Brief Definitive Report

ENHANCEMENT OF DELAYED HYPERSENSITIVITY REACTION WITH VARIETIES OF ANTI-CANCER DRUGS

A Common Biological Phenomenon

By MITSUYOSHI GOTO, AKIO MITSUOKA,* MASATOSHI SUGIYAMA, and MORIHISA KITANO

From the Departments of Hospital Pharmacy and Thoracic Surgery, Tenri Hospital, Tenri 632, Japan

Anti-cancer drugs generally have immunosuppressive effects (1), which presents a serious clinical problem. Under selected conditions, cyclophosphamide (CPM) has an immunopotentiating effect on the delayed hypersensitivity reaction (DHR; 2–4). This effect has been attributable to the interruption of a feedback control to the effector T cells by B cells (2, 5) and/or by suppressor T cells (4, 5). Recently, similar phenomena have been reported in other cell-mediated (6, 7) and tumor immunities (8–10). Immunopotentiating effects of CPM appear to take place preferentially in vivo, suggesting that such effects may be due to a biological consequence of diffuse cell damage with CPM, rather than to a unique biochemical action on selected populations of lymphoid cells.

The presence of naturally occurring suppressor T cells (11) and the effect of CPM on them have been reported previously (4, 12). Here, we report that anti-cancer agents other than CPM also exhibit potentiating effects on the DHR when they are administered according to schedules of current clinical anti-cancer therapy.

Materials and Methods

Mice. Inbred C57BL/6Cr mice were purchased from the Shizuoka Cooperative Association for Experimental Animals (Hamamatsu, Japan).

Antigen. Methylated human serum albumin (MHSA) prepared by the methanol-hydrochloric acid method (13) was kindly donated by Dr. S. Morikawa, Shimane Medical University.

Anti-Cancer Drugs. CPM and vincristine (VCR) were purchased from Shionogi, Osaka, Japan; carbazylquinone (CQ) was obtained from Sankyo, Tokyo, Japan; nitrogen mustard-*N*-oxide (NM-*N*-O) was purchased from Yoshitomi, Osaka; mitomycin c (MMC), adriamycin (ADM), and 5-fluorouracil (5-FU) were purchased from Kyowa Hakko, Tokyo; methotrexate (MTX) was obtained from Lederle Japan, Tokyo; cyclocytidine (Cyclo C) was purchased from Yamanouchi, Tokyo; and N,N′,N″-triethylenethiophosphoramide (thio-TEPA) was obtained from Sumitomo Kagaku, Osaka. Drugs were given to mice intraperitoneally before sensitization. As described in the previous work with CPM (12), the dose of every anti-cancer drug was fixed to ⅝ of LD_{50}: CPM, 150 mg/kg; CQ, 1.5 mg/kg; NM-*N*-O, 30 mg/kg; MMC, 2 mg/kg; thio-TEPA, 7.5 mg/kg; ADM, 5 mg/kg; 5-FU, 100 mg/kg; MTX, 40 mg/kg; Cyclo-C, 1,500 mg/kg; and VCR, 1 mg/kg. In an experiment of intermittent treatments with CPM or 5-FU, each drug was administered once, twice, or three times at 1-wk intervals with each dose being ⅝ of LD_{50}. In an experiment of consecutive treatments with the drugs, CPM (10 mg/kg) or 5-FU (7 mg/kg) was given every evening for 14 consecutive days to make doses equivalent to ⅝ of LD_{50}.

* To whom correspondence should be addressed at the Laboratory of Immunobiology, National Cancer Institute, National Institutes of Health, Building 37, Room 2B23, Bethesda, Md. 20205.

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TABLE I

Effect of Various Alkylating Drugs on the DHR

Drug	DHR on day 13 (0.1 mm)	Body weight
		g
—	5.3 ± 1.6	25.1 ± 0.7
CPM	9.7 ± 1.3	24.1 ± 0.5
CQ	15.6 ± 1.8*	26.3 ± 0.3
Thio-TEPA	15.2 ± 1.8*	25.7 ± 0.5
NM-*N*-O	13.5 ± 2.1‡	25.5 ± 0.4

Male C57BL/6Cr mice were given alkylating drugs by ⅛ of LD_{50}, intraperitoneally, 4 d before sensitization. Mice were 3 mo old at sensitization. Each group consisted of six animals. The data are shown as mean ± SE.

* $P < 0.01$ compared with drug (—).

‡ $P < 0.02$ compared with drug (—).

TABLE II

Effect of Various Anti-cancer Drugs on the DHR

Classification of drug	Drug	DHR on day 13 (0.1 mm)	Body weight
			g
	—	3.8 ± 1.6	19.0 ± 0.4
CCNS drug	MMC	13.8 ± 2.4*	19.1 ± 0.7
	ADM	13.0 ± 2.3‡	19.8 ± 0.8
CCS drug	5-FU	14.2 ± 1.3*	19.7 ± 0.4
	VCR	11.8 ± 1.8‡	19.4 ± 0.7
	Cyclo-C	11.2 ± 0.7*	20.6 ± 0.2
	MTX	10.4 ± 1.6§	19.6 ± 0.4

Female C57BL/6Cr mice were given various anti-cancer drugs by ⅛ of LD_{50}, intraperitoneally, 4 d before sensitization. Mice were 3 mo old at sensitization. Each group consisted of five animals.

* $P < 0.01$ compared with drug (—).

‡ $P < 0.02$ compared with drug (—).

§ $P < 0.05$ compared with drug (—).

Sensitization. Sensitization for DHR was carried out by subcutaneous injection into the hind footpad with 0.05 ml of emulsion (5 mg/ml MHSA solution in phosphate-buffered saline, pH 7.1, and complete Freund's adjuvant, including 3 mg of dead H37RV tubercle bacilli/ml; 14). The sensitization was performed 4 d after the last drug treatment.

DHR Assay. The difference in footpad thickness before challenge and at 24 h after challenge was measured and expressed as DHR in 0.1-mm units. Challenge with 0.02 ml of 0.1 mg/ml MHSA solution in phosphate-buffered saline was performed around the 11th–13th d of sensitization (14).

Statistical Analysis. All data are presented as means ± standard errors. Statistical comparisons were performed using the Student's *t* test.

Results

When the effects on the DHR of several alkylating agents including CPM were examined, there was a significant enhancement of DHR (Table I). CQ, thio-TEPA, and NM-*N*-O were greater in their potency in DHR enhancement than CPM. These alkylating drugs are now categorized as cell cycle nonspecific (CCNS) drugs (15). To examine whether the observed effects are specific for CCNS, we employed both CCNS and cell cycle specific (CCS) drugs (Table II). Essentially similar results were obtained with CCS drugs, suggesting that potentiation of DHR with anti-cancer drugs is a common phenomenon. A transfer of 2×10^7 thymus cells from untreated mice eliminated the DHR-enhancing effect of 5-FU (a CCS drug; data not shown). The

TABLE III

Effect of Intermittent High Dose Treatments with CPM or 5-FU on the DHR

Drug	Number of injections	DHR on day 11 (0.1 mm)	Body weight
			g
—	0	9.0 ± 1.1	25.7 ± 0.7
CPM	1	14.8 ± 1.3*	21.2 ± 0.7
CPM	2	14.5 ± 1.6‡	26.2 ± 0.9
CPM	3	20.3 ± 0.6§	25.3 ± 0.9
5-FU	1	13.0 ± 1.3‖	22.7 ± 0.6
5-FU	2	14.5 ± 1.8‖	24.7 ± 1.0
5-FU	3	13.5 ± 3.8	24.3 ± 0.8

Male C57BL/6Cr mice were given CPM or 5-FU by ⅝ of LD_{50}, intraperitoneally once, twice, or three times at 1-wk intervals. Sensitization was on 4 d after the last drug treatment. Mice were 3.5-mo-old at sensitization. Each group consisted of six animals.

* $P < 0.01$ compared with drug (—).

‡ $P < 0.02$ compared with drug (—).

§ $P < 0.001$ compared with drug (—).

‖ $P < 0.05$ compared with drug (—).

TABLE IV

Effect of Consecutive Smaller Dose Treatments with CPM or 5-FU on the DHR

Drug	Interval between the last treatment and sensitization	DHR on day 12 (0.1 mm)	Body weight
	d		*g*
—	—	5.3 ± 0.7	20.9 ± 0.5
CPM	1	4.5 ± 0.8	20.4 ± 0.4
CPM	4	7.3 ± 0.9	20.1 ± 0.2
CPM	7	5.0 ± 0.9	22.1 ± 0.6
5-FU	1	6.0 ± 1.4	20.8 ± 0.3
5-FU	4	5.3 ± 0.6	21.6 ± 0.3
5-FU	7	4.6 ± 0.8	21.6 ± 0.3

Female C57BL/6Cr mice were given CPM or 5-FU intraperitoneally for 14 consecutive days. The total dose was equivalent to ⅝ of LD_{50} for each drug. Mice were 3.5-mo-old at sensitization. Each group consisted of 10 animals.

primary target of these drugs might therefore be naturally occurring suppressor T cells, in keeping with our previous results, which showed that CPM (a CCNS drug) eliminated suppressor T cell activity involved in DHR (4, 12).

To examine whether the immunopotentiating effects of these drugs depend on the protocols of the treatments, we compared the effects of intermittent high dose treatments and those of consecutive smaller dose treatments with drugs on the DHR (Tables III and IV). The data clearly demonstrated that the intermittent high dose treatments are effective in enhancement of DHR (Table III), whereas the consecutive smaller dose treatments have no effects on the DHR, although the total dose given to each animal was ⅝ of LD_{50} (Table IV).

Discussion

Anti-cancer drugs are generally classified as either CCS or CCNS on the basis of their effects on cells (15, 16). CCS drugs are usually schedule dependent and require maintenance of drug concentration for a period of time sufficient to affect tumor cells. CCNS drugs are reported to be most effective when given in intermittent high

doses because of the greater sensitivity of tumor cells compared with that of normal cells. CPM was once classified as a CCS drug (16), but was later considered to be a CCNS drug (15). In any case, superiority of treatment with intermittent high doses is accepted in CPM anti-cancer therapy (17, 18). The intermittent high dose therapy was also reported to be valid for some CCS drugs such as 5-Fu (19) and MTX (20, 21). Our present study clearly demonstrated that the intermittent high dose therapy with anti-cancer drugs is effective in immunopotentiation.

We previously proposed that the principal reason the suppressor activity was eliminated after CPM treatment in vivo is that suppressor T cells are recovered more slowly or more insufficiently from damage than the other cells, effector T cells, and macrophages (4, 12). This would suggest that a clue for the differential elimination is based on the biological properties of the host rather than on a biochemical property of CPM. The present work, together with others (22–25), supports this idea because many other cytotoxic agents besides CPM exhibited similar effects on the DHR. It is quite likely that some, if not all, suppressor cells or their precursors might be more or less sensitive to cytotoxic agents such as CPM (26).

CPM is often used as an immunopotentiator in the studies on cellular immunology (2–7) and tumor immunology (8–10). The activation of CPM by microsomal enzymes in the liver is necessary for the action as a cytotoxic agent (27). The enzyme activity for the activation depends on the strain and age of the mouse (M. Goto, A. Mitsuoka, M. Sugiyama, and M. Kitano, unpublished data). In addition, these enzymes can be induced by various pretreatments (28). The relative level of the active form(s) in CPM-treated animals is undetermined. Other anti-metabolic agents reported in this paper have similar effects on the DHR. These drugs do not require the conversion into the active form as does CPM, and thus may be readily assessed as cytotoxic agents for immunoregulation.

Summary

Delayed hypersensitivity reaction in mice was commonly enhanced with various anti-cancer agents administered as single or intermittent high doses but not consecutive divided doses. The effect of anti-cancer agents on the delayed hypersensitivity reaction was thought to be due to elimination of suppressor T cell activity.

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