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The effects of the EW dipeptide optical and chemical isomers on the CFU-S population in intact and irradiated mice

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Abstract

The influence of Glu–Trp (EW) synthetic dipeptide isomers on hemopoietic progenitor cells and certain immune response reactions is determined by their optical and chemical properties. Thus, the all L-amino acid containing dipeptides L-Glu–L-Trp and L-γGlu–L-Trp have no effect on proliferation of committed and pluripotent CFU-S in intact bone marrow. The optical isomers of the Glu residue are an essential determinant of the EW dipeptide biological activity. The inversion of the amino acid optical form imparts suppressor properties: D-Glu–D-Trp, D-γGlu–D-Trp, D-Glu–L-Trp and D-γGlu–L-Trp inhibit proliferation of hemopoietic progenitors in intact bone marrow. The type of the peptide bond between L-Glu and Trp is another important factor for the biological activity of the L-Glu-containing peptides. Unlike L-Glu–D-Trp with α-peptide bond, the dipeptide L-γGlu–D-Trp with γ-peptide bond stimulates CFU-S-8 proliferation in intact bone marrow. The diverse effects of the EW optical isomers on hemopoietic progenitors underlie the radioprotective properties of the D-Glu-containing dipeptides and the radiotherapeutic ones of the L-Glu dipeptides. In animals, pre-irradiation injection of D-Glu–D-Trp, D-γGlu–D-Trp, D-Glu–L-Trp, D-γGlu–L-Trp, or post-irradiation injection of L-Glu–L-Trp, L-γGlu–L-Trp promoted regeneration of the hemopoietic progenitor population.

Keywords: Peptide isomers; Reciprocal activities; Immunosuppression; Immunostimulation

1. Introduction

Creation of a new generation of drugs — non-toxic synthetic peptide immuno- and hemoregulators — is an important challenge. In the past decade, evidence has been accumulated on the importance of amino acid sequence and chain length of synthetic immunoregulatory peptides for their functional activity. Far less research, however, has sought to evaluate the role of optical isomerism in the biological activity of amino acids and peptides. There have been relatively few studies on the effects of synthetic peptides consisting of D- or L-amino acid residues on the development of graft-versus-host disease, formation of antibodies, angiogenesis [1–3]. Most of the relevant publications report that peptides comprised of only D- or L-amino acid residues (D- or L-peptides) have unidirectional effects, although D-peptides are more peptidase-resistant *in vivo*. Scarce information is available on the change of the

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immunoreactive and antineoplastic properties of such peptides upon replacement of one or more L-amino acid residues with D- ones [4,5]. And finally, practically unexplored are the effects of D- or mixed L- and D-peptides at the initial stages of hemopoiesis, i.e. on stem cells that are the progenitors of all morphologically identifiable blood and immune cells.

Using the Glu–Trp dipeptides as an example, we have demonstrated the dependence of peptide biological activity the optic form of each constituent amino acid residue. In our earlier publications, we reported that L-Glu–L-Trp (EW, Thymogen) and its structural and optical D-isomer D-γGlu–D-Trp (γEW, Thymodepressin) have different effects on hemopoietic progenitor cells *in vivo*: the L–L-peptides have hemostimulating properties, while the D–D-peptides — hemoinhibiting ones [6]. Thymogen and Thymodepressin are patented in several countries of Europe, America and Asia and approved in Russia for medical use: Thymogen as immunomodulator [7] and Thymodepressin as immunosuppressor [8].

Taking into consideration the available data and our own findings on the significance of amino acid optical isomers in synthetic dipeptides for regulatory processes and intercellular interaction, it would be reasonable to expect that a change in the optical orientation of even one amino acid residue may impart new properties to mixed dl or ld dipeptides. This study is devoted to the effects of synthetic D–D, L–L, D–L and L–D-isomers of the dipeptides EW (α-peptide bond) or γEW (γ-peptide bond) on the number, proliferating capacity and other properties of hemopoietic progenitor cells in normal animals, as well as upon exposure to ionizing radiation.

2. Materials and methods

2.1. Mice

Test animals were C57B1/6 and (CBA×C57Bl6) F1 female mice, 2–3 months, 20–22 g, from the "Stolbovaya" Animal Breeding Center.

2.2. Peptides

The EW and γEW peptides under study were synthesized at the Department of peptide chemistry, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS.

Abbreviation of the peptides under the study: dE–dW — (D-Glu–D-Trp), (dd); dE–lW — (D-Glu–L-Trp), (dl); lE–dW — (L-Glu–D-Trp), (ld); lE–lW — (L-Glu–L-Trp), (ll); d-γE–dW — (D-γGlu–D-Trp), (γ-dd); d-γE–lW — (D-γGlu–L-Trp), (γ-dl); l-γE–dW — (L-γGlu–D-Trp), (γ-ld); l-γiE–lW — (L-γGlu–L-Trp), (γ-ll).

The peptides were synthesized in solution using the classical method with maximum functional group protection. The products were purified using high performance liquid chromatography in a 0.1% AcOH/ethanol buffer gradient (5–30%). The purity and structure of the final products were controlled by TLC, HPLC, high-resolution NMR- and MS spectroscopy.

2.3. Irradiation

Test animals were irradiated with ^{60}Co gamma-rays at a dose rate of 0.8 Gy per min. Bone marrow recipients were irradiated with a dose of 8 Gy, donors — with 4 Gy. The suspension of bone marrow cells of intact animals was irradiated with 1 Gy, 5–10 min prior to injection to lethally irradiated recipients.

2.4. Determination of CFU-S

The colony forming capacity of bone marrow was measured according to Till and McCulloch [9]. The effects of the peptides under study to form colonies of bone marrow *ex vivo* were evaluated as follows: the suspension was dispensed into weighing bottles, 10 ml each; treated with the peptides, 0.2 μg/ml; incubated at 37 °C for 60 min; washed and injected to lethally irradiated recipients. For *in vivo* studies, donor mice received varying intraperitoneal doses of the peptides, from 1 to 10 000 μg/kg 48 h *prior* to bone marrow extraction. Bone marrow suspension was prepared from femoral marrow of intact and irradiated donors. The peptide effects on the CFU-S proliferation were evaluated in the "thymidine suicide" test [10]. Donor animals were intact or irradiated (4 Gy) mice. Bone marrow from irradiated donors was taken on day 7 following irradiation. Some of them received an intraperitoneal injection of a peptide under study, 10 μg/kg, 48 h *prior to* bone marrow extraction. Intact donors also received peptide injections 48 h *prior to* bone marrow extraction. The bone marrow suspension (3×10^6 cells per ml, 2 ml) was incubated with ^{3}H-thymidine, 200 μCi, at 37 °C for 20 min, diluted to desired concentration and injected to lethally irradiated recipients. Control samples were incubated with the aliquots of "cold" thymidine. Marrow repopulation ability (MRA) was assayed by the modified method [11]. Bone marrow from normal or treated with the peptides donors were injected into primary recipients and after 12 days aliquots of their femoral cells were injected into secondary recipients. Spleen colonies in the secondary recipients were counted 12 days later.

In a series of tests, bone marrow cells were irradiated (1 Gy) *in vitro*, at 4 °C, and injected to recipients 5–7 min after the exposure. After 30 min, the recipients received additional intraperitoneal injections of the peptides in doses 1, 10, 100, 1000 and 10 000 μg/kg.

2.5. Cytofluorometric analysis

The cytofluorometric assay of cells bearing the CD34 was performed as follows: donor mice received the peptides 48 h prior to organ extraction; extracted bone marrow cells were incubated with monoclonal antibodies specific for CD34 and CD45 ("PharMingen Becton Dickinson", USA). Stained samples were analyzed using a "FACS Vantage" cytometer,

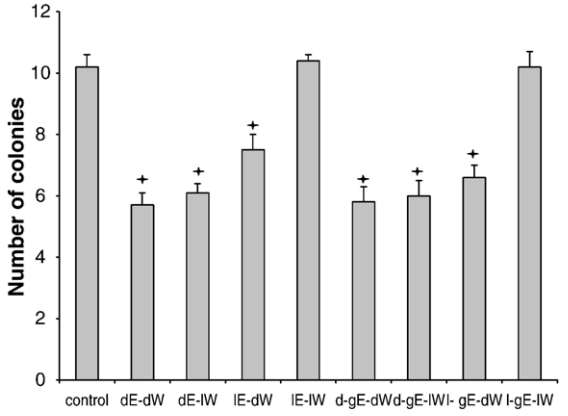

Fig. 1. Colony formation following *ex vivo* treatment of intact bone marrow with EW isomers. The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. *Significantly different ($p<0.05$) from control.

"Becton Dickinson Immunocytometry Systems", USA, for the percent of CD34+ $CD45^{low}$ cells within gate of lymphoid cells, selected by forward and side scattering, and the intensity of CD34 fluorescence on surface of the positive cells.

2.6. Statistical analysis

Statistical analysis was performed using the Student *t*-test.

3. Results

3.1. Ex vivo effects of EW and γEW dipeptide isomers on CFU-S of intact mice

As we have published earlier, EW dipeptides consisting of L-amino acid residues differ from D-isomers in their action on bone marrow hemopoietic progenitor cells of intact mice. While L-dipeptides have no effect on colony formation of intact cells, the D-isomers reduce the yield of exogenous spleen colonies [6]. This test system was used for preliminary screening of mixed isomers of the EW and γEW dipeptides. In the case of *ex vivo* treatment of bone marrow suspension (37 °C, 60 min), the dipeptides containing at least one D-amino acid residue inhibited spleen colony formation, while ll and γ-ll dipeptides had no such effect (Fig. 1).

3.2. In vivo effects of EW and γEW dipeptide isomers on CFU-S of intact mice

Taking into consideration the publications of Magli et al., and Baines and Visser [12,13], describing pool of hemopoietic progenitors as developing dynamic system consisting of subpopulations with various committed level and repopulation ability we have decided to test the influence of EW and γEW peptides on the process of 8- and 12-days exocolonies as representatives of higher and lover committed hemopoietic progenitors correspondently.

The relative number (per 10^5 injected bone marrow cells) of CFU-S-8 and CFU-S-12 in the bone marrow of donor mice, injected with the L–L, D–D, D–L-, or L–D-dipeptide in a dose of 1, 10, 100, 1000 or 10 000 μg/kg 48 h before the organ extraction has been determined. Dose-dependent inhibition of colony growth, as assessed on day 8, was observed following the use of dE–dW, d-γE–dW (Fig. 2) and dE–lW, d-γE–lW (Fig. 3), while no effect was detected following the use of lE–lW, lE–dW, l-γE–lW (Table 1). Injection of l-γE–dW dose-dependently increased the number of colonies by more than 40% (Table 1, Fig. 4). Evaluation of the effects of the EW dipeptide isomers on pool of pluripotent hemopoietic progenitors (CFU-S-12) showed that both dE–dW and d-γE–dW not only statistically significant increase the yield of spleen colonies on day 12 in primary recipient, but in secondary recipient the amount of CFU-S-12 has increased almost two folds as well (Tables 1 and 2). The other peptides, with at least one L-amino acid residue, had no effect on this parameter (Table 1). Since the lE–lW, lE–dW and l-γE–lW dipeptides have no effect on progenitors in intact bone marrow, it was decided to study their effect on affected CFU-S. As a damaging factor, irradiation with a dose of 1 Gy was used. Bone marrow suspension was irradiated *ex vivo*; the peptides were injected to recipients intraperitoneally, in the doses of 1, 10, 100, 1000 or 10 000 μg/kg, 15–30 min after transplantation of the irradiated cells. As the result, lE–lW, l-γE–lW (Fig. 5) and lE–dW (Fig. 6) dose-dependently restored the population of CFU-S-8 affected by irradiation, almost to the level of intact control. Based on the obtained results, the dose of 10 μg/kg was selected as optimal for further *in vivo* studies.

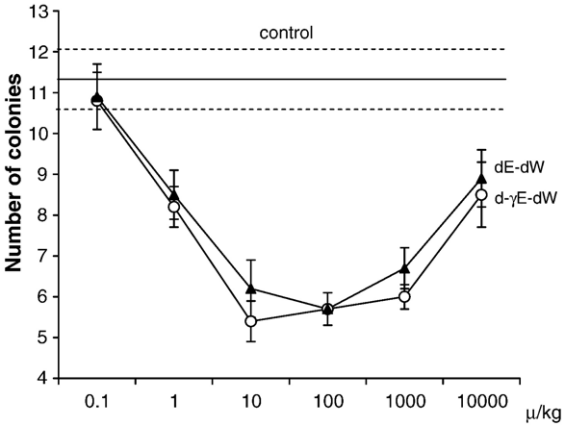

Fig. 2. The dose dependent effects of d-γE–dW and dE–dW (injected 48 h *prior to* bone marrow extraction) on the number of spleen colonies (CFU-S-8). The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. Control level: solid line — the mean number of colonies; dotted line — ±SEM.

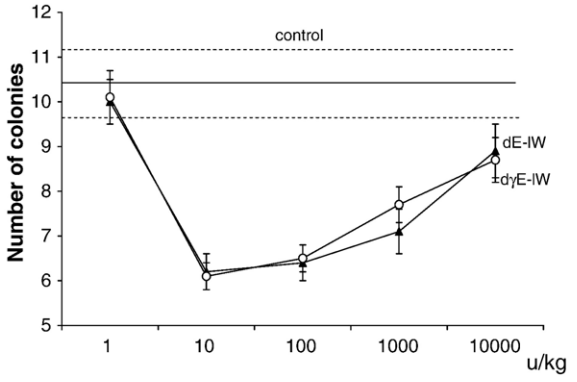

Fig. 3. The dose dependent effects of d-γE–lW and dE–lW (injected to donor 48 h *prior to* bone marrow extraction) on the number of spleen colonies (CFU-S-8); The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. Control level: solid line — the mean number of colonies; dotted line — ±SEM.

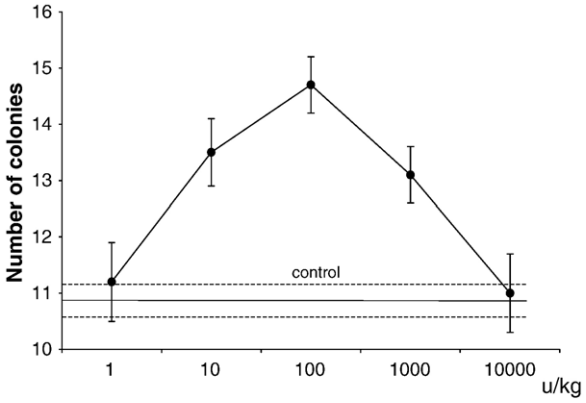

Fig. 4. The dose dependent effect of l-γE–dW (injected to donor 48 h *prior to* bone marrow extraction) on the number of spleen colonies (CFU-S-8); The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. Control level: solid line — the mean number of colonies; dotted line — ±SEM.

3.3. The effects of EW and γEW peptides on the expression of membrane molecules on the surface of bone marrow and spleen cells in mice

Flow cytometry was used to evaluate the effects of EW and γEW isomers on the relative number of CD34+ cells (hemopoietic progenitors) and the surface marker density in the bone marrow of mice. The peptides were injected to donors. On the second day following the injection, the relative number of CD34+bone marrow cells has been evaluated. Each of the tested peptides statistically significantly decreased the parameter as compared to control. The only D-Glu–D-Trp or D-γGlu–D-Trp increased the marker density ($p<0.05$) and the percent of pluripotent progenitors in the bone marrow (Table 3).

3.4. The effects of the EW and γEW dipeptide isomers on CFU-S proliferation in normal and irradiated donor mice

The effects of the EW and γEW dipeptides were studied using the "thymidine suicide" method in two test systems, with intact and irradiated donors (Figs. 7 and 8). In the first case, intact donor mice received peptide injections 48 h *prior* to bone marrow extraction. As evident from the results (Fig. 7), only l-γE–dW stimulated proliferation of CFU-S-8 in intact donors. This effect was probably responsible for the increased number of spleen colonies formed by the partially committed CFU-S-8 (Table 1).

The other peptides had no effect on the number of CFU-S-8 in the S-phase of the cycle in this test system. However,

Table 1
In vivo effects of the EW dipeptide isomers on the relative number of CFU-S-8 and CFU-S-12

Donor treatment (10 μg/kg)	Relative number of CFU-S-8 (M±SEM)	% suppression (↓) or stimulation (↑)	Relative number of CFU-S-12 (M±SEM)	% stimulation (↑)
–	10.0±0.6 (30)		9.8±0.5 (20)	
dE–dW	6.2±0.5[a] (20)	41(↓)	13.7±0.3[a] (20)	40(↑)
dE–lW	6.1±0.3[a] (20)	41(↓)	10.0±0.6 (20)	0
lE–dW	11.4±0.5 (20)	0	10.3±0.8 (20)	0
lE–lW	10.7±0.4 (20)	0	9.9±0.7 (20)	0
d-γE–dW	5.4±0.5[a] (30)	46(↓)	14.3±0.6[a] (20)	46(↑)
d-γE–1w	6.1±0.3[a] (30)	40(↓)	10.3±0.7 (20)	0
l-γE–dW	13.3±0.8[a] (30)	33(↑)	10.9±1.0 (20)	0
l-γE–lW	10.1±0.5 (30)	0	10.3±0.9 (20	0

Bone marrow from normal or treated with the peptides (intraperitoneal, 48 h *prior* to bone marrow extraction) donors were injected into lethally irradiated recipients. Spleen colonies in the recipients were counted 8 or 12 days later.

[a] $p<0.01$ relative to control; number of mice is indicated in brackets.

Table 2
The effects of the dE–dW and d-γE–dW dipeptides on absolute number of CFU-S-12 in primary and secondary recipients

Donor treatment (10 μg/kg)	Absolute number of CFU-S-12 in primary recipients per femur, M±SEM	Absolute number of CFU-S-12 in secondary recipients per femur, M±SEM
–	2219±110 (25)	285±31 (25)
dE–dW	2979±64[a] (25)	589±50[a] (25)
d-γE–dW	3146±83[a] (25)	514±39[a] (25)

Bone marrow from normal or treated with the peptides (intraperitoneal, 48 h *prior* to bone marrow extraction) donors were injected into primary lethally irradiated recipients and after 12 days aliquots of their femoral cells were injected into secondary lethally irradiated recipients. Spleen colonies in the secondary recipients were counted 12 days later.

[a] $p<0.001$ relative to control; number of mice is indicated in brackets.

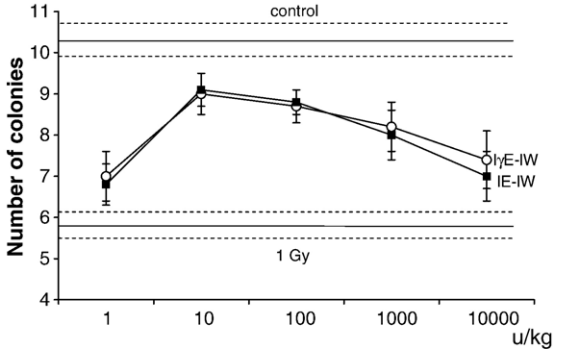

Fig. 5. The dose dependent effects of l-γE–lW and lE–lW on the number of spleen colonies (CFU-S-8), formed by *ex vivo* irradiated (1 Gy) bone marrow (peptides being injected to recipients 30 min *following* injection of bone marrow suspension); The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. Control: solid line — the mean number of colonies, formed by normal bone marrow; dotted line — ±SEM. 1 Gy: solid line — the mean number of colonies, formed by *ex vivo* irradiated (1 Gy) bone marrow; dotted line — ±SEM.

it is difficult to judge the specific effect of the peptides on proliferation in this system, because intact bone marrow contains as little as 3 to 10% CFU-S in the S-phase, the remaining cells being at relative rest. For capturing more significant changes, the cells in the S-phase must account for at least 40%. This is the case with the mice bone marrow

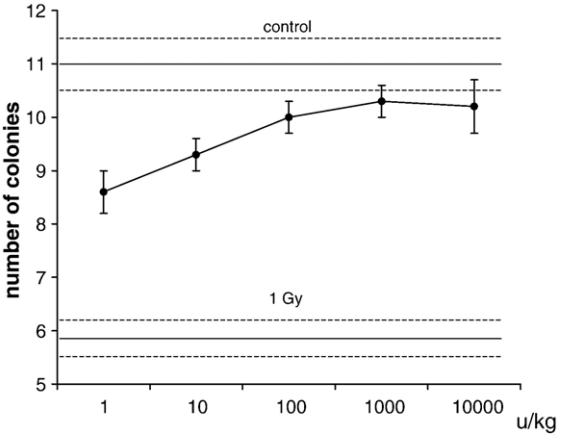

Fig. 6. The dose dependent effects of lE–dW on the number of spleen colonies (CFU-S-8), formed by *ex vivo* irradiated (1 Gy) bone marrow (peptides being injected to recipients 30 min following injection of bone marrow suspension). The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. Control: solid line — the mean number of colonies, formed by normal bone marrow; dotted line — ±SEM. 1 Gy: solid line — the mean number of colonies, formed by *ex vivo* irradiated (1 Gy) bone marrow; dotted line — ±SEM.

Table 3
The effects of the EW dipeptide isomers on the expression and fluorescence intensity of CD34+ cells in bone marrow

Donor treatment (10 μg/kg)	CD34+ cells (%), M±SEM	Mean fluorescence intensity of CD34+ cells±SEM
–	3.8±0.3	55.1±3.8
dE–dW	2.6±0.1 $p=0.01$	88.6±5.9 $p=0.006$
dE–lW	2.5±0.4 $p=0.02$	56.3±4.7
lE–dW	1.9±0.2 $p=0.0002$	55.2±6.4
lE–lW	1.6±0.3 $p=0.00002$	56.9±3.7
d-γE–dW	2.4±0.2 $p=0.01$	89.8±3.8 $p=0.008$
d-γE–lW	2.3±0.4 $p=0.02$	58.7±3.8
l-γE–dW	1.5±0.1 $p=0.00001$	54.4±3.7
l-γE–lW	1.2±0.2 $p=0.00001$	56.5±5.3

Donor mice received the peptides (intraperitoneal) 48 h *prior to* organ extraction; 15 mice per group.

on day 7 after irradiation with the dose of 4 Gy. In the alternative test system donor mice were irradiated with 4 Gy; on day 5 post irradiation the animals received an injection of one of the peptides under study, and 2 days later (on day 7 post irradiation) their bone marrow was analyzed for CFU-S-8 in the S-phase of the cycle using the method of "thymidine suicide". The results (Fig. 8.) show that the dGlu-containing peptides (dE–dW, dE–lW and d-γE–dW, d-γE–lW) had similar effects on the actively proliferating bone marrow: the peptides reduced the percent of CFU-S in the S-phase of the cell cycle to the control level. The effects of L-Glu-containing peptides (lE–dW, lE–lW and l-γE–dW, l-γE–lW) were also similar: they neither reduced, nor increased statistically significant the number of CFU-S in the cycle.

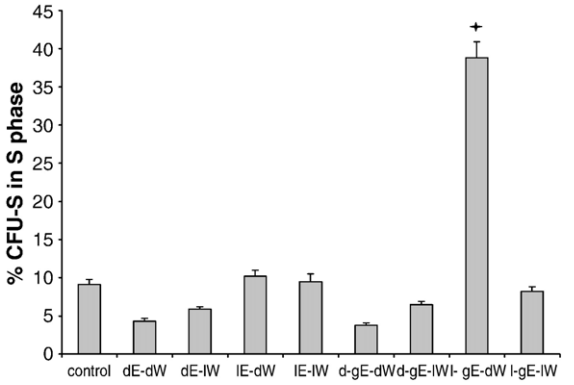

Fig. 7. The effects of the EW dipeptide isomers on the percentage of CFU-S-8 in the S-phase in intact donor bone marrow (10 μg/kg). The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. *Significantly different ($p<0.001$) from control.

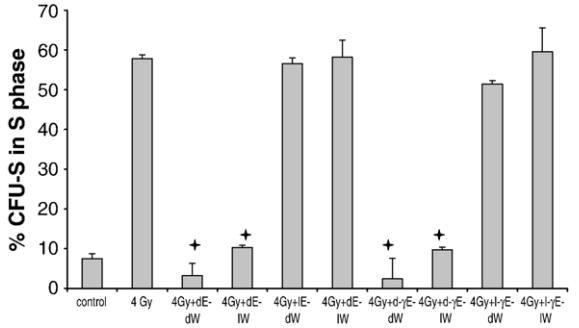

Fig. 8. The effects of the EW dipeptide isomers on the percentage of CFU-S-8 in the S-phase in irradiated donor (4 Gy) bone marrow (10 μg/kg). The results are means±SEM of three independent experiments, each with ten mice/group assayed individually. *Significantly different ($p<0.001$) from 4 Gy.

3.5. The effects of γEW dipeptide isomers on the population of hemopoietic progenitors in irradiated mice

In the first set of tests, the dipeptides were used to protect the initial stages of hemopoiesis against the effect of ionizing radiation. Peptide injections were given to mice 48 h *prior to* irradiation (4 Gy). Bone marrow was extracted on day 8 post irradiation and the number of CFU-S-8 was assessed by the capacity to form spleen colonies. It was found (Table 4) that administration of the D-Glu-containing peptides dE–dW, dE–lW and d-γE–dW, d-γE–lW before exposure to ionizing radiation statistically significantly increased both relative and absolute numbers of CFU-S in the mice bone marrow on day 8 after irradiation, but not reaching the initial intact level. l-γE–dW decreased only the relative content of CFU-S in bone marrow, while lE–dW, lE–lW and l-γE–lW were ineffective.

Table 4
The effects of EW dipeptide isomers used 48 h prior to irradiation (4 Gy) on the absolute and relative number of CFU-S in mouse bone marrow

Donor treatment (10 g/kg)	Donor irradiation (4 Gy)	Relative number of CFU-S-8 (per 10^5 bone marrow cells) M±SEM	Absolute number of CFU-S-8 per femur M±SEM
–	–	11.2±0.5	2164±87
–	+	3.3±0.4 [a]	405±55 [a]
			**$p<0.01$
dE–dW	+	5.6±0.3 [a, b]	916±69 [a, b]
dE–lW	+	5.3±0.5 [a, b]	641±60 [a, b]
lE–dW	+	3.4±0.7 [a]	425±87 [a]
lE–lW	+	3.9±0.8 [a]	514±106 [a]
d-γE–dW	+	6.0±0.5 [a, b]	1059±84 [a, b]
d-γE–lW	+	5.4±0.4 [a, b]	674±63 [a, b]
l-γE–dW	+	2.1±0.6 [a]	465±60 [a]
l-γE–lW	+	4.3±0.7 [a]	414±46 [a]

Peptide injections were given to mice 48 h *prior to* irradiation (4 Gy). Bone marrow was extracted on day 8 post irradiation and the number of CFU-S-8 was assessed for the capacity to form spleen colonies. The results are means±SEM of three independent experiments, each with ten mice/group.
[a] $p<0.05$ in comparison to non-irradiated mice.
[b] $p<0.05$ in comparison to irradiated (4 Gy) mice.

Table 5
The effects of EW dipeptide isomers injected 24 h *post* irradiation (4 Gy) on the absolute and relative number of CFU-S in mouse bone marrow

Donor treatment (10 μg/kg)	Donor irradiation (4 Gy)	Relative number of CFU-S-8 (per 10^5 bone marrow cells) M±SEM	Absolute number of CFU-S-8 per femur M±SEM
–	–	10.2±0.5	2223±107
–	+	3.1±0.4 [a]	446±63 [a]
dE–dW	+	2.6±0.5 [a]	448±78 [a]
dE–lW	+	2.9±0.6 [a]	441±91 [a]
lE–dW	+	5.1±0.4 [a, b]	678±52 [a, b]
lE–lW	+	5.7±0.4 [a, b]	1265±89 [a, b]
d-γE–dW	+	2.7±0.3 [a]	468±50 [a]
d-γE–lW	+	3.2±0.4 [a]	448±56 [a]
l-γE–dW	+	4.9±0.5 [a, b]	656±48 [a, b]
l-γE–lW	+	5.3±0.3 [a, b]	1250±65 [a, b]

Peptide injections were given to mice 24 h *post* irradiation (4 Gy). Bone marrow was extracted on day 8 post irradiation and the number of CFU-S-8 was assessed for the capacity to form spleen colonies. The results are means±SEM of three independent experiments, each with ten mice/group.
[a] $p<0.05$ in comparison to non-irradiated mice.
[b] $p<0.05$ in comparison to irradiated (4 Gy) mice.

The second set of tests evaluated the radiotherapeutic properties of the peptides. Each of the peptides was injected to irradiated (4 Gy) donor mice 24 h *after* exposure to the ionizing radiation. As in the previous tests, bone marrow was analyzed for relative and absolute content of CFU-S on day 8 post irradiation. It was found (Table 5) that D-Glu-containing peptides injected after the exposure had no effect on the regeneration of the compartment of hemopoietic progenitors. lE–lW and l-γE–lW doubled relative as well as absolute content of CFU-S in bone marrow as compared to irradiated control. lE–dW and l-γE–dW had a similar, although less marked effect.

4. Discussion

According to the results of our previous *in vivo* studies of chemical and optical isomers of the natural EW dipeptide, the isomers composed of natural L-amino acids usually have immuno- and hemostimulating effects, while peptides made of D-amino acids have immuno- and hemosuppressive properties [6].

In vivo studies of the biological effects of chemical and optical isomers of the natural EW dipeptide (D–D, L–L, D–L and L–D isomers) showed that a change of the optical orientation of even one of the constituent amino acids causing new biological properties. L-dipeptides (L-Glu–L-Trp, L-γGlu–L-Trp) are inert to committed

and pluripotent CFU-S of intact bone marrow, but restore the cells population affected by ionizing irradiation. D-Glu-containing dipeptides (D-Glu–D-Trp, D-Glu–L-Trp D-γGlu–D-Trp, D-γGlu–L-Trp) have an inhibiting effect on the pool of partially committed CFU-S-8, and only D-Glu–D-Trp or D-γGlu–D-Trp can increase the population of pluripotent hemopoietic progenitors (CFU-S-12) increasing the repopulation ability of bone marrow. The effects of the mixed dipeptides L-Glu–D-Trp and L-γGlu–D-Trp on the CFU-S population differ: L-Glu–D-Trp restores the number of CFU-S collected from 1 Gy irradiated bone marrow and at the same time, like L-Glu–L-Trp, has no effect on *in vivo* colony formation by intact bone marrow cells. L-γGlu–D-Trp stimulates the proliferation of CFU-S-8, increasing the number of colonies by over 40% as compared to control. Thus, the change of the α-peptide bond for the non-natural γ-peptide bond in the EW dipeptide imparts a new property to the l-γE–dW peptide — the capacity to increase the number of CFU-S in intact bone marrows. Injected to donors, each of the peptides under study (EW or γEW) caused a statistically significant reduction of the relative number of CD34+ bone marrow cells as compared to control. However, only D-Glu–D-Trp or D-γGlu–D-Trp increased the marker density ($p<0.05$). The obtained data suggest that the peptides act directly on the CD34+ cells.

Spleen colony formation is a cooperative process, which involves not only the hemopoietic stem cell, but also a set of cellular and innate factors of the microenvironment. Accessory T-lymphocytes are one of the essential components at the initial stages of hemopoiesis [14]. These cells control the proliferation of CFU-S. A removal of T-cells while enriching bone marrow with stem cells or while treating the suspension with anti-mouse brain serum would slacken the CFU-S proliferation [15,16].

As demonstrated in our previous publication [6], inhibition of colony formation by d-γE–dW can be reversed by injecting the mice with intact thymocytes, which indicates to an indirect, via accessory T-cells, action of the peptide on CFU-S. We would suggest that the change in the number of spleen colonies on day 8 under the effect of other D- and L- isomers of the EW peptide is also caused not only by a decrease in the relative number of CD34+hemopoietic progenitors, but also by the effect of the peptides on the hemopoietic microenvironment, specifically on accessory T-lymphocytes that are necessary for hemopoietic stem cell proliferation.

Therefore, one might expect that the peptides would change the rate of CFU-S proliferation and this was demonstrated in two test systems: using the "thymidine suicide" test in intact and irradiated donors. The D-Glu-containing dipeptides inhibited proliferation of CFU-S-8 (in intact as well as in proliferating bone marrow), declining the percent of cells in the S-phase of the cell cycle below the intact level. We believe that this inhibition of CFU-S-8 proliferation decreased the number of committed CFU-s-8 after treatment by dE–dW and dγE–dW (Figs. 7 and 8) resulting in accumulation of more pluripotent CFU-s-12 in bone marrow.

L-Glu–L-Trp, L-γGlu–L-Trp and L-Glu–D-Trp had no such effect, while L-γGlu–D-Trp enhanced proliferation of CFU-S in normal bone marrow, increasing the percent of cells in the S-phase of the cell cycle by about 40% as compared to 10% in control.

Thus, D–D, L–L and mixed isomers of the EW dipeptide differ in their biological action on hemopoietic progenitors in intact bone marrow. The D-Glu-containing dipeptides, irrespective of the optical form of Trp, acting toward inhibition of 8-day spleen colonies both *ex vivo* and *in vivo* and slowing down the proliferation of CFU-S-8.

The L-Glu-containing dipeptides do not inhibit the colony formation. Moreover, the mixed dipeptide l-γE–dW enhances both the proliferation and the number of CFU-S. The dipeptides lE–lW, l-γE–lW and lE–dW are "inert" to the compartment of hemopoietic progenitors in the intact organism. The obtained data suggest that the inhibitory or stimulatory effects of the EW optic and chemical isomers on intact stem cells depend on the optical orientation and the type of the peptide bond of the Glu residue.

Based on the effects of the EW dipeptides on the initial stages of hemopoiesis, it was reasonable to evaluate their potential use as agents reducing the detrimental effect of ionizing radiation on hemopoiesis. According to our findings, the D-Glu-containing peptides have a radioprotective activity, while the L-Glu- ones — radiotherapeutical properties. The injection of D-Glu–D-Trp, D-γGlu–D-Trp or D-Glu–D-Trp, D-γGlu–D-Trp *prior to*, and L-Glu–L-Trp, L-Glu–D-Trp or L-γGlu–D-Trp, L-γGlu–L-Trp *after* irradiation intensified the regeneration of CFU-S.

The D-Glu-containing dipeptides are capable to inhibit the proliferation of CFU-S and have a radioprotective effect on the cell population. The peptides reduce the number of progenitors in the cycle, and hence the radiation acts on "resting" cells that more resistant to damage. The L-Glu-containing dipeptides l-γE–dW and l-γE–lW are only effective when used after irradiation: they intensify the regeneration of the population of hemopoietic progenitors as compared to irradiated control. In this case as well the optical orientation (D- or L-) of the Glu residue determines the effect of the EW isomers on the population of irradiated stem cells.

Thus, the results of the study illustrate the great significance of the spatial structure and optical orientation of a dipeptide molecule for its biological properties.

A change in the spatial orientation of a diastereomer can alter not only the magnitude, but also the direction of its biological effects. Our findings about such changes in the fine structure of the EW dipeptide isomers and the associated changes in the magnitude and direction of their biological effects suggest that at least the EW dipeptides should be considered not just as a minimal sequence peptide consisting of two different amino acids and having specific biological properties, but as an integral organic molecule, whose general chemical structure, optical and spatial orientation determines the magnitude and the nature of its biological effects.

Based on the obtained data, the dipeptide molecules can be considered as a sort of unique "bridge" between polypeptides and proteins having broad spectrum biological activity, on the one hand, and low-molecular organic compounds (small molecules), that are the active substances of most modern synthetic drugs, on the other hand. We believe that the established relationships between the optical and chemical structures of the EW dipeptides and their biological properties will help in the search for new peptide drug candidates.

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