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A Phase I Study of the Left-shifting Agent BW12C79 plus Mitomycin C and the Effect on the Skeletal Muscle Metabolism Using ^{31}P Magnetic Resonance Spectroscopy

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ABSTRACT

BW12C79 stabilizes the oxyhemoglobin molecule resulting in a reversible left-shift of the oxygen saturation curve. The activity of a number of bioreductive anticancer drugs, such as mitomycin C, may be enhanced under hypoxic conditions. Twenty-four patients with various malignancies received BW12C79 and mitomycin C. BW12C79 was administered i.v. with a loading dose (20–50 mg/kg) over 1 h followed by a maintenance infusion of 4 mg/kg/h for 5 h. Percentage modification of the oxyhemoglobin (degree of left-shift) was dose related with maximum modification of 56% and was maintained for the duration of maintenance infusion of BW12C79. Hemoglobin electrophoresis showed a fast moving band consistent with the BW12C79-oxyhemoglobin complex. Side effects at the top dose level comprised headache, nausea/vomiting, vein irritation, and myocardial ischemia. One other patient suffered from an acute encephalopathy of unknown etiology a few days following BW12C79. ^{31}P magnetic resonance spectroscopy of exercising calf muscles showed increased breakdown of high energy phosphate stores and a greater reduction in pH. Recovery of the high energy phosphate stores after exercise was slow. These results were consistent with reduced oxygen supply due to either a left shift of the oxygen saturation curve and/or reduced muscle blood flow. BW12C79 did not interfere with the pharmacokinetics of mitomycin C. In conclusion, this phase I study demonstrates the feasibility of achieving a significant left shift in the oxygen saturation curve in cancer patients which is maintained for at least 5 h with acceptable toxicity. The maximum tolerated dose of BW12C79 was 50 mg/kg loading infusion followed by a maintenance infusion of 4 mg/kg/h. Magnetic resonance spectroscopy results were consistent with reduced supply of oxygen to exercising skeletal muscle. BW12C79 may be of potential benefit as an adjunct to bioreductive drugs in the treatment of solid tumors.

INTRODUCTION

Drug resistance precludes the successful treatment of the majority of solid tumors. Various mechanisms of resistance have been identified in experimental systems. Hypoxic tumor cells are resistant to ionizing radiation and to the cytotoxic effect of several anticancer drugs (1). Nevertheless, under hypoxic conditions, a number of cytotoxic agents (bioreductive drugs) undergo metabolic activation to reactive metabolites that will damage tumor DNA or interfere with essential macromolecules (2).

BW12C79 (5-[2-formyl-3-hydroxyphenoxyl] pentanoic acid) is the most potent of a series of substituted benzaldehydes specifically designed to increase the affinity of hemoglobin for oxygen (3). The drug binds preferentially to the oxygenated molecule at the NH_2-terminal α-amino groups (4). This stabilizes the oxyhemoglobin molecule and results in a dose-dependent left-shift of the OSC[3] which is more marked at lower partial pressures of oxygen. The effect of BW12C79 on hemoglobin is stoichiometric and the resultant OSC is biphasic, being a conjugate of curves corresponding to the two functionally different hemoglobin molecules having different affinities for oxygen. This allows the percentage of BW12C79-modified high-affinity hemoglobin to be determined (3). Repeated administration of BW12C79 is required in order to prolong the duration of left shift because of its relatively short half-life of 3 h (5). Although BW12C79 and its analogues were initially designed for the treatment of sickle cell anemia, their tissue oxygen-lowering properties make them potential agents in the treatment of solid tumors because they may enhance the activity of bioreductive drugs (6). Studies in normal subjects, patients with sickle cell disease, and cancer patients have shown good tolerance to the drug, with the main side effects being headaches, vein irritation, tachycardia, and vomiting (5, 7). Studies in animals bearing tumor xenografts have shown that BW12C79 enhances the cytotoxic effect of certain bioreductive drugs. It will also protect normal tissue against radiation induced damage and will mimic mechanically induced tumor hypoxia (6, 8). In addition, studies in animal tumor models have shown an increase in the proportion of necrotic tumor cells after treatment with BW12C79 (6).

MMC is the prototypical bioreductive alkylating agent that preferentially kills hypoxic cells in *vitro* and *in vivo* (9) and has a relatively broad spectrum of activity against solid tumors. There are several enzymes that activate MMC into reactive species. Reduction by cytochrome P-450 reductase is rate limiting under normal oxygen tension and lower activities of this enzyme have been detected in some resistant cell lines (10). Following bioreductive activation, MMC can produce a number of cytotoxic lesions in DNA including intrastrand and interstrand cross-links and monoadducts (11). Using the Chinese hamster ovary cell line, CHO-K1, it was shown that resistant sublines (CHO-MMC^r) which were associated with reduced cytochrome P-450 reductase activity did not exhibit resistance to MMC under hypoxic conditions (10).

In skeletal muscle, creatine kinase catalyses the transfer of high energy phosphate from PCr to ADP supplementing ATP production when oxidative phosphorylation and anerobic glycolysis cannot meet demand. Any alteration in ATP synthesis by these pathways can be assessed by comparing the extent of the change in PCr concentration during exercise at the same workload under different conditions. The metabolic acid production within the cell, as measured by a change in intracellular pH, is almost completely due to lactic acid accumulation occurring from anerobic production of ATP. ^{31}P-MRS allows rapid measurement *in vivo* of intracellular pH and the concentrations of PCr, Pi, and calculation of ADP concentration in skeletal muscle *in vivo*. Hence anerobic and, indirectly, aerobic metabolism can be reliably

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[3] The abbreviations used are: MMC, mitomycin C; PCr, phosphocreatine; Pi, inorganic phosphate; MRS, magnetic resonance spectroscopy; OSC, oxygen saturation curve.

Table 1 *Patient characteristics*

Characteristic	
Number entered	24
Median age	49 years (range, 26–71)
Male:female	15:9
Performance status (ECOG)	
0–1	23
2	1
Diagnoses	
Ca[a] unknown Primary	4
Malignant Melanoma	4
Soft tissue sarcoma	1
Prostatic ca	1
Pancreatic ca	2
Lung ca	1
Cervical ca	1
Small bowel ca	1
Colorectal ca	5
Stomach ca	1
Carcinoid tumor	1
Breast ca	1
Renal cell ca	1
Mesothelioma	1
Hepatoma	1
Previous chemotherapy	12 (50%)
Disease sites	
Liver	10
Abdomen/pelvic mass	8
Bone	2
Brain	1
Soft tissues	9
Lungs	6
Pleural cavities	2

[a] Ca, carcinoma.

compared using ^{31}P-MRS. In an animal tumor model *in vivo* there was a small increase in intracellular Pi upon infusion of BW12C79 (12).

The aims of this phase I study were to: (*a*) evaluate the safety of sequential loading and maintenance infusions of BW12C79 in patients with solid tumors; (*b*) determine the effect of BW12C79 on the OSC and hemoglobin electrophoresis; (*c*) determine the effect of BW12C79 on the pharmacokinetic profile of MMC; (*d*) determine safety of the combination of mitomycin C and BW12C79; and (*e*) determine influence of BW12C79 on skeletal metabolism using MRS. To achieve this, BW12C79 was given as an i.v. bolus infusion over 1 h followed by an i.v. maintenance infusion over 5 h to prolong the duration of the left shift. MMC was administered at the completion of the loading infusion. Serial blood samples were obtained to determine the changes in the OSC, hemoglobin electrophoresis, and MMC pharmacokinetics. ^{31}P-MRS of calf muscles was performed before and during the infusion of BW12C79.

MATERIALS AND METHODS

Patients. Twenty-four patients, 15 males and 9 females, took part in this phase I study. Median age was 49 years with a range of 26 to 71 years. All patients had histologically proven advanced cancer with objective evidence of progressive disease (Table 1). Patients had a life expectancy of greater than 3 months with a median Eastern Cooperative Oncology Group performance status of 1. All patients with symptomatic ischemic heart disease or cerebrovascular disease were excluded from the study. One-half of the patients had been previously treated with chemotherapy but none had received MMC. A normal full blood count was one of the eligibility criteria for entry into the study: hemoglobin $\geq$ 10 g/dl; total WBC count $\geq 3.5 \times 10^9$/liter; and platelets $\geq 100 \times 10^9$/liter. All patients had normal renal function and liver biochemical profile (unless due to malignancy). Approval to conduct this study was granted by Central Oxford Research Ethical Committee and each patient provided an informed written consent.

Drugs and Administration. MMC was initially administered as a single agent followed 2 weeks later by single agent BW12C79. The combination of BW12C79 and MMC was administered a week later and repeated every 3 weeks to a total of 4 courses of each drug.

BW12C79 (Wellcome Research Laboratories, Beckenham, United Kingdom) was administered via a controlled i.v. infusion through an antecubital vein. A loading dose was dissolved in 250 ml of isotonic saline and infused over 1 h followed by a maintenance infusion of 4 mg/kg/h in 1 liter isotonic saline over 5 h. The loading dose of BW12C79 was commenced at 20 mg/kg and subsequently escalated to 25, 30, 40, and 50 mg/kg. A minimum of 3 patients were treated at each dose level and no intrapatient escalations were permitted.

Mitomycin C (Mitomycin C Kyowa; Martindale Pharmaceuticals Ltd., Romford, England) was administered as an i.v. bolus (15 min) injection of 10 mg every 3 weeks. The timing of the injection was at the completion of the loading infusion of BW12C79 to coincide with peak modification of OSC (7).

Antiemetic medication comprised either metoclopramide 10 mg p.o. four times daily q.d.s. or prochlorperazine 10 mg four times daily p.o.

Oxygen Saturation Curve. Five ml of venous blood were obtained prior to the BW12C79 infusion, at the end of the loading infusion, 1 h into the maintenance infusion, mid-maintenance infusion, end of the maintenance infusion, and 1 h after the completion of the maintenance infusion. Blood samples were stored on ice pending analysis within 24 h of their collection. The percentage peak modification of the oxygen saturation curve was determined by the method of Beddell *et al.* (3). The peak modification of OSC represents the proportion of oxyhemoglobin converted to a high affinity form by complexing with BW12C79. Analyses were performed at 37°C on an automated Hem-O-Scan analyser (Aminco, Maryland, USA). After equilibration, the ratio of oxyhemoglobin to hemoglobin was monitored continuously as the proportion of oxygen in a humidified oxygen-carbon dioxide atmosphere was slowly increased. Each post-BW12C79 infusion curve was investigated by comparison with a series of artificially computer generated curves (template method) and the percentage modification was calculated according to the best fit.

Hemoglobin Electrophoresis. Five ml of venous blood in an EDTA containing tube were obtained from 4 patients prior to administering BW12C79 and repeated 1 and 5 h from the end of the maintenance infusion. Hemoglobin electrophoresis was performed using a standard technique. A lysate was obtained by washing the whole blood in isotonic saline and adding carbon tetrachloride. The lysate was then applied on a cellulose acetate strip. After complete separation strips were stained with Ponceau S (BDH, Dorset, United Kingdom) and the excess stain was removed by immersion in 3% acetic acid.

^{31}P Magnetic Resonance Spectroscopy of Exercising Muscle. Seven patients (6 males, 1 female) were investigated by ^{31}P-MRS. Subjects were positioned in a 1.9 Tesla superconducting magnet (Oxford Magnet Technology, Oxford, United Kingdom) interfaced with a Bruker Biospec spectrometer (Bruker Spectrospin Ltd, Coventry, United Kingdom) with the right calf muscle overlying a single 6 cm diameter surface coil tuned to 32.7 MHz. Spectra from the gastrocnemius were collected during rest, exercise, and recovery from exercise as described by Hands *et al.* (13). Exercise (up to 13 min) was performed by plantar flexion of the right foot at a rate of 30 per min using a foot pedal to raise a weight of 20% lean body mass (light exercise) for 9 min followed by 32% lean body mass (heavy exercise) for 4 min. This protocol was performed by subjects both before and 4 h after the commencement of BW12C79 infusion (30–50 mg/kg loading dose levels).

Cytosolic Pi and PCr (μmol/liter cell water) were calculated from the signal intensity ratios of Pi/β-ATP and PCr/β-ATP, intracellular pH from the chemical shift of Pi relative to PCr, and free ADP concentration (μmol/liter cell water) from the intracellular pH and PCr measurements as described by Arnold *et al.* (14). Recovery half-times of PCr were determined by graphical interpolation.

Assessment of Toxicity. The toxicity profile of BW12C79, MMC, and the combination of MMC and BW12C79 was investigated. The adverse effects to BW12C79 were monitored by determining the physical and vital signs and electrocardiography during and after BW12C79 infusion. Hematological and biochemical indices were determined prior to each treatment and repeated at weekly intervals. Adverse reactions were graded according to the WHO criteria (0–4 scale).

Determination of MMC Pharmacokinetics. Plasma MMC pharmacokinetics were investigated in 6 patients without and with the accompanying administration of BW12C79. Ten ml of heparinized blood samples were obtained at the following time points from the end of the bolus MMC i.v. injection: 5, 15, 30, 60, 120, and 180 min. Plasma was separated by centrifugation and stored at −20°C pending analysis by reverse-phase high performance liquid chromatography (7). The pharmacokinetic parameters of MMC

were determined using nonlinear regression analysis using Ultrafit curve fitting package (Biosoft, Cambridge, United Kingdom) for the Apple Macintosh computer. Plasma concentrations of MMC were fit into a two-compartment model and the area under curve was calculated by the log trapezoidal rule and calculated to infinity.

Assessment of Tumor Response. All patients underwent the appropriate imaging investigations at entry in order to obtain baseline tumor measurements. Investigations were repeated after 2 cycles of MMC and BW12C79 in order to determine tumor response. Standard criteria for the determination of objective tumor response were adopted.

Statistical Analysis. The two-tailed *t* test was used to determine the statistical difference between paired samples using StatView 512+ package for the Macintosh computer. Statistical significance was represented by *P* values of 0.05 or less.

RESULTS

Oxygen Saturation Curve. The modification of oxyhemoglobin was determined in 23 patients during the first course of BW12C79. There was a dose-related increase in the percentage modification of the oxygenated hemoglobin following treatment with BW12C79 (Fig. 1). Peak modification was attained 1 h from the start of the loading dose of BW12C79 and was maintained throughout the duration (5 h) of the maintenance infusion of BW12C79. The mean percentage (± SE) modification of the OSC after 1 h from the start of the loading infusion of BW12C79 were as follows: 19.0% ± 1.1 at 20 mg/kg; 21.5% ± 1.5 at 25 mg/kg; 28.7% ± 2.2 at 30 mg/kg; 34.3% ± 1.9 at 40 mg/kg; and 44.7% ± 2.8 at 50 mg/kg.

Adverse Effects. All patients were eligible for acute toxicity assessment. Table 2 shows the number of patients entered into each dose level of BW12C79, the total number of BW12C79 exposures at each dose level, and the frequency and severity of the side effects to BW12C79 alone.

Patients in the first 4 cohorts (20–40 mg/kg) of BW12C79 experienced minimal side effects. There was a dose-related increase in nausea and/or vomiting, irritation of the vein, and tachycardia. Irritation of the cannulated vein was maximal during the infusion of the loading dose but was not followed by any signs of phlebitis. The grading of the venous irritation in Table 2 refers to the intensity of the pain experienced by the patient. Headache and nausea were transient and patients recovered within one to six hours from completing the BW12C79 infusion. Tachycardia was dose related but no patient actually complained of palpitations despite recording markedly accelerated heart rates in a number of patients. The range of percentage of change of the heart rate at the top dose level was 36–92%.

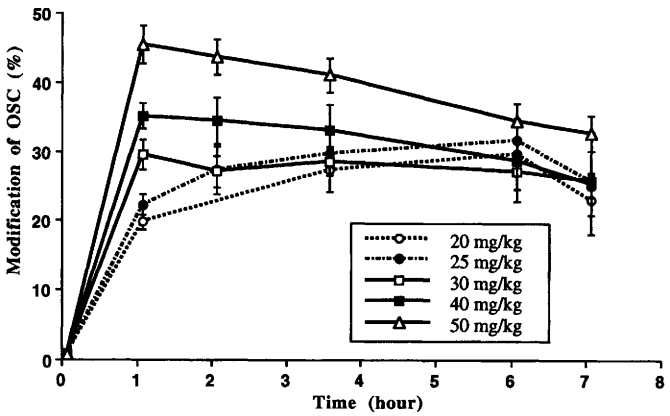

Fig. 1. Percentage modification (mean ± SE) of OSC in 23 patients at various dose levels of BW12C79. The following loading i.v. doses were used: 50, 40, 30, 30, 25, and 20 mg/kg. Loading dose was followed by an i.v. maintenance infusion of BW12C79 at 4 mg/kg/h for 5 h.

Treatment was discontinued in 3 patients at the 50 mg/kg dose level due to early toxicity following the first dose of BW12C79. One patient (26 years) with hepatoma developed encephalopathy 5 days after receiving the first dose of BW12C79 and lapsed into a deep coma. Extensive investigations during the acute phase of the illness did not show any evidence of central nervous system infection or metastases, metabolic abnormalities, or cerebrovascular catastrophe. Full consciousness was regained after 5 days and the patient was not rechallenged with BW12C79. A 71-year-old male patient with pretreatment electrocardiogram suggestive of myocardial ischemia experienced severe angina pectoris and hypotension within 1 h of starting the BW12C79 infusion but responded promptly to the discontinuation of the drug. The patient was included in the study despite an abnormal electrocardiogram because of absence of any history of symptomatic myocardial ischemia. Another female patient (64 years) with non-small cell lung cancer developed an acute episode of nausea and vomiting, hypotension and S-T segment depression within 1 h of starting the loading dose of BW12C79. Symptoms were alleviated by the discontinuation of the treatment with a return of S-T segment to the isoelectric line. This lady with a normal electrocardiogram at presentation and absent history of symptomatic myocardial ischemia had the highest modification of oxyhemoglobin, 56 and 57% at 1 and 2 h, respectively. In both patients serial electrocardiograms and serum cardiac enzymes assays showed no evidence of myocardial infarction.

The side effects to the BW12C79 and MMC combination were not greater than those expected following single agent MMC when used at the current dose and schedule of administration. In particular the use of the combination was not associated with neutropenia, thrombocytopenia, or treatment-related deaths. This indicated that the administration of BW12C79 did not significantly potentiate the adverse effects to MMC despite the marked left shift of the OSC.

^{31}P Magnetic Resonance Spectroscopy. No effect of BW12C79 was noted in the resting skeletal muscle. Relative concentrations of PCr, Pi, and ATP as well as intracellular pH were unaffected (data not shown). During exercise at the lower workload of 20% lean body mass, the infusion of BW12C79 did not affect either net PCr breakdown or intracellular pH significantly (Table 3). However, BW12C79 did cause a significant increase in net PCr breakdown and a significantly greater decrease in intracellular pH when the load was increased to 32% lean body mass. Exercise duration was also reduced by BW12C79 in 2 of the 7 patients. There was no correlation between the increased breakdown of PCr and the proportion of modified hemoglobin (data not shown). PCr recovery, an oxygen-dependent process, was slower when patients were on the infusion of BW12C79 as shown by the increased half-time recovery. This was in spite of similar end of exercise ADP concentration (Table 3).

Hemoglobin Electrophoresis. Hemoglobin electrophoresis was performed on samples from 4 patients who received BW12C79 with a loading dose of 50 mg/kg. A fast moving band which was seen in samples obtained 1 h from the start of BW12C79, consistent with the oxyhemoglobin-BW12C79 complex. A similar pattern persisted in blood samples at 5 h. When electrophoresis was repeated in one patient 1 day later, the abnormal band had disappeared. This was consistent with a short biological half-life of BW12C79.

Mitomycin C Pharmacokinetics. Table 4 gives the pharmacokinetic parameters of MMC determined in 6 patients who underwent therapy with MMC and BW12C79 at the top loading dose of 50 mg/kg. There was no significant change in any of the pharmacokinetic parameters when comparisons were made between pharmacokinetics of MMC with and without the concomitant administration of BW12C79.

Tumor Response. Response to the combination of MMC and BW12C79 was determined in 20 patients who had completed 2

Table 2 *The frequency and severity of side effects to different dose levels of BW12C79 using the WHO scale (0–4). Figures under each toxicity represent the number of courses associated with that particular side effect and the severity of each episode*

Loading dose (mg/kg)	No. of patients	No. of courses	Frequency and grade of toxicity				
			Headache	Nausea	Tachycardia	Venous irritation	Tiredness
20	4	8	1				
25	2	4				1	
30	3	6	1			1,2	
40	3	9	1,1,1			1,1,2	2
50	12	25	1,2	2,2,2,2,3	1,1,2	1,1,1,1,1 2,2,2,3,3,4	
			Other toxicity				
			Acute myocardial ischemia			Encephalopathy	
			50			2	
			50			1	

Table 3 *Results of the ^{31}P magnetic resonance spectroscopy of exercising calf muscle prior to and during the infusion of BW12C79. Values represent means ± SE (n = 7)*

	Preinfusion	On infusion	P (t test)
End of light exercise (20% of lean body mass)			
PCr/(PCr + Pi)	0.65 ± 0.04	0.59 ± 0.07	0.07
pH	6.97 ± 0.03	6.96 ± 0.04	0.71
ADP (μmol/liter)	48.0 ± 6.0	47.0 ± 8.0	0.78
End of heavy exercise (32% of lean body mass)			
PCr/(PCr + Pi)	0.55 ± 0.04	0.45 ± 0.05	0.02
pH	6.93 ± 0.02	6.80 ± 0.03	0.01
ADP (μmol/liter)	52.0 ± 6.0	45.0 ± 6.0	0.19
PCr recovery			
Half-time (min)	0.43 (± 0.02)	0.71 (± 0.04)	0.02

Table 4 *Pharmacokinetic parameters of MMC (n = 6; mean ± SE) from patients who received MMC with and without BW12C79. Statistical analysis was performed using a two-tailed t test for paired samples*[a]

Pharmacokinetic parameter	MMC only	MMC plus BW12C79	P
$t_{1/2\alpha}$ (min)	8.36 ± 0.63	6.35 ± 0.97	0.24
$t_{1/2\beta}$ (min)	53.52 ± 0.73	47.67 ± 5.76	0.31
$AUC_{0-\infty}$[b] (μg/ml/min)	16.82 ± 0.86	16.11 ± 1.17	0.59
Cl (ml/min)	600.0 ± 28.0	638.0 ± 48.0	0.46

[a] $t_{1/2}$, half-time.
[b] $AUC_{0-\infty}$, area under the curve calculated to infinity.

courses of mitomycin C and BW12C79. Four patients were not eligible for assessment because of the early discontinuation of treatment after the first dose of BW12C79. There was partial remission of hepatic metastases from a patient with metastatic small bowel adenocarcinoma which lasted 7 months. Two other patients were shown to have stable disease after four cycles of treatment, but the rest had disease progression.

DISCUSSION

The rationale for incorporating left-shifting agents in the treatment of cancer patients is the potential of those drugs to enhance the antitumor activity of bioreductive drugs. Tumor circulation is relatively inefficient compared to that of normal tissues because the newly formed tumor vessels have no smooth muscle and therefore unlike the normal vasculature are unable to regulate the blood flow in the tumor tissue in response to systemic hypoxia. This implies that any therapeutic procedure which will decrease tissue oxygenation, such as a left shift of the OSC, will also result in circulatory adjustments which will favor the oxygenation of normal tissues at the expense of tumor deposits (steal phenomenon) thereby exaggerating tissue hypoxia resulting from the left shift. Studies with animals having tumor xenografts have shown a variable effect of BW12C79 on the tumor blood flow (15, 16). Preclinical studies have also shown that tumor growth was delayed in mice bearing human colon xenografts treated with BW12C79 and misonidazole (8).

In our study the degree of modification of the left shift induced by BW12C79 was represented by the percentage modification of oxyhemoglobin. We have demonstrated that BW12C79 modifies the oxygenated hemoglobin in a dose-related fashion over the range of doses used in this study. The maximum modification was achieved within 1 h of starting a loading dose of the drug. It was also possible to maintain the modification of OSC for the duration of BW12C79 administration with no additional toxicity (Fig. 1).

Adverse effects resulting from normal tissue hypoxia have been demonstrated with the higher doses of BW12C79 (50 mg/kg) manifested by headache and tachycardia. The occurrence of symptoms of myocardial ischemia in 2 patients within 1 h of starting the BW12C79 infusion suggests that the peak modification of the OSC was a dose-limiting factor. Serious cardiac toxicity is, however, unlikely to occur in patients without preexisting coronary artery disease. The risk of precipitating myocardial ischemia would therefore be greatly reduced by excluding patients with clinical or electrocardiographic evidence of myocardial ischemia. Clinicians involved in the administration of BW12C79 should therefore be aware of the risk of acute myocardial ischemia and preferably monitor the patients for the first hour of the infusion.

The relationship between the treatment with BW12C79 and subsequent encephalopathy in one patient remained obscure. There was no evidence of any metabolic derangement and both CT scanning of the brain and cerebrospinal fluid examination were normal. Nevertheless, the timing of the episode and its reversibility suggested major pharmacological/metabolic components to the acute illness of the patient. It is possible that there was release of toxic metabolites from the tumor deposits due to the necrosis of tumor cells which might have been enhanced by BW12C79.

MMC pharmacokinetic parameters were similar to other studies (7). The mean terminal half-life of MMC was significantly less than the duration of the left shift in this study. The left-shift of the OSC can be maintained over 5 half-lives of MMC and therefore maintained over 95% of the exposure to MMC. Lack of pharmacokinetic interactions between BW12C79 and MMC is probably due to differences in the metabolic pathways handling these two drugs. Plasma pharmacokinetics of MMC will not provide any information on its intracellular metabolism in target cells and it would have been unlikely for the hepatic metabolism of MMC to be influenced by changes in OSC. Further studies are indicated to determine the influence of the left shift of OSC on the cellular metabolism of MMC in tumor deposits.

Our study on skeletal muscle metabolism *in vivo* by using MRS provides evidence of substrate supply limitation upon BW12C79 in-

fusion. It is likely that this limitation was due to a reduction of oxygen supply to the mitochondria of skeletal muscle which in turn may have been due to either reduced blood flow or to decreased release of oxygen from the hemoglobin molecule or a combination of these factors. In the seven patients studied we were unable to demonstrate a correlation between the decrease in PCr concentration and the proportion of modified hemoglobin. This means that we cannot exclude diversion of blood supply from the exercising skeletal muscle to other organs playing a role in the decrease of oxygen available to skeletal muscle. Similar reduction of oxygenation of tumor cells would be beneficial in enhancing the activity of hypoxically activated drugs such as mitomycin C.

The efficacy of the bioreductive agents when used in combination with left-shifting agents will ultimately depend on the balance between their metabolic activation inside tumor cells and the tumor bioavailability of these drugs. Response to BW12C79 and MMC in this phase I trial was low. The majority of patients had tumor types which were resistant to chemotherapy and 50% had been previously exposed to cytotoxic therapy. Further randomized clinical trials are needed to investigate the role of BW12C79 in enhancing the efficacy of bioreductive drugs in specific tumor types (*e.g.*, breast cancer). Lack of potentiation of toxicity to MMC at the current dose and schedule of administration indicates that it would be feasible to use higher doses of MMC in combination with BW12C79 in future phase II studies. Combinations of BW12C79 and the more selective bioreductive drugs, such as RSU 1069 and tirapazamine (17), should also be investigated for their antitumor activity *in vivo*.

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