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Clinical, Toxicological, and Pharmacological Studies of Combination Chemotherapy of Adenocarcinoma with Adriamycin and Baker's Antifolate*

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Summary. *Ten patients with disseminated adenocarcinoma were treated with combination chemotherapy employing Adriamycin and Baker's Antifolate (BAF). There were seven patients with lung adenocarcinoma, two of whom achieved partial remission while the remaining five had their disease stabilized. Drug toxicity to the bone marrow, gastrointestinal mucosa, and skin was dose-limiting and was greater than the known toxicities of the individual drugs. Pharmacological studies of both drugs were performed on five patients to determine whether abnormal pharmacokinetics could explain this collateral toxicity. Adriamycin plasma concentrations and disappearance seemed to be unaffected by BAF. However, BAF levels were prolonged, apparently due to an Adriamycin effect on the plasma elimination of BAF, resulting in a prolonged exposure of sensitive tissues and organs to BAF. Consequently, when BAF and Adriamycin are used in combination, appropriate dose and schedule changes must be made to avoid any potentially serious side effects.*

Introduction

Baker's Antifolate (BAF) (NSC 139105) is a folate antagonist synthesized by the late Baker (Baker, 1971) to bypass cellular resistance to methotrexate caused by a defective mechanism for its transport. It was chosen for initial clinical trials because of its activity against Walker 256 carcinosarcoma and Dunning leukemia in the rat. Our early pharmacological studies in experimental animals demonstrated significant biliary excretion of the compound, and human studies showed prolonged drug plasma concentrations in patients with impaired liver function (Benjamin, 1975; Benjamin et al., 1975). Clinical phase I studies of BAF conducted at our institution have shown that the major toxicities of this agent are on bone marrow, skin, and gastrointestinal mucosa (Rodriguez et al., 1976); these were enhanced in patients with abnormalities of liver function. In a phase II study with BAF, tumor regression or stability was seen in 13 of 31 patients with lung adenocarcinoma (Rodriguez et al., 1977), suggesting the potential clinical application of BAF against this neoplasm.

An agent of considerable versatility, Adriamycin is also active against lung adenocarcinoma, with response rates of 8–37%, and of 15% when all the studies are combined (Selawry, 1975). Median response durations were 6–8.5 months, with responding patients surviving two to four times longer than those not responding. These observations and the reported synergism of Adriamycin with Methotrexate in an experimental tumor system (Goldin and Johnson, 1975) prompted us to study BAF-Adriamycin combination chemotherapy in patients with lung adenocarcinoma. The study also included three patients with adenocarcinomas of other sites.

Since Adriamycin and BAF are significantly excreted in the bile (Benjamin, 1975; Riggs et al., 1977; Benjamin et al., 1975), we were concerned about a possible drug interaction or competition for hepatic elimination. Therefore, we examined the plasma concentrations and pharmacokinetics of both drugs in five patients. Dosage and schedule adjustments were subsequently made based on drug kinetics and liver function evaluations.

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Patients and Methods

Clinical Studies

Ten patients with disseminated adenocarcinoma were entered in the study; seven with adenocarcinomas of the lung, one with adenocarcinoma of unknown primary, and two with gastrointestinal adenocarcinomas. Informed consent was obtained from each patient according to institutional policy before the therapeutic and pharmacologic studies. Pretreatment evaluation included a complete history and physical examination, a complete blood count, urinalysis, serum chemistries, chest x-ray, electrocardiogram and appropriate radiologic and isotopic scan studies to document and quantitate the extent of the tumor.

Adriamycin was administered intravenously in 50 ml of 5% dextrose in water over 5–15 m at an initial dose of 60 mg/m^2. BAF was administered intravenously in 100–250 ml of 5% dextrose in water over 1 h at 250 mg/m^2/day for 3 consecutive days. Therapy was repeated at 3-week intervals after recovery from toxicity. On subsequent courses appropriate dosage adjustments of both drugs were made on the basis of the toxicities and complications from the previous course.

Evaluation during treatment included complete blood counts twice to three times weekly, with repeated serum chemistries before each course of therapy. Tumor response was evaluated by chest x-rays and other radiologic or isotopic studies every two courses of therapy. Responses to therapy were defined as follows: 1) partial remission (PR), a 50% decrease in the product of the longest perpendicular diameters of all measurable lesions, lasting at least 4 weeks without the appearance of new lesions; 2) stable disease (S), a steady state of response less than PR, without evidence of progressive disease for at least 8 weeks; 3) progressive disease (PD), an increase of any measured lesion by over 25% or the appearance of any new lesions.

Pharmacology Studies

Plasma pharmacokinetic studies of Adriamycin and BAF were performed in five patients. Heparinized blood samples of 10 ml each were collected before therapy and serially for 24 h after drug administration. Adriamycin and its metabolites were determined fluorometrically after acid-alcohol extraction from plasma (Benjamin et al., 1973). Patients who participated in the pharmacology studies received 20 mg (250 μCi) BAF-4,6 ^{14}C. Plasma BAF concentrations were determined by counting in a liquid scintillation counter and comparison with appropriate standards. Since BAF was not appreciably metabolized initially, the total radioactivities were expressed in terms of μg/ml of the unchanged drug. Plasma half-lives, concentration by time (C × t), and metabolic clearance rate (MCR) values were determined from best curve fitting nonlinear regression analysis.

Results

Clinical Response and Toxicity

Ten patients received 36 courses of BAF and Adriamycin. The number of courses at each dose level is shown in Table 1. Five patients had subsequent dose excalations and two required a dosage reduction because of excessive toxicity. The patients' clinical characteristics are shown in Table 2.

The antitumor effects of this drug combination were evaluable in nine patients (Table 3). One patient expired 3 weeks after treatment from progressive tumor and related complications, and was not considered evaluable for response. Two patients with lung adenocarcinomas achieved partial remissions but expired, while in remission, from drug-related toxicity and associated complications, 2 months after therapy. The other lung carcinoma patients achieved stable disease for 3–12 months (median 7). Two other patients (one with adenocarcinoma of the gallbladder and one with unknown primary) achieved stable disease for 6 months.

Sites of major drug toxicity were the gastrointestinal (GI) tract mucosa, the skin, and the bone marrow. The incidence and severity of these toxicities were unpredictable and not apparently related to the dose or the patient's liver function status (Tables 1 and 2). In two patients severe toxicity suddenly developed after multiple courses at similar doses with impunity. Gastrointestinal toxicity became manifest in mucositis, which included the oral and anal mucosa and occured 6–7 days after drug administration, lasting 7–10 days. Two pa-

Table 1. Adriamycin, Baker's antifolate toxicity related to dose

Adriamycin (mg/m^2) Baker's antifol (mg/m^2)[a]	No. courses evaluable	Courses with toxicity			
		Mucositis	Skin	Granulocytes < 1000/μl	Platelets < 100,000/μl
75/300	7	4	1	2	–
75/200	1	1	–	–	–
60/300	6	–	–	–	–
60/250	16	5	2	3	4
60/200	3	1	1	1	2
60/150	1	–	1	–	–
60/125	1	–	–	–	–
50/200	1	1	–	–	–

[a] Dose given daily times three

Table 2. Patients clinical characteristics and toxicity

Patients		Adenocarcinoma diagnosis	Prior therapy	Liver function[a]	Mucositis	Toxicity[b]	
age	sex					Skin	Bone marrow
67	F	Lung	Radiation	Normal	++		++
62	F[c]	Lung	Radiation	Normal	+++	+	+++
48	M	Lung	Radiation	Normal	+	+	+
63	M[c]	Lung	None	Normal	+++		++
68	M	Lung	None	Alk. ptase (87)	+++	++	+
34	M	Lung	None	Normal	+		+
55	M	Lung	Surgery	Alk. ptase (114)	++	+	+
51	F	Unknown primary	None	Normal			+
63	M	Gallbladder	Surgery	Alk. ptase (100)	+		+
56	M[d]	Rectal and pancreatic primaries	Surgery	Normal		+	++

[a] Alkaline phosphatase values are in parentheses in mU/ml (normal is 30–85)
[b] For mucositis: + = mild soreness and redness without ulceration; ++ = mild to moderate ulceration; +++ = moderate to severe ulceration or necrosis. For rash: + = mild asymptomatic erythema; ++ = moderate erythema with pruritis; +++ = desquamation or exfoliative dermatitis. For bone marrow: + = platelets 50,000–100,000 μl or granulocytes 1000–2000/μl, ++ = platelets < 50,000/μl or granulocytes < 1000/μl; +++ = platelets < 50,000/μl and granulocytes < 1000/μl
[c] Died with severe granulocytopenia and septicemia with gastrointestinal tract ulceration
[d] Died of progressive tumor after one course of therapy

Table 3. Therapeutic results with Adriamycin and Baker's antifolate

Adenocarcinoma diagnosis	Number evaluable	Response[a]	
		PR	S
Lung	7	2[b] (2, 2)	5 (12, 7, 7, 3, ?)
Gallbladder	1		1 (6)
Unknown primary	1		1 (6)

[a] Number of patients who responded with duration of response in months in parentheses
[b] Both patients expired of drug-related causes while in partial remission

tients had severe GI tract mucosal ulceration and expired from septicemia associated with granulocytopenia. At autopsy extensive necrosis was seen in the small bowel, and probably this was the source of continued bacteremia. Skin toxicity in this study was similar to that seen in patients treated on the BAF phase I study, and varied from an asymptomatic, erythematous, macular rash to an exfoliative dermatitis. It occurred 1–2 weeks after therapy and resolved in 1–2 weeks. Two patients had skin hyperpigmentation in the areas affected after the rash resolved. This was particularly prominent in areas of prior radiotherapy. Myelosuppression was seen in all patients and was severe in four (Tables 1 and 2). This toxicity contributed to mortality in two patients, but the other two patients recovered without complications.

After the severe toxicity and drug-related deaths in two cases, all patients who were then receiving the drug combination were admitted to the hospital and studied pharmacologically to determine whether there was a collateral toxic drug interaction and to estimate the appropriate drug dose for each patient. Prior BAF pharmacology studies had shown that prolonged plasma drug concentrations resulted in excessive toxicity (Benjamin et al., 1975).

Pharmacology

Adriamycin and its metabolites were determined together by total fluorescence. Plasma total fluorescence of Adriamycin and its metabolites in our patients were similar to those previously determined in patients not receiving BAF. The mean plasma concentrations of total fluorescence (in Adriamycin equivalents) were 0.14 μg/ml 1 h after drug administration and fell to 0.06 μg/ml at 24 h. The Adriamycin plasma drug concentration was no higher or longer-lasting in our patients than in earlier patients who did not receive BAF (Benjamin et al., 1973). Nor was there any evidence that BAF affected Adriamycin plasma levels or disappearance. Although no abnormalities of total anthracycline fluorescence were found, we cannot exclude a specific effect of BAF on the metabolism or intracellular action of Adriamycin, which could enhance toxicity.

Pharmacokinetic measurements of total plasma ^{14}C radioactivity were used to assess BAF disappearance in

Table 4. Pharmacokinetic parameters of Baker's antifolate administered with Adriamycin

Patient[a]			Plasma terminal $t_{1/2}$ (h)	Terminal Cxt (μg-h/ml)	Metabolic clearance ml/kg/min
No.	Age	Sex			
1	68	M	9.6	12.4	3.8
2	55	M	6.4	10.5	3.4
3	67	F	7.2	11.9	4.4
4	63	M	6.1	14.6	3.1
5	48	M	16.5	37.1	2.0
5	48	M[b]	16.5	24.5	2.7

[a] BAF 250 mg/m², Adriamycin 60 mg/m²
[b] Repeat study without Adriamycin

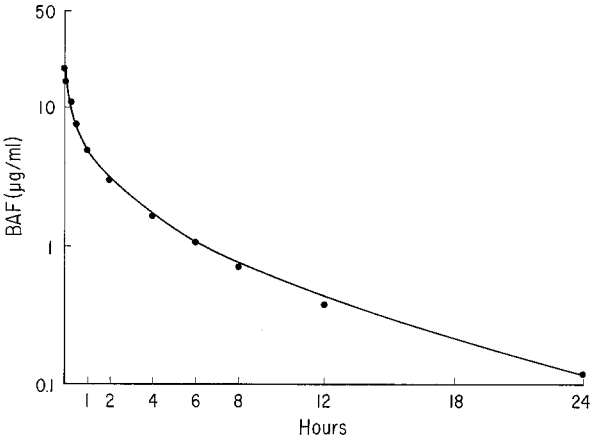

Fig. 1. Mean plasma disappearance curve of BAF in four patients who received Adriamycin 60 mg/m² and BAF 250 mg/m²

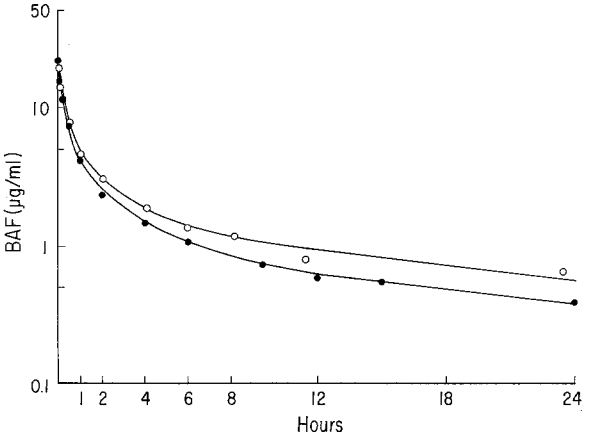

Fig. 2. Plasma disappearance of BAF, 250 mg/m², in one patient, when given with and without Adriamycin 60 mg/m²; ○ with Adriamycin; ● without Adriamycin

five patients. Our previous pharmacology studies, in man and dogs (Benjamin et al., 1975), suggested minimal metabolism of BAF, and these studies correlated well with the drug's toxic effects. The pertinent pharmacokinetic information is presented in Table 4. The mean of the plasma terminal half-lifes ($t_{1/2}$) was 10.4 h, with a mean terminal concentration by time (C × t) of 18.5 μg-h/ml and a mean metabolic clearance (MCR) of 3.3 ml/kg/min. One patient had a plasma $t_{1/2}$ and a C × t two to three times those of the others and a MCR appreciably smaller. If he is excluded the mean plasma $t_{1/2}$ is 7.3 h, the mean C × t is 12.4 μg-h/ml, and the mean MCR is 3.7 ml/kg/min. The mean plasma disappearance curve for ^{14}C-BAF in these four patients is shown in Figure 1. In our previous studies of patients who received only BAF the terminal plasma $t_{1/2}$ was 5.3 h and the terminal C × t was 11.4 μg-h/ml in five patients with normal liver function, but 8.3 h and 102 μg-h/ml in three patients with markedly abnormal liver function as evidenced by elevated serum bilirubin and alkaline phosphatase (Benjamin et al., 1975). Because of the marked abnormal pharmacokinetics in patient no. 5, he was treated aggressively with citrovorum factor after his dose of BAF, and experienced no toxicity. The BAF pharmacology study was repeated in this patient 3 weeks later without Adriamycin. The two plasma BAF disappearance curves are shown in Figure 2. The terminal plasma BAF $t_{1/2}$ without Adriamycin was 16.5 h, identical with that of the previous study with Adriamycin. However, the terminal C × t was 24.5 μg-h/ml and the MCR was 2.7 ml/kg/min, as compared with 37.1 μg-h/ml and 2.0 ml/kg/min with Adriamycin. The prolongation of the terminal C × t and the decrease in MCR of BAF when given with Adriamycin suggests that Adriamycin prolonged the exposure of tissues to BAF and increased toxicity. We have previously observed that the same total dose of BAF is less toxic when administered over 3 days than when given over 5 days (Rodriguez et al., 1976).

Discussion

Adriamycin-BAF combination chemotherapy showed definite antitumor activity. Two of seven patients (29%) with lung adenocarcinoma achieved partial remissions and five (71%) showed disease stabilization. However, toxicity and morbidity seemed greater than expected from those recorded with the two drugs separately.

The toxicities of the drug combination were striking. In the phase I trial of BAF only 20–30% of patients had platelet counts lower than 200,000/μl or leukocyte counts lower than 4,000/μl (Rodriguez et al., 1976). In the phase II study 30% of the patients experienced

myelosuppression and these were mostly the patients who also developed dermatitis or mucositis (Rodriguez et al., 1977). There was additive myelosuppression of Adriamycin with BAF in our study, which could not be predicted from one course of therapy to the next. Another major toxity of the drug combination involved the mucous membranes and GI tract mucosa, with irritation, ulceration, and necrosis. It occurred to some degree in all patients at some time during the therapy, and was difficult to predict from one course of therapy to the next. It subsided when the BAF dose was reduced. Skin toxicity occurred in 50% of the patients and generally presented as an erythematous, maculopapular skin rash, which progressed in some cases to an exfoliative dermatitis. Both drugs when given alone are known to cause radiation "recall" skin reactions (Rodriguez et al., 1977; Gottlieb et al., 1977) and this may be more prominent when the drugs are given together.

The total toxicity pattern suggested the predominance of BAF in this regard. The one patient with markedly abnormal plasma BAF pharmacokinetics was given citrovorum factor because extensive toxicity was predictable. The citrovorum factor abated all potential toxicity. Subsequent courses were given without toxicity, by decreasing the BAF dose 50% and giving the Adriamycin 1 day after the last dose of BAF. In other patients it was also found that decreasing the BAF dose reduced toxicity.

The pharmacological studies detected no alteration in plasma Adriamycin levels, but BAF pharmacokinetics were altered when it was given with Adriamycin. These included a longer terminal $C \times t$ or $t_{1/2}$ and a diminution in MCR, which may be attributable to minimal abnormalities of alkaline phosphatase (present in three of our five patients); these could result in abnormal biliary elimination of BAF or Adriamycin, which is also primarily excreted in the biliary tree, effecting BAF elimination. It is possible that both of these mechanisms were operative. In two patients serum alkaline phosphatase was elevated during Adriamycin-BAF therapy, probably due to a drug effect. No evidence of liver metastases was found in these patients, however. Only one of our patients took part in BAF pharmacological studies with and without Adriamycin. He showed markedly different BAF pharmacokinetics. There was a decrease in the $C \times t$ and an increase in the MCR but the plasma half-life remained unchanged when Adriamycin was eliminated. This patient had a normal liver scan, Bromsulphalein retention time, serum bilirubin, liver enzymes, and renal function tests, and the reason for his different BAF pharmacokinetics is obscure. However, there may be other patients like him and this could explain the unpredictability of the drug's toxicity. In the other patients Adriamycin appeared to prolong the half-life and the exposure of tissues to BAF as manifested by prolongation of the terminal $C \times t$ and the reduction in MCR.

Previous combination chemotherapy regimens employing Adriamycin with Methotrexate showed additive toxicity (Ahmann et al., 1975; Eagan et al., 1975). The toxicity was expressed primarily as myelosuppression with mild mucositis. Because of myelotoxicity, dose reductions of both drugs are necessary and the combination is tolerated at the reduced doses. Methotrexate, as opposed to BAF, is excreted primarily by the renal route (Huffman et al., 1973); since Adriamycin is excreted by the biliary tree (Benjamin et al., 1975; Riggs et al., 1977) this could diminish any possible competition for elimination. These differences in Methotrexate and BAF toxicity and pharmocology appear to determine each drug's contribution to toxicity when used in combination with Adriamycin.

The anticancer responses seen in this study, with the combination of Adriamycin and BAF, are promising. However, caution must be exercised in future studies to avoid severe toxicity. We recommend to start at lower doses with both drugs and to give Adriamycin 24 h after BAF when there has been significant excretion of BAF from the body. It would also be desirable to evaluate BAF pharmacokinetics, particularly in patients with any abnormalities of alkaline phosphatase. This will determine whether they will have prolonged drug plasma levels and an increased risk of toxicity.

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