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Anti-inflammatory Effect of *Scutellariae Radix*

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Abstract - This research is the basic research to develop new anti-inflammatory medicine by feeding *Scutellariae Radix* extract to lipopolysaccharide (LPS) exposed rats, and analyzed it's effect on inflammatory response by LPS derivation. As a result, Plasma interleukin-1β (IL-1β) and Plasma interleukin-6 (IL-6) concentration showed the highest point at 5h after LPS injection, and in this time, the concentration of IL-1β and IL-6 in the *Scutellariae Radix* extract groups at 200mg/kg and 300mg/kg showed lower values than that of control group. Plasma tumor necrosis factor-α (TNF-α) concentration after LPS injection showed the highest point at 2h and showed similar level till at 5h. TNF-α concentration at 2h after LPS injection showed the low value only in the *Scutellariae Radix* extract 300mg/kg group compared to others, and in 5h, the all *Scutellariae Radix* extract groups showed lower value than that of the control group. Plasma interleukin-10 (IL-10) concentration increased at 2h after LPS injection and reached the highest at 5h. After LPS injection the IL-10 concentration at 2h, the *Scutellariae Radix* extract injection group at 300mg/kg showed higher value than that of the others, and in 5h after LPS injection, *Scutellariae Radix* extract 200mg and 300mg groups showed higher value than the control group. Concluding from the above results, in inflammatory response by LPS derivation, the *Scutellariae Radix* gives positive effect.

Key words - *Scutellariae Radix*, Lipopolysaccharide, Anti-inflammatory, Cytokine

Introduction

The *Scutellariae Radix* is a perennial herb group's *labitate*, which bark of the root of *Scutellaria Baicalensis Georgi* is removed. The major components are compounds of flavonoid types, and there were around 30 elements found (Kim, 2001). Until now, the major biological functions of *Scutellariae Radix* are antihistamine effect (Middleton and Drzewiecki, 1985), anti liver toxin (Wagner,1986), antibiosis (Middleton and Harborne *et al.*, 1988), anticancer (Middleton and Harborne, 1986), anti bacteria activation (Kubo and Kimura *et al.*, 1981), anti-inflammatory effect (Kubo and Matusuda, 1984) and etc. These major functions are mostly from the flavonoid in *Scutellariae radix*, and in the dextran sodium sulfate (DSS) exposed animals, baicalein showed a high anti-inflammatory effect same as sulfasalazine (Tie and Jin, 2002). Also, Lee *et al.* (2004) reported that in the inflammatory model animals derived from DSS, *Scutellariae Radix* had increased immunity system, the interest on the *Scutellariae Radix* immune control and it's development possibility as replaced anti-inflammatory medicine had grown. On the other hand, presently used clinically, the ulcer medication glucocorticoid and sulfasalazine had been reported of it's an adverse effects (Boumpas and Chrousos, 1993), that development of anti-inflammatory medicine with less an adverse effects and better cure effect is needed. Therefore, in this research being the basic of developing new anti-inflammatory medicine, to investigate the details of *Scutellariae Radix* on anti-inflammatory effect, after administration *Scutellariae Radix* alcohol extract to rats, by injection LPS, the effect of *Scutellariae Radix* extract on anti-inflammatory effect had been compared and analysed among the treatment groups.

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Materials and Methods

Animals and treatments

Eighty-four Sprague-Dawley male rats of 176.35 $\pm$ 4.51g average body weight had been adapted to basal diet for one week, then on similar average weight, it was divided into control group (normal, saline 100mg/kg), *Scutellariae Radix* extract 100mg/kg group, *Scutellariae Radix* extract 200mg/kg group, and *Scutellariae Radix* extract 300mg/kg group, and each groups were placed 21 rats in random.

Diet and water

Dietary (Table 1) and water were *ad libitum* provided for 4

weeks of experiment period.

Table 1. Composition of experimental diet

Ingredients (%)	Basal diet
Casein	20.0
α-Corn starch	35.0
Sucrose	11.0
Lard	4.0
Corn oil	1.0
Mineral mix[1)]	3.5
Vitamin mix[2)]	1.0
Cellurose powder	23.5
DL-methione	0.3

[1)]Mineral mix. (g/kg diet): $CaCO_3$, 29.29; $CaHPO_4 \cdot 2H_2O$, 0.43; KH_2PO_4, 34.30; NaCl, 25.06; $MgSO_4 \cdot 7H_2O$, 9.98; Feric citrate hexahydrate, 0.623; $CuSO_4 \cdot 5H_2O$, 0.516; $MnSO_4 \cdot H_2O$, 0.121; $ZnCl_2$, 0.02; KI, 0.005; $(NH_4)_6 MO_7O_{24} \cdot 4H_2O$, 0.0025.
[2)]Vitamin mix (mg/kg diet): Thiamine-HCl, 12; Riboflavin, 40; Pyrodoxin-HCl, 8; Vitamin-B_{12}, 0.005; Ascorbic acid, 300; D-biotin, 0.2; Menadione, 52; Folic acid, 2; D-calcium pantothenate, 50; P-aminobenzoic acid, 50; Nicotinic acid, 60; Cholin choloride, 2000 (IU/kg diet); Rethinyl acetae, 5000 (IU/kg diet); Cholecalciferol, 250 (IU/kg diet).

Scutellariae Radix ext.

Scutellariae Radix 500g (dried weight) was divided and extracted 3 times at 5hours each in cooling water reflux cistern, and decompression concentrated, and made MeOH extract 120g. *Scutellariae Radix* ext. administration was placed orally using Jones tube at 5pm everyday. The control group was given normal saline in the same form.

LPS injection

After 4 weeks of experiment period was finished, the LPS was injected in abdominal cavity in same method to all groups at the level of 5mg/kg.

Sampling and analysis

The blood sampling was taken at the end of experiment of 4 weeks per each group, dividing into 3 groups of 7 rats, 7 rats right before LPS injection, and other 14 rats after LPS injection, and at 2h and 5h, each 7 rats were blood sampled in ether anesthesia with cardiac puncture method. Blood samples were centrifuged (5℃, 1800G) and were stored at -80℃ frozen. Plasma cytokine (IL-1β, TNF-α, IL-6 and IL-10) concentrations were determined by enzyme linked immunosorbent assay (ELISA), using commercial Kits (Biosource International, USA). The minimum detectable concentration of TNF-α is 0.7pg/ml, and the rest cytokines are 3-8 pg/ml.

Statistical analysis

Results were one-way ANOVA examined by using SPSS package, and each group's significance examination was done in the level of $P<0.05$ by Duncan's multiple range test.

Result and Discussion

Plasma cytokine measurement was done at before LPS injection (0h), 2 hours (2h), and 5 hours after LPS injection (5h), and the results were showed in Table 2,3,4 and 5. After LPS injection, the plasma cytokine measurement time was made in sampling time that was fit to examine shock of endotoxin of the rat through many experiment results (Mathiak and Grassother, 2000), and LPS injection concentration was 5mg/kg. This concentration was based upon another experiment results that report that it gives shock to rat endotoxin to increase the cytokine concentration of the liver and blood (Aono and Isobe, 1997; Barton and barton, 2001; Harry and Anand *et al.*, 1999; Sang and Wallis, 1999; Corral and Muller, 1996). The concentration of Plasma IL-1β (Table 2) and Plasma IL-6 (Table 3) showed the highest values at 5h after LPS injection, and in this time, the concentration of IL-1β and IL-6 in the *Scutellariae Radix* extract groups at 200mg/kg and 300mg/kg showed lower values than that of control group. IL-1β is a proinflammatory

Table 2. Effect of *Scutellariae Radix* ext. on plasma IL-1β concentration in lipopolysaccharide-exposed rats

Treatment	IL-1β (pg/ml), Time (h)*		
	0h	2h	5h
Control (saline, 100mg/kg)	12.43 ± 2.82^{NS}	58.86 ± 10.37^{NS}	238.29 ± 25.73^{b}
Scutellariae Radix ext. (100mg/kg)	13.86 ± 2.34^{NS}	55.71 ± 8.69^{NS}	246.71 ± 26.47^{b}
Scutellariae Radix ext. (200mg/kg)	14.14 ± 3.18^{NS}	53.00 ± 7.39^{NS}	158.43 ± 27.86^{a}
Scutellariae Radix ext. (300mg/kg)	14.57 ± 3.36^{NS}	47.71 ± 6.26^{NS}	156.00 ± 28.71^{a}

*0h, 2h and 5h after LPS injection.
[a,b]Means in the same column with different superscripts are significantly different ($p<0.05$).
[NS]Not significantly different ($P>0.05$).

Table 3. Effect of *Scutellariae Radix* ext. on plasma IL-6 concentration in lipopolysaccharide-exposed rats

Treatment	IL-6 (pg/ml), Time (h)*		
	0h	2h	5h
Control (saline, 100mg/kg)	21.43 ± 3.74^{a}	175.86 ± 37.67^{NS}	803.57 ± 78.75^{b}
Scutellariae Radix ext. (100mg/kg)	25.29 ± 4.27^{ab}	172.00 ± 20.07^{NS}	777.00 ± 90.16^{b}
Scutellariae Radix ext. (200mg/kg)	24.86 ± 3.58^{ab}	148.29 ± 19.37^{NS}	557.57 ± 55.98^{a}
Scutellariae Radix ext. (300mg/kg)	27.14 ± 3.13^{b}	155.29 ± 14.77^{NS}	601.57 ± 60.64^{a}

*0h, 2h and 5h after LPS injection.
[a,b]Means in the same column with different superscripts are significantly different ($p<0.05$).
[NS]Not significantly different ($P>0.05$).

cytokine that has been implicated as a mediator of LPS toxicity in vivo and in vitro. The biological properties of IL-1β are remarkably similar to those of TNF-α, and synergism between effects of these two molecules is evident in several models. In the result of this experiment after LPS injection it showed the highest value at 5h, it was similar to other research reported that it showed the peak of IL-1β concentration at 4-6h after LPS injection (Mathiak and Grass, 2000). IL-6 is an important proinflammatory cytokine produced by monocytes/macrophages, and in the liver mainly by Kupper cells. In this experiment, showing the peak at 5h after LPS injection, it was similar to the reported result that the plasma IL-6 concentration shows the peak at 4-6 hours after LPS injection (Mathiak and Grass, 2000). Plasma IL-1β and IL-6 concentration showing low at 5h after LPS injection, that is antiinflammatory effect of *Scutellariae Rradix* extract. Plasma TNF-α concentration (Table4) showed the peak at 2h after LPS injection and maintained similar level until 5h. In the plasma TNF-α concentration changes of each groups, after 2h of LPS injection of the peak, the *Scutellariae Radix* extract 300mg/kg group had only shown lower value compared to others, but in 5h, all the *Scutellariae Radix* extract groups showed lower value than the control group. TNF-α is a peptide mediator released by monocytes and macrophages in response to various stimuli including bacterial LPS (Chamulitrat and Blazka, 1995). It has been hypothesized to be the principal mediator of deleterious effects of endotoxin (Harbrecht and DiSilvio *et al.*, 1994). TNF-α by LPS shock, it is secreted from Kuffer cell, and causes hepatic injury and hepatocyte apoptosis (Hamada and Nishida, 1999), and TNF-α over production is associated with a wide range of pathologic conditions

Table 4. Effect of *Scutellariae Radix* ext. on plasma TNF-α concentration in lipopolysaccharide-exposed rats

Treatment	TNF-α (pg/ml), Time (h)*		
	0h	2h	5h
Control (saline, 100mg/kg)	7.29 ± 2.69^{NS}	560.00 ± 75.64^{b}	506.57 ± 88.52^{c}
Scutellariae Radix ext. (100mg/kg)	7.14 ± 1.86^{NS}	557.29 ± 61.59^{b}	405.43 ± 65.67^{b}
Scutellariae Radix ext. (200mg/kg)	7.71 ± 1.80^{NS}	534.57 ± 68.07^{b}	335.14 ± 48.56^{ab}
Scutellariae Radix ext. (300mg/kg)	7.86 ± 1.22^{NS}	396.57 ± 65.26^{a}	274.86 ± 44.07^{a}

*Oh, 2h and 5h after LPS injection.
[a,b,c]Means in the same column with different superscripts are significantly different ($p<0.05$).
[NS]Not significantly different ($P>0.05$).

Table 5. Effect of *Scutellariae Radix* ext. on plasma IL-10 concentration in lipopolysaccharide-exposed rats

Treatment	L-10 (pg/ml), Time (h)*		
	0h	2h	5h
Control (saline, 100mg/kg)	15.43 ± 3.74^{NS}	49.14 ± 7.08^{a}	72.29 ± 19.20^{a}
Scutellariae Radix ext. (100mg/kg)	13.57 ± 4.28^{NS}	52.71 ± 8.26^{a}	80.71 ± 17.18^{a}
Scutellariae Radix ext. (200mg/kg)	15.14 ± 3.08^{NS}	54.43 ± 12.16^{a}	129.57 ± 24.35^{b}
Scutellariae Radix ext. (300mg/kg)	11.86 ± 3.48^{NS}	110.71 ± 30.77^{b}	136.00 ± 17.72^{b}

*0h, 2h and 5h after LPS injection.
[a,b]Means in the same column with different superscripts are significantly different ($p<0.05$).
[NS]Not significantly different ($P>0.05$).

and has therefore led much recent effort to find ways to down-regulate its production or inhibit its effects in vivo (Marriot and Westby *et al.*, 1998). In this experiment, by showing TNF-α concentration decrease as the increased amount of *Scutellariae Radix* extract was added, it suggest that the *Scutellariae Radix* extract interferes with antiinflammatory function. The concentration of Plasma IL-10 (Table 5.) increased at 2h after LPS injection and reached the peak at 5h. Each group changes showed that after LPS injection, only the *Scutellariae Radix* extract 300mg/kg, after 2h of LPS injection, showed higher value than the others, but after 5h, the 200mg and 300mg groups of *Scutellariae Radix* extract showed higher value than the control group. IL-10 is the potent produced by lympocytes and macrophages, and it is pleiotropic anti-inflammatory cytokine (Tompson and Trowern *et al.*, 1998). This controls the synthesis of inflammations cytokine like IL-6 and TNF-α and it decreased the T-cell activation in vitro and in vivo (Sang and Wallis, 1999; Moreira and Sampaio,1993). In this experiment, 2h after LPS injection and 5h, all showed IL-10 concentration increase as the *Scutellariae Radix* extract increase, it can be thought to have effected the changes of the concentration of other cytokines.

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