Abrogation of the Suppressive Effect of 12-*O*-Tetradecanoylphorbol 13-Acetate and 7,12-Dimethylbenz[*a*]anthracene on Footpad Reaction of Mice by Indomethacin and Some Inhibitors of Tumor Promotion

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Influences of indomethacin, which has been known as an inhibitor of the production of prostaglandins, on the suppression of footpad reaction (FPR) of BALB/c mice against sheep red blood cells by the painting of 12-*O*-tetradecanoylphorbol 13-acetate (TPA, a typical tumor promoter) were studied. The temporary suppressive effect by the painting of TPA (8 nmol) was abrogated by the painting of indomethacin (7—70 nmol) 60 min before TPA treatments. The lasting suppressive effect by TPA treatment (8 nmol/d) for 7 d following the painting of 7,12-dimethylbenz[*a*]anthracene (DMBA, 400 nmol), which is a typical tumor initiator, also disappeared when indomethacin (7 nmol) was painted 30—90 min before each TPA treatment.

Influences of some inhibitors of tumor promotion on the lasting suppressive effect of FPR by DMBA and TPA were also tested. Painting of 0.6 μmol of 1-phenyl-2-pyrazolidone, 8.2 nmol of salcophytol A, 17 nmol of retinoic acid, 5.6 μmol of 3(2)-*tert*-butyl-4-hydroxyanisol, and 3 μmol of quercetin 45 min before each TPA treatment decreased the suppressive effect on the footpad reaction.

Keywords footpad reaction; 12-*O*-tetradecanoylphorbol 13-acetate; 7,12-dimethylbenz[*a*]anthracene; indomethacin; 1-phenyl-2-pyrazolidone; sarcophytol A; retinoic acid; 3(2)-*tert*-butyl-4-hydroxyanisol; quercetin

Introduction

In preceding papers, we reported that footpad reaction (FPR) against sheep red blood cells (SRBC) in BALB/c mice was temporarily (about 24 h) suppressed by painting of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a typical tumor promoter,[1,2] and that the suppressive effect became lasting (not less than 2 weeks) when TPA was painted every day for 4—7 d following 7,12-dimethylbenz[*a*]anthracene (DMBA), a typical tumor initiator.[2] The lasting effect was caused by the induction of antigen-nonspecific T suppressor cells (Thy-1$^+$, Lyt-2$^+$), and the first step of the induction of T suppressor cells was shown to be related to Ia$^-$ macrophages.[3] The suppression of FPR under such conditions might be advantageous to the growth of tumors because delayed-type hypersensitivity, which causes FPR, has been presumed to be one of the immunosurveillance systems against tumors.[4-7]

In this paper, to clarify the mechanism of the suppression of FPR by TPA painting, the influences of indomethacin, which has an inhibitory effect on the production of prostaglandins (PG), on the effects of TPA described above were studied. Indomethacin was painted on the same region before each TPA treatment and FPR against SRBC was estimated. The influences of some inhibitors of tumor promotion on the lasting suppressive effect on FPR were tested also.

Materials and Methods

Animals Male BALB/c mice (6—8 weeks) were obtained from the Sankyo Labo Service Co. (Shizuoka, Japan), and given the diet CE-2 (the Clea Japan Inc., Tokyo, Japan).

Reagents DMBA and TPA were obtaind from Sigma (U.S.A.). Indomethacin, 1-phenyl-2-pyrazolidone(phenidone), retinoic acid, 3(2)-*tert*-butyl-4-hydroxyanisol (BHA) and quercetin were obtained commercially. Sarcophytol A was a present from Dr. H. Fujiki (National Cancer Center).

Assay of FPR FPR was assayed by the method described in the preceding paper.[1] After 13 d of immunization of mice by s.c. injection of 10^8 of SRBC, 10^6 SRBC were injected into the footpads of the right legs. The thickness of the footpads was measured before and 24 h after the second injection of SRBC, and the difference in thickness was recorded as the swelling of the footpads. Data was described as means ± S.E. of 5 mice.

Painting DMBA (400 nmol) in 50 μl of the mixture of benzene and ethanol (1 : 1), TPA (8 nmol) in 50 μl of ethanol, or the test compounds of in 12.5 μl of ethanol were painted on the dorsal regions. In the groups described as (−), equal volumes of the solvents were painted in place.

Results

Influence of Indomethacin on the Temporary Suppression of FRP by the Painting of TPA In the experiments of FPR assay, FPR was suppressed when TPA was painted 24 h before the second treatment with SRBC, but the suppression was not observed when the time interval between the TPA treatment and second injection was prolonged to 72 h (temporary suppression).[1,2] We studied the influences of the pretreatment by indomethacin on the suppression of FPR by the TPA treatment 24 h before the second injection of SRBC.

Table I shows that the suppression of FPR was abrogated by painting of indomethacin (7—70 nmol) on the same region 60 min before the TPA treatment. As shown in Table II, the suppression of FPR by TPA was not influenced when

TABLE I. The Influences of the Pretreatment with Indomethacin on the Temporary Suppression of FPR by TPA

Treatments		Dose of indomethacin (nmol)	FPR (mm)	FPR % of the control
Indomethacin	TPA			
−	−	—	0.810 ± 0.026 (Control)	100.0
−	+	—	0.497 ± 0.059[a]	61.4
+	+	7	0.926 ± 0.068	114.3
+	+	18	0.763 ± 0.033	94.2
+	+	35	0.730 ± 0.029	90.1
+	+	70	0.799 ± 0.025	98.6

a) $p < 0.05$ (against the control).

Table II. The Change of the Order of the Treatments with Indomethacin and TPA

1st treatment	2nd treatment	FPR (mm)	FPR % of the control
—	—	0.793±0.018 (Control)	100.0
Indomethacin	—	0.758±0.015	104.6
TPA	—	0.443±0.053[a]	55.9
Indomethacin	TPA	0.720±0.047	90.8
TPA	Indomethacin	0.395±0.025[a]	49.8

Indomethacin: 7 nmol, TPA: 8 nmol. *a*) $p<0.05$ (against the control).

Table III. The Influences of the Region on Which Indomethacin was Painted

Regions		FPR (mm)
Indomethacin	TPA	
Back	—	0.642±0.030[a]
—	Back	0.184±0.074[b]
Back	Back	0.579±0.017
Flank	Back	0.429±0.071[b]

Indomethacin: 7 nmol, TPA: 8 nmol. *b*) $p<0.05$ (against *a*)).

Table IV. The Influences of the Treatment with Indomethacin before TPA Painting on the Lasting Suppression of FPR by DMBA Plus TPA

Treatments			Time intervals between indomethacin and TPA (min)	FPR (mm)	FPR % of the control
DMBA	Indomethacin	TPA			
−	−	−	—	0.975±0.039 (Control)	100.0
+	−	+	—	0.509±0.057[a]	52.2
+	+	+	5	0.687±0.035[a]	70.5
+	+	+	15	0.775±0.061[a]	79.5
+	+	+	30	0.933±0.050	95.7
+	+	+	60	0.888±0.036	91.1
+	+	+	90	0.876±0.045	89.8

Before each TPA treatment, 7 nmol of indomethacin was painted. *a*) $p<0.05$ (against the control).

the order of the treatments with indomethacin and TPA was reversed. When indomethacin was painted in the flank whereas TPA was painted on the back, abrogation of the suppression was not complete (Table III). Therefore, pretreatment with indomethacin counteracts the temporary suppression of FPR and it is suggested that the mode of action may be local.

Influence of Indomethacin on the Lasting Suppressive Effect of FPR by the Painting of DMBA and TPA When TPA was painted on immunized mice 4—7 times following treatment with DMBA once, suppression of FPR continued not less than 2 weeks (lasting suppression).[2] The effect of the lasting suppression can be estimated by measuring the FPR by the second injection 3 d after the last TPA painting. We studied the influences of the pretreatment with indomethacin before each TPA painting in the experiments involving the lasting suppression of FPR.

On the first day, mice were immunized with SRBC, and 400 nmol of DMBA was painted on the back of each mouse. On days 4—10, 8 nmol of TPA was painted on each mouse every day. On 13 day, the second injection of SRBC was performed and FPR was estimated. Before each TPA treatment, 7 nmol of indomethacin was painted on the same regions. The time intervals between the treatments with indomethacin and TPA were altered in the range of 5—90 min. As shown in Table IV, the suppressive effect on FPR was abrogated when the time interval was greater than 30 min. This data shows the lasting suppression of FPR by the DMBA plus TPA is also counteracted by the pretreatment of indomethacin before TPA painting.

Table V. The Influences of the Treatments with the Inhibitors of Tumor Promotion before TPA Painting on the Lasting Suppression of FPR by DMBA Plus TPA

Exp. No.	Treatments			dose of inhibitors (μmol)	FPR (mm)	FPR % of the control
	DMBA	Inhibitors	TPA			
1	−	—	−	—	0.742±0.023 (Control)	100.0
	+	—	+	—	0.389±0.021[a]	45.7
	+	Phenidone	−	0.6	0.848±0.029	114.3
	+	Phenidone	+	0.6	0.473±0.032[a]	63.7
	+	Sarcophytol A	−	0.0082	0.767±0.041	103.4
	+	Sarcophytol A	+	0.0082	0.690±0.042	93.0
	+	BHA	−	5.6	0.703±0.082	94.7
	+	BHA	+	5.6	0.564±0.045	76.0
2	−	—	−	—	0.871±0.079 (Control)	100.0
	+	—	+	—	0.300±0.038[a]	34.4
	+	Retinoic acid	−	0.017	0.610±0.055	70.0
	+	Retinoic acid	+	0.017	0.696±0.080	79.9
3	−	—	−	—	0.735±0.060 (Control)	100.0
	+	—	+	—	0.346±0.029[a]	47.5
	+	Quercetin	−	3	0.750±0.026	102.0
	+	Quercetin	+	3	0.846±0.053	115.1

45 min before each TPA treatment, the doses shown were painted. *a*) $p<0.05$ (against the control).

Influence of Other Inhibitors of TPA on the Suppressive Effect of FPR by DMBA and TPA In the experiments described above, phenidone, retinoic acid, sarcophytol A, BHA or quercetin was painted 45 min before each TPA treatment. The results are shown in Table V. The treatments with these inhibitors decreased the lasting suppression of FPR by DMBA and TPA.

Discussion

The data in this paper shows that both the temporary suppressive effect of TPA and the lasting suppressive effect of DMBA plus TPA on FPR in BALB/c mice was abrogated by the treatment with indomethacin before TPA painting. On the other hand, several inhibitors of tumor promotion, the action of which are different from each other, namely phenidone (a prostaglandin synthesis inhibitor),[8] retinoic acid,[9] sarcophytol A,[10] BHA (an antioxidant)[11] and quercetin (a calmodulin antagonistic flavonoid),[12] were also shown to restrain the lasting suppressive effect on FPR by DMBA plus TPA.

It has been known that indomethacin can either enhance or inhibit tumor promotion by TPA depending on the dose. Verma, Ashedel and Boutwe[11,13] reported that the induction of epidermal ornithine decarboxylase (ODC) and tumor promotion by TPA CD-1 mice were markedly

inhibited by treatment with 280 nmol of indomethacin before each painting of TPA. The inhibitory effects on ODC induction were most remarkable when the mice were treated with indomethacin 2—4 h before TPA painting. However, Fischer, Gleason, Mills and Slaga[14] showed that tumor promotion by TPA in SENCAR mice was greatly enhanced by treatment with TPA plus 10—50 μg (28—140 nmol) of indomethacin although the promotion was inhibited when 100 μg (280 nmol) of indomethacin was used. In this work, the abrogation of the suppression of FPR by TPA was observed following pretreatment with 7—70 nmol each of indomethacin, and this was not in agreement with the condition in which tumor promotion was inhibited. It is possible that the abrogation by indomethacin can be related to the inhibition of prostaglandin synthesis[15] because some researchers have suggested that the prostaglandins can act as regulatory factors of immunity, namely in the inhibiting the function of lymphocyte[16,17] and inducting the induction of antigen nonspecific T suppressor cells[18] and that TPA can stimulate the production of prostaglandins.[19-22]

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