RESEARCH COMMUNICATION

Therapeutic Effects of Combination of Paeoniflorin and Albiflorin from *Paeonia Radix* on Radiation and Chemotherapy-induced Myelosuppression in Mice and Rabbits

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Abstract

The aim of this study was to investigate the therapeutic effects of the combination of paeoniflorin and albiflorin (CPA) extracted from *Paeonia radix* on radiation and chemotherapy induced myelosuppression in two animal models: mice and rabbits. Mice were exposed to X-ray radiation (400 Roentgen), and both mice and rabbits were intraperitoneally injected with cyclophosphamide (100.0 mg/kg) and cytarabine chloride (92.7 mg/kg), respectively, for 3 days to induce myelosuppression. CPA was subsequently administrated intravenously at low (15.0 mg/kg for mice, 6.00 mg/kg for rabbits), intermediate (30.0 mg/kg for mice, 12.0 mg/kg for rabbits) and high (60.0 mg/kg for mice, 24.0 mg/kg for rabbits) doses, as well as orally (60.0 mg/kg for mice, 24.0 mg/kg for rabbits) for 7 days. Shenqi tablets were used as positive controls (oral administration of 936.0 mg/kg for mice, 336.0 mg/kg for rabbits). The administration of CPA significantly ameliorated myelosuppression in all cases. For the X-ray irradiated mice and the chemotherapy treated mice and rabbits, high dosages of CPA resulted in the recovery of, respectively, 94.4%, 95.3% and 97.7% of hemoglobin content; 67.7%, 92.0% and 94.3% of platelet numbers; 26.8%, 137.1% and 107.3% of white blood cell counts; as well as a reversal in the reduction of peripheral differential white blood cell counts. There was also a recovery of 50.9%, 146.1% and 92.3%, respectively, in the animals' relative spleen weight. Additionally, a recovery of 35.7% and 87.2% in the number of bone marrow nucleated cells was observed in the radio- and chemotherapy treated mice, respectively. Bone marrow white blood cell counts also resumed to normal levels. These results substantiate the marked therapeutic effects of CPA to ameliorate myelosuppression induced by radio and chemotherapy.

Keywords: Paeoniflorin - albiflorin - myelosuppression - hematopoietic - thrombocytopoietic - leukocytotic

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Introduction

Paeonia radix, the dried root of *Paeonia lactiflora* Pall, is one of the most important herbs used in Traditional Chinese Medicine (TCM), and has been prescribed in various formulated preparations for over 1500 years. In the ancient Chinese medical literature, such as Shennong's Herbal, *P. radix* was regarded as a bitter and sour medicinal herb with a slightly "cold property", which was usually indicated for tonifying the blood as well as for several gynecopathies, blood deficiencies, the so-called imbalances in the liver energy and for treating those prone to infection by pathogens (a condition traditionally referred to in China as exterior deficiency syndrome).

Modern research has identified an active fraction extracted from *P. radix*, the total glucosides of paeony (TGP), which seems to underlie many of its therapeutic properties. Among these literature, TGP has been shown to possess anti-inflammatory effects (Yamahara et al. ,1982) as well as anti-coagulant (Ishida et al., 1987), cognition-enhancing (Ohta et al., 1993), anti-hyperglycemic (Hsu et al., 1997), neuroprotective (Liu et al., 2005), analgesic (Kobayashi et al., 1990), anti-oxidative (Okubo et al., 2000), immune regulatory (Wu et al., 2007; Xu et al. ,2007), a neuromuscular blocking action (Kimura et al., 1985) and a protective effect against hepatic injury (Wu et al., 2007; Xu et al., 2007). In 1998, the use of TGP as a disease modifying drug was approved by the Chinese State Food and Drug Administration (SFDA).

Extensive efforts have been devoted to elucidate the mechanisms underlying the therapeutic and pharmacological properties of TGP. To date, at least eight major active constituents have been identified: paeoniflorin (PF) (Shibata and Nakahara, 1963), albiflorin (AF), oxypaeoniflorin, benzoylpaeonifiorin (Kaneda et al., 1972), oxybenzoylpaeoniflorin, benzoyloxypaeoniflorin,

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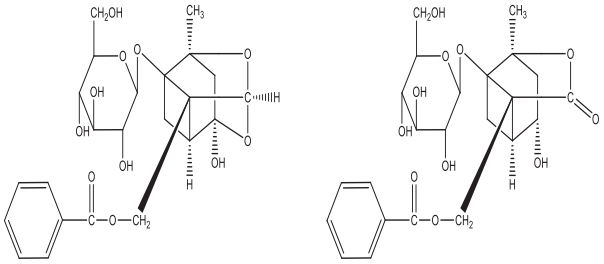

Figure 1. Chemical Structures of a) Paeoniflorin and b) Albiflorin

paeonilactinone, and lactinolide (Murakami et al., 1996). Still, most studies involved a single constituent, with very few examining the pharmacological effects of the combination of two or more active compounds.

In a previous study we established a protocol for the extraction and preparation of the combination of paeoniflorin and albiflorin (CPA) (Figure 1) (You et al., 2002). Here we explore the potential of CPA as an adjunctive drug in the treatment of cancer by investigating its ameliorative effects on myelosuppression induced by radio and chemotherapy in two animal models.

Materials and Methods

Preparation of CPA

CPA were prepared and quantified according to a previously established protocol (You et al., 2002). Dried roots of *P. lactiflora* Pall (5.0 kg, purchased from Bozhou city, Anhui Province, China) were powdered and extracted twice (2 h in each case) in boiling water (50 L). The combined water extract was filtered and concentrated to 1/20 volume (4.5 L), then transferred into a pot, stilled for 10 h, and filtered again. The filtrate was extracted with water saturated ethyl acetate (4.5L) twice. The combined ethyl acetate layer was concentrated under reduced pressure at 60°C and desiccated in vacuum to give a brown residue (102.2 g). The residue was dissolved in 50% EtOH (500 ml), absorbed on Al2O3 (100-200 mesh 800g) and seperated on a silica gel column (7.5×120 cm, 200-300 mesh,1000 g) using EtOH (70%) as eluent. The CPA-containing fraction (6 L) was concentrated under reduced pressure at 60 °C and desiccated in vacuum to give white powder (45.3 g). This purified powder (45.3 g) was dissolved in water (450.0 ml) and decolorized with activated carbon (1%), then filtered. The filtrate was concentrated under reduced pressure at 60 °C and desiccated in vacuum to give the final white powder of CPA. Compound structures of paeoniflorin and albiflorin were identified by 1H-NMR, 13C-NMR, and quantified by reversed phase high performance liquid chromatography (RP-HPLC) (paeoniflorin+albiflorin > 92% by HPLC, paeoniflorin : albiflorin = 7:3).

Drugs and chemical reagents

Cyclophosphamide, cytarabine chloride and all other reagents were obtained from Aldrich-Sigma (St. Louis, MO, USA). Shenqi tablets, widely prescribed to treat leukopenia induced by chemo or radiotherapy in patients with cancer, were purchased from Jilin Henghe Pharmaceutical Co., China.

Animals

Balb/c mice (6 weeks old, 18-22 g) and New Zealand rabbits (1.8-2.2 kg) were obtained from the Animal Experiment Center of China Medical University and maintained at controlled temperature (22 ± 2 °C) and humidity (50 ± 10%) with a 12 h light/ dark cycle. All protocols were approved by the Ethics Committee of Shenyang Pharmaceutical University, in accordance with the Principles of Laboratory Animal Care and Use in Research (Ministry of Health, Beijing, China). Animals were allowed access to food and tap water ad libitum throughout the acclimatization and experimental stages.

X-ray radiation (mice)

After a week of acclimatization, mice were assigned to seven groups (n=10 mice per group, 5 males and 5 females), as follows: (1) control group (NC), (2) x-ray treated group (XT, negative control), (3) low-dose treatment group (LT: 15.0 mg/kg CPA administered intravenously, i.v.), (4) intermediate-dose treatment group (IT: 30.0 mg/kg CPA, i.v.), (5) high-dose treatment group (HT: 60.0 mg/kg CPA, i.v.), (6) oral treatment group (OT: 60.0 mg/kg CPA administered orally, p.o.) and (7) Shenqi tablet treatment group (ST: 936.0 mg/kg, p.o., positive control). All mice except the control group (1) were exposed to X-ray radiation (400 Roentgen) once. From the day of radiation, CPA was administrated once a day for 7 days using the doses and administration routes specified above (see also Table 1). Shenqi tablets were administered to the positive control group (ST) using the same regimen.

Cyclophosphamide treatment (mice)

Mice were grouped in the same way as that employed for those mice exposed to the x-ray treatment (item 2.4). All mice except the control group (NT) were injected intraperitoneally with cyclophosphamide at a dose of 100.0 mg/kg/day for 3 consecutive days. Starting on the last day of cyclophosphamide injection, CPA was administrated once a day for 7 consecutive days to mice from all groups except NT (control group) and CT (cyclophosphamide treated group, negative control) using the same dosages and administration routes as those described for the X-ray treatment. Shenqi tablets (936.0 mg/kg p.o.) were administered to the positive control group (ST) using the same regimen.

Cytarabine chloride treatment (rabbits)

After a week of acclimatization, rabbits were assigned to seven groups (n=10 rabbits per group, 5 males and 5 females), as follows: (1) control group (NT), (2) negative control group (XT), (3) low-dose treatment group (LT: 6.0 mg/kg CPA, i.v.), (4) intermediate-dose treatment group (IT: 12.0 mg/kg CPA, i.v.), (5) high-dose treatment group (24.0 mg/kg, i.v.), (6) oral treatment group (24.0 mg/kg, p.o.) and (7) Shenqi tablet treatment group (336.0 mg/kg, p.o., positive control). All rabbits except those in the control group (NT) were injected intraperitoneally (i.p.) with cytarabine chloride at a dose of 92.7 mg/kg/day for 3 consecutive days. From the last day of cytarabine chloride injection, CPA was administrated once a day for 7 days

using the doses and administration routes described above (see also Table 3). Shenqi tablets (336.0 mg/kg p.o.) were administered to the positive control group (ST) using the same regimen.

Peripheral blood parameter measurements

24 hours after the administration of the final CPA dose, blood samples from all mice and rabbits were taken from their petro-orbital plexus and ear vein, respectively, and placed into heparinized capillary tubes. Counts of total white blood cells, blood platelets, and differential white blood cells (neutrophils, lymphocytes and monocytes) were manually determined for each sample. Peripheral hemoglobin content was examined using the hemoglobin cyanide (HiCN) method (International Committee for Standardization in Haematology 1965).

Bone marrow measurements

Twenty four hours after the administration of the final CPA dose, all mice and rabbits were sacrificed. Their thigh bones were then removed to enable the preparation and count of nucleated bone marrow cells (Xu et al. 2002). Bone marrow was milked using forceps and mixed with serum, and the count of differential bone marrow white blood cells was conducted manually.

Spleen index

All subjects and their spleens were weighed 24 hours after the final administration of CPA and a spleen index (spleen weight/animal weight) calculated for each animal.

Statistical analyses

Results are expressed as mean ± S.D. Statistically significant differences were determined by one-way analyses of variance and Student's t-tests. A p value < 0.05 indicates a statistically significant difference.

Results

Following the administration of CPA, significant erythropoietic (hematopoietic), thrombocytopoietic and leukocytotic effects were observed in subjects from all treatment groups (radio-and chemo-treated mice and rabbits), as described below.

Amelioration effects of CPA on myelosuppression induced by X-ray irradiation in mice

Table 1 describes the results of the irradiation treatment and subsequent administration of CPA. The hemoglobin content of blood samples from irradiated mice was on average 25.8% lower than that in the control group (NT). The average increase in the hemoglobin content achieved with the intravenous administration of CPA at low, intermediate and high doses was, respectively, 6.43%, 6.50% and 20.2% when compared to the negative control group (XT), which was irradiated but not treated with CPA. The oral administration of CPA (group OT) also increased hemoglobin content by approximately 10.6% as compared to that in group XT, whereas the oral administration of the Shenqi tablet resulted in an average increase of 6.04% in the hemoglobin content of subjects from group ST. At a same dosage of 60.0 mg/kg, CPA had a stronger hematopoietic effect when administered intravenously (HT) than orally, resulting in a recovery of approximately 94.4% of the hemoglobin content when compared to the normal control group (NC). X-ray exposure resulted in an average decrease of 30.5% in the count of platelets as compared to the normal control group. When subsequently administered at low and intermediate doses, CPA increased platelet counts by approximately 41.1% and 46.4%, respectively. The oral administration of CPA also increased the number of platelets by about 47.3%, whereas the oral administration of Shenqi tablets

Table 1. Amelioration Effects of CPA on Myelosuppression Induced by Exposure of Mice to X-ray Radiation

Group	Treatment	n	Dosage (mg/kg)	Peripheral blood parameter					
				Hemoglobin (g/L)	Platelet (x 10^9/L)	WBC (x 10^9/L)	Differential White blood cells count (%) Neutrophil	Lymphocyte	Monocyte
1	NC	10		152.3±7.11	649.6±135.9	5.61±0.80	17.2±3.21	72.0±2.70	8.74±3.01
2	XT	10		113.1±7.26##	198.3±52.0##	0.72±0.21##	24.4±2.92##	54.8±3.01##	18.4±4.21##
3	LT	10	15 i.v.	122.9±11.6*	267.0±136.6*	1.14±0.31**	23.8±3.81	66.0±4.61**	8.14±3.41*
4	IT	10	30 i.v.	123.0±4.31**	301.5±135.7*	1.33±0.40**	19.1±2.41*	71.6±1.32**	7.51±1.64**
5	HT	10	60 i.v.	143.8±7.92**	439.8±122.5**	1.54±0.41**	16.8±3.01**	75.5±2.24**	6.74±2.41**
6	OT	10	60 p.o.	129.3±9.74**	307.3±143.4*	1.22±0.42**	23.6±9.14	62.0±7.03**	11.8±2.30**
7	ST	10	936 p.o	122.3±6.52**	284.4±117.6*	1.63±0.71**	23.4±3.71	60.4±5.21**	14.1±4.72

Bone marrow blood parameter				Spleen Index (mg/g)
BMNC (x 10^9/L)	Differential White Blood cells count (%) Granulocyte	Lymphocyte	Monocyte	
8.51±2.16	43.9±3.81	15.7±1.31	3.63±1.01	5.36±0.85
2.15±0.92##	73.9±6.92##	9.63±1.90##	7.42±3.20##	1.46±0.21##
2.27±0.65*	68.8±6.31**	12.9±1.31*	5.53±1.31	2.49±0.98*
2.59±0.24*	63.2±3.51**	14.3±1.30**	5.21±1.34	2.65±1.65*
3.04±0.69*	61.1±7.24**	16.0±3.22**	4.74±1.21*	2.73±0.54*
2.46±0.42	69.2±6.53	11.1±1.23	5.93±1.31	1.94±0.52*
2.51±0.70	67.4±7.82	11.5±1.31*	6.12±0.91	2.31±1.14*

##P < 0.01 (compared to normal control group, NC); *P < 0.05, **P < 0.01 (compared to X-ray treatment group); NC, normal control group; XT, X-ray treatment group, negative control; LT, low dosage treatment group; IT, intermediate dosage treatment group; HT, high dosage treatment group; OT, Oral administration treatment group; ST, Shenqi tablet treatment group, positive control

Table 2. Amelioration Effects of CPA on Myelosuppression induced by Treatment of Mice with Cyclophosphamide

Group	Treatment	n	Dosage (mg/kg)	Peripheral blood parameter					
				Hemoglobin (g/L)	Platelet (x 10^9/L)	WBC (x 10^9/L)	Differential White blood cells count (%) Neutrophil	Lymphocyte	Monocyte
1	NC	10		150.2±6.71	855.8±89.2	7.30±1.31	13.4±1.78	79.9±2.42	4.42±1.67
2	CT	10		118.1±8.45##	403.1±33.1##	3.24±0.82##	24.4±2.89##	51.4±5.31##	18.8±4.03##
3	LT	10	15 i.v.	126.0±4.56*	671.3±33.7**	5.67±2.32**	21.3±2.74*	66.2±3.90**	8.23±3.17**
4	IT	10	30 i.v.	135.6±6.81**	712.7±51.2**	6.37±1.67**	17.4±2.33**	73.8±3.52**	6.16±2.26**
5	HT	10	60 i.v.	143.2±7.47**	787.1±66.6**	10.0±5.49**	14.4±2.56**	78.3±2.61**	4.24±1.61**
6	OT	10	60 p.o.	124.2±7.63	493.0±44.4**	4.73±0.62**	23.9±4.09	65.9±3.89**	7.94±2.16**
7	ST	10	936 p.o	127.6±8.78*	694.5±29.0**	7.44±5.01**	24.9±4.43	57.4±6.56*	14.4±4.72*

Bone marrow blood parameter				Spleen Index (mg/g)
BMNC (x 10^9/L)	Differential White Blood cell count (%) Granulocyte	Lymphocyte	Monocyte	
8.44±1.49	63.5±4.41	15.8±2.23	3.74±1.45	8.05±2.40
2.29±0.39##	81.3±4.40##	5.93±1.32##	2.43±1.17##	2.99±0.80##
5.44±1.51**	78.6±5.02	9.22±1.67**	2.53±1.03	5.21±1.63**
6.08±0.95**	78.2±4.89	10.6±2.31**	2.52±1.13	7.59±3.85**
7.36±2.18**	69.3±5.27**	13.4±1.89**	2.34±0.89	11.8±5.75**
5.26±2.34**	80.1±4.78	8.82±1.92**	1.72±0.51	3.76±0.45*
5.66±1.38**	76.7±7.53	9.81±2.90**	2.82±1.73	5.42±2.50**

##P < 0.01 (compared to normal control group, NC); *P < 0.05, **P < 0.01 (compared to cyclophosphamide treatment group, CT); NC, normal control group; CT, Cyclophosphamide treatment group, negative control; LT, low dosage treatment group; IT, intermediate dosage treatment group; HT, high dosage treatment group; OT, Oral administration treatment group; ST, Shenqi tablet treatment group, positive control

resulted in an increase of approximately 13.2%. In the group receiving a high dose of CPA (HT), there was an average increase in the number of platelets of 67.7% compared to the negative control group (XT), indicating that CPA had a moderate thrombocytopoietic effect.

When compared to the normal control group, the X-ray radiation caused an average decrease of approximately 12.5% in the count of peripheral white blood cells. Conversely, intravenous administration of CPA at 15.0, 30.0 and 60.0 mg/kg increased such count by about 19.6%, 23.2% and 28.6%, respectively. The oral administration of CPA and Shenqi tablets also led to an increase in the number of white blood cells as compared to group XT, of approximately 21.4% and 28.6%, respectively. Overall, these results suggest that CPA had a slight leukocytotic effect.

Although relative proportions of peripheral neutrophils, lymphocytes and monocytes were affected by the X-ray treatment, the administration of CPA caused the reversal of these changes to some extent, depending on the dosage and administration route employed (see Table 1). It is noticeable that white blood cell proportions similar to the normal control group were nearly achieved with the high dose of CPA. The results shown in Table 1 also indicate a slight increase in the number of nucleated bone marrow cells following the X-ray treatment, which was partially maintained even after the administration of the high dose of CPA.

The relative proportion of bone marrow white blood cells was also affected by the X-ray exposure. In all treatment groups the CPA administration resulted in a moderate tendency towards the same proportions of granulocytes, lymphocytes and monocytes observed in the control group (NC). Again, in the high dose treatment (HT), these proportions were nearly the same as those of the control group.

The administration of CPA was also responsible for a slight increase in the spleen index, which were reduced by the X-ray radiation. In the high dose treatment group (HT), a 50.8% recovery in the spleen index was achieved, whereas in the ST group (Shenqi tablets) an average recovery of only 43.1% was obtained.

The dose-dependent effects of CPA were clearly visible within the range examined (15.0 to 60.0 mg/kg). At the same dosage of 60.0 mg/kg, the intravenous administration of CPA produced a stronger effect than its oral administration. Shenqi tablets were only more effective in the recovery of the number of peripheral white blood cells. These results substantiate the hematopoietic as well as strong regulating effects of CPA on the proportion of peripheral white blood cells.

3.2 Amelioration effects of CPA on myelosuppression induced by cyclophosphamide in mice

The effect of CPA on hemoglobin content following the cyclophosphamide therapy (Table 2) was similar to that observed following the x-ray radiation (Table 1). On average, the administration of CPA intravenously at a high dose (HT, Table 2) and Shenqi tables (ST) resulted in a recovery of approximately 95.3% and 85.0%, respectively, in the hemoglobin content of the blood samples analyzed. A reduction of 52.9% in the platelet count was observed following the cyclophosphamide treatment. Yet, recovery levels as high as 92.0% and 81.2% were achieved with the administration of CPA at a high dose and Shenqi tablets, respectively, revealing the strong thrombocytopoietic effect of this compound.

Despite a decrease in the white blood cell count of approximately 56.1% due to the cyclophosphamide treatment, a strong reversal of such reduction was

Table 3. Amelioration Effects of CPA on Myelosuppression Induced by Treatment of Rabbits with Cytarabine Chloride

Group	Treatment	n	Dosage (mg/kg)	Peripheral blood parameter					
				Hemoglobin (g/L)	Platelet (x 109/L)	WBC (x 109/L)	Differential White blood cells count (%) Neutrophil	Lymphocyte	Monocyte
1	NC	10		144.8±4.04	487.3±25.9	7.36±2.42	17.8±1.16	78.0±2.72	2.24±1.04
2	YT	10		117.5±5.76##	300.5±32.7##	1.66±0.73##	31.5±5.80##	53.5±3.08##	11.2±6.10##
3	LT	10	6 i.v.	127.4±4.24**	395.4±27.7**	4.22±1.27**	20.1±2.88**	70.9±1.44**	7.14±1.66
4	IT	10	12 i.v.	135.7±4.29**	407.5±27.7**	4.75±1.30**	16.1±2.16**	76.2±1.57**	4.81±1.69
5	HT	10	24 i.v.	141.5±5.01**	459.7±32.7**	7.88±1.27**	14.0±2.40**	82.0±2.82**	1.98±0.91**
6	OT	10	24 p.o.	125.4±4.37**	333.3±17.5*	5.84±2.45**	26.4±1.18*	58.1±2.34**	12.8±1.84
7	ST	10	336 p.o	130.8±5.76**	421.4±25.9**	3.66±0.61**	23.0±1.10**	62.5±2.17**	12.1±1.55

Bone marrow blood paramete Differential White Blood cells count (%)			Spleen Index (mg/g)
Granulocyte	Lymphocyte	Monocyte	
38.8±1.78	19.6±3.82	2.25±0.79	0.97±0.12
51.0±3.79##	13.1±1.88##	5.80±1.68##	0.50±0.16##
46.9±3.59*	19.4±2.89*	2.47±1.08**	0.65±0.14*
44.0±4.50**	21.8±3.98**	2.15±0.62**	0.73±0.14**
39.6±3.88**	25.8±4.12**	1.80±0.63**	0.90±0.16**
48.8±4.79	14.6±4.29	4.10±1.54*	0.64±0.18
42.5±4.88**	18.8±2.57**	2.65±0.47**	0.71±0.22*

##P < 0.01 (compared to normal control group, NC); *P < 0.05, **P < 0.01 (compared to cytarabine chloride treatment group, YT); NC, normal control group; YT, cytarabine chloride treatment group, negative control; LT, low dosage treatment group; IT, intermediate dosage treatment group; HT, high dosage treatment group; OT, Oral administration treatment group; ST, Shenqi tablet treatment group, positive control

observed in those groups treated with CPA. Here, oral treatment with CPA resulted in an average recovery in the number of white blood cells of 64.4%, with full recovery achieved in the Shenqi tablet group, and in the HT group an increase of about 37.0% in the number of white blood cells above the levels observed in the control group. These results substantiate the robust leukocytotic effects of CPA following chemotherapy with cyclophosphamide.

Similar trends were observed in the analysis of the proportion of peripheral neutrophils, lymphocytes and monocytes, which revealed the ability of CPA (especially when administered intravenously using a high dose) to modulate those proportions towards levels observed in the absence of treatment with cyclophosphamide.

A marked increase in the nucleated bone marrow cell count was maintained, however, even after the administration of CPA at a high dose, which led to a recovery of approximately 87.2% (still higher than the 67.1% recovery achieved following the oral administration of Shenqi tablets). The proportion of bone marrow white blood cells (granulocytes, lymphocytes and monocytes) was also affected by the cyclophosphamide treatment. The subsequent administration of CPA in all treatment groups was accompanied by a relatively strong tendency towards the original proportions. Again, the high dose treatment led to the highest level of recovery.

In the CPA treatment groups, there was a sharp increase in the spleen index as compared to the negative control group (CT). In the high dose treatment group, a recovery of 46.0% above the index of the control group (NC) was achieved, whereas in the ST group a lower recovery level, of approximately 67.3%, was observed.

Similarly to the results shown for the radiation treatment, it was also possible to clearly observe here the dose-dependent effects of the CPA administration. At a same dose of 60 mg/kg, the intravenous administration of CPA was more effective than its oral administration. The use of Shenqi tablets was not as effective as the high intravenous dose of CPA for all parameters analyzed. These results underscore the potential use of CPA owing to its hematopoietic, thrombocytopoietic effects and marked leukocytotic effects, as well as its regulating effect in the modulation of the relative proportion of peripheral and bone marrow white blood cells.

Amelioration effects of CPA on myelosuppression induced by cytarabine chloride in rabbits

To explore the putative ameliorative effects of CPA on myelosuppression induced by chemotherapy in larger animal models, New Zealand rabbits were exposed to a cytarabine chloride treatment.

The effect of CPA on hemoglobin levels following the cytarabine chloride treatment was similar to those observed for the mice model, with an average recovery of 97.7% and 90.3% in hemoglobin content observed in the high dose and Shenqi tablet treatment groups.

A 38.3% drop in the number of platelets was observed after the cytarabine chloride treatment. Still, recovery levels as high as 94.3% and 86.5% were observed in the high dose (HT) and Shenqi tablet (ST) treatment groups, indicating the marked thrombocytopoietic effect of CPA. Despite a decrease in the white blood cell count of about 77.4% resulting from the chemotherapy, a strong reversal of such reduction was observed in the groups treated with CPA. Oral treatment with CPA resulted in a recovery in the number of white blood cells of about 79.3%, with half recovery achieved in the ST group, and an increase of 7.07% above the levels in the control group observed following the administration of CPA at a high dose, suggesting a robust leukocytotic effect of CPA.

Similar tendencies were observed in the analysis of the proportion of peripheral neutrophils, lymphocytes and monocytes after treatment with CPA, indicating the ability of this combined compound (especially when administered intravenously using a high dose) to modulate those proportions towards levels observed in the absence of chemotherapy.

The proportion of bone marrow white blood cells (granulocytes, lymphocytes and monocytes) was also affected by the cytarabine chloride treatment. The subsequent administration of CPA administration in all treatment groups was accompanied by a relatively strong

tendency towards the original proportions of these cells. Again, the high dose treatment led to the highest level of recovery. After the CPA administration, there was a sharp increase in the spleen index compared to the negative control group (CT). In the high dose treatment group, a recovery of 92.3% was achieved, whereas under the Shenqi tablet treatment a lower recovery level, of approximately 72.6%, was obtained.

Again, the effects of CPA depended on the dose administered. At the same dosage of 24.0 mg/kg, the intravenous administration was more effective than the oral administration. Shenqi tablets were less effective than the high intravenous dose of CPA for all parameters analyzed. These findings once more emphasize the potential therapeutic uses of CPA owing to its hematopoietic, thrombocytopoietic and leukocytotic effects, as well as its ability to regulate the proportion of peripheral and bone marrow white blood cells.

Discussion

Myelosuppression is a common side-effect of radio- and chemotherapy, characterized by a decrease in the ability of bone marrow to produce blood cells. In addition to anemia, leucopenia and thrombocytopenia directly result from myelosuppression. In particular, neutropenia (a decrease in the number of white blood cells known as neutrophils) can increase the risk of infection in cancer patients. Although mild myelosuppression is usually normalized within a few weeks, it may greatly weaken the effects of cancer treatments, since patients cannot receive full dose treatments due to resulting effects such as fatigue or excessive bleeding.

Presently, the injection of growth factors is a commonly employed procedure to stimulate the production of blood cells in the bone marrow. Erythropoietin stimulates red blood cell production, which may decrease the need for transfusion and improve the life quality of patients. The granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (G-CSF and GM-CSF, respectively) can also speed the recovery in the number of neutrophils, but their side effects include rashes, fever, nausea, and bone pain. Interleukin 11 is a protein that can also increase platelet numbers, but may cause red eyes, fluid retention and rapid heartbeat. In addition to these side effects, all growth factors that can ameliorate cancer therapy are expensive and require more than one injection.

In several Asian countries, however, many plants (especially their parts or extracts, as those used in Traditional Chinese Medicine) are commonly used to alleviate the myelosuppressive effects caused by radio and chemotherapy. If effective, their active compounds could provide alternative adjunctive treatments with greatly reduced clinical costs.

Many medicinal herbs used in TCM, such as *Radix sanguisorbae* (Di Yu), *Rhizoma polygonati* (Huang Jing), *Radix scutellariae* (Huang Qi) and *Fructus ligustri lucudi* (Nu Zhen Zi) have been extensively used to improve the recovery of cancer patients following radio and chemotherapy. In this research, the Shenqi tablet, as the positive control, was a major OTC used for the same purpose.Although several of the therapeutic effects of TGP have been demonstrated, there were only few references to the potential amelioration effects of *P. lactiflora* on myelosuppression. To our knowledge, the present study is the first to report the hematopoietic, thrombocytopoietic and leukocytotic effects resulting from the combined use of paeoniflorin and albiflorin.

In X-ray treated mice, a high intravenous dose of CPA had a strong hematopoietic effect, moderate thrombocytopoietic effects and mild leukocytotic effects. Similarly, the intravenous administration of CPA following chemotherapy with cyclophosphamide resulted in marked hematopoietic and thrombocytopoietic effects, as well as robust leukocytotic effects, in mice. The same pattern was observed in cytarabine chloride treated rabbits, for which a high dose of intravenous CPA led to strong hematopoietic and thrombocytopoietic effects and robust leukocytotic effects.

The observed increase in the spleen index achieved with CPA was additionally responsible for a positive immunoregulatory effect, a finding that may also broaden its pharmacological applications.

In conclusion, The results of the present study indicate that CPA has a potent ameliorative effect on radiation-induced myelosuppression in mice, and chemotherapy-induced myelosuppression in both mice and rabbits. We also showed that CPA is more effective in reversing the effects of chemotherapy than of radiotherapy, a result that might be related to underlying differences in the nature of the damage produced by each of these therapies.

Further pharmacological and pharmacodynamic studies should be conducted to determine the mechanisms underlying the therapeutic effects of CPA, and therefore its potential use as a myelosuppressive drug therapy,

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