Study of the amitozyn effect on the cells of ovary teratoblastoma of human (RA-1 line cells)

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Summary. The dynamics of penetration and action of preparation amitozyn on RA-1 line cells (cells of ovary teratoblastoma of human) was studied and its intrinsic fluorescence was investigated.

Amitozyn was found to penetrate into RA-1 line cells, causing a considerable cytotoxic action and thus revealing its ability to fluorescence.

Amitozyn is an antitumor compound, which combines a high antitumor action with minimal toxicity, reveals no inhibition of sanguification and immunity [1]. This drug is a sum of thiophosphamide derivatives of Chelidonium majus L. alkaloids. These alkaloids have a special property of intrinsic fluorescence in ultra-violet rays (with a wavelength 330-360 nm.). This fluorescence is mainly of different shades of yellow (barberyn, sanguinarine) or blue (chelidonine) [2]. Due to this property, preparations of Chelidonium majus L, including amitozyn, are fluorescence capable. [3].

Amitozyn has a wide spectrum of antitumor action in the experimental and clinical oncology.

The aim of the present work was to study the antitumor action of amitozyn on RA-1 line cells (cells of ovary teratoblastoma of human) and its fluorescence ability.

RA-1 culture was obtained form the Russian collection of cell cultures (Saint Petersburg). The cells of this line are epithelial-like in their morphology [5]. Cells were grown in nutrient Dulbecco's modified Eagle's medium (DMEM, Cellgro, USA) with the addition of embryonic serum of calf. (10 %, Sangva, Lviv, Ukraine).

Before using, the serum was inactivated by heating for 30 min at 56C. Penicillin at 100units/ml and streptomycin (Kievmedpreparat, Ukraine) at 100 mg/ml were added to the medium.

In re-inoculation the cells were detached by a solution of 0,25% tripsin and 0,02 % versen in 1:1 ratio.

The cells were cultivated at 37 C in the atmosphere containing 5% CO_2 [6].

Luminescent and microscopical picture of the cells was studied using a luminescent microscope ML-2("LOMO", Russia).

Preparation amitozyn (Ukrain) at concentration 1mg/ml, the preparation solvent, distilled water, RA-1 line cells (cells of ovary teratoblastoma of human) were used.

The cells were seeded and 24 hours after incubation were treated with the preparation at operating concentration 1mg/ml.

In the experiment 2 controls were used: cells, treated with the preparation solution and those treated with distilled water.

The preparation was observed to penetrate into tumor cells of RA-1 line and first it located near the nucleus. At concentration 1mg/ml amitozyn showed a substantial cytotoxic effect. The fluorescence of most cells was observed 10 min. after the cells with amitozyn were incubated.

After 4 hours of cells incubation with the preparation, a half of the cells died and after 24 hours incubation, the number of living cells was no more than 10%.

In the experiment, the fluorescence of dead cells was observed. By its character the fluorescence of dead cells differed from that of the cells treated with the preparation at initial stages. Dead cells showed a uniformed fluorescence while the tumor cells, treated with amitozyn showed a bright pulsatile one.

In both controls the fluorescence of living RA-1 line cells was not observed, the cell death had no pronounced character.

Conclusion

In the experimental study of amitozyn antitumor action, we determined that amitozyn at concentration 1mg/ml (at 24h exposure) showed a considerable cytotoxic effect on cells of ovary teratoblastoma of human (cells of RA-1line). The preparation penetrates into tumor cells first localizing near the nucleus and then causing their destruction and death.

The data obtained suggest further prospects for studying and application of antitumor preparation amitozyn for the control of the selectivity of its action and tumors diagnostics.

References

1. L.I.Petlichnaya, A.I. Potopalsky, S.V.Ivasivka. Inventors certificate № 1007353T N^{I},N^{II},N^{III}-three[2—(N- berberino) ethylamide] thiophosphoric acid, possessing antitumor activity– 23.11.82.

2. Kovar J., Simek K., Kozouskova E., Klukanova H., Slavik J. Fluorescence properties of some isoquinoline alkaloids // Coll. Czech Chem. Commun. – 1985. –N 50. – P. 1312-1328.

3. A.I. Potopalsky, L.I.Petlichnaya, , S.V.Ivasivka. Berberis and its preparations in biology and medicine. - Kiev: Naukova dumka, 1989. – p.115.

4. A.I. Potopalsky. Chelidonium majus L. preparations in biology and medicine. - Kiev: Naukova dumka, 1992. – p. 102-108; p. 140-183.

5. Catalogue Russian cell culture collection (RCCC). - St. Peterburg; OMSK, 1999.- 80 p.

6. R. Adams. Methods of cell culture for biochemists: Trans. from English. Ph.D. (biol) M.A.Ivanova /Edited by Ph.D. (biol) V.Yu. Polyakova. Moscow, Mir, 1983. - p.263.