J. Pharm. Pharmacol. 1998, 50: 351–354
Received September 18, 1997
Accepted December 5, 1997

Shenjincao (*Palhinhaea cernua*) Injection for Treatment of Experimental Silicosis of Rats

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Abstract

Shenjincao injection is a traditional Chinese medicine prepared from *Palhinhaea cernua* (L.) A. Franco et Vasc. by ultrafiltration. Its anti-silicosis action has been investigated both as a prophylactic and for treatment of the disease.

Wistar rats were injected intra-tracheally with quartz dust and then divided randomly into groups—treatment and control prophylactic groups and treatment and control disease groups. After five days or eight weeks, respectively, the silica-exposed rats of the two treatment groups were injected intraperitoneally three times a week with shenjincao injection, dose 2·0 mL, for five weeks or 11 weeks, respectively. The rats were then dissected, and the ceruloplasmin content of the serum and the fresh weight, dry weight, collagen content and pathological grade of the lungs were measured. Compared with the corresponding exposed control groups for the same treatment periods the values of these parameters were reduced by 62·8% to 30·7% for rats in the prophylactic treatment group ($P < 0.01$ for all) and by 50·8% to 30·2% for the diseased group ($P < 0.01$ for all). The values for the disease-treatment group were also reduced by 37·9% to 25·9% compared with values for the exposed control group before treatment ($P < 0.01$ or $P < 0.05$). The effective coefficients for prophylactic treatment were 82·6% to 56·0%; for disease treatment they were 68·8% to 39·8%.

These results show that shenjincao injection is efficacious against experimental silicosis not only when used prophylactically but also when used to treat the disease.

Shenjincao is a traditional Chinese medicine, the botanical origins of which include *Lycopodium japonicum* Thunb. (*L. clavatum* L.), *Palhinhaea cernua* (L.) A. Franco et Vasc. (*Lycopodium cernuum* L.) and others (Yang 1981, 1985; Dai & Ye 1991). It has a slightly bitter and pungent taste, mild properties and the functions of expelling pathogenic wind to clear away cold, eliminating dampness to diminish swelling and relaxing muscles and tendons to promote blood circulation. In the practice of traditional Chinese medicine, shenjincao is used to treat rheumatism caused by wind, cold and dampness as combined pathogenic factors, aching joints, unfavourable crooking and stretching, injuries from falls, fractures, contusions, and strains, and herpes zoster, hepatitis, jaundice and dysentery, etc. It has been reported that shenjincao has other pharmacological action, for example as an anti-pyretic (Nikonorow 1939; Marier & Bernard 1948), as an anti-hypertensive (Marier & Bernard 1948) and as an analgesic (Zhang & Nan 1988). To investigate the anti-silicosis action of shenjincao, we prepared shenjincao injection by ultrafiltration and used it in a pharmacodynamic experiment with two methods of administration—as a prophylactic and for treatment of the disease.

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Materials and Methods

Male Wistar rats, approximately 200 g, were obtained from the Institute of Blood Transfusion, the Chinese Academy of Medical Science. Standard quartz dust used for exposure to silica was obtained from the Institute of Health, the Chinese Academy of Preventive Medicine. The free silica content of the dust was >97% and 99·9% of the particles were $< 5\,\mu m$. Shenjincao injection was prepared with a hollow-fibre ultrafilter, the intercepting molecular weight of which was 6000 Da. The crude drug used was *Palhinhaea cernua* (L.) A. Franco et Vasc., as verified by the Chengdu Institute for Drug Control.

Experiments were performed on six groups of rats; groups 1–3 were used to study prophylactic treatment, groups 4–6 for study of treatment of the disease. The rats used for the two types of treatment were killed approximately 6 and 19 weeks, respectively, after the beginning of the experiment. Twelve rats were selected randomly before exposure to dust and divided into two equal groups. These were used as intact control groups for the prophylactic treatment group (group 3) and the disease-treatment group (group 6), respectively.

The other rats were injected intra-tracheally, while under ether anaesthesia, with a suspension (1 mL) of standard quartz dust (45 mg) and penicillin (30 000 units), according to the method described by Goldstein et al (1962). The surviving rats were injected intraperitoneally with penicillin (50 000 units) for three days and then divided randomly into four groups. The prophylactic treatment group (group 1) was injected intraperitoneally with shenjincao injection for six weeks starting five days after exposure to the dust. The disease-treatment group (group 4) was injected intraperitoneally with shenjincao injection for 11 weeks starting eight weeks after exposure to the dust. Injections (2·0 mL) were administered three times a week. The two other groups were the exposed control group for prophylactic treatment (group 2) (this group was also used concurrently as the exposed control group for the disease-treatment group before treatment) and the exposed control group for the disease-treatment group (group 5).

At the end of treatment the rats of the prophylactic treatment and disease-treatment groups were killed by exsanguination from the femoral artery under thiopental sodium anaesthesia and the lungs were removed. The condition of the lungs was assessed by methods described by Xing & Fu (1985) and Dauber et al (1980). Values for the ceruloplasmin content of the serum and the fresh weight, dry weight, and collagen content of the lungs were measured. Three histological sections were cut from the areas of the left lung where pathological changes were most severe and two lobes of right lung were taken from each rat, stained with haematoxylin and eosin (H & E), and graded according to four criteria for pathological changes in experimental silicosis: grade I, cellulous node; grade II, fibro-cellulous node; grade III, cello-fibrous node; grade IV, fibrous node. Normal sections were regarded as grade 0.

All measurement data are expressed as means ± s.d. The significance of differences between means for a treatment group and for the corresponding exposed control group was determined by use of the unpaired *t*-test. The ranked data are expressed as numbers of sections with the grades 0–IV, from which the average grades were calculated; the significance of differences between a treatment group and the corresponding exposed control group was analysed by the ranked-order-value method (Sun 1987). $P < 0{\cdot}05$ was considered as indicative of statistical significance. The reduction in each parameter for the treatment groups was calculated according to $D = 100\% \times (b-a)/b$, where a is the mean for the treatment group and b the mean for the corresponding exposed control group. The effective coefficient for each type of treatment was calculated according to $E = 100 \times (b'-a)/(b'-c)$, where a is the mean parameter for a treatment group, b′ is the mean for the exposed control group for the same period, and c is the mean for the intact control group for the same period.

Results

The silica-exposed rats were without vigour and had poor appetite; one or two rats suffered from nasal haemorrhage and stridor after exposure to the dust, although later their condition improved. The eating, defecation and urination of the rats were basically normal, but a small number of rats died during the experiment. One rat which was noticed, after dissection, to be suffering from a pulmonary abscess was rejected from the statistical data. The lungs of the two intact control groups were pink and soft, but those of two exposed control groups were obviously larger and some parts of the lobes were greyish-white and hard. The condition of the lungs of the treatment groups were between those of the intact and exposed control groups. Details of grouping and administration of medicine are summarized in Table 1. The values obtained for ceruloplasmin content, fresh weight, dry weight and collagen content and the results from grading of the histological sections for all groups of rats, and the effective coefficient of treatment values (E values) calculated, are summarized in Table 2. The statistical results from comparison of each treatment group with its exposed control group for the same period, and those from comparison of the disease-treatment group (group 4) with its exposed control group before treatment (group 2) are presented in Table 3.

These results show that shenjincao injection is efficacious against experimental silicosis in rats, not only for prophylactic treatment but for treatment of the disease. The effective coefficients for prophylactic treatment and for treatment of the disease were 82·6% to 56·0% and 68·8% to 39·8%, respectively. Compared with the corresponding exposed control group for the same period, the values of above mentioned parameters for the

Table 1. The grouping of the rats and the doses of medicine administered.

Group	Name	Number of rats			Single dose (mL)	Total (mL)
		Per group	Dissected	Statistical analysis		
1	Prophylactic treatment	12	6	6	2·0*	29·0
2	Exposed control	11	11	11	0	0
3	Intact control	6	5	0	0	
4	Disease-treatment	10	6	6	2·0	68·0
5	Exposed control	9	8	8	0	0
6	Intact control	6	4	3	0	0

*First dose 1·0 mL.

Table 2. Results of physical and chemical measurements, of pathological grading and from calculation of E values.

Group	Name	Ceruloplasmin		Fresh weight		Dry weight		Collagen content		Pathological grade					Mean	E
		(units)	E	(g)	E	(g)	E	(mg)	E	0	I	II	III	IV		
1	Prophylactic treatment	472 ± 208	66·1	2·00 ± 0·38	82·6	0·37 ± 0·08	74·0	46·7 ± 10·3	81·8	3	10	3	1	1	1·28	56·0
2	Exposed control	681 ± 85	–	4·38 ± 1·11	–	0·74 ± 0·19	–	125·7 ± 42·3	–	0	0	8	20	5	2·91	–
3	Intact control	365 ± 153	–	1·50 ± 0·20	–	0·24 ± 0·04	–	29·1 ± 5·0	–	15	0	0	0	0	0	–
4	Disease-treatment	498 ± 134	68·8	2·72 ± 0·52	59·7	0·50 ± 0·11	61·8	93·2 ± 16·5	61·5	1	8	5	1	3	1·83	39·8
5	Exposed control	714 ± 137	–	4·50 ± 1·18	–	0·84 ± 0·22	–	189·6 ± 52·9	–	0	4	4	3	13	3·04	–
6	Intact control	400 ± 90	–	1·52 ± 0·10	–	0·29 ± 0·01	–	32·8 ± 2·0	–	9	0	0	0	0	0	–

prophylactic treatment group and for the disease-treatment group were reduced by 62·8% to 30·7% and by 50·8% to 30·2%, respectively; the values for the disease-treatment group were reduced by 37·9% to 25·9% compared with its exposed control group before treatment. These differences were statistically significant ($P < 0·05$). These results prove that shenjincao injection not only inhibits the progress of silicosis but also reverses the pathological changes which result from the disease.

Discussion

Silicosis is a progressive lung disorder caused by inhalation of silica-containing dust; it is an extremely serious occupational disease which endangers the health of industrial workers exposed to the dust. To date there has been no reliable drug for treatment of silicosis. Our experiments have proved that treatment with shenjincao injection was efficacious against silicosis in rats, not only prophylactically when given during the course of development of the disease after exposure to the dust, but also for treatment of the disease when given as medicine when silicosis has developed after several weeks exposure to the dust. The ceruloplasmin content of the serum and the fresh weight, dry weight, collagen content and pathological grade of the lungs, classical parameters for measuring the extent of pathological changes resulting from experimental silicosis, not only decreased markedly but were even tending towards normal values after the treatment. This provides a reliable therapeutic basis for the clinical use of shenjincao injection. Shenjincao contains alkaloids (Burnell & Mootoo 1961; Ayer et al 1964), steroids (Cambie & Parnell 1970; Cai & Pan 1987), anthraquinones (Cai et al 1991), etc.; the identities of the active principles of shenjincao remain a question for study.

Results of toxicological experiments have shown that the largest intravenous dose of shenjincao injection which mice can tolerate is 40 mL kg^{-1}. Routine blood examination, liver function testing, renal function testing and histopathological inspection of the lungs, heart, liver, kidneys and testes have shown no evident toxic effect in rats to which shenjincao injection had been administered intraperitoneally for one year. A study of clinical effects of shenjincao injection on silicosis patients is in progress.

Ultrafiltration is a membrane-separation technology which has developed rapidly in recent years. It can be used for removal of a variety of particles, including colloids, bacteria and pyrogens, and for separation of solutes of different molecular

Table 3. Statistical results for treatment groups compared with corresponding exposed control groups.

		Groups compared		
		1 and 2	4 and 5	4 and 2
Ceruloplasmin	Reduction (%)	30·7	30·2	26·9
	t value	2·97	2·95	3·47
	Probability *P* value	< 0·01	< 0·01	< 0·01
Fresh weight	Reduction (%)	54·3	40·0	37·9
	t value	5·03	3·43	3·43
	Probability *P* value	< 0·01	< 0·01	< 0·01
Dry weight	Reduction (%)	50·0	40·9	32·4
	t value	4·50	3·45	2·82
	Probability *P* value	< 0·01	< 0·01	< 0·01
Collagen content	Reduction (%)	62·8	50·8	26·9
	t value	4·44	4·27	1·79
	Probability *P* value	< 0·01	< 0·01	< 0·05
Pathological grade	Reduction (%)	56·0	39·8	37·1
	U value*	4·71	2·78	3·33
	Probability *P* value	< 0·01	< 0·01	< 0·01

*Calculated by ranked-order-value method.

weight. Its application in the preparation of pharmaceuticals, especially in the preparation of traditional Chinese medicine injections, is gradually expanding (He 1995). The results of this experiment have also proved that the preparation of shenjincao injection by ultrafiltration is feasible. In addition, we have studied the conditions and technological process of ultrafiltration when preparing the injection and have developed a new method of refining the injection, by double-ultrafiltration, which improves the quality of the injection and reduces losses of the active principles. This study has been reported in detail elsewhere (He 1996). Some rats in all the groups except group 2 died during the course of this experiment, so the sample size for statistics had decreased noticeably by the end of the experiment. In addition the rats' resistance to disease and tolerance of the drug declined markedly as a result of the lungs being attacked by another, external, pathogen after exposure to the dust; this was probably because of the relatively long duration of the experiment and the imperfect experimental conditions under which the animals were maintained.

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