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Original Research

Flavored Guilu Erxian decoction inhibits the injury of human bone marrow mesenchymal stem cells induced by cisplatin

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Abstract: To examine the exact role of flavored Guilu Erxian decoction, a Traditional Chinese Medicine (TCM) in the treatment of cisplatin-induced side-effects in bone marrow mesenchymal stem cells (BM-MSCs). BM-MSCs were isolated from bone marrow collected from SD rats and identified by flow cytometry. Cells were cultivated in MEM alpha medium containing 5% (TCM-L), 10% (TCM-M) and 20% (TCM-H) dosages of flavored Guilu Erxian decoction with or without cisplatin. Cell viability was determined through CCK-8 and thymidine analog 5-ethynyl-2′-deoxyuridine (EdU) staining assay. Flow cytometry was used to determine cell cycle and apoptosis. The expression of p21 and cleaved-caspase-3 were examined using Western blot assay. The PI3K-AKT-mTOR pathway associated proteins, including p-PI3K, p-AKT and p-mTOR, were also examined by Western blot assay. CCK-8 and EdU staining assay demonstrated that cisplatin could inhibit cell proliferation in BM-MSCs in a dose and time dependent manner. Further, cisplatin could induce apoptosis through increasing G0/G1 cell cycle arrest, p21 and cleaved-caspase-3 expression. However, these phenomena would be significantly alleviated when adding the serum containing flavored Guilu Erxian decoction. Furthermore, the PI3K-AKT-mTOR pathway activation could be inhibited by cisplatin in BM-MSCs, while flavored Guilu Erxian decoction treatment successfully abrogated this effect. Combination of flavored Guilu Erxian decoction and cisplatin could reduce the damage to BM-MSCs. This indicates that the flavored Guilu Erxian decoction can enhance the possibility of BM-MSCs repairing and rehabilitating the normal function of injured tissues induced by cisplatin, which could provide a new direction for therapeutic applications.

Key words: Flavored Guilu Erxian decoction; Traditional Chinese Medicine (TCM); Cisplatin; BM-MSCs; Proliferation; Apoptosis.

Introduction

Cisplatin, cisplatinum, also known as *cis*-diamminedichloroplatinum (II) (CDDP), is a famous chemotherapeutic agent. It has been clinically proven to combat different kinds of malignant tumors including bladder, ovarian, lung, and testicular cancers(1). However, the clinical use of cisplatin is often limited by drug resistance and multitudinous side-effects in normal tissues such as bone marrow suppression, allergic reactions, peripheral neuropathy, ototoxicity, and nephrotoxicity(2, 3). It is also reported that cisplatin-induced nephrotoxicity primarily occurs in renal vasoconstriction and renal profiles with worsening inflammation, oxidation, anoxia, and apoptosis, neurotoxicity in the upper and lower extremities, and ototoxicity within the mechanosensory hair cells of the cochlea, or inner ear(4, 5). Therefore, to alleviate the side-effects of cisplatin, a strategy to tackling multi-cellular levels is essential. Several advantages of treating many disorders by cell therapy such as Acute Kidney Injury (AKI) have been demonstrated, especially in combination with drug therapy(6). Moreover, cisplatin treatment could induce DNA lesions, which can lead to cell cycle arrest and apoptotic death(7, 8). However, these usable treatment prescriptions are still not the best. Thus, it is advisable to explore ameliorated therapeutic strategies for using cisplatin.

In recent years, stem cell therapy is thought to be an efficient and alternative therapeutic strategy, providing the possibility of rehabilitating and renewing the normal function of damaged tissues(9). The bone marrow stem cell, as one of the transplanted stem cells which has been well studied, contains a variety of cell types, such as bone marrow mesenchymal stem cells (BM-MSCs) and hematopoietic stem cells. Mesenchymal stem cells (MSCs) which can differentiate into chondrocytes, osteocytes, adipocytes and fibroblasts *in vitro*, have drawn attention for having a role in several therapeutic applications, such as for cardiovascular, bone and cartilage, and also cancer progression(10-17). Previous studies have shown that bone marrow-derived mesenchymal stromal cells could improve the function and structure of the ovaries, which were injured by cyclophosphamide(18, 19). Several potential advantages through MSCs therapy over specific drugs such as in treating AKI have also been proved(6, 20), but the effect of cisplatin on BM-MSCs is still unknown.

As an important complementary and alternative medication system, Chinese herbal, originated from plants, has been widely used for thousands of years and is a key component of Traditional Chinese medicine (TCM).(21). Accumulative evidence has revealed that a large proportion of natural agents from TCM herbs exert chemopreventive properties against carcinogenesis (25). Flavored Guilu Erxian decoction is composed of *Colla cornus cervi*, *Tortoise plastron*, *American ginseng*, *Fructus Lycii*, *Colla corii asini*, *Radix adenophorae*, *Astragalus*, *Angelica* and *Notoginseng*. The effect of each component was assessed independently. In the treatment of intractable anemia, *Colla cornus cervi* increased the platelet and enhanced the hemoglobin concentration(33). *Tortoise plastron* promoted cell differential of neurons after mesenchymal stem cell transplantations in cerebral ischemia of rats(34). *Fructus Lycii* was addressed to exhibit anti-aging properties and is effective against oxidative stress(35). *Colla Corii Asini* was recently characterized with antioxidant activity(36). It was also found that a polysaccharide purified from *Radix adenophorae* promoted cell activation and pro-inflammatory cytokine production macrophages(37). Astragalus polysaccharides regulated the immune system against the postburn sepsis and the progression of tumors (38, 39). Effect of *Astragalus membranaceus* and *Angelica sinensis* combined with Enalapril was found for the treatment of rats with obstructive uropathy(40). Recent findings have demonstrated that *Panax notoginseng saponins* contributed to a therapeutic effect on severe acute pancreatitis through the regulation of mTOR/Akt and caspase-3 signaling pathway by upregulating miR-181b expression in rats(41).

Here, flavored Guilu Erxian decoction are made by the combination of multiple TCM herbs. The effects of Flavored Guilu Erxian decoction for cisplatin-induced side-effects in the bone marrow stem cells are detected by CCK-8 and Flow cytometry. Moreover, the cellular mechanism of TCM regulated the proliferation, cell cycle and apoptosis of BM-MSCs, influenced by cisplatin, is further explored by Western Blot. The results achieved would be a good reference value for clinicians and offer further research for candidates, since doctors ordinarily combine several medicines to cure a single disease.

Materials and Methods

Bone marrow derived mesenchymal stromal cells isolation and culture

The centrifuge gradient method was used to isolate MSCs from bone marrow (BM) collected from the SD rats. BM aspirates were cultured in MEM alpha medium (Life Technologies, USA) with 10% FBS (Gibco, USA). After 24 hours, the suspension cells were removed, and adherent cells were washed with phosphate buffered saline. Adherent cells were cultured for about 10 days with twice medium changes (PBS). The cells were cultured and used for experiments when about 75% confluence was achieved.

Identification of BM-MSCs by flow cytometry

The International Society for Cellular Therapy defined the criteria for MSCs (42), and various sources of MSCs express different CD markers. In BM-MSCs, the markers CD31, CD34 or CD45 were not expressed, while CD44, CD90 and CD105 were expressed. Flow cytometry were used to identify BM-MSCs surface markers. BM-MSCs were collected and re-suspended with PBS then seeded in 96-well microtiter plates. Monoclonal antibodies specific to PE-CD31, FITC-CD34, PE-Cy5-CD45, PE-CD44, PE-Cy5-CD90, PE-CD105 (BD Biosciences, USA) were added to the wells and incubated according to the manufacturer's instructions. Samples were analyzed by FACS flow cytometer (Becton Dickinson, USA). Data was analyzed by Flow Plus software.

Preparation of serum containing drug and blood-serum pharmacology

Flavored Guilu Erxian decoction was composed of 15 g melted *Colla cornus cervi*, 50 g *Tortoise plastron* which needed frying for 40 minutes beforehand, 15 g *American ginseng*, 15 g *Fructus lycii*, 15 g melted *Colla corii asini*, 30 g *Radix adenophorae*, 30 g *Astragalus*, 6 g *Angelica*, 10 g *Notoginseng*. The raw materials were purchased from the outpatient pharmacy of Zhujiang hospital of the Southern Medical University. The equivalent dose rate of the human and rats was calculated by the proportion of the surface area (70kg)/ rats (200g) = 1/0.018, and the daily dose of the rat was 8.42 g. The serum containing the drug was diluted 10 times in the culture medium, and the amount of gastric irrigation in the rats was 2mL/100g each time. The total volume of the rat was about 4mL/time, and the total amount of irrigation was 8mL per day. As a result, the concentration of the lowest decoction in this experiment was 10.5 g/L, and the minimum decoction concentration was 10.5 g/L, 21 g/L, 42g/L, 84 g/L and 168 g/L respectively. Preparation of the water decoction from the previous day was kept at 4°C within a refrigerator.

The SD rats were randomly divided into 5 groups, which received the gavage of Chinese medicine in a dosage of 10.5 g/L, 42g/L and 168g/L respectively for 5d, and the last one given the drug was after fasting with water for 12 h. At the end of the final time, 1 h after injection, blood was taken from the heart in aseptic conditions and placed in a sterile anticoagulant after an abdominal cavity injection of 10% hydrate chloral injection according to a 3.5ml/kg dose and anesthesia. After having been stored at room temperature for 30min, the blood coagulates and the serum precipitated. After 15min of 3000r/min, it was absorbed to be a serum containing drug or blank. Group serum was mixed with 0.22 μm of microporous membrane filter in addition to bacteria, and stored at 20°C. In this study, we chose 5% (v/v) drug serum (TCM-L), 10% (v/v) drug serum (TCM-M) and 20% (v/v) drug serum (TCM-H) for subsequent experiments.

Cell proliferation assay

CCK-8 and thymidine analog 5-ethynyl-2′-deoxyuridine (EdU) staining were used to detect cell proliferation. Briefly, BM-MSCs were seeded in 96-well plates (corning, USA) and cisplatin (DDP) alone or DDP and Serum containing medicine were used together to treat cells. After 24 hours or 48 hours, the old media was removed and fresh media was added to each well.

Then, CCK-8 solution (CCK-8, Japan) was added into each well and incubated following the instructions. The absorbance was measured at 450 nm using the Spectra-Max M5 microplate reader (Molecular Devices, USA). EdU was used as a marker of cell proliferation. BM-MSCs were treated with 50 μM EdU (Ribobio, China) for 2 hours. Then, cells were collected and stained with the Apollo®643 EdU labeling kit (Ribobio, China) and the nuclei were stained with DAPI (Sigma-Aldrich, USA). EdU staining was performed according to manufacturer's instructions. Following this step, cells were photographed by a fluorescence confocal microscopy (Olympus FV1000). The scale bar is 100 μm.

Cell cycle Assay

BM-MSCs were exposed to DDP alone or DDP combined with serum containing medicine for 48 hours, and cells were collected and stained according to the manufacturer's guidelines. Cells were counted immediately with FACScan flow cytometer and analyzed as described previously.

Apoptosis assay

To determine the effect of DDP or Serum containing medicine treatments on apoptosis, flow cytometry was performed. BM-MSCs were seeded in 6-well plates and treated with DDP alone or DDP combined with Serum containing medicine for 48 hours. BM-MSCs were collected and stained with Annexin V-FITC/PI apoptosis detection kit (Sigma-Aldrich, USA) according to instructions. Cells were counted immediately with FACS can flow cytometer. Data was analyzed as described previously.

Western blot analysis

The BM-MSCs were treated with DDP alone or DDP combined with serum containing medicine for 48 hours and the expression of related proteins was detected by western blot. This was then incubated with antibodies against Phospho-PI3 Kinase (CST, 1:1000), Phospho-Akt (CST, 1:1000), Phospho-mTOR (CST, 1:1000), p21 (CST, 1:1000), Cleaved Caspase-3(CST, 1:1000) and GAPDH (CST, 1:1000). HRP-conjugated goat anti-rabbit secondary antibodies (CST, 1:3000) were used and the band was visualized using an ECL chemiluminescence substrate (Amersham Biosciences, USA).

Statistical Analysis

All experiments were repeated at least three times. The data is presented as the mean ± SD. Student's t-test or one-way ANOVA with the Newman–Keulspost-test was performed. Statistical analysis was carried out by GraphPad Prism Version 6.0 (San Diego, USA). Differences were considered significant at $P < 0.05$.

Results

Identification of BM-MSCs surface markers.

The International Society for Cellular Therapy defined the criteria for MSCs (42), the expression of cell surface markers can be used to confirm the type of stem cells. As various sources of MSCs express different CD markers(42), flow cytometry were used to identify BM-MSCs surface markers. The results showed that

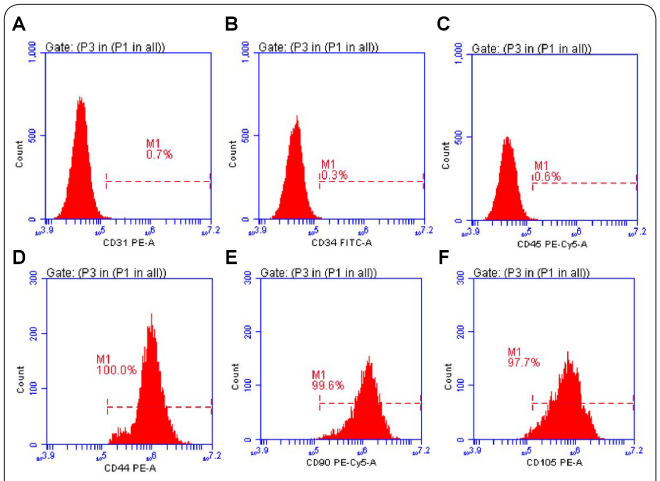

Figure 1. Surface markers of BM-MSCs. (A-F) The expression of CD44, CD90, CD105, CD31, CD34 and CD45 was detected by flow cytometry.

BM-MSCs did not express the markers CD31, CD34 or CD45 (Figure 1A-C), while CD44, CD90 and CD105 were expressed (Figure 1D-F). These results demonstrated that we have obtained BM-MSCs from human bone marrow successfully.

TCM partially restored the proliferation capacity of BM-MSCs which inhibited by cisplatin.

Cisplatin is widely used to treat varieties of solid malignant tumors such as lung, ovarian and bladder cancers though inducing cells apoptosis and inhibiting proliferation(43). Hence, we examined the effect of cisplatin on BM-MSCs proliferation through CCK-8. Results indicated that cisplatin inhibited BM-MSCs proliferation in a dose-dependent manner. The half maximal inhibitory concentration (IC50) was 20.2 μg/ml (Figure 2A), and we chose the 20.2 μg/ml as the cisplatin concentration in the subsequent experiment.

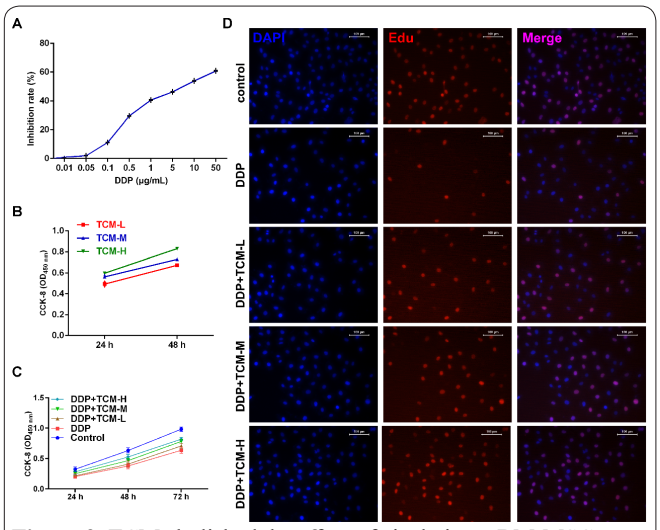

Figure 2. TCM abolished the effect of cisplatin on BM-MSCs proliferation. (A) BM-MSCs were cultured in the presence of various concentrations of cisplatin for 24 hours, and CCK-8 assay was performed. (B) BM-MSCs were treated with different percentages of drug serum (TCM-L, TCM-M, TCM-H) for 24 hours or 48 hours, and the effects of TCM on cell proliferation were determined via CCK-8. The effect of Cisplatin (20.2 μg/ml) alone or cisplatin combined with different concentrations of TCM on cell proliferation were determined via CCK-8 (C) and Edu stain (D), respectively. DDP: cisplatin.

The effect of TCM on BM-MSCs was detected by CCK-8. BM-MSCs were cultured in MEM alpha medium with different percentages of drug serum for 24 hours or 48 hours. The drug serum consisted of as follows: 5% (v/v) drug serum (TCM-L), 10% (v/v) drug serum (TCM-M) and 20% (v/v) drug serum (TCM-H). The CCK-8 results showed TCM up-regulated the proliferation of BM-MSCs at 48 h rather than 24 h (Figure 2B). Previous results indicated that cisplatin inhibited BM-MSCs proliferation. Hence, we examined the effect of cisplatin combined with TCM on the proliferation of BM-MSCs by CCK-8 and Edu stain. The results showed that the proliferation capacity of BM-MSCs inhibited by cisplatin showed an upward trend accompanied by the increasing concentration of TCM therapy, especially at 48 hours (Figure 2C). BM-MSCs were treated by vehicle, cisplatin (20.2 μg/ml) or cisplatin (20.2 μg/ml) combined with TCM (TCM-L, TCM-M, TCM-H) respectively for 48 hours. Further, we detected the changes in DNA synthesis ability after being treated with cisplatin combined with different concentrations of TCM. As shown in Figure 2D, the results demonstrated that the DNA synthesis ability inhibited by cisplatin was gradually rescued by the increasing concentration of TCM therapy. All the results demonstrated that cisplatin inhibition of MSCs proliferation was attenuated effectively by TCM in concentration and time dependent manners.

Cisplatin induced G0/G1 cell cycle arrest was abolished by TCM

It is known that cell proliferation is closely related to cell cycle, and arresting the cell cycle at the G1 phase has become a major objective for the inhibition of cell proliferation. As the proliferation of BM-MSCs inhibited by cisplatin was attenuated effectively by TCM, we wonder whether cisplatin or TCM influences the cell cycle distribution. The cell cycle assay results demonstrated that a cisplatin (20.2 μg/ml) treatment could increase the percentage of G0/G1 phase by 15.69% and decrease the percentage of the S phase by 13.29% (Figure 3A, B, F), while the cell cycle arrested by cisplatin was abolished in a dose-dependent manner when treated

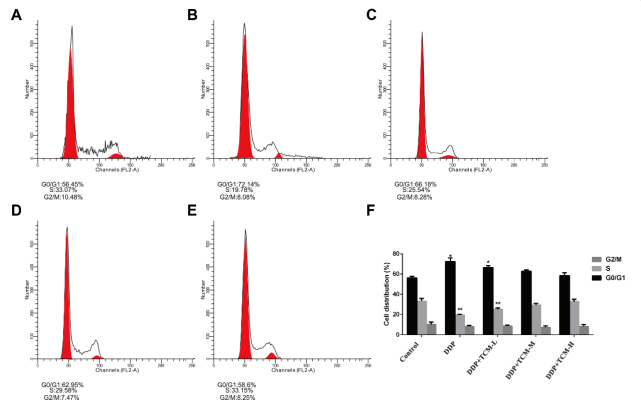

Figure 3. Cisplatin induced G0/G1 cell cycle arrest was abolished by TCM. (A-E) The cell cycles of BM-MSCs were detected by flow cytometry after exposure to cisplatin (20.2 μg/ml) or cisplatin (20.2 μg/ml) combined with TCM for 48 hours. The percentage of G1, S and G2 phase cells was shown. (F) The data presents the Mean ± SEM. *P<0.05, **P<0.01 VS control; **P<0.01, ***P<0.001 VS DDP. One representative from three experiments is shown. DDP: cisplatin.

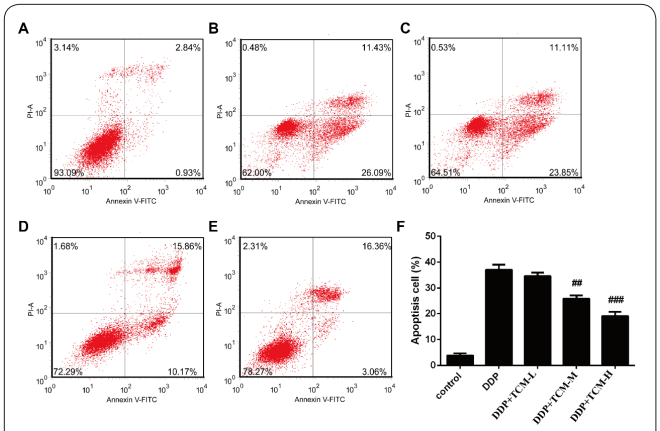

Figure 4. Cisplatin-induced BM-MSCs apoptosis was decreased by TCM. (A-F) Cell apoptosis of BM-MSCs were detected by flow cytometry and TUNEL after exposure to cisplatin (20.2 μg/ml) or cisplatin (20.2 μg/ml) combined with TCM for 48 hours. The percentage of cell apoptosis was shown. (F) The data presented the Mean ± SEM. $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 VS DDP. DDP: cisplatin.

with TCM (Figure 3C, D, E). All results demonstrated that TCM abolished the G1 cell cycle arrest induced by cisplatin in BM-MSCs.

TCM decreased the effect of Cisplatin on BM-MSCs apoptosis

The results showed that apoptosis of BM-MSCs treated with cisplatin (20.2 μg/ml) increased significantly compared with the control group (Figure 4A, B, F), while cisplatin induced apoptosis was decreased in a dose-dependent manner by TCM (Figure 4C, D, E).

Expression of PI3K-AKT-mTOR pathway proteins and apoptosis proteins

The phosphatidylinositol-3 kinase/AKT/mammalian target of rapamycin (PI3K-AKT-mTOR) signaling pathway is a classical intracellular signaling pathway that has been identified in various aspects of cellular maintenance, including cellular proliferation, survival, migration, adhesion and metabolism(44, 45). To further explore the role of the PI3K-AKT-mTOR pathway in cellular apoptosis, BM-MSCs were treated with cisplatin (20.2 μg/ml) or TCM, the expression of p-PI3K, p-AKT, p-mTOR, p21 and cleaved-caspase-3 were detected by a western blot. The results showed that the expression of p-PI3K, p-AKT and p-mTOR decreased significantly, while p21 and cleaved-caspase-3 were up-regulated by cisplatin (Figure 5A, B). TCM partially abolished the effect of cisplatin on PI3K-AKT-mTOR inhibition and decreased the expression of p21 and cleaved-caspase-3 in BM-MSCs (Figure 5A, B).

Discussion

The major finding of this study is that it is the first time to clarify the protective effect of flavored Guilu Erxian decoction in BM-MSCs injury induced by cisplatin. This study further suggests that flavored Guilu Erxian decoction can stratify and target intervention to improve the effect of cisplatin.

Cisplatin is the most frequently used chemotherapeutic drug for the treatment of cancer. However, despite efficacious cytotoxicity of cisplatin in the initial treatment, the cancer cells eventually develop acquired cisplatin

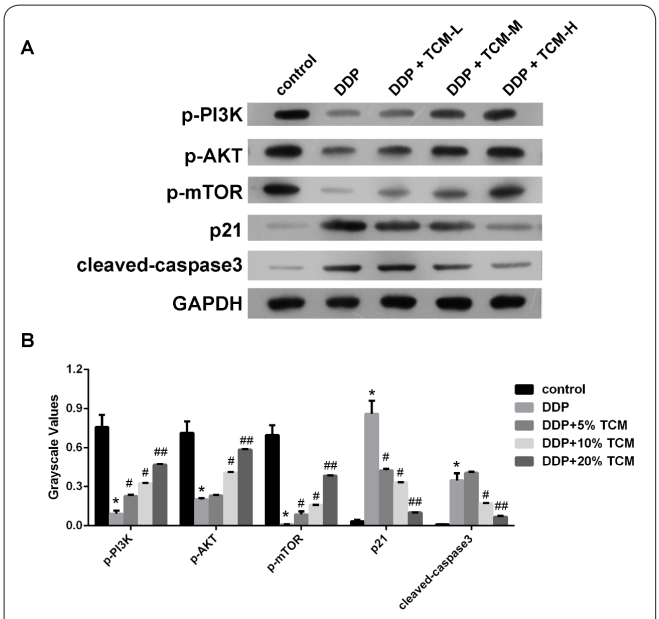

Figure 5. Expression of PI3K-AKT-mTOR pathway proteins and apoptosis proteins. (A, B) BM-MSCs were cultured in the presence of cisplatin (20.2 μg/ml) or cisplatin (20.2 μg/ml) combined with TCM for 48 hours, the expression of phospho-PI3K, phospho-Akt, phospho-mTOR, p21and cleaved Caspase-3 were analyzed by the western blot. GAPDH was used as an internal control. All experiments were repeated at least three times. *P<0.01, **P<0.001 VS Control. #P<0.05, ##P<0.01 VS DDP. DDP: cisplatin.

resistance as a result of repeated use which can lead to cisplatin-based treatment failure(46). For example, ovarian cancer is very responsive to multiple chemotherapeutic agents, but the majority of responsive patients relapse due to the development of resistance(47). And the unique way to conquer the resistance of cancer cells to cisplatin is to increase the dose, but injury may occur at higher dosages to normal body cells. Previous studies have shown that the combination of TCM with cisplatin can improve the efficacy of chemotherapeutic drugs and/or inhibit their toxicity(50, 51). Origanum majorana Attenuates Nephrotoxicity of Cisplatin Anticancer Drug through Ameliorating Oxidative Stress(52). Therefore, some of the renoprotective approaches have been evaluated to seek alternative strategies with greater efficacy and fewer toxic effects. In the present study, we showed that the serum acquired from SD rats which suffered the intragastric administration of the flavored Guilu Erxian decoction, significantly suppresses cisplatin-induced BM-MSCs injury by attenuating apoptosis. The possibility of the flavored Guilu Erxian decoction to promote the growth of MSCs which significant decreased by treating with cisplatin and cell division were also detected in our study.

The ability of BM-MSCs to self-renew and regenerate has become an ideal strategy for the treatment of disease and tissue regeneration. The strategies of improving the therapeutic efficiency of BM-MSC cell therapy is the key to promote its clinical application. However, no ideal marker of BM-MSCs has been identified. In our study, FACS analysis showed BM-MSCs expressed CD44, CD90 and CD105, but not CD31, CD34 and CD45, which is in agreement with Stagg et al(53).

It has been reported that cisplatin can induced the apoptosis or necrosis of granulose cells *in vitro*(54). The cytotoxicity depending on the concentration of cisplatin was believed based on oxidative stress, energy metabolism injury, DNA damage and other factors(55, 56). Thus, a previous study showed that co-culture with BM-MSCs could reduce ovarian damage induced by cisplatin(57). However, much less is known at present about the impact of cisplatin on BM-MSCs. In the present study, we found that cisplatin could inhibit the proliferation of BM-MSCs in a dose and time dependent manner and induce BM-MSCs apoptosis. But, these phenomena had been significantly alleviated when adding the serum containing flavored Guilu Erxian decoction. Therefore, we propose that the flavored Guilu Erxian decoction can enhance the possibility of BMSCs repairing and renovating the normal function of damaged tissues induced by cisplatin, which could provide a new direction for therapeutic applications.

The application of cisplatin in tumor chemotherapy is often through inhibiting the DNA replication, cell cycle arrest and cell death(58, 59). Previous studies have showed that cisplatin could produce predominantly intrastrain cross-links DNA and therefore imparts unique genotoxic and cytotoxic activities against cancer cells, which resulting in the activation of multiple signaling pathways and finally culminating through apoptosis pathway(56, 60). Earlier studies have pointed out cisplatin induces cytotoxicity through activation of p53 which blocks the cell cycle of cancer cells by activating of p21(61-63). Our findings indicate that cisplatin produces cytotoxicity through causing cell cycle arrest by activation of p21 in BM-MSCs, while TCM could decrease the p21 expression. It is also reported that the cytotoxicity induced by cisplatin decreased the expression level of Ki67 which is a cell cycle proliferation marker in APL cells (64), but this requires further proving in BM-MSCs. Previous research has showed that cisplatin-induced cytotoxicity eventually activates the apoptosis through caspase 3 activation in cancer cells (55, 60, 65). Our results also support the findings of cisplatin inducing apoptosis in BM-MSCs by up-regulation of caspase 3 expression. We also proved that flavored Guilu Erxian decoction abolished the effect of cisplatin on apoptosis in BM-MSCs.

In the current study, accumulating evidence shows that the PI3K-AKT-mTOR signaling pathway plays an important role in cell cycle regulation, cell proliferation, migration, invasion, and survival(44, 45). As a key driver of carcinogenesis in several cancer types, an increasing number of anti-neoplastic therapies against the PI3K-AKT-mTOR pathway are used for cancer treatment, either in combination with chemotherapy or other targeted therapies such as stem cell therapy. Cisplatin-induced cytotoxicity presents the decreasing viability of cells, elevating apoptosis of cells through ROS accumulation, inhibition of survival signaling pathway PI3K/Akt, suppression of mTOR, production of proinflammatory cytokines, activation of apoptotic pathways JNK1 and autophagy(48, 49). Notably, phosphorylation of PI3K and Akt significantly ameliorates apoptosis in cisplatin-treated cells, while mTOR restricts the autophagy. Similarly, in this study, flavored Guilu Erxian decoction treatment successfully abrogated the side-effects of cisplatin via the activation of the PI3K-AKT-mTOR pathway. Our data, for the first time, reported the efficacy of combination therapy of Guilu Erxian decoction

and cisplatin which decreased the side effect on bone marrow mesenchymal stem cells (BM-MSCs). However, the active compounds in flavored Guilu Erxian decoction and the underlying roles of active compounds of flavored Guilu Erxian decoction in cisplatin-induced BM-SMCs injuries remains to be further clarified.

We have demonstrated that flavored Guilu Erxian decoction has a protective effect on cisplatin-induced BM-MSCs injuries though anti-apoptotic action via a down-regulation of caspase 3 and anti-cyclical regulation via reduction of p21, while involving the PI3K-AKT-mTOR pathway in these two functions was up-regulated.

Acknowledgments

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Conflict of Interest

The authors declare that there is no conflicts of interest regarding the publication of this article.

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