

Original Article

Preventive effects of rabies vaccination with Zagrab regimen on persons with high-risk exposure to rabies virus

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Abstract: Objective: To explore the effects of rabies vaccination produced by Chengda Biotechnology with Zagrab regimen on persons with high-risk exposure to rabies virus. Methods: According to the disposal specification for exposure to rabies made by former Ministry of Health, people with high-risk exposure to rabies virus were divided into groups of grade II and grade III. People in grade II group were injected with rabies vaccination under Zagrab regimen only, and people in grade III group were injected with both rabies vaccination and immunoglobulin. Serum specimens of those persons were detected for the neutralizing antibody against rabies, and analysis on the effect was conducted between two groups using the seroconversion rate and geometric mean titers of rabies neutralizing antibody. Results: The persons with high-risk exposure to rabies were effectively protected by injecting rabies vaccination with Zagrab regimen. It showed that the conversion rate of neutralizing antibody of 29 persons in two groups was 100%. The geometric mean titre of neutralizing antibodies in grade III group (injection of rabies immune globulin) and grade II group (none of the injection of rabies immune globulin) were similar, and there was no statistical significance between two groups ($t = 1.16$, $P > 0.05$). No statistical significances in different age groups ($F = 1.15$, $P > 0.05$) and in different gender groups ($t = 1.19$, $P > 0.05$) were observed. Conclusion: Combined with correct wound treatment, rabies vaccination with the Zagrab regimen can play a preventive role for persons exposed to rabies virus.

Keywords: Rabies vaccination, zagrab regimen, prevention, analysis

Introduction

Rabies is an acute fatal disease which is caused by rabies virus invading into the central nervous system. It has the world's highest fatality rate in all the human infectious diseases. Once the disease is found, the mortality is almost 100%. So far, only one cured patient was reported [1, 2]. Lishui City is a rabies endemic area in Zhejiang Province [3, 4]. In the 10 years between 2004 and 2013, there were 14 cases having been bitten by dogs and other animals without vaccine injection leading to rabies caused death. In 2013 there was one case that bitten by a wild animal ferret badger without vaccination leading to rabies caused deaths [5-9]. Today, post-exposure prophylaxis

is the most effective ways for prevention and control of rabies occurrence [10].

Currently WHO recommends post-exposure immune program including five stitches and 2-1-1 program [11]. Lishui City, which is in Zhejiang Province, adopted a traditional 5-pin vaccination program in the past, since September 2011 WHO recommended 2-1-1 program has been used. Until now the use of 2-1-1 program in the city has reached 4.6 million copies. People with dog injured exposure adopted 2-1-1 program, and there was no case of vaccination occurred while achieved a good preventive effect. Between 2012-2013, Lishui City there were seven identified dog rabies virus carried injured people events leading to 29 people at high risk

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of exposure. We used 2-1-1 program for the high-risk exposure to immunization, and succeeded in achieving the purpose of prevention. Now the results are analyzed as follows:

Materials and methods

General information

Between 2012-2013, in four counties of Suichang, Songyang, Jingning, Yunhe in Lishui City, there were seven events of dogs carrying rabies virus with a total of 29 cases with high-risk exposure including 16 men and 13 women; the minimum age is 6 years old and the oldest was 77 years old. The average age of male was 41.36, and the average age of females was 43.44, while the difference was not significant ($t = 1.32, P > 0.05$). In 29 cases of high-risk exposure, 19 people were in grade III exposure, with an average age of 43.12 years and 10 people were in grade II exposure with an average age of 40.72 years, while the difference was not significant ($t = 1.56, P > 0.05$).

Methods

Materials: Liaoning Chengda produced human rabies vaccine (Vero cells) and Sichuan Yuanda Shuyang Pharmaceutical produced rabies immune globulin were used.

Disposal methods after high risk exposure: As for III level exposure, adopted standard wound debridement + anti-rabies immunoglobulin + rabies vaccines were adopted. As for II level of exposure, standard wound debridement + rabies vaccine were adopted; vaccination was performed using 2 1-1 program, that is on first day, one vaccine was inoculated to the left and right upper arm deltoid respectively, and then on the 7 and 21 days one vaccine inoculation was performed respectively.

Testing methods for dogs carrying rabies: Brain tissue was used for direct immunofluorescence (DFA) to detect rabies virus-specific antigen. Dog foramen magnum fast sampling method was used to collect dog brain tissue Through printing, drying and acetone fixed, PBS solution with 1% BSA (bovine serum albumin) were added to dilute 70 μ L rabies virus monoclonal fluorescent antibody (CHEMICON) at the rate of 1:50 on the printing. And then they were incubated at 37°C for 30 min. Take them out and slowly rinse for 3-5 s. PBS was used for

vibrating wash. And then they were vibrated washed for twice with distilled water, each time for 2 min. Then they were dried by air; then they were mounted by 90% glycerol and then observed under a fluorescence microscope (Olympus CX41).

Crowd of anti-rabies serum neutralizing antibody detection method: When people were attacked by canine which carried rabies virus, they were at high risk of exposure. The 2-1-1 full program and adequately inoculate rabies vaccine. 45 days after the first first dose, serum was collected for rapid fluorescent focus inhibition test (RFFIT). That is: the fixed-dose adaptive cultured standard attacked strain cells (CVS strain) were incubated with serial dilutions of test sera and the standard serum which the titer is known; When serum and virus were mixed and incubated, they were inoculated to susceptible cells and cultured for 24 h. Cell monolayers were fixed with acetone. Fluorescein-labeled anti-rabies virus nucleoprotein antibody staining was used to detect residual and non-neutralized virus (that was a focal distribution of fluorescent spots). We calculated the highest serum dilution for neutralizing 50% viral load and then calculated titer according to the standard samples' titer [12]. Discriminate criteria: anti-rabies virus neutralizing antibodies less than 0.5 IU/ml was considered negative [13].

Observed indicators: In grade II group, after injection of rabies immunoglobulin and rabies vaccine, neutralizing antibody titers were detected. In grade III group, after injection of rabies vaccine, neutralizing antibody titers were detected. The mean titer and seroconversion rates were calculated respectively.

Statistical methods: SPSS software was used; for measurement data (the mean antibody titer), two sets of data were compared by geometric mean t test; multiple sets of data were compared using the geometric mean F test; count data (seroconversion rate) were compared using chi-square test.

Results

High-risk debunkers' exposed parts

There were 19 people in grade III group, and 10 people in grade II group. High-risk exposure

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Table 1. 21 case of high-risk debunkers' exposed parts

Exposed parts	Number of grade II exposed part	Number of grade III exposed part	Total
Head	0	1	1
Trunk	2	4	6
Upper limb	4	7	11
Lower limb	4	9	13
Total	10	19	29

individuals' exposed parts were shown in **Table 1**.

Test results of dogs carrying rabies virus

Direct fluorescence assay (DFA) were performed on brain tissue of the suspected canine who attacked 29 people and the antigen detection were all strongly positive. From the vision of fluorescence microscope we can see the fluorescence flashed and Nicky's body was clear and wide-ranging; while a strongly positive image and a negative control image were shown as in **Figure 1**.

All the high risk debunker tided the incubation period

Through observation, all the debunker tided the rabies incubation period (1-3 months) [14], while seven people were safely tided eight months, and six people tided nine months. There were four people tided 12 months, six people for 21 months, and six people for 26 months.

Rabies neutralizing antibody positive seroconversion conditions in high risk debunker

Neutralizing antibody seroconversion rates in high-risk debunker: Serum were collected from 29 cases of high-risk debunker at 45 days after the first dose and RFFIT was used to detect. The neutralizing antibody positive rate was 100%, while the geometric mean antibody titer was 7.94 IU/ml.

Antibody titers situation in different exposure levels: Grade II debunkers were only vaccinated with a total of 10 people, and all of serum neutralizing antibody seroconversion average antibody titer reached 7.31 IU/ml; There were 19 people in grade III group. They were given rabies

vaccine and anti-rabies immunoglobulin. Serum neutralizing antibodies were all positive with an average titer of 8.27 IU/ml. Both of the two applied geometric mean statistical tests, and the difference was not significant ($t = 1.16$, $P > 0.05$), shown in **Table 2**.

Comparisons of seroconversion and antibody titers in high-risk groups with different gender: There were 16 male high-risk debunkers with an average titer of 6.38 (IU/ml) and 13 female with an average titer of 9.86 (IU/ml). Through the geometric mean statistical analysis, it showed no significant difference ($t = 1.19$, $P > 0.05$), as shown in **Table 2**.

Comparisons of seroconversion and antibody titers in high-risk groups with different age: The high-risk debunkers were divided age and the groups were youth group, young group and older group with the antibody titers were: 10.09 IU/ml, 7.26 IU/ml, and 7.23 IU/ml. By the statistical test, the difference was not significant ($F = 1.15$, $P > 0.05$), as shown in **Table 2**.

Discussion

WHO recommended classic immune program of exposure after canine injury is 5-needle method, which is also called "Essen" program and started in 1965. Taking into account of the cost of the vaccine, as well as the supply of antisera and compliance costs and other factors, it proposed a number of alternatives to reduce vaccinated times. "2-1-1" program is also called "Zagreb" program, which was developed [15] in the former Yugoslavia. In our country, this program was formally approved in October 2010.

Li Shui is a rabies epidemic area in Zhejiang province. By using the 2-1-1 program, 29 cases of high risk of rabies virus debunkers were vaccinated with Chengda human rabies vaccine, and the rabies neutralizing antibody seroconversion rate was 100%. Its antibody seroconversion conditions were similar to that of the traditional 5-pin [16]. High-risk debunkers successfully tided the incubation period and achieved the goal of prevention. The results of protective effect were in consistency with the 2-1-1 vaccination program, which was applied in Thailand for 100 cases of debunkers caused by rabid dog [17].

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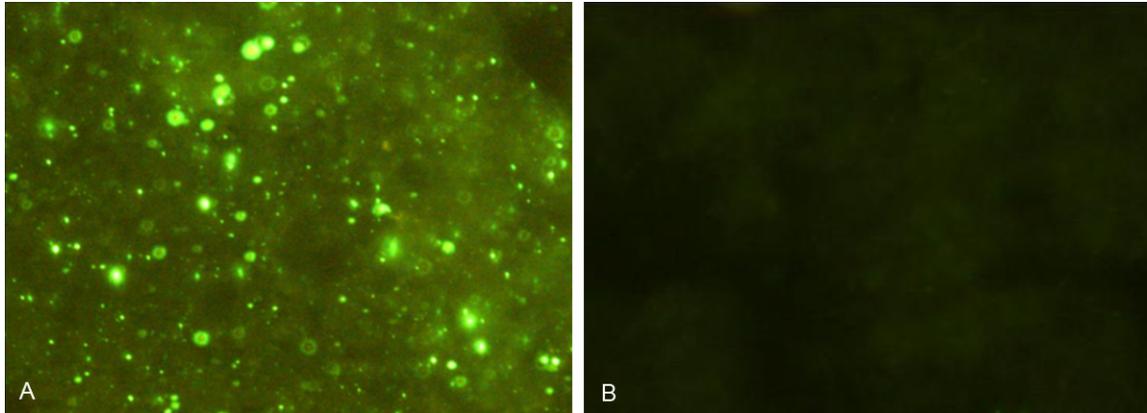


Figure 1. A: Image from fluorescence microscope with strongly positive; B: Image of the negative control.

Table 2. Seroconversion rate and mean titer conditions of different gender, age and level of exposure

Group		Total (n)	Positive (n, %)	Mean antibody titer (IU/ml)
Exposure level	Grade II	10	10 (100)	7.31
	Grade III	19	19 (100)	8.27
	Total	29	29 (100)	7.94
Sex	Male	16	16 (100)	6.38
	Female	13	13 (100)	9.86
	Total	29	29 (100)	7.94
Age	≤ 15	7	7 (100)	10.09
	16-60	18	18 (100)	7.26
	≥ 61	4	4 (100)	7.23
	Total	29	29 (100)	7.94

19 people receiving grade III high-risk exposure were vaccinated against rabies using anti-rabies immune globulin + 2-1-1 program. The neutralizing antibody seroconversion rate was 100% and the average titer was 8.27 IU/ml, reaching the level of protection (greater than 0.5 IU/ml as positive). Ministry of Health stipulates that the people exposed to III grade of high-risk exposure should be treated with anti-rabies immunoglobulin (20 IU/kg) and the first vaccine at the same time. If 7-8% of body weight is blood [18] and the specific gravity of blood [18] is 1.05-1.06, when anti-rabies immune globulin is injected, the body's blood is about 0.30 IU/ml, so the passive immunization makes the concentration of about 0.30 IU/ml. Combining the reaction of antibody and antigen in the body as well as the half-life, immune globulin injection had little effect on neutralizing antibody average titers (8.27 IU/ml), and did not produce positive results. This is consistent with

the report of foreign literature that 2-1-1 did not cause rabies immunoglobulin (HRIG)-induced immune suppression [15].

10 people receiving grade II high-risk exposure were vaccinated against rabies using wound debridement + 2-1-1 program; The mean titer was 7.31 IU/ml, also achieving the purpose of immunoprophylaxis (greater than 0.5 IU/ml as positive). There were no statistically significant differences in geometric mean antibody titers between people receiving grade II and III exposure ($t = 1.16$, $P > 0.05$), further illustrating that the antibody titers of people exposed to grade III exposure (8.27 IU/ml) was irrelevant with the emergency injection of anti-rabies immunoglobulin. Injection of rabies immune globulin does not affect the production of neutralizing antibody and titer level, which is consistent with previous studies [19].

The immunogenicity of 2-1-1 program to different genders is basically the same, which is basically consistent with the reported 5-needle results [20, 21]. The observed 16 males and 13 females were treated with 2-1-1 program; the seroconversion rate reached 100%, and there was no statistically significant difference in seroconverted anti-rabies antibody titer between males and females (male titer was 6.38 IU/ml, and female titer was 9.86 IU/ml, $t = 1.183$, $P = 0.252$), reflecting that immune response of different gender to 4-pin (2-1-1 program) was basically the same.

The immunogenicity of 2-1-1 program to different ages is basically the same, which is basi-

cally consistent with the reported 5-pin results [22, 23]. The 21 exposed people were divided into three groups: youth group, young group, and older group; neutralizing antibody seroconversion rate of the three groups had reached 100%, and the antibody titers of the three groups reached 12.91 IU/ml, 6.22 IU/ml and 9.22 IU/ml, without significant differences ($F = 1.15$, $P > 0.05$), showing that people of different age groups had the same immunogenic response to 2-1-1 program essentially.

Since September 2011, 46,000 expositors have received vaccination using Liaoning Chengda 2-1-1 program in Lishui City, especially 29 cases with high-risk exposure to rabies virus; we believe that the immune effect of Liaoning Chengda 2-1-1 program is consistent with that of 5-needle method; it can be used for the prevention of grade II and III rabid expositors; For grade III expositors, the combined used of vaccine and rabies immunoglobulin antibody did not affect the production of antibody [24], which can achieve the desired prevention effect for the exposed person. In addition, the 2-1-1 program can reduce the number of clinic visits and vaccination, improve patient compliance [25, 26], and reduce the workload of doctors, and save time and cost, which should be introduced.

Rabies neutralizing antibodies are effective protective antibodies; in this paper, Rabies Testing Center, Wuhan Institute of Biological Products measured the neutralizing antibody titers; here we express our sincere gratitude! In china, currently the laboratories able to detect antibodies were few, so it is difficult to meet the actual needs of the grassroots prevention of rabies; the new technologies of detection need further study by scientists.

Disclosure of conflict of interest

None.

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