

EFFICACY AND MECHANISM OF ACTION OF YIN LAI TANG (LUNG-STOMACH TREATMENT) IN DYSPEPSIA MOUSE INFECTED BY FM1 VIRUS

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Abstract: The aim of this study was to assess the efficacy and elaborate the mechanism of action of Yin Lai Tang (Lung-Stomach Treatment) on dyspepsia mouse infected by FM1 virus. Ninety male, 4 week old Kunming mouse with 12-14 g weight, were randomly divided into 9 groups, i.e., normal, infected, dyspepsia, ribavirin, Shuanghuanglian, Children's indigestion tablet, YinLaiTang high dose, YinLaiTang middle dose and YinLaiTang low dose, and these groups had been treated by according drugs to get objectives. Compared with normal group, lung index significantly ($p < 0.01$) increased in all groups except ribavirin group where lung index obviously ($p < 0.05$) increased. There was non-significant ($p > 0.05$) difference in the values of lung homogenate virus titer between dyspepsia group and other groups. Compared to normal group, there was variable degree of inflammatory cell infiltrations in respiratory tract structures in the animals of other groups, and there was a significant ($p < 0.01$) increase in the level of serum IL-6, IL-10, and TNF- α in infected and dyspepsia group and significant ($p < 0.01$) decrease in the level of serum IFN- γ was observed. Compared with single clearing stomach method and single clearing lung approach, lung-stomach treatment reduced the level of IL-6 with non-significant difference ($p > 0.05$) and increased the level of IL-10 obviously, and compared with the single clearing lung method, there was a significant difference ($p < 0.05$). Compared with the single clearing stomach method and the single clearing lung method, the lung-stomach treatment method had a better efficacy and showed effects on the expression of pro-inflammatory factor and anti-inflammatory factor.

Keywords: cytokines, dyspepsia, FM1 virus, lung-stomach treatment

With the development of society, change of diet structure and accelerating pace of life, internal thermal of Yang Ming has become a normal pathological state in clinical settings, especially in infants. According to the pediatric physiological and pathological characteristics, diet disorder and other factors, it is easy to cause the infant suffering from gastrointestinal intrinsic heat and gastrointestinal function disorder, leading to the internal environment turbulence (1-6).

According to research of lung-stomach relationship from basic theories and clinical experiences in recent years, there is a tendency that lung-stomach treatment has been used in treating some diseases. At the same time, more attention has been focused on studying the relevance of lung and stomach (1, 3, 7).

Yin Lai Tang, consisting of Jinyinhua (*Flos loniceræ*), Lianqiao (*Fructus forsythiae*), Huangqin (*Scutellaria baicalensis*), Yuxingcao (*Herba houttuyniae*), Qianhu (*Radix peucedani*), Laifuzi (*Semen raphani*), and Gualou (*Fructus trichosanthes*) with the effects on clearing lung and stomach, and promoting digestion and eliminating stagnation, is commonly used formula for children lung-heat syndrome (2).

The experiment was designed to observe the efficacy, mechanism of action and protective functions of Yin Lai Tang (lung-stomach treatment) in dyspepsia mouse infected by FM1 virus on molecular level by testing the content of cell factor IL-10, IL-6, TNF- α , IFN- γ in serum and to detect its capability to kill virus.

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MATERIALS AND METHODS

Materials and Instruments

The experiment reagents included ether, sterile saline, and 75% ethanol. The experimental instruments included biological safety cabinet binder, electric constant temperature drying oven DG20-002, electronic balance JA3003 (Shanghai Yueping Scientific Instrument Co., Ltd., and micro-Adjustable Pipette (DRAGON, China).

Model virus

The FM1 viruses were provided by Pathogenic Immune Laboratory, China and the hemagglutination titer was 2-7 from allantoic fluid after two generations in chick embryo allantoic cavity and the LD₅₀ of mouse was 10-3.7.

Experimental drugs

The experimental drugs are given in table below.

Experimental animals, their grouping and feed

The ethical approval for this study was obtained from Beijing University of Chinese Medicine, China. This study was carried out according to the guidelines of Good Clinical Practice and approved guidelines for animal use in experiments. Ninety male, 4 week old Kunming mouse with 12-14 g weight were provided by Beijing Weitong Lihua Experimental Animals Company, China for this study. These 90 animals were randomly divided into 9 groups with 10 mice in each group, i.e., normal group (Ng), infected group (Ig), dyspepsia group (Dg), ribavirin group (Rg), Shuanghuanglian group (Sh), Children's indigestion tablet group (Ci), YinLaiTang high dose group (Yh), YinLaiTang middle dose group (Ym) and YinLaiTang low dose group (Yl).

Standard mice pellet feed was provided by Beijing Weitong Lihua Experimental Animals Company, China. It was a high-heat and high-protein

feed made from milk powder, soybean meal, dried fish floss and flour (1 : 2 : 1 : 1) mixed homogeneously with water to be shaped as the normal mice pellet feed. Milk solution (52%) and milk powder were purchased from Beichen Shopping Center, China. Mouse were feed in Animal Laboratory, Beijing University of Chinese Medicine, China with temperature 22 ± 2°C, humidity 50-60%, well ventilated, natural lighting, free food and enough water. All groups were given this feed, gavaged with 52% milk solution at 2nd day, 0.2 mL/10 g of animal weight/day, and given the same dose of physiological saline. After four days, normal group was given physiological saline by nasal inhalation, while other groups were given 4LD₅₀ flu virus droplets by nasal inhalation, 0.05 mL/mice and drugs were given to every group after 1 h.

Drug administration

The dose of ribavirin particle, children's indigestion tablet and Shuanghuanglian oral liquid was counted according to clinical dosage, and medium dose of Yin Lai Tang was counted according to adult clinical dose by Professor XiaoGong Gu, two times for high dose, half for low dose.

1. Normal control group (Ng): physiological saline lavage, each 0.3 mL/day
2. Infected control group (Ig): physiological saline lavage, each 0.3 mL/day
3. Dyspepsia group (Dg): physiological saline lavage, each 0.3 mL/day
4. Ribavirin group (Rg): ribavirin lavage, each 0.3 mL/day, 0.075 g/kg/day
5. Shuanghuanglian group (Sh): ShuangHuangLian oral liquid lavage, each 0.3 mL/day, 20 mL/kg/day
6. Children's indigestion tablet group (Ci): Children's indigestion tablet lavage, each 0.3 mL/day, 2 g/kg/day
7. YinLaiTang high dose group (Yh): Yin Lai Tang water decoction lavage, each 0.3 mL/day, 28.3 g/kg/day

No.	Experimental drugs	Dose of adult in clinical treatment	Supplier
1	Yin Lai Tang (Lung-Stomach Treatment)	85 g/60 kg	Guoyitang of Beijing University of Chinese Medicine, China
2	Shuanghuanglian oral liquid (lung-clearing method)	20 mL/60 kg	Sanjing Pharmaceutical Co.Ltd.; Harbin Pharm. Group
3	Children's indigestion tablet (promoting digestion and eliminating stagnation method)	3 g/60 kg	Beijing Tong Ren Tang, China
4	Ribavirin particle (positive control drug)	0.5 mg/60 kg	Sichuan Baili Pharmaceutical Company, China

Table 1. Lung index and lung index inhibition percentage ($x \pm s$, 100%) as well as comparison of lung homogenate virus titer ($x \pm s$, LN)

Group	Lung index and lung index inhibition percentage ($x \pm s$, 100%)			Comparison of lung homogenate virus titer ($x \pm s$, LN)	
	N	Inhibition ratio	Lung index	N	Lung homogenate virus titer
Ng	12	—	0.00689 ± 0.00044	6	—
Dg	10	—	0.01684 ± 0.00893**	6	5.0106 ± 0.7593*
Rg	10	32.6	0.01135 ± 0.00192*ΔΔ	6	—
Sh	9	13.6	0.01455 ± 0.00508**	5	4.8951 ± 0.6815*
Ci	10	17.8	0.01384 ± 0.00434**	6	4.7333 ± 0.6199
Yh	10	2.0	0.01651 ± 0.00398**	6	4.8720 ± 0.5799*
Ym	10	2.4	0.01643 ± 0.00469**	5	4.3148 ± 0.4948
Yl	10	23.3	0.01292 ± 0.00453**	6	4.0348 ± 0.3870Δ

*Compared with normal group, $p < 0.05$, **Compared with normal group $p < 0.01$; Δ compared with Dg $p < 0.05$, ΔΔ compared with Dg $p < 0.01$.

Table 2. Values of IL-6, IL-10, TNF- α and IFN- γ ($x \pm s$, pg/mL).

Group	IL-6		IL-10		TNF- α		IFN- γ	
	N	Values	N	Values	N	Values	N	Values
Ng	10	55.7764 ± 11.6604ΔΔ	9	234.7271 ± 11.7102ΔΔ	10	68.94907 ± 8.4850ΔΔ	9	205.6863 ± 69.7587ΔΔ
Ig	9	90.5716 ± 31.3267**	10	57.7868 ± 18.3344**	8	87.6194 ± 6.4025**	10	129.8040 ± 41.7970**
Dg	9	99.6824 ± 38.4430**	9	67.8504 ± 29.6688**	9	90.1337 ± 11.9643**	9	137.4748 ± 32.6493**
Rg	10	57.0943 ± 17.9954ΔΔ	8	93.5169 ± 52.64524**	9	73.7879 ± 12.5762ΔΔ	8	144.3214 ± 52.7568*
Sh	9	71.3391 ± 28.7344Δ	10	67.6338 ± 21.6426**	10	80.4818 ± 7.0670**Δ	10	129.6298 ± 53.0480**
Ci	10	72.1999 ± 17.9284Δ	11	9.4589 ± 21.6662**	10	77.9227 ± 13.0713*ΔΔ	11	140.4104 ± 34.8137**
Yh	9	76.9943 ± 14.1824*Δ	9	75.7903 ± 35.1301**	10	84.3632 ± 4.9756**	9	124.3470 ± 35.3442**
Ym	10	69.9608 ± 21.7305ΔΔ	11	92.9905 ± 13.7413**	10	72.7277 ± 8.7500ΔΔ	11	157.3534 ± 53.1474*
Yl	11	64.0977 ± 13.7557ΔΔ	10	104.0490 ± 15.6989**Δ	10	72.4486 ± 7.4084ΔΔ	10	149.2835 ± 54.5268*

*Compared with normal group, $p < 0.05$, ** Compared with normal group, $p < 0.01$; Δ Compared with Dg, $p < 0.05$, ΔΔ Compared with Dg, $p < 0.01$.

- YinLaiTang middle dose group (Ym): Yin Lai Tang water decoction lavage, each 0.3 mL/day, 14.2 g/kg/day
- YinLaiTang low dose group (Yl): Yin Lai Tang water decoction lavage, each 0.3 mL/day, 7.1 g/kg/day.

Observation indexes

The effects of Yin Lai Tang (lung-stomach treatment) on lung tissue morphology and virus titer in dyspepsia mouse infected by FMI virus were elaborated by checking lung homogenate virus titer, lung tissue pathological formation and lung index and lung index inhibition percentage. Ten lung tis-

sues were taken from each group and lung homogenate virus titer was detected by chicken erythrocyte suspension. Six lung tissues (at the right lung hilum) from each group were cut to observe the lung tissue pathological formation. To determine lung index and lung index inhibition percentage (weighed 7 days after infection), the following formulas were used (4-7):

$$\text{Lung index} = \frac{\text{Lung weight (g)}}{\text{body weight of mice (g)}} \times 100\%$$

Lung index inhibition percentage = [(average lung index of model groups – the average lung index of experimental groups) / average lung index of model groups] × 100%

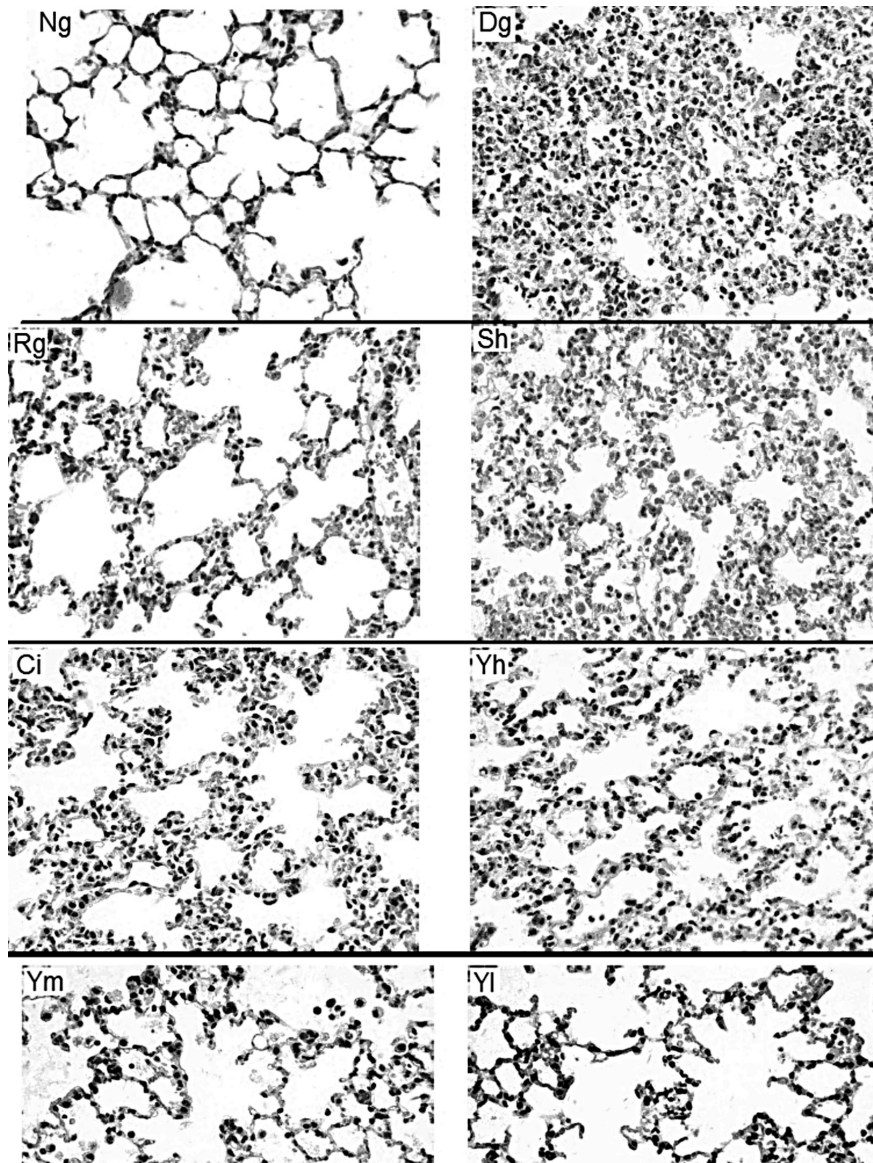


Figure 1. Comparison of alveolar sac structure

The effects of Yin Lai Tang (lung-stomach treatment) on cell factors in dyspepsia mouse infected by FM1 virus were also studied. Serum interferon γ (IFN- γ), tumor necrosis factor- α (TNF- α), interleukin 6 (IL-6), interleukin 10 (IL-10) in serum and lung tissue homogenate were measured by a quantitative enzyme-linked sandwich immunoassay (ELISA), according to instructions on respective boxes.

Statistical methods

The experimental results were analyzed by SPSS version 17.0 and all of data were shown by the

mean \pm standard deviation ($\bar{x} \pm s$). T-value or χ^2 test were used to test the difference i.e., obvious difference ($p < 0.05$) and significant ($p < 0.01$) difference among groups.

RESULTS AND DISCUSSION

Effect on lung index and lung homogenate virus titer (virus titer)

Compared with Ng, lung index significantly ($p < 0.01$) increased in all groups except Rg where lung index obviously less significantly, ($p < 0.05$) increased (Table 1). After intervention and com-

pared with Dg, there was significant difference ($p < 0.01$) increase in lung index in Rg.

There was non-significant ($p > 0.05$) difference in the values of lung homogenate virus titer between Dg and other groups (Table 1). While there was an obvious killing effect on virus in Yl, but no obvious ($p < 0.05$) blood clotting phenomenon in Rg. There was an obvious ($p < 0.05$) difference in the values of lung homogenate virus titer among Yl and Sh, and Yh.

Effect on lung tissue morphology under light microscope

Compared to Ng, there was large number of inflammatory cell infiltration in alveolar sac struc-

ture in the animals of Dg, Sh, Ci, Yh, Ym, and Yl (Figure 1).

In respiratory bronchiole structure, there was a high number of inflammatory cells infiltration in cavity and surrounding stroma in comparison to that of Ng. No inflammatory cells infiltration was in pipe cavity, a small amount of inflammatory cell infiltration was in surrounding stroma and a small amount of vascular congestion in respiratory bronchioles in Rg, Ci, and Yh, however, large amount of inflammatory cell infiltration was observed in surrounding stroma in Sg. In the respiratory bronchioles of Ym, there was no inflammatory cells infiltration in pipe cavity and a small amount of inflammatory cell

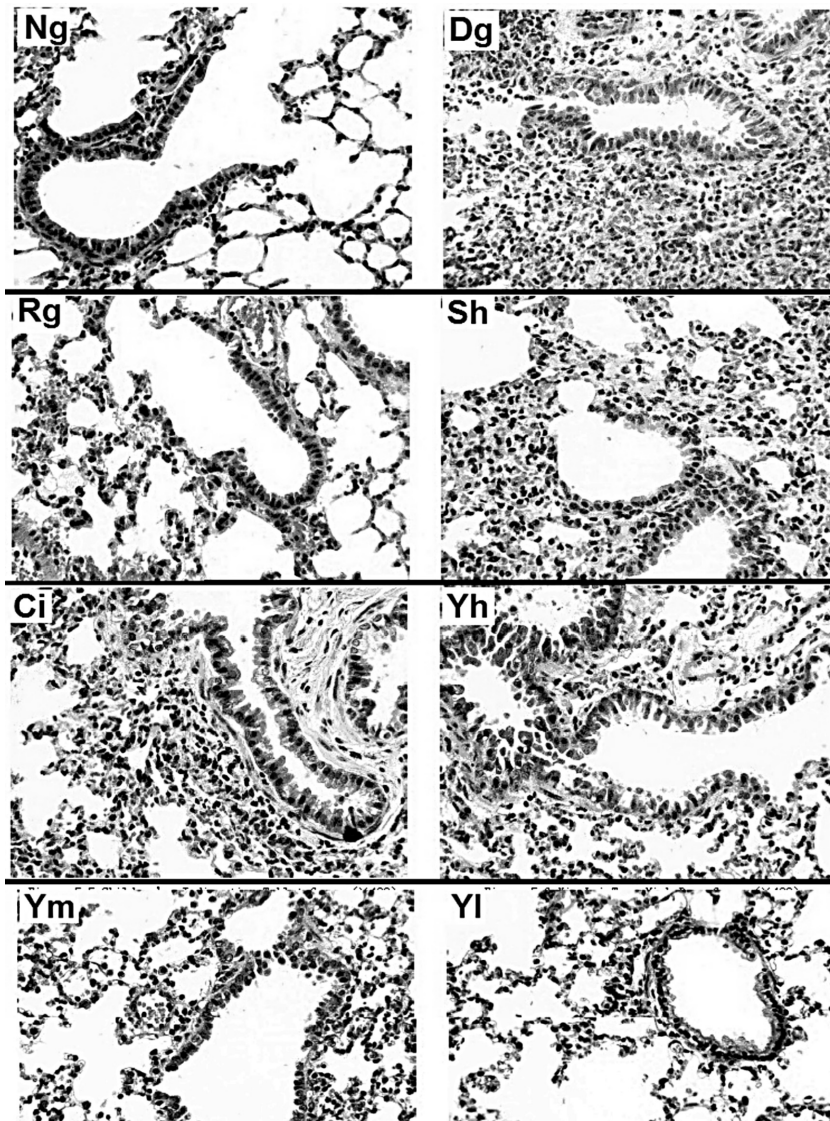


Figure 2. Comparison of respiratory bronchiole structure

infiltration was observed in surrounding stroma. No inflammatory cells infiltration was in pipe cavity, and inflammatory cells infiltration was not apparent in surrounding stroma accompanied with vascular congestion in the respiratory bronchioles of YI (Figure 2).

Compared to the terminal bronchi of Ng (Table 2), there was a large number of inflammatory cell infiltrations in pipe cavity and interstitial lung around in Dg and Sh, but there was no inflammatory cells infiltration in the cavity and small amount of inflammatory cell infiltration in surrounding stroma in that of Rg, Ym, and YI. In terminal bronchi of Ci

and Yh, there was no inflammatory cell infiltration in cavity, and there was a lot of inflammatory cell infiltration in surrounding stroma accompanied with vascular congestion. In bronchus structure of Dg, there were many nuclear giant cells and there was a large number of inflammatory cell infiltrations in the cavity and interstitial lung around in comparison to that of Ng. The bronchus of Rg, Sh, and Ci showed some pseudostratified ciliated columnar epithelium fell off having no inflammatory cells infiltration in cavity, but there was inflammatory cell infiltration in surrounding stroma with a small amount of vascular congestion. In bronchus struc-

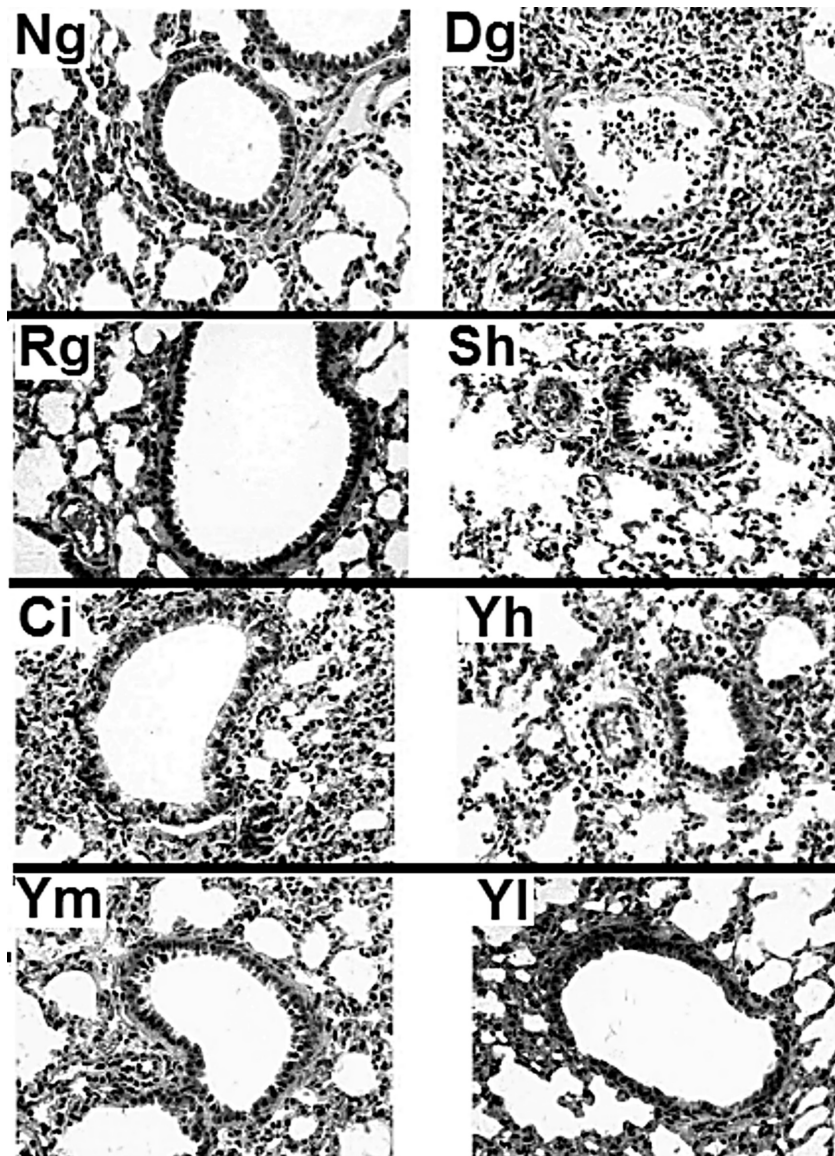


Figure 3. Comparison of terminal bronchi

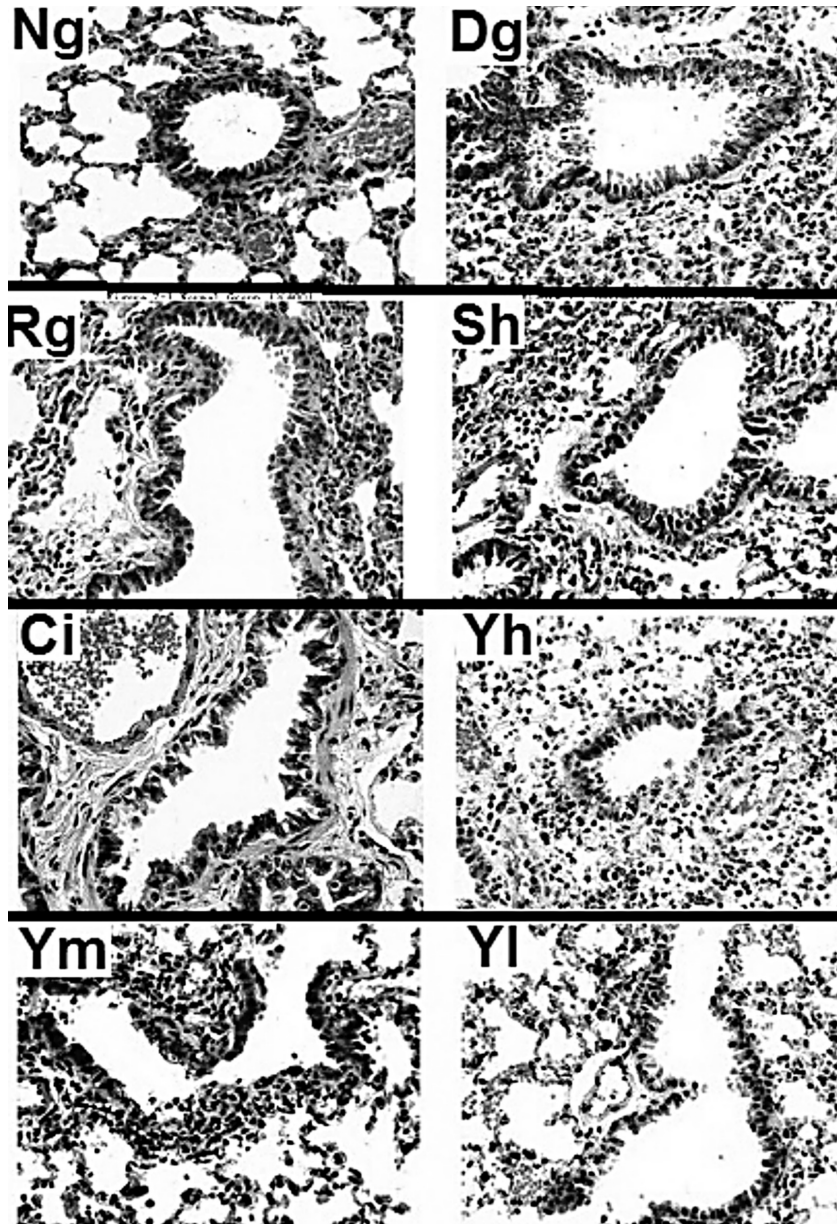


Figure 4. Comparison of bronchus

ture of Yh, there was a small amount of inflammatory cell infiltration in pipe cavity, and there was many inflammatory cells infiltration in surrounding stroma accompanied with vascular congestion. There was a small amount of goblet cells and there was a small amount of inflammatory cell infiltration in cavity and surrounding stroma in bronchus structure of Ym. In bronchus structure of Yl, there was not obvious inflammatory cells infiltration in the cavity and surrounding stroma accompanied with vascular congestion.

Effects on cell factors (IL-6, IL-10, TNF- α , and IFN- γ)

Compared with Ng, there was a significant ($p < 0.01$) increase in the level of serum IL-6, IL-10, and TNF- α in Ig and Dg whereas significant ($p < 0.01$) decrease in the level of serum IFN- γ was observed (Table 2). After intervention and compared with Dg, there was (i) significant ($p < 0.01$) decrease in the level of serum IL-6 and TNF- α in Rg, Ym, and Yl, as well as (ii) non-significant ($p > 0.05$) increase in the level of serum IL-10 and IFN- γ in Rg, Sg, Ci, Yh, and Ym was observed.

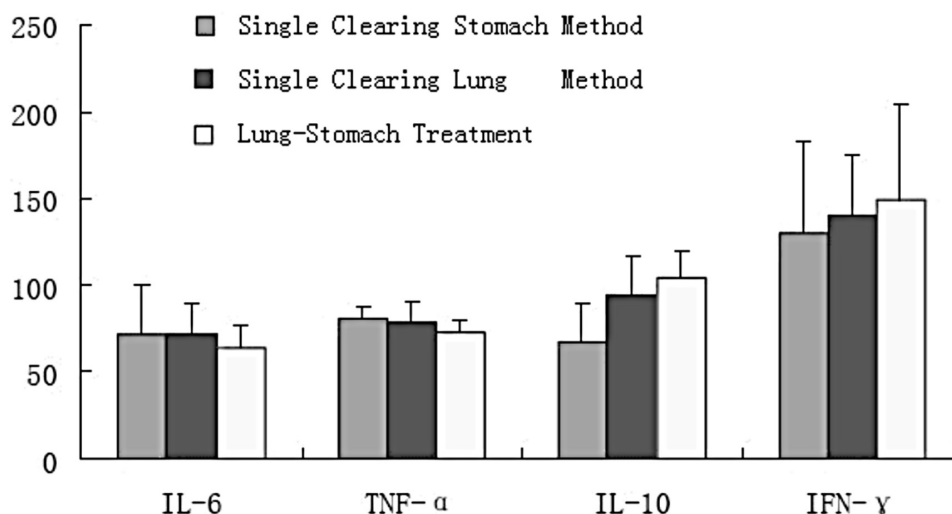


Figure 5. Effects of lung-stomach treatment, single clearing stomach method and single clearing lung method on cell factors in FM1 infected mouse

Compared with single clearing stomach method and single clearing lung approach, lung-stomach treatment reduced the level of IL-6 with non-significant difference ($p > 0.05$) and increased the level of IL-10 obviously, and compared with the single clearing lung method, there was a significant difference ($p < 0.05$). Regarding its effect on inhibiting the TNF- α increase, there was a significant ($p < 0.05$) difference between lung-stomach treatment and the single clearing stomach method ($p < 0.01$) and there was an obvious ($p < 0.05$) difference between lung-stomach treatment and the single clearing lung method. The trend of increasing the level of IFN- γ was as follows: single clearing stomach method < single clearing lung method < lung-stomach treatment with non-significant ($p > 0.05$) difference among these groups (Figure 5).

The experimental results showed that, after FM1 virus infection, lung volume increased with congestion and edema, and pulmonary index increased significantly in model group and drug group. Compared with normal control group, there were significant differences in other groups. There were significant differences between the Rg lung index and Dg, which revealed that there was a strong inhibiting effect of ribavirin on lung lesions caused by virus. Compared with Dg, there was a obvious trend of decrease in lung index in Yin Lai Tang low dose group ($p = 0.06$) indicating that Yin Lai Tang low dose group had some effect on lung weight by virus, but deep researches are needed.

Lung viral load is often determined by lung homogenate hemagglutination titer method (4).

Compared with Dg, there was a certain degree of inhibition on viral multiplication in other groups. Ribavirin showed a great killing virus effect inhibiting the viral multiplication and blood congeal titer was zero. There was an effective function of inhibiting virus in Yin Lai Tang low dose group; the inhibiting effect of viral multiplication in children's indigestion tablet group and Shuanghuanglian group was worse; the effect in Yin Lai Tang medium dose group and high dose group was worst. In comparison of various parts in lung pathological form, there was obvious injury in lung after virus infection, lungs integral structure significantly damaged, and there was a certain degree of improvement on the change of structure in drug groups.

At all levels of structure, there was an obvious change of lung in ribavirin group and low dose group with clear lung internal structure, less inflammatory cell infiltration, and improvement of congestion. Experimental results showed that there were effects on virus infection in drug groups, but in different aspects, such as improvement of inflammatory cell infiltration, effect on the vascular congestion situation, and guarantee of the integrity of cell structure.

Promoting inflammation cell factors like TNF- α , IL-6, and IL-8, mainly mediated inflammatory reaction and caused tissue damage; anti-inflammatory cell factors like IL-4 and IL-10, mainly inhibited the release of promoting inflammation media and prevented the out of control promoting inflammation (5).

From experiment results, lung-stomach treatment, single clearing stomach method group and

single clearing lung method group could reduce the level of serum IL-6 and TNF- α , controlled promoting inflammation reaction, increased the level IL-10 and IFN- γ , and protected the lung injury and especially there was a significant adjustment in promoting inflammation reaction and inhibiting inflammation reaction balance in lung-stomach treatment, revealing that lung-stomach treatment could regulate the level of cell factors in dyspepsia mouse infected by virus, maintain the stability of internal environment, and improve the ability of fighting diseases.

CONCLUSION

In summary, YI had an obvious virus killing effect on dyspepsia mouse infected by virus which could significantly improve the pathological damage in lung and could maintain balance between promoting inflammation and anti-inflammatory factors after infection. Lung-stomach treatment was better than single clearing stomach method and single clearing lung method in treating dyspepsia virus infected mouse in death protection, virus killing, repairing pathological damage, and maintaining balance of promoting inflammation factors and anti-inflammation factors showing a better treating effect.

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