Ginseng-Derived Panaxadiol Saponins Promote Hematopoiesis Recovery in Cyclophosphamide-Induced Myelosuppressive Mice: Potential Novel Treatment of Chemotherapy-Induced Cytopenias

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ABSTRACT Objective: To investigate the potential efficacy of panaxadiol saponins component (PDS-C), a biologically active fraction isolated from total ginsenosides, to reverse chemotherapy-induced myelosuppression and pancytopenia caused by cyclophosphamide (CTX). Methods: Mice with myelosuppression induced by CTX were treated with PDS-C at a low- (20 mg/kg), moderate- (40 mg/kg), or high-dose (80 mg/kg) for 7 consecutive days. The level of peripheral white blood cell (WBC), neutrophil (NEU) and platelet (PLT) were measured, the histopathology and colony formation were observed, the protein kinase and transcription factors in hematopoietic cells were determined by immunohistochemical staining and Western blot. Results: In response to PDS-C therapy, the peripheral WBC, NEU and PLT counts of CTX-induced myelosuppressed mice were significantly increased in a dose-dependent manner. Similarly, bone marrow histopathology examination showed reversal of CTX-induced myelosuppression with increase in overall bone marrow cellularity and the number of hematopoietic cells (P<0.01). PDS-C also promoted proliferation of granulocytic and megakaryocyte progenitor cells in CTX-treated mice, as evidenced by significantly increase in colony formation units-granulocytes/monocytes and -megakaryocytes (P<0.01). The enhancement of hematopoiesis by PDS-C appears to be mediated by an intracellular signaling pathway, this was evidenced by the up-regulation of phosphorylated mitogen-activated protein kinase (p-MEK) and extracellular signal-regulated kinases (p-ERK), and receptor tyrosine kinase (C-kit) and globin transcription factor 1 (GATA-1) in hematopoietic cells of CTX-treated mice (P<0.05). Conclusions: PDS-C possesses hematopoietic growth factor-like activities that promote proliferation and also possibly differentiation of hematopoietic progenitor cells in myelosuppressed mice, probably mediated by a mechanism involving MEK and ERK protein kinases, and C-kit and GATA-1 transcription factors. PDS-C may potentially be a novel treatment of myelosuppression and pancytopenia caused by chemotherapy.

KEYWORDS panaxadiol saponins component, ginsenosides, chemotherapy-induced myelosuppression, mitogen-activated protein kinase, extracellular signal-regulated kinase, receptor tyrosine kinase, globin transcription factor 1, Chinese medicine

Cancers, such as aggressive lymphoma and leukemia, are frequently treated with intensive chemotherapy with or without radiotherapy in an attempt to achieve a cure, complete remission or prolonged disease free survival. Post high-dose chemotherapy, the patients almost invariably suffer severe pancytopenia. The severe neutropenia often leads to serious, potentially life-threatening infections. Post-chemotherapy marked thrombocytopenia may lead to serious bleeding. Significant pancytopenia may also delay commencement of the next cycle of chemotherapy.1,2 There is clearly a clinical need to enhance early recovery of chemotherapy-induced severe neutropenia and thrombocytopenia and if
possible, to prevent or to reduce the severity of chemotherapy-induced cytopenias. Granulocyte-colony stimulating factor (G-CSF) is often administered to reduce/prevent chemotherapy-induced neutropenia and to hasten its recovery.\(^{(3)}\) Thrombopoietin receptor (TPO-R) agonists (romiplostim and eltrombopag) may potentially be used to prevent or treat chemotherapy-induced thrombocytopenia.\(^{(4)}\) However, there is no compound as yet that could be used to treat chemotherapy-induced pancytopenia, in particularly neutropenia and thrombocytopenia simultaneously.

Chinese ginseng has been used in China for over thousand years to treat a number of medical conditions including blood disorders. Ginseng is known to possess functions of strengthening the body resistance to eliminate pathogenic factors, improve immunologic function, and reduce the side effects of chemotherapy drugs. Ginseng extract contains characteristic ring-structure compounds known as ginsenosides, many literature reported that ginsenosides have the effect for anti-tumor.\(^{(5-7)}\) Since 1992, we have extracted and isolated total saponins of panax ginseng (TSPG) from ginseng herb, containing multiple ginsenosides,\(^{(8-12)}\) further purified TSPG using macroporous resin to yield a fraction, termed panaxadiol saponins component (PDS-C) which contains 5 ginsenosides. PDS-C was formulated into capsule named as Pai-Neng-Da (派能达), for which we have been granted a patent by China Food and Drug Administration (CFDA) in 2010 to treat pancytopenia from various causes.\(^{(13)}\) Subsequently, CFDA has now grant approval for us to carry out placebo-controlled phase II clinical trials for treatment of primary immune thrombocytopenia (ITP) and chronic neutropenia. Our previous studies have demonstrated that PDS-C enhanced proliferation and differentiation of human CD34+ hematopoietic stem/progenitor cells,\(^{(14)}\) but its efficacy in enhancing recovery of chemotherapy-induced neutropenia and thrombocytopenia has not reported. In this study, we used a murine model of chemotherapy-induced myelosuppression to investigate the enhancement by PDS-C in promoting hematopoiesis following myelosuppression induced by cyclophosphamide (CTX).

**METHODS**

**Preparation Dry Fine Powder of PDS-C**

Purified PDS-C dry fine powder was isolated from total saponins of *Panax ginseng* in The First Affiliated Hospital of Zhejiang Chinese Medical University. It contains 5 panaxadiol saponins monomers (ginsenosides) with a purity of 92.44% analyzed by high-performance liquid chromatography (HPLC) using specific ginsenosides as reference standards (purchased from the China Institute of Food and Drug Test).\(^{(13,15,16)}\) The positive control drug was leucogen, made by Jiangsu Jiebeier Pharmaceutical Company (China, batch No. 150107).

**Murine Model of Chemotherapy-Induced Myelosuppression**

Kunming mice of male and female were purchased from the Shanghai Si-lai-ke Experimental Animals Company in China, with certification No. SCXK (Hu) 2013-0016. The murine model with chemotherapy-induced myelosuppression was established,\(^{(17)}\) a dose of 100 mg/kg of CTX was injected intraperitoneally for 3 days; the peripheral blood white blood cells (WBC), neutrophil (NEU) and platelet (PLT) counts were detected.

**Treatment of CTX-Induced Myelosuppressive Mice with PDS-C**

Sixty mice were randomized into 6 groups of 10 mice each. The first group consisted of normal control mice, which were not treated with CTX nor PDS-C. Except for the normal control group, mice in other groups were all treated with 100 mg/kg of CTX, injected intraperitoneally for 3 days. Then, model mice were treated with normal saline, PDS-C (20, 40, 80 mg/kg) or leucogen (10 mg/kg), respectively for 7 days. The peripheral blood samples of the mice were collected for counting WBC, NEU and PLT numbers by Blood Cell Counter of ABX Pentra XL (Horiba, France) on 3, 5 and 7 days, respectively.

**Bone Marrow Culture of Colony Formation**

After treated by PDS-C for 7 days, the bone marrow mononuclear cells were acquired from mouse femurs of each group, and plated onto semisolid culture system of colony forming assay of units-granulocyte/monocyte (CFU-GM) and -megakaryocyte (CFU-MK) as previously described.\(^{(15,18)}\) The numbers of CFU-GM colony (≥40 cells) were counted using an inverted microscope after culturing for 5 days. The murine megakaryocytes within CFU-MK colonies were identified by acetylcholinesterase staining, and the numbers of CFU-MK colony (≥4 cells) were counted using an inverted microscope after 7-day culture.

**Bone Marrow Histopathological Examination**

Mice of each group were sacrificed by cervical...