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Dithranol (Anthralin) and 10-Butyryl Dithranol (Butantrone) Do Not Morphologically Transform Cultured C3H 10T1/2 C18 Mouse Embryo Fibroblasts

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The ability of dithranol and 10-butyryl dithranol to induce morphological cell transformation was studied in cultured C3H 10T1/2 C18 fibroblasts. The cells were incubated with different concentrations of the test compounds for 48 h and cultured for 5 weeks thereafter. At the end of the culture period the cultures were fixed, stained and examined for the presence of transformed foci. Dithranol and 10-butyryl dithranol did not increase the formation of transformed foci, while the positive control compound, 7,12-dimethylbenz(a)anthracene (DMBA), induced a high frequency of transformations significantly different from controls. Thus the in vitro cell transformation model with uninitiated C3H 10T1/2 C18 fibroblasts is not able to detect the weak tumorigenic action of dithranol and 10-butyryl dithranol which has been observed in mouse skin. ***Key words: Cell transformation; Carcinogenesis; In vitro.***

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Dithranol is a widely used and effective antipsoriatic drug, which has not been shown to cause skin cancer in psoriasis patients (1–3). However, it is a well-known tumor promoter in in vivo experimental models, like mouse skin (4–9), and it has also been shown to act as a cocarcinogen when administered simultaneously with benzo(a)pyrene (BaP) (7). 10-butyryl dithranol (butantrone), an acutely less irritating derivative of dithranol, has a lower tumor-promoting activity than dithranol in mouse skin (8, 9). Both compounds have also shown a weak direct tumorigenic activity by inducing squamous cell papillomas even without initiation with 7,12-dimethylbenz(a)anthracene (DMBA) (4–6, 8, 9). In addition, there is evidence that dithranol and, to a lesser extent, 10-butyryl dithranol are able to interact with DNA. Dithranol was reported to induce DNA strand breaks in human leucocytes in vitro (10). Dithranol and 10-butyryl dithranol were mutagenic in *Salmonella typhimurium* TA1537 both with and without metabolic activation. They also showed a clastogenic activity by increasing the number of chromosomal gaps and breaks in human lymphocytes in vitro (11).

The purpose of this study was to examine if dithranol and butantrone show a direct transforming potential in cultured C3H 10T1/2 C18 mouse embryo fibroblasts. This test system allows the expression of transformed foci of dense multilayered cells on a non-transformed contact-inhibited monolayer.

MATERIALS AND METHODS

Test compounds

Dithranol (mol. wt 226.2) was obtained from Bayer (Leverkusen, Germany) and purified by recrystallizing from a mixture of acetic acid and water (90:10). 10-butyryl dithranol (mol. wt 296.3) was synthesized according to Mustakallio et al. (12) and recrystallized from a mixture of isopropanol and acetonitrile. The test substances were dissolved in dimethylsulphoxide (DMSO) immediately prior to the treatment.

Cell culture

C3H 10T1/2 C18 cells at the passage number of 11–15 were grown in Dulbecco's modified essential medium with Earle's salts (DMEM) containing 5% fetal calf serum (Gibco, Grand Island, NY, USA) and gentamycin (25 mg/ml). Cells were maintained at exponential growth conditions in a humidified incubator containing 10% CO_2 in air at 37°C.

Cytotoxicity and cell transformation assays

The concentrations for the transformation assays were selected in preliminary cytotoxicity tests, where cell survival and colony forming efficiency were measured. In the final transformation assays, a solvent control (0.1% DMSO), five concentrations (0.05–0.5 μg/ml) of the test compounds and a positive control (DMBA, 0.05 μg/ml) were used. The final concentration of DMSO was 0.1%. The cells were plated on 60 mm Petri dishes (2000 cells/dish, 15–30 replicates), exposed for 48 h and cultured for 5 weeks. The growth medium was changed twice weekly. At the end of the culture period the cultures were fixed with methanol, stained with 5% aqueous Giemsa and scored for the presence of morphologically transformed type II and type III foci (∅ > 2 mm) according to Reznikoff (13). Statistical significance of the treatments was calculated using Fisher exact test.

RESULTS

Cytotoxicity of dithranol and 10-butyryl dithranol, expressed as colony-forming efficiency, is shown in Fig. 1. Dithranol was slightly more toxic to C3H 10T1/2 C18 cells than 10-butyryl dithranol.

The numbers of transformed foci observed after the culture period of 5 weeks are presented in Table I. Dithranol and 10-butyryl dithranol did not significantly or dose-dependently increase the formation of transformed foci, while the positive control DMBA induced a high frequency of transformations, significantly different from controls.

DISCUSSION

Both the therapeutic and toxic effects of dithranol are related to the formation of free radicals during dithranol autoxidation (14–18). In addition to the highly reactive dithranol-free radical (10-anthranyl radical), reactive oxygen species (singlet oxygen, superoxide anion radical, hydroxyl radical) are the biologically most significant radical species formed during dithranol

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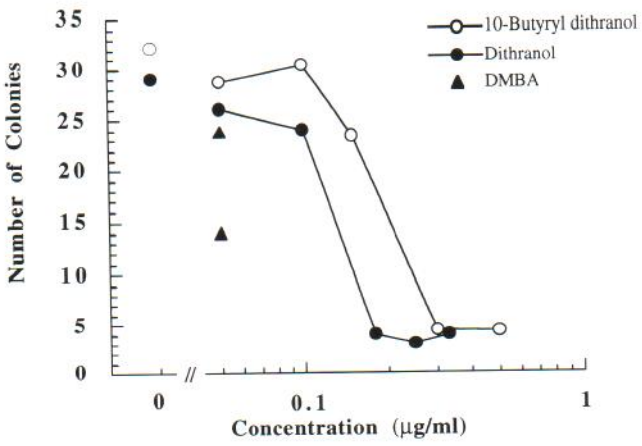

Fig. 1. Cytotoxicity of dithranol and 10-butyryl dithranol to C3H 10T1/2 C18 cells. Two hundred cells were plated on 60 mm Petri dishes, treated with test compounds for 48 h, and incubated thereafter for 7 days. Mean numbers of colonies (containing at least 50 cells) per dish, 5 replicates.

autoxidation. The ability of dithranol and 10-butyryl dithranol to react with DNA (10, 11) is obviously related to the formation of the free radicals. However, the compounds were not able to transform uninitiated C3H 10T1/2 C18 cells in this study.

Dithranol has not uniformly shown genotoxic activity in different models for genotoxicity. Dithranol was unable to induce chromosomal mutations in yeast (*Saccharomyces cerevisiae*) or ascomycete (*Ophistoma multiannulatum*) (19). Similarly, it did not induce forward mutagenesis, sister chromatide exhanges or structural chromosomal changes in V79 Chinese hamster lung fibroblasts (20). In some cases the use of too high (toxic) concentrations has confused the interpretation of the results (21, 22).

In previous cell transformation studies dithranol has been shown to be active when initiated cells are used (in vitro substitutes of tumor promotion studies). Dithranol transformed virally infected Swiss 3T3 mouse fibroblasts to form colonies in soft agar (23). Baturay & Trombetta (24) showed that dithranol transformed Balb/c-3T3 cells after initiation with either BaP, which needs metabolic activation for its carcinogenic action, or β-propiolactone (BPL), a direct-acting carcinogen. Moreover, when the cells were treated with one of the initiators and dithranol at the same time, the cells were transformed with BaP, but not with BPL. In this study, however, without initiation or simultaneous treatment with initiators, no cell transformation was observed. Therefore, the in vitro cell transformation model with uninitiated C3H 10T1/2 C18 cells does not predict or is not sensitive enough to detect the observed weak direct tumorigenic action of dithranol and 10-butyryl dithranol in mouse skin.

Table I. *The effect of dithranol and 10-butyryl dithranol on the induction of transformed foci in C3H 10T1/2 C18 cells*

The cells were treated with the test compounds for 48 h and cultured for 5 weeks thereafter (15–30 dishes per concentration).

Treatment	Concentration		Number of transformed foci			
	μg/ml	(μM)	Type			Total foci/dish
			II	III	II+III	
Control 1	0	(0)	7	0	7	0.30
Dithranol	0.05	(0.221)	5	1	6	0.25
	0.10	(0.442)	5	0	5	0.26
	0.18	(0.796)	3	0	3	0.20
	0.25	(1.105)	16	0	16	0.89
	0.33	(1.459)	5	0	5	0.33
DMBA 1	0.05	(0.195)	19	21	40	2.22*
Control 2	0	(0)	7	0	7	
10-butyryl dithranol	0.05	(0.169)	5	0	5	0.17
	0.10	(0.337)	9	0	9	0.36
	0.15	(0.506)	12	0	12	0.43
	0.30	(1.012)	5	0	0	0.24
	0.50	(1.687)	4	1	5	0.29
DMBA 2	0.05	(0.195)	24	42	66	3.30*

Statistics: $*p < 0.0005$, Fisher exact probability test.

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