ON THE MECHANISM OF THE ANTITUMOR EFFECT OF THIO-TEPACOMBINED WITH ESTROGENS IN RAT MAMMARY CANCER

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As preceding investigations have demonstrated, the reactivity of our first generations [7] of transplantable cancer of rat mammary glands (RMK-1) is similar with respect to hormonotherapy methods that differ in mechanism of action to the reactivity of human mammary cancer [8]. This made it possible to use RMK-1 as an experimental model for elaborating new methods of hormonotherapy, in particular in combination with chemotherapy.

The reactivity of mammary cancer to Thio-Tepa, which is a chemotherapeutic preparation from the ethylenimine group, has presently been established in the clinic [3, 14, 17, 21, 23]. There is also information available that the combined use of Thio-Tepa and hormones in the treatment of patients with advanced forms of breast cancer has a more pronounced antitumor effect than each of these preparations separately [11, 13, 19, 20, 21]. According to the data in the literature, the enhancement of the antitumor effect in the combined use of chemo-preparations and hormones is due to summation of their inhibiting effect on the tumor.

It is known that Thio-Tepa acts as a cytotoxic agent directly on tumor cells [6]. It is also established that Thio-Tepa affects the entire organism, and particularly the organs of the endocrine system [1, 4, 15, 16]. For example, data are available on the damaging action of Thio-Tepa on ovaries [1, 15, 22]; it was established in our laboratory that Thio-Tepa stimulates the follicle-stimulating function of the rat hypophysis [4]. On the basis of these data it was hypothesized that Thio-Tepa enhances proliferative processes in breast tumors, and, consequently, increases the sensitivity of the proliferating tumor cells to its cytotoxic effect. Therefore, we can assume that the antitumor effect of Thio-Tepa in mammary cancer is due not only to cytotoxic action, but also to the indirect effect through the hypophysis [4].

In connection with this we assumed that Thio-Tepa, simultaneously with stimulating the follicle-stimulating function of the hypophysis, elevates the sensitivity of the hypophysial cells producing the follicle-stimulating hormone (FSH) to estrogens which regulate the production of this hormone in the hypophysis. As a result of this, in the combined use of Thio-Tepa and estrogens the latter should induce a more pronounced suppression of the production of FSH.

It is known that large doses of estrogens suppress the follicle-stimulating function of the hypophysis, and we established that the mechanism of the antitumor action of estrogens in breast cancer leads to suppression of the production of FSH [10] as one of the hormones determining the proliferative processes in the mammary gland [12]. Hence, it follows that an enhancement of the antitumor effect of estrogens in combination with Thio-Tepa is possible because under these conditions estrogens more markedly suppress the follicle-stimulating function of the hypophysis.

The purpose of this study was to verify the expressed hypothesis of the mechanism of the antitumor effect of a combination of Thio-Tepa with estrogens in mammary cancer.

Antitumor Effect of Thio-Tepa in Combination with Synestrol in RMK-1

Experimental Conditions	Generation				Index of follicle-stimulating activity of hypophyses
	7th		10th		
	No. of rats	Inhibition of growth (in %)	No. of rats	Inhibition of growth (in %)	
Control	10	0	10	0	3.5
Thio-Tepa (2 mg/kg per day)	10	56	10	23	4.9
Synestrol (0.2 mg per day)	11	83	10	13	3.0
Thio-Tepa (2 mg/kg per day) + synestrol (0.2 mg per day)	11	98	11	58	2.3

METHOD

The work was carried out on the 7th and 10th generations of RMK-1 transplanted on female weanlings. Treatment of the tumors began two weeks after transplantation and continued on the average four weeks. Thio-Tepa was injected intraperitoneally in a physiological salt solution in a dose of 2 mg/kg per day and the estrogens (synestrol) subcutaneously as a suspension in a physiological salt solution in a dose, therapeutic for rats, of 0.2 mg per day. The antitumor effect was evaluated by the formula commonly used in chemotherapy.

To confirm the assumed mechanism of action of the combination of Thio-Tepa with synestrol (enhancement of the suppression of the follicle-stimulating function of the hypophysis), in addition to the antitumor effect we investigated the follicle-stimulating function of the hypophysis in experimental rats and the morphology of their ovaries.

The follicle-stimulating activity of the hypophyses of the rats was determined by the change in the weight of the uterus of the infantile mice [2] that had been injected with acetone-dried rat hypophyses as a suspension in a physiological salt solution based on 1 hypophysis per mouse. The results were evaluated as an index representing the ratio of the weight of the uterus and ovaries of mice multiplied by 1000 to their body weight. Five rat hypophyses were tested in each experimental group.

The histological preparations of the ovaries were prepared by the usual method: the ovaries were fixed in a 20% solution of formalin, imbedded in paraffin, and stained with hematoxylin-eosin.

RESULTS

It follows from the table that in the 7th generation with the injection of Thio-Tepa alone or synestrol alone, inhibition of the growth of RMK-1 was respectively 56 and 83% and with their simultaneous injection it increased to 98%, i.e., there were practically no tumors. During subsequent reinoculations a certain decline in the therapeutic effect of Thio-Tepa and synestrol, as well as of their combination, was observed owing to the gradual decrease in the reactivity of RMK-1 with respect to the hormonal effects [9]. For example, in the 10th generation with the combined effect of Thio-Tepa and synestrol, inhibition in the growth of RMK-1 was only 58%.

Changes in the index of the follicle-stimulating activity of the hypophyses in experimental rats were as follows (over-all for the 7th and 10th generations). If after the injection of the hypophyses of control rats with an inoculated tumor the index was 3.5, then after the injection of the hypophyses of rats that received Thio-Tepa alone it increased to 4.9, and after the injection of the hypophyses of rats treated with synestrol alone, it dropped to 3.0. However, after the injection of the hypophyses of rats that received Thio-Tepa in combination with synestrol, the index dropped to 2.3. If we take into account that the control infantile mice which were injected only with a physiological salt solution, the index was 1.8, then it becomes evident that the treatment with Thio-Tepa in combination with synestrol markedly reduces the content of the follicle-stimulating hormone in the hypophysis.

In the histological investigation of the ovaries of rats with tumors treated with Thio-Tepa alone, we did not observe a primary cytotoxic effect on the granulosa cells of the developing follicles, and we could record only a negligible increase in the number of atresic follicles in comparison with the control. More pronounced was the cytotoxic effect in the proliferating cells of the theca interna in which a sparser arrangement of the cells, as well as disintegration of the nuclei and cells, was noted. There is the opinion that the theca tissue of the ovary is the main source of

estrogens [5, 18, 24], consequently its damage from Thio-Tepa apparently causes a reduction in the secretion of estrogens by the ovaries. The interstitial tissue of the ovaries, which is well expressed in rodents and which also secretes estrogens, in the rats treated with Thio-Tepa alone is morphologically functionally active and hardly differs from the control, which in turn can serve as a morphological index of the presence of FSH in the body.

In the ovaries of rats that received synestrol alone, the follicular apparatus changed little. The number of atresic follicles and corpora lutea in them did not differ from the control. At the same time we noted a pronounced suppression of the functional activity of the cells of the interstitial tissue: there was little protoplasm in the cells and it was unvacuolated, which can indicate a drop in the content of FSH in the hypophyses.

In the ovaries of rats that received Thio-Tepa and synestrol, the cytotoxic effect and the degree of atresia of the follicles were similar to the effect of Thio-Tepa alone. However, primordial follicles were almost completely absent and there was very little interstitial tissue, which indicates the absence of FSH in the body.

Thus, the data on the antitumor effect of a combination of Thio-Tepa with estrogens agrees with the data on the change in the follicle-stimulating activity of the hypophyses in these rats and with the morphology of their ovaries. This confirms the hypothesis that the mechanism of the antitumor action of Thio-Tepa in combination with estrogens in mammary cancer is associated with a more pronounced suppression of the production of FSH owing to an increase in the sensitivity of the hypophysial cells to the suppressing action of large doses of estrogens.

It is necessary to point out that if when using Thio-Tepa alone its stimulating and suppressing effect is manifested in tumors, then in combination with synestrol the main seat of the demonstration of the stimulating and suppressing action of both preparations is transferred to the hypophysis, i.e., to an organ whose hormones regulate the proliferative processes in mammary tumors. With such a mechanism of the combined action of Thio-Tepa and synestrol the possibility is not precluded, of course, of a direct cytotoxic action of Thio-Tepa on the tumor cell.

These data permit the conclusion that the combined use of Thio-Tepa and estrogens is advantageous in advanced forms of mammary cancer. At the present time the achievements of combined Thio-Tepa and hormonotherapy of mammary cancer have already been tested and confirmed in the clinic [10, 11, 18-21].

At the same time our data permit us to mention the new possibility of a complex chemo-and hormonotherapy of mammary cancer, assuming that the chemo-preparations can be used in combination with hormones not only as inhibitors of tumor growth, but also as factors sensitizing the function of the endocrine glands regulating the growth of this tumor to the effect of the hormones.

SUMMARY

Treatment of rats with inoculated cancer of the mammary glands (RMK-1) by means of Thio-Tepa (2 mg/kg daily) in combination with hexoestrol (0.2 mg daily) intensified the antiblastic effect, provoked a marked reduction of the follicle-stimulating hormone level in the hypophysis and changes in the ovaries, indicating a reduction of follicle-stimulating hormone in the organism. The mechanism of enhanced antiblastic effect produced by a combination of Thio-Tepa with estrogens in cancer of the mammary gland is associated with a greater depression by estrogens of the follicle-stimulating hypophysis hormone production as one of the hormones determining the proliferative processes in the mammary gland tissue. The data obtained indicate a possibility of a combined chemo-and hormone-therapy, in which the chemopreparations may be used in combination with hormones not only as inhibitors of the tumor growths, but also as stimulants of their growth.

LITERATURE CITED

1. N. S. Artem'eva and L. M. Farutina, Byull. éksper. biol. (1962), No. 1, p. 98.
2. M. G. Belova, Abstracts of the Fourth Summary Scientific Conference of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1961), p. 8.
3. N. G. Blokhina, Transactions of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1960), 2, p. 427.
4. I. M. Valueva, Abstracts of the Fourth Summary Scientific Conference of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1961), p. 16.
5. M. F. Glazunov, Ovarian Tumors [in Russian], Leningrad (1961).
6. G. L. Zhdanov, Transactions of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1960), 2, p. 137.

7. V. P. Konoplev and N. D. Lagova, Byull. éksper. biol. (1960), No. 7, p. 79.
8. N. D. Lagova, Probl. éndokrinol. (1960), No. 5, p. 3.
9. N. D. Lagova, Byull. éksper. biol. (1962), No. 5, p. 108.
10. N. D. Lagova and V. I. Samoilov, Abstracts of the Reports of the Eighth International Anti-Cancer Congress [in Russian], Moscow (1962), p. 341.
11. V. I. Samoilov, Byull. éksper. biol. (1963), No. 1, p. 104.
12. O. V. Svyatukhina, Abstracts of the Fourth Summary Scientific Conference of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1961), p. 80.
13. P. I. Stradyn' and R. K. Tseplite, Klin. med. (1958), No. 7, p. 135.
14. L. M. Farutina, Abstracts of the Reports of the Conference of Young Scientists of the Institute of Experimental and Clinical Oncology, AMN SSSR [in Russian], Moscow (1960), p. 28.
15. V. A. Chernov, A. A. Grushina, and Zh. F. Zakharova, Vopr. onkol. (1959), No. 9, p. 350.
16. J. Bateman, New Engl. j. Med. (1955), V. 252, p. 879.
17. G. Corner, Physiol. Rev. (1938), V. 18, p. 154.
18. R. Grinberg and L. Jaroslavsky, Sem. méd. (B. Aires) (1957), V. 111, p. 715.
19. H. Holden and B. Sundstrup, Med. j. Aust. (1959), V. 2, p. 671.
20. A. Miller, Brit. med. J. (1961), N5226, p. 619.
21. R. Payne and T. L. Whitsett, Proc. Soc. exp. Biol. (N. Y.), (1961), V. 106, p. 189.
22. R. Schell and B. Hall, Surg. Gynec. Obstet. (1958), V. 106, p. 459.
23. M. Wallet, L'hyperfolliculinie, Paris (1946).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
