A CYTOPHOTOMETRIC AND KARYOMETRIC STUDY OF THE ACTION OF ThioTEPA AND PROTAMINE ON TUMOR CELLS IN TISSUE CULTURE

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The results of previous investigations conducted in the Laboratory of Medical Cytology at the Central Postgraduate Medical Institute showed that a more marked depression of mitotic activity is produced in tissue cultures by the combined action of ThioTEPA and protamine then by ThioTEPA alone. With the combined use of the two preparations, degeneration of many more of the cells was also observed. In addition, some degree of selectivity of the action of protamine, whether alone or combined with ThioTEPA, was observed on diploid Wy38 cells.

The object of the present investigation was to study the cytochemical changes in cultures of tumor cells exposed to the action of these preparations alone or in combination.

EXPERIMENTAL METHOD

Transplantable strains of human tumors HEp-2 and HeLa and human diploid Wy38 cells were used. In accordance with earlier findings, ThioTEPA was used in doses of 6.25 and 12.5 μg/ml and protamine in doses of 25 and 50 μg/ml. The required concentrations of the test substances were prepared in medium No. 199. The action of the preparations on a three-day growth was studied. The results were read 24 h after addition of the preparations. For the cytological investigations a monolayer of cells grown on glass slides grown on cover slips was used. In cytochemical investigations the RNA content in the cells was determined by Brachet's method, the DNA by Feulgen's method, and the total protein by Mazia's method. A karyometric study was made of an HEp-2 culture after treatment with ThioTEPA alone, with protamine, and with both together, of a culture of HeLa cells after contact with a combination of ThioTEPA and protamine, and of a culture of human diploid Wy38 cells. In each preparation no fewer than 100 nuclei were measured. The nuclei were drawn by means of a drawing apparatus and measured with a planimeter. The volume of the nuclei was calculated taking into account the enlargement of the drawing apparatus and the planimeter. Part of the karyometric study of these cultures was undertaken in the Laboratory of Cytology of the N. F. Gamaleya Institute, under the direction of Ya. E. Khesin, to whom the authors are grateful.

The cytophotometric investigation of the DNA content was carried out with a single-beam probe cytophotometer at wave lengths of 498 and 540 mμ [1]. No fewer than 100 nuclei were measured in each histological preparation. The photometrically investigated nuclei were photographed and projections of the nuclei were obtained by means of an enlarger, their outlines were traced, and then measured with a planimeter, after which the volume of the nuclei was calculated. The DNA content was determined as the product of the optical density of the nucleus and the volume of the same nucleus. All the results of karyometry and cytophotometry were subjected to statistical analysis. The significance of the results obtained were determined by means of the χ^2 criterion.

EXPERIMENTAL RESULTS

DNA was demonstrated in the nuclei of the control cultures of HEp-2 and HeLa cells in the form of scattered tiny granules and of larger granules uniformly filling the nucleus. An agglomeration of material giving a positive Feulgen reaction was found in the perinucleolar zone and around the periphery of the

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TABLE 1. Changes in the Size of the Nuclei of HEp-2 Cells Under the Influence of ThioTEPA and Protamine, Alone and in Combination

group no.	Dose of preparation		Mean size of nuclei	increase in size of nucleus	χ^2
	thio-TEPA	protamine			
1	—	—	64	—	—
2	12,5	—	65	—	<05
3	—	50	70	—	<05
4	6,25	25	104,8	63	>01
5	12,5	50	114	78	>01

TABLE 2. Changes in the DNA Content in HEp-2 Cells Under the Influence of ThioTEPA and Protamine, Alone and in Combination

Dose of preparation (in μg/ml)		Mean DNA content (in conventional units)	χ^2
thioTEPA	protamine		
—	—	603	—
12,5	—	405	>01
—	50	470	<01
12,5	50	311	>01

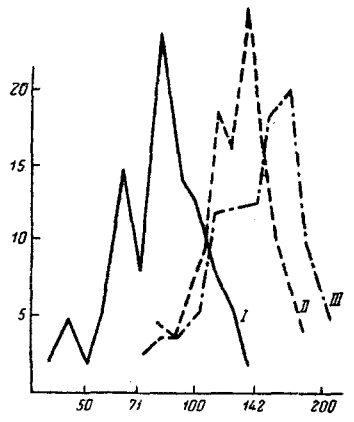

Fig. 1. Effect of a combination of ThioTEPA with protamine changes in the column of the nuclei of HEp-2 cells: I) control; II) 6.25 μg ThioTEPA + 25 μg protamine; III) 12.5 μg ThioTEPA + 50 μg protamine. Abscissa – volume of nucleus (in μ^2); ordinate – number of nuclei.

nuclei. Under the influence of ThioTEPA the large granules of chromatin disappeared from the nucleus, the staining of the perinucleolar zone was weaker, and the general staining of the nucleus became less intensive. Protamine caused pycnosis of some of the nuclei. The Feulgen reaction was strongly positive in these nuclei. In the nuclei not affected by pycnosis the intensity of the reaction as seen by the naked eye was indistinguishable from that in the control. After treatment with thiotepa in conjunction with protamine, the intensity of staining of the nuclei was reduced still further and the number of degenerating cells was increased.

The RNA content of the cells was not appreciably affected by ThioTEPA, protamine, or a combination of both. The intensity of the reaction for total protein in the cytoplasm of the cells of both tumor strains fell slightly after treatment with these preparations alone or in combination. The intensity of straining of the nuclei and nucleoli was unaffected. These results showed that the two preparations, whether separately or together, had the most marked action on the DNA content in the HEp-2 and HeLa cells.

The next stage of the investigation was the karyometric study of the same cultures after treatment with ThioTEPA, protamine, and both preparations together. The results of karyometry of the HEp-2 culture are given in Table 1.

It is clear from Table 1 that the very slight increase in the size of the nuclei after treatment with protamine alone and ThioTEPA alone was not statistically significant*. The increase in the mean volume of the nuclei after treatment with a combination of ThioTEPA and protamine was quite significant. This increase in size was due to disappearance of the small nuclei and the appearance of new classes of large nuclei (Fig. 1).

The same tendency toward an increase in the size of the nuclei under the combined influence of ThioTEPA and protamine was also observed with the HeLa culture, although it was less marked (34%). In the culture of diploid Wy38 cells the mean volume of the nuclei after treatment with 12.5 μg ThioTEPA and 50 μg protamine was unchanged. In both the experimental and the control series it was 50 μ^2.

The cytophotometric investigation was conducted on cells of the HEp-2 strain (Table 2).

The results given in Table 2 show that the DNA content fell by the greatest amount after the combined administration of ThioTEPA and protamine (the result was statistically significant).

*The increase in the volume of the nuclei observed in tumors of animals [2, 3] took place after the repeated administration of ThioTEPA in relatively large doses.

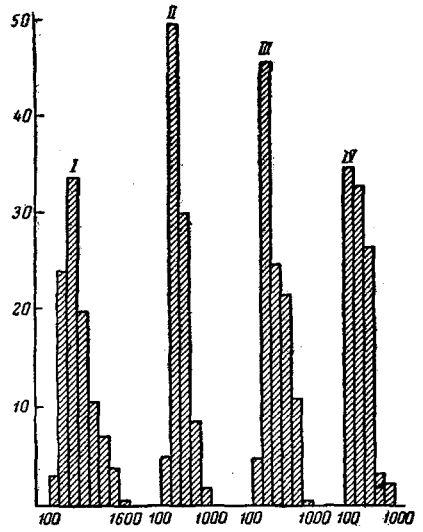

Fig. 2. Histogram of changes in the relative number of cells with different DNA contents under the influence of ThioTEPA, protamine, and a combination of both. I) control; II) TEPA; III) protamine; IV) ThioTEPA + protamine. Abscissa – DNA content (in conventational units); ordinate – number of cells.

It is clear from Fig. 2.that the nuclei of the control cultures contained from 200 to 1600 conventional units of DNA, and that the largest number of nuclei contained 200-1000 conventional units of DNA. After treatment with ThioTEPA the nuclei of the tumor cells contained from 100 to 1000 conventional units of DNA, and the largest number of nuclei contained 200-800 conventional units of DNA. Under the influence of protamine the DNA content in the nuclei fell by a lesser degree, and although the nuclei of the HEp-2 culture also contained 100-1000 conventional units of DNA was much larger in this case than following the action of ThioTEPA. After the combined action of ThioTEPA and protamine, the DNA concentration in the nuclei fell sharply, and in the largest number of nuclei the DNA content was 100-600 conventional units.

After treatment with 12.5 μg ThioTEPA and 50 μg protamine, the DNA content in the nuclei of the human diploid cells not only failed to show a decrease, but it actually increased from 367 conventional units (control) to 448 conventional units (experiment), but this difference is not statistically significant.

As these investigations showed, ThioTEPA, in combination with protamine, had a more injurious action on the HEp-2 tumor cells than these same preparations used separately. Following the combined action of the preparations the DNA content in the cell fell sharply and the volume of the nuclei increased. Human diploid cells were essentially unchanged after treatment with the same doses of ThioTEPA in combination with protamine. It may be concluded from these results that a further study of the natural origin is necessary in order to increase the effectiveness of their antitumor action.

LITERATURE CITED

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