EXPERIMENTAL BIOLOGY

Effects of Structural and "Mixed" Isomers of Glu-Trp Dipeptide on Normal Hemopoietic Stem Cells

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We studied the effects of optical (dd-, ll-, dl-, and ld-dipeptides with α-bond, EW) structural isomers and cyclic (dd-, ll-, dl-, and ld-dipeptides with γ-bond, iEW) analogs of Glu-Trp synthetic dipeptide on the population of normal hemopoietic stem cells. Dipeptides containing lGlu (lGlu-lTrp, lGlu-dTrp) injected to mice were inert towards committed bone marrow CFU-S; dGlu-containing dipeptides (dGlu-dTrp, dGlu-lTrp) inhibited the growth of CFU-S-8; and LiGlu-dTrp stimulated these cells. Inhibitory or stimulatory effects of optical and chemical isomers of Glu-Trp dipeptide are determined by optical orientation and nature of peptide bond of Glu residue. The effects of cyclic and mixed peptides towards colony formation are similar to those of the corresponding linear dipeptides.

Key Words: *CFU-S; mice; optical and structural isomers of EW dipeptide*

Discovery of the important role of thymic peptides in the regulation of immuno- and hemopoiesis gave grounds for the synthesis of numerous peptides and creation of drugs on the basis of these peptides. Glu-Trp dipeptide occupies a special place. lGlu-lTrp dipeptide consisting of l-amino acids (thymogen) proved to be an effective immunostimulator [1], while its optical and structural isomer (diGlu-dTrp) thymodepressin produces an immunoinhibitory effect [2]. We previously demonstrated [3] different effects of these peptides on the population of hemopoietic stem cells: thymogen exhibited hemostimulatory effects, while thymodepressin proved to be a hemoinhibitory agent. Hence, modification of optical and spatial orientation of the peptides leading to emergence of new biological characteristics of the compounds is a perspective method for the creation of new drugs. Moreover, introduction of amino acids of artificial configuration (d-) more resistant to enzymatic cleavage [4] into synthetic molecule can solve the problem of poor stability and short-lasting effects of the peptides in the body, which is the main obstacle for their clinical use. Another solution of this problem is synthesis of peptides with cyclic structure.

We studied the effects of optical and structural isomers of synthetic Glu-Trp (EW) dipeptide on the population of normal hemopoietic stem cells. Cyclic analogs of EW peptide containing pyroglutamic instead of glutamic acid residue (pyroEW) of natural and artificial optical configuration (l or d) were also tested.

MATERIALS AND METHODS

Peptides for the study were synthesized in Center Peptos Company. We used EW, iEW, pyroEW, and pyroiEW peptides containing d- and l-amino acid residues: dGlu-dTrp — (dd); dGlu-lTrp — (dl);

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lGlu-dTrp — (ld); lGlu-lTrp — (ll); diGlu-dTrp — (idd); diGlu-lTrp — (idl); liGlu-dTrp — (ild); liGlu-lTrp — (ill), pyro-dGlu-dTrp — (pyrodd), pyro-dGlu-lTrp — (pyrodl), pyro-lGlu-dTrp — (pyrold), pyro-lGlu-lTrp — (pyroll). The peptides were synthesized by the classical method in solution using the strategy of maximum protection of functional groups. The final products were purified by HPLC in 0.1% AcOH/ethanol buffer (5-30%) gradient. The purity and structure of compounds were confirmed by thin-layer chromatography, HPLC, and high resolution NMR spectroscopy.

Experiments were carried out on 2-3-month-old female C57Bl/6 and (CBA×C57Bl/6)F_1 mice (20-22 g) from Stolbovaya Breeding Center. Bone marrow recipient mice were exposed to γ-rays (^{60}Co) in a dose of 8 Gy on a Luch-1 device (dose power of 0.8 Gy/min). Colony-forming activity of the bone marrow was evaluated as described previously [5]. Bone marrow cells were washed from the femoral bone with medium 199. For evaluation of the effects of the test peptides on colony-forming activity of the bone marrow *in vitro*, the cell suspension was poured into weighing bottles (10 ml), peptides (0.2 μg/ml) were added, the mixture was incubated for 60 min at 37°C, and after washout was injected to lethally irradiated recipients. For evaluation of *in vivo* effects of dipeptides, bone marrow donors received the preparations in a dose of 10 μg/kg intraperitoneally or orally 48 h before bone marrow isolation. Splenic colonies were counted on day 8 after injection of bone marrow cells. The results were statistically processed using Origin software (MicroCal Software).

RESULTS

We previously showed that peptides of the EW family with α-bond (Trp residue attached to Glu α-carboxyl) consisting of only l- or d-amino acid residues differed by their effects on the population of normal murine bone marrow stem cells. 1-Dipeptides did not modify colony formation by hemopoietic cells in the spleens of irradiated mice, while d-peptides suppressed this process [3]. Evaluation of the effects of "mixed" dipeptides showed that *in vitro* treatment of the bone marrow suspension with dipeptides containing at least one d-amino acid residue led to inhibition of splenic colony formation. The same regularity was observed in experiments with dipeptides containing γ-bond: EW and iEW peptides containing at least one d-amino acid residue inhibited colony formation after *in vitro* treatment of bone marrow cells (Table 1).

In vivo studies (donor mice treatment with the preparations 2 days before removal of the bone marrow) showed that EW dipeptides containing lGlu (lGlu-lTrp, lGlu-dTrp) were inert towards committed bone marrow CFU-S. Dipeptides containing dGlu (dGlu-dTrp, dGlu-lTrp) inhibited the pool of CFU-S-8 (Table 2). In contrast to inert lGlu-dTrp, liGlu-dTrp dipeptide injected to mice stimulated CFU-S-8. Hence, the inhibitory or stimulatory effect of optical and chemical isomers of Glu-Trp di-

TABLE 1. Effect of *In Vitro* Treatment of Intact Bone Marrow with Linear and Cyclic Isomers of Synthetic EW Peptide on Colony Formation ($M \pm m$)

Peptide	Number of mice	Mean number of colonies per 10^5 cells	Inhibition, %
Control	30	10.3±0.5	
dE-dW	20	5.7±0.4*	45
dE-lW	20	6.1±0.3*	41
lE-dW	20	7.5±0.5*	27
lE-lW	20	10.4±0.2	0
diE-dW	20	5.8±0.5*	43
diE-lW	20	6.0±0.5*	41
liE-dW	20	6.6±0.4*	35
liE-lW	20	10.2±0.5	0
Pyro-dE-dW	20	8.4±0.6*	21
Pyro-dE-lW	20	7.0±0.4*	34
Pyro-lE-dW	20	6.6±0.4*	38
Pyro-lE-lW	30	10.5±0.7	0

Note. Here and in Tables 2, 3: *$p<0.05$ compared to the control.

TABLE 2. *In Vivo* Effects of Linear and Cyclic Isomers of Synthetic EW Peptide on Colony Formation (*M*±*m*)

Peptide administered to donors	Number of mice	Relative number of CFU-S-8	Suppression (↓) or stimulation (↑), %
Control	40	10.4±0.6	
dE-dW	20	6.2±0.5*	41(↓)
dE-lW	20	6.1±0.3*	41(↓)
lE-dW	20	11.4±0.5	0
lE-lW	20	10.7±0.4	0
diE-dW	30	5.4±0.5*	46(↓)
diE-lW	30	6.1±0.3*	40(↓)
liE-dW	30	13.3±0.1*	33(↑)
liE-lW	30	10.1±0.5	0
Pyro-dE-dW	20	7.5±0.3*	28(↓)
Pyro-dE-lW	20	7.9±0.5*	26(↓)
Pyro-lE-dW	30	11.9±0.7	0
Pyro-lE-lW	30	10.7±0.5	0

TABLE 3. *In Vivo* Effects of Linear and Cyclic Isomers of Synthetic EW Peptide on Colony Formation after Oral Treatment (*M*±*m*)

Donor treatment	Number of mice	Relative number of CFU-S-8	Suppression, %
Control	20	10.6±0.3	
dE-dW	20	10.8±0.4	0
dE-lW	20	10.7±0.6	0
lE-dW	20	10.6±0.5	0
lE-lW	20	10.3±0.5	0
Pyro-dE-dW	20	8.4±0.7*	20
Pyro-dE-lW	20	7.1±0.7*	33
Pyro-lE-dW	20	10.0±1.0	0
Pyro-lE-lW	30	10.5±0.8	0

peptide on intact stem cells is determined by optical orientation and type of Glu residue peptide bond.

Cyclic analogs of EW dipeptides containing pyroglutamic (instead of glutamic) acid residue of natural and artificial optical configuration (l or d) were studied. Cyclization provides higher stability of the resultant compounds with the same toxic profile. The effects of pyro-lE-lW, pyro-dE-dW, and mixed (pyro-dE-lW, pyro-lE-dW) peptides on colony formation *in vivo* and *in vitro* were similar to those of the corresponding linear dipeptides, but less pronounced (Tables 1, 2). Intraperitoneal injection of pyro-dE-dW or pyro-dE-lW (100 μg/kg, 2 days before collection of the bone marrow) dipeptides containing dextra-rotating Glu decreased the number of hemopoietic colonies in the spleens; pyro-lE-dW and pyro-lE-lW virtually did not change their number in comparison with the control. Inhibition of colony formation after *in vitro* treatment of bone marrow cell with cyclic dipeptides depended on the presence of dextra-rotating amino acid residue in the molecule. Differences in the effects of linear EW and pyroEW peptides were observed, when they were administered orally (100 μg/kg). Linear peptides were ineffective in these experiments, while pyro-dE-dW and pyro-dE-lW reduced the number of colonies, similarly as after intraperitoneal injection (Table 3).

Hence, modification of the structure of EW peptide by cyclization and introduction of d-amino acid residues into the molecule improved enzyme resistance of the compound and does not fundamentally modify its properties. Low-molecular-weight peptides are nontoxic, cause no side effects, and the spectrum of their use is wide; this suggests further search for active compounds among them and improvement of the drugs created on the basis of these peptides.

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