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12-O-Tetradecanoylphorbol-13-acetate (TPA)-induced increase in depressed white blood cell counts in patients treated with cytotoxic cancer chemotherapeutic drugs

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ABSTRACT **Fifty-two patients with solid tumors had depressed white blood cell and neutrophil counts because of prior treatment with cytotoxic cancer chemotherapeutic drugs. These patients were given one or more i.v. infusions of 0.125–0.25 mg of 12-O-tetradecanoylphorbol-13-acetate (TPA), and this treatment increased the low white blood cell and neutrophil counts toward the normal range. The average white blood cell and neutrophil counts were 2.55×10^9/liter and 1.76×10^9/liter, respectively, before treatment with TPA. After one or more i.v. infusions of TPA, the white blood cell and neutrophil counts increased to peak values of 5.92×10^9/liter and 4.76×10^9/liter, respectively, within a few days. Most patients had increased levels of white blood cells and neutrophils by 24 hr after a single i.v. infusion of 0.25 mg TPA. Elevated levels were observed for at least 3 days. This study demonstrates that treatment with parenteral TPA is feasible with useful biological activity. Only mild and reversible side effects were observed.**

12-O-Tetradecanoylphorbol-13-acetate (TPA) has a broad range of cellular and potentially useful pharmacological effects (1–3), but it was only recently studied in humans. In a pilot study, we found a therapeutic effect of intravenous infusions of TPA in patients with myelocytic leukemia who were refractory to other chemotherapeutic drugs (4). Myeloblasts in the bone marrow and peripheral blood were decreased, and several remissions were obtained (4). During the course of this earlier study in leukemia patients, we observed that TPA administration increased white blood cell (WBC) counts and neutrophils in several of the patients who previously had low WBC counts. Because granulocyte colony-stimulating factor (G-CSF) and granulocyte–monocyte colony-stimulating factor (GM-CSF) were not available to many patients, we obtained permission from the Central Hospital (Nan Yang, Henan) and the People's First Hospital of Nan Yang (Nan Yang, Henan) in the People's Republic of China to study the effects of i.v. infusions of TPA in patients who had low WBC counts because of prior treatment of solid tumors with one or more cytotoxic cancer chemotherapeutic drugs. In the present study, we show that i.v. infusion of TPA causes a rapid increase in WBC counts and neutrophils in the peripheral blood.

METHODS

TPA. Sterile ampules of TPA [0.25 or 0.5 mg in 2 ml of ethanol/saline (65:35)] were prepared as described in our accompanying paper in this issue of the *Proceedings* (4). The ampules of TPA were supplied by Xichuan Pharmaceutical Co. (Nan Yang, Henan). Appropriate amounts of TPA were added to 200 ml of sterile saline for intravenous infusion. The intravenous infusion solutions were administered very slowly over a 1-hr interval.

Peripheral Blood. Hemoglobin (Hb), WBC, red blood cells, neutrophils, lymphocytes, and platelets in peripheral blood were determined by routine clinical methods.

Clinical Tests Used for Assessing Potential TPA Toxicity. Electrocardiogram evaluations were routinely undertaken, and pulmonary function was assessed by measurement of respiratory rate, tidal volume, vital capacity, and maximal voluntary ventilation. Liver enzyme testing included the measurement of alanine aminotransferase, gamma glutamyl transferase, and aspartate aminotransferase. Kidney function was evaluated by the measurement of blood urea nitrogen and/or blood creatine and creatinine concentrations. Routine clinical tests were used for measuring hemoglobin and protein in the urine.

Patients with Low WBC and Neutrophil Counts. Fifty-two patients with esophageal cancer, stomach cancer, breast cancer, lung cancer, bile duct cancer, neck cancer, colon cancer, thyroid cancer, or brain cancer had been treated previously with myelosuppressive cytotoxic chemotherapeutic drugs. The patients had low WBC counts [2.55 ± 0.13 (SE) $\times 10^9$ cells/liter] and low neutrophil counts [1.76 ± 0.09 (SE) $\times 10^9$ cells/liter], and they had a high risk of developing serious infection if treatment with cytotoxic chemotherapeutic drugs was continued. Because G-CSF and GM-CSF were not available, we evaluated the effect of i.v. infusions of TPA on WBC and neutrophil counts in these patients. Statistical analyses were done with the Student's *t* test.

RESULTS

Effect of TPA on WBC Counts in Patients with Solid Tumors Treated with Cancer Chemotherapeutic Agents. We investigated the effects of TPA on WBC counts in patients with solid tumors and low WBC counts who had an increased risk of infection because of prior treatment with myelosuppressive cancer chemotherapeutic drugs. Each of these patients had stopped taking chemotherapeutic drugs just before TPA administration. Without further treatment, WBC counts would not have increased during the subsequent 2 weeks of the study

Abbreviations: WBC, white blood cells; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–monocyte CSF; TPA, 12-O-tetradecanoylphorbol-13-acetate or phorbol 12-myristate 13-acetate (PMA).

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(historical controls; see below). Administration of one or more doses of TPA to 52 patients who had been treated previously with cytotoxic cancer chemotherapeutic drugs and who had low WBC counts resulted in a marked increase in WBC counts (Table 1). The average WBC count in 52 patients before TPA administration was $2.55 \pm 0.13 \times 10^9$ cells/liter, and this was increased 132% to a peak of $5.92 \pm 0.28 \times 10^9$ cells/liter after TPA administration ($P < 0.01$; Table 1). Both neutrophils and lymphocytes were measured in 50 of the 52 patients, and both were increased by treatment of the patients with TPA. The average neutrophil count before TPA administration was $1.76 \pm 0.09 \times 10^9$ cells/liter, and this was increased 170% to

Table 1. TPA-induced increases in white blood cell counts in patients with solid tumors undergoing chemotherapy

Patient no.	Dose of TPA	WBC, 10^9/liter		Neutrophils, 10^9/liter	
		Before TPA	After TPA	Before TPA	After TPA
1	0.25 mg once (WBC measured 1 day later)	3.0	4.6	1.41	2.81
2	0.25 mg once (WBC measured 1 day later)	1.9	5.7	0.99	4.10
3	0.25 mg once (WBC measured 1 day later)	2.9	10.1	2.41	9.60
4	0.25 mg (twice on days 1 and 3) (WBC measured 1 day after second TPA dose)	3.4	8.0	2.86	6.08
5	0.375 once (WBC measured 3 days after TPA)	2.3	5.4	1.29	4.48
6	0.125 mg twice (separated by 3 days) (WBC measured 2 days after first dose)	3.3	6.0	2.38	4.86
7	0.125 mg twice (1 day apart) (WBC measured 1 day after second dose)	3.6	4.8	2.20	3.79
8	0.125 mg once (WBC measured 1 day later)	3.1	10.5	1.40	9.45
9	0.125 mg twice (1 day apart) (WBC measured 1 day after second dose)	3.3	5.7	2.61	4.50
10	0.125 mg three times (on days 1, 2, and 16) (WBC measured 1 day after second dose)	3.0	8.2	1.98	6.40
11	0.125 mg four times (on days 1, 2, 3, and 4) (WBC measrued 1 day after third dose)	3.4	5.6	2.62	4.20
12	0.125 mg twice (1 day apart) (WBC measured 1 day after first dose)	2.7	10.7	1.59	8.67
13	0.125 mg twice (1 day apart) (WBC measured 1 day after second dose)	3.7	6.0	2.52	4.98
14	0.25 mg twice (1 day apart) (WBC measured 1 day after second dose)	2.5	4.2	2.00	3.44
15	0.25 mg twice (on days 1 and 9) (WBC measured 1 day after second dose)	3.3	7.6	2.11	5.47
16	0.25 mg once (WBC measured 1 day after TPA)	3.4	3.4	2.62	2.62
17	0.2 mg three times (on days 1, 3, and 9) (WBC measured 1 day after third dose)	2.8	4.4	2.10	3.52
18	0.25 mg on day 1 and 0.375 mg on day 8 (WBC measured 4 days after second dose)	2.6	5.3	1.69	4.29
19	0.25 mg once (WBC measured 2 days after TPA)	2.1	6.4	1.51	5.76
20	0.25 mg once (WBC measured 1 day after TPA)	2.3	5.1	1.70	4.18
21	0.25 mg twice on days 1 and 3 (WBC measured 1 day after first dose of TPA)	3.0	6.4	2.31	5.70
22	0.25 mg once (WBC measured 3 days after TPA)	3.2	4.7	2.02	3.90
23	0.25 mg once (WBC measured 1 day after TPA)	2.4	4.0	1.61	3.32
24	0.25 mg once (WBC measured 1 day after TPA)	3.0	5.8	2.01	4.52
25	0.25 mg 3 times on days 1, 11, and 20 (WBC measured on day 21)	2.1	5.5	1.81	5.01
26	0.25 mg once (WBC measured 8 days after TPA)	1.7	5.3	1.50	3.18
27	0.25 mg once (WBC measured 1 day after TPA)	3.0	10.7	2.31	9.52
28	0.25 mg once (WBC measured 5 days after TPA)	2.8	6.0	2.02	5.58
29	0.125 mg once (WBC measured 4 days after TPA)	2.8	5.3	2.44	4.61
30	0.25 mg twice (on days 1 and 7) (WBC measured 2 days after second dose)	2.3	4.2	1.79	3.57
31	0.25 mg twice (on days 1 and 2) (WBC measured 10 days after TPA)	2.3	3.2	1.40	2.59
32	0.25 mg on day 1 and 0.125 mg on days 3, 5, and 7 (WBC measured on day 15)	2.2	7.0	1.23	5.60
33	0.125 mg on days 1–4 (WBC measured on day 9)	2.7	6.9	1.24	5.66
34	0.25 mg twice (on days 1 and 3) (WBC measured on day 10)	1.5	9.2	0.51	8.74
35	0.25 mg twice (on days 1 and 2) (WBC measured on day 11)	2.3	7.2	—	—
36	0.125 mg twice (on days 1 and 2) (WBC measured on day 10)	3.2	8.1	1.92	6.89
37	0.25 mg four times during a 1-week period (WBC measured 1 day after last TPA)	0.7	6.8	0.60	5.64
38	0.25 mg once (WBC measured 1 day later)	3.0	4.5	2.43	3.42
39	0.25 mg twice (on days 1 and 2) (WBC measured 1 day later)	0.9	2.5	0.62	2.00
40	0.25 mg once (WBC measured 1 day later)	3.8	8.0	—	—
41	0.25 mg once (WBC measured 1 day later)	2.4	7.1	1.99	6.67
42	0.25 mg once (WBC measured 1 day later)	2.4	5.2	1.61	4.52
43	0.25 mg twice (separated by 4 days) WBC measured 4 days after second dose	2.0	4.4	1.70	3.78
44	0.25 mg twice (1 day apart) WBC measured 1 day after second dose	2.4	4.0	1.51	2.40
45	0.25 mg twice (1 day apart) WBC measured 1 day after second dose	2.9	5.1	2.20	4.18
46	0.25 mg on days 1 and 3 and 0.50 mg on days 5, 7, and 9 (WBC measured on day 11)	0.7	2.7	0.20	2.30
47	0.25 mg on days 1 and 2 and 0.50 mg on days 3, 5, 6, and 8 (WBC measured on day 9)	1.1	1.5	0.80	1.20
48	0.25 mg once ((WBC measured 7 days after TPA)	1.9	7.6	1.69	6.23
49	0.25 mg once (WBC measured 4 days after TPA)	2.3	3.9	1.20	1.72
50	0.25 mg once (WBC measured 1 day after TPA)	1.1	5.3	0.80	3.18
51	0.25 mg once (WBC measured 1 day after TPA)	2.1	6.4	1.51	5.76
52	0.25 mg once (WBC measured 2 days after TPA)	3.6	5.6	2.88	4.54
	Mean ± SE	2.55 ± 0.13	5.92 ± 0.28*	1.76 ± 0.09	4.76 ± 0.28*

Patients with solid tumors previously treated with myelosuppresive cytotoxic anticancer drugs were given i.v. infusions of TPA as indicated. Peripheral WBC and neutrophil counts were determined at the indicated times after TPA. When multiple blood samples were obtained after TPA, we have presented data for peak WBC counts.

*Statistically different from before TPA ($P < 0.01$).

a peak of 4.76 ± 0.28 × 10^9 cells/liter after TPA administration ($P < 0.01$; Table 1). The average percentage of neutrophils and lymphocytes before TPA administration in 50 patients where both were measured was 69% and 31%, respectively. After TPA administration, the average percentage of neutrophils and lymphocytes was 80 and 20%, respectively. The TPA-induced increase in the proportion of neutrophils to lymphocytes was statistically significant ($P < 0.01$). Administration of TPA did not influence the number of platelets or the concentration of hemoglobin in the peripheral blood at the time of TPA-induced peak changes in WBC and neutrophil counts (data not presented). TPA administration increased WBC counts substantially (>40%) in all but one patient, and TPA administration increased WBC to the normal range (WBC > 4.5 × 10^9/liter) in 77% of the patients studied (Table 1). Neutrophil counts were increased to the normal range (neutrophils > 3.0 × 10^9/liter) in 84% of the 50 patients studied where both neutrophils and lymphocytes were measured (Table 1).

In an additional study in which historical controls were obtained by using hospital records, 21 patients with solid tumors who had depressed WBC and neutrophil counts because of treatment with cytotoxic chemotherapeutic drugs were treated for 10–14 days with pentoxyl (a frequently used treatment for depressed WBC counts in China). This treatment had no effect on WBC or neutrophil counts (data not presented). These studies in a control population of patients with similar tumors and chemotherapy and from the same hospitals as in the present study indicate that without TPA administration the WBC and neutrophil levels remain depressed for at least 10–14 days.

We examined the time course for the increase in WBC and neutrophils in patients who received a single i.v. infusion of 0.25 mg TPA, and these data are described in Fig. 1. Peak levels of WBC and neutrophils were achieved by 1–2 days after TPA administration (Fig. 1). The mean WBC count in 24 patients before TPA treatment was 2.55 ± 0.11 (SE) × 10^9/liter, of which neutrophils were 1.80 ± 0.11 (SE) × 10^9/liter. At 24 hr after administration of TPA to these patients, the mean WBC and neutrophil counts were increased to 4.50 ± 0.43 ($P < 0.01$) and 3.86 ± 0.41 ($P < 0.01$), respectively. Seventy-one percent of the 24 subjects had a TPA-induced increase in WBC counts at 24 hr after TPA administration. Some of the patients who did not respond to TPA within 24 hr did respond on subsequent days. The results (Fig. 1) indicate that WBC and neutrophil counts were significantly increased at 24 hr after an i.v. infusion of 0.25 mg TPA, and these values remained elevated for at least 3 days.

Four patients received only a single i.v. infusion of 0.125 mg TPA. The WBC and neutrophil counts before TPA administration were 3.14 ± 0.10 × 10^9/liter and 2.21 ± 0.24 × 10^9/liter, respectively, and these values were increased to 6.10 ± 1.15 × 10^9/liter ($P < 0.05$) and 5.03 ± 1.05 × 10^9/liter ($P < 0.05$) at 1–2 days after the 0.125 mg dose of TPA. These data suggest that an i.v. infusion of 0.125 mg TPA may be just as effective as an i.v. infusion of 0.25 mg TPA, but this must be confirmed in a larger group of patients. Administration of 0.125 mg TPA to five additional patients on 2 consecutive days increased WBC from 3.16 ± 0.15 before TPA administration to 7.08 ± 1.06 at 1–2 days after the second dose of TPA ($P < 0.01$), and neutrophils were increased from 2.06 ± 0.17 to 5.62 ± 0.86 ($P < 0.01$).

Adverse Effects of TPA. In our earlier studies with leukemia patients (4), we administered intravenous infusions of 1.0 mg, 0.5 mg, or 0.25 mg of TPA, and side effects were noted with the higher doses. These side effects consisted of brief and reversible shortness of breath, proteinuria, hemoglobinuria, fever, chills, and irritation of the vein at the infusion site. In the present series of 52 patients with solid tumors using i.v. doses of only 0.125 mg or 0.25 mg of TPA, about 40% of the patients had a brief period of mild fever (0.5–2.0°C increase in temperature) and/or mild chills after the administration of TPA, and about 40% of the patients had irritation of the vein at the infusion site. One patient had shortness of breath for 5 min and hemoglobin in the urine for 1 day. All side effects observed were short-lived and reversible. The low doses of TPA used in the present study had no overt effects on cardiac, pulmonary, renal, or hepatic function as measured by clinical or laboratory evaluation (as described in *Methods*).

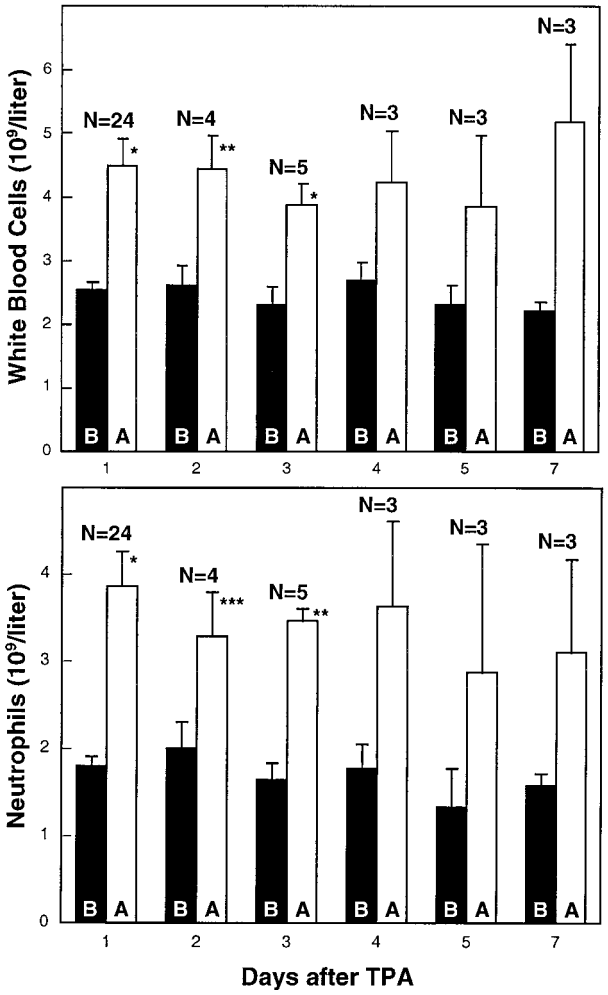

FIG. 1. Effect of a single i.v. infusion of TPA on white blood cells and neutrophils in the peripheral blood of patients previously treated with cytotoxic chemotherapeutic drugs. Patients with solid tumors who had been treated with cytotoxic chemotherapeutic drugs were given a single i.v. infusion of 0.25 mg TPA. Total white blood cells and neutrophils in the peripheral blood were measured before (B) and at 1–7 days after (A) TPA administration. N represents the number of subjects for which WBC and neutrophil values were determined both before and on the indicated day after TPA administration. Each value represents the mean ± SE. *, Statistically different from values before TPA ($P < 0.01$). **, Statistically different from values before TPA ($P < 0.05$). ***, Statistically different from values before TPA ($P < 0.10$).

DISCUSSION

The present study utilized patients with solid tumors who had been treated with cytotoxic anticancer drugs that resulted in bone marrow suppression and decreased WBC counts and neutrophils in the peripheral blood. Because G-CSF and GM-CSF were not available and because our earlier study in leukemia patients suggested that TPA treatment may elevate depressed WBC counts (4), we investigated the effect of TPA

on the levels of WBC and neutrophils in the above patients with solid tumors who had depressed WBC and neutrophil counts.

Intravenous infusion of 0.125 or 0.25 mg of TPA increased WBC counts and neutrophils in patients with depressed levels of these cells in the peripheral blood. Treatment with TPA resulted in increased lymphocytes and neutrophils toward the normal range. A single i.v. infusion of 0.25 mg TPA increased WBC counts and neutrophils within 24 hr, and the WBC and neutrophils remained elevated for at least 3 days. Administration of TPA increased the levels of both neutrophils and lymphocytes in the peripheral blood, and it was of considerable interest that there was a preferential increase in neutrophils.

It should be noted that the present group of patients had only moderately lowered neutrophil counts (mean = 1.76×10^9/liter) before TPA treatment. It will be of interest to investigate the effects of TPA administration in patients with more severely depressed neutrophil counts ($<0.5 \times 10^9$/liter).

Further research is needed to determine whether TPA-induced increases in peripheral WBC and neutrophils are effective for preventing infections and to determine the mechanisms of the TPA-induced increases. It will be important to determine whether TPA administration increases the formation of new WBC and neutrophils or causes the redistribution of WBC and neutrophils from tissues. *In vitro* studies indicate that TPA has a mitogenic effect on peripheral blood lymphocytes (predominantly an effect on T cells) and that TPA enhances chemotaxis, activates the initial stages of phagocytosis, and stimulates superoxide formation in polymorphonuclear leukocytes (neutrophils) (reviewed in ref. 1). These observations suggest that the increased levels of WBC and neutrophils observed in the present study may be effective in fighting infections.

Although increased levels of neutrophils may be effective in fighting infections, increased levels of neutrophils (and associated reactive oxygen species) in the lung or in other tissues may cause an inflammatory response in these tissues. The i.v. administration of TPA to rabbits or sheep has been reported to cause respiratory distress and to cause an inflammatory response in the lung (5–7). In the present study, we observed a 5-min period of shortness of breath in only 1 of 52 patients who received a 0.125- to 0.25-mg i.v. infusion of TPA. In an earlier study, we observed a transient period of shortness of breath in six of seven patients who received a high, 1-mg i.v. dose of TPA. In all cases, the effect of TPA in causing shortness of breath was transient, and TPA did not influence lung function as measured by respiratory rate, tidal volume, vital capacity, and maximal voluntary ventilation (this paper and ref. 4).

Additional studies are needed to determine the pharmacokinetics of TPA and the optimal dosing regimen for effective treatment of patients with lowered WBC and neutrophil counts. Preliminary data from the present study suggest that i.v. administration of 0.125 mg of TPA may be as effective as the i.v. administration of 0.25 mg of TPA. Further studies with lower single and multiple doses of TPA are needed. More research also is needed to compare the effects of TPA with those of G-CSF and GM-CSF as well as to explore the possibility of synergistic effects of TPA together with G-CSF or GM-CSF in refractory patients.

In summary, patients with solid tumors were treated with cytotoxic anticancer drugs, and they had depressed WBC counts and neutrophils in the peripheral blood. The i.v. administration of 0.125–0.25 mg of TPA to these patients resulted in increased WBC and neutrophil counts toward the normal range, and this effect occurred within 24 hr in many of the patients. WBC counts and neutrophils remained elevated for at least 3 days. Side effects observed with the low doses of TPA used in the present pilot study were fever, chills, and irritation of the vein at the site of injection in about 40% of the patients. Shortness of breath (for 5 min) and hemoglobin in the urine during the first day of treatment were observed in one patient. Although our studies in patients did not reveal serious toxic effects of TPA at the low-dose levels used and all side effects were transient, more detailed toxicity studies are needed.

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