ACTION OF TAMOXIFEN ON THE REPRODUCTIVE ORGANS OF GUINEA PIGS

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The use of synthetic nonsteroid antiestrogens (in particular, tamoxifen), whose mechanism of action can be mainly explained by blocking of estrogenic receptors in target tissues, is a new tendency in the treatment of diseases of hormone-dependent organs. Tamoxifen has been used successfully in the treatment of breast cancer [3, 6, 8] and, in recent years, in the treatment of carcinoma of the endometrium, the prostate gland, and so on [7, 14]. There are grounds for considering the use of tamoxifen in the treatment of myomas of the uterus [2]. Yet there is virtually no information in the literature on changes in the body arising during prolonged administration of these substances.

The object of this investigation was an experimental study of the effectiveness and mechanism of action of the antiestrogen tamoxifen (from the Research Institute of Pharmaceutical Chemistry, Hungary) in guinea pigs with estrogen-induced benign tumors of the uterus.

EXPERIMENTAL METHOD

Tumors of the uterus which, in their histological structure, were identical with myomas of the human uterus, were induced in guinea pigs by prolonged administration of dihydrostilbestrol [4, 5].

Experiments were carried out on 32 guinea pigs weighing initially 509 ± 9 g. The animals were divided into five groups, three of which were controls (intact guinea pigs, animals receiving tamoxifen or dihydrostilbestrol), and two were experimental (guinea pigs receiving dihydrostilbestrol and tamoxifen simultaneously, or receiving dihydrostilbestrol first, and tamoxifen later). The duration of exposure in groups 2, 3, 4, and 5 was 5 months. In group 4 tamoxifen and dihydrostilbestrol were administered simultaneously from the beginning and until the end of the experiment, in group 5 dihydrostilbestrol was given throughout the experiment, and tamoxifen during the last 2-3 months. Dihydrostilbestrol was given until the end of the experiments, for if it was stopped the tumors induced by it underwent spontaneous regression. The drugs were given daily in the form of a suspension in 5% alcohol in physiological saline: tamoxifen in a dose of 0.4 mg/kg, dihydrostilbestrol in a dose of 1 mg/kg.

During the last (5th) month sex cycles of 22 guinea pigs were studied by the vaginal smear method. At the end of the experiment the animals were decapitated, and after autopsy the uterus and ovaries were removed and weighed. The content of three estrogen receptors (ER) and glucocorticoid receptors (GR) and of common (free and bound) progesterone receptors (PR) in the cytosol from the uterine cornua was determined by methods described previously [1]. The ovaries and fragments of the uterus were fixed in formalin and subjected to microscopic investigation. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Tamoxifen was shown to have no toxic action, as was confirmed by the good general condition of the animals and the absence of pathological changes in their internal organs. As Table 1 shows, the body weight of the guinea pigs increased during administration of the

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TABLE 1. Body Weight, and Weight of Uterus and Ovaries of Guinea Pigs during Long-Term Administration of Tamoxifen and Dihydrostilbestrol (M ± m)

Group No.	Conditions	Number of animals	Weight of			
			body, g		uterus, mg	ovaries, mg
			initial	final		
1	Control (intact animals)	5	545±25	643±36	1220±264	85±3
2	Tamoxifen	10	505±23	645±42	1175±65	93±9
3	Dihydrostilbestrol	7	488±23	463±21	3530±255	98±9
4	Dihydrostilbestrol and tamoxifen together	5	489±30	455±41	2690±343	68±9
5	Dihydrostilbestrol (5 months) and tamoxifen (last 3 months)	5	529±17	519±25	2890±195	91±21

TABLE 2. Content of Cytoplasmic Steroid Hormone Receptors in Uterus of Guinea Pig during Long-Term Administration of Tamoxifin and Dihydrostilbestrol (M ± m)

Group No.	Conditions	Content of receptors, fmoles/mg protein		
		ER	PR	GR
1	Control (intact animals)	389±54	666±130	78,9±28,9
2	Tamoxifen	22,8±5,5	497±80	10,5±3,4
3	Dihydrostilbestrol	1,4±0,9	832±128	19,5±8,9
4	Dihydrostilbestrol and tamoxifen together	2,9±1,1	829±106	18,8±4,4
5	Dihydrostilbestrol (5 months) and tamoxifen (last 3 months)	9,1±0,1	632±73	11,2±3,4

drug by 28%, whereas in the control during the same period it rose by 18% ($P < 0.1$). In animals receiving dihydrostilbestrol, either alone or together with tamoxifen, the body weight decreased by 5-6%, but in group 5 it decreased by 2%.

Tamoxifen is known to have an antiestrogenic action. Under these experimental conditions, during its long-term administration together with dihydrostilbestrol (groups 4 and 5) the weight of the uterus doubled, whereas in animals receiving dihydrostilbestrol alone its weight was trebled compared with the control. Administration of tamoxifen alone had practically no effect on the weight of the uterus in the guinea pig.

Marked hyperplasia and mucoid degeneration of the prismatic epithelium of the body of the uterus and of the stratified squamous epithelium of the vagina, and also hyperplasia of the endo- and myometrium, sometimes ending in the formation of polyps and myomas of the uterus, were discovered. It must be emphasized that the severity of the hyperplastic processes in groups 4 and 5 was significantly less than in group 3 (animals receiving dihydrostilbestrol). Meanwhile, in animals receiving tamoxifen alone, no polyps or tumors of the uterus were observed.

Comparison of the results of weighing the uterus and of histological investigation revealed the antitumor action of tamoxifen.

When tamoxifen was given alone or in combination with dihydrostilbestrol, either simultaneously or successively, anestrus was observed in all the animals, in agreement with the results of the microscopic investigation, which demonstrated the presence of considerable mucus formation in the cytoplasm of cells of all layers of the vaginal epithelium. As a rule no corpora lutea were present in the ovaries of guinea pigs receiving dihydrostilbestrol alone or together with tamoxifen. Administration of tamoxifen alone inhibited ovulation by a lesser degree: corpora lutea were found in the ovaries of 60% of animals receiving tamoxifen alone, but as a rule they were fewer in number than in intact animals. The weight of the ovaries was unchanged in all groups except the 4th.

As Table 2 shows, receptors of all three classes of steroid hormones (estrogens, progesterone, glucocorticoids) were found in the uterine cytosol of the intact animals.

Administration of dihydrostilbestrol, alone or together with tamoxifen, led to a sharp decline in the level of free cytoplasmic ER. If the animals were given tamoxifen for 3-4 months the level of free ER in the cytosol rose considerably higher than in the group receiving dihydrostilbestrol alone, to reach on average 22.8 ± 5.5 fmoles/mg protein.

Administration of tamoxifen or dihydrostilbestrol had no significant effect on the PR content in the uterine cytosol ($P > 0.1$). However, there was a very slight tendency for the PR level to fall in the uterus of guinea pigs receiving tamoxifen alone for long periods, and for it to rise in animals receiving dihydrostilbestrol either alone or together with tamoxifen simultaneously.

In guinea pigs receiving tamoxifen, dihydrostilbestrol, or both of these drugs the mean GR content in the uterine cytosol fell appreciably. During prolonged administration of tamoxifen the GR level was extremely low.

The most widely held view on the mechanism of action of antiestrogens suggests competitive relations between them and ER and the formation of a complex which, retained in the nuclei, blocks synthesis of cytoplasmic ER [10, 11]. The method of determination which we used could reveal only free cytoplasmic ER. The virtual absence of cytoplasmic ER in the uterus of animals receiving dihydrostilbestrol was probably the result of binding of exogenous hormone with the receptors.

Synthesis of PR is known to depend on the presence of ER. A high PR level indicates that functionally active ER must be present in the cells [9, 10]. The low level of free ER in groups 2-5 can probably be explained on the grounds that they could be in the form of a complex with exogenous hormone, and since the total PR level in the uterine cytosol did not differ significantly in all the groups, this suggests that the total ER level must also be quite high.

Prolonged administration of estrogens to guinea pigs is known to cause the development of hyperplasia and benign tumors of the uterus [5], in agreement with the present results. Tamoxifen also is known to inhibit growth of the uterus in laboratory rodents [12]; the antiestrogenic action of the drug is explained under these circumstances by its competition with estrogens for binding with ER in target tissues. The present experiments showed that administration of tamoxifen leads to a decrease in ER, probably due to binding of the drug with the receptors. However, the ER–tamoxifen complex is deficient in biological activity, as is shown by the absence of any increase in weight of the uterus (group 2) and a tendency for the RP level to fall. Similar results were obtained by Koseki et al. [13]. It should be pointed out that in earlier investigations the animals used were ovariectomized or sexually immature, and tamoxifen was administrated for only 5-7 days [10-13]. In the present experiments guinea pigs with intact ovaries were used and they received tamoxifen for a long period — 3-5 months.

The presence of GR in the cytoplasm of the uterus is evidence of possible participation of adrenocortical hormones in regulatory processes of this hormone-dependent organ. The fall observed in the GR level, most marked when tamoxifen was given, suggests that the antitumor action of the drug may be realized through its action not only on ER, but also on GR.

This experimental study thus showed that prolonged administration of tamoxifen has no toxic action on guinea pigs with induced benign uterine tumors, it partially inhibits ovulation in guinea pigs, it inhibits estrogen-induced growth of the uterus and tumors, does not cause the development of polyps or tumors of the uterus, and leads to a sharp decline in the content of free cytyoplasmic ER and GR and a very small decrease in the content of PR.

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