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Subchronic toxicity studies of *Radix Astragali* extract in rats and dogs

Shu-Yi Yu, Hong-Tao OuYang, Ju-Yun Yang, Xiao-Liang Huang, Ting Yang, Ju-Ping Duan, Jun-Ping Cheng, Yu-Xiang Chen *, Yong-Jia Yang, Pang Qiong

School of Biosciences and Technologies of Central South University, 172 Tongzipo Road, Changsha 410013, China

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Abstract

Radix Astragali extract (RAE) is obtained from *Astragalus membranaceus*. It consists of *Astragalus* polysaccharide and *Astragalus membranaceus* saponins. In the study, we observed the subchronic toxicity of RAE in Sprague–Dawley rats and beagle dogs to evaluate the safety dosage range in clinical application. These subjects were daily administered of RAE by intra-peritoneum or vein for three consecutive months. General index were observed such as food-intake, behavior, body weight, hematological parameters, etc. Body weight, the weight of principal organ and hematology index are normal in experimental groups and control groups. The hematological biochemistry examination and histopathology examination of experimental groups are similar to control groups. In conclusion, our studies clearly demonstrated that RAE was safe without any distinct toxicity and side effects, the safety dosage range is 5.7–39.9 g/kg for rats and 2.85–19.95 g/kg for beagle dogs, which is equal to 70 or 35 times of that of human (0.57 g/kg, say, average BW 70 kg), respectively.

Keywords: RAE; Subchronic toxicity; Rat; Dog

1. Introduction

Radix Astragali is a dried root of *Astragalus membranaceus (Fisch) Bge.* or *Astragalus membranaceus (Fisch) Bge. var. mongholicus (Bge.) Hsiao*, known as *Huangqi* in China, harvested in spring and autumn. It is used to replenish the vital energy for the treatment of lacking strength, anorexia and loose stools, prolapse of uterus and anus, spontaneous sweating, and chronic nephritis with edema and proteinuria, and to dispel pus and accelerate the healing of chronic ulcers (Pharmacopoeia of the People's Republic of China, 2005 edition). The modern pharmacological studies have been shown to contain triterpene saponins, flavanone, isoflavonoids, pterocarpan glycosides, polysaccharides, and some trace elements (Lin et al., 2000; Ma et al., 2002a,b, 2003, 2004; Wu et al., 2005), possessed immunostimulant, tonic (adaptogenic), hepatoprotective, diuretic, antidiabetic, analgesic, expectorant, protects from ischemic brain injury, parasite, aging (Schinella et al., 2002; Ma et al., 2002a,b; Lee et al., 2003; Li et al., 2003; Luo et al., 2004; Lee and Jeon, 2005; Yang et al., 2005). The extensive phytochemical and pharmacological studies on Radix Astragali have proved the flavonoids, cyclolanostane-type saponins and polysaccharides to be the main bioactive principles (Gariboldi et al., 1995; Verotta et al., 2001; Luo et al., 2004; Lee and Jeon, 2005), astragaloside IV, one of the cyclolanostane-type saponins, has been chosen as "marker compound" for the chemical evaluation or *Radix Astragali* quality control. It was reported that astragaloside IV is a strong scavenger for superoxide radicals (IC50 < 0.023 mg/ml) and hydroxyl radicals (IC50 < 0.053 mg/ml) in vitro studies (Ma and Yang, 1999) and has the effect protecting from the ischemic brain injury (Luo et al., 2004), improving the cardiac function in ischemic rats and reducing excessive accumulation of intracellular calcium within myocardial cells (Zi and Cao, 2002). *Astragalus membranaceus* saponins could be safely used as adjuvant with low or non-haemolytic effect (Yang et al., 2005). *Astragalus* polysaccharide (APS) could activate mouse B cells and macrophages (Shao et al., 2004), stimulate macrophages to express iNOS gene through the activation of NF-κB/Rel (Verotta et al., 2001), promote both humoral and cellular immune responses and would be expected as the component drug of a new-type immunopotentiator (Kong et al., 2004; Wang et al., 2005). In addition, scientific investigation in the latest years has revealed much pharmacological functions of APS, such as anti-virus, decrease in blood glucose, protection

* Corresponding author. Tel.: +86 731 2650521; fax: +86 731 4327545.
E-mail address: chenyx@xysm.net (Y.-X. Chen).

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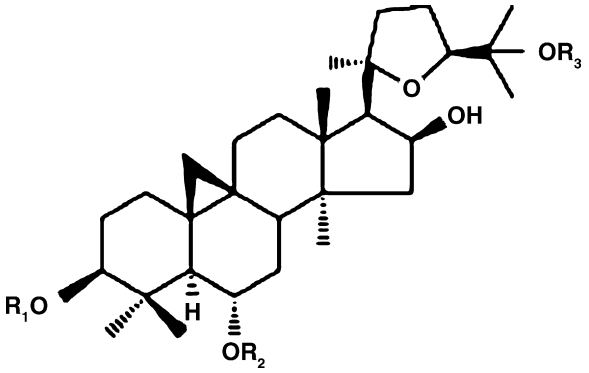

Fig. 1. The molecular structure of cycloastragenol.

Table 1
The constituents and the molecular structure of saponins in RAE

Saponins	Aglycone	R1	R2	R3
Astragaloside I		2,3-di-O-Ac-Xyl	Gle	H
Isoastragaloside I		2,4-di-O-Ac-Xyl	Gle	H
Astragaloside II		2-O-Ac-Xyl	Gle	H
Isoastragaloside II	Cycloastragenol	3-O-Ac-Xyl	Gle	H
Astragaloside IV		Xyl	Gle	H
Isoastragaloside IV		Xyl	H	Gle
Acetylastragaloside I		2,3,4-tri-Ac-Xyl	Gle	H

Ac: acetyl; Glc: β-D-glucopyranosyl; Xyl: β-D-xylopyranosyl.

of vessel, anti-cancer, etc. RAE is a refine production made of extract of *Astragalus membranaceus*. *Astragalus* polysaccharide and *Astragalus membranaceus* saponins are the constituents of RAE, *Astragalus* polysaccharide contains 88.96% of glucose, saponins include astragaloside IV, isoastragaloside IV, astragaloside I, isoastragaloside I, isoastragaloside II, astragaloside II, and acetylastragaloside I. The constituents and the molecular structure of saponins in RAE have been showed in Fig. 1. The dosage used in the experiment was based on the MED in rats. The dosage was set according to the standard protocol for subchronic toxicity test (Guidelines for chronic toxicity testing of nature medicine and TCM, issued by State Food and Drug Administration of China, 2005). In the experiment, the high dosage (39.9 g/kg) is equal to 12 times of the MED in rats, which is 70 and 35 times high, respectively, than that of human (0.57 g/kg, say, average BW 70 kg). The low dosage is little high than of MED, and the medal dosage is the square root of cross product of high dosage and low dosage. The dosages used in the experiment were all expressed by crude drug (0.5 g crude drug equal to 1 mg RAE). The results are reported as follows (Table 1).

2. Materials and methods

2.1. Plant material

Radix Astragali were collected from Sichuan province of China in July 2004. A voucher specimen was deposited in the Herbarium of Hunan province TCM institution, which was authenticated by professor Liang Jianguo with the Hunan Institute of Pharmaceutical Control of China.

2.2. Methods

Radix Astragali was powdered and extracted with organic solvent. The extract was purified and freeze-dry to made into lyophilized powder, 25 mg/bottle (equal to 0.5 g crude drug/mg), which was reconstructed in sterile saline before medication at different concentration. Rats or dogs were randomly assigned to four different groups (30 rats/group, 4 dogs/group). Isovolum RAE were administrated at three different dosage of 39.9, 15.08 and 5.7 g/kg, respectively, in rats (1.0 ml/100 g) by intraperitoneal injection, and 19.95, 7.54 and 2.85 g/kg, respectively, in beagle dogs (5 ml/kg) by intravenous injection, once a time per day, for 3 months, at the same time, the isovolum vehicle was given the rats and beagle dogs for control. Subchronic toxicities of RAE were assessed according to the standard protocol for subchronic toxicity test (Guidelines for chronic toxicity testing of nature medicine and TCM, issued by State Food and Drug Administration of China, 2005). Sprague–Dawley (7-week-old) rats and beagle dogs (8-month-old) of both sexes used in the experiment received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 86-23, revised 1986).

3. Statistics

In the studies above, a two-way analysis of variance was performed on appropriate data sets using the general linear models procedure in the Statistical Analysis System SPSS 10.0. The measurement data include the hematological and hemato-biochemical parameters, and absolute weight of organs expressed by mean ± S.D., Student's *t*-test was used to evaluate significance between mean values. The differences of necropsy and histopathology were analyzed by *Ridit* test. $p < 0.05$ was accepted as statistically significant.

4. Results

The increase of body weight and the absolute and relative organ weights had no difference for dog and rat experiment. There were no abnormal clinical signs and hematological parameters during the period of observation and no drug-related deaths. The weight, size, shape, or color of various tissues between treated animals and the control showed no significant difference.

4.1. In the rat study

Hemato-biochemical analysis results indicated that γ-GT of high-dose group (39.9 g/kg) was higher than the control (Table 2, $p < 0.05$) for rats, but within normal limits, no correlation with dosage. The high (39.9 g/kg), low (5.7 g/kg) dosage and control rats with treatment of 13 weeks had 3, 4 and 3 cases having light-medium pulmonary cirrhosis, respectively. Whereas, for 17 weeks, moderate-dose (15.08 g/kg) and control group had 4 and 3 cases having the same pathogenesis, respectively. However, all the data analyzed by *Ridit* test indicated that the pathogenesis in the lung was non-associated with the drug RAE.

Table 2
The influence on rats' hemato-biochemical administered RAE for 3 months by intraperitoneal injection

Observation	High dosage	Moderate dosage	Low dosage	Control
ALT (U/l)	56.30 ± 21.75	52.50 ± 12.55	50.50 ± 12.42	54.20 ± 8.16
TP (g/l)	90.58 ± 5.53	91.79 ± 4.57	90.40 ± 4.15	92.71 ± 5.06
ALB (g/l)	48.66 ± 6.60	52.94 ± 4.81	49.50 ± 8.82	50.21 ± 10.20
GLO (g/l)	41.92 ± 7.88	38.85 ± 4.53	40.90 ± 5.56	42.50 ± 7.55
TBIL (g/l)	0.99 ± 0.17	0.88 ± 0.25	1.00 ± 0.22	1.53 ± 2.08
AST (g/l)	229.60 ± 44.59	234.20 ± 42.55	217.80 ± 29.31	242.00 ± 43.33
γ-GT (U/l)	15.40 ± 2.80**	13.70 ± 2.26	12.40 ± 2.46	12.90 ± 2.33
AKP (U/l)	148.10 ± 64.42	164.50 ± 76.21	169.70 ± 91.44	157.30 ± 70.69
BUN (mmol/l)	11.69 ± 2.55	10.87 ± 2.39	10.71 ± 3.10	12.80 ± 2.54
TC (mmol/l)	1.66 ± 0.43	1.85 ± 0.34	1.75 ± 0.32	1.66 ± 0.41
Cr (μmol/l)	92.00 ± 20.95	84.50 ± 20.74	86.60 ± 13.20	100.30 ± 23.54

Mean ± S.D., $^{**}p < 0.01$ comparison with control.

Table 3
The influence on beagle dogs' hemato-biochemical administered RAE for 13 weeks by intravenous injected

Observation	High dosage		Moderate dosage		Low dosage		Control	
	13 weeks	17 weeks	13 weeks	17 weeks	13 weeks	17 weeks	13 weeks	17 weeks
ALT (U/l)	27.65 ± 7.41	32.00 ± 1.41	26.98 ± 6.42	43.00 ± 9.90	23.27 ± 6.47	42.00 ± 1.41	23.27 ± 3.18	43.50 ± 14.85
TP (g/l)	64.95 ± 5.65	65.75 ± 2.47	63.53 ± 5.11	66.75 ± 0.78	60.68 ± 6.01	61.25 ± 3.61	65.08 ± 3.99	63.70 ± 4.24
ALB (g/l)	29.73 ± 2.96	32.15 ± 1.91	29.40 ± 1.39	33.15 ± 1.34	27.35 ± 2.47	32.05 ± 1.48	30.45 ± 2.73	30.65 ± 0.92
GLU (g/l)	3.46 ± 0.329	4.07 ± 0.007	3.79 ± 0.955	3.99 ± 0.134	3.46 ± 0.97	4.82 ± 0.431	3.42 ± 0.518	4.85 ± 1.124
TBIL (g/l)	1.017 ± 0.512	0.80 ± 0.141	0.817 ± 0.397	0.75 ± 0.354	1.05 ± 0.472	0.95 ± 0.212	0.90 ± 0.494	0.80 ± 0.283
AST (g/l)	36.45 ± 4.70	29.00 ± 1.41	36.67 ± 6.60	33.00 ± 7.07	38.20 ± 7.57	30.50 ± 3.54	37.42 ± 7.27	27.50 ± 6.36
γ-GT (U/l)	2.19 ± 0.033	2.16 ± 0.028	2.14 ± 0.019	2.13 ± 0.021	2.16 ± 0.024	2.16 ± 0.028	2.13 ± 0.017	2.13 ± 0.007
AKP (U/l)	271.02 ± 33.17	46.00 ± 14.14	318.23 ± 78.19	70.00 ± 7.07	325.77 ± 83.68	80.50 ± 14.14	270.80 ± 48.74	72.50 ± 19.09
BUN (mmol/l)	7.75 ± 0.684	7.31 ± 0.686	7.82 ± 1.773	7.01 ± 1.004	7.51 ± 1.257	7.05 ± 0.297	8.38 ± 1.689	8.45 ± 0.445
TC (mmol/l)	4.24 ± 0.397	4.27 ± 0.368	3.95 ± 0.532	4.07 ± 0.940	3.63 ± 0.752	4.03 ± 0.502	4.42 ± 0.372	3.39 ± 0.714
Cr (μmol/l)	47.98 ± 3.59	68.50 ± 4.95	46.78 ± 8.35*	78.00 ± 2.83	46.68 ± 4.61	86.50 ± 4.95	48.25 ± 4.78	82.50 ± 2.12

Mean ± S.D., $^{*}p < 0.05$ comparison with control.

4.2. In the beagle dog study

Beagle dog's electrocardiogram and the observations of urine were normal prior to dosing, at weeks 6 and 13. Each group had no remarkable differences before or after dosing. Hemato-biochemical analysis indicated that TBIL for high (19.95 g/kg), moderate (7.54 g/kg) groups after RAE for 6 weeks (dada not shown), urea and creatine for moderate group treatmented for 6 weeks (dada not shown) or 13 weeks, were higher than the control, the changes had significant difference (Table 3, $p < 0.05$) without no clinical significance. Histomorphological examination indicated that high, moderate dosage and control groups treatmented with RAE for 13 weeks each had one case with light-medium pulmonary cirrhosis, while high-dose and control groups after withdrawing drug 4 weeks (week 17) each had one case. All the data analyzed by *Ridit* test indicated that the pathogenesis in the lung was non-associated with the drug RAE.

5. Discussion

Radix Astragali (*Huangqi*), a traditional Chinese medicine, has been widely used for several thousand years with a significant efficacy and low-toxicity. In order to get pure active compounds *Astragalus polysaccharide* and *Astragalus membranaceus* saponins, which we extracted from crude *Radix Astragali* (*Huangqi*) and made into a lyophilized powder dosage form. RAE has super merits to the *Radix Astragali* (*Huangqi*) and its pharmacological effects RAE as clear as mentioned above. Furthermore, RAE is of high purity, rapid onset, less side effect, stability and easy to application in clinic.

The subchronic toxicity test of RAE indicated that in the rat and beagle dog study, behavior activity and general condition are normal, hematological, hemato-biochemical parameters, absolute weight and relative weight of major organs did not have significant difference compared with the control after 3 months treatment and recovery period of 4 weeks. The routine urine and electrocardiogram of beagle dogs had not remarkable difference compared with the control. So we reasoned that rats and beagle dogs did not show obvious toxicity after intra-peritoneum or vein injection of RAE for 13 weeks, at the dosage of 39.9, 15.08 and 5.7 g/kg and 19.95, 7.54 and 2.85 g/kg, respectively. Meanwhile these results should provide direction for clinical application and suggested the technology of the manufacturing is fairly reasonable.

In conclusion, our studies clearly demonstrated that RAE was safe without any distinct toxicity and side effects, the safety dosage range is 5.7–39.9 g/kg for rats and 2.85–19.95 g/kg for beagle dogs, which is 70 and 35 times high, respectively, than

that of human (say, average BW 70 kg). Since response for the drug has difference between animals and human, the further study in the clinic is needed.

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