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EFFECT OF ORGANOFLUOROSILICON COMPOUNDS ON DEVELOPMENT OF CERTAIN TUMORS IN MICE

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Every year sees an increase in the number of publications on the synthesis of organosilicon compounds possessing various antitumor properties. The most interesting of them have been examined in a review [4]. Of all the different derivatives of organosilicon compounds preference is awarded today to silsesquioxanes of the $[O_{1;5}\ Si(CH)_{2n}CH(COOM)_2]$ type, in which n is a number from 0 to 4, and M = H denotes an alkali metal [3]. Injection of these compounds into mice with an Ehrlich's ascites tumor prolongs their survival by 1.5-2 times compared with the control [8]. Japanese workers have shown that silsesquioxanes containing a perfluorinated radical of the $[CF_3CF_2CF_220CF(CF_3)\ COO(CH_2)_3Si]_2\ O_3$ type are the most active and prolong the survival of mice by 2.5-3 times [9].

We have studied for the first time the action of organosilicon compounds containing a fluorinated radical of the $(CF_3CHX_1CHX_2)n - SiR^1R^2R^3$ type, where n = 3 $X_1 = X_2 = H$, $R^1 = OH$ [3], $R^1 = OH$ [4], when $n = 1$, $X_1 = X_2 = H$, $R^1 = R^2 = R^3 = OC_2H_5$ [1], $R^1 = R^2 = OC_2H_5$, $R^3 = CH_3$ [2], when $n = 3$, $X_1 = X_2 = H$, $R^1 = O\ (CH_2CH_2CF_3)_3$ [5], when $n = 1$ $X_1 = X_2 = H$, $R^1 = R^2 = R^3 = (OCH_2CH_2)_3N$ [6], and when $n = 1$, $X_1 = H$, $X_2 = Cl$, $R^1 = R^2 = R^3 = (OCH_2CH_2)3N$ [7], on the cytotoxic activity and on the development of a virus-induced Rauscher leukemia and tumors induced by MCh-11 cells, in mice.

EXPERIMENTAL METHOD

Cytotoxic activity was studied in experiments with human ovarian carcinoma cells (line CaOv) in culture. The indicator of activity was inhibition of incorporation of ^3H-thymidine into the cell DNA. The cells were cultured in a monolayer on medium 199 with 10% bovine serum. The substances were tested in concentrations of 5×10^{-4}, 1×10^{-4}, and 1×10^{-5} M. Exposure of the cells to the substances lasted 24 h, after which the cells were incubated for 1 h in medium with ^3H-thymidine (37 mBq/ml). After washing to remove radioactivity and after removal of the acid-soluble fraction from the cells by hydrolysis in 10% $HClO_4$ at 80°C

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TABLE 1. Action of Organofluorosilicon Compounds on Incorporation of ^{3}H-Thymidine into DNA of CaOV Cells Obtained from Human Ovarian Carcinoma (exposure 24 h)

No. of compound	Inhibition of ^{3}H-thymidine incorporation in $0.5 \cdot 10^{-3}$ M, %	CE_{50}	
		μg/ml	μmoles/ml
1	31,0	1000	3,8462
2	16,0	1000	4,3478
3	93,0	40±5	0,1250
4	83,1	15±2	0,0446
5	30,0	1000	2,7548
6	27,5	1000	3,6900
7	12,2	1000	3,2787

for 20 min, nucleotides were extracted. Incorporation of ^{3}H-thymidine was measured in ZhS-8 fluid on an 'Intertechnique CL-4000' scintillation counter (France). Radioactivity of the samples was recorded in counts per minute (cpm). For each concentration of the substance the mean level of ^{3}H-thymidine incorporation was calculated relative to the control (in %). On the basis of the data the half-effective dose (EC_{50}) was determined graphically, with calculation of the 95% confidence interval, in micrograms/ml, and EC_{50} also was calculated in micromoles/ml (Table 1). Full details of the method are given in [2].

The effect of these compounds on tumor growth was determined on two models: on a model of virus-induced Rauscher leukemia in BALB/c mice sensitive to this virus, and on solid and ascites sarcomas induced by transplantable MCh-11 cells in C57BL/6 mice. The experiments on the model of virus-induced Rauscher's leukemia were carried out as follows. BALB/c mice aged 1.5-2 months were given an injection of tenfold dilutions of Rauscher leukemia virus. The test preparations, in the corresponding doses, were injected into the mice 1 day before injection of the virus. Animals receiving the preparation and different dilutions of virus constituted the experimental group. Mice receiving an injection only of the virus or only of the test preparation served as the control. For each dilution no fewer than 7-10 mice were used. The virus was injected intraperitoneally and the preparations intramuscularly. The animals were killed on the 21st day and the spleen weighed (to detect splenomegaly) [12]. Depending on the degree of splenomegaly, the titer of the virus was determined in mice receiving virus alone and mice receiving the preparation plus the same dilutions of the virus. The titer of the virus was calculated by the method of Reed and Muench [7]. The action of the test preparations on development of Rauscher leukemia in mice was estimated from the change in titer of the virus in the experimental group. The effect of the test preparations on development of a tumor induced by MCh-11 cells was studied as follows. C57BL/6 mice were given an injection of 10^6 MCh-11 cells in a volume of 0.2 ml subcutaneously or intraperitoneally. The animals were given intramuscular injections of the test preparations 1 day after injection of the tumor cells in the corresponding doses, either once or repeatedly. The effect of the preparations on tumor development was assessed on the basis of the times of death of the animals. Significance was calculated by Student's t test [6].

The compounds 1 and 2 used in the work were synthesized by the method in [5], compounds 3-5 were synthesized as described in [10], and compounds 6 and 7 as described in [1].

EXPERIMENTAL RESULTS

Data on the cytotoxic activity of the organofluorosilicon compounds are given in Table 1. They show that of the seven compounds tested, only compounds 3 and 4 exhibited high cytotoxic activity (83 and 95%, respectively). It is interesting to note that compounds containing different numbers of fluorinated radicals 3 (1RF) and 5 (6Rf) possess identical cytotoxicity (31 and 30%, respectively). The molecular structure of the organofluorosilicon compound in a given series — a linear (1, 2, 5) or cyclic (6) molecule, has no significant effect on cytotoxicity (31, 30, and 27.5%). Compound 7 had lower cytotoxicity (12.2%) than compound 6 (27.5%). These compounds differ in the chlorine atom in the radical. However, according to some workers [11], introduction of a chlorine atom into an organic radical sometimes increases the cytotoxicity of the compound. The results of the study of cytotoxicity led us to investigate the effect of organofluorosilicon compounds on the development of certain types of tumors: on virus-induced Rauscher leukemia and on tumors induced by MCh-11 cells (Table 2). It follows from the data in Table 2 that under the influence of compounds 1 and 3, and 4, 5, and 6, in a dose of 100 μg per mouse, mortality among the animals on the 27th day was reduced by almost 1.5 times compared with the control. Injection of compound 3 also led to a general increase in the survival of the animals by 3 days. These data correlate with results obtained for this compound on CaOV cells. In the study

TABLE 2. Effect of Organofluorosilicon Compounds on Development of a Tumor Induced by MCh-11 Cells (based on length of survival of the animals)

No. of compound	Concentration, μg/mouse	No. of animals dying by the 27th day		Length of survival of animals, days
		abs.	%	
1	100	10	66,6	34
2	100	11	73,3	31
3	100	9	60,0	37
4	100	9	60,6	34
5	100	8	53,3	34
6	100	8	53,3	31
7	100	12	80,0	34
MCh-11	Control	12	80,0	34

of the effect of compounds 1-7 in a dose of 100 μg per mouse on the development of Rauscher erythroleukosis these compounds were found to have no effect on development of the leukemia, for titers of Rauscher virus were not statistically significantly different in the control and experiment. Accordingly, since compound 1 in a concentration of 0.0446 mmole/ml had considerable cytotoxic activity on CaOV cells, we studied the effect of this compound in a dose of 1000 mg/kg (20 mg per mouse) on an ascites tumor induced in C57BL/6 mice by MCh-11 tumor cells. The results indicate that this compound, in the dose stated, has no action on development of the tumor in animals (the time of death of the animals was the same in the control and experimental groups).

The results thus show that, of all the organofluorosilicon compounds tested, compounds 3 and 4 have the highest cytotoxic activity, which was confirmed in experiments in vivo.

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