ACTION OF DIMETHYL SULFOXIDE ON MORHOLOGY AND MULTIPLICATION OF NGUK-1 TUMOR CELLS

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Research workers have been busily engaged in recent years seeking inducing agents capable of differentiating tumor cells. The list of inducing agents includes cAMP derivatives and polar solvents: dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), etc. As a result of the action of small doses of DMSO on cells *in vitro*, some properties characterizing the phenotype of the transformed cells are modified. Changes in their morphology, the rate and character of their growth, loss of ability to grow in semisolid agar, and depression of their tumorigenicity [3-5] have been reported.

It has been shown that DMSO stimulates synthesis of substances specific for normal tissues in the cells of various tumors: synthesis of albumin in mouse hepatoma cells [8], of α-and β-globin, hemoglobin, and membrane proteins specific for normal erythrocytes in mouse erythroleukemia cells, and so on [6, 7]. The aim of this investigation was to study the action of various doses of DMSO on morphology and multiplication of tumor cells of rat neurinoma NGUK-1.

EXPERIMENTAL METHOD

Experiments were carried out on transplantable cell line NGUK-1 obtained from a neurinoma of the rat gasserian ganglion [2]. Tumor cells were cultured in Eagle's medium with the addition of 15% bovine blood serum, 15% calf blood serum, and 5% chick embryonic extract. Dimethyl sulfoxide (from Serva, West Germany) was sterilized by autoclaving at 1 atm. Working solutions were prepared in Eagle's medium.

A cell suspension for electron-microscopic investigation was centrifuged at 1500 rpm for 3 min and fixed with 1.5% glutaraldehyde in 0.1 M cacodylate buffer, dehydrated, stained with 0.5% uranyl acetate in 70° alcohol, and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-III Ultrotome, stained with lead citrate by Reynolds' method, and examined in the IEM-100B electron microscope with accelerating voltage of 80 kV.

The autoradiographic investigations were conducted by the usual methods [1]. ^{3}H-Thymidine of USSR origin (specific radioactivity 22 Ci/mmole), in a final concentration of 1 μCi/ml, was added to the culture medium for 20 min (pulse label) 24 and 48 h after treatment of the cells with DMSO. Autoradiographs were prepared by coating the section with type M emulsion and exposing them at 4°C for 4 to 6 days. The number of labeled cells in 3000 cells and the number of mitoses in 1000 cells were counted. A cell was considered to be labeled if no fewer than five grains of silver were present above its nucleus. The scheme of all the experiments was the same. DMSO was added to the experimental plaques containing cells growing on cover-slips, 1 day after seeding, in a final concentration of 1, 2, 3, 5, 6 or 7%, and then incubated at 37°C for 24 and 48 h. Samples were taken at these times for morphological and autoradiographic analysis.

EXPERIMENTAL RESULTS

DMSO inhibited multiplication of the NGUK-1 tumor cells, as shown by a fall in the index of ^{3}H-thymidine-labeled cells and the mitotic index. The inhibitory effect of DMSO depended

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TABLE 1. Changes in Number of NGUK-1 Cells Synthesizing DNA after Incubation with DMSO in Various Concentrations

DMSO concentration, %	Index of labeled cells, %	
	After 24 h	After 48 h
0	53,74±3,24	28,13±3,84
1	48,2±3,14	22,03±2,01
3	34,36±3,18	14,91±3,02
5	5,65±0,91	0,88±0,3

Legend. DMSO in concentrations of 6% and 7% had a toxic effect.

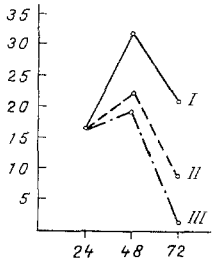

Fig. 1. Changes in mitotic index in culture of NGUK-1 cells incubated with different doses of DMSO. Abscissa, duration of culture (in days); ordinate, mitotic index (in %). I) Control cultures; II, III) cultures with 3% and 5% DMSO respectively.

both on its concentration and on the duration of its action of the cells. Table 1 shows that 24 h after addition of 3% DMSO, the number of cells entering the period of DNA synthesis was reduced by 32%, whereas after addition of 5% DMSO the number fell by 80%. On subsequent incubation of the cells in medium with DMSO its inhibitory effect was even stronger. After 48 h in 5% DMSO entry of the cells into the phase DNA synthesis was blocked virtually completely.

Data of the action of DMSO on mitotic activity of the culture indicates that 3% DMSO reduced mitotic activity of the tumor cells by 1.5 and 2.1 times after 24 and 48 h respectively (Fig. 1). The greatest inhibition of mitotic activity (by 12.3 times) was observed after treatment with 5% DMSO for 48 h. Irrespective of the duration of exposure to 1% DMSO no inhibitory action was observed either on mitotic activity or on the index of labeled cells.

Electron-microscopic investigation showed that 3% and 5% DMSO caused the appearance of dilated cisterns of the rough endoplasmic reticulum (RER), filled with finely granular material of low electron density, evidence of stimulation of synthesis of a protein substrate in RER. The nucleoli in most cells had a foam-like structure (Fig. 2a, b). Under the influence of 6% DMSO a cytotoxic effect was observed: vacuolation of the cytoplasm as a result of an increase in size of the dilated, translucent cisterns of RER and destruction of the mitochondrial cristae. Vacuolation took place in cells both with electron-dense cytoplasm and an increased number of free mon- and polysomes, and also in cells with a translucent matrix of their cytoplasm (Fig. 3).

Under the influence of 7% DMSO nearly all the cells were in a state of destruction after 24 h.

It can be concluded from these results that DMSO, in concentrations of between 3% and 5%, inhibits the entry of cells into the S period of the cell cycle.

It was shown previously [10] that DMSO, in low concentrations, prevents entry of cells into the S period, delaying them in the G_1 phase of the cell cycle. As a result of the G_1/S block the cell doubling time was increased [3], and this was evidently one cause of the

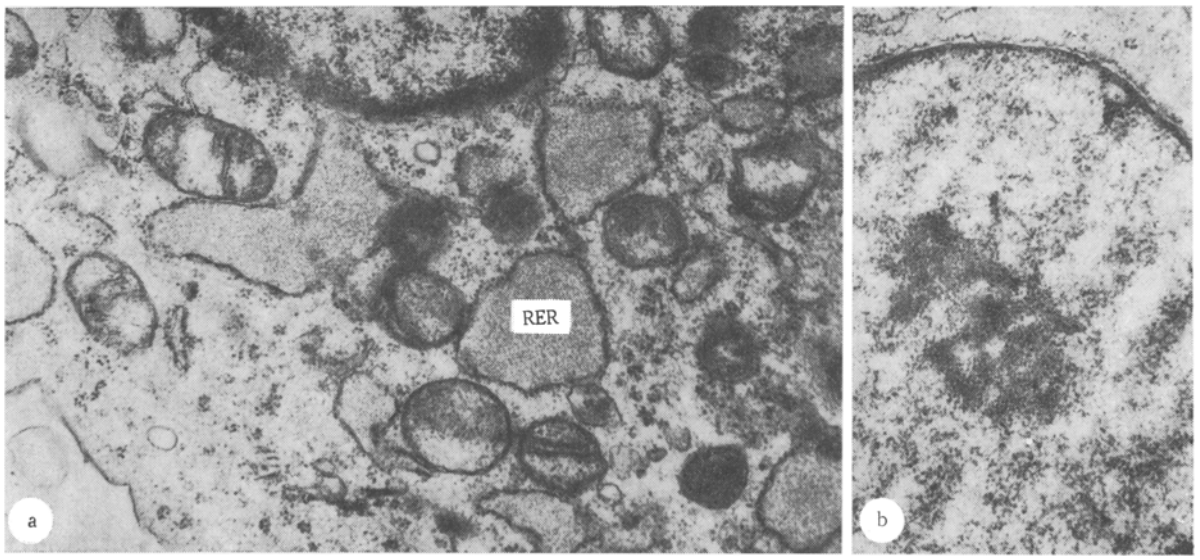

Fig. 2. NGUK-1 cells after culture for 24 h in the presence of 5% DMSO. a) Dilated cisterns of rough endoplasmic reticulam (RER), filled with finely granular material; b) foam-like structure of nucleolus. 20,000 ×.

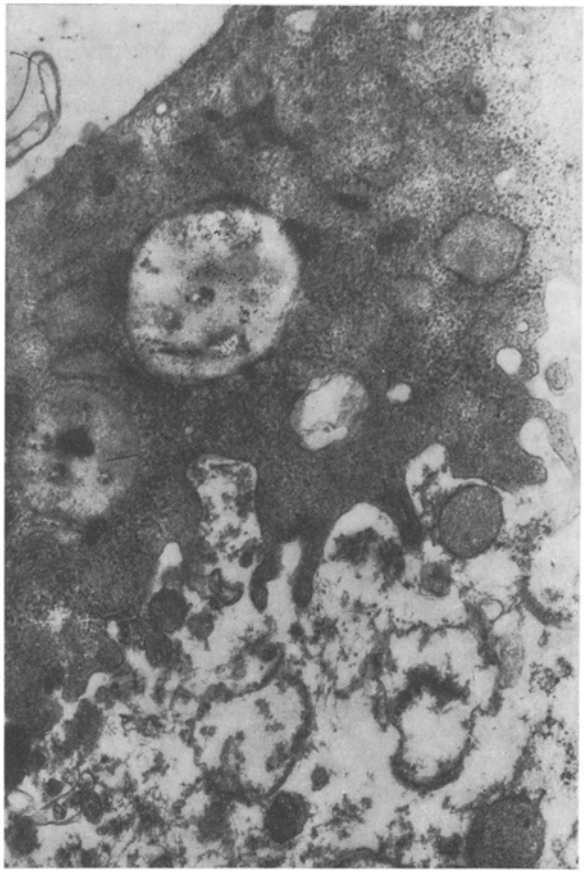

Fig. 2. NGUK-1 cells after culture for 48 h in the presence of 6% DMSO. Cytotoxic effect (20,000 ×).

reduction in the rate of their proliferation. This effect was revealed as a rule not less than 20 h after the beginning of exposure to DMSO, which coincides in time with various events characterizing the intial stages of cell differentiation [9, 11]. It is considered [10] that the most important of these in this period is synthesis of regulatory proteins, which play a key role

in the control of gene expression. Meanwhile, other workers also have found an increase in the total protein concentration in mouse hepatoma cells under the influence of DMSO [8].

The effect of DMSO on cells thus depends on its concentration and on the duration of its action. Optimal concentrations causing definite changes in tumor cells, which suggest the onset of cellular differentiation, are 3% and 5% of DMSO.

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