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The protective effects of Baypamun®HK in mice experimentally infected with *Toxoplasma gondii*

Efeitos do Baypamun®HK em camundongos infectados experimentalmente com *Toxoplasma gondii*

Eva Laurice Pereira CUNHA[1];
Rodrigo Costa da SILVA[1];
Aristeu Vieira DA SILVA[1];
Hélio LANGONI[1]

1- Núcleo de Pesquisas em Zoonoses (NUPEZO) do Departamento de Higiene Veterinária e Saúde Pública da Faculdade de Medicina Veterinária e Zootecnia da Universidade Estadual Paulista, Botucatu - SP

Abstract

The present work aimed to verify the effects of Baypamun®HK on the survival of albinic mice, experimentally infected with *Toxoplasma gondii* RH strain, treated or not with sulfadiazine-pirimetamine, as well as on the formation and the number of brain cysts. Four groups of 20 mices were inoculated with 10^5 tachizoites, subcutaneously, and undergone to different treatments. Serum tests were carried out by indirect immunofluorescence and samples of brain, liver and lung were collected from animals who died for cytological and brain cysts examinations. After 60 days, all survivors were sacrificed. Isolated Baypamun®HK didn't present a protective action against the infection by *T. gondii,* but its association sulfadiazine-pirimetamine, a specific treatment caused a higher survival of animals and a more intense and longer antibody responses.

Key-words:
Toxoplasma gondii.
Baypamun®HK.
Indirect immunofluorescence.
Mice.

Correspondence to:
HELIO LANGONI
Núcleo de Pesquisas em Zoonoses (NUPEZO)
Departamento de Higiene Veterinária e Saúde Pública
Faculdade de Medicina Veterinária e Zootecnia
Universidade Estadual Paulista
Campus de Botucatu
Distrito de Rubião Jr., s/n
18618-000 – Botucatu – SP
hlangoni@fmvz.unesp.br

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Introduction

Non-specific immunological stimulation is named paraimmunization and corresponds to an immunological status independent from the contact of the immune system with specific pathogens.[1,2] The activation of systems involved in paraimmunity may occur naturally by the contact with infectious agents or generated artificially by the administration of paraimmunity inductors.[3]

Paraimmunizer agents activate the production of humoral factors, like the interferons, mediators like interleukin and the tumour necrosis factor, the complement systems and lyzosime and also cellular factors, like macrophages, natural killer cells, T and B lymphocytes, being that many of these components are involved in the establishment of the specific immunity.[2,4,5,6] Thus the induction of paraimmunity is indicated in all cases in which immunoprophylaxis or chemiotherapy aren't enough to control infections.[2]

The utilization of BCG seems not to improve the response to the infection by *Toxoplasma gondii*. Dubey[7] studied the reexcretion of oocysts of *T. gondii* in superinfected cats, where the administration of BCG before the infection seemed ineffective over the re-excretion of the oocysts. Rezai et al.[8] paraimmunized mice with BCG and, 17 days after, inoculated a virulent strain of *T. gondii.* Animals presented incomplete resistance, but survived considerably longer than non-paraimmunized controls.

Intravenous pre-treatment of mice with *Corynebacterium parvum* showed a temporary protection in animals challenged with non-virulent strains of *T. gondii*, but not when infected with virulent strains. Peritoneal macrophages were activated,

destroying *Toxoplasma in vitro*, being that the population of activated macrophages persisted even in animals in which protection was temporary.[9] McLeod and Remington[10] studying the ability of peritoneal macrophages of mice in responding to the infection by *T. gondii* verified that in mice treated with *C. parvum* these cells efficiently destroyed the parasite.

Parapoxvirus ovis (strain D1701), isolated agent of epitelial pustules of sheep, is recognizably an activator of paraimmunity, being the active component of Baypamun®HK (Baypamun®HK, Bayer Saúde Animal), a largely used product used nowadays in prophylaxis and as a coadjuvant in the specific treatment of a number of infectious diseases and immunodepressive conditions, such as stress and neoplasias.

There is no register in literature of the use of Baypamun®HK as a paraimmunizer in infections by *T. gondii*. The present work aimed to verify the effects of Baypamun®HK on the survival of albinic mice experimentally infected with *T. gondii* RH strain, treated and no treated with sulfadiazine-pirimetamine, as well as on the formation of tissue cysts of *T. gondii* in the brain.

Materials and Methods

Eighty Swiss mice 21-day-old were used, divided into 4 groups of 20 animals, being: group 1 (Gl), control, represented by animals treated neither with Baypamun®HK* nor with sulfadiazine-pirimetamine; Group 2 (G2), treated with Baypamun®HK only; Group 3 (G3), treated with sulfadiazine-pirimetamine only; and Group 4 (G4), treated with Baypamun®HK and sulfadiazine-pirimetamine.

For the experimental infection *T. gondii* RH strain was used and tachizoites were obtained by mice peritoneal flushes seven days after inoculation. The inoculum obtained from this flushes was adjusted to a concentration of 1 x 10^5 tachizoites/mL.

Two days after blood collection, via orbital sinus for serological examination, mice were subcutaneously inoculated with 1 mL of the inoculum, containing 1 x 10^5 tachizoites.

Animals from groups G2 and G4 were orally treated with sulfadiazine-pirimetamine (Fanzidar®), in the dose of 20 mg of sulfadiazine and 1 mg of pirimetamine plus 1 g of sodium bicarbonate ($NaHCO_3$) to each 100 mL of drinkable water, between the days two and fifteenth after infection, according to Villard et al..[11]

Mice were observed twice a day for sixty days and blood samples were collected, via orbital sinus, for serological examination at 0, 3, 10, 17, 24, 31, 38, 45, 52 and 59 after the infection. Indirect fluorescent antibody test (IFAT) was carried out according to Camargo[12], up 1/16 dilution and in ratio 4 until the extinction of the serological reaction. From animals that died and from the ones that were sacrificed at the end of the observation period, samples of liver, lungs and brain were collected for cytological examination by Giemsa coloration.

One half of each brain collected was crushed and homogenized with 0,5 mL of phosphate buffered saline (PBS), being 25 mL examined between glass slides of 22x22 mm to count the total number of tissue cysts. The average of the total number of cysts counted in three laminas was multiplied by the correction factor 20.[13]

The results of the serological examination of groups G3 and G4 were analyzed by the Mann-Whitney test and data obtained in cytological and brain cysts counting examinations, as well as the survival of the animals, were compared by the Kruskal-Wallis test.[14]

Results and Discussion

Animals from groups G1 and G2 died between seven and nine days and three and eight days after the inoculation with *T. gondii*, with an average from 7.70 and 6.85 days, respectively. In groups G3 and G4 only seven and two animals died, respectively and, among these, no one presented signs of the

disease.

There was no statistical significance between groups G1 and G2 ($p>0.05$) and between G3 and G4 ($p>0.05$) regarding to the animal survival, indicating that this higher number of animals of groups G3 (average 49.90 days) and G4 (55.65 days) was due to the effects of the specific treatment.

Serological examinations carried out in animals from groups G1 and G2 showed that none of these were serum converted. There was no statistical significance between the serological response of mice from groups G3 and G4. However, it must be emphasized that, in G3, only 50% of animals were serum converted and, from these ones, 40% presented serological reaction in more than one sample, while, in G4, 75% of animals were serum converted, with 73.3% of serological reactions in more than one sample, indicating a possible stimulation of Baypamun®HK over the formation of antibodies.

The cytological examination of the tissue samples of mice from groups G1 showed different degrees of infection with average from 0.45, 1.65 and 19.20 tachizoites in 20 microscope fields in the brain, lung and liver, respectively. For the group G2 the average were 1.15, 9.40 and 28.50, respectively, in the same organs (Table 1). In the groups G3 and G4 parasitic forms were not found in none of the organs examined, even in those which died before the end of the observation period.

The direct examinations of the crushed brain of mice who died were negative in all 40 animals of groups G1 and G2. On the other hand, the cyst counting of *T. gondii* in the brain of the mice of groups G3 and G4 revealed not significantly different ($p>0.05$) (Table 2).

Baypamun®HK did not show a protective effect over the infection of mice with *Toxoplasma gondii*; however, when associated to the specific treatment with sulfadiazine-pirimetamine it increased the survival of animals, inducing the formation of specific antibodies in higher number of mice. It shows a more effective and intense cellular stimulation, once these animals also presented higher antibodies titles when compared with the ones of the group which received only sulfadiazine-pirimetamine.

Table 1
Average and median of the infection degree (number of tachizoites in 20 microscopic fields) of mice of groups G1 and G2, in the different organs evaluated. Botucatu, 2003

	G1		G2	
Organs	Average	Median	Average	Median
Brain	0.45	0.00	1.15	1.00
Lung	1.65	1.00	9.40	7.00
Liver	19.20	14.00	28.50	28.50

Table 2
Average and median of the number of tissue cysts of *Toxoplasma gondii*(corrected value) in brains of mice from groups G3 and G4. Botucatu, 2003

	Tecidual cysts	
Groups	Average	Median
3	13	0
4	10	0

The cellular activity and the production of citocines was not studied at this moment, but the serological result shows the possibility of Baypamun®HK as a potentiator in the treatment of toxoplasmosis and, also, as a possible adjuvant of vaccines against this parasite. In conclusion, Baypamun®HK, alone, did not show a protective effect in mice experimentally infected with *T. gondii*, RH strain; however, when associated with sulfadizine-pirimetamine, it provided a higher animal survival and a more intense and longer antibody responses.

Resumo

O presente trabalho teve como objetivo verificar os efeitos do Baypamun®HK sobre a sobrevida de camundongos albinos, experimentalmente infectados com *Toxoplasma gondii* amostra RH, submetidos ou não ao tratamento com sulfadiazina-pirimetamina, bem como sobre a formação e número de cistos cerebrais. Quatro grupos de 20 camundongos foram inoculados com 10^5 taquizoítos, via subcutânea, e submetidos a diferentes tratamentos. Os testes sorológicos foram realizados pela técnica de imunofluorescência indireta, e, dos animais que sucumbiram, amostras de cérebro, pulmão e fígado foram retiradas para exame citológico e de cérebros para a pesquisa de cistos. Ao final de 60 dias todos os sobreviventes foram sacrificados. Isoladamente o Baypamun®HK não apresentou uma ação protetora contra a infecção pelo *T. gondii,* mas sua associação com o tratamento específico pela sulfadiazina-pirimetamina promoveu maior sobrevida dos animais e uma resposta de anticorpos mais intensa e duradoura.

Palavras-chave:
Toxoplasma gondii.
Baypamun®HK.
Imunofluorescência indireta.
Camundongos.

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