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Effect of Xymedon and Mexidol in Combination with Antineoplastic Drugs on Spermatogenesis Indicators and Functional State of Spermatozoa in Rats with Walker-256 Carcinoma

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We compared the effect of Xymedon (100 mg/kg), Mexidol (50 mg/kg), and their combination on spermatogenesis indicators and functional state of spermatozoa in rats with Walker-256 carcinoma treated with doxorubicin (4 mg/kg) and cyclophosphamide (45 mg/kg) (once intraperitoneally on day 11 after tumor cells transplantation). Xymedon and Mexidol were injected intramuscularly for 10 days starting from day 11 of the experiment. The studied parameters were evaluated on experimental days 14 and 21. We have established that gonadoprotective effect of Xymedon developed gradually and persisted longer than that of Mexidol. It manifested in an increase in the number of epithelial spermatogenesis cells (spermatogonia by 3.2 times, early spermatids by 2.2 times, late spermatids by 2.9 times, and Leydig cells by 4 times) in the testes and also the proportion of viable progressively and non-progressively motile epididymal spermatozoa (by 2 times). The combination of Xymedon and Mexidol stimulated spermatogenesis (with restoration of the initial level of spermatocytes, an increase in the number of early spermatids by 65.5 and 99% in comparison with Xymedon alone and Mexidol alone, respectively) and increased the number of viable epididymal spermatozoa more effectively than Xymedon and Mexidol alone by 54 and 60%, respectively.

Key Words: *Xymedon; Mexidol; cytostatics; spermatogenesis; spermatozoa*

Modern advances in antitumor therapy of malignant neoplasms and an increase in the number of cured patients of reproductive age highlight the problem of maintaining their quality of life, including preservation of fertility [12]. Cytostatics produce pronounced gametotoxic effect that largely depends on the chemical structure determining properties of medications and administration regimen. Spermatogenesis disorders are associated with the ability of antineoplastic drugs to damage rapidly proliferating cells. Thus, alkylating cytostatics possess marked gametotoxicity and can cause long-term azoospermia. Anthracyclines also reduce male fertility, especially in combined chemotherapy [13]. At the same time, cytostatic activate oxidative processes in the testicles, which contributes to the development of testicular dysfunction and suppression of spermatogenesis [7]. ROS accumulation reduces the proliferative and differentiation potential of the spermatogenic epithelium, suppresses testosterone produc-

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tion by Leydig cells, destabilizes the membranes and DNA of spermatozoa thereby impairing their mobility and inducing apoptosis [14]. This necessitates studying of the effectiveness of drugs with antioxidant activity for reducing gonadotoxicity of antiblastomic chemotherapy.

Myeloprotective and cardioprotective effects of pyrimidine (Xymedon) and 3-hydroxypyridine (Mexidol) derivatives during antitumor chemotherapy under experimental conditions were previously shown [9,10]. However, the effects of Xymedon and Mexidol on spermatogenesis and functional state of mature spermatozoa under conditions of antiblastomic chemotherapy remain unstudied.

Here we analyzed the effect of combined use of Xymedon, Mexidol, doxorubicin (DOX), and cyclophosphamide (CP) on changes in the cellular composition of the spermatogenic epithelium and functional state of spermatozoa in rats with Walker-256 carcinoma (W-256).

MATERIALS AND METHODS

The study was carried out on 10-12-week-old male Wistar rats (n=94) weighing 150-250 g (affiliated branch of Stolbovaya nursery, Research Center of Biomedical Technologies, Federal Medical-Biological Agency of Russia). The animals were kept under standard vivarium conditions at natural illumination and free access to water and food (standard ration). All manipulations were carried out in accordance with the rules of the European Convention for the Protection of Vertebrates used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study was approved by the Local ethics committee of the Mordovia State University.

A suspension of spontaneously metastasizing W-256 carcinoma (10^6 cells) was injected under the skin of the tail; the neoplasm was verified histologically by light microscopy. Officinal dosage forms of DOX (Pharmachemie) in a dose of 4 mg/kg and CP (Baxter Oncology) in a dose of 45 mg/kg were administered once intraperitoneally (in isotonic NaCl) on day 11 after tumor cells transplantation. These doses of cytostatics corresponded to 0.4 LD_{50} and were determined in accordance with published data [3,6,9].

Xymedon (N-(2-hydroxyethyl)-4,6-dimethyl-2-dehydropyrimidone) in the form of a substance (Kristall Research Institute) in a dose of 100 mg/kg (10% solution in isotonic NaCl) was injected intramuscularly for 10 days starting from day 11 after tumor cells injection. Mexidol (ethylmethylhydroxypiridine succinate; officinal dosage form 5% solution; Farmasoft) was injected intramuscularly in a dose of 50 mg/kg for 10 days starting from day 11. The applied doses of Xymedon and Mexidol were isotoxic (6% LD_{50}) [2,8] and therapeutically effective [9,10].

The animals were divided into 7 groups (10-14 rats per group): intact animals (group 1); rats with transplanted W-256 carcinoma not receiving treatment (group 2, control); rats with W-256 carcinoma receiving DOX (group 3); rats with W-256 carcinoma receiving DOX+CP (group 4); rats with W-256 carcinoma receiving DOX+CP+Xymedon (group 5); rats with W-256 carcinoma receiving DOX+CP+Mexidol (group 6); rats with W-256 carcinoma receiving DOX+CP+Xymedon+Mexidol (group 7).

The functional state of the reproductive system of male rats was assessed on experimental days 14 and 21 (*i.e.* days 3 and 10 after cytostatic treatment, respectively). The rats were sacrificed (6-7 animals from each group) by cervical dislocation under sodium thiopental anesthesia (50 mg/kg). The suspension obtained from the epididymis in 2 ml of isotonic NaCl was mixed with a rubber tube at room temperature [11]. Spermatozoa were counted in a Goryaev's chamber and the numbers of progressively motile spermatozoa, non-progressive motile spermatozoa, and immobile spermatozoa were counted [1]. The viability of spermatozoa and the number of degenerative cell forms were also assessed [1].

Changes in spermatogenesis were assessed by the quantitative cytological method using smears of the cell suspension of testicular tissues. To this end, 0.1 g tissue was placed in 0.2 ml of isotonic NaCl and the tubules were carefully destroyed by a single turn of a fluoroplastic pistil; then, 0.8 ml isotonic NaCl was added and the cells were stained after Romanovsky—Giemsa). Stained cells were examined under a microscope using oil immersion (×1000). Spermatogenic epithelial cells, Leydig and Sertoli cells (total of 500) were counted, and a spermatogram (percentage of different types of spermatogenic epithelial cells) was compiled. The number of spermatogenic epithelial cells in 1 g of testis tissue in absolute values was calculated using mathematical proportions taking into account the absolute number of spermatozoa counted in the Goryaev's chamber [5].

Statistical analysis was performed using Statistica 6.0 software (StatSoft, Inc.). The arithmetic means (M) and errors of the means (m) were calculated. The significance of differences was estimated using Mann—Whitney U test. The differences were significant at $p<0.05$.

RESULTS

In control rats, a decrease in the level of progressively motile spermatozoa (by 46.2 and 59% on days 14 and 21, respectively, Fig. 1) was accompanied by a de-

crease in their viability by 37.7% on experimental day 21 ($p<0.01$).

DOX administration led to a decrease in the number of progressively motile spermatozoa by 2.5 times ($p<0.01$) and non-progressive motile spermatozoa by 29.8% ($p<0.05$) on the day 14 of the experiment (Fig. 1). The total number of sperm cells decreased from $40.1\pm1.6\times10^6$ to $31.0\pm2.8\times10^6$ per ml (by 23%, $p<0.05$). The level of viable cells decreased by 48% with a 2-fold increase in the number of degenerative forms of spermatozoa (mainly with a tail loop) ($p<0.01$) relative to intact rats. On the day 21 of the experiment, the number of progressively motile spermatozoa decreased by 2.9 times and the number of non-progressive motile spermatozoa decreased by 48% ($p<0.01$) in comparison with intact control. The total number of spermatozoa decreased by 26.5% ($p<0.01$), the proportion of viable cells decreased by 2.7 times ($p<0.001$), and the number of degenerative forms increased by 42% ($p<0.05$).

The administration of DOX in combination with CP led to a decrease in the number of progressively motile spermatozoa by 3.8 times ($p<0.001$) on day 14 relative to intact control. At the same time, the number of non-progressive motile spermatozoa decreased by 41% ($p<0.01$), the proportion of viable cells decreased by 4 times ($p<0.001$), and the number of immobile cells increased by 64% ($p<0.01$) relative to intact rats. The number of degenerative forms of spermatozoa increased by 2 times relative to the intact control ($p<0.01$). On day 21, the number of progressively motile and non-progressive motile spermatozoa decreased by 4.6 and 2.4 times, respectively, in comparison with intact rats ($p<0.001$, Fig. 1). The number of immotile spermatozoa and degenerative forms exceeded the initial levels by 61% ($p<0.05$) and 72% ($p<0.01$), respectively; viability of spermatozoa decreased by 2.6 times ($p<0.001$).

The administration of Xymedon in combination with cytostatics was accompanied by improvement in the morphofunctional properties of spermatozoa: on day 14, the number of viable spermatozoa increased by 2 times ($p<0.01$) in comparison with rats not treated with Xymedon; on day 21, the number of spermatozoa with progressive movement increased by 2.15 times ($p<0.05$) and with non-progressive movement by 2.2 times ($p<0.01$, Fig. 1). The total number of spermatozoa increased by 29% ($p<0.01$) and the number of viable spermatozoa increased by 70% ($p<0.05$) relative to the group treated with cytostatics alone.

Similar changes were observed in animals treated with Mexidol: on day 14, we observed an increase in the number of viable spermatozoa by 73% and a decrease in the number of degenerative forms of cells by 44% relatively to rats receiving cytostatics without

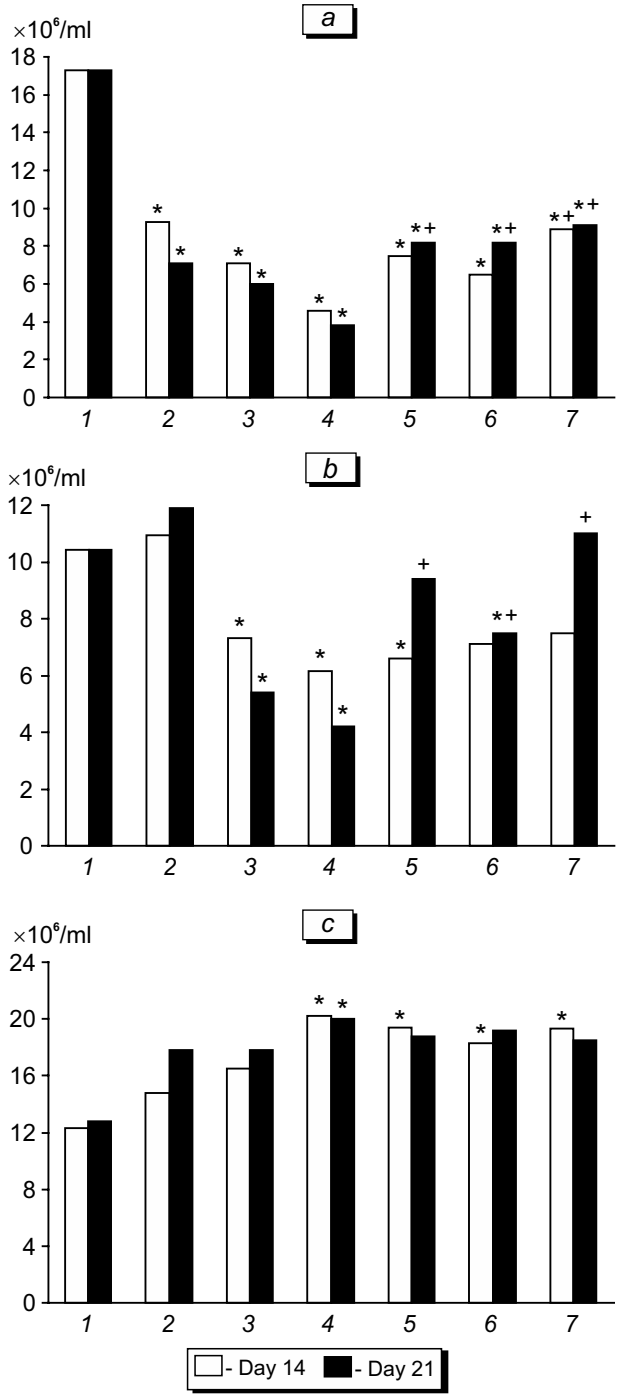

Fig. 1. The effect of combined treatment with Xymedon, Mexidol, DOX, and CP on a number of progressively motile (*a*), non-progressive motile (*b*), and immobile spermatozoa (*c*) in rats with W-256 carcinoma. *1*) Intact rats; *2*) W-256 carcinoma; *3*) W-256+DOX; *4*) W-256+DOX+CP; *5*) W-256+DOX+CP+Xymedon; *6*) W-256+DOX+CP+Mexidol; *7*) W-256+DOX+CP+Xymedon+Mexidol. $p<0.05$ in comparison with *intact rats, ⁺therapy with DOX and CP.

Mexidol ($p<0.05$). By the day 21, the number of progressively motile spermatozoa increased 2.15 times ($p<0.05$) and non-progressive motile spermatozoa by

79% ($p<0.01$, Fig. 1). At the same time, we observed an increase in the total number of spermatozoa by 25%, sperm viability by 66%, and a decrease in degenerative cell forms by 31% ($p<0.05$) in comparison with the group treated with cytostatics only.

Combined treatment with Xymedon and Mexidol led to a 2-fold increase in the number of spermatozoa with progressive movement ($p<0.05$, Fig. 1) in comparison with rats receiving only cytostatics as soon as on day 14 of the experiment, the total number of spermatozoa remained at the level of intact control. The number of viable spermatozoa increased by 3 times ($p<0.001$) and the number of degenerative forms a decreased by 44% ($p<0.05$) relative to group treated with cytostatics alone. On day 21, the number of spermatozoa with progressive and non-progressive movement increased by 2.4 and 2.6 times ($p<0.01$), respectively; cell viability increased by 2.5 times ($p<0.001$) and the number of degenerative forms of spermatozoa decreased by 28.7% ($p<0.05$) in comparison with the group receiving cytostatics alone.

The tumor process in male rats receiving no treatment was accompanied by impaired spermatogenesis: on day 14, the number of spermatogonia in the testicles significantly decreased by 2.2 times, early and late spermatids by 32 and 58.6%, respectively, and Leydig cells by 2.2 times ($p<0.05$) relative to intact rats (Table 1). On day 21, a similar picture was observed.

The administration of DOX was accompanied by a significant decrease in the number of spermatogonia in the testicles by 4.3 times, early and late spermatids by 2.5 times, and Leydig cells by 6.7 times on day 14 of the experiment relative to intact control (Table 1). On day 21, the number of spermatogonia decreased by 7.5 times, spermatocytes by 35.5%, early and late spermatids by 3 times, and Leydig cells by 12 times relative to the initial parameters.

The administration of DOX+CP led to more significant disturbances in spermatogenesis on day 14 of the experiment: the number of spermatogonia decreased by 17.4 times ($p<0.01$), spermatocytes by 2.6 times ($p<0.001$), early and late spermatids by 6.3 and 4 times, respectively ($p<0.01$), Leydig cells by 23 times ($p<0.01$), and spermatozoa by 21.3% ($p<0.05$) relative to intact control. On day 21, the number of spermatogonia decreased by 20 times ($p<0.01$), spermatocytes by 3 times ($p<0.001$), early and late spermatids by 7.5 and 4.3 times ($p<0.01$), Leydig cells by 47 times ($p<0.01$), spermatozoa by 34.5% ($p<0.01$, Table 1).

The administration of Xymedon in combination with cytostatics led on day 14 of the experiment to an increase in the number of early spermatids by 88% and late spermatids by 2.2 times ($p<0.05$) in comparison with the group receiving antitumor agents only. On day 21, more positive dynamics was observed: the number of spermatogonia significantly increased by 3.2 times ($p<0.01$), early and late spermatids by 2.2 and 2.9 times ($p<0.05$), Leydig cells by 4 times ($p<0.01$), spermatozoa by 51% (to the initial level) in comparison with the group receiving cytostatics (Table 1).

Administration of Mexidol in combination with cytostatics led to an increase in the number of spermatogonia in the testicular tissue by 3.5 times ($p<0.05$), spermatocytes, early and late spermatids by 2 times ($p<0.05$), spermatozoa by 36% ($p<0.05$) (to an intact level) on day 14 of the experiment relative to rats receiving only cytostatics (Table 1). It is noteworthy that the number of spermatocytes was 49.4% ($p<0.05$) higher than that in rats receiving Xymedon. However, on day 21, we observed a deterioration in the dynamics of spermatogenesis parameters, and they did not differ from those in rats who received a combination of cytostatics without Mexidol (Table 1).

Combined use of Xymedon and Mexidol was accompanied by an increase in the number of spermatogonia by 2.6 times, early and late spermatids by 2.4 and 1.7 times, Leydig cells by 2 times on day 14 of the experiment in comparison with rats receiving a combination of cytostatics without Xymedon and Mexidol (Table 1). The number of spermatozoa in the testicular tissue increased by 31.6% and did not differ from the level of intact control. On day 21, the number of spermatogonia was higher by 3.7 times ($p<0.05$) than in the group treated with cytostatics alone, the number of spermatocytes was higher by 2.1 times ($p<0.01$), early and late spermatids by 3.6 and 2.1 times, respectively ($p<0.05$), and Leydig cells by 5.6 times ($p<0.01$). The number of sperm in the testicular tissue increased by 83% and did not differ from the level of intact control. The number of early spermatids was higher by 81 and 74% ($p<0.01$) than in groups with separate administration of Xymedon and Mexidol, respectively. The number of Leydig cells was higher by 2.6 times ($p<0.05$) than that in the group with Mexidol (Table 1).

Thus, significant inhibition of spermatogenesis at all terms of the study after administration of DOX and CP was accompanied by significant disturbances in the morphofunctional characteristics of mature epididymal spermatozoa in experimental rats.

Mexidol, in contrast to Xymedon, on day 14 of the experiment (day 3 after cytostatics administration) reduced the number of degenerative forms of epididymal spermatozoa and preserved the number of non-progressive motile cells, and also prevented suppression of spermatogenesis by increasing the number of spermatogonia (by 3.5 times) and spermatocytes (by 2 times) in the testis of rats with W-256 carcinoma receiving DOX+CP.

The combination of Xymedon and Mexidol, in contrast to their separate use, on day 14 of the experi-

TABLE 1. Effect of Combined Use of Xymedon, Mexidol, DOX, and CP on Cell Composition of Spermatogenic Epithelium in Rats with W-256 Carcinoma (*M*±*m*)

Group		Spermato-gonia	Spermato-cytes	Spermatids		Spermato-zoa	Leydig cells
				early	late		
Intact rats (*n*=10)		16.1±3.2	58.3±5.0	108.8±9.6	54.9±10.5	55.0±3.8	8.1±1.5
W-256 (control) (*n*=14)	day 14	6.9±0.9	48.2±9.3	73.5±6.2	22.7±3.7	57.8±5.2	3.6±0.5
		p_1<0.05		p_1<0.05	p_1<0.05		p_1<0.05
	day 21	5.6±1.4	53.0±5.1	87.8±18.1	40.4±5.6*	56.4±3.0	3.0±0.9
		p_1<0.05					p_1<0.05
W-256+DOX (*n*=14)	day 14	3.7±0.5	45.8±7.1	44.8±6.0	20.8±3.9	51.0±7.0	1.2±0.3
		$p_{1,2}$<0.05	p_1<0.01	$p_{1,2}$<0.05	p_1<0.05		$p_{1,2}$<0.01
	day 21	2.1±0.4*	37.6±6.0	34.3±4.7	19.0±4.0	44.0±3.7	0.7±0.1
		p_1<0.01	p_1<0.05	$p_{1,2}$<0.05	$p_{1,2}$<0.05	p_2<0.05	p_1<0.01
W-256+DOX+CP (*n*=14)	day 14	0.92±0.10	22.6±4.9	17.2±3.2	13.6±0.9	43.3±3.9	0.3±0.1
		p_{1-3}<0.01	$p_{1,3}$<0.05	p_{1-3}<0.01	p_1<0.01	$p_{1,2}$<0.05	p_{1-3}<0.05
	day 21	0.8±0.2	21.8±5.5	14.4±4.0	13.0±3.4	36.0±3.0	0.17±0.07
		p_{1-3}<0.05	$p_{1,2}$<0.01	p_{1-3}<0.05	$p_{1,2}$<0.01	$p_{1,2}$<0.01	p_{1-3}<0.05
W-256+DOX+CP+ Xymedon (*n*=14)	day 14	1.7±0.5	31.4±4.0	32.5±3.9	29.4±5.4	47.0±6.6	0.30±0.06
		p_{1-3}<0.05	p_1<0.05	$p_{1,2}$<0.001	p_4<0.05		p_{1-3}<0.05
				p_4<0.05			
	day 21	2.7±0.3	29.0±2.3	31.6±3.2	37.7±9.7	53.0±2.5	0.7±0.1*
		$p_{1,4}$<0.01	$p_{1,2}$<0.01	$p_{1,2,4}$<0.05	p_4<0.05	p_4<0.05	$p_{1,4}$<0.05
W-256+DOX+CP+ Mexidol (*n*=14)	day 14	3.2±0.8	46.9±5.2	35.5±4.5	28.2±5.2	59.0±4.0	0.5±0.1
		p_1<0.01	$p_{4,5}$<0.05	$p_{1,2}$<0.01	p_4<0.05	p_4<0.05	$p_{1,2}$<0.01
		$p_{2,4}$<0.05		p_4<0.05			
	day 21	1.4±0.2	30.0±5.3*	26.3±5.9	19.3±3.3	52.0±6.8	0.37±0.1
		p_1<0.01	$p_{1,2}$<0.05	$p_{1,2}$<0.05	$p_{1,2}$<0.05		$p_{1,2}$<0.05
		$p_{2,5}$<0.05					
W-256+DOX+CP+ Xymedon+Mexidol (*n*=14)	day 14	2.4±0.3	32.4±2.8	41.5±5.1	22.5±1.7	57.0±4.6	0.7±0.1
		$p_{1,2,4}$<0.01	p_1<0.01	$p_{1,2,4}$<0.01	$p_{1,4}$<0.05	p_4<0.05	$p_{1,2}$<0.01
							$p_{4,5}$<0.05
	day 21	3.2±0.8	45.7±4.4*	52.3±4.1	27.5±4.4	66.0±4.0	0.96±0.2
		$p_{1,4}$<0.05	p_{4-6}<0.05	p_1<0.001	p_4<0.05	p_{3-5}<0.05	$p_{1,4}$<0.01
				p_{3-6}<0.05			p_6<0.05

Note. Subindexes 1-6 show significant differences from the corresponding group; *p<0.05 in comparison with day 14 of the experiment.

ment, increased the number of epididymal spermatozoa with progressive movement (by 93.5%), as well as the number of Leydig cells (by 2 times) in the testicular tissue after combined treatment with DOX+CP.

Later, on day 21 of the experiment (day 10 after cytostatics administration), the efficiency of Xymedon and Mexidol in improving the morphofunctional characteristics of epididymal spermatozoa was comparable. At the same time, Xymedon, unlike Mexidol, promoted stimulation of spermatogenesis by increasing the number of spermatogonia (by 3.2 times), early and late spermatids (by 2.2 and 2.9 times, respectively), as well as Leydig cells (by 4 times) after combined administration of DOX and CP.

The combination of Xymedon and Mexidol more effectively than their individual administration restored the number of viable epididymal spermatozoa (by 54 and 60% relative to Xymedon and Mexidol separately, respectively), increased the number of spermatocytes (by 58 and 52%), early spermatids (by 65.5 and 99% relative to Xymedon and Mexidol, respectively) in the testes, which can attest to more rapid and efficient

restoration of spermatogenesis. Thus, gonadoprotective effect of Mexidol under conditions of chemotherapy with DOX and CP is characterized by rapid development, but a short duration, while the effect of Xymedon is characterized by gradual development and greater stability, which is probably due to features in the mechanism of action of drugs affecting different links of cytoprotection.

Thus, gonadoprotective effect of Xymedon and Mexidol during antitumor therapy with DOX and CP is realized due to not only improvement of the morphofunctional state of mature spermatozoa, but also acceleration of effective restoration of spermatogenesis. These findings extend our understanding of the pharmacodynamics of Mexidol and Xymedon also on the greater efficiency of their joint use in comparison with separate. The efficiency of combined use was higher than the efficiency of individual administrations of these drugs.

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