PHARMACOLOGY AND TOXICOLOGY

Effect of Afobazole on Genotoxic Effects of Tobacco Smoke in the Placenta and Embryonic Tissues of Rats

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The DNA comet assay was used to evaluate the severity of genotoxic changes in embryonic tissues and placenta of rats daily exposed to tobacco smoke *per se* or in combination with an anxiolytic agent afobazole. The exposure to tobacco smoke (4 cigarettes containing 13 mg tar and 1 mg nicotine per 72 dm^3) for 20 min on days 1-13 of pregnancy increased the degree of DNA damage and elevation of apoptotic DNA comets in cells of the placenta and embryo from pregnant rats. Afobazole (1 and 10 mg/kg orally) reduced the genotoxic effect of tobacco smoke and decreased the amount of apoptotic DNA comets in placental tissue and embryonic tissue from rats.

Key Words: *tobacco smoke; embryo; DNA damages; antigenotoxic effects; afobazole*

There is a strong evidence that smoking and/or tobacco combustion products produce the genotoxic and mutagenic effects on people (including nonsmoking pregnant women) [4,9]. This problem was recently reviewed [3,7]. Published data indicate that many substances (*e.g.*, medical products) exhibit antimutagenic properties [5]. They include anxiolytic drug afobazole, which produced the antigenotoxic and antiteratogenic effect in experiments with cyclophosphamide [2].

Here we studied the severity of genotoxic changes in embryonic tissues and placenta of rats that were regularly exposed to tobacco smoke during pregnancy. The efficiency of afobazole in modulating these processes was evaluated.

MATERIALS AND METHODS

Experiments were performed on female outbred albino rats weighing 200-250 g. The animals were maintained in a vivarium (Research Laboratory, V. V. Zakusov Institute of Pharmacology) with the 12:12-h light/dark cycle and had free access to water and food (standard pelleted food). Each group consisted of 4-6 specimens.

Males and females were housed in pairs in individual cages. The day of detection of spermatozoa in vaginal smears from rats was considered as day 1 of pregnancy. On days 1-3 of pregnancy, the animals were exposed to an atmosphere of tobacco smoke. They were maintained in plastic chambers (60×40×30) equipped with a special device.

Five animals were placed into each chamber. Tobacco smoke of 4 filtered cigarettes (13 mg tar and 1 mg nicotine) was successively blown into the chamber for 5 min to produce smoke. Then the animals were maintained in closed chambers for 15 min. The in-chamber air was continuously fanned over a 20-min period. Control animals were subjected to the same procedure with no delivery of tobacco smoke. The animals were removed from this chamber after 20 min and maintained in a vivarium under standard conditions.

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An aqueous solution of afobazole (1 and 10 mg/kg) was given *per os* immediately before the procedure of forced smoking. On day 13 of pregnancy, the animals were killed by cervical dislocation (30 min after smoking). Each group consisted of 5 animals.

Female rats (n=4) receiving cyclophosphamide (20 mg/kg, 20 h) on day 13 of pregnancy were used as a positive control.

DNA damage in cells of the placenta and embryos was studied by the DNA comet assay (alkaline method) according to the recommendations [1].

After autopsy, 4 placentas and 4 embryos were taken from each female. Each embryo was cut to separate the head and body (except for embryos of the positive control group). The cell suspension of the embryonic head, embryonic body, and placenta was placed in tubes with 1% low melting point agarose, placed on agarose-coated slides, covered with a cover glass, and maintained on ice. After solidification of agarose, the glasses were put in a lysing buffer of 10 mM Tris-HCl (pH 10), 2.5 M NaCl, 100 mM EDTA-Na_2, 1% Triton X-100, and 10% DMSO and incubated at 4°C for at least 1 h. By the end of lysis, these samples were put in a chamber with electrophoresis buffer (300 mM NaOH and 1 mM EDTA-Na_2, pH>13) and incubated for 20 min. Electrophoresis was conducted for 20 min (field strength 1 V/cm, current strength ~300 mA). After electrophoresis, the samples were fixed in 70% ethyl alcohol for 20 min, dried, and stored at room temperature.

The samples were stained with SYBR Green I (1:10,000 in TE-buffer; Invitrogen). They were examined under a Mikmed-2 12T epifluorescence microscope (Lomo) equipped with a high-performance digital camera (VEC-335; EVS) at a magnification of ×200. Digital images of DNA comets were obtained from microscopic slides and analyzed with CASP v. 1.2.2 software. At least 100 DNA comets were analyzed in each sample of the placenta, embryonic head, and embryonic body. The percentage of comet tail DNA (% tail DNA) was used as a criterion of DNA damage. DNA comets with a tail DNA percentage of more than 50% were classified into a special group. They were usually presented by DNA comets with a wide diffuse tail and nearly undetected head (apoptotic comets). The percentage of apoptotic DNA comets was estimated by visual examination. The results were analyzed by Student's t test.

RESULTS

We studied the effect of afobazole on genotoxic effects of passive smoking in cells of the placenta and embryo from experimental animals (Table).

The degree of spontaneous DNA damage in placental cells from control animals was 2.2±0.2% tail DNA. This parameter in cells of the embryonic head and body was 2.8±0.6 and 3.6±0.9%, respectively. Administration of cyclophosphamide to animals of the positive control group was followed by a significant increase in the degree of DNA damage in placental cells (23.7±3.1% tail DNA) and embryonic cells (16.0±3.8% tail DNA).

In animals exposed to tobacco smoke, the degree of DNA damage in the placenta significantly increased (14.9±2.9% tail DNA). These changes were accompanied by an increase in the degree of DNA damage in cells of the embryonic head (by 4.2 times, 11.6±0.9% tail DNA) and body (by 3.4 times, 12.4±1.5% tail DNA).

Administration of afobazole in a dose of 1 mg/kg reduced the degree of tobacco-smoke induced DNA damage in the placenta, embryonic head, and embryonic body. The percentage of tail DNA in embryos was decreased and did not differ from that in animals of the negative control group (cells of the embryonic head: 3.7±0.7 and 2.8±0.6%, respectively; cells of the

TABLE 1. Effect of Afobazole (AF) on DNA Damage in Cells of the Placenta and Rat Embryos after Exposure to Tobacco Smoke (TS; M±SD)

Group	n/n_1			
	percentage of tail DNA	placenta	embryonic head	embryonic body
Control	5/20	2.2±0.2	2.8±0.6	3.6±0.9
Cyclophosphamide, 20 mg/kg	4/16	23.7±3.1*	16.0±3.8$^+$	
TS	5/20	14.9±2.9*	11.6±0.9$^+$	12.4±1.5*
TS+AF, 1 mg/kg	5/20	5.2±0.7^x	3.7±0.7^x	4.1±1.0^x
TS+AF, 10 mg/kg	5/20	6.8±0.4^x	5.2±0.7^x	6.6±0.6^x

Note. Here and in Table 2: n/n_1, number of animals/embryos. p<0.001: *compared to the control; $^+$compared to the control (comparison of mean values for the embryonic head and body); ^{x}compared to TS.

TABLE 2. Effect of Afobazole (AF) on the Level of Apoptotic DNA Comets in Cells of the Placenta and Rat Embryos after Exposure to Tobacco Smoke (TS; *M*±*SD*)

Group	n/n_1			
	percent of apoptotic DNA comets	placenta	embryonic head	embryonic body
Control	5/20	3.0±1.2	1.1±0.2	1.9±0.8
Cyclophosphamide, 20 mg/kg	4/16	40.3±3.4*	8.5±3.6+	
TS	5/20	21.8±6.4*	10.9±3.0*	11.7±3.3*
TS+AF, 1 mg/kg	5/20	3.4±0.9x	0.6±0.3x	1.5±0.5x
TS+AF, 10 mg/kg	5/20	6.9±3.2x	1.3±0.6x	2.7±0.5x

embryonic body: 4.1±1.0 and 3.6±0.9%, respectively). The degree of tobacco-smoke induced DNA damage in placental cells was reduced by 2.9 times under the influence of afobazole.

Afobazole in a dose of 10 mg/kg was also potent in reducing the degree of tobacco-smoke induced DNA damage. We revealed a decrease in the percentage of tail DNA in placental cells (from 14.9±2.9 to 6.8±0.4%) and cells of the embryonic head (from 11.6±0.9 to 5.2±0.7%) and body (from 12.4±1.5 to 6.6±0.6%).

We studied the effect of afobazole on tobacco smoke-induced apoptotic DNA comets in the placenta and embryos of experimental animals (Table 2).

In control animals, the ratio of apoptotic DNA comets in cells of the placenta, embryonic head, and embryonic body was 3.0±1.2, 1.1±0.2, and 1.9±0.8%, respectively. Administration of cyclophosphamide was followed by an increase in the ratio of apoptotic DNA comets in placental and embryonic cells to 40.3±3.4 and 8.5±3.6%, respectively. The ratio of apoptotic DNA comets was significantly increased in samples of the placenta, embryonic head, and embryonic body from tobacco smoke-exposed animals (21.8±6.4, 10.9±3.0, and 11.7±3.3%, respectively).

The ratio of apoptotic DNA comets in cells of the placenta, embryonic head, and embryonic body from animals receiving afobazole (1 mg/kg) and exposed to tobacco smoke was 3.4±0.9, 0.6±0.3, and 1.5±0.5%, respectively. No significant differences were found between these animals and control rats. These data attest to a decrease in the percentage of apoptotic DNA comets to a level observed in the negative control level.

Afobazole in a dose of 10 mg/kg also reduced the percentage of tobacco smoke-induced apoptotic DNA comets. The percent of apoptotic DNA comets in placental cells decreased by more than 3 times (from 21.8±6.4 to 6.9±3.2%). The percentage of apoptotic DNA comets in cells of the embryonic head and body was shown to decrease and did not differ from the negative control level.

We conclude that the exposure of pregnant rats to tobacco smoke increases the degree of DNA damage in cells of the placenta and developing embryo. Our results are consistent with published data that smoking has a genotoxic effect on blood cells of rat embryo [6,8].

Afobazole diminishes the genotoxic effect of tobacco smoke in embryonic tissues and placenta, which is consistent with published data on the antimutagenic and antiteratogenic properties of this agent in relation to chemical mutagens and teratogens [2,5]. It should be emphasized that the protective effect was most pronounced after treatment with afobazole in a dose of 1 mg/kg.

Our findings indicate that tobacco smoke causes DNA damage in cells of the embryo and placenta. The genotoxic effect of tobacco smoke on embryonic cells is abolished after administration of afobazole in therapeutic doses.

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