250 mg/m² for subsequent administrations. In regime B, the dose of DTIC was 500 mg/m² throughout the entire treatment plan. In regime C, the dose of DTIC was 800 mg/m² throughout the entire treatment plan. In regime D, the dose of DTIC was 500 mg/m² but the sequence was repeated on day 8. Dose reduction was applicable in case of severe (grade IV) or prolonged (> 2 weeks delay) toxicity. Of note, in the reference regime (alternated combination) due to delayed haematotoxicity the drug was administered as follows: fotemustine 100 mg/m² day 1 + 8 and DTIC 250 mg/m² day 15 to 18, followed after a 5-week rest period by fotemustine 100 mg/m² day 1 and DTIC 250 mg/m² day 2 to 5, every 3–4 weeks. All measurable sites and toxicity were evaluated following the WHO recommendations.

Table 1. Patients' characteristics

Total number of patients	107
Tumour type	Melanoma
Male/female	62/45
Median age (years)	55 (19–75)
Median performance status	1 (0–2)
Pretreatment	
Surgery	100
Radiotherapy	16
Chemotherapy	10
Median number of courses	2 (1–8)
Cerebral metastases	21
Visceral metastases	69
Non-visceral metastases	17

RESULTS AND TOXICITY

Results

The salient clinical data are presented in Table 2.

The activity ranging from 0 to 41% with a mean overall reponse rate of 27% and a median duration of response of 17 weeks is in the range of the alternated combination. On lung metastases the activity was impressive with a cumulated response rate of 23/42 (48%) and more importantly 10/42 (24%) complete responses for that site. Activity was also observed at the cerebral and non-visceral sites with no clear benefit compared to the reference regime.

We demonstrated that DTIC was able to increase the alkylating power of fotemustine giving a similar overall response rate with only half of the two drug doses in regime B compared to the alternated regime. The advantage of regime B is essentially the observation of complete responses in lung and liver metastases.

Toxicity

Haematotoxicity was clearly dose related to DTIC with grade IV platelets toxicity ranging from 0% with regime A (lowest DTIC dose) to 42% in regime D (highest DTIC dose intensity). In regime D we observed one case of prolonged aplasia leading to the patient's death. White blood cell (WBC) toxicity was also related to the DTIC dose ranging from 0 to 19% but no case of fatal septicaemia was reported.

Hepatotoxicity was clinically significant in regime C and D only with the occurrence of 16% grade III–IV transaminases or alkaline phosphatase toxicity and 2 cases of chemical hepatitis.

In these two last regimes, excessive haemato- and hepatotoxicity led to a bad compliance. Only 60% of the planned dose intensity could be given. In regime A no relevant toxicity nor activity could be observed.

In 6 patients (Table 3), lung toxicity developed as a rapidly progressing alveolar shadowing first considered as an interstitial pneumonitis of infectious origin (Figs 1–3). In 2 cases there was also a left heart enlargement. The patients were not leucopenic

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Revised 6 July 1992; accepted 20 July 1992.

Table 2. Salient clinical data

Regime	A	B	C	D	Mean results of sequential administration	Reference treatment (alternated administration)
DTIC (mg/m²)	400/250	500	800	500 × 2	—	250 × 4
every 4 weeks	(day 1)	(day 1)	(day 1)	(day 1 + 8)		(D15,16,17,18)
Fotemustine (mg/m²)	100	100	100	100 × 2	—	100 × 2 (D1 + 8)
Evaluable patients	14	42	22	29	107	103
Overall response rate (RR%)	0%	31%	41%	24%	27%	27%
Duration (weeks)	—	18	12	20	17	21
(No) of target sites RR% (CR%)						
Cerebral	(4) 0% (0%)	(5)20% (0%)	not entered	(8)25% (0%)	(17)15% (0%)	(19)31% (15%)
Visceral	(10) 8% (0%)	(38)24% (13%)	(21)24% (5%)	(26)23% (8%)	(95)20% (7%)	(58)14% (3%)
Lung	(5)16% (0%)	(20)60% (40%)	(9)33% (0%)	(8)50% (25%)	(42)48% (24%)	(23)13% (5%)
Liver	(3) 0% (0%)	(11)28% (18%)	(7)14% (14%)	(12) 8% (0%)	(33)15% (9%)	(28)14% (3%)
Non-visceral	(8)10% (10%)	(33)36% (12%)	(22)40% (20%)	(15)14% (0%)	(78)25% (10%)	(76)31% (14%)
WBC/Plt grade IV	0%/0%	5%/10%	19%/38%	19%/42%	11%/23%	4%/7%
Median day of nadir	22/22	15/21	22/22	21/22	22/22	42/32
Hepatotoxicity*	0%/0%	6%/0%	16%/4%	4%/4%	7%/2%	4%/0%
Lung toxicity	0	2	2	2	6	0

*Grade III + IV transamisases or alkaline phosphatase/bilirubin.
Main participating institutions to trials: A = DNR, Oslo (N),Dr Aamdal; Inst. Bordet, Brussels (B), Dr Kerger.
B = DNR, Oslo (N), Dr Aamdal; Christie Hospital, Manchester (UK),Drs Thatcher/Lee/Radford.
C = Christie Hospital, Manchester (UK), Drs Thatcher/Lee/Radford UZA, Antwerp (B),Dr Becquart; A.Z. Groningen (NL),Dr Mulder; St. James University Hospital, Leeds (UK), Dr Selby.
D = AVL, Amsterdam (NL), Dr Israëls; Regina Elena, Roma (I), Dr Calabresi; Westfal. Wilhelm Univers. Hautklinik, Munster (RDA), Dr Bröcker; DNR, Oslo (N), Dr Aamdal.

Table 3. Clinical data about patients with fatal lung toxicity

	Patients					
Characteristics						
Age	61	62	47	50	65	50
Sex	M	F	M	M	F	M
Performance status	0	1	0	0	0	0
Site of metastases	Lung	Lymph nodes	Lung	Pharynx	Lung, skin, viscera	Lung, skin, bone, lymph nodes
Pretreatment (radio- or chemotherapy)	0	0	+	0	0	0
Treatment						
Regime	B	B	C	C	D	D
No. courses	3	3	3	2	3	2
Cumulated dose (mg/m²)						
DTIC/fotemustine	1500/300	1500/300	2800/375	1600/300	2650/530	2000/400
Toxicity						
Interval between onset of treatment to death (weeks)	13	14	14	10	13	10
Toxicity (max grade)						
WBC/plt	3/2	0/0	2/3	4/4	1/4	2/4
Alkaline phosphatase bilirubin	0/0	3/0	1/0	0/0	1/0	2/0
Ser. creatinin	0	0	0	2	0	0
Clinical response						
Overall	CR	PR	PR	PR	PR um	PD
Pulmonary site	CR	—	PR	—	CR	Not evaluable

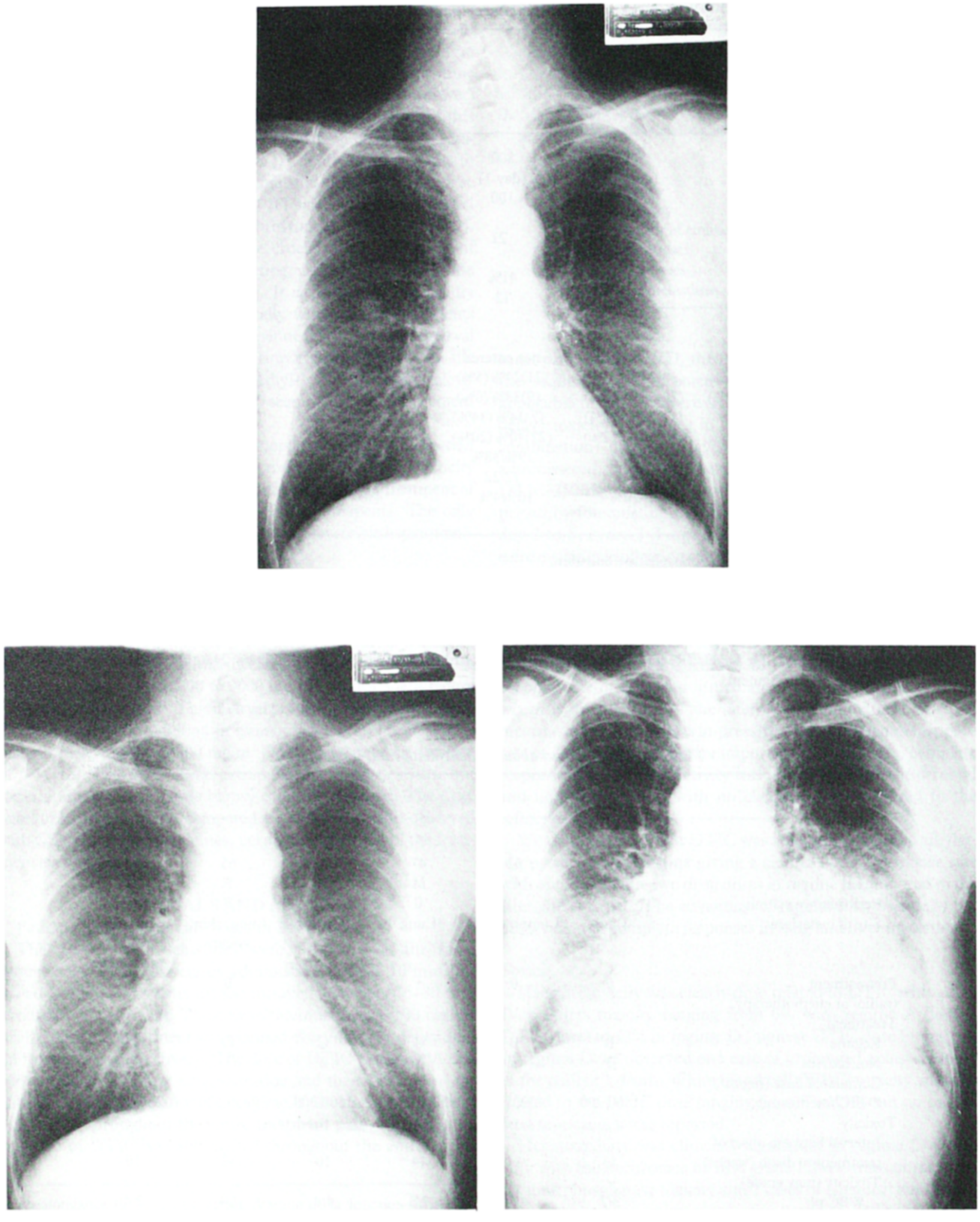

Fig. 1. (a) Radiological features at entry (presence of two metastases) of the right lobe. (b) After two chemotherapy courses (complete response). (c) One week before death.

when the symptoms developed starting with cough and increasing dyspnoea. 2 of them were subfebrile.

They had all received a minimum of 1500 mg/m² cumulated dose of DTIC within 2 or 3 chemotherapy cycles for a median time of onset for symptoms of 10 weeks after entry in the study. Only 1 patient had received prior chemotherapy with carboplatin and etoposide followed by cyclophosphamide, doxorubicin and vindesine. This patient had also received radiotherapy 50 Gy on the right lung, 1 year before. 5 out of 6 patients were responding to antitumour therapy.

In 1 patient a grade 2 renal insufficiency also developed one week after onset of the symptoms. All patients failed to respond to antibiotherapy and/or corticotherapy. Pulmonary insufficiency increased within 1–3 weeks leading to death in all 6 cases despite adequate intensive care. Autopsy was permitted in 3 cases and showed signs of adult respiratory distress syndrome (ARDS) with hyaline membranes formation and foci of interstitial fibrosis.

Necropsy report no. 1 from Christie Hospital Manchester (U.K.): Dr Banerjee (Fig. 4)

Macroscopic findings. The pleural surface of the right lung was covered by fibrinous exudates and in places fibrous tissue. On slicing, both lungs appeared extremely oedematous and the entire right lung and the lower lobe of the left lung felt solid and in places fibrotic. In addition a few peripheral branches of the right pulmonary artery contained thromboemboli but there was no evidence of any infarction. No further deposits were seen in the lungs.

Microscopic findings. Sections of both lungs show marked oedema, congestion of capillaries in the interstitial tissue, hyaline membrane formation, proliferation of type II pneumocytes (some containing bizarre nuclei) and patchy interstitial fibrosis. Many pulmonary vessels show thickening of their walls. The features are those of adult respiratory distress syndrome with interstitial fibrosis. There is no evidence of any infection.

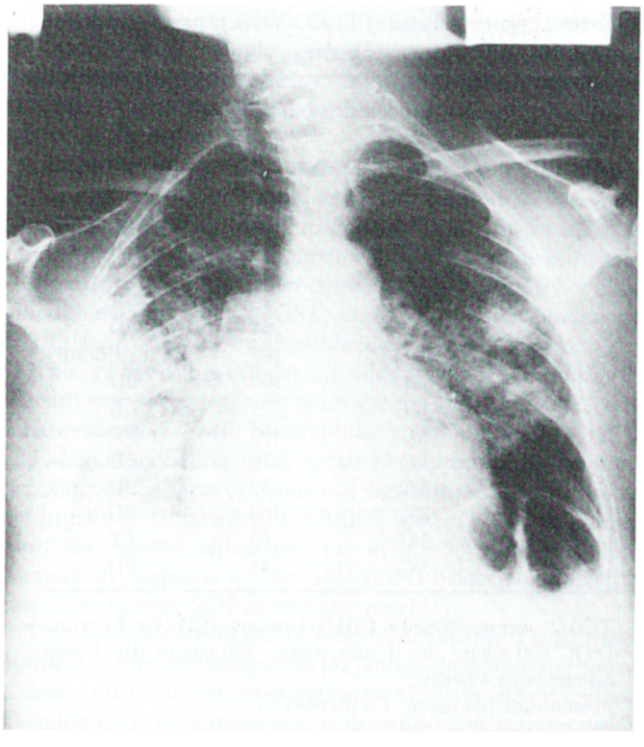

Fig. 2. Bilateral interstitial pneumonitis (see necropsy report no. 1).

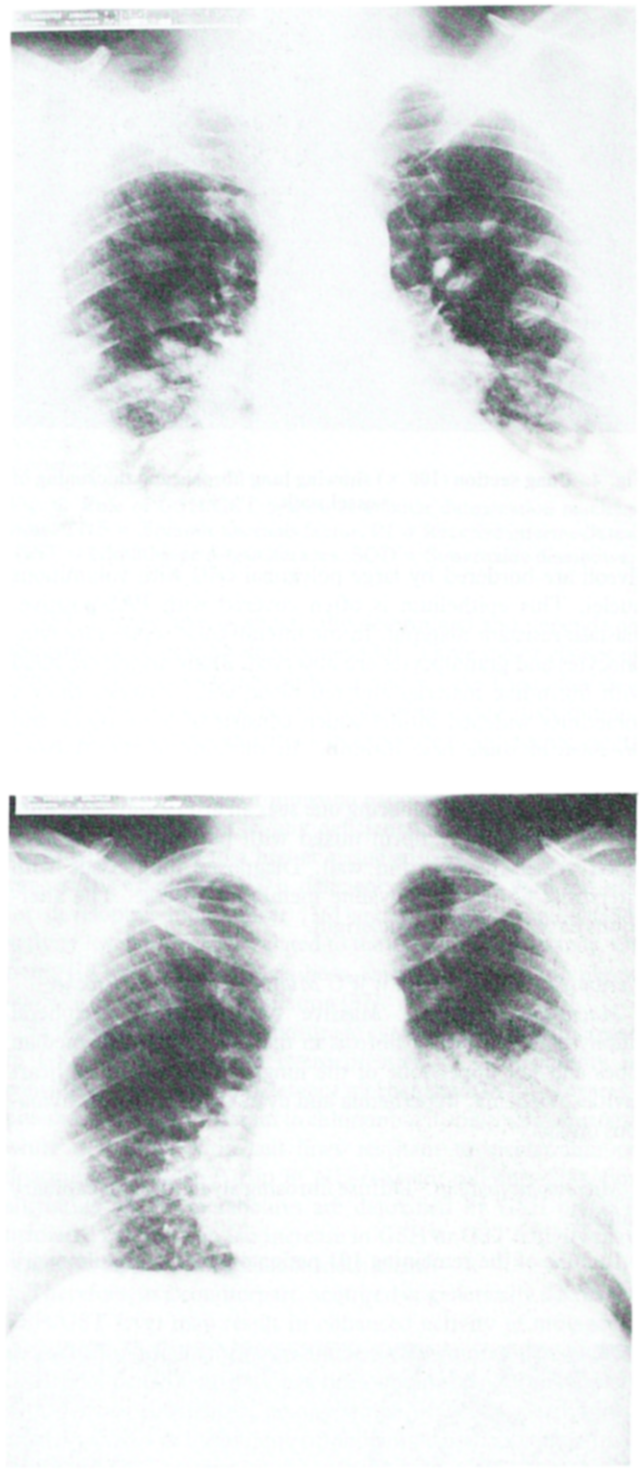

Fig. 3. (upper panel) Massive metastatic involvement at entry. (lower panel) Partial response after three courses and left lung pneumonitis (see necropsy report no. 2).

Necropsy report no. 2 from AZU Antwerp (B); Dr Goovaerts (Fig. 5)

Microscopic findings. The lung tissue shows a disturbed architecture. Alveoli are mostly fusiform. The interalveolar septa are enlarged and consist of loose connective tissue. The

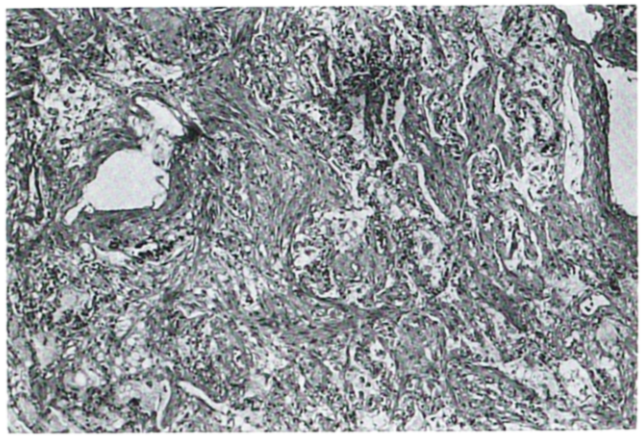

Fig. 4. Lung section (100 ×) showing lung fibrosis and thickening of vessel walls.

alveoli are bordered by large polygonal cells with voluminous nuclei. This epithelium is often covered with PAS-positive, diastase-resistant material. In the interalveolar septa, rare lymphocytes and granulocytes are observed. Many alveoli are filled with fibrin-like material and red blood cells. Arteries show a sometimes widened intima which consists of loose tissue and presence of some new thrombi. In the cuts of the Masson-colouring, the periodic acid-Schiff (PAS) colouring and the Sirius haematoxylin colouring one sees an artery and branching that contains locally fibrin mixed with polynuclears. Venous blood vessels have a thin wall. Diagnosis: lung tissue with interstitial fibrosis and hyaline membrane disease. The alterations have possibly a toxic origin.

Necropsy report no. 3 from WWU Munster (RDA); Dr Bröcker

Macroscopic findings. Massive lung oedema, peripheral emphysema and foci of fibrosis in the right upper and median lobes and left upper lobe of the lungs. Dilatation of all heart cavities. Oedema, hyperhemia and cyanosis of all parenchymatous organs.

Microscopic findings. Diffuse fibrosing alveolitis with capillary damage.

In none of the remaining 101 patients, could any pulmonary insufficiency be clinically detected. The respiratory function parameters were monitored in a subsequent trial.

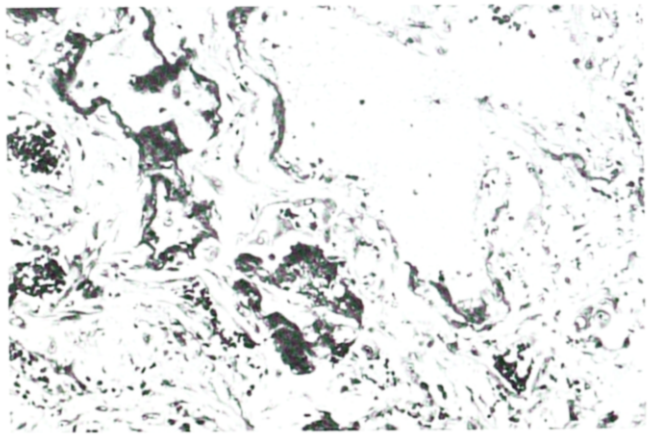

Fig. 5. Lung section with PAS staining (200 ×) showing hyaline membranes boardering the alveoli.

DISCUSSION

DTIC or fotemustine used alone have not been reported to cause pulmonary toxicity: so far, more than 1200 patients have been treated with the single agent fotemustine or in alternated combination with DTIC for an average cumulated dose of 400 mg/m² fotemustine (range 300–1.700 mg/m²) [13, 16] or in combination with other cytostatics and interferons, or even at high doses before bone marrow transplantation.

None of our patients had pre-existing lung disease, and only one of them had previous chemotherapy and radiotherapy, recognised as major prognostic factors [17, 18]. Smoking habits were not reported. Infection was not evident in any case. As recommended by some authors [19], the testing of the respiratory function before each administration was started during regime D and a pilot study (C−) not reported here (same dose as regime C for the first cycle plus dexamethasone day 0 to 8, followed by a maintenance treatment with decreased dose of DTIC 250 mg/m² 4 h before fotemustine 100 mg/m² every 4 weeks). Out of 15 patients, the capacity for carbonoxide transfer, corrected for the vital capacity (TLCO/VC), was significantly altered in 5 cases (decrease > 25%) (Table 4). These patients had complete response (1), stabilised (1) and progressive disease (3) but no pulmonary side-effect of clinical relevance so far. On the other hand, in a patient with normal lung X-ray and normal respiratory tests, dyspnoea and cough appeared only 4 days after the last assessment and the patient died of acute respiratory failure 8 days later despite adequate supportive care. In another patient, normal values of respiratory tests were observed 3 weeks before the onset of the dyspnoea. In the study by O'Driscoll, 2 patients with pulmonary fibrosis induced by BCNU had also normal chest X-ray and computed tomography (CT) scan [20]. In a series of 81 patients with monitored respiratory functions,

Table 4. Minimal value of TLCO/VC *in percentage of initial value*

Patient no.	No of courses/ regime	TLCO/CV* (%)	Response
1	2/C⁻	80	PD
2	3/C⁻	100	PR/toxic death
3	5/C⁻	84	PR
4	9/C⁻	100	MR
5	7/C⁻	93	CR
6	2/C⁻	64	PD
7	2/D	100	PR/toxic death
8	2/D	100	PD
9	6/D	73	CR
10	1/C⁻	100	PD
11	3/C⁻	76	PD
12	2/C⁻	57	PD
13	2/C⁻	100	PD
14	3/C⁻	63	ST
15	3/C⁻	46	PD

Trial C⁻ was performed at CHUV Lausanne (CH),Dr. Leyvraz.
DTIC 800 mg/m² day 1 first course, 250 mg/m² day 1 next courses every 4 weeks.
Fotemustine 100 mg/m² 4 h after DTIC.
Dexamethasone day 0 to 8 first course.
*TLCO/CV: carbonoxide transfer, corrected for vital capacity.

the respiratory tests also failed at predicting bleomycin-induced lung toxicity [21].

The monitoring of the respiratory function was, therefore, abandoned. Of note, the concomitant administration of high dose steroids also proved to be insufficient with the occurrence of a further case of pneumonitis in the pilot study C- previously mentioned. The clinical trials were stopped.

All these observations support the view that, at least in some tissues and with the sequence used, DTIC causes a potentiation of the pharmacological effects of fotemustine. This raised one essential question: why does the pulmonary toxicity only occur with the sequential administration of DTIC prior to fotemustine?

Synergism in the production of interstitial pneumonitis has already been recognised for some associations of antineoplastic drugs, concomitant O_2 administration, irradiation and even non-antineoplastic drugs [17, 22].

We consider that the activity and toxicity of this sequential regime is due to an enhanced alkylating activity through the decrease of at least two major cellular defence mechanisms against these types of xenobiotics: the O⁶AT repair system and the glutathione system. The alteration of the O⁶AT repair system has been well documented in patient's blood lymphocytes after a single dose of DTIC or a methylating agent [15, 23]. Accordingly DNA damage induced by fotemustine is more severe and longer lasting after DTIC pretreatment in tumour cells as well as in normal cells depending on the importance of the depletion. This will be related to the dose of the depletor and the basal activity level of O⁶AT system.

The O⁶AT level is genetically determined. Its amount is highly variable from one individual to another as well as in the different organs and in normal versus tumour cells of the same tissue [2, 24]. The amount of O⁶AT could even be related to the individual sensitivity to mutagenic agents with higher risk for developing a lung cancer if O⁶AT has genetically a low level [25, 26].

If we consider that some patients may have already the Mer^- phenotype, the depletion in O⁶AT alone cannot explain the occurrence of toxicity which could, therefore, also occur with fotemustine used as single agent. However, these patients and those having a deep decrease in O⁶AT after DTIC administration could be at higher risk for developing toxicity if a second defence mechanism is depressed.

Indeed glutathione plays a crucial role in the cell defences. Glutathione is a tripeptide (glu–cys–gly) present in most organs. The balance between its reduced form (GSH) and its oxidised form (GSSG) is actively maintained by glutathione-reductase (GSR). GSH reacts as cellular detoxifier, spontaneously or after glutathione-*S*-transferase (GST) catalysed covalent binding or by oxidation catalysed by glutathione peroxidases (Fig. 6).

It plays a key role in cellular antioxidant capacity and ability to detoxify various endogenous or exogenous electrophilic reactive intermediates [27–28]. Electrophilic compounds like CENU and various alkylating agent metabolites, products of the lipid peroxidation by some cytostatics or cigarette smoke condensate are usually metabolised by GSH/GST system [29, 30]. Therefore, the cellular sensitivity, i.e. ability to eliminate these compounds, depends on the GSH-GSSG balance and activity rate of the various GSH-related enzymes [31].

The protective role of GSH has been demonstrated *in vitro* and *in vivo* after bleomycin exposure [32] and in clinical application to decrease the renal toxicity of high-dose cisplatin and cyclophosphamide [33]. Its interference with antitumour activity needs further testing.

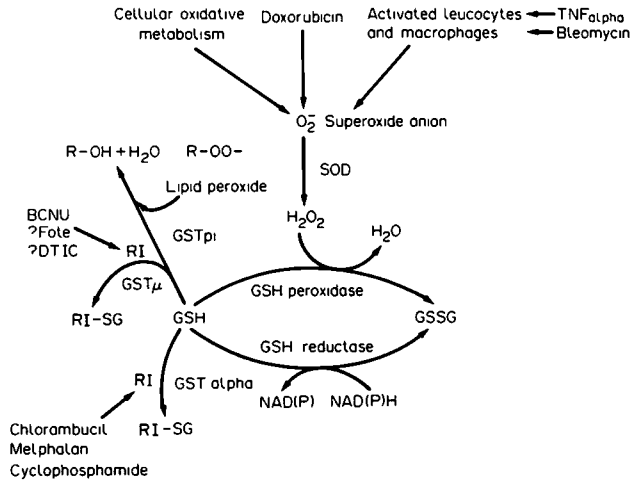

Fig. 6. Role of GSH/GST system in cellular detoxication mechanisms. TNF = Tumour necrosis factor. RI = Reactive intermediates. GST = Glutathione *S*-transferases. SOD = Superoxide dismutase.

GST activity level is genetically determined and depends on the amount of specific isoenzymes [34]. Three major classes of cytosolic GST isoenzymes alpha, μ and π have been identified with various degrees of activity following tissue distribution, (pre)malignant status and previous exposure to mutagenic agents [35]. Since their activity rate has to play a role in the defence mechanisms against environmental exposure and dietary ingestion of mutagenic agents, any deficiency in specific isoenzyme could be correlated to a higher tissue susceptibility to develop cancer: for example, GST μ deficiency correlates with the risk for developing lung cancer [36] and in brain tumours GST activity level is inversely related to the degree of malignancy. Of note, GST μ deficiency has been reported to occur in about 40–50% of the human population [37].

In the case of previous exposure to cytotoxic drugs, an increase in GSH level and/or an overexpression of GST activity is considered as an acquired defence mechanism: GST α is overexpressed in cell lines resistant to chlorambucil or nitrogen mustard while it is GST π in cell lines resistant to cisplatinum or doxorubicin and GST μ in BCNU-resistant cell lines [38]. For alkylating agents metabolites are detoxified by GSH or GST mediated conjugation, an increase in GSH or GST activity may represent a potential resistance mechanism.

Therefore, as a counterpart, acquired or genetically decreased GSH/GST level may result in enhanced activity of mutagenic and alkylating agents. *In vitro* and *in vivo*, acquired depletion in GSH pool or GST activity has been obtained by pretreatment with various substances, among which imidazole derivatives, ethacrynic acid or buthionine sulphoximine used as competitive inhibitors, GST activity inhibitor or inhibitor of GSH-synthesis, producing increased alkylating power of a subsequent alkylating agent.

In addition to the decreased elimination of reactive intermediates, alkylation and/or oxidation of sulphydryl groups, when a severe depletion in GSH is maintained, may also impair the activity of crucial thiol-containing proteins altering the calcium homeostasis and causing cell death by impairing the repair of membrane lesions due to lipid peroxidation [39].

Therefore, a transient depletion in GSH due to the detoxication of DTIC reactive intermediates could play a role through some decrease in the detoxication of fotemustine reactive intermediates. In addition, DTIC could also transiently deplete the

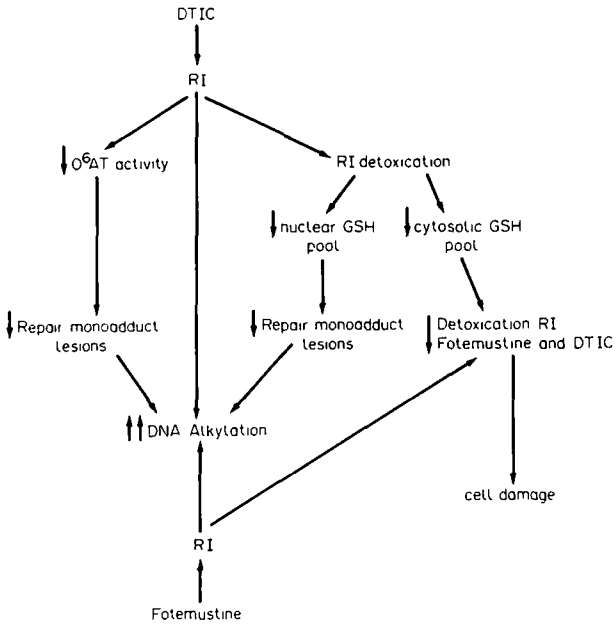

Fig. 7. Possible mechanisms of synergy between DTIC and fotemustine sequentially given.

nuclear GSH pool leading to less repair of monoadduct lesions [40]. Such depletion has been documented within 30 min after doxorubicin + BCNU administration and was shown to be able to increase activity of melphalan [41]. Fotemustine metabolism is still incompletely known but in contrast to BCNU, it seems not to reduce glutathione reductase activity [42, 43]. Its chemical structure (chloroethylnitrosourea) and the identification of some of its metabolites combined with GSH in rat urine [44] permit postulation regarding a crucial role for GSH/GST (μ?) in its detoxication. DTIC metabolism has been poorly investigated but its chemical structure leading to the formation of methylcarbocations and formaldehyde makes it also highly probable that GSH/GST system plays a role in its metabolism. Identification of S metabolites is underway to document this proposal.

In conclusion, in addition to the depletion in O⁶AT, it is probable that DTIC leads to a decrease in cytosolic and/or nuclear GSH pool. Consequently, more active fotemustine carbonium ions will be present at the nuclear site where cumulated decreased repair capacity of the monoadduct lesions will result in enhanced antitumour activity, while competition for the same detoxifying pathways may lead to acute toxicity through an excess in some DTIC and/or fotemustine metabolites with higher risk for patients with low GSH pool, GST μ deficiency and/or MER-phenotype (Fig. 7).

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The Identification of Informative Parameters in the Flow Cytometric Analysis of Breast Carcinoma

John Lawry, Kenneth Rogers, John L. Duncan and Christopher W. Potter

DNA ploidy and the measurement of proliferation or S-phase fraction are both of prognostic significance in breast cancer, yet clinical use is minimal in the U.K. Immunohistochemistry is, however, used to aid diagnosis, so a panel of antibodies were analysed by flow cytometry to assess their predictive value for prognosis, tumour stage and grade. Of 10 parameters tested on 202 breast tumour samples, tumour cell proliferation and DNA ploidy were the two most informative; cytokeratin staining, natural killer and B-cell infiltration also proved to be of value but there was no prognostic value in measuring tumour infiltrating monocytes, helper/suppressor T-cell ratios, tumour cell reactivity with carcinoembryonic antigen or human milk fat globulin antibodies. For each of the informative parameters, scores numerically weighted towards a poorer prognosis were derived which when combined, correlated with tumour grade, stage and prognosis. Such data interpretation is objective, and can be transposed to other human tumours.
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INTRODUCTION

NUMEROUS REPORTS have suggested that flow cytometric parameters parallel clinicopathological observations in tumours of the breast, and can thus be used as indicators of tumour stage and prognosis; in particular, tumour cell ploidy [1–4] and the percentage of dividing cells [5, 6] have proven to be valuable markers. In contrast, other proposed parameters remain controversial; these include the expression of carcinoembryonic antigen (CEA) [7, 8] and human milk fat globule (HMFG) [9], and the measurement of tumour infiltrating lymphocytes, natural killer (NK) cells and monocytes. Thus, there remains a need to identify and confirm which of the many cytometric parameters proposed are of clinical value, and to devise a method by which these measurements can be interpreted.

In order to identify the cytometric measurements which correlate with histological grade, pathological stage and survival, 202 primary breast carcinoma samples, together with samples of normal tissue, were examined by cytometry using 10 separate