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EFFECT OF EPHEDRINE AND ITS DERIVATIVES IN KML CELL CULTURE AND ON TUMOR STRAINS OF ANIMALS

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The cytotoxic activity and the ability to inhibit growth of experimental animal tumors of about 30 ephedrine and pseudoephedrine derivatives were studied in KML cell culture. It was found that the chemical structure and the biological activity are related. Four active chlorinated ephedrine and pseudoephedrine derivatives, including N-(β-chloroethyl)- and N-nitroso-groups, were found in in vitro *and* in vivo *systems and may be promising for antitumor preparations.*

Key words: ephedrine, ephedrine derivatives, tumor-cell culture, cytotoxic test, toxicity test, tumor strains of animals.

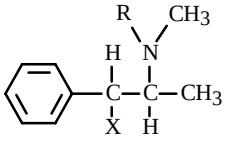
Ephedrine (EP) and its diastereomer pseudoephedrine (PEP) are used in modern clinical practice to treat cancerous diseases, i.e., they in combination with other preparations relieve arterial spasms, neurotoxic reactions after radiation therapy and chemotherapy, etc. [1, 2]. However, we previously demonstrated that certain EP and PEP N-β-chloroalkylamine derivatives possess a different spectrum of anti-cancer activities [3].

Our goal was to determine the cytotoxic and antitumor activities of EP and its derivatives.

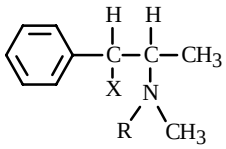
Antitumor compounds were initially selected using the new murine melanoma cell line KML [5], which is highly sensitive to vincristine and sarcolysine [6].

We studied the cytotoxic action of vinblastine and colchamine and demonstrated that this cell line is highly sensitive to the action of plant alkaloids. The CE_{50} index of the above-mentioned preparations was 1 mg/mL and less.

The action of about 30 EP and PEP derivatives with various groups, namely, N-(β-ethyl)chloro, N-(β-chloroethyl)chloro, N-(nitroso)chloro, N-(nitroso), N-(β-propyl), N-(β-hydroxypropyl), N-(β-hydroxyethyl), N-(N′-methylthiocarbamoyl), N-(N′-ethylthiocarbamoyl), and N-(β-amidoethyl), on this line of tumor cells was studied. The structural formulas of EP, PEP, and their derivatives are given below:



1, 3, 5, 7



2, 4, 6, 8

1, 2: X = H, R = H;
3, 4: X = Cl, R = CH_2CH_3;
5, 6: X = Cl, R = CH_2CH_2Cl;
7, 8: X = Cl, R = NO

The experimental procedure for studying the activity of these compounds in a cytotoxicity test is described in the Experimental section.

The doses of the compounds were 1, 10, and 100 μg/mL of nutrient medium. KML cells without compounds were used as a control. Table 1 presents the results for EP and PEP derivatives that were active in the cytotoxicity test.

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TABLE 1. Cytotoxic and Anti-Tumor Activity of Ephedrine and Pseudoephedrine Derivatives

Compound	CE_{50}, mg/mL	LD_{50}, mg/kg	Strains of animal tumor lines (% inhibition)	
			EAC	S-180
1	>100			
2	>100			
3	11.0±1.0			
4	45.0±3.5			
5	16.0±1.0	16.0±1.2	80.0±4.2	84.0±4.5
6	27.0±2.5	20.0±1.5	54.0±3.0	56.0±3.6
7	17.0±1.5	40.0±2.4	75.0±3.9	65.0±3.7
8	33.0±2.0	46.0±2.6	70.0±3.5	62.0±3.3

*Compounds active in vivo are given;

****7**-**10** were inactive for tumor strain NK/Ly;

***inhibited growth of Walker carcinoma by 67.0 ± 3.8%.

EP and PEP derivatives including the Cl-containing groups N-(β-ethyl)chloro, N-(β-chloroethyl)chloro, and N-(nitroso)chloro were active in the cytotoxicity test for ^{3}H-thymidine incorporation. The concentrations causing 50% cytotoxicity were in the range 11.0-45.0 μg/mL (Table 1, **3**-**8**). However, EP chloro-derivatives (**3**, **5**, **7**) were more active than the PEP ones. This was evidently due to differences in the spatial structure of the diastereomers.

EP, PEP, and the remaining derivatives were inactive.

Then we studied the antitumor activity of **5**-**8** in animals with xenografts of Erlich ascitic cancer (EAC), sarcoma S-180, NK/Ly, and Walker carcinoma using common methods [9]. The antitumor effect was estimated in percent versus the control from the increased lifespan of tumor-bearing animals; the toxicity of the compounds, from LD_{50} values (50% lethal dose) for mice upon i.p. administration of the compounds [9].

Compounds investigated *in vitro* suppressed growth of these tumor strains to various degrees. The highest antitumor activities were found for **5** vs. EAC, S-180, and Walker carcinoma (80, 84, and 67%, respectively, inhibition of tumor growth); **6**, EAC and S-180 (54 and 56%, respectively); **7**, EAC and S-180 (75 and 65%, respectively); **8**, EAC and S-180 (70 and 62%, respectively). A slight dependence between *in vitro* cytotoxicity and ability to suppress growth of tumor strains *in vivo* (**7**-**10**) was noted. EP and its diastereomer were inactive in both systems.

Thus, about 30 EP and PEP derivatives yielded 4 active chloro-derivatives: N-(β-chloroethyl)chloroephedrine, N-(β-chloroethyl)chloropseudoephedrine, N-(nitroso)chloroephedrine, and N-(nitroso)chloropseudoephedrine, in a cytotoxicity test on KML cell culture and in tests on tumor strains of animals. Therefore, these compounds may be promising for antitumor preparations.

EXPERIMENTAL

KML cell culture was obtained from murine B-16 melanoma strain [5]. EP, PEP, and their derivatives were characterized as before [3, 4].

Cells were innoculated into penicillin vials (1×10^5 cells/mL) containing nutrient medium (3 mL, RPMI-1640 with 10% fetal calf serum, 200 mM glutamine, antibiotics) and were cultured in a CO_2-incubator at 37°C. Cells were adjusted to concentrations of 1.0-100.0 μg/mL 24 h after innoculation. Cells were exposed to compounds for 24 h. Then ^{3}H-thymidine (10 μCi per vial) was added for 1 h, after which labeled DNA precursor was removed and the culture was washed three times with Hanks' solution. Next versene solution (1 mL, 0.2%) was added for 3-5 min. Cells were suspended, transfered onto GFC filters, fixed, and washed with TCA solution (5%) for 30 min to remove unbound label and for 30 min with H_2O, dried, and counted in a β-counter in scintillant-106.

The cytotoxic effect of the compounds on cells was estimated by determining the extent of DNA synthesis using ^{3}H-thymidine incorporation [6, 7]. The results were expressed in percent vs. the control. Then CE_{50} values, i.e., the preparation concentration at which the obtained value could be halfed (50% cellular effect), were determined by analyzing curves for the effect as functions of dose for each preparation [8]. A preparation was considered active if a dose of less than 100 mg/mL was required to reach CE_{50}.

The antitumor effect was estimated in percent vs. the control from the increased lifespan of tumor-bearing animals by the common method [9].

The toxicity of compounds was determined from the LD_{50} value using the literature method [9].

Experiments, including the controls, were repeated three times. The controls were cells or animals with tumors and without preparations.

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