ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2014, Vol. 40, No. 1, pp. 1–8. © Pleiades Publishing, Ltd., 2014.
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Antioxidant and Detoxifying Activities of Analogues of the Delta Sleep Inducing Peptide

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Received April 23, 2013; in final form, August 19, 2013

Abstract—Sixteen peptides differing in structure from the delta sleep inducing peptide (DSIP) by one to two substitutions of amino acid residues have been synthesized to study the possibility of their application in oncology. The antioxidant properties of the peptides in vitro and their detoxifying activity in vivo have been examined on a model of toxicosis caused by cisplatin, a cytostatic drug widely used in the therapy of tumor diseases. It has been shown that almost all DSIP analogues examined exhibit a direct antioxidant activity, with the activity of the ID-6 analogue being higher than that of DSIP and comparable with that of vitamin C and β-carotene. This analogue shows the most pronounced detoxifying activity toward the action of cisplatin, which manifests itself as a decrease in the death of animals from acute toxicity to 17% compared to 50–67% in the control and the restoration of some biochemical parameters of blood, in particular, a decrease in the activity of the enzymes, aspartate and alanine aminotransferases, and the concentration of the end products of nitrogen exchange: creatinine and urea. Thus, the peptides of the DSIP family may appear promising agents for decreasing the toxic effects of cytostatics used in oncology.

Keywords: delta sleep inducing peptide, analogues, antioxidant activity, detoxifying activity, peptides, synthesis, lipid peroxidation, cisplatin

DOI: 10.1134/S1068162014010087

INTRODUCTION

The delta sleep inducing peptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu) is an endogenous regulatory peptide, which has a polyfunctional prolonged action on the organism [1, 2]. The results of studies of DSIP and its analogues over a period of 30 years since its discovery enable one to state with confidence that DSIP is a unique member of the family of peptide neuromodulators. It exhibits a pronounced stress-protective action and decreases stress-induced metabolic and functional disorders in human and animal organisms exposed to stresses of different natures. The effects of the peptide are accomplished through the modulating action on central regulatory processes, owing to the systemic antioxidant action, the modulating influence on the activity of GABAergic, glutamatergic, and other neuronal systems, on the expression of early response genes in brain structures, and on the activity of biosynthetic and proteolytic processes [3–6]. The studies of DSIP are also continuing to date since many questions regarding the mechanism and the spectrum of its biological action still remain unanswered.

Earlier, it was found in a study of oncoprotective properties of DSIP and some of its analogues that a 10-unit cyclic DSIP analogue enhanced the antitumor resistance of animals with inoculated L-1210 leukemia cells under stress [7]. The peptide and its cyclic analogue considerably restricted or abolished (the analogue) a stress-induced decrease in the activity of normal killer cells toward tumor target cells, thereby restoring the efficiency of the natural antitumor protection component [8]. Later, it was shown that DSIP (60 μg/kg, intravenously) substantially inhibited the dissemination of the Lewis carcinoma in mice under enormous stress, a combination of surgical removal of the tumor with emotional and pain stress [9]. A decrease in the number and volume of metastases with a more than a fourfold reduction in the index of metastasis stimulation were found.

It was shown in studies on mice with dissemination Lewis carcinoma that DSIP and some its analogues

Abbreviations: AlT, alanine aminotransferase; AsT, aspartate aminotransferase; DIEA, diizopropylethylamine; DSIP, delta sleep inducing peptide; cHex, cyclohexyl; Fmoc, fluorenylmethyloxycarbonyl; GABA, γ-aminobutyric acid; HBTU, 2-(1-hydroxybenzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate; MDA, malonic dialdehyde; TFA, trifluoroacetic acid; AOA, antioxidant activity; LP, lipid peroxidation.

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1 2 3 4 5 6 7 8 9

Trp-Ala---Gly-Gly-Asp-Ala-Ser-Gly-Glu (DSIP)

Trp-**Pro**---Gly-Gly-Asp-Ala-Ser-Gly-Glu (ID-6)

Trp-***D*-Ala**-Gly-Gly-Asp-Ala (ID-10)

Trp-**Leu---Pro**-Gly-Asp-**Thr-Thr**-Gly-Glu (ID-16)

Trp-**Pro**---Gly-Gly-Asp-Ala (ID-18)

Trp-**Hyp**---Gly-Gly-Asp-Ala-Ser-Gly-Glu (ID-19)

Ac-Trp-Ala---Gly-Gly-Asp-Ala-Ser-Gly-Glu (ID-25)

Trp-Ala---Gly-Gly-Asp-Ala-**Pro**-Gly-Glu (ID-30)

Trp-**Lys**---Gly-Gly-Asp-Ala-Ser-Gly-Glu (ID-35)

Trp-**His**---Gly-Gly-Asp-Ala-Ser-Gly-Glu (ID-36)

Gly-Pro-Trp-Ala---Gly-Gly-Asp-Ala-Ser-Gly-Glu (ID-37)

Trp-**ßAla-His**--Gly-----Ala-Ser-Gly-Glu (ID-38)

Trp-**Pro-Gly**---Gly-**Glu**-Ala-Ser-Gly-Glu (ID-39)

Tyr-*D*-Val-Gly-**Thr**-Asp-Ala-Ser-Gly-Glu (ID-42)

Trp-**Lys**---Gly-Gly-**Asn**-Ala-Ser-Gly-Glu (ID-43)

Trp-**βAla-His**--Gly-Asp-Ala-Ser-Gly-Glu (ID-44)

DSIP analogues. Substituted amino acid residues are shown in bold type in comparison with DSIP. The synthesis of compounds ID-10, ID-16, ID-18, ID-19, ID-37 was described earlier by other methodical schemes in [11–12].

substantially reduced the number and volume of metastases, inhibited the invasion of tumor cells, diminished the adhesiveness of the cell monolayer, and activated the natural mechanisms of antimetastatic protection that were inhibited during tumor growth [10, 11].

The results of the studies indicate that the search for more effective DSIP analogues as the basis for the design of novel drugs for the application in oncological practice (e. g., for the inhibition of dissemination of tumor, a decrease in the toxicity of cytostatics, and restriction of disorders of the neuroendocrine status) holds promise.

We synthesized DSIP and a series of its analogues (figure) with the aim to find novel pharmacologically active derivatives promising for the application in the therapy of tumor diseases and, together with the researchers of the Hertsen Oncology Research Institute, examined their antioxidant and detoxifying properties.

RESULTS AND DISCUSSION

Among the analogues we synthesized (see below), there are new peptides and several peptides that we had previously synthesized using other methodical schemes [11–13]. The group of analogues examined in this work has substitutions in positions 2, 3, 4, 5, and 7 and involves compounds with the residues of D-amino acids (Ala, Val, or ßAla) in position 2 (ID-10, ID-42, ID-38, ID-44), a derivative with the attached *N*-terminal dipeptide unit Gly-Pro (ID-37), and an *N*-acetylated DSIP derivative, which were obtained to increase the resistance of the peptide to aminopeptidases (ID-25).[2] Two DSIP analogues (ID-10 and ID-18) are truncated from the *C*-terminus to six residues in the chain. We also synthesized the previously identified nine-membered peptide homologous to DSIP (ID-16) [11]; this peptide is a fragment of the

[2] The code ID and the through numbering of peptides are used in this study and in our other works concerned with the properties of DSIP analogues (see, e. g., [11, 12]).

Table 1. Protocol of solid-phase synthesis of DSIP analogues by the Fmoc scheme

Step number	Solvent/reagent	Volume/amount	Number of washings	Time, min
1	20% 4-Methylpiperidine in DMF	10 mL	1	1
		10 mL	1	20
2	DMF	10 mL	5	1
3	Protected amino acid HBTU DIEA	2 equivalents in 4 mL of DMF 2 equivalents in 4 mL of DMF 4 equivalents in 4 mL of DMF		
4	Stirring for 120 min, 20–25°C			
5	DMF	10 mL	3	1

sequence 14–22 of the human immunoglobulin precursor (the cappa chain, V-3 region), which, similar to DSIP, contains residues Trp^1, Gly^4, Asp^5, Gly^8, and Glu^9 (figure).

All peptides were obtained by the solid-phase method on an automated peptide synthesizer using 2-chlorotrityl chloride resin and Fmoc derivatives of amino acids according to the protocol presented in Table 1. The side functions of trifunctional amino acids were blocked using the following protecting groups: Boc for Lys, OBu^t for Asp and Glu, Trt for His and Asn, and Bu^t for Ser, Thr, and Tyr. The *N*-terminal amino group of Trp, Tyr, and Gly was protected using the Boc-group. The elongation of the polypeptide chain on a polymer carrier was carried out using the condensing agent, HBTU. The reagents were used in a twofold excess relative to the content of reactive groups on resin. The peptides were cleaved from the polymer with simultaneous deprotection by the treatment with 98% aqueous TFA.

After the cleavage from the resin and deprotection, the resulting peptides were dissolved in 0.05% TFA and purified by reverse-phase HPLC using a gradient of acetonitrile concentration. The preparative HPLC was controlled by analytical HPLC. The peptides were characterized by the data of analytical HPLC and mass spectrometry; the characteristics are given in Table 2.

The antioxidant activity of DSIP and its analogues was assessed from their ability to inhibit LP in a mouse liver homogenate in vitro (Table 3) using vitamin C and β-carotene as reference samples.

It should be noted that the test used estimates only the direct antioxidant effect of compounds, i. e., their ability to act as targets for free radical products. Earlier experiments revealed no significant direct antioxidant effect of DSIP and some of its analogues. The compounds of this group are characterized by a very high indirect AOA mediated through the effect on the activity of some antioxidant enzymes [14, 15]. However, it was of interest to estimate the direct AOA since it may indicate a possible detoxifying activity of peptides because there is evidence that direct antioxidants produce a protective effect in the case of the therapeutic application of some cytostatics [16]. As is evident from the data in Table 3, all peptides used exhibit the AOA. The specific AOA was calculated as the inverse of the molar concentration of a sample necessary for the 50% inhibition of LP and expressed in arbitrary units (arb. un.). The found values of the AOA of the peptides were in the range from 0.9 to 2.2. arb. un., which is comparable with the activity of vitamin C and β-carotene. The maximum AOA (2.2. arb. un.) of peptide ID-16 is 1.3 higher than that of the activity of DSIP.

The detection of antioxidant properties of DSIP and some of its analogues led to the suggestion that the peptides of the DSIP family have detoxifying properties.

The detoxifying effect of peptides in vivo was estimated in mice on a model of toxicosis caused by the cytostatic cisplatin, which is widely used in clinic and belongs to alkylating agents [16]. The antitumor effect of cisplatin is accomplished through the formation of free radical products, which intensify LP in cell membranes of the liver, lungs, kidneys, and other organs. In the final analysis, cisplatin breaks the structure and function of these organs, by inducing a complex of various toxic reactions. The main type of toxicity during its application at therapeutic doses is nephrotoxicity. However, when this drug is used in high doses, the spectrum of its toxic reactions becomes broader, and their intensity increases, resulting in the death of animals from acute toxicity. Therefore, to study the detoxifying effect of peptides on the toxic reactions of cisplatin, we used the dose that induces a 50% death of experimental animals.

Table 4 shows the data on the effect of the peptides on the severity of the toxic action of cisplatin. A single intravenous injection of cisplatin at a semilethal dose of 15 mg/kg led to the death of approximately 50% of animals from acute toxicity. The injection of DSIP analogues ID-16, ID-18, ID-19, ID-25, ID-30, ID-36,

Table 2. Yields, retention times in analytical HPLC, and molecular weights of synthetic peptides

Analogue	Molecular weight		Retention time, min	Purity according to HPLC data*, %	Yield**, %
	calculated	according to MS data			
ID-6	874.8	875.2 $[M + H]^+$	17.8	97.7	39
ID-10	575.7	576.6 $[M + H]^+$	18,6	95.7	32
ID-16	975.1	975.3 $[M + H]^+$ 997.3 $[M + Na]^+$ 1013.3 $[M+ K]^+$	16.7	98.8	48
ID-18	601.3	602.0 $[M + H]^+$	18.1	98.8	21
ID-19	890.9	891.3 $[M + H]^+$	16.2	97.5	18
ID-25	890.8	913.2 $[M + Na]^+$	16.0	98.1	24
ID-30	859.0	859.3 $[M + H]^+$	15.4	98.4	44
ID-35	906.1	906.4 $[M + H]^+$ 928.4 $[M + Na]^+$	12.7	98.3	48
ID-36	915.0	915.3 $[M + H]^+$ 937.3 $[M + Na]^+$	16.2	97.5	51
ID-37	1003.0	1003.6 $[M + H]^+$	14.2	97.8	52
ID-38	813.5	814.5 $[M + H]^+$ 836.4 $[M + Na]^+$	12.9	98.5	56
ID-39	889.0	*889.3* $[M + H]^+$ *911.3* $[M + Na]^+$	14.6	97.6	24
ID-43	906.1	*906.4* $[M + H]^+$ *928.4* $[M + Na]^+$	12.7	99.3	55
ID-42	897.3	898.1 $[M + H]^+$ 920.1 $[M + Na]^+$ 936.1 $[M + K]^+$	14.2	96.8	37
ID-44	928.5	929.5 $[M + H]^+$	12.9	99.5	41
DSIP	848.8	849.5 $[M + H]^+$	17.9	98.8	43

* Determined by normalizing the peaks on HPLC chromatogram.
** Yield calculated for the content of the initial amino acid on resin or for the initial DSIP in the case of the ID-25 analogue.

ID-38, ID-39, and ID-42 (series 2 and 4) at a single dose of 50 μg/kg for four days one day after the injection of cisplatin (the course dose of the peptide 200 μg/kg; other doses were not examined) did not contribute to the decrease in cisplatin toxicity, and the death rate of animals was from 50 to 67%. The injection of peptides ID-10, ID-35, and ID-37 (series 2) at the same course dose decreased the lethal effect of cisplatin to 33%. Peptides ID-6, ID-43, and ID-44 had the most pronounced positive influence among all compounds examined; their application against the background of cisplatin led to a two- to threefold decrease in lethality, which was 17–30% against 50–67% in the control (series 1, 2, and 3). A comparison of the detoxifying effects of DSIP and one of the most active peptides ID-6 against the toxic action of cisplatin showed that ID-6 has a more pronounced positive effect. Its fourfold application equally decreased the death of animals from the acute toxicity of the cytostatic to 17% at both a single dose of 50 and 250 μg/kg. DSIP had a

similar effect only when the single dose was increased abruptly from 50 to 250 μg/kg (Table 4, series 1).

It is interesting to note that the acetylation of the N^{α}-amino group (ID-25, series 2) somewhat enhanced the toxicity; i. e., the DSIP analogue showed the effect opposite to the action of DSIP itself. An analysis of the relationship between the structure of DSIP and its analogues and their detoxifying effects showed that significant and even minor changes in their primary structure lead to the disappearance of detoxifying properties or a slight enhancement of toxic effects of both ID-25, ID-38, ID-39, ID-42 (series 2) and ID-16, ID-30, ID-36 (series 4). Only particular substitutions at position 2 (substitution of Pro, Lys, βAla, or D-Ala for Ala) and at position 5 (substitution of Asn for Asp) affected favorably the detoxifying activity. Thus, when we compare the activities of the analogues ID-38 and ID-44, which differ only by the presence of the Asp residue at position 5 in the active peptide ID-44 and the deletion of this residue in the inactive ID-38, and the activities of analogues ID-6 and ID-39 (substitution of Asp5 for Glu), the importance of Asp5 in the sequence of the active peptide becomes evident. At the same time, Asp can be equally readily substituted for by Asn with the additional replacement of residue Ala2 by Lys2 (peptide ID-43), which agrees well with our hypothesis that the peptide ID-43 (Trp-**Lys**-Gly-Gly-**Asn**-Ala-Ser-Gly-Glu, earlier we called it KND) is a putative endogenous prototype of DSIP [13, 17]. The activity of this peptide was found to be significantly higher than that of DSIP. If only the Ala residue in the DSIP molecule is substituted for Lys at position 2, as in the case of ID-35, the activity of the peptide also increases compared with DSIP, although is lower than the activity of ID-43.

However, the data presented in this paper were obtained on a limited number of compounds and, for the most part, with the use of only one single and course dose. In this case, many aspects of the structure—function relationship in the series of DSIP analogues remain unclear. Nevertheless, the screening of the effect of individual amino acid substitutions in the primary structure of DSIP on the activity revealed several peptides (ID-6, ID-35, ID-43, and some others), which seem promising for further studies.

To obtain biochemical evidence of the antitoxic effect of peptides, we examined the blood of surviving animals that received a semilethal dose of cisplatin. In Table 5, the biochemical parameters of blood after the injection of cisplatin and the peptides DSIP and ID-6 are given. The injection of the cytostatic at the LD_{50} dose led on day 10 to a substantial increase in the activity of the cytoplasmic enzymes AlT and AsT to 135 ± 16 and 246 ± 21 IU/L, respectively, and an abrupt increase in the concentration of total bulirubin (more than twofold), creatinine (to 102 ± 12 μM), and urea (to 11.1 ± 0.9 mM). The combined use of cisplatin and ID-6 in the range of course doses of 200–1000 μg/kg led to a 35% decrease in the activity of AlT and AsT and a 20–35% decrease in the level of bilirubin and the products of nitrogen exchange compared with the "cisplatin" group. As the dose of ID-6 was decreased to 100 μg/kg, no significant effect of the peptide was observed. In groups of animals receiving DSIP at a course dose of 1000 μg/kg, a significant decrease in the concentration of AlT, AsT and the final products of nitrogen exchange: creatinine and urea was also noted on day 10 of observation; however, at a course dose of 200 μg/kg, the changes were not reliable.

Table 3. Antioxidant activity of DSIP and its analogues

Sample	AOA *	
	$C_{1/2}$ (mol/mL)	Arbitrary unit of activity
ID-6	4.6×10^{-4}	2.2
ID-10	6.9×10^{-4}	1.5
ID-18	1.2×10^{-3}	0.8
ID-19	7.0×10^{-4}	1.4
ID-25	1.1×10^{-3}	0.9
ID-35	8.1×10^{-4}	1.2
ID-36	1.1×10^{-3}	0.9
ID-37	7.3×10^{-4}	1.4
ID-38	1.0×10^{-3}	1.0
ID-39	1.1×10^{-3}	0.9
DSIP	5.9×10^{-4}	1.7
β-Carotene (reference material)	3.5×10^{-4}	2.9
Vitamin C (reference material)	3.9×10^{-4}	2.6

* See the Experimental section, part "Determination of AOA in a biological system in vitro."

Thus, the study of the detoxifying effect of the peptides on the model of toxicosis showed that DSIP and its analogues ID-6, ID-10, ID-37, ID-43, and ID-44 contributed to a decrease in the toxicity of cisplatin, which manifested itself in a diminished death of animals caused by the injection of the semilethal dose of the cytostatic, and led to a reduction in its hepato- and nephrotoxicity (DSIP and ID-6). Of all DSIP analogues examined, ID-6 and, probably, the so far less studied ID-43 are of particular interest from the standpoint of their possible application as therapeutic detoxifying preparations. It also makes sense to further study the detoxifying action of the peptides of this group with the variation of their doses and the protocol of application on a model of toxicosis induced by different cytostatic preparations; both a decrease in the

Table 4. Effect of DSIP analogues on the toxicity of cisplatin*

Series of experiments	Compound	Death of animals from acute toxicity of cisplatin, %
1. In groups of six animals	Cisplatin	67
	DSIP	50
	DSIP**	17
	ID-6	17
	ID-6**	17
2. In groups of six animals	Cisplatin	50
	ID-6	17
	ID-10	33
	ID-18	50
	ID-19	50
	ID-25	67
	ID-35	33
	ID-36	67
	ID-37	33
	ID-38	50
	ID-39	67
3. In groups of ten animals	Cisplatin	60
	ID-43	30
	ID-44	30
4. In groups of five animals	Cisplatin	40
	ID-16	60
	ID-30	60
	ID-42	60

* Cisplatin was injected intravenously singly at a dose of 15 mg/kg in all experiments, and after 24 h peptides at a single dose of 50 μg/kg were injected intravenously for four days.

** Peptides were injected at a single dose of 250 μg/kg for four days.

toxicity of cytostatics by the action of peptides and the retention or the potentiation of their therapeutic efficacy are of interest.

EXPERIMENTAL

Amino acid derivatives and reagents used in the study were from Reanal (Hungary), Aldrich (United States), Iris Biotech (Germany), Novabiochem (Germany), and Khimmed (Russia). All peptides were synthesized by the solid-phase method on a Beckman 990B peptide synthesizer (Beckman, United States), which we adapted for the synthesis by the Fmoc scheme according to the protocol presented in Table 1. Gel filtration was carried out using Sephadex LH-20 (Pharmacia, Sweden). Preparative HPLC was performed on a Beckman 343 chromatograph (Beckman, United States) on a Luna C-18(2) column (250 × 21.2 mm, 5 μm; Sweden) using a concentration gradient of solution B (90% acetonitrile in 0.2% AcOH) in solution A (0.2% AcOH in water) at a flow rate of 6 mL/min; detection was at 220 nm. Analytical HPLC was carried out on a System Gold chromatograph (Beckman, United States) on a Phenomenex C-18 column (2 × 250 mm, 5 μm; Sweden) using a concentration gradient of solution B (80% acetonitrile in 0.05% TFA) in solution A (0.05% TFA in water) from 0 to 100% B for 30 min at a flow rate of 0.25 mL/min with detection at 220 nm. The structures of the peptides were confirmed by MALDI mass spectrometry on an Ultraflex TOF/TOF device (Bruker, United States).

Coupling of the first amino acid to 2-chlorotrityl chloride resin. Dry 2-chlorotrityl chloride resin (0.7 g) was added to a solution of Fmoc-Glu(OBu^t)-OH (0.61 g, 1.44 mmol) or Fmoc-Ala-OH (0.45 g, 1.44 mmol) in chloroform (5 mL) in the presence of DIEA (0.5 mL, 2.88 mmol) at a nominal load of 1.6 mmol/g resin, and the solution was stirred for 90 min at room temperature. Unreacted active groups on the polymeric carrier were blocked by adding MeOH (4 mL), and the reaction mixture was kept for 30 min under stirring. The polymer was washed with DMF (4 × 10 mL), and an aliquot (about 20 mg) was taken, washed additionally with EtOH (3 × 2 mL), dried in vacuo, and the load of the first free amino acid on resin was determined by the method of Kaiser [18]; the load was found to be 0.5–0.7 mmol/g.

Elongation of the amino acid chain of all peptides was carried out in situ according to the general protocol presented in Table 1 with the indication of the sequence of operations, reagents and solvents, their amounts, as well as the duration and the number of treatments.

Cleavage of peptides from resin with simultaneous deprotection was carried out by holding the peptidyl polymer in 25 mL of a 98% TFA solution in water for 2 h under stirring. The resin was filtered off and washed with 98% TFA (2 × 20 mL). The filtrate was evaporated on a rotor evaporator in vacuo at 30 mmHg, and the resulting product was dissolved in 0.05% TFA solution (1.5 mL) and purified by preparative reverse-phase HPLC. The yields of peptides per initial load of the first amino acid on resin, the times of the retention of peptides in analytical HPLC, and molecular weights, as determined from mass spectrometry data, are listed in Table 2.

Acetylation of DSIP. DSIP (50 mg, 58.9 mmol) and acetylimidazole (29 mg, 262 mmol, 4 equivalents) were stirred in a mixture of tetrahydrofuran and water (2 : 1) for 1 h. The course of the reaction was monitored by analytical HPLC. The reaction mixture was neutralized with a 0.1 M aqueous ammonia solution, and the product was purified by gel filtration on an

Table 5. Biochemical parameters of the blood of animals[1]

Experimental group	AsT, IU/L*	AlT, IU/L	Total bilirubin, μmol/L	Creatinine, μmol/L	Urea, mmol/L	Total protein, g/L
Cisplatin	246 ± 21**	135 ± 16**	8.7 ± 2.1**	102 ± 12**	11.1 ± 0.9**	53 ± 7
Cisplatin + DSIP(200)	227 ± 16	133 ± 21**	8.7 ± 1.3**	94 ± 13**	10.4 ± 0.8**	60 ± 6
Cisplatin + DSIP(1000)	201 ± 15	99 ± 15***	8.0 ± 1.2**	77 ± 10***	8.5 ± 0.9**	58 ± 7
Cisplatin + ID-6 (100)	254 ± 19**	146 ± 19**	7.3 ± 1.0	106 ± 16	10.2 ± 0.9	58 ± 9
Cisplatin + ID-6 (200)	166 ± 21***	88 ± 18***	7.5 ± 0.4	86 ± 15**	7.1 ± 0.9***	61 ± 10
Cisplatin + ID-6 (1000)	177 ± 17	96 ± 11***	8.3 ± 1.1**	81 ± 9**	7.5 ± 0.5	63 ± 11
Control	145 ± 12	48 ± 8	3.9 ± 1.1	35 ± 5	4.7 ± 1.0	55 ± 5

[1] Material was taken on day 10 after the injection of cisplatin. Each group involved ten BDF1 female mice. Cisplatin was injected intravenously singly at a dose of 15 mg/kg. After 24 h, DSIP was injected intravenously at single doses of 50 and 250 μg/kg for four days (course doses of 200 and 1000 μg/kg, respectively) and ID-6 was injected intravenously at single doses of 25, 50 and 250 μg/kg for four days (course doses of 100, 200 and 1000 μg/kg, respectively).

* IU, international unit of activity.

** A biologically significant difference from the group "control" ($p < 0.05$).

*** A biologically significant difference from the group "cisplatin" ($p < 0.05$).

LH-20 column (25 × 700 mm) equilibrated with a 0.1 N solution of acetic acid and preparative HPLC.

Determination of AOA in a biological system in vitro. The method is based on the ability of a substance under study to inhibit LP in a mouse liver homogenate. The intensity of LP was estimated from the concentration of MDA by the thiobarbituric method [19]. The results were expressed in percent of the control and represented graphically in a system of coordinates, where the abscissa is the concentration of a sample in a test (mol/mL) and the ordinate is the percent of the inhibition of MDA formation in the test. The molar concentration of a substance ($C_{1/2}$) at which it inhibits LP by 50% was determined from plots. The AOA was calculated as the inverse of the molar concentration of the sample under study necessary for the 50% inhibition of LP and expressed in arbitrary units.

Evaluation of the detoxifying effect on a model of toxicosis induced by cisplatin. Cisplatin at a single dose of 15 mg/kg was injected singly intravenously to BDF1 female mice. All peptides tested were injected intravenously at a single dose of 50 μg/kg for 4 days (the course dose 200 μg/kg) 24 h after the injection of cisplatin. In the case of the ID-6 peptide and DSIP, a fivefold single dose of 250 μg/kg was also used under the same conditions (the course dose of 1000 μg/kg). The death of animals from acute toxicity of cisplatin was recorded, and the biochemical parameters of the blood of surviving animals were determined on day 10 of the experiment. Blood was taken from the cut tail of animals and treated by conventional methods. The biochemical examination of the blood involved the determination of the activity of AsT and AlT, the concentration of urea, creatinine, total protein, and the content of total bilirubin in the blood plasma of experimental animals. The biochemical parameters of the blood were determined using standard biochemical kits (Diakon, Russia) on a Screen Master Plus analyzer (Italy). The biochemical parameters of the blood of intact animals were used as a control.

ACKNOWLEDGMENTS

This work was supported by the Department of Science and Industrial Policy of Moscow under the scientific and technical program "Development and Practical Application of Novel Methods and Drugs for the Prophylaxis, Diagnostics, and Therapy of Oncological, Infectious, and Other Dangerous Diseases in Public Health" for 2010–2012.

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Translated by S. Sidorova