Insulin-like growth factor-1 fused with thrombopoietin mimetic peptide effectively increase platelets count in vivo

Hai-Tao Zhang, PhD, *Zheng-Wu Li,* PhD, *Chang-Hong Guo,* PhD, *Xiu-Qin Zhang,* PhD, *Yu-Jun Chen,* PhD, *Hui-Cheng Li,* PhD.

ABSTRACT

الأهداف: دراسة مدى فعالية إدماج البروتينات التي تحتوي على واحد أو اثنان من الببتيد المحاكي لهرمون الثرومبوبويتين.

الطريقة: أُجريت هذه الدراسة التجريبية في مركز هاربين للأبحاث والصيدلة، هاربين، الصين وذلك خلال الفترة من يونيو 2009م إلى يناير 2010م، حيث شملت 48 فأراً وتم تقسيمها إلى 4 مجموعات. لقد قمنا بتصميم البروتين من خلال إدماجه بالنهاية الكربونية في عوامل النمو المشابهة للأنسولين وبواسطة الرابط الببتيدي المرن وذلك بإتباع الاختبارات التالية: اختبار دامي لتكاثر الخلايا، واختبار تكوين المستعمرات، وتحليل الصفائح الدموية أيضاً وذلك من أجل إثبات الفرضية التي نريد طرحها.

النتائج: أشارت نتائج الدراسة إلى أن إنتاج البروتينات المدمجة في الإشريكية القولونية (BL21) قد وصل إلى أعلى من 26% من مجموع البروتينات الخلوية. وأظهرت اختبارات الأنشطة الحيوية بأن اندماج البروتينات كان عالياً مقارنةً بالثرومبوبويتين المأشوب، كما وأظهرت البروتينات المدمجة في الثرومبوبويتين المحاكي للببتيد وعوامل النمو المشابهة للأنسولين نشاطاً حيوياً أعلى من الثرومبوبويتين في اختبار دامي لتكاثر الخلايا، واختبار تكوين المستعمرات، وكذلك اختبارات الإشعاع النخاعي التي بإمكانها زيادة عدد الصفائح الدموية.

خاتمة: أكدت الدراسة بأن التجارب على الفئران وإجراء اختبارات الأنشطة الحيوية قد يكون لهما دوراً فعالاً في إثبات التطبيقات الصيدلانية من أجل علاج قلة الصفائح الدموية.

Objectives: To design the expression of fusion proteins containing one or 2 thrombopoietin mimetic peptide (TMP).

Methods: This study was conducted at Harbin Pharmaceutical Group Research and Development Center, Harbin, China from June 2009 to January 2010. We designed the protein that was fused to the C-terminus of insulin-like growth factors (IGF-1) by a flexible peptide linker by Dami cell proliferation assay, colony-forming assay, and analysis of platelet in mice to prove our hypothesis. The total number of mice used was 48 in all 4 groups.

Results: The fusion proteins were produced in *Escherichia coli* BL21 (DE3) at up to 26% of the total cell proteins. Subsequent biological activity assays showed that the fusion proteins exhibited higher potency than recombinant human thrombopoietin (TPO). Our results showed that the fusion proteins IGF-1-TMP exhibited higher biological activities than TMP in Dami cell proliferation, human cord blood cell colony-forming assays, and in experiments on acute myeloid radiation sickness mice, which can effectively increase the number of platelets.

Conclusion: Experiments in mice and biology activity assay, which can effectively increase the number of platelets, indicated that it has a potential role in pharmaceutical applications for the treatment of thrombocytopenia.

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From the Key Laboratory of Molecular Cytogenetics and Genetic Breeding of Heilongjiang Province (Zhang H, Guo), College of Life Science and Technology (Chen), Harbin Normal University, and Harbin Pharmaceutical Group Research and Development Center (Zhang H, Li Z, Zhang X, Li H), Harbin, China.

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Address correspondence and reprint request to: Prof. Chang-Hong Guo, College of Life Science and Technology, Harbin Normal University, Harbin 150025, China. Tel. +86 (1) 3845186709. Fax. +86 (4) 5187135699. E-mail: kaku3008@yahoo.com.cn

With the recent new discovery of thrombopoietin (TPO), we try to enhanced understanding of both hematopoiesis and platelet production. Thrombopoietin is an important cytokine, which supports hematopoietic stem cell survival and expansion, as well as promoting all aspects of megakaryocyte development.[1] The TPO receptor was discovered in 1991, and TPO was purified in 1994.[2] Unfortunately, the development of a clinically

useful TPO was hampered by the appearance of neutralizing antibodies to some forms of recombinant TPO.[2] However, thrombopoietin mimetic peptide (TMP) is a 14-mer (IEGPTLRQWLAARA) peptide acting as an agonist to the TPO receptor.[3,4] There were 2 new drugs approved by the Food and Drug Administration in 2008 that mimic the effect of TPO, and became available to treat thrombocytopenia.[2] Insulin-like growth factor-1 (IGF-1) is present in α granule of platelets, and its receptor is expressed on the platelet surface. It may contribute to the amplification of platelet responses and pathogenesis of cardiovascular disease. The IGF-1 activates the IGF receptor/insulin receptor substrate, phosphoinositide-3 kinase, protein kinase pathway, and that PI3Kα is essential for the potentiatory effect of IGF-1 on platelet responses. The IGF-1 can also improved megakaryotes proliferation.[5] The IGF-1 and TPO are critical cytokines in megakaryopoiesis and hematopoiesis.[6,7] In liquid cultures of murine progenitors, the addition of IGF-1 to TPO greatly increased the proliferation and maturation of megakaryocytes (MK) progenitor cells. In combination with IGF-1, TPO augments the proliferation of various types of hematopoietic progenitors in vitro. Thus, it is expected that cytokine combinations including IGF-1 and TPO may be used to improve the efficiency of peripheral progenitor collection, due to their ability to maintain, and expand the number of hematopoietic stem cells ex vivo.[8,9] Some groups having fused stem cell factor (SCF) and TMP showed higher potency.[9] In the present study, we designed the expression of the fusion proteins containing one or 2 TMP, which was fused to the C-terminus of IGF-1 by a flexible peptide linker, or was fused to both of the C-terminus and N-terminus of IGF-1 by the same linker.

Methods. The IGF-1/TMP gene sequences were synthesized by Shanghai Bioengineering Company, Shanghai, China. The NBS Bioreactor was made by New Brunswick Scientific Co. Inc, Edison, NJ, USA and Q-Sepharose Fast Flow columns were purchased from Amersham BioSciences, Buchs, Switzerland, and Millipore ultrafiltration system was purchased from Millipore Co., Billerica, MA, USA. The TMP was provided by San Sheng Pharmaceutical Factory, Shenyang, China. Dami cells were obtained from American Type Culture Collection (ATCC), Manassas, VA, USA. Mouse IGF-1 mAb was purchased from Advtech, Hong Kong, China. Blood routine analyzer was offered by Northeast Agricultural University, Harbin, China. The Balb/c mice were purchased from the Weitong Lihua Laboratory Animal Co., Beijing, China.

Construction of the pET-3a-IGF1/TMP fusion plasmid. Human IGF1 cDNA and TMP cDNA were linked with each other through DNA sequence coding for a 5-mer linker (GSGSG) peptide, which was named IGF-1/TMP (IT) fusion protein. The human IGF-1 cDNA, and TMP cDNA sequence were also linked with each other through GSGSG peptide, which was named TMP/IGF-1/TMP (TIT) fusion protein. Plasmid pET-3a-IT and pET-3a-TIT were constructed as shown in **Figure 1**. The primers used in this procedure were as follows; P1: 5'-CAT ATG ACT CTG TGC GGT GCT GAA CTG-3' (NdeI site underlined); P2: 5'-GGA TCC TTA GGC ACG AGC CGC CAG CCA CTG GCG TAA GTA CGG GCC CTC GAT GCC AGA GCC AGA ACC AGC AGA TTT AGC CGG TTT C-3' (BmaHI site underlined); P3: 5'-CAT ATG ATT GAA GGT CCG TAT CTG CGT CAG TGG TTA GCC GCA CGC GCG GGT TCT GGC TCT GGC ATG ACT CTG TGC GGT GCT GAA CTG-3' (NdeI site underlined). The DNA fragment encoding IGF-1 was amplified from pET-3a-IGF-1 by polymerase chain reaction (PCR) with primers P1 and P2. The PCR was carried out using the following amplification conditions: an initial denaturing step at 94°C for 3 minutes, 30 cycles at 94°C for 40 seconds (s), 56°C for 40 s, 72°C for one minute, and a final extension at 72°C for 10 minutes. The TMP-linker-IGF-1-linker-TMP fragment was obtained by overlap PCR using primers P2 and P3. Ligation of the above 2 fragments was performed by applying splicing by overlapped extension (SOEing) techniques. The amplified products were digested with NdeI and BamHI, and inserted into the same digested pET-3a to obtain pET-3a-IT. A similar strategy was used to build plasmid pET-3a-TIT (TMP/IGF-1/TMP) (**Figure 1**). This study was conducted from June to July 2009 in Harbin Pharmaceutical Group Research and Development Center, Harbin, China.

Expression and purification of the fusion proteins. The *Escherichia coli* (*E. coli*) BL21 (DE3) cells containing the recombinant plasmid were grown at 37°C in 3.6 L minimal salts (M9) + Luria-Bertani (LB) medium supplemented with 0.5 g ampicillin, 1.2 g magnesium sulfate (MgSO4), and one ml antiblowing agent. When OD600 reached 6, 0.6 g isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce the expression of the fusion proteins. After a further 5 hours (hrs) of cultivation, the cells were harvested, and subjected to ultrasonic cell disruptor. The inclusion bodies thus obtained, were washed 3 times with 20 mM Tris/hydrogen chloride (HCl) (pH; 8.2), and 0.5% (v/v) Triton X-100, and then dissolved in a denaturing buffer (6 M guanidine-HCl, 5 mM ethylene diamine tetraacetic acid [EDTA], 10 mM DL-dithiothreitol (DTT), 20 mM Tris/HCl, pH;8.2). The denaturing

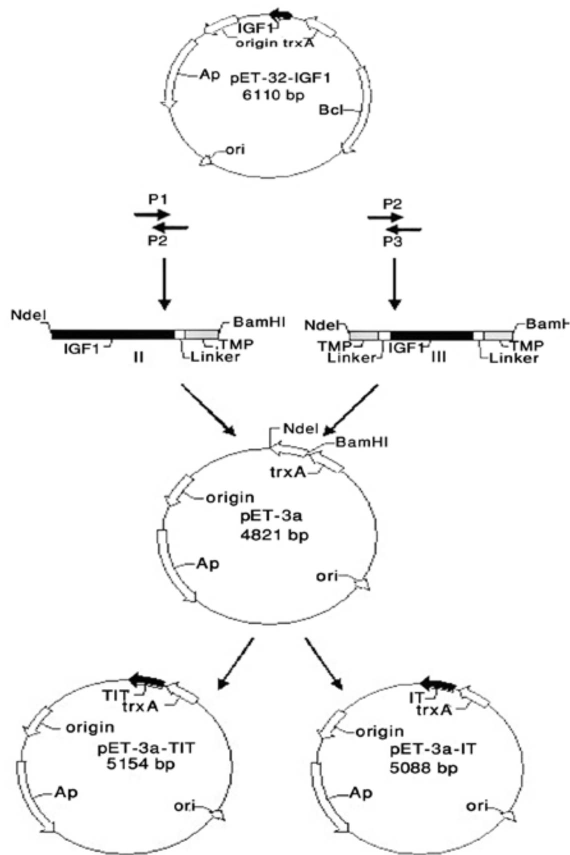

Figure 1 - Cloning strategy and construction of plasmid pET-3a-insulin-like growth factor (IGF1) - thrombopoietin mimetic peptide (TMP) (IT) and plasmid pET-3a-TMP-IGF1-TMP (TIT). The DNA fragment containing IGF1-linker-TMP and TMP-linker-IGF1-linker-TMP obtained by SOEing reaction digested with NedI and BamHI enzymes and inserted into the same digested plasmid pET-3a. bp - base pair

liquid was slow dilution to refolding buffer (2 M urea, 20 mM Tris/HCl, 0.25 mM glutathione disulfide [GSSG], pH;8.5) by volume ratio of 1:100 at 4°C. This was left standing for 20 hrs, and then was ultra-filtrated to one-tenth of the total volume through ultra-filters with 3 kDa pore size membranes. Subsequently, the protein solution was loaded onto a Phenyl, SP Sephadex Q-Sepharose ® Fast Flow chromatographic column. Elution of the fusion proteins was carried out with a salt concentration (0.3 M NaCl in 20 mM Tris/HCl, pH;8.0). The eluted fusion protein fractions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. The study was conducted at Harbin Pharmaceutical Group Research and Development Center from July to August 2009.

Dami cell proliferation assay. Dami cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 containing 10% (v/v) fetal bovine serum (FBS), one mM glutamine, 50 U penicillin/ml, 50 µg streptomycin/ml, and 8 ng recombinant human granulocyte macrophage colony-stimulating factor (rhGM-CSF) (R&D)/ml. After deprivation of rhGM-CSF for 24 hrs, $4x10^4$ Dami cells were plated in 100 µl RPMI-1640/10% (v/v) FBS per well in a 96-well microtiter plate supplemented with TMP, IT, control (medium), and TIT in the same molar concentration. After 3 days incubation at 37°C in a fully humidified atmosphere containing 10% CO_2, the number of viable cells was determined by 5-diphenyltetrazolium bromide (MTT) method. Statistical significance was determined using Student's t-test. The study was conducted at Harbin Pharmaceutical Group Research and Development Center from August to October 2009.

Colony-forming assay. Mononuclear cells (MNC) were isolated from human umbilical cord blood samples by Ficoll centrifugation. Each of $1x10^5$ MNC was resuspended in 2 ml Iscove's Modified Dulbecco's Media (IMDM), 30% (v/v) FBS, 10 ng IL-3 (R and D)/ml and 1% (v/v) methylcellulose, and plated on a 35 mm culture dish. All cultures were supplemented with 5 nM TMP, IT, and TIT. The assay was performed in triplicate at 37°C in a humidified incubator with 5% CO_2. After 14 days of culture, the number of colony forming unit-granulocyte and macrophage (CFU-GM) colonies was scored by inverted microscope. Statistical significance was determined using Student's t-test. The study was conducted at Harbin Pharmaceutical Group Research and Development Center in October 2009.

Analysis of platelet in mice. This is a one-time uniform whole body irradiation with 60Co γ-rays to Balb/c mice, absorbed doses of 5 Gray (Gy), distance of 1.5 m, absorbed dose rate of 31.02-31.98 cGy/min, through the cobalt 60 illuminate, to destroy mouse platelet production system, construct animal models, and to build a mouse model of acute myeloid radiation sickness. The Balb/c mice were randomly divided into 4 groups (A, B, C, D) (n=12 for each group). Mice were treated with 400 µg/kg of IT (group A), TIT (group B), TMP (group C), or saline injection (group D [control group]). The IT, TIT, TMP were each diluted with 0.2 ml saline. The next day, mice in group D was given the same amount of saline solution. On day 6, 8, 10, 12, 14, 16, and 18 of the experiment under sterile conditions, each mouse tail vein blood were collected (10 µl) with the routine blood test instrument, a buffer of 500 µl diluent, and fully mixed. With the Coulter automated blood cell analyzer, we measured white blood cell, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet total for 8 indicators.

Statistical significance was determined using Tukey's test by Statistical Package for Social Sciences version

12 software (SPSS Inc, Chicago, IL, USA). These animal experiments were evaluated and approved by the Experiments Animal Ethics Committee of Harbin Pharmaceutical Group Research and Development Center, and conducted according to the National Guidelines for the Use and Care of Laboratory Animals. This study was conducted from October 2009 to January 2010 at the Harbin Pharmaceutical Group Research and Development Center.

Results. ***Expression and purification of IGF1/TMP fusion proteins.*** As shown in **Figure 2**, the recombinant fusion proteins were produced in *E. coli* to seize 26% of the total cell proteins, and most fusion proteins were detected as inclusion bodies. To reduce the effect of contaminants on the refolding yield, inclusion bodies were washed extensively with Tris/HCl and Triton X-100 to reach over 80% purity. The dissolved inclusion bodies were then subjected to slow dilution to refolding buffer, which could inhibit protein aggregation, resulting in an enhanced refolding yield.[10] The final product of IT was 83 mg from one L cell culture, and the TIT was 89 mg. The purity of the fusion proteins was over 95% as detected by SDS-PAGE (**Figure 2**, Lanes 2 & 3). The molecular weight of IT was 9.1 kDa, and TIT was 11 kDa, which were consistent with the sizes deduced from their coding sequence. Western blot analysis showed that both IT and TIT reacted with monoclonal mouse antibodies against human IGF1 (**Figure 3**).

Biological activities of IGF1/TMP fusion proteins. The biological activities of the fusion proteins were evaluated by proliferation assays of Dami cell, which expresses both IGF1 and TPO receptors. As shown in **Figure 4**, IT induced a higher level of proliferation in Dami cell than TMP at 2.7 and 6.8 nM (versus control). The TIT shows lightly lower level of proliferation in Dami cell than TMP at 2.7, but it was higher than TMP at 6.8 nM. We also investigated the abilities of the IGF1-TMP to stimulate colony-formation on human cord blood mononucleated cells (MNC).[9] The number of CFU-Meg colonies was 45+11 with IT, 42±11 with TIT, and 25±7 with TMP at day 14 (**Figure 5**) These results showed that both IT and TIT fusion proteins obtained enhanced biological activity compared to TMP, but no significant difference between IT and TIT was observed.

Analysis of platelet in mice. The Balb/c mice platelet test in vivo results show that the 5Gy γ-ray irradiation of mice during the first 16 days or so, to reach the minimum value of peripheral blood platelet count (<50,000 cells/μl), then the platelet count gradually reaching the incipient number. Starting from the first 10 days after the IT, TIT, and TMP treated mice were compared to the control group, the peripheral blood platelet count had increased amplitude of which the IT group had the most significant increase of platelet, and fastest platelet recovery rate (**Figure 6**). At the same time, the blood routine test results suggest this experiment without any effect on normal blood cells. The Tukey's test multiple comparison shows that IT was statistically significantly higher than the control, TMP, and TIT to effectively increase platelet counts. The TMP and TIT has no statistical significant difference. The IT fusion protein has obvious therapeutic effect on radiation injury induced thrombocytopenia, and the role of the fusion protein better than TMP and TIT.

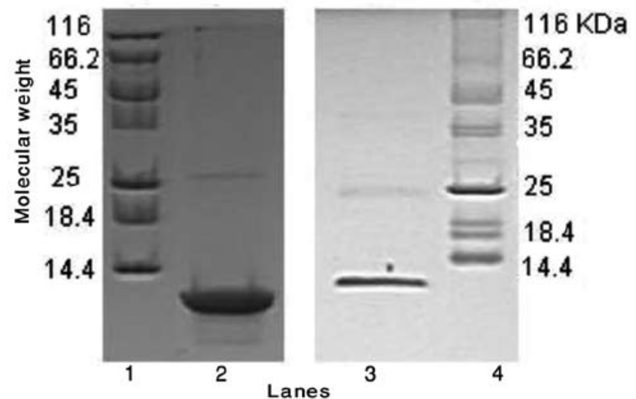

Figure 2 - Coomassie-stained 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (non-reduced) analysis of recombinant fusion proteins showing: Lane 1 - low molecular weight standards; Lane 2 - purified insulin-like growth factor (IGF)1- thrombopoietin mimetic peptide (TMP) protein; Lane 3 - purified TMP-IGF1-TMP protein; Lane 4 - low molecular weight standards. KDa - kiloDalton

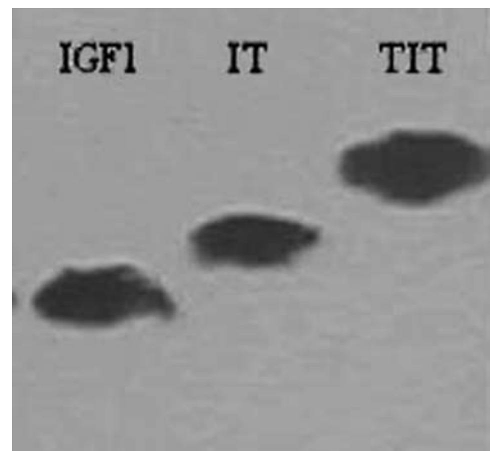

Figure 3 - Insulin-like growth factor 1 (IGF)-thrombopoietin mimetic peptide (TMP) and TMP-IGF1-TMP (TIT) proteins detected by Western blot analysis. Lane 1 - shows the IGF1 proteins; Lane 2 - refers to IT proteins; and Lane 3 shows the TIT proteins.

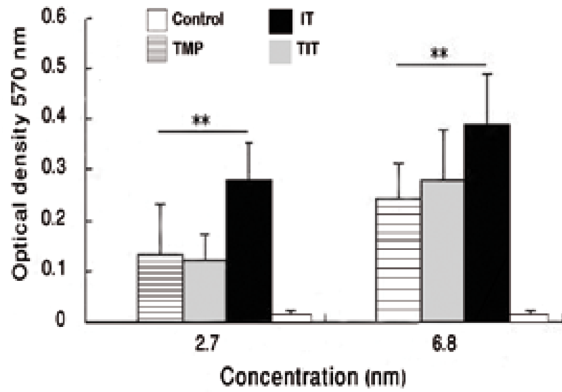

Figure 4 - Proliferation measurement of Dami cells during a 72-hour incubation with thrombopoietin mimetic peptide (TMP), insulin-like growth factor (IGF)1-TMP control (medium), or TMP-IGF1-TMP. Statistical significance was determined using Student's t-test. The error bars indicate standard error. (***p*<0.01 versus control). IT - insulin-like growth factor-thrombopoietin mimetic peptide, TIT - thrombopoietin mimetic peptide-insulin-like growth factor-thrombopoietin mimetic peptide.

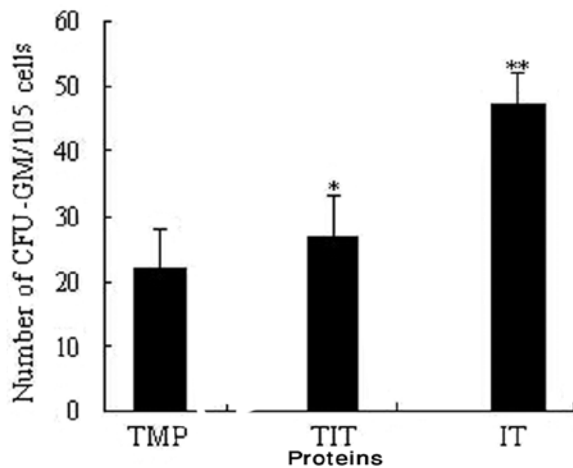

Figure 5 - Colony stimulating activity of the purified insulin-like growth factor (IGF)1-thrombopoietin mimetic peptide (TMP) (IT) and TMP-IGF1-TMP (TIT) showing that IT can effective enhance the number of colony forming unit-granulocyte and macrophage (CFU-GM). Each data point was sampled in triplicate. Statistical significance was determined using Student's t-test. The error bars indicate standard error. (**p*<0.05; ***p*< 0.01 versus control).

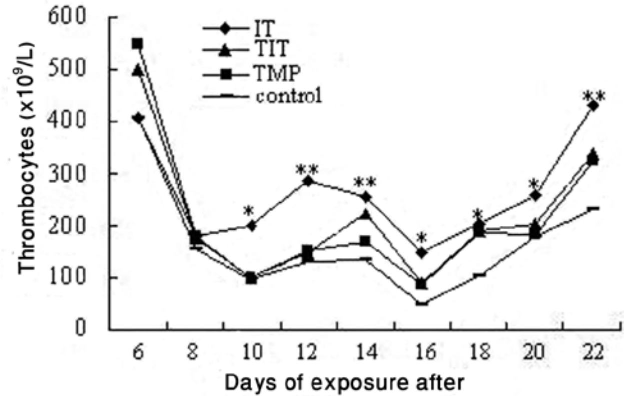

Figure 6 - Comparison and analysis of mice peripheral blood platelet after 5Gray γ-ray irradiation (groups A, B, C compared to group D). A line chart of different time points and blood platelet counts was obtained in mice peripheral. Statistical significance was determined using Tukey's and Student's t test (*mean *p*<0.05; **mean *p*<0.01 versus control).

Discussion. Fusion proteins consisting of complementary cytokines have a higher activity than the simple combination of cytokines.[11,12] However, the large size of the fusion cytokines may easily cause immunologic neutralization.[13] In this study, the TMP was fused to IGF-1. It was anticipated that the cytokine/mimetic peptide fusion proteins would induce less immunogenic response than the fused cytokines. Studies had shown that the N-terminal PEGylation improved the potency of TMP,[14] indicating that the attachment of a large molecule to the N-terminus does not prevent the interaction of TMP with its receptor. Therefore, TMP was fused through its amino termini to the IGF-1 by a flexible peptide linker. Compared with TPO, TMP was approximately 250-fold less potent in an in vitro cell proliferation assay. This may be partially due to its short half-life, and the thermodynamically unfavorable dimerization.[9] One way to overcome this problem is to fuse mimetic peptides with a protein, which can form a dimer.[15] The majority of IGF-1 exists as a monomer under physiological conditions, however, IGF-1 functions as a non-covalent homodimer to bind IGF1-R, and initiate signal transduction. Through extensive polar and nonpolar interactions, the 2 IGF-1 molecules formed a slightly bent dimer, thus facilitating and stabilizing the dimerization of TMP.

To further enhance the potency of TMP, we designed the expression of the fusion protein containing IGF-1 and 2 TMP (TIT), in which 2 TMP were linked by a GSGSG linker. Our results showed that the fusion proteins IGF-1-TMP exhibited higher biological activities than TPO mimetic peptide in Dami cell proliferation and human cord blood cell colony-forming assays and in experiments on acute myeloid radiation sickness mice, which can effectively increase the number of their platelets. While dimerization was reported to increase the biological activity of TMP dramatically, TIT was no more efficacious than IT. The reason for this lack of superiority may be partly ascribed to the IGF-1 protein activity area that was blocked in the TIT fusion protein. In previous studies, the TMP was usually linked through its C-terminus to an acid amino group of a lysine residue,[3] given that linker length and flexibility significantly affected biological potencies of bifunctional cytokine fusion proteins.[16] We speculated

that the GSGSG linker might compromise the ability of each monomer to interact with its receptor, resulting in a decrease of efficiency for the dimer. Moreover, the attachment of TMP to the C-terminus and N-terminus of IGF-1 block the activity area of the TIT, which may be important for the interaction with its receptor.[17]

In summary, we have shown that the human IGF-1 and TMP can be expressed as a fusion protein in *E. coli* in an inclusion body form, and the protein can be refolded into an active form. The fusion proteins present higher activity than TMP. This study may present a new strategy to enhance biological activities of natural factors, and effectively increase the blood platelet count. More experiments in different animal models to prove further fusion protein of biological activity has to be carried out, and fusion protein mechanism needs further study.

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References

1. Geddis AE, Linden HM, Kaushansky K. Thrombopoietin: a pan-hematopoietic cytokine. ***Cytokine and Growth Factor Reviews*** 2002; 13: 61-73.
2. Kuter DJ. Thrombopoietin and thrombopoietin mimetics in the treatment of thrombocytopenia. ***Annu Rev Med*** 2009; 60: 193-206.
3. Liem-Moolenaar M, Cerneus D, Molloy CJ, End D, Brown KH, de Kam ML, et al. Pharmacodynamics and pharmacokinetics of the novel thrombopoietin mimetic peptide RWJ-800088 in humans. ***Clin Pharmacol Ther*** 2008; 84: 481-487.
4. Hers I. Insulin-like growth factor-1 potentiates platelet activation via the IRS/PI3Kalpha pathway. ***Blood*** 2007; 110: 4243-4252.
5. Aro AL, Savikko J, Pulkkinen V, von Willebrand E. Expression of insulin-like growth factors IGF-I and IGF-II, and their receptors during the growth and megakaryocytic differentiation of K562 cells. ***Leukemia Research*** 2002; 26: 831-837.
6. Kuter DJ, Begley CG. Recombinant human thrombopoietin: basic biology and evaluation of clinical studies. ***Blood*** 2002; 100: 3457-3469.
7. Klusmann JH, Godinho FJ, Heitmann K, Maroz A, Koch ML, Reinhardt D, et al. Developmental stage-specific interplay of GATA1 and IGF signaling in fetal megakaryopoiesis and leukemogenesis. ***Genes Dev*** 2010; 24: 1659-1672.
8. Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, et al. Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. ***Blood*** 2001; 97: 3075-3085.
9. Su L, Chen S, Yang K, Liu C, Liang Z. Cloning and expression of human stem cell factor fused with thrombopoietin mimetic peptide in *Escherichia coli*. ***Biotechnol Lett*** 2006; 28: 857-862.
10. Gu Z, Weidenhaupt M, Ivanova N, Pavlov M, Xu B, Su ZG, et al. Chromatographic methods for the isolation of, and refolding of proteins from *Escherichia coli* inclusion bodies. ***Protein Expr Purif*** 2002; 25: 174-179.
11. Gillies SD, Lan Y, Brunkhorst B, Wong WK, Li Y, Lo KM. Bi-functional cytokine fusion proteins for gene therapy and antibody-targeted treatment of cancer. ***Cancer Immunol Immunother*** 2002; 51: 449-460.
12. Battaglia A, Fattorossi A, Pierelli L, Bonanno G, Marone M, Ranelletti FO, et al. The fusion protein MEN 11303 (granulocyte-macrophage colony stimulating factor/erythropoietin) acts as a potent inducer of erythropoiesis. ***Exp Hematol*** 2000; 28: 490-498.
13. Schellekens H. Immunogenicity of therapeutic proteins: clinical implications and future prospects. ***Clin Ther*** 2002; 24: 1720-1740.
14. de Serres M, Yeager RL, Dillberger JE, Lalonde G, Gardner GH, Rubens CA, et al. Pharmacokinetics and hematological effects of the PEGylated thrombopoietin peptide mimetic GW395058 in rats and monkeys after intravenous or subcutaneous administration. ***Stem Cells*** 1999; 17: 316-326.
15. Kuai L, Wu C, Qiu Q, Zhang J, Zhou A, Wang S, et al. Plasminogen activator inhibitor-1 fused with erythropoietin (EPO) mimetic peptide (EMP) enhances the EPO activity of EMP. ***J Pept Res*** 2000; 56: 59-62.
16. Vidal M, Liu WQ, Lenoir C, Salzmann J, Gresh N, Garbay C. Design of peptoid analogue dimers and measure of their affinity for Grb2 SH3 domains. ***Biochemistry*** 2004; 43: 7336-7344.
17. Qiu H, Belanger A, Yoon HW, Bunn HF. Homodimerization restores biological activity to an inactive erythropoietin mutant. ***J Biol Chem*** 1998; 273: 11173-11176.

Related topics

Dalcik C, Yildirim GK, Dalcik H. The effects of ethanol on insulin-like growth factor-I immunoreactive neurons in the central nervous system. ***Saudi Med J*** 2009; 30: 995-1000.

Gad HI. The potential osteogenic effects of systemic leptin and insulin administration in streptozotocin-induced diabetic female rats. ***Saudi Med J*** 2007; 28: 1185-1190.

Ozguner IF, Savas C, Ozguner M, Delibas N. Effects of nonstrangulated small bowel obstruction on intestinal histology, insulin-like growth factor-I level, antioxidants, and lipid peroxidation in rats. ***Saudi Med J*** 2006; 27: 405-407.