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Effect of Resveratrol on Coxsackie Virus B3m-induced Myocarditis in Mice

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ABSTRACT

To observe the intervening effect of resveratrol on coxsackie virus B3m-induced myocarditis in Balb/c mice and explore the mechanism of intervening effect.

Using an animal model of viral myocarditis induced by coxsackie virus B3m (CVB3m), with Ribavirin and Astragalan as comparison, to examine the changes of general condition, mortality, the weights of heart, liver and spleen, serum MDA and NO levels, and cardiac histology in Balb/c mice.

By comparison with Ribavirin and Astragalan, it was found that in the mice model of viral myocarditis induced by coxsackie virus B3m resveratrol significantly improved the changes of general condition, mortality, the weights of heart, liver and spleen, serum MDA and NO levels, and cardiac histology.

It suggested that resveratrol may have some chemopreventive and chemotherapeutic effects in the treatment of viral myocarditis.

Key words: coxsackie virus, Balb/c mice, myocarditis, animal model, resveratrol, Ribavirin, Astragalan injection, Astragalan decoction, chemopreventive effect, chemotherapeutic effect

INTRODUCTION

It have been demonstrated by vast literatures that resveratrol has the characteristics of prevention and treatment of cardiovascular disease.[1-14] Whereas all these evidences, we have studied the the intervening effect of resveratrol on coxsackie virus B3m-induced mycocarditis in Balb/c mice and explore the mechanism of intervening effect, in order to submit a primary result of finding a new method to prevent and cure coxsackie virus induced myocarditis clinically.

MATERIALS AND METHODS

Materials

Animal: Totally 268 male and female Balb/c mice of 4～7 week instar, which are provided by Center of Experimental Animal of Medical College Affiliated to Fu Dan University and Shanghai Center of Experimental Animal of the Academy of Sciences of China, are divided into three batchs.

Virus: Coxsackie virus B3m (CVB3m, nancy strain) of $10^{5.5}TCID_{50}$ is provided by Key Laboratory of Viral Heart Disease of Ministry of Public Health of Zhong Shan Hospital affiliated to Medical Collage of Fu Dan University.

Drug: Resveratrol (content: 98%) is produced by Shanxi Huike Botanical Development CO., Ltd; Ribavirin Injection (100 mg/2 mL) is produced by Shanghai Hefeng Pharmaceutical CO., Ltd. Batch NO is 010902; Astragalan Injection (4 g/2 mL) is produced by Chengdu Diaojiouhong Pharmaceutical Factory. Batch NO is

0102065; Astragalan Decoction is obtained from water added with Astragalan after boiling 30 minutes later. Astragalan is provided by Shu Guang Hospital affiliated to Shanghai University of Traditional Chinese Medicine.

Treatment experiment

Animal model: Mice (9±2 g) of 4 week instar are inoculated abdominally with 0.1 mL venom of coxsackie virus B3m for establishing animal model of viral myocarditis. Mice of the normal control are injected abdominally with 0.1 mL culture fluid.

Grouping and medication: Normal Control (28), Negative Control (28), Ribavirin Injection (28), Resveratrol (28). According to random principle.

Medication is administered abdominally 72 h later after inoculation. Group of Ribavirin Injection: Mice are injected abdominally with 0.1 mL mixed solution (1 portion of Ribavirin Injection plus 9 portion of Normal Saline). Group of Resveratrol: Mice are injected abdominally with 0.1 mL resveratrol solution (resveratrol (100 mg/kg) plus Normal Saline). Normal Control and Negative Control: Mice are injected abdominally with 0.1 mL Normal Saline. Mice are injected once a day and are fed with normal forage till 3 weeks.

Dose and effect experiment (*Omission*)

Prevention and treatment experiment (*Omission*)

Testing items and testing methods

General condition: Including activity, color pattern, body weight, ingestion and survival rate, etc.

Appearances and weights of heart, liver, spleen and thymus gland: Including size, texture, color and weight of heart, liver, spleen and thymus gland, etc.

Serum MDA and NO levels: Including MDA (nmol/mL), NO (μmol/L). Testing according to direction of the product (reagent bought from Nanjing Jiancheng Bioengineering Company).

Cardiac histology: Including conventional HE chromoscopy and Philips Tecnai-12 Transmission Electron Microscope testing.

Statistic

SPSS software is used for deal with the data. Course of One-Way ANOVA is used for quantitative date and Course of Chi-Square is used for categorical date.

RESULTS

The comparison of mortality of animal model of male and female mice

Death is begun at male mice 4～5 days later after inoculation. No matter what drug they are used, the mortality of male mice are higher than female mice (Table 1).

Treatment experiment

General condition: After 2～3 days later of inoculation, mice of Group of Negative Control appear weight loss,

Table 1. The comparison of mortality of animal model of male and female mice in dose-effect experiment and prevention-treatment experiment

Items	Mortality of M.M.	Mortality of F.M.	Chi-Square	P
D-E Exp.	44.44 (24/54)	26.67 (08/30)	4.5	0.034
P-T Exp.	52.63 (20/38)	25.00 (10/40)	10.051	0.002

fur dim, activity decreasing and ingestion reduction; mice of Group of Ribavirin Injection appear weight loss, fur dim, activity decreasing, ingestion reduction, hydroposia increasing and urination augmentation; mice of Group of Resveratrol appear lighter body weight in comparison with the former two groups, and they also show fur luster, activity increasing and ingestion amelioration (Table 2).

Appearances and weights of heart, liver and spleen: In Group of Normal Control the hearts, livers and spleens of mice are full of red and luster. In Group of Negative Control the hearts of mice are dim, the livers are enlargement, the spleens are enlargement and dim. In Group of Ribavirin Injection the hearts, livers and spleens of mice are like those of the mice of Group of Negative control. In Group of Resveratrol the hearts are not dim obviously, the livers are not enlargement manifestly and the spleens are more enlargement than those of the mice of Group of Negative control (Table 4).

Serum MDA and NO levels: Resveratrol has the effects of decreasing serum MDA and NO levels (Table 5).

Table 2. the comparison of body weights of the survival mice of every group in treatment experiment (g)

Groups	Body Weights
Normal Contral (A)	20.6±2.48
Negative Contral (B)	20.0±1.25
Ribavirin Injection (C)	18.4±3.07
Resveratrol (D)	$14.9\pm1.56^{*\triangle\blacktriangle}$

Notice: comparison with A, $^{*}p<0.05$; comparison with B, $^{\triangle}p<0.05$; comparison with C; $^{\blacktriangle}p<0.05$.
Death is begun at mice of every group 4～5 days later after inoculation and stop till 10th days. The group of Resveratrol has the lowest mortality (2/28 (7.14%), death happen at 2th and 3th days after inoculation). The group of Ribavirin Injection has the same mortality as The group of Negative Contral (14/28 (50%) and 16/28 (57.14%)). The mortalitys of every group of the treatment experiment are not same (Chi-Square shows $p<0.05$) (Table 3).

Table 3. The comparison of mortalitys of Negative Control, Ribavirin Injection and Resveratrol in treatment experiment (%)

Groups	Mortalitys
Negative Contral (B)	Male (10/18) Female (6/10) Total (16/28)
Ribavirin Injection (C)	Male (12/18) Female (2/10) Total (14/28)
Resveratrol (D)	Male (02/18) Female (0/10) Total $(02/28)^{*\triangle}$

Notice: comparison with B, $^{*}p<0.05$; comparison with C, $^{\triangle}p<0.05$.

Table 4. The comparison of weights of heart, liver and spleen and rates of the survival mice of every group in treatment experiment (g, %)

Groups	Heart	Liver	Spleen	H/BW	L/BW	S/BW	H/L	H/S	L/S
Normal Contral (A)	.122 ±.023	.954 ±.063	.101 ±.024	.648 ±.095	5.091 ±.772	.532 ±.089	.129 ±.025	1.240 ±.231	9.888 ±2.753
Negative Contral (B)	.112 ±.010	1.261 ±.098*	.088 ±.019	.557 ±.043	6.294 ±.238*	.443 ±.120	.088 ±.007*	1.341 ±.367	15.150 ±4.119*
Ribavirin Injection (C)	.113 ±.010	1.115 ±.193	.108 ±.020	.632 ±.134	6.051 ±.222*	.597 ±.122	.105 ±.022*	1.092 ±.293	10.508 $\pm2.147^{\triangle}$
Resveratrol (D)	.093 $\pm.018^{*\triangle\blacktriangle}$	.938 $\pm.160^{\triangle\blacktriangle}$	.183 $\pm.051^{*\triangle\blacktriangle}$	.622 ±.069	6.287 ±.605*	1.229 $\pm.302^{*\triangle\blacktriangle}$	.099 ±.012*	.540 $\pm.164^{*\triangle\blacktriangle}$	5.395 $\pm1.315^{*\triangle\blacktriangle}$

Notice: comparison with A, $^{*}p<0.05$; comparison with B, $^{\triangle}p<0.05$; comparison with C, $^{\blacktriangle}p<0.05$.

Table 5. The comparison of serum MDA and NO levels of the survival mice of every group in treatment experiment (nmol/mL, μmol/L)

Groups	MDA	NO
Normal Contral (A)	8.20±1.285	93.8±13.14
Negative Contral (B)	15.19±1.139*	192.43±25.89*
Ribavirin Injection (C)	15.1±6.998*	185.57±21.02*
Resveratrol (D)	13.93±8.967*△▲	164.92±22.08*△

Notice: comparison with A, *$p<0.05$; comparison with B, △$p<0.05$; comparison with C, ▲$p<0.05$.

Cardiac histology

Light microscope Heart tissue of the Group of Normal Control: Cadiocytes present short cast appearance, with endochylema dyed red abundance. Nucelus present orbicular-ovate, and situated in the center of the cell. Lamellar connective tissue are between cadiocytes and the nucleus present fusiform shape. Transverse striations are seen under high power lens ambiguously. Epicardium is composed mainly by mesothelial cells with nucelus thin and flat and sticks to cadiocytes tightly.

Heart tissue of the Group of Negative Control: Small amounts of chronic inflammatory cells infiltrate in the myocardium degeneration and necrosis focus. Connective tissue proliferate obviously.

Heart tissue of the Group of Ribavirin Injection: Pathological changes are similar to the group of negative control. Chronic inflammatory cells infiltration and connective tissue hyperplasia can also be seen.

Heart tissue of the Group of Resveratrol: Pathological changes are better than the group of negative control. Chronic inflammatory cells infiltration and connective tissue hyperplasia can not be seen clearly.

Electron microscope Heart tissue of the Group of Normal Control: Cellular structure is clear. Bioblasts are abundance, endocytoplasmic reticulum can be seen, thin and thick myofilaments are in good order, Z lines and M lines are clear.

Heart tissue of the Group of Negative Control: Engorgement and cavitation of bioblasts are manifest, some nuclear chromatins are concentration and edge collection, thin and thick myofilaments are ambiguous, Z lines and M lines are not clear, virus particles can be found accidently.

Heart tissue of the Group of Ribavirin Injection: Pathological changes are similar to the group of negative control.

Heart tissue of the Group of Resveratrol: Engorgement and cavitation of bioblasts are rarely, thin and thick myofilaments can be seen, Z lines and M lines are not clear enough, no virus particles can be found.

Dose and effect experiment (*Omission*)

Prevention and treatment experiment (*Omission*)

DISCUSSION

In our research, we use Ribavirin Injection as contrast for western medicine and use Astragalan Decoction and Astragalan Injection as contrast for traditional chinese medicine. Resveratrol significantly improved the changes of general condition, mortality, the weights of heart, liver and spleen, serum MDA and NO levels, and cardiac histology. The amelioration effect of resveratrol can be refered to the cardiac histology of the paper.[15] On the one hand, viral myocarditis is caused by virus which can hurt the heart tissue directly, on the other hand, viral myocarditis is caused by immunological reaction, inflammatory reaction and oxidizing reaction, these reactions

which correlate intimately are incurred by virus infection. Compared with the Ribavirin and Astragalan, the resveratrol has the structure of phenol which can enable the resveratrol to act as an antioxidant. Further more, the resveratrol can inhabit virus reproduction by suppress protein tyrosine kinases (PTKs). Immunological reaction, inflammatory reaction, oxidizing reaction, virus reproduction and cell proliferation correlate intimately. Resveratrol has the effect of curing viral myocarditis probably by the mechanism of antioxidation, antiinflammation, anti-immunization, antivirus and antiproliferation. Generally speaking, the heart weights are greater in diseases with manifest connect tissue proliferation such as chronic viral myocarditis, many kinds of myocardiopathy etc., and the percentages of heart weights are also greater. In our studies we find that the heart weights in mice of the group of resveratrol are lower, and the percentages of heart weights are also lower. The bigger and weighter of spleen and thymus gland may probably mean that the drug has the function of modulating immunity. In our studies the spleens and thymus glands of mice in the group of resveratrol show bigger and weighter. We also reveal that in some experiments the mice weight of the group of resveratrol are relatively lower, this may attribute to its mechanisms of antioxidation which can enable the mice function normal and metabolism normal, modulation abnormality of blood lipid metabolism which are also the liability factor for coronary atherosclerotic heart disease, and estrogen like function which provide regulation for endocrine system etc.

Our experiments show that the resveratrol can decrease serum malonaldehyde (MDA) and nitric oxide (NO) levels. Oxidative damage is an important molecular mechanism of tissue damage. Lipid such as unsaturated fatty acid are likely to be attacked by the free radical and therefore lipid peroxidation are formed. MDA is an important breakdown product of the lipid peroxidation. MDA is often used as an index of lipid peroxidation. NO is not only a kind of free radical but also a kind of mediators of inflammation. NO can form many kinds of substance to kill microorganism by reaction with active oxygen. High level of NO can decrease microorganism and can also cause cell damage. NO has double biological effects in experimental viral myocarditis. Low level of NO can inhibit virus replication and cell apoptosis, thereby protecting cardiac muscle cell. High level of NO have cytotoxic action.[16] From the experiments we can conclude that the resveratrol have the function of curing viral myocarditis through its mechanism of antioxidation and antiinflammation.

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