Effect of intravenous infusions of 12-O-tetradecanoylphorbol-13-acetate (TPA) in patients with myelocytic leukemia: Preliminary studies on therapeutic efficacy and toxicity

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ABSTRACT Studies by several investigators have shown that 12–0-tetradecanoylphorbol-13-acetate (TPA) is an extraordinarily potent stimulator of differentiation of cultured human promyelocytic leukemia cells in vitro. In the present study, TPA was administered to humans by i.v. infusion without irreversible toxicity, and it was shown to have pharmacological activity for the treatment of myelocytic leukemia in patients refractory to cytosine arabinoside (Ara C), retinoic acid, and other antileukemic drugs. Marked decreases in bone marrow myeloblasts as well as temporary remission of disease symptoms were observed when TPA was administered alone or in combination with vitamin D₃ and Ara C. Additional studies with TPA after the determination of optimum dosing regimens are needed to determine whether long-lasting or permanent remissions of myelocytic leukemia can be achieved. Transient and reversible side effects were observed after a 1-mg i.v. dose of TPA, but these adverse effects became less intense or disappeared when a lower dose of TPA was used. The results of this study indicate a therapeutic effect of TPA in patients with myelocytic leukemia.

Croton tiglium L. is a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. The seed oil (croton oil) obtained from this plant or its major active constituent, 12–0-tetradecanoylphorbol-13-acetate (TPA), is an irritant and inflammatory agent that has been used widely as a tumor promoter on the skin of mice previously initiated with 7,12-dimethylbenz[a]anthracene or other polycyclic aromatic hydrocarbons (1–6). Topical application of TPA alone to mouse skin twice a week for several months either has no tumorigenic effect or results in only an occasional nonmalignant papilloma.

TPA is an extraordinarily potent stimulator of differentiation in HL-60 human promyelocytic leukemia cells in vitro (7–10). Concentrations of 0.1–15 nM TPA have been reported to stimulate differentiation and inhibit DNA synthesis or cell replication in cultured HL-60 cells (7, 9, 10). Additional studies revealed that TPA stimulated differentiation in vitro when added to freshly obtained peripheral leukemia cells from patients with acute myelocytic leukemia (11, 12). Because of the extraordinarily potent effect of TPA in stimulating the differentiation of cultured human leukemia cells in vitro, we calculated that it might be possible to achieve therapeutically effective blood levels without serious toxicity by intravenous infusion of TPA into patients with leukemia. After doing acute toxicity studies in animals, we obtained permission from several hospitals in the People’s Republic of China, including the District People’s Hospital of Shang Qiu, (Shang Qiu, Henan), Central Hospital, (Nan Yang, Henan), People’s First Hospital of Nan Yang, (Nan Yang, Henan), People’s People’s Republic of China; ‡Central Hospital, Xin Shiang, Henan 453000, People’s Republic of China; §People’s First Hospital, Ping Ding San, Henan 467000, Peoples Republic of China; and **Laboratory for Cancer Research, Department of Chemical Biology, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020

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MATERIALS AND METHODS

Source of TPA. TPA was obtained from Xichuan Pharmaceutical Co. (Nan Yang, Henan). The purity of TPA was >99% as measured by HPLC, NMR, IR and mass spectrometry. The preparation of sterile TPA ampules was done at Xichuan Pharmaceutical Co. Briefly, TPA (0.25 or 0.50 mg) was dissolved in 1.3 ml ethanol. Saline (0.7 ml) was added and mixed. The solution was filtered through a bacteriological filter and stored in sealed sterile ampules. The content of each ampule, which contained 0.25 or 0.50 mg of TPA per 2 ml, was added to 200 ml of sterile saline for intravenous infusion. The intravenous infusion solutions were each administered over a 1-hr interval. For patients who received 1 mg TPA, 5 mg of dexamethasone was either injected i.v. 10 min before TPA or included in the infusion solution. Patients who received lower doses of TPA were not treated with dexamethasone.

Peripheral Blood and Bone Marrow. Hemoglobin, white blood cells, red blood cells, neutrophils, lymphocytes, and platelets in peripheral blood were determined by routine clinical methods. The proportion of myeloblasts in the peripheral blood and in the bone marrow were also determined by routine clinical methods.

Clinical Tests Used for Assessing Potential TPA Toxicity. Electrocardiography, pulmonary function (tidal volume, vital capacity, and maximal voluntary ventilation), measurement of hepatic enzymes (alanine aminotransferase, gamma glutamyl transferase, and aspartate aminotransferase), measurement of
and peripheral blood analyses were done 1–2 days after the last Ara C or TPA administration (or as indicated in the text). A dash indicates that

**Patients with Leukemia.** Five patients (numbers 1, 6, 8, 11, and 12) had acute myelocytic leukemia as described in ref. 13, six patients (numbers 2–5, 9, and 10) had chronic myelocytic leukemia with blast crisis, and one patient (number 7) had a myelodysplastic syndrome with refractory anemia and excess blasts. All patients except no. 11 had been treated unsuccessfully with other chemotherapeutic drugs before TPA administration. Patients 1–7 received weekly combined treatment with TPA, Ara C, and vitamin D3 (or 1α, 25-dihydroxyvitamin D3). Patients 8–12 received TPA only. A detailed description of their treatments is provided in the text.

**RESULTS**

**Therapeutic Effect of TPA in Patients with Myelocytic Leukemia.** TPA was administered to 11 patients with acute myelocytic leukemia, chronic myelocytic leukemia with blast crisis, or a myelodysplastic syndrome who had failed to have a beneficial response from treatment with other chemotherapeutic drugs. TPA was administered as initial therapy to one patient with acute myelocytic leukemia. The chemotherapeutic drugs used before TPA administration included Ara C, daunomycin, homoharringtonin, α-interferon, busulfan, hydroxyurea, etoposide (VP16), and all-trans retinoic acid. After the lack of a beneficial response to therapy with one or more of the above drugs, six of the patients were treated with a combination of TPA, Ara C, and vitamin D3 (TPA/Ara C/VD3). One patient was treated with TPA/Ara C/1α,25-dihydroxyvitamin D3. The five other patients were treated with TPA alone. In only one patient (number 3) was bone marrow hypoplasia observed after treatment.

Many of the 12 patients treated with combination therapy or TPA alone demonstrated a reduction in the number of leukemic cells in blood or bone marrow. In some patients this reduction in leukemic cells corresponded to a remission that was associated with an improvement in performance status. Some of the patients who were seriously ill and bedridden before treatment were able to leave the hospital and lead a normal lifestyle after treatment with TPA alone or in combination with Ara C and VD3.

**Detailed Description of Effects of TPA Treatment in 12 Leukemia Patients.** A detailed description of each patient is given below and the results are summarized in Table 1.

Patient no. 1 was a 32-year-old male with a refractory acute myelogenous leukemia (M5) who had previously been treated with two courses of combination chemotherapy with daunomycin and Ara C and one course of homoharringtonin and Ara C. These treatments resulted in bone marrow hypoplasia, but leukemic cells persisted. He subsequently was treated with combination TPA/Ara C/VD3 administered over a 2-week period. TPA (1 mg) was administered i.v. on days 4 and 11, Ara C (40 mg) was administered i.v. on days 3–14, and vitamin D3 (6 × 103 units) was administered by i.m. injection on days 1, 2, 8, and 9. In conjunction with the TPA/Ara C/VD3 treatment, the patient had a marked reduction in the number of myeloblasts in blood and bone marrow (Table 1) and an improvement in the white blood cell count (from 1.0 × 109/liter to 2.8 × 109/liter) and platelet count (from 135 ×

Table 1. Summary of clinical efficacy of TPA in patients with myelocytic leukemia who were refractory to chemotherapy

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose of TPA</th>
<th>Bone marrow myeloblasts (% of total cells)</th>
<th>Peripheral myeloblasts (% of white cells)</th>
<th>Patient condition after TPA</th>
<th>Duration of remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mg per week for 2 weeks</td>
<td>Before TPA 30</td>
<td>After TPA 2.5</td>
<td>Before TPA 5</td>
<td>After TPA 0</td>
</tr>
<tr>
<td>2</td>
<td>1 mg per week for 5 weeks</td>
<td>36</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1 mg per week for 7 weeks</td>
<td>90</td>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1 mg per week for 7 weeks</td>
<td>67.5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1 mg per week for 2 weeks</td>
<td>27.5</td>
<td>0.5</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1 mg per week for 3 weeks</td>
<td>48</td>
<td>3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.5 mg twice a week for 11 weeks</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0.5 mg twice a week for 3 weeks</td>
<td>81</td>
<td>17</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>0.25 mg given once</td>
<td>—</td>
<td>—</td>
<td>4*</td>
<td>0†</td>
</tr>
<tr>
<td>10</td>
<td>0.5 mg once and 0.25 mg 2 days later</td>
<td>TPA treatment increased megakaryocytes in bone marrow more than 10-fold; no effect on % blasts</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>0.25 mg every other day for five doses</td>
<td>77</td>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>1 mg once</td>
<td>80</td>
<td>60</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Patients 1–6 received TPA, Ara C, and vitamin D3. Patient 7 received TPA, Ara C, and 1α,25-dihydroxyvitamin D3. Patients 8–12 received only TPA. Five patients (1, 6, 8, 11, and 12) had acute myelocytic leukemia, six patients (2–5, 9, and 10) had chronic myelocytic leukemia with blast crisis, and one patient (no. 7) had myelodysplastic syndrome with refractory anemia. A description of each patient is given in the text. Bone marrow and peripheral blood analyses were done 1–2 days after the last Ara C or TPA administration (or as indicated in the text). A dash indicates that no data were available.

*Data were obtained 2 days after TPA administration.
†Data were obtained 5 days after TPA administration.
10^9/liter to 283 \times 10^9/liter). There was no bone marrow hypoplasia. The patient’s performance status improved dramatically. The patient, who was very sick and bedridden before therapy, had a remission and was able to get out of bed and to leave the hospital after therapy. The patient was able to return to an almost normal lifestyle. The remission lasted 5 months.

Patient no. 2 was a 30-year-old male with chronic myelocytic leukemia who developed a blast crisis while being treated with busulfan and hydroxyurea. Eight days after stopping drug treatment he was treated with TPA/Ara C/VD3. TPA (1 mg) was administered i.v. on days 4, 11, and 18. Ara C (40 mg) was administered i.v. on days 3–14, and vitamin D3 (6 \times 10^9 units) was administered i.m. on days 1, 2, 8, and 9. In conjunction with treatment, his spleen size diminished (from 6 cm below the costal margin to 0.5 cm below the costal margin), the number of myeloblasts in blood and bone marrow decreased (Table 1), and his platelet count improved (63 \times 10^9/liter to 94 \times 10^9/liter). His white blood cell count (9.8 \times 10^9/liter pretreatment, 4.9 \times 10^9/liter posttreatment) and hemoglobin (94 g/liter pretreatment, 104 g/liter posttreatment) remained stable. No bone marrow hypoplasia was observed. This patient also had a marked improvement in performance status. The remission lasted 3 months.

Patient no. 3 was a 42-year-old male with chronic myelocytic leukemia who developed a blast crisis while being treated with busulfan and hydroxyurea. Two days after stopping drug therapy he was treated with TPA/Ara C/VD3 according to the same treatment plan used to treat patient no. 2. In conjunction with the treatment there was a marked reduction in the number of myeloblasts in blood and bone marrow (Table 1) with a reduction in the total number of white blood cells (from 27.5 \times 10^9/liter to 2.2 \times 10^9/liter) and an improvement in the platelet count (21 \times 10^9/liter to 70 \times 10^9/liter) and hemoglobin (70 g/liter to 96 g/liter). His performance status improved markedly. This patient was very sick and bedridden before treatment with TPA/Ara C/VD3. After the 3-week treatment, the patient had a remission, went home, and was able to work and lead a normal lifestyle. The benefits of treatment were short-lived, however, because features of the blast crisis reappeared within 26 days.

Patient no. 4 was a 25-year-old male with chronic myelocytic leukemia that progressed to a blast crisis during treatment with hydroxyurea and busulfan. Four days after stopping treatment he was treated with TPA/Ara C/VD3 according to the same treatment plan utilized for patients no. 2 and 3. In conjunction with treatment, the number of myeloblasts in blood and bone marrow (Table 1) and 210 \times 10^9/liter posttreatment, but the hemoglobin fell (87 g/liter to 45 g/liter) during treatment. Eighteen days after the completion of therapy there was further reduction in the white blood cell count (to 8.4 \times 10^9/liter) and an improvement in the hemoglobin (to 100 g/liter). No bone marrow hypoplasia was noted. The patient improved from a blast crisis to the chronic phase of the disease, and the patient also had an improvement in performance status with a remission that lasted 3 months.

Patient No. 5 was a 38-year-old male with chronic myelocytic leukemia that progressed to a blast crisis while being treated with busulfan, a-interferon, and hydroxyurea. Two days after stopping treatment with these drugs, he was treated with TPA/Ara C/VD3 according to the treatment plan described for patient no. 1. Immediately after the completion of treatment, the number of myeloblasts in blood and bone marrow was improved (Table 1) and his white blood cell count was increased (from 36.6 \times 10^9/liter to 273 \times 10^9/liter) possibly as a consequence of the establishment of the chronic phase of chronic myelocytic leukemia. The hemoglobin level (84 g/liter) did not change in conjunction with treatment, and the platelet count remained in the normal range (290 \times 10^9/liter pretreatment and 170 \times 10^9/liter posttreatment). The patient had an improvement in performance status with a remission that lasted at least 1 month. Although the patient was still in remission, he then received 10 days of treatment with a-interferon and occasional treatment with hydroxyurea. The remission continued for an additional 8 months.

Patient no. 6 was a 57-year-old male with acute myelogenous leukemia (M3, acute promyelocytic leukemia) whose disease had failed to achieve a remission despite combination therapy with daunomycin and Ara C, homoharringtonin and Ara C, and all-trans retinoic acid. He subsequently was treated with TPA/Ara C/VD3 administered over a 3-week period. TPA (1 mg i.v.) was given once a week for 3 weeks, Ara C (40 mg i.v.) was given three times a week for 3 weeks, and vitamin D3 (6 \times 10^9 units i.m.) was given twice a week for 2 weeks. In conjunction with therapy there was a marked decrease in the number of myeloblasts in the blood and bone marrow (Table 1) and improvement in the white blood cell count (0.4 \times 10^9/liter pretreatment to 4.1 \times 10^9/liter posttreatment), hemoglobin (60 g/liter pretreatment to 118 g/liter posttreatment), and platelet count (40 \times 10^9/liter pretreatment to 80 \times 10^9/liter posttreatment). The patient’s performance status improved markedly, and the remission lasted 8 months.

Patient no. 7 was a 67-year-old male with a myelodysplastic syndrome (refractory anemia with excess blasts) who previously had been unsuccessfully treated with orally administered etoposide (VP16). He subsequently was treated with TPA, Ara C, and 1α,25-dihydroxyvitamin D3. TPA (0.5 mg i.v.) was administered twice a week for 11 weeks, Ara C (40 mg i.v.) was administered seven times a week for 3 weeks, and 1α,25-dihydroxyvitamin D3 was administered orally once a daily schedule for 1 week at a dose of 0.5 µg and for an additional week at a dose of 0.25 µg. There was a reduction in his spleen size (3 cm below the costal margin before treatment and 0.5 cm below the costal margin after treatment), and there were decreases in the number of myeloblasts in blood and bone marrow after treatment (Table 1). The patient’s anemia (hemoglobin levels of 36 g/liter pretreatment and 42 g/liter posttreatment) and thrombocytopenia (29 \times 10^9 platelets/liter pretreatment and 34 \times 10^9 platelets/liter posttreatment) did not improve.

Patient no. 8 was a 36-year-old male with acute myelogenous leukemia (M3, acute promyelocytic leukemia) whose disease did not respond to all-trans retinoic acid (80 mg/day administered orally for 50 days). Seven days after discontinuing the all-trans retinoic acid he was treated with TPA (0.5 mg i.v. twice a week for 3 weeks). In conjunction with treatment there was an improvement in the number of myeloblasts in blood and bone marrow (Table 1), white blood cell count (1.0 \times 10^9/liter pretreatment, 2.2 \times 10^9/liter posttreatment), hemoglobin concentration (45 g/liter pretreatment, 66 g/liter posttreatment), and platelet count (35 \times 10^9/liter pretreatment and 223 \times 10^9/liter posttreatment). The patient had a marked improvement in performance status. The patient was very sick and bedridden before treatment with TPA, and he was able to lead an almost normal lifestyle after treatment with TPA. These improvements lasted 2 months.

Patient no. 9 was a 26-year-old female with chronic myelocytic leukemia who was refractory to treatment with homoharringtonin and Ara C. She was subsequently treated with a single dose of TPA (0.25 mg i.v.). Although there was a decrease in myeloblasts in the blood, there is not enough available clinical or laboratory information to further assess the response to treatment.

Patient no. 10 was a 40-year-old female with chronic myelocytic leukemia who had developed a blast crisis that was unresponsive to treatment with daunomycin, Ara C, homoharringtonin, vincristine, and endoxan. She was subsequently treated with TPA (0.5 mg i.v. followed 2 days later by 0.25 mg i.v.). There was no change in the percentage of myeloblasts in
the bone marrow, but megalakaryocytes in the bone marrow were increased (Table 1). There is not enough clinical or laboratory information to assess other criteria of response.

Patient no. 11 was a 28-year-old male with acute myelocytic leukemia (M4) and bleeding who received TPA (0.25 mg i.v. on alternate days for a total of 5 doses) as initial therapy for his disease. The patient had extreme anxiety before and during treatment. There was a reduction in the number of myeloblasts in bone marrow in conjunction with therapy (Table 1); however, no additional clinical or laboratory information is available.

Patient no. 12 was a 34-year-old female with refractory acute myelocytic leukemia (M5b) who had received previous treatment with two courses of daunomycin + Ara C and a single course of daunomycin + Ara C + etoposide (VP 16). She subsequently was treated with a single dose of TPA (1 mg i.v.). One week later there was a small decrease in the number of myeloblasts in the bone marrow, and there was also an increase in cell size suggesting a possible effect of the TPA infusion on differentiation. There is insufficient additional clinical and laboratory information to further assess the patient’s response to treatment.

Adverse Effects of TPA in Patients. Patients 1–6 and 12 were treated with 1 mg of TPA by i.v. infusion. One hour after the infusion, all of the patients except patient no. 4 experienced chills (lasting 30 min) followed by fever (37.5°–39.5°) and diaphoresis. The fever and diaphoresis lasted for 3–5 h. Several patients experienced 10–20 min of shortness of breath (dyspnea) 40–90 min after the TPA. A local irritation of the vein used for the i.v. infusion was also noted in most patients. Hemoglobinuria, proteinuria, and neutropenia were detected on the day of TPA administration and persisted for an additional day. Bleeding from the nose, gums, and stomach was observed in one patient treated with 1 mg of TPA, but it is not known whether TPA caused these effects. There was no apparent effect of this dose of TPA on the cardiac, pulmonary, hepatic, or renal tests that were undertaken.

Patients 7, 8, and 10 were given i.v. infusions of 0.5 mg TPA. These patients also experienced adverse effects (fever, chills, local irritation at the infusion site, mild dyspnea, hemoglobinuria, and proteinuria) but to a lesser extent than those experienced by the patients who received the 1-mg dose.

Patients 9, 10, and 11 received a lower dose of TPA (0.25 mg i.v.) and they had only chills and fever. They had no dyspnea, and no hemoglobin or protein was found in the urine.

In summary, adverse effects of TPA observed in patients after i.v. infusion of 1 mg included a short period of dyspnea, a short period of fever and chills, bleeding (one patient), hemoglobinuria, proteinuria, and venous irritation at the infusion site. All of these adverse effects were reversible. The extent of adverse effects was correlated with the dose, being much milder in conjunction with the 0.5-mg dose and even milder with the 0.25-mg dose. No effects of TPA on cardiac, hepatic, renal, or pulmonary measurements were observed.

**DISCUSSION**

Earlier studies have shown that TPA is a potent stimulator of differentiation of human promyelocytic leukemia HL-60 cells in vitro via a macrophage-like pathway and that DNA synthesis and cell proliferation are also inhibited (7–10). Concentrations of TPA as low as 0.1–15 nM TPA had a strong stimulatory effect on differentiation of cultured HL-60 cells. Additional studies showed a stimulatory effect of TPA in vitro on the differentiation of freshly collected peripheral white blood cells from leukemia patients (11, 12).

This is the first report describing the administration of TPA to humans. In this report, we evaluated the toxicity and therapeutic effect of intravenous infusions of TPA in patients with myeloid malignancies. Most of the treated patients had disease that was refractory to prior therapy with a variety of chemotherapeutic agents. In many of the patients, treatment with TPA/Ara C/VD₃ or TPA alone resulted in an improvement of hematologic parameters with criteria for complete or partial remission being documented in several patients. Clinical and hematologic improvements were even seen in patients with disease states (e.g., chronic myelogenous leukemia in blast crisis and refractory acute myelogenous leukemia) that are notoriously refractory to treatment. These responses were seen in the absence of severe or irreversible adverse effects.

It is likely that TPA contributed to the beneficial effects seen in many of the patients treated with TPA/Ara C/VD₃. Some of these patients had been treated previously with higher doses of Ara C and others had disease states (chronic myelogenous leukemia in blast crisis) generally considered to be refractory to low, intermediate, or high doses of Ara C administered as a single agent. Nevertheless, it is possible that the triple drug combination has a unique impact on the malignant cells that depended on an interaction of the effects of the agents.

The significance of the remissions induced by TPA/Ara C/VD₃ or TPA alone is highlighted by the fact that many patients had an improvement in their performance status and several of the patients being treated had diseases that are particularly refractory to treatment. For example, chronic myelogenous leukemia in blast crisis is generally a very refractory disease, and even intensely myelosuppressive therapy is often unsuccessful in reestablishing the chronic phase of the disease. A therapy capable of reestablishing the chronic phase of the disease in the absence of bone marrow hypoplasia would be a major advance in the treatment of these patients. Similarly, acute myelogenous leukemia that is refractory to standard cytotoxic therapy or relapses after therapy is almost uniformly fatal unless treated with high-dose chemotherapy alone or together with radiation administered in conjunction with a bone marrow transplant. The results reported in this study provide the background clinical information that should motivate a more detailed clinical research effort to define the maximally tolerated dosing regimen of TPA, the pharmacodynamics of TPA, and the potential efficacy of TPA as a component of combination therapy for the treatment of myeloid malignancies. These studies should be undertaken in appreciation of the potential therapeutic impact of a nonmyelosuppressive therapy for these clinically aggressive diseases.

In the present study, intravenous infusions of TPA have been administered to patients without irreversible toxicity. It is possible that TPA-induced fevers, chills, and dyspnea are due to TPA-induced increases in tissue cytokines. Bleeding was observed in one patient, and local irritation at the infusion site was also noted. The dyspnea was most apparent at the highest infusion dose of TPA (1 mg; about 20 μg/kg) and may be due to a pulmonary inflammatory response, as was seen in earlier studies with rabbits and sheep. In the animal studies, an intravenous infusion of TPA (5 μg/kg) in sheep resulted in altered lung mechanics, increased neutrophils and thromboxane B₂ in the lungs, and decreased leukocytes in blood (14–16). Similarly, an intravenous infusion of TPA (40 μg/kg) in rabbits resulted in respiratory distress, a rapid decrease in the number of neutrophils in blood (which lasted for at least 90 min), intravascular pulmonary accumulation of neutrophils and platelets as well as foci of alveolar hemorrhage (17). The data suggested TPA-induced enhanced permeability and/or occlusion of the pulmonary microvasculature. The inflammatory reaction observed in sheep lungs may be caused partially by the TPA-induced formation of arachidonic acid metabolites (16, 18, 19), because a nonsteroidal anti-inflammatory agent partially inhibited the appearance of the TPA-induced changes (19). The physiologic basis, clinical significance, and impact of pharmacologic therapy on the dyspnea seen after TPA infusion in humans will need to be a focus of future clinical studies.
During the course of the present investigation, we observed increased white blood cell counts in several subjects with low white cell counts who had been treated with TPA (patients 1, 6, 7, and 8). Because of this observation, we initiated a study on the effect of TPA administration on low white cell and neutrophil counts observed in patients with solid tumors who had been treated previously with cytotoxic chemotherapeutic drugs. The results demonstrated a TPA-induced rapid increase in white cells and neutrophils in the peripheral blood of these patients (see accompanying paper; ref. 20).

Unpublished observations in our laboratory indicate that i.p. injections of TPA (100 μg/kg) once a day for 7 days to mice inhibited the growth of transplanted sarcoma 180 by 37–48% in two experiments. The results of our studies indicating that TPA can be administered to patients without overt serious toxicity suggests the possible use of TPA for the treatment of solid tumors. TPA (1–15 nM) has been shown to enhance differentiation, increase apoptosis, and, or to inhibit DNA synthesis and cell proliferation in studies with cultured human breast cancer MCF-7 cells (21), human colon cancer VACO 10MS cells (22), a human non-small-cell lung cancer cell line (23, 24), the human LNCaP prostate cancer cell line (25–28), and some human melanoma cell lines (29) in vitro. The possibility of effective treatment of patients with these solid tumors by the use of TPA alone or in combination with other chemotherapeutic drugs should be considered.

In summary, this report describes the administration of TPA to humans. We present preliminary observations on the toxicity and potential therapeutic efficacy of i.v. infusions of TPA in patients with myeloid malignancies. The preliminary results described in this study demonstrate that TPA is likely to have pharmacologic activity at doses that generally are well tolerated without irreversible adverse effects. Although many of the therapeutic responses that were seen were of short duration, they are of particular significance in the context of the refractoriness of the disease states, the association with clinical benefit to the patient, their occurrence in the absence of bone marrow hypoplasia (which suggests that the responses are occurring as a consequence of differentiation), and the brief duration of treatment. The preliminary information obtained from the treatment of these patients with TPA alone or in combination with Ara C and vitamin D₃ will provide the basis for future, detailed clinical studies designed to define the pharmacokinetics, pharmacodynamics, maximum tolerated dose, optimal dose, and potential therapeutic efficacy of TPA when administered alone or as a component of combination therapy. More detailed molecular and genetic analyses during these clinical studies may allow a better definition of the mechanisms contributing to disease response. The broad range of biological effects of TPA at the cellular level (30–32) and the potential broad range of pharmacological effects of TPA in vivo also may provide other active areas of investigation during these clinical studies.

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