# CHANGES IN RADIOSENSITIVITY OF CAVE CELLS AFTER REPEATED EXPOSURE TO β-MERCAPTOPROPYLAMINE (MPA)

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As a result of the repeated action of the radioprotector β-mercaptopropylamine on cells of the CAVE line, a variant $CAVE_{K-10V}$ was obtained, the cells of which are more resistant to high concentrations of the radioprotector and to ionizing radiation. These features persisted unchanged during prolonged (about 2 years) cultivation of the cells under ordinary conditions.

Many different chemical compounds with a radioprotective action are now known. The mechanism of action of radioprotective substances at the level of the living cell and of the organism is due to their active intervention in biochemical processes and to sharp changes in the principal radiosensitive metabolic reactions [4].

The absolute majority of radioprotectors exert a protective action only if they are given in subtoxic doses, inducing severe changes in biochemical and physiological systems [3-5]. It is also well known from the literature that the protection of biological objects by the use of radioprotectors takes place only in their presence and only for a very short time, limited to a few hours, after their administration.

The object of the present investigation was to develop a method of obtaining cell variants resistant to high concentrations of radioprotectors and to study the radioresistance of such cells.

## EXPERIMENTAL METHOD

Cells of line CAVE* were used, because previous work with them showed that the cells of this line have minimum adhesiveness and after death they do not break up so quickly into fragments. These properties enable more precise results to be obtained when the cells are counted and they facilitate the work considerably. In addition, cells of line CAVE are more resistant to unfavorable factors.

The radioprotector chosen was β-mercaptopropylamine (MPA), one of the most effective of the aminothiol compounds used to prevent radiation damage [4]. This compound was chosen also because it is localized mainly in the hyaloplasm and to a much lesser degree (10 times less) in the nuclei [2].

The cells were grown in bacteriological tubes on medium No. 199 with the addition of 10% bovine serum. After the cells (50,000-75,000 per ml nutrient medium) had been seeded into the tubes, they were incubated at 37°C.

* The CAVE line of cells was obtained by Dobrynin and Dirgulyan in 1961 from the epithelium of a gastric carcinoma in a woman [1].

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To determine the maximum concentration of MPA tolerated by the cells, the compound diluted with nutrient medium to a concentration of 0.0125-0.2% was added in a volume of 1 ml to each tube on changing the medium after the cultures had grown for 3 days. The maximum tolerated concentration was 0.05%. The first treatment of the cell culture with MPA took place in that concentration.

As a rule, 3 days after continuous contact between the compound and cells, cells from the tubes (varying from 1111 to 7777 living cells not staining with 1% trypan blue solution) were seeded in the usual way into Carrel's dishes to determine the number of viable cells. Cells treated once with MPA in a concentration of 0.05% gave definite growth (1-7 colonies; $M \pm m = 3.0 \pm 0.8$) only after 1-1.5 months. Cells treated with the compound in a concentration of 0.025% after seeding gave growth until the 14th-21st day (from 18 to 32 colonies per plate; $M \pm m = 23.0 \pm 2.86$). The number of viable cells was determined from the number of colonies growing on the plate.

After treatment of the cells with MPA in a concentration of 0.0125%, the rate of propagation when they were transferred to ordinary conditions was indistinguishable from that of the control culture.

The dividing cells were treated again with MPA.* In this way the cells were subjected to this procedure 10 times.

The study of the survival rate of the cells in relation to the number of procedures showed that cells which survived and subsequently divided after primary treatment with the compound in a concentration of 0.05%, survived a second treatment only in a concentration of 0.0125%, they survived after a third treatment in a concentration of 0.025%, but they survived after a 5th treatment of the original concentration (0.05%). After 10 procedures the number of surviving cells was considerably larger (from 12 to 20 colonies per plate; $M \pm m = 13 \pm 1.73$), whereas after the first treatment with MPA in the same concentration (0.05%), as mentioned above the number was small (1-7 colonies per plate). The difference between the mean number of colonies was significant ($P < 0.0001$). However, when the concentration of the compound was doubled (0.1%), even cells subjected to 10 treatments died. This suggests that in this case either cells more resistant to this compound had been selected, or some of the cells when treated with MPA were able to adapt their biochemical processes in a direction which enabled them to survive, i.e., they acquired increased resistance to this particular radioprotector (chemical resistance).

Death of the cells after the first treatment with the compound in the same, or even a lower concentration, was probably due to sensitization of the cells to this compound, i.e., to chemical sensitization.

Parallel with the method described above, another method used by most investigators, in which the chemical compound is permanently present in the nutrient medium, also was tested. This method proved ineffective for the present purpose, because cells grown in low concentrations of the compound did not acquire increased resistance to ionizing radiation, and when larger doses of the compound were added to the nutrient medium, the cells did not adhere to the glass and they died.

Cells treated repeatedly with MPA differed in a number of features from the original line, and they were described as variant $CAVE_{K-10V}$.

When CAVE cells were seeded into tubes, a triangular monolayer was formed, while the variant obtained in these experiments grew in a narrow strip or thread. Microscopic examination showed that the variant consisted of polygonal cells, larger than those of the original CAVE line. The cell nuclei also were larger, and the intercellular spaces were wider. During prolonged observation the variant $CAVE_{K-10V}$ acquired the appearance of a continuous sheet of epithelium, growing more loosely than cells of the CAVE line. Giant cells and, in particular, elongated cells, were much more common.

The mean results obtained from a study of the coefficient of proliferation (CP), mitotic index (MI), and the number of dead (ND) and of multinuclear cells (NC) are given in Table 1.

As Table 1 shows, CP for the variant was almost 50%, and MI approximately 35% lower than in the original line. Counting the number of mitoses in the individual phases showed that the differences between the mean percentages of individual phases of mitosis in line CAVE and the variant were very small. The number of multinuclear cells, on the other hand, was almost twice as high in the variant as in the original

* Each successive treatment of the cells with the compound was carried out after not less than 2-3 subcultures of the cells under ordinary conditions.

TABLE 1. Comparative Characteristics of Cells of Line CAVE and Variant $CAVE_{K-10V}$

| Culture of cells | CP | MI (in %) | ND (in %) | MC (in %) | Number of nuclei per cell | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 2 | 3 | 4 | 5 and over |
| CAVE | 7,30 | 18,73 | 7,0 | 8,87 | 6,45 | 1,40 | 0,97 | 0,05 |
| $CAVE_{K-10V}$ | 3,93 | 14,28 | 5,0 | 15,44 | 8,80 | 1,90 | 1,85 | 2,89 |

line. Cells with 5-10 nuclei or more were particularly numerous in the $CAVE_{K-10V}$ variant. Statistical analysis of the results showed that these differences are significant.

A study of the radioresistance of the $CAVE_{K-10V}$ variant showed that after $\gamma$-ray ($Co^{60}$) irradiation* in a dose of 6000 R all the cells of the intact CAVE line died, whereas some cells (from 20 to 45%) of the $CAVE_{K-10V}$ variant survived. Cells were regarded as viable if they did not die in the course of 15 subcultures.

The results thus show that the newly obtained variant differs from the original CAVE line by the larger number of cells resistant to high concentrations of MPA, by lower sensitivity to ionizing radiation, and also in certain other features. These changes are stable, for they persisted during prolonged (about 2 years) cultivation of the cells under normal conditions.

This suggests that the newly obtained variant of the cells arose through mutation. However, it is not yet known whether it was present in the original population and arose as the result of simple selection, or whether it arose through the action of MPA.

Differences between the newly obtained variant and the original line with respect to radiosensitivity and resistance to the toxic action of MPA are of definite interest from the radiobiological point of view, notably for radioprotection.

They indicate that, in principle, it is possible to increase the resistance of the body tissues to radiation by the suitable use of pharmacological protective agents.

In the writers' view, a promising result of this investigation is the long duration of the action of radioprotectors on the tissues, which may lead to a lasting increase in the radioresistance of the tissues and, consequently, of the organism as a whole.

## LITERATURE CITED


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* Activity about 1000 Ci, distance 50 cm, dose rate 48 R/min.