MEDICAL SCIENCES

URINOPROTECTIVE EFFECTS OF THE PEPTIDE IPH VGA

Ivko X.O.
The research scientist, Open Institute of Human and Nature, Lithuania, Vilnius
Bocharova Ks.A.
The research scientist, Belgorod National Research University
Olenskaya T.L.
The research scientist, Vitebsk State Medical University
Polev A.V.
The research scientist, The clinic of medical innovations «Medinnova», Moscow
Satardinova E.E.
The research scientist, Irkutsk State Medical Academy of postgraduate education

Abstract

The article describes the result of biological and clinical protective effects of the peptide IPH VGA. The peptide IPH VGA is a high biological activity to the control the normal formation of urine system, the restoration of the urinary system, as well as confirm information about the cytostatic and oncoprotective properties of the peptide IPH VGA in relation to the human urinary system according to the expression of biological molecules on cell culture. The article proves that the peptide IPH VGA has a normalizing effect on the functional activity of the bladder detrusor cells, has a cytoprotective and anti-ischemic effect on the bladder according to experimental studies. The article describes that the application of the peptide IPH LGA has a regulatory effect on the functional activity of the cells of the bladder wall and detrusor, contributes to the normalization of urination function, reduces the number of daily urination and brings relief of urination, which together leads to improved quality of life and normalization of moral status in patients with overactive bladder according to clinical studies.

Keywords: peptide, urinary bladder, the bladder wall, protective effects, biological effects.

Introduction.

The study of the effects of peptides has a great interest today. Peptides have the same structure as proteins, but the size of these molecules is smaller. It is also important that short peptides are a natural metabolic product in the body and they can`t be detected in blood or urine. That`s why, it is interesting to study the effects of peptides on cell cultures [1, 2, 5].

The peptide IPH VGA contains a complex of low molecular weight peptides and has a normalizing effect on the cells of the bladder wall and detrusor [3, 4, 6].

The results of experimental studies have shown that the peptide IPH VGA has a selective tissue-specific effect on the cells of the bladder wall and sphincter, improves its trophism and has a regulating effect on the metabolic processes in the bladder wall and sphincter, promotes the normalization of functional and morphological changes in the testes, reducing the risk of various pathological processes. This suggests the effectiveness of the use of IPH VGA peptide to restore bladder function in patients suffering from urinary disorders of various origins [1, 7, 8].

The aim of this study was to identify biological and clinical urinoprotective effects of the peptide IPH VGA.

Material and methods.

We conducted 3 areas of research to identify the biological and clinical effects of the peptide IPH VGA:

1. The study of the effects of the peptide IPH VGA on the cell.

2. The study of the effects of the peptide IPH VGA in the experiment.

3. The clinical study of the effects of the peptide IPH VGA.

In a cell-based study we selected embryonic stem cells to study the cytostatic, immunomodulatory, geroprotective and oncoprotective properties of the peptide IPH VGA in relation to the urine-reproductive system. These cells belong to the pluripotent type, which means that they can be differentiated into all three primary germ sheets: ectoderm, endoderm and mesoderm. Organs and glands of the urine-sexual and other systems are formed from these primary germ sheets in the future. The human embryo transformes the blastocyst stage in 5-6 days after fertilization. The stem cells are obtained from the blastocyst.

We carried out the expression of the SRY, SOX9, WT1, gene encoding steroidogenic factor SF1 gene, which responsible for the differentiation of the gonads and the ontogeny of the urinary system.

We also assessed the biological active markers. We used immunofluorescence technique using primary antibodies to Ki67 (1:75, Abcam), p53 (1:50, Abcam).

We have created the following groups for the study: 1 group – the study of molecular expression before the study; 2 group-control (we added the culture medium, incubation with serum albumin); 3 group – we added the control dipeptide Glu-Trp at the concentration of 100 micrograms (mcg); 4 group – we added the peptide IPH VGA at a concentration of 100 micrograms (mcg). We selected the peptide Glu-Trp with the immune properties and well described in the literature as a control.

The PCR method was used to measure the level of gene expression using Novocasta's reagents and sets of monoclonal antibodies produced by Biosource (Belgium). We used confocal microscope Olympus

FluoView FV1000 with indicator of 200, 400, 600. We conducted the measurement of the expression in %.

We have chosen the most commonly used species of laboratory animals for the study for the experiment recommended by the Ministry of Health of the Russian Federation in the Manual for preclinical studies of drugs - rats.

We created an experimental model of the development hyperfunction of urinary bladder detrusor in rats to study the properties of the peptide IPH VGA. These changes in rats are generated by the deterioration of metabolic processes. It is necessary to increase the secretion of insulin and insulin-like growth factor, prolactin, violation of the glycemic index, also inducing the development of prostate adenoma in combination with hyperactivity of the bladder detrusor with an increase in the frequency of urination. The rats were kept for 3 months on a high-calorie diet. We used complete feed for rodents with additional feeding in the form of fruits and vegetables, and we added the butter to the standard feed in a ratio of 1:4.

We was studied 40 rats at the age of 13,5±0,7 months and weighing 387,5±5,7 g. All procedures of animal keeping and testing were carried out in accordance with standards ISO 10993-1-2003 and GOST RISO 10993.2-2006. The rats were divided into 2 groups – the control (n=20) and the main group (n=20). The rats of the main group were given orally through a pipette-dispenser a solution consisting of water for injection in a dosage of 1 ml, in which the lyophilized powder of IPH VGA peptides was dissolved in a concentration of 0.59 micrograms (mcg) per rat body weight per day for 30 days. A pipette-dispenser allowed to control the volume and the fact of liquid consumption.

The rats were killed after 30 days. Then the urine bladder was removed, fixed by immersion in a solution of 4% paraformaldehyde in phosphate buffer (PBS pH = 7.3) for 24 hours at a temperature of 4 °C. We produced slices with a thickness of 20 µm using cryotome of Leica CM 1510S model (Germany). Then the sections were mounted on a slide and stained with hematoxilin and eosin. We used the Olympus IX81 microscope for the study. The Danet criterion was used to assess the reliability of the difference in the results obtained in the groups before the use of the peptide, compared with the groups after the application of the peptide IPH VGA.

The clinical studies of the peptide IPH VGA were conducted in 69 patients aged 37 to 69 years (mean age was 61.2±1.4 years) with the overactive bladder.

We conducted studies the effectiveness of peptides in the dosage of 50 µg (n= 65 people) and 150 µg (n=63 people) to assess the effectiveness of the dose of 100 µg (n=69 people) for the peptide IPH VGA. All patients were exposed to diagnoses and appropriate treatment carried out in accordance with the recommendations of the Committee for standardization of terminology of the International society for urinary retention (ICS).

The peptide IPH VGA was administered orally: 1 capsule (100 µg peptide) 1 time per day for 30 days, then 30 days a break in the medication. And repeat the same course for another 30 days, again 30 days a break in the medication - and the third course for 30 days. The total course was 6 months (3 courses of 30 days and 3 a break in the medication of 30 days). We studied the effectiveness of the improved management scheme of such patients using the peptide IPH VGA after 3 and 6 months. The control values was selected the results before the study.

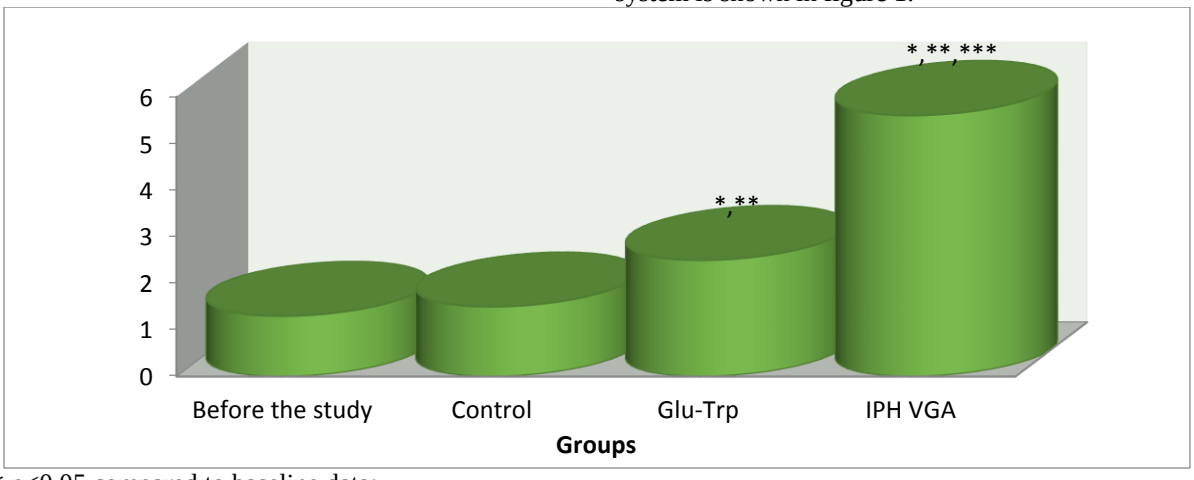
The efficacy of the peptide IPH VGA was evaluated on the basis of the dynamics of patients' complaints, the degree of abdominal pressure during urination and the nature of the urine stream, fluorometric index and quality of life assessment.

We used standard statistical methods of medical and biological research.

Results and discussion.

The biological analysis of urinoprotective effects of the peptide IPH VGA on cell culture

The effect of the IPH VGA peptide on the expression of the SRY, SOX9, WT1, gene encoding steroidogenic factor SF1 gene, which responsible for the differentiation of the gonads and the ontogeny of the urinary system is shown in figure 1.

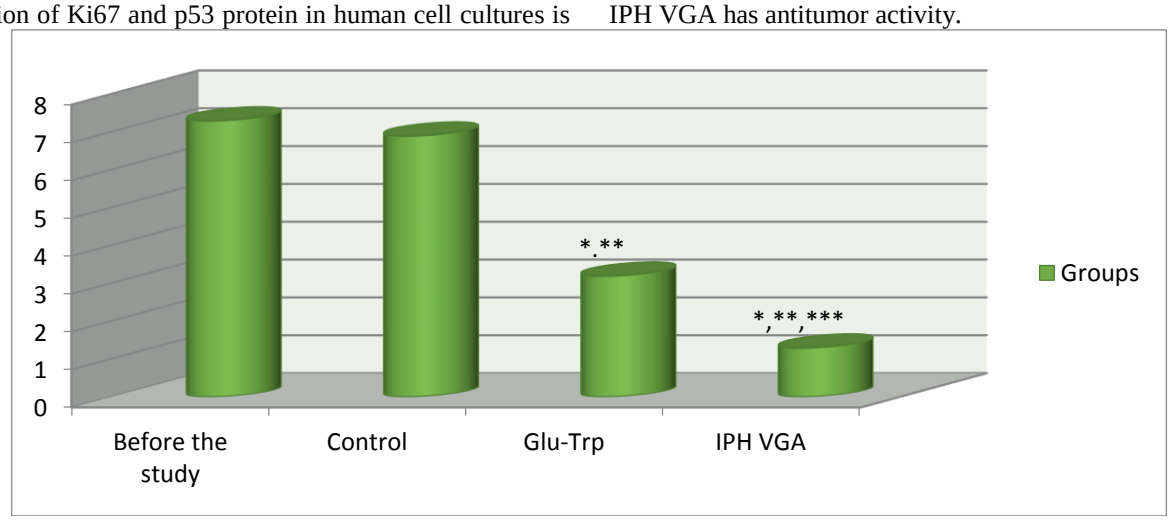


* p<0.05 compared to baseline data;
** p<0.05 compared to control;
*** p<0.05 between the indicators of the level of expression used Glu-Trp and the peptide IPH VGA.

Figure 1. The expression of the SRY, SOX9, WT1, gene encoding steroidogenic factor SF1 gene.

It was found that the IPH VGA peptide significantly increases the "cascade" of signal molecules in human cell culture, which is necessary to activate the processes of proliferation and differentiation of stem cells into cells of the urinary system.

The effect of the peptide IPH VGA on the expression of Ki67 and p53 protein in human cell cultures is shown in figure 2 and 3. It was found that the application of the peptide IPH VGA reduces the expression of Ki67 in 5,6 times from the baseline. Ki67 protein can be an important marker for assessing the decrease in proliferative activity of cells and the degree of involutive processes in the urine bladder. Thus, the peptide IPH VGA has antitumor activity.

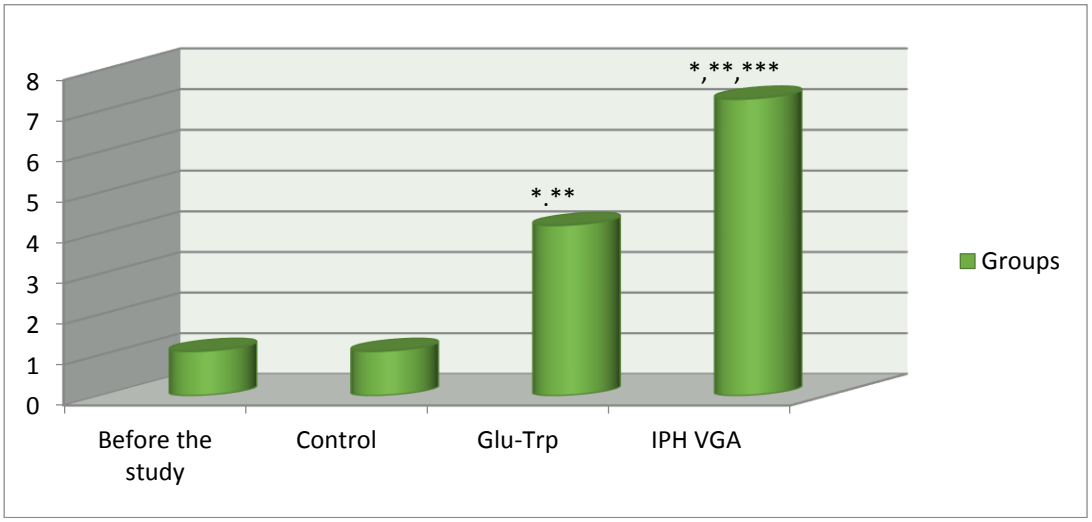


* p<0.05 compared to baseline data;
** p<0.05 compared to control;
***p<0.05 between the indicators of the level of expression used Glu-Trp and the peptide IPH VGA.

Figure 2. The effect of the peptide IPH VGA on the expression of the Ki67 protein in the cell culture.

Consequently, the application of the peptide IPH VGA is protected against cancer, in particular, against malignant tumors of the urine-reproductive system according to the level of expression of the marker Ki67 protein on cell culture.

The effect of the peptide IPH VGA on the expression of p53 protein in cell cultures is presented in figure 3. The application of the peptide IPH VGA increases the production of protein p53, which is a transcriptional factor and acts as a suppressor of malignant tumor formation by the way of activating apoptosis in the tissues. This results lead to the conclusion about the antitumor properties of the peptide IPH VGA.



* p<0.05 compared to baseline data;
** p<0.05 compared to control;
***p<0.05 between the indicators of the level of expression used Glu-Trp and the peptide PRO VGA.

Figure 3. The effect of the peptide IPH VGA on the expression of p53 in the cell culture.

P53-dependent apoptosis also avoids the accumulation of mutations. In the case when mutations have already arisen, p53-dependent apoptosis allows to eliminate this potentially dangerous cells. On this fact we can make a conclusion about the cytoprotective effect of the peptide IPH VGA.

The peptide IPH VGA had a high onco-protective activity in relation to the cells of the urine system according to the expression of biological molecules in cell culture.

Biological analysis of urinoprotective effects of the peptide IPH VGA in an experimental model

We found that in the control group, the bladder microstructure had a sharply hypertrophied muscle layer in 4.6 times larger in the distribution area than in the main group. This is due to difficulty urinating.

In the sections of the bladder of rats of the control group, the ischemia area was found in the volume of 48.4±1.6% of the total area. While in rats of the main group the ischemia area was found in 1.3 times significantly less than in rats of the control group, which was 36.1±0.9% of the total area, $p<0.05$ compared to the control group.

The functional state of the bladder is reflected in table 1, which are obtained by infusion cystometry.

Table 1.

The functional state of the urine bladder.

Indicators	The control group (n=20)		The main group (n=20)	
	Before the experiment	Max. filling the urine bladder	Before the experiment	Max. filling the urine bladder
Detrusor pressure (sm. water column)	5,2±1,2	42,1±2,2	5,4±1,2	28,5±1,9*
Peak C1 (mOm)	25,1±2,2	72,2±3,1	25,4±2,2	106,7±3,9*

*$p<0.05$ compared to control.

Thus, the average value of detrusor pressure during urination in rats in the main group was in 1.5 times significantly lower than in the control group, which indicates a positive effect of the peptide IPH VGA on the cells of the bladder detrusor.

The cardiac peak C1, characterizing the state of blood supply to the bladder wall during its filling, was in 1.5 significantly higher in rats after the application of the peptide IPH VGA compared with the rats of the control group (blood supply was impaired due to the developed diseases), $p<0.05$ compared with the indicator in the control group. These data indicate a positive effect of the peptide IPH VGA on the compensatory mechanisms of the cells of the bladder detrusor and the ability to normalize the blood circulation in the pathologies of the urine bladder according to the experimental model.

Thus, the peptide IPH VGA has a tissue-specific effect on the normalization of the functional ability of the urine bladder detrusor.

Clinical analysis of urinoprotective effects of the peptide IPH VGA

According to table 2, which reveal the urodynamics in patients with overactive bladder after the application of the peptide IPH VGA, it should be noted that the urinary retention time significantly decreased in 1.3 times after 3 months of the application of the peptide and in 2 times in the long-term study compared to the baseline, $p<0.05$ between indicators compared with the control group (before the study), $p<0.05$ between indicators after 6 months and 3 months.

Table 2

The urodynamics in patients with overactive bladder after the application of the peptide IPH VGA

Indicator	Before the study	In 3 months			In 6 months		
		The dosage of the peptide IPH VGA			The dosage of the peptide IPH VGA		
		50 μg	100 μg	150 μg	50 μg	100 μg	150 μg
Urination delay time	4,5±0,6	4,4±0,6	3,3±0,4*,#	3,4±0,4*	4,5±0,6	2,2±0,3*,**,#	2,1±0,3*,**
Number of urinations: - during the day - at night	 8,7±0,2 3,7±0,3	 8,5±0,2 3,6±0,3	 7,1±0,3*,# 2,8+0,2	 7,2±0,3* 2,8+0,2	 8,8±0,2 3,6±0,3	 6,2±0,1*,**,# 2,0+0,4	 6,1±0,1*,** 2,1+0,4
The degree of abdominal pressure points	3,2±0,4	3,3±0,4	2,8±0,3	2,6±0,3	3,4±0,4	2,2±0,3	2,1±0,3
The character of the urine stream (scores)	3,4±0,4	3,3±0,4	2,3±0,3*,#	2,2±0,3*	3,6±0,4	2,1±0,3*,#	2,2±0,3*

* $p<0.05$ compared to the control group (before the study).
** $p<0.05$ between 3 months and 6 months.
# $p<0.05$ between 50 μg and 100 μg.

Also noteworthy is a significant reduction in the number of urinations in the daytime after the application of IPH VGA peptides in 1.2 times in 3 months and in 1.4 times in 6 months compared to the baseline, $p<0.05$ between the indicators compared with the control group (before the study), $p<0.05$ between the indicators after 6 months and 3 months, which probably also affected the quality of life of such patients.

The results of a clinical study of the peptide IPH VGA showed that pollakiuria (increased urination) completely ceased to bother 88.3% of patients with chronic prostatitis, 93.2% of patients lost the need for night urination. Stranguria (difficult urination) ceased to bother 74.8% of patients, 26.7% of patients noted a marked increase in urine flow and relief of urination (table 3).

Table 3.

The dynamics of fluorometry indices after the application of IPH VGA peptide in patients with overactive bladder

Indicator	Before the study	In 3 months			In 6 months		
		The dosage of the peptide IPH VGA			The dosage of the peptide IPH VGA		
		50 μg	100 μg	150 μg	50 μg	100 μg	150 μg
The average urine flow rate, ml/s	18,4±1,2	18,3±1,2	22,4±1,5	22,3±1,5	18,2±1,2	26,7±1,7*,#	26,8±1,7*
Maximum speed urination, ml/s	19,5±1,4	19,6±1,4	22,6±1,6	22,2±1,6	19,6±1,4	28,4±1,9*,#	28,6±1,9*
The time to reach peak flow rate, ml/s	3,7±0,7	3,6±0,7	2,5±0,6*,#	2,6±0,6*	3,8±0,7	1,1±0,1*,#	1,2±0,1*

* $p<0.05$ compared to the control group (before the study).
** $p<0.05$ between 3 months and 6 months.
# $p<0.05$ between 50 μg and 100 μg.

Thus, the results of the study indicate the therapeutic efficacy of the peptide IPH VGA and the feasibility of its application in the complex treatment of urine disorders in overactive bladder.

We also assessed the quality of life using the scale EuroQol EQ-5D. The data are given in table 4.

Table 4.

The assessment of quality of life using the questionnaire EuroQol EQ-5D (M±m, points)

Indicator	Before the study	In 3 months			In 6 months		
		The dosage of the peptide IPH VGA			The dosage of the peptide IPH VGA		
		50 μg	100 μg	150 μg	50 μg	100 μg	150 μg
Mobility	3	3	5	5	3	5	5
Self service	3	3	5	5	3	5	5
Habitual daily activities	2	2	5	6	2	5	5
Pain/ Discomfort	2	2	3	3	2	4	4
Anxiety/ Depression	1	1	3	3	1	4	4
Visual analog scale, mm	30	30	80	80	30	80	80

Thus, the quality of life of patients has improved for each parameter, as much as possible in the terms of the usual daily activities, mobility and self-service.

It is important to emphasize that the level of anxiety and depression decreased in 3 times after the application of the peptide IPH VGA after 3 months and remained throughout the study.

It should be noted that the subjective assessment of quality of life on a visual-analog scale increased in 2,7 times in 3 months after the use of the peptide IPH VGA and remained throughout the study.

We haven`t found significant differences between the results in the application of 100 mcg and 150 mcg in all the studied parameters like in 3 months as in 6 months. Also, we have not found significant differences between the indicators in the application of 50 mcg and before the study on all parameters. This fact proves that the effective optimal dosage for the peptide IPH VGA is 100 mcg.

Conclusion.

The studies confirm the high biological activity of the peptide IPH VGA in relation to the control of the normal formation of the urinary system in humans at the genetic level, the restoration of the urinary system, as well as confirm information about the cytostatic and oncoprotective properties of the peptide IPH VGA in relation to the human urinary system according to the expression of biological molecules on cell culture.

It has also been proved that the peptide IGF VGA has a normalizing effect on the functional activity of the bladder detrusor cells, has a cytoprotective and anti-ischemic effect on the bladder according to experimental studies

We have found that the application of the peptide IPH VGA has a regulatory effect on the functional activity of the cells of the bladder wall and detrusor, contributes to the normalization of urination function, reduces the number of daily urination and brings relief of urination, which together leads to improved quality of

life and normalization of moral status in patients with overactive bladder according to clinical studies.

The application of the peptide IPH VGA is recommended as a supplement and in combination with any drugs of etiological, symptomatic and pathogenetic therapy, which used to treat urine disorders, in the most effective dosage of 100 μg per day as a supplement of regulating the functional activity of the urine bladder.

With the support of «Ideal Pharma Peptide GMBH», Ferdinandstr. 11 61348 Bad Homburg.

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