

Baypamun[®] action in hamsters experimentally infected with *Leptospira interrogans* serogroup Canicola

Ação do Baypamun[®] em hamsters experimentalmente infectados com *Leptospira interrogans*, sorogrupo *canicola*

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SUMMARY

Baypamun[®] is an immunity modulator recommended as prophylactic and therapeutic use. In the present study, this product was used in the hamsters experimentally infected with *Leptospira interrogans* serogroup *canicola*. The animals were divided in 5 groups, with 20 animals each. This product was used as a therapeutic, prophylactic and as adjuvant. As a therapeutic use all the animals died. Despite of eight (40.0%) survivors, the prophylactic use wasn't also totally favorable due to leptospire recovering from kidneys of those animals. Based on the analysis of antibodies levels among animals that received vaccine and vaccine with Baypamun[®] as adjuvante, this product didn't conferred superior humoral answer. However, other studies should be accomplished utilizing this product in a longer period as well as verify the cellular answer by Baypamun[®] use as adjuvant in the infectious disease.

UNITERMS: Immunomodulators; *Leptospira*; Immunity.

INTRODUCTION

Baypamun[®] is an immune modulator, produced with *Parapoxvirus ovis* D1701 strain, that stimulates the unspecific immune response and is recommended for prevention and treatment of infectious diseases. This product, studied in several animal species, improved the concentration of antibodies in the milk of primiparous sows, and increased the survival of piggies¹⁴. In the same specie, the treatment of reproductive diseases and respiratory syndromes with Baypamun[®] reduced the mortality and increased the body weight rate⁷.

In spite of the prophylactic and therapeutic action of Baypamun, it presented poor or completely negative results in the occurrence of respiratory diseases complex in felines¹⁰ but the paraimunization was effective in leucemics cats⁹. Ovinos with diarrhoea recovered in a few days after treatment⁴. Rabbits with traveling diseases caused by *Pasteurella multocida*, *Bordetella bronchiseptica* or *Mycoplasma* sp., and induced by different stress factors, had their occurrence reduced with Baypamun treatment¹.

There are no reports about the influence of Baypamun[®] over the course of Leptospirosis, however, Alves et al.² found that BCG enhanced the resistance of hamsters experimentally infected with serovar *pomona*, Ris and Hamel¹², observed that Freund's complete adjuvant was better than aluminum hydroxide in commercial vaccines produced with serovar *pomona*. The present work aimed to verify the influence of Baypamun as a paraimmunizer in hamsters experimentally infected with *Leptospira interrogans* serogroup *Canicola*.

MATERIAL AND METHOD

One hundred female hamsters (*Mesocricetus auratus*) were used with weights varying 60 to 80 gm. The animals were conditioned in plastic boxes, 20 per group, observed twice a day, for 21 days, with daily annotations of the clinical alterations.

Before the inoculations, animals' serums were examined to verify the presence of antileptospira agglutinins. The blood was obtained through heart puncture and the serum was stored in flasks type "vaccines" maintained in freezer (-20°C). Microscopic agglutination test (MAT) was used to serological analysis, at the screening dilution of 1:100⁵.

The experimental infection was performed with a virulent strain of *Canicola* serogroup, cultivated in Flecther medium. For each animal the infectious inocula was 0,5 ml, with 10⁶ microorganisms/ml by intra-peritoneal route¹³. The animals that reached the agonic state of the disease as well as the survivors were killed and examined searching for macroscopic alterations. Before the sacrifice, it was collected blood samples for anti-leptospire agglutinins determination study.

The animals were divided in five groups, with 20 animals each. The Group 1 was the infectious inoculum control, and received only leptospire culture. The Group 2 was treated with 0,5 ml doses of Baypamun[®] applied on four and two days before experimental infection. The Group 3 received two vaccine doses using Baypamun[®] as adjuvant, 0,5 ml of each one, into interval of 15 days, challenged 15 days after the last inoculation. Likely to Group 3, the Group 4 only didn't receive Baypamun[®]. In the Group 5, this product was applied four and six days after experimental infection, with doses of 0,5 ml each.

The vaccine was prepared according to Avila et al.³ starting from leptospire liquid culture. The inactivation was done by 0.1% formalin solution, and the culture was centrifuged at 10,000 rpm during two hours and resuspended in physiologic saline solution containing 0.18% red phenol, and adjusted for 4.0 x 10⁸ leptospire/ml.

After performing the necropsies exams it were collected liver and kidneys samples, which were macerated separately. Saline solution with neomycin and nitrofurantoin in the concentration of 25 µg/ml of each drug¹¹, was added in the proportion of 1/10. Serial decimal dilutions until 10⁻³ was cultivated using Fletcher medium. Pasteur pipette technique was also utilized for organs cultivation⁸. The flasks were incubated at 30°C, during 60 days, for the observation of Dinger's zone.

The macerated tissue was examined under dark field microscopic of searching for leptospire. Serological results was statistical analysed by Mann-Whitney Test, with $\alpha = 0.05$ significant level.

RESULTS AND DISCUSSION

All the serum samples previously obtained were MAT negative. Antibodies titres ranged from negatives, in animals died until six days after infection, to 12,800 in the next days, in the Groups 1, 2 e 5.

In the Groups 3 and 4, respectively, titres ranged from 200 to 1,600 and 200 to 3,200. There was no statistical differences between two immunization schedules employed, with $p > 0.05$.

In the Group 1, seven (35.0%) animals died in the 6th day, eight (40.0%) in the 7th day, three (15.0%) in the 8th day, and two (10.0%) in the 9th day after infection. In the Group 2, eight (40.0%) animals survived, two (10.0%) died in the 6th day, four (20.0%) in the 7th day, two (10.0%) in the 8th day, two (10.0%) in

the 9th day and two (10.0%) in the 12th day after the experimental inoculation. In the Group 5, 10 (50.0%) animals died in the 6th day, two (10.0%) in the 7th day, six (30.0%) in the 8th day, and two (10.0%) in the 9th day after infection. All the animals survived in the Groups 3 and 4.

The clinical signals observed were apathy, anorexia, dehydration, bristly hair, incoordination and moderate jaundice. The symptoms also were present in the eight (40.0%) survivors animals from Group 2, but they recovered and survived. Alterations were not observed in the Groups 3 and 4, which presented no clinical signs.

The reisolation was obtained from all the animals from Groups 1, 2 and 5, including the eight (40.0%) survivors from Group 2. Positive culture was not obtained in the Groups 3 and 4.

Liver and kidneys were congested and hemorrhagic at necropsy. Petechiae and suffusions were observed in the lungs surface and moderate jaundice in epiplon and abdominal musculature.

Pasteur pipette technique was more effective for recovering leptospire, once the cultivation by serial dilution was positive in only one animal from Group 1. The first technique presents higher contamination risk, but with the necessary care can be avoided.

Baypamun® used as therapeutic form didn't give protection against leptospire infection in Group 5. In spite of eight (40.0%) animals that survived from Group 2, the prophylactic wasn't also totally favorable, due to recovering leptospire from kidneys of these animals, which means they became kidney carriers of leptospire.

Based on analysis of antibodies levels among animals from Groups 3 and 4, Baypamun® didn't increased humoral response. However, other studies should be accomplished utilizing this product in a longer period as well as verifying the cellular response.

RESUMO

Baypamun® é um imunomodulador recomendado na profilaxia e terapêutica de enfermidades. No presente estudo, este produto foi usado em hamsters experimentalmente infectados com *Leptospira interrogans*, sorogrupo *canicola*. Estes animais foram divididos em 5 grupos com 20 animais cada. O produto foi usado como terapêutico, profilático e como adjuvante. Quando usado de forma terapêutica, todos os animais vieram a óbito. Quando usado na profilaxia, 8 animais (40%) sobreviveram e foi possível recuperar o agente a partir dos rins desses animais. Baseado na análise do título de anticorpos nos animais que receberam ou não o Baypamun como adjuvante vacinal, o produto não elevou a resposta humoral. Os resultados sugerem, entretanto, outros estudos que utilizem o produto por períodos mais prolongados, além de se verificar a resposta celular para se avaliar o seu efeito como adjuvante nas infecções.

UNITERMOS: Imunomoduladores; *Leptospira*; Imunidade.

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