REFERENCES

1. D. A. Ambruster, *Clin. Chem.*, **39**, 81-95 (1993).
2. W. J. Catalona, D. S. Smith, and T. L. Ratliff, *N. Engl. J. Med.*, **324**, 1156-1161 (1991).
3. E. H. Cooper, *Scand. Lab. Invest. Suppl.*, **206**, 42-51 (1991).
4. H. Lilja, *Urol. Clin. North Am.*, **20**, 42-46 (1993).
5. A. A. Luderer, Y.-T. Chen, T. F. Soriano, *et al.*, *Urology*, **46**, 187-194 (1995).
6. R. T. McCormack, T. J. Wang, H. G. Rittenhouse, *et al.*, *Ibid.*, **45**, pp. 729-744.
7. J. E. Oesterling, *J. Urol.*, **145**, 907-923 (1991).
8. T. A. Stamey, H. Yang, A. R. Hay, *et al.*, *N. Engl. J. Med.*, **317**, 909-916 (1987).
9. T. J. Wang, T. M. Till, R. L. Sokoloff, *et al.*, *Prostate*, **28**, 10-16 (1996).
10. M. C. Wang, L. A. Valenzuela, G. P. Murphy, *et al.*, *Invest. Urol.*, **17**, 159-163 (1979).

Relationship between Tobacco Smoke and Uterotropic Effect of Estrogen

L. M. Bershtein, E. V. Tsyrlina, O. G. Kryukova, S. V. Dzhumasultanova, and O. S. Kolesnik

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 9, pp. 331-333, September, 1997
Original article submitted January 17, 1997

Female rats aged 3-3.5 months were daily exposed for 2 h to 2.25% tobacco smoke in a 20-liter chamber for 3 weeks. A week after the beginning of the experiments, all rats were subjected to bilateral oophorectomy. Some of them were injected with estradiol in a daily dose of 2 μg or diethylstilbestrol in a daily dose of 1 μg for 10 days. Tobacco smoke exposure led to an increase in the uterus weight in oophorectomized rats, and combined exposure to tobacco smoke and estrogens resulted in an increase in the proportion of intact (twisted) DNA in the uterus. These results can be explained by a relatively short term of tobacco smoke exposure (stimulation stage).

Key Words: *estrogens; uterus; DNA; tobacco smoke*

Tobacco smoke is an environmental factor which affects both the endocrine system and increases the incidence of some malignant tumors. In addition to the risk of cancer, females with a many-year history of tobacco smoking earlier develop the menopause and intense postmenopausal osteoporosis; the incidence of endometriosis, fibromyoma, and cancer of the corpus uteri is decreased in smoking women [1,3]. These signs of estrogen deficiency are not supported by data on blood estrogen levels, which are, as a rule, normal in the female tobacco smokers [6]. There are reports about increased 2-hydroxylation of estrogens in intense tobacco smoking [9]. On the other hand, accumulation of 2-hydroxyestrogens prevents inactivation (methoxylation) of 4-hydroxyestradiol and 4-hydroxyestrone, which increases the total genotoxic (DNA-damaging) potential of estrogens [8]. Clinical manifestations of estrogen insufficiency in smoking women, the spectrum of tumors they develop, and the important role of estrogen genotoxicity in the induction of neoplastic transformation [8] led us to a hypothesis that tobacco smoke attenuates the specific hormonal effects of estrogens and increases the estrogen capacity to damage the DNA [1,4]. In this study we tested this hypothesis, which is important for analysis of the mechanisms of hormonal carcinogenesis, and other estrogen-dependent processes [1,4].

MATERIALS AND METHODS

Experiments were carried out on 3-3.5-month female rats from the *Rappolovo* breeding center. At the be-

N. N. Petrov Institute of Oncology, Ministry of Health of Russia, St. Petersburg

0007-4888/97/0009-0914$18.00

ginning of experiment, the rats weighed 140-160 g. During the first week, half of the animals (each group consisted of 4-6 females) were exposed to tobacco smoke in a 20-liter plastic chamber. The smoke was collected from a Prima cigarette (class V, 29 mg tobacco resin and 1.3 mg nicotine per cigarette) with Janet's syringe and rapidly pumped into the chamber through special opening, which was hermetically sealed after creating a 2.25% concentration of tobacco smoke. The animals were left in this atmosphere for 10 min, after which the chamber was aired for 3-4 min and then a new portion of smoke was let in for 10 min. The total duration of such "smoking" was 120 min a day.

On day 8 of experiment, after measuring body weight, all animals (exposed to tobacco smoke and sham-exposed) were subjected to bilateral oophorectomy under ether narcosis. On the same day, the animals were divided into 4 groups: 1) controls, 2) exposed to tobacco smoke, 3) to estrogens, and 4) to tobacco smoke+estrogens. Rats of groups 2 and 4 were exposed to tobacco smoke for 10 and more days. To animals of groups 3 and 4, estradiol (Sigma) in a daily dose of 2 μg or diethylstilbestrol (Aldrich) in a daily dose of 1 μg was injected at 11:00-12:00. On day 19 of experiment, the animals were weighed and sacrificed by chloroform overdose. The uteruses were separated from the adjacent tissues, weighed, and kept in liquid nitrogen until measuring progesterone receptors (as estrogen-inducible proteins) and degree of DNA twisting (as a parameter characterizing liability to DNA chain rupture) [11]. The content of progesterone receptors was assessed by the carbon-dextrane radioligand method [10]. DNA twisting in uterine tissue homogenates was assessed by fluorimetry by the method of H. Birnboim and J. Jevcak modified by N. I. Ryabchenko *et al.* [2]. A total of 79 rats were used in 2 experiments. The results were processed by the parametrical method based on the Student's t and P tests.

RESULTS

Exposure of rats to tobacco smoke decelerated body weight increment after oophorectomy similarly as estrogen injection. A moderate weight-reducing effect of passive tobacco smoking was observed as early as during the first week of experiment, i.e., before oophorectomy (Table 1), and therefore, it should be regarded as a reaction not mediated by estrogens. Unexpectedly, tobacco smoke slightly stimulated the increment of the weight of the uterus in rats administered no estrogens (Table 1), which is confirmed by significantly ($p<0.05$) increased ratio of uterine (mg) to body (g) weight in group 2 (0.47±0.01) in comparison with the control (0.43±0.01). Although exposure to tobacco smoke resulted in a decrease in the uterus weight in estradiol-treated rats (244±14 mg *vs.* 298±24 mg in group 3, $p=0.06$), in general tobacco smoking did not influence the estrogen-induced increase in the uterus (Table 1).

Despite certain but insignificant differences between the groups of animals injected estradiol or diethylstilbestrol, a similar conclusion can be drawn regarding the receptor-inducing capacity of estrogens: in our experiments, exposure of rats to tobacco smoke did not modify this parameter. On the other hand, combined exposure to tobacco smoke and estradiol/diethylstilbestrol led, unexpectedly, to an increase in the content of twisted DNA in the uterus in comparison with the animals not exposed to tobacco smoke (Table 2).

TABLE 1. Body and Uterus Weight in Experimental Animals ($M\pm m$)

Group	Body weight, g				Weight of uterus, g
	initial	before oophorectomy	end of experiment	changes throughout experiment	
Control (n=20)	146.2±1.8	158.6±2.1	194.0±3.0	47.8±4.7	74.7±2.8
Tobacco smoke (n=19)	149.4±2.1	154.2±1.8	174.3±2.1*	24.9±2.9*	80.2±2.5
Estradiol (n=4)	159.0±1.2	174.0±6.1	191.0±7.3	32.0±5.7*	298.0±24.0*
Diethylstilbestrol (n=16)	150.2±3.1	160.0±3.1	173.0±5.3*	22.8±3.2*	223.3±8.0*
Tobacco smoke+estradiol (n=4)	150.0±4.9	151.0±3.6	165.0±3.6*	15.0±5.2*	244.0±14.0*
Tobacco smoke+diethylstilbestrol (n=16)	154.3±2.1	160.0±2.1	176.0±2.0*	21.7±3.4*	236.4±6.0*
Estrogens (estradiol+diethylstilbestrol) (n=20)	152.0±2.6	162.8±2.6	176.6±4.4*	24.6±2.7*	238.2±6.7*
Tobacco smoke+estrogens (estrogen+diethylstilbestrol) (n=20)	153.4±1.8	158.2±1.8	173.8±1.7*	20.4±2.9*	237.9±5.7*

Note. *$p<0.05$ *vs.* the control.

TABLE 2. Content of Progesterone Receptors and DNA Twisting in the Rat Uterus (*M±m*)

Group	Progesterone receptors, fmol/mg protein	DNA twisting (proportion of intact twisted DNA), %
Control	90.4±13.5 (8)	15.7±3.7 (4)
Tobacco smoke	91.0±18.0 (8)	15.7±2.5 (4)
Estradiol	165.0±66.0 (2)	11.5±1.5 (2)
Diethylstilbestrol	203.7±31.3* (6)	15.8±0.8 (5)
Tobacco smoke+estradiol	216.0±89.0 (2)	22.0±3.0 (2)
Tobacco smoke+diethylstilbestrol	171.8±44.7 (6)	19.8±3.2 (5)
Estrogens (estradiol or diethylstilbestrol)	194.0±23.4* (8)	14.6±1.1 (7)
Tobacco smoke+estrogens (estrogen+diethylstilbestrol)	182.9±33.5* (8)	20.4±2.3** (7)

Note. Number of measurements is given in parentheses; uteruses of several animals were united for some measurements if necessary. $p<0.05$: *vs. the control, **vs. the estrogen group.

Thus, instead of the expected [1,4] alleviation of the specific (uterotropic) effect of estrogens and increased DNA damage caused by tobacco smoke, we observed its moderate stimulating effect on the weight of the uterus in oophorectomized rats and an increase in the proportion of intact (twisted) DNA in uterine tissue of estrogen-treated rats. Before trying to explain these seemingly paradoxical results, we should like to note that the mean content of twisted DNA in the uterus of oophorectomized rats is almost two times lower than in the liver of intact rats [11] and that the choice of estrogen dose in our experiments was impeded by the "divergence" of the purpose: the chosen concentration could be too high for assessing the modifying effect of tobacco smoke on the uterotropic effect of estrogens [7] but insufficient for inducing DNA injuries [5]. We cannot neglect the fact that body weight loss under the effect of tobacco smoke could partially reflect its toxic action.

However, our data are not accidental and can be interpreted as follows. In our previous studies of hormonal and neuroendocrine parameters in tobacco smokers aged under 40-50 years we revealed more signs of activation of these functions than of their abatement, whereas in many-year tobacco smokers aged over 50-60 years an opposite effect of tobacco smoking on these parameters was observed. We denoted this regularity as the "stimulation-depression principle" [1]; it can be applied with a high degree of probability to the interpretation of the present results; the short term of passive tobacco smoking and can be regarded as the "stimulation stage." Further long-term experiments will confirm or reject this assumption; it will be then possible to assess the role of time factor and animal age in the modifying action of tobacco smoke on uterotropic effect of estrogens.

This study was financed by the Russian Foundation for Basic Research (grant No. 97-04-48022).

REFERENCES

1. L. M. Bershtein, *Oncoendocrinology of Smoking* [in Russian], St. Petersburg (1995).
2. N. I. Ryabchenko, S. Ya. Proskuryakov, B. R. Ivannik, and A. I. Kutmin, *Radiobiologiya*, **24**, 154-157 (1984).
3. J. A. Baron and E. R. Greenberg, *Semin. Reprod. Endocrinol.*, **7**, 335-343 (1989).
4. L. M. Berstein, *Cancer J.*, **9**, 106 (1996).
5. Z. Han and J. G. Liehr, *Carcinogenesis*, **15**, 997-1000 (1994).
6. T. J. Key, M. C. Pike, J. A. Baron, *et al.*, *J. Steroid Biochem.*, **39A**, 529-534 (1991).
7. S. Li, C. Martel, S. Dauvois, and F. Labrie, *Endocrinology*, **134**, 1352-1357 (1994).
8. J. G. Liehr, M. J. Ricci, C. R. Jefcoate, *et al.*, *Proc. Natl. Acad. Sci. USA*, **92**, 9220-9224 (1995).
9. J. J. Michnovicz, R. J. Hershcopf, N. P. Hally, *et al.*, *Metabolism*, **38**, 537-541 (1989).
10. M. Saez, P. Martin, and C. D. Chouvet, *Cancer Res.*, **38**, 3468-3473 (1978).
11. M. Taningher, R. Bordone, P. Russo, *et al.*, *Anticancer Res.*, **7**, 669-680 (1987).