PILOT STUDY OF BIOLOGICAL
ACTIVITY OF DRUG X

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Materials and methods

Pilot study of the biological activity of the drug X were performed at a certified cell culture of human pancreatic carcinoma (acinar cell clone, the bank Institute of Cytology RAS).

Cells were cultured in 24-well plates (Sostar) at 37°C in an atmosphere with 5% $C0_2$ in a medium RRMI 1640 ("Flow") supplemented with 10% calf serum embryos ("Sigma"), L-gputamina (300 ug / ml; "Flow"), Hepes buffer (0.02 M; " Serva ") and gentamicin (100 ug / ml; "Farmahim"). Initial concentration was 106 cells / ml. Each sample was put in duplicate.

Populations of cells were cultured with drug X. The cells were 4 passages. Drug X was added to the medium at each passage of cells in three doses: 1 ng / ml, 10 ng / ml, 100 ng / ml.

The biological activity of the drug X was determined by the expression of these signaling molecules:

chromogranin A (a marker of secretory activity of cells, antibodies from Novocastra , 1:250),

the regulator protein proliferative Ki-67 (antibodies from Dako, 1:150),

apoptosis regulatory protein p53 (antibodies from Dako, 1:150).

digital microscopy and morphometric study was conducted using a system of computer analysis of microscopic images of Nikon Eclipse E400 and the software "VIDEOTEST-Morphology 5.0.

In each case, analyzed 10 fields of view with increasing 400h. Determines the area of expression, which is the ratio of the area occupied by immunopositive cells to the total area of cells in the field of view,

expressed in percentages. This parameter reflects the intensity of synthesis of signaling molecules in cells.

The results were processed by a computer program STATISTICA 5.0 (Statsoft).

Results of research

studies showed that the drug X can both weaken and strengthen the functional secretory activity of acinar cells of human pancreas, having a pronounced dose efficiency. For example, at a dose of 10 ng / ml of the drug 2 times reduces the expression of chromogranin A, while a dose of 100 ng / ml, it is a factor of 3 increases the expression of this marker.

The results show that drug X has a pronounced antiapoptotic activity - in doses 1 ng / ml and 100 ng / ml, it prevents apoptosis in 100% of cells in the population.

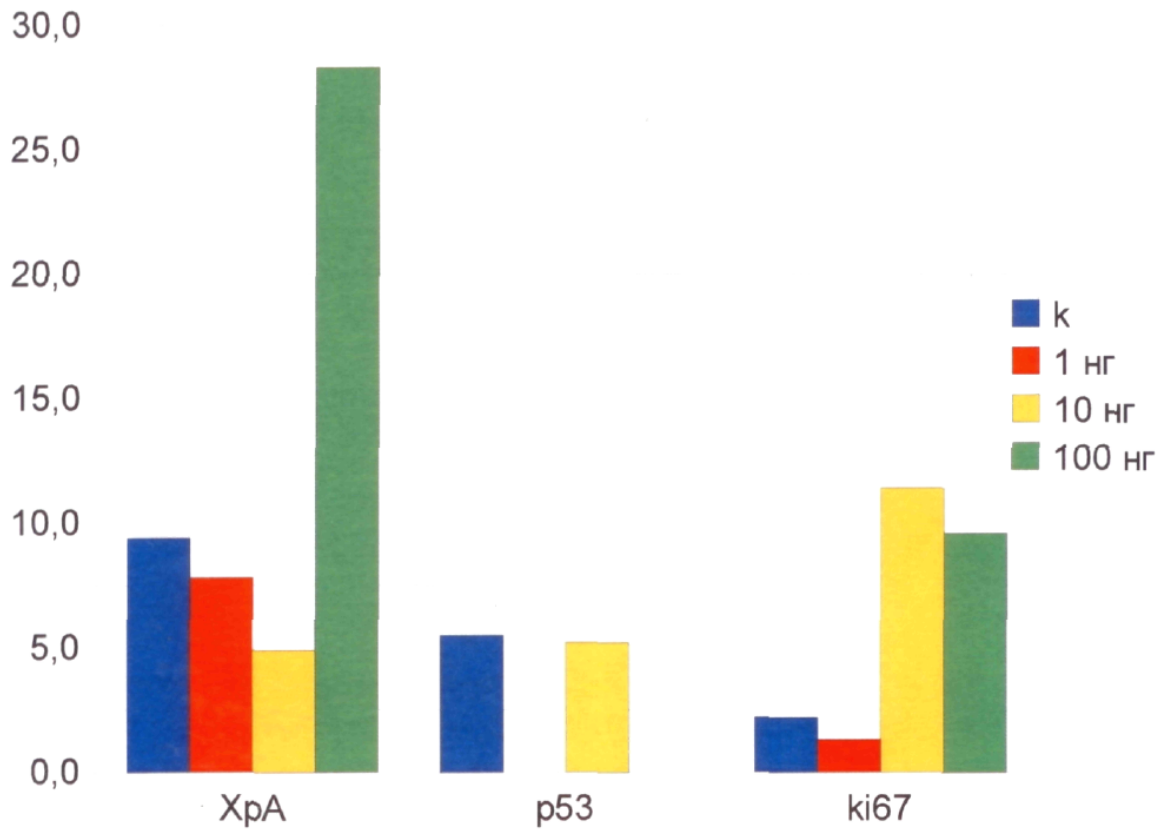
The data also indicate efficacy of the dose of X with respect to cell proliferation. At a dose of 1 ng / ml of drug X reduces cell proliferation by 1.8-fold, and in doses of 10 and 100 ng / ml, enhances it. The most effective dose is 10 ng / ml - with her drug enhances cell proliferation by about a factor of 4.

Conclusion

Thus, the results of our investigations allow us to consider the drug X as a highly effective drugs with diverse biological and pharmacological action, capable of stimulating / a debilitating effect on the synthesis and secretion of hormones that prevent cell death and stimulate / inhibit cell proliferation.

Multidirectional revealed dose efficacy allows you to extend its scope by changing the dose.

The results suggest further in-depth studies of biological and pharmacological effects of the drug X certainly promising.



	By	1 ng	10 ng	100 ng
XpA	9,4%	7,8%	4,9%	28,3%
p 53	5.5%	0%	5,2%	0%
Ki 67	2.19%	1.3%	11, 4%	9,6%