DETECTABILITY OF TUMOR PROMOTERS BY THEIR INHIBITORY ACTION ON INTERCELLULAR EXCHANGE OF LUCIFER YELLOW

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An urgent problem in preventive oncology is the development of an approach to the more rapid detection of tumor promoters – compounds which potentiate and accelerate the action of carcinogens. Experience with the testing of chemical carcinogens suggests that to detect tumor promoters with a pleiotropic action on cells, it is necessary to create a battery of several tests. One component of such a test system could be methods estimating the inhibitory action of promoters on intercellular exchange of metabolites or dyes [1, 9, 12].

The aim of this investigation was to study the effect of seven promoters of different chemical structure and different organ affinity on the cell-to-cell spread of Lucifer yellow (LY) in a culture of transformed Jungarian hamster fibroblasts.

EXPERIMENTAL METHOD

Experiments were carried out on a culture of Jungarian hamster fibroblasts of line DM-15, transformed by virus SV-40, and obtained by E. S. Kakpakova (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR) by the method described previously [2]. The cells were cultured on coverslips (10^5-$2 \cdot 10^5$ cells per penicillin flask) in Eagle's medium with 10% bovine serum (USSR). The promoters were added to the culture medium 24-48 h after seeding of the cells. The compounds 12-O-tetradecanoylphorbol-13-acetate – TPA (from Sigma, USA), mezerein (ICN, USA), A23187 (Sigma), butylhydroxytoluene – BHT (Research Institute of Rubber and Latex Goods, Ministry of the Oil Industry of the USSR) were dissolved in ethyl alcohol, dichlorodiphenyltrichloroethane – DDT (Geigy, USA), and anthralin (ICN, USA) was dissolved in acetone, and phenobarbital – PB (Serva, West Germany) was dissolved directly in the culture medium. The final concentration of the solvents in the medium did not exeeed 1%. To prevent LY from overflowing, the slides with cells were washed to remove the promoters and transferred into a chamber with a constant circular flow of lactalbumin solution (volume of the system 15 ml), placed on the stage of a luminescent microscope (LYUMAM). A saturated solution of LY (Sigma) in distilled water was injected iontophoretically into the cells by means of glass microelectrodes (diameter of tip 0.5 μ). The measurements were made at room temperature. The number of stained recipient cells was counted 1 min after the beginning of the injection.

EXPERIMENTAL RESULTS

Under normal conditions DM-15 cells are well interconnected [2]. The mean number of stained recipient cells in the control at early passages was 6-8, and at later passages 12-14. The intensity of cell-to-cell spread of LY was independent of the time after subculture (2-4 days), of the density of the culture, and of temperature within the range from 18 to 36°C. Incubation of the cells with solvents such as alcohol and acetone (1% in the medium) did not affect the spread of LY.

Tumor promoters such as TPA, mezerein, A23187, DDT, and BHT were found to be powerful inhibitors of cell-to-cell exchange of LY: under optimal incubation conditions they caused

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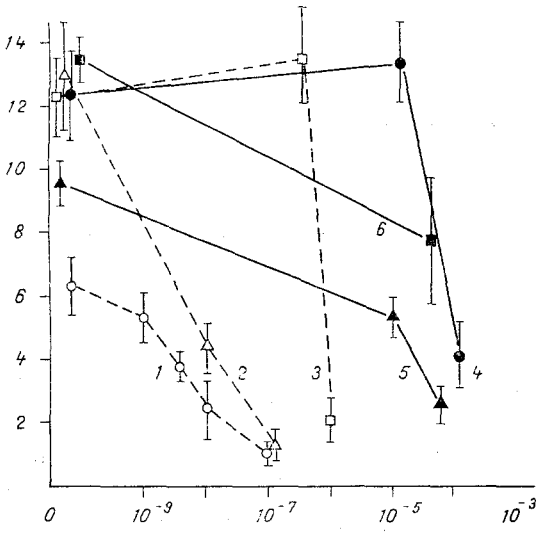

Fig. 1

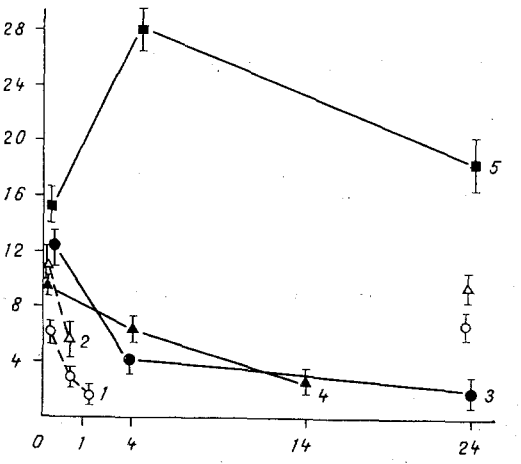

Fig. 2

Fig. 1. Effect of concentration of promoters on spread of LY between DM-15 cells. Abscissa, concentration (in g/ml); ordinate, number of stained cells (x ± m). 1) TPA, 2) mezerein, 3) A23187, 4) DDT, 5) BHT, 6) anthralin. Each point is the mean result of 10 to 30 microinjections. Duration of incubation with promoters: TPA, mezerein, and A23187 30 min; anthralin and DDT 4-5 h; BHT 14 h. Here and in Fig. 2, results of separate experiments are given.

Fig. 2. Effect of duration of incubation of DM-15 cells with promoters of cell-to-cell transfer of LY. Abscissa, duration of incubation (in h); ordinate, number of stained cells (x ± m). 1) TPA, 2) mezerein, 3) DDT, 4) BHT, 5) PB. Each point is the mean result of 8-30 microinjections. Concentration of promoters: TPA and mezerein 10^{-7} g/ml; DDT and BHT 10^{-4} g/ml; PB 10^{-3} g/ml.

a decrease in the number of stained cells by 3-6 times compared with the control (Figs. 1 and 2). Anthralin (50 μg/ml, incubation for 5 h) had a weak inhibitory action on cell-to-cell transfer of LY in DM-15 cells. In three of four experiments it caused a decrease in the number of stained cells by 1.7-2.5 times, and in one case it had no action. In two more experiments, to judge by the change in cell morphology, anthralin was toxic in a concentration of 50 μg/ml, and in lower concentrations it was ineffective. PB had a paradoxical action on the intercellular junctions: it potentiated linkage between the cells. It increased intercellular transfer of LY most effectively (by 1.8 times) in a concentration of 1 μg/ml during incubation for 5 h.

The effectiveness of the action of the promoters on intercellular transfer of LY depended on their concentration and on the duration of incubation of the cells with these substances. TPA and mezerein reduced the number of stained cells, starting with a dose of 0.003-0.01 μg/ml. A23187 effectively disconnected the cells within a comparatively narrow concentration range (0.2-2 μg/ml). DDT, BGT, and anthralin affected intercellular exchange of LY in considerably higher concentrations (10-100 μg/ml).

Essentially, an increase in the duration of incubation of the cells with the promoters could lead to opposite effects (Fig. 2). For instance, in the case of BHT and DDT, increasing the duration of incubation from 4 to 14 h (for BHT) or to 24-48 h (for DDT) led to strengthening of the uncoupling action of the promoters. Meanwhile TPA and mezerein ceased to uncouple the cells if the duration of their incubation was increased up to 20-24 h. This was demonstrated previously for TPA [6, 12]. The loss of sensitivity of the cells to the uncoupling action of TPA and mezerein is evidently based on proteolytic degradation of protein kinase C – the phorbol ester receptor [3]. It is an interesting fact that the effectiveness of action of PB also decreased with an increase in the incubation time to 24 h. An increase in the incubation time of the cells with A23187 led to their death.

The action of all the promoters on intercellular junctions was reversible. The number of stained cells began to rise 4-24 h after rinsing (in the case of PB, it began to fall), to approach the control level (Table 1). Connection between cells treated with TPA, mezerein and A23187 was restored most quickly of all. The reversibility of action of the promoters on

TABLE 1. Reversibility of Action of Promoters on Cell-to-Cell Exchange of LY in DM-15 Cells

Treatment	Number of stained cells (x ± m)			
	0	duration of rinsing, h		control
		4—6	24	
TPA (0.01 μg/ml, 30 min)	3,0±1,0 (8)	7,5±1,6 (8)	—	7,0±0,8 (14)
Mezerein (0.01 μg/ml, 30 min)	1,4±0,5 (19)	10,5±1,7 (8)	—	13.0±1,7 (15)
A23187 (1 μg/ml, 30 min)	2,6±0,8 (17)	10,8±1,8 (14)	—	12.0±1,3 (12)
Anthralin (50 μg/ml, 5 h)	5,4±0,6 (15)	—	11,0±1,1 (10)	9,8±1,3 (12)
DDT (100 μg/ml, 4 h)	4,2±1,1 (19)	—	13,1±1,2 (15)	12,6±1,4 (26)
PV (1 mg/ml, 5 h)	30,6±2,4 (15)	—	16,0±1,6 (13)	16.4±1,6 (19)
BHT (50 μg/ml, 14 h)	5,9±0,7 (13)	11,6±0,9 (13)	—	14,1±1,0 (15)

Legend. Cells treated with promoters, rinsed 3 times with lactalbumin, the medium changed, and after the specified time, cell-to-cell spread of LF was determined. Results of separate experiments are given. Number of measurements in parentheses.

TABLE 2. Effect of Promoters on Cell-to-Cell Exchange of LY in DM-15 Cells

Promoter	Target in vivo	Minimal acting concentration in vitro, g/ml	Inhibition of intercellular junction	Reproducibility of results (number of experiments in which an effect was found/total number of experiments)
TPA	Skin [4, 8]	$3 \cdot 10^{-9}$	++	16/16
Mezerein	" [4, 8]	10^{-8}	++	4/4
A23187	» » [8]	10^{-6}	++	5/5
Anthralin	» » [4]	$5 \cdot 10^{-5}$	±	3/6
DDT	Liver [3, 7]	10^{-4}	+	12/12
PB	" [4]	10^{-3}	Exchange intensified LY +	4/4
BHT	Lungs [7, 11], Urinary bladder	10^{-5}		5/5

the spread of LY is waiting, if indirect, proof of the specific effect of the promoters, unconnected with any toxic action, on intercellular junctions.

Thus six of the seven promoters tested inhibited exchange of LY. Allowing for the effectivenes of their action, the active concentrations, and the optimal incubation time these compounds could be divided into strong (TPA, mezerein, A23187) and medium strong (DDT and BHT) inhibitors of intercellular exchange of LY (Table 2). Anthralin was classed as a weak inhibitor of LY exchange. Incidentally, a similar relationship of effectiveness of action of TPA, mezerein, BHT, DDT, and anthralin on intercellular junctions was discovered in experiments to study inhibition of metabolic cooperation [9, 10]. Judging by our own results it can be postulated that the action of PB on intercellular junctions is periodic, and at a certain stage it potentiates intercellular communication. This hypothesis could explain the contradictory data obtained by different workers who have studied the effect of PB on metabolic cooperation [5, 9, 10].

The following conclusions can be drawn from comparison of the action of the above-mentioned promoters in vivo and their effect on LY exchange in vitro. First, the uncoupling

action of the promoters in a culture of Jungarian hamster fibroblasts is exhibited irrespective of the organotropism of their action in vivo. Second, the promoters inhibited LY exchange independently of the character of their promoting action in vivo. In fact, TPA, anthralin, DDT, and BHT are powerful complete promoters [4, 7, 8, 11], whereas A23187 and mezerein are weak promoters of stages I and II, respectively [4, 8] and exhibit their activity only when their action is combined with that of other promoters.

The experiments thus showed that, in principle, tumor promoters with different types of action and with different degrees of organotropism can be detected by a method based on estimation of intercellular exchange of Lucifer yellow. The method has definite advantages that are essential for screening methods: the experimental results are highly reproducible (Table 2), only a few cells are needed for the test, and the results can be obtained within 1 to 2 days. The further study of the sensitivity and specificity of this method of detection of tumor promoters is important.

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CHANGES IN TISSUE GLYCOGEN RESERVES OF TUMOR-BEARING RATS AS A SIGN OF THE HYPOGLYCEMIC STRESS SYNDROME

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No systematic study has yet been made of changes in tissue glycogen levels in tumor-bearing animals. Data in the literature are difficult to compare and often reflect only the terminal periods of tumor growth.

This paper describes an attempt to study the glycogen content in the brain, skeletal muscles, and liver of animals during growth of malignant tumors. To analyze the particular

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