Clinical Investigation

Treatment of Advanced Malignancy With Plasma Perfused Over Staphylococcal Protein A

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A total of 14 extensively pretreated patients with advanced and progressive malignancy were given 140 infusions of autologous plasma that had been perfused over staphylococcal protein A bound to an agarose gel (Sepharose). Infusions ranged in volume from 35 ml to 260 ml (mean, 70 ml), and the quantity of protein A used ranged from 1 to 30 mg per 100 ml of plasma (mean, 10 mg). Acute toxic reactions included fever (21%), chills (18%), nausea (17%), vomiting (8%), pain (9%) and bronchospasm (2%). Four patients did not have an acute toxic reaction and no chronic or cumulative toxic effects were identified. In two patients there was objective tumor regression and in five there was stabilization of disease lasting from 4 to 12 weeks. Further study of this treatment modality is warranted.

There has been considerable interest in the role of serum "blocking factors" in the pathogenesis of malignancy.[1-4] The finding that these factors are immune complexes or immunoglobulins led to the concept that their removal by nonspecific[5] or specific[6,7] means might prove useful in the treatment of malignancy. The observation that staphylococcal protein A nonspecifically binds IgG and its complexes via the Fc receptor[8] led to its use as an "immunoadsorbent" in the treatment of various malignant diseases of cats,[6] dogs[7,9,10] and humans.[11-15] Terman and co-workers[15] noted that small volumes of protein A-treated autologous plasma produced tumor regression in humans with significant but manageable acute toxic effects, which observation led us to undertake an evaluation of this novel treatment modality. We report our initial phase I experience in treating advanced malignancy with autologous plasma perfused over staphylococcal protein A covalently bound to an agarose gel (Sepharose).

Patients, Materials and Methods

Patients with biopsy-proved malignancy who had progressive cancer following standard therapy (including at least one course of chemotherapy) and for whom no established therapy nor any potential cure could be offered were eligible for this study. Informed consent for participation was obtained according to institutional policies.

Patients received no other therapy during the study and had received no therapy for at least four weeks before study entry, except for radiotherapy to locally symptomatic or threatening disease sites. These irradiated sites were not considered in assessing response to plasma therapy.

Autologous plasma was obtained by phlebotomy using standard blood donor sets (450-ml capacity, with citrate-dextrose-adenosine anticoagulant) and plasma transfer packs. Red blood cells were reinfused on the day of phlebotomy. Plasma was treated by centrifugation at 3,000 G for 30 minutes, followed by passage over a column of Sepharose-bound protein A (Pharmacia, Inc, Piscataway, NJ). The quantity of protein A varied from 1.0 to 30 mg per 100 ml of plasma. Treated plasma was filtered through a 0.22-micron filter, packaged in standard blood product administration sets and either administered fresh or stored frozen

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at −70°C until use. From one to four (median three) separate packages of treated plasma were prepared from each unit of blood. For patients on stable doses (see below), equal aliquots of plasma were packaged, whereas for patients still undergoing dose escalation, a typical preparation would be packaged in volumes of 50, 100 and 150 ml. Plasma processing was done in a laminar flow hood at ambient temperature, and sterility was tested on aliquots of treated plasma using standard blood culture media. No growth was observed in any cultures.

In the absence of detailed information about the mechanism of action of this therapy, the definition of "dose" is problematic. We assumed that the potential toxic or antitumor activity of the treated plasma was directly related to the volume of plasma and to the ratio of protein A to plasma used in the preparation of the treated plasma. Based on the report by Terman and associates,[15] we chose 50 ml of autologous plasma treated at a ratio of 1.0 mg of protein A per 100 ml as a starting dose. For the ninth and subsequent patients, the planned starting dose was 50 ml of plasma treated at 4.0 to 5.0 mg of protein A per 100 ml of plasma. After each infusion without a significant toxic reaction, dosage was escalated by 50% to 100% for the next treatment. We considered the dose at each treatment to be the product of treated plasma volume (ml) × (mg of protein A per 100 ml of plasma), and dose escalation consisted of increasing either the volume of plasma or the amount of protein A used in its preparation.

The study plan was to infuse treated plasma twice a week, increasing the dose at each treatment until an easily measured acute toxic effect was observed, and then to continue at this maximally tolerated dosage for a total of two months or until disease progression. For patients living at a considerable distance for whom twice-a-week visits were not possible, the treatment schedule was modified to include three infusions of treated plasma on days 1, 3 and 5, and to repeat the cycle (with dose escalation as indicated) on day 15.

Treated plasma was infused over 5 to 15 minutes. Vital signs were monitored at intervals of 15 to 30 minutes and subjective reports of a toxic reaction recorded for two hours thereafter or until stable. Fever and chills were treated by giving acetaminophen and diphenhydramine hydrochloride and for patients receiving a stable (nonescalating) dosage, these medications were used prophylactically if warranted by previous symptomatic toxic reactions.

Patients were evaluable for toxic reactions after one infusion and for response after four infusions of treated

TABLE 1.—*Clinical Characteristics of Patients Studied*

Patient	*Diagnosis*	*Age/Sex*	*Weight (kg)*	*Karnofsky Status*	*Disease Sites**	*Prior Therapy†*	*Schedule Planned‡*
1	Adenocarcinoma, lung	30 ♂	50	20	L	Chemotherapy (3)	I
2	Adenocarcinoma, breast	44 ♀	48	40	HO	Chemotherapy (3), radiation, surgical	I
3A§	Adenocarcinoma, breast	43 ♀	200+	40	LO	Chemotherapy (3), radiation, surgical	I
3B§	Adenocarcinoma, breast	43 ♀	200+	40	LO	Chemotherapy (3), radiation, surgical	I
4	Squamous carcinoma, lung	77 ♂	78	40	LP	Chemotherapy (1)	II
5	Squamous carcinoma, lung	53 ♂	52	70	DO	Chemotherapy (1), radiation, surgical	I
6	Squamous carcinoma, lung	54 ♂	66	80	L	Chemotherapy (1), radiation, surgical	II
7	Squamous carcinoma, head and neck	65 ♂	61	80	DN	Chemotherapy (1), radiation, surgical	II
8	Paraganglioma	53 ♀	45	50	LOP	Chemotherapy (1), radiation, surgical	I
9	Squamous carcinoma, lung	66 ♂	82	60	LO	Chemotherapy (1), radiation, surgical	II
10	Renal carcinoma	44 ♂	54	40	DLO	Chemotherapy (2), radiation, surgical	I
11	Melanoma	55 ♂	68	50	N	Chemotherapy (3), radiation, surgical	I
12	Adenocarcinoma, colon	54 ♀	50	40	HL	Chemotherapy (1), radiation, surgical	I
13	Adenocarcinoma, colon	35 ♀	98	70	H	Chemotherapy (1), radiation, surgical	I
14	Adenocarcinoma, breast	70 ♀	47	50	P	Chemotherapy (2), surgical	I

*Disease sites are: D=skin, H=liver, L=lung, N=nodes, O=osseous, P=pleura.
†Numbers in parentheses indicate the number of chemotherapy regimens previously used.
‡Schedule I consists of twice-a-week infusions. Schedule II consists of three infusions every other week.
§Patient 3 had two courses of therapy separated by five weeks.

TABLE 2.—*Acute Toxic Effects of Plasma Perfused Over Protein A Agarose Gel (Sepharose)*

Dosage Range (ml×mg protein A/100 ml)	*Doses Number*	*Fever**	*Rigor*	*Nausea*	*Broncho-spasm*	*Local Pain*	*Hypo-tension*
		Number of Patients					
35- 100	17	6	6	6	..	..	..
101- 200	22	4	3	1	..	..	..
201- 300	16	1	1	1	..	1	..
301- 500	30	5	4	8	..	5	1
501-1,000	18	6	5	5	2	4	..
1,001-1,500	17	3	2	2	1	2	..
1,501-2,500	8	3	3	..	..	..	..
2,501-3,500	2	1	1	1	..	..	..

*Fever is defined here as elevation of oral temperature exceeding 37.8°C.

plasma. One patient (case 7) was nonevaluable because of lack of measurable disease.

Results

Of the initial 14 patients, all are evaluable for toxic reactions and 12 are evaluable for response. The nonevaluable patients include one who died early and one who had no evaluable disease. The initial clinical characteristics of these patients are shown in Table 1. The volumes of plasma for individual infusions ranged from 35 ml to 260 ml (mean, 70 ml), and the ratios of protein A per 100 ml of plasma ranged from 1.0 to 30 (mean, 10). Dosage ranged from 35 to 3,000 arbitrary units. Significant toxic effect was seen at doses as low as 100 units, but some patients had no toxic reaction at the highest level attained. All toxic reactions except hypercalcemia were evident within an hour. There was no evidence that toxicity was cumulative or schedule dependent for the two schedules used. Freshly prepared plasma and stored frozen plasma were equitoxic. About 80% of treatments were given with previously frozen treated plasma. Table 2 summarizes the observed toxic effects as a function of dosage. The most common toxic reactions were fever and chills. These were generally not associated with life-threatening changes in blood pressure or respiratory state, though patients 4 and 14 (Table 1) had a total of three episodes of bronchospasm. Subcutaneous administration of epinephrine was required for relief in one case. In most cases, chills were noted within about 30 minutes of beginning the infusion, and maximal temperature was reached in 30 to 90 minutes. In only one patient did a treatment-associated fever persist for more than two hours.

Other toxic reactions included a single episode of hypotension, which required no specific therapy and resolved within ten minutes. Three patients had nausea following therapy (cases 6, 8 and 14, Table 1). There were two episodes of treatment-related diarrhea. Only one patient (case 8) had exacerbations of local pain at tumor sites with therapy.

In one patient with breast cancer and extensive bone metastasis, hypercalcemia developed on two occasions 72 to 96 hours after an infusion of treated plasma (Figure 1). In both instances, control of the serum calcium level required admission to hospital for infusions of saline and mithramycin. Table 3 gives a summary of the course of each patient, including data on doses of treated plasma, fever, duration of therapy and clinical response.

As this was a phase I study, its objective was to determine toxicity of treatment. Significant antitumor effects were observed in 2 of 12 evaluable patients, however, and an additional five patients had stabilization of disease for four to eight weeks. The other five patients had definite or probable progression of tumor within the first four weeks of treatment. One patient with progressive disease and renal carcinoma (case 7) received dexamethasone for spinal cord compression during therapy with protein A-treated plasma, and this may have interfered with toxic effects or therapeutic response (or both). Two patients (cases 2 and 3) for technical reasons received rather low doses of plasma, and the latter had a toxic reaction only once at the highest dosage used, after which she refused further therapy. Only one patient (case 6) had both fever and chills with treatment and prompt progression of tumor.

A brief description of responses in two patients will show the quality of tumor regression seen.

Patient 4 was a 77-year-old man with squamous

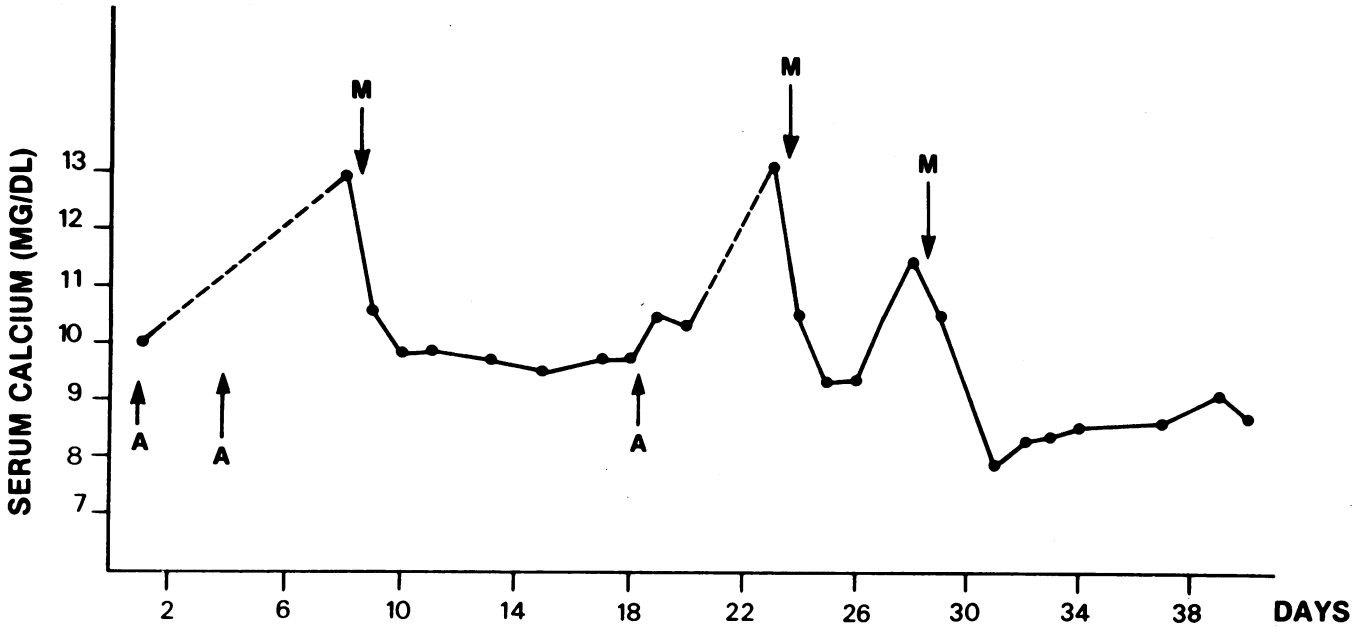

Figure 1.—Serial measurements of serum calcium in patient 2 are shown. Arrows indicate times of administration of treated plasma **(A)** or mithramycin **(M)**.

carcinoma of the lung. Figure 2 shows a series of x-ray films taken over the first six weeks of his therapy. His pleural effusion resolved and collapsed segments of lung reexpanded. During therapy a central abscess developed in a large right upper lobe mass, which was the site of his primary tumor. Ultimately, an associated pneumonia led to his death after 65 days of plasma therapy.

Patient 8, a 54-year-old woman with paraganglioma, had a left retroorbital mass, multiple pulmonary nodules, bone metastasis with pathologic fractures and bilateral pleural effusions. During plasma therapy, there was reduction of proptosis and recovery of the ability to read and embroider, which had been impossible because of diplopia and blurring of vision. Pleural effusions resolved, new bone formation was noted in an area of pathologic fracture and multiple pulmonary nodules remained stable for 12 weeks.

Discussion

In any phase I study, the central goal is to define toxic effects and to establish suitable dose-schedule relationships that will allow subsequent studies to delineate the spectrum of antitumor activity of a new therapy. The uncertainty about the mechanism of action of this therapy, however, makes the definition of dose problematic. We defined arbitrary "units" of dosage based on the assumption that effects would be related to volume of plasma infused and quantity of protein A used. Starting doses were based on the limited literature available regarding dosage[12,15] and were escalated progressively. Despite this dose escalation,

TABLE 3.—*Treatment Data for Patients Studied*

Patient	*Number of Treatments*	*Duration of Therapy (Days)*	*Initial Dose (units)*	*Initial Amt Protein A (mg/100 ml plasma)*	*Maximum Dose (units)*	*Maximum Amt Protein A (mg/100 ml plasma)*	*Maximum Temperature (°C)*	*Clinical Response*
1	2	3	60	2.0	120	2.0	37.4	NE
2	3	26	35	2.5	85	2.5	37.7	PD
3A	8	26	85	2.0	400	8.0	37.5	S
3B	1	1	..	..	800	8.0	37.9	NE
4	12	65	200	5.0	720	16.0	39.7	PR
5	8	30	100	6.2	200	6.2	39.1	PD
6	15	60	100	5.0	2,400	50.0	38.7	S
7	10	31	120	6.1	750	16.0	38.6	NE
8	27	84	100	5.0	1,100	40.0	37.2	MR
9	10	42	250	12.5	3,000	90.0	38.5	S
10	6	12	200	12.0	1,050	12.0	37.4	PD
11	5	14	660	20.0	1,250	29.0	36.9	PD
12	5	9	250	12.0	1,000	34.0	38.3	PD
13	14	42	200	11.0	2,000	60.0	38.6	S
14	14	60	250	12.0	1,500	35.0	38.7	S

NE=not evaluable; PD=progressive disease; S=stable; PR=partial regression of more than 50%; MR=minor response.

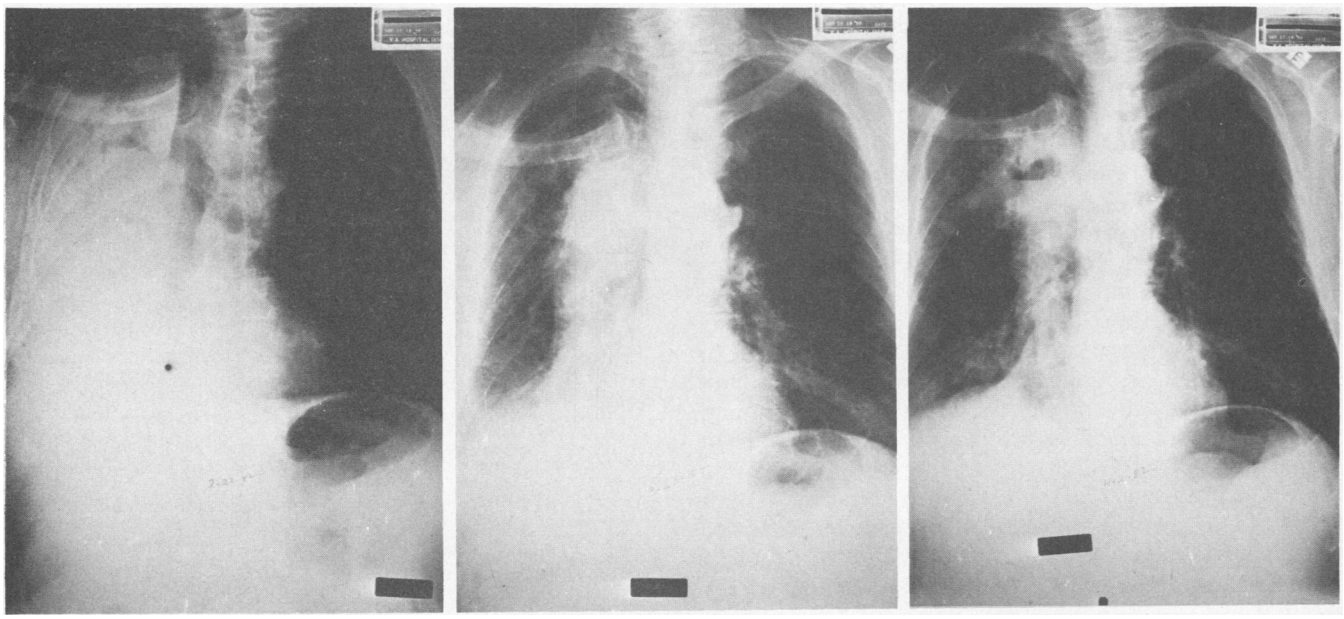

Figure 2.—Left, Chest radiograph of patient 4 at the start of plasma therapy. **Center,** After four weeks of therapy, pleural effusion has decreased, showing collapsed segments of lung. **Right,** After six weeks of therapy, some collapsed portions of lungs have reexpanded and the primary tumor shows an air-fluid level.

four patients had no observable or subjective toxic reactions. The toxic effects described were generally acute, well tolerated and easily managed. This treatment regimen is suitable for phase II trials in refractory malignant conditions. Based on our initial experience, we recommend beginning therapy with plasma treated with Sepharose-protein A at a ratio of 4 mg of protein A per 100 ml of plasma. An initial infusion of 50 ml of treated plasma can be followed at one- to three-day intervals by volumes of 100 ml and 150 ml from the initial phlebotomy. If no toxic reaction is encountered, protein A ratios can be successively doubled for subsequent phlebotomies and similar volume escalations used at each protein A-to-plasma ratio. Because of the wide range of doses associated with toxic reactions in our patients, we think that individual patients may have pronounced differences in their maximal tolerated doses. Because of the possibility of a relationship of response and toxic reaction, low starting doses with rapid escalation may be necessary in phase II or III studies of this treatment modality.

Understanding the mechanism of action of the therapy will enhance our ability to define dosage and to select appropriate patients for therapy based on in vivo or in vitro predictive tests. The observation of antitumor effects after infusion with small volumes of treated plasma[15] or after infusion of larger volumes treated inadequately to produce complete immunoadsorption[11,13,14] suggests that the removal of "blocking factors" is unlikely to explain the effects observed. When Holohan and colleagues[7] measured blocking activity in dogs with breast cancer before and after protein A-treated plasma infusions, they concluded that "complete removal of blockers is neither necessary nor sufficient" for tumoricidal effects. Terman and co-workers[15] found immunoglobulins and complement on the surface of tumor cells in biopsy specimens taken after treated plasma infusions. If this finding can be confirmed and correlated with response, it could provide the basis for an in vivo "predictive" test for efficacy in patients who have lesions in which a biopsy is easily done, giving us useful insight into the mechanism of action of the therapy. Many patients have circulating antitumor antibodies and Clq binds to antibody-protein A complexes.[8] If antitumor antibodies bind to Clq during perfusion of plasma over protein A, these complexes may be competitively removed from protein A by passage of plasma in excess of the protein A-binding capacity and subsequently infused into a patient. Such IgG-Clq complexes could be responsible for initiation of complement activation at the tumor cell surface and thus account for many of the biologic effects observed, including local pain and hyperemia,[15] fever, chills and tumoricidal effects. Other explanations that have been put forth to explain the activity of protein A-treated plasma include an alteration of the balance of opposing immunologic activities[6] or selective adsorption of immune complexes leading to high titers of cytotoxic antibody.[6] Nonspecific leaching of material from the protein A column[16] seems unlikely in view of the wide range of active protein A-containing materials that have been used[6-15] and the chemical stability of the covalent linkages in the preparations we used. The "blocking factor" hypothesis may still be an appropriate explanation for the therapeutic results of plasmapheresis.[11] Of note is that plasmapheresis or plasma exchange does not produce the spectrum of toxic reactions[11] that we and others[15] observed with infusion of small volumes of protein A-treated plasma.

In summary, the distinct biologic effects, including antitumor effects, that we and others[6-15] have observed using protein A-treated plasma suggest that we do indeed have a new therapeutic modality for use in the treatment of cancer. Phase II studies of protein A-treated plasma infusions for a wide range of refractory malignant conditions can be undertaken based on the preliminary evidence of broad efficacy that is developing. Elucidating the mechanism of the antitumor effects produced by this ex vivo perfusion of plasma over protein A should enable us to optimize and standardize plasma treatment and administration. Developing suitable in vitro predictive tests should also be possible and the use of this modality in randomized trials with standard therapies, initially for advanced malignancy and later into treatment programs where there is a curative intent (such as adjuvant chemotherapy for breast cancer), should become possible.

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