Braz. J. vet. Res. anim. Sci.,
São Paulo, v.33, n.3, p.139-143, 1996.

Quantitative evaluation of tuberculosis evolution in hamsters submitted to an eight-week Trichlorfon treatment and infected with *Mycobacterium bovis*, strain AN5

Avaliação quantitativa da evolução da tuberculose em hamsters tratados 8 semanas consecutivas com Triclorfon e infectados com *Mycobacterium bovis*, cepa AN5

José Soares FERREIRA NETO [1]; Sonia Regina PINHEIRO [1]; Zenaide Maria MORAES [1]; Luiz Carlos Sá ROCHA [1]; Idércio Luiz SINHORINI [1]; Fumio Honma ITO [1]; Silvio Arruda VASCONCELLOS [1]

CORRESPONDENCE TO:
José Soares Ferreira Neto
Departamento de Medicina Veterinária Preventiva e Saúde Animal
Faculdade de Medicina Veterinária e Zootecnia da USP
Av. Prof. Dr. Orlando Marques de Paiva, 87 - Cidade Universitária Armando de Salles Oliveira
05508-900 - São Paulo - SP - Brasil

1 - Faculdade de Medicina Veterinária e Zootecnia da USP - SP

SUMMARY

The evolution of disease by intraperitoneal inoculation of *Mycobacterium bovis* (strain AN5) was evaluated in hamsters (*Mesocricetus auratus*) treated daily, from Monday to Friday, with 30 mg/kg of Trichlorfon given subcutaneously, for a period of 8 consecutive weeks. The criteria used for the evaluation were post infection survival time and quantitative cultures of mycobacteria from spleen, kidney, liver and lung taken at 1, 15, 29 and 45 days after inoculation. Histopathologic examinations were also performed at each scheduled time. Treated and control animals developed progressive disease. The results of quantitative cultures and survival time after inoculation were not influenced by Trichlorfon as related to the evolution of tuberculosis.

UNITERMS: Hamsters; *Mycobacterium bovis*; Trichlorfon.

INTRODUCTION

Tuberculosis is an important disease of dairy cattle in many countries and Trichlorfon is a parasiticide widely used for these animals. The influence of Trichlorfon on immune response of vertebrate hosts is a controversial subject.

Shtenberg *et al.* (1947) *apud* World Health Organization[12] (1986) reported that oral daily doses of 5-7 mg/kg of Trichlorfon, administered during a not specified period, supressed haemagglutination levels in rats immunized against sheep red blood cells.

Krustev *et al.*[6] (1976) observed that guinea pigs treated with 100 mg/kg of Trichlorfon for 60 consecutive days did not present alterations of either the gammaglobulin levels or the phagocytic activity of spleen and liver cells.

Hermanowicz; Kossmann[5] (1984) verified that human beings occupationally exposed to organophosphorus compounds, including Trichlorfon, presented decrease of neutrophil chemotaxis but not an increase of infectious diseases that affect other organs besides the higher respiratory tract. The authors concluded that such complications of the higher respiratory tract occurred due to inhalation of pesticides and solvents.

Reiss *et al.*[9] (1987) observed that the administration of Trichlorfon with drinking water (175 mg/ml), for 2 consecutive weeks, did not induce cellular or humoral immune response alterations in rats.

Garcia Castaño[4] (1991) observed that daily doses of Trichlorfon (30 mg/kg), administered from Monday to Friday, for 8 consecutive weeks, did not interfere with the result of tuberculin test; however, it caused a decrease of the diameter of the granuloma induced by BCG in the pad of hamsters. Expansion and morphology of the tuberculous granuloma induced by mycobacteria are regulated by sensitized lymphocytes and macrophages 7, the two important cells of the host immune system. These observations suggest a possible influence of Trichlorfon on the mycobacterium-hamster relationship.

An interesting way for the study of the influence of a variable on the immune system of vertebrate hosts is through the quantitative evaluation of a disease evolution using controlled animal models.

Bermudez *et al.*[1] (1992) described an animal model of *Mycobacterium avium* complex infection using oral doses and studied some factors that possibly affect the host-parasite relationship. Through quantitative cultures of samples taken from liver, spleen and blood of infected animals, it was observed that ingestion of ethanol is associated with a significant increase in the number of mycobacteria isolated from the organs compared to those in controls. Whether the observed increase is due to enhanced passage of *M. avium*

across the intestinal wall (secondary to alcohol-induced-irritation) or to ethanol effects on the host immune system remains unknown.

The present work is designed to examine if treatment with Trichlorfon interferes on the course of tuberculosis in hamster, using an animal model described by Ferreira Neto *et al.*[3] (1994).

MATERIAL AND METHOD

The biologic effects of Trichlorfon have been previously evaluated in 6 hamsters treated subcutaneously with 30 mg/kg and in 6 hamsters treated with placebo. Twenty minutes after injection, the animals were anesthetized by chloroform inhalation, sacrificed and the acetylcholinesterase activity was measured in plasma and in brain[2]. Treated animals presented 78.07% and 75.22% of cholinesterase activity, respectively, in plasma and in brain.

Thirty hamsters were treated with a daily subcutaneous dose of Trichlorfon (30 mg/kg), from Monday to Friday, during 8 consecutive week[4]. Other 30 animals received apyrogenic sterile water at the same conditions.

At the 1st day of the 5th week of the Trichlorfon treatment, all 60 hamsters were inoculated intraperitoneally with 1.0 mg of cultures of *Mycobacterium bovis*, strain AN5 (net weight). At 1, 15, 29 and 45 days after inoculation, 5 animals of each group were anesthetized, sacrificed and histopathologic and bacteriologic examinations were performed[3]. The 20 remaining hamsters were maintained in strict observation until death.

Right medial haepatic lobe, left kidney, 2/3 of the ventral spleen portion and left pulmonary lobe were used for the bacteriologic examinations. These organs were submitted to 3 consecutive washings with sterile physiologic saline solution (SPSS), weighed, ground, diluted at 1:10 (weight/volume) with SPSS and filtered. The filtrate was 10^{-1} dilution, and from this the 10^{-3}, 10^{-5} and 10^{-7} was obtained. Each dilution was inoculated into 2 culture media (Petragnani). The volume of the inoculum was 0.2 ml per tube.

At the 34th day of incubation at 37°C the colonies counts were done adopting the following criterion (adapted from Pinheiro *et al.*[8], 1992): none Colonie Forming Unit (CFU): - ; below 50: (the number effectively counted); from 51 to 100 CFU: 1+; from 101 to 200 CFU: 2+; from 201 to 500 CFU: 3+; up to 500 CFU: 4+. The average of the two counts per dilution was obtained by transformation of counts in average number: - : 0.0 CFU; ( ): the number effectively counted; 1+: 75 CFU; 2+: 150 CFU; 3+: 350 CFU; 4+: 500 CFU.

The histopathologic examinations were performed with right caudal pulmonary lobe left lateral haepatic lobe, right kidney and the rest of the spleen. From these organs histologic preparations of 5 micra were obtained and stained by hematoxilin-eosin and Ziehl-Nielsen method for microscopic observation.

The CFU counts were analyzed by Mann-Whitney's "u" test[10]. The acetylcholinesterase determinations and the survival time after inoculation were analyzed by Student's "t" test[11]. The significance level was 0.05.

RESULTS

The results of bacterial counts of all scheduled times were organized in Tab. 1 and 2, and statistically significant difference among the intoxicated and control animals has not been observed.

Main macroscopic alterations were: at 1 day after inoculation, animals of both groups did not present any macroscopic alteration; at 15 days after inoculation, animals of both groups presented splenomegaly; at 29 days after inoculation, animals of both groups presented splenomegaly, yellowish point lesions at the surface of the spleen, kidneys, liver, lungs, epiploon, mesentery and peritoneum, being more evident in spleen and kidneys. Adherences were also found in the abdominal cavity. At 45 days after inoculation, animals of both groups presented splenomegaly and the same kind of lesions described above for animals of the 29 days after inoculation, though being slightly bigger. Adherences were present in the abdominal cavity.

At each scheduled time, animals of both groups presented the same microscopic alterations, beginning with a mononuclear cell infiltration (1 day after infection), progressing to reactions characterized by the predominance of macrophagic cells (15 days after inoculation), then showing the typical tuberculous granuloma structure (29 and 45 days after infection).

For all animals sacrificed at 29 and 45 after inoculation, the histologic preparations stained by Ziehl-Nielsen method presented positive results.

Tab. 3 contains the results of survival time after inoculation. There was no statistically significant difference between the treated and control animals.

DISCUSSION

The results observed in the experiment did not show any influence of Trichlorfon on the evolution of tuberculosis experimentally induced by AN5 strain of *Mycobacterium bovis* in hamster, although Garcia Castaño[4] (1991) has found a minor granuloma induced by BCG in the pad of hamster

Table 1

Mycobacterium bovis CFU mean number recovered from hamsters experimentally infected with *Mycobacterium bovis* (strain AN5) and sacrificed at 1 and 15 days after inoculation according to dilutions, experimental groups and organs. São Paulo, 1992.

SCHEDULED TIMES	1 DAY AFTER INFECTION								15 DAYS AFTER INFECTION							
DILUTIONS	10^{-1}		10^{-3}		10^{-5}		10^{-7}		10^{-1}		10^{-3}		10^{-5}		10^{-7}	
GROUPS ORGANS	A*	B**	A	B	A	B	A	B	A	B	A	B	A	B	A	B
LIVER	150.0	112.5	8.5	8.0	-	-	-	-	500.0	150.0	350.0	14.5	6.0	0.5	-	-
	14.0	112.5	0.5	3.5	-	-	-	-	150.0	500.0	10.0	150.0	-	2.5	-	-
	21.0	20.0	0.5	0.5	-	-	-	-	350.0	150.0	47.0	4.0	1.0	-	-	-
	35.0	10.5	4.5	0.5	-	-	-	-	350.0	500.0	75.0	150.0	2.0	3.0	-	-
	60.0	1.5	1.0	-†	-	-	-	-	500.0	150.0	112.5	11.0	5.0	-	-	-
STATISTICAL DECISION	NS† †		NS						NS		NS		NS			
SPLEEN	350.0	350.0	75.0	49.5	8.0	-	-	-		500.0	350.0	75.0	21.0	1.5	-	-
	350.0	350.0	51.5	112.5	3.0	5.5	-	-		500.0	112.5	150.0	1.5	13.5	0.5	-
	C@	150.0	61.5	100.0	3.5	1.5	-	-	500.0	350.0	150.0	75.0	3.5	2.5	-	-
	350.0	350.0	150.0	75.0	7.5	-	-	-	500.0	350.0	150.0	112.5	12.0	1.0	-	-
	150.0	150.0	22.0	32.0	-	-	-	-	350.0	500.0	75.0	150.0	1.0	9.0	-	-
STATISTICAL DECISION	NS		NS		NS				NS		NS		NS		NS	
KIDNEY	75.0	43.0	10.0	2.0	-	-	-	-	350.0	75.0	75.0	1.5	2.0	-	-	-
	30.0	150.0	0.5	9.0	-	-	-	-	150.0	75.0	39.0	5.5	-	-	-	-
	75.0	1.0	2.0	-	-	-	-	-	150.0	150.0	6.0	5.0	-	-	-	-
	C	75.0	32.0	5.0	0.5	-	-	-	150.0	350.0	3.0	62.5	-	-	-	-
	75.0	4.0	1.0	-	-	-	-	-	150.0	75.0	60.5	3.5	-	-	-	-
STATISTICAL DECISION	NS		NS		NS				NS		NS		NS			
LUNG	55.0	21.5	-	-	-	-	-	-	500.0	75.0	112.5	7.0	1.5	-	-	-
	3.5	60.5	-	0.5	-	-	-	-	150.0	150.0	17.5	62.0	-	0.5	-	-
	4.0	5.0	-	-	-	-	-	-	150.0	150.0	6.0	3.0	-	0.5	-	-
	44.0	38.0	2.5	-	-	-	-	-	425.0	500.0	60.0	75.0	0.5	0.5	-	-
	30.0	0.5	-	-	-	-	-	-	150.0	350.0	40.0	11.0	-	-	-	-
STATISTICAL DECISION	NS		NS						NS		NS		NS			

*A: treated with Trichlorfon

**B: control

† -: none CFU

† † NS: statistically not significant (Mann-Whitney test, $\alpha = 0.05$)

Table 2

Mycobacterium bovis CFU mean number recovered from hamsters experimentally infected with *Mycobacterium bovis* (strain AN5) and sacrificed at 29 and 45 days after inoculation according to dilutions, experimental groups and organs. São Paulo, 1992.

SCHEDULED TIMES	29 DAYS AFTER INFECTION								45 DAYS AFTER INFECTION							
DILUTIONS	10^{-1}		10^{-3}		10^{-5}		10^{-7}		10^{-1}		10^{-3}		10^{-5}		10^{-7}	
GROUPS ORGANS	A*	B**	A	B	A	B	A	B	A	B	A	B	A	B	A	B
LIVER	500.0	500.0	150.0	350.0	8.0	20.5	-†	0.5	500.0	500.0	350.0	150.0	40.0	10.0	1.5	0.5
	350.0	350.0	75.0	75.0	1.0	0.5	-	-	500.0	500.0	150.0	350.0	21.0	60.0	-	1.5
	350.0	350.0	75.0	112.5	3.5	4.0	-	-	500.0	500.0	350.0	150.0	75.0	7.0	7.0	0.5
	500.0	500.0	350.0	350.0	5.5	75.0	0.5	3.5	350.0	500.0	75.0	350.0	1.0	21.5	-	-
	500.0	350.0	150.0	75.0	6.0	3.0	0.5	-	500.0	500.0	350.0	350.0	26.0	150.0	0.5	20.0
STATISTICAL DECISION	NS††		NS		NS		NS		NS		NS		NS		NS	
SPLEEN	500.0	500.0	350.0	350.0	24.5	75.0	1.0	5.0	500.0	500.0	350.0	350.0	150.0	45.5	5.5	2.0
	500.0	500.0	350.0	350.0	20.5	15.5	1.0	0.5	500.0	500.0	350.0	350.0	75.0	75.0	2.5	3.0
	500.0	500.0	350.0	350.0	33.5	17.5	0.5	0.5	500.0	500.0	500.0	350.0	150.0	62.5	19.0	1.5
	500.0	500.0	350.0	350.0	36.0	75.0	0.5	11.0	500.0	500.0	150.0	350.0	17.0	150.0	1.0	7.0
	500.0	500.0	350.0	350.0	25.5	17.5	2.5	1.5	500.0	500.0	350.0	350.0	75.0	150.0	6.0	17.5
STATISTICAL DECISION	NS		NS		NS		NS		NS		NS		NS		NS	
KIDNEY	350.0	500.0	150.0	350.0	5.5	45.0	-	1.5	350.0	350.0	62.5	70.5	3.0	1.0	-	-
	350.0	350.0	75.0	20.0	-	-	-	-	350.0	350.0	58.5	150.0	2.0	4.5	-	-
	350.0	350.0	150.0	75.0	1.5	4.0	-	-	350.0	350.0	350.0	150.0	19.5	4.5	1.5	0.5
	350.0	500.0	112.5	350.0	3.5	24.5	-	0.5	350.0	350.0	60.5	150.0	0.5	11.5	-	-
	350.0	350.0	16.0	150.0	0.5	9.5	-	-	350.0	500.0	75.0	350.0	3.0	62.0	-	3.5
STATISTICAL DECISION	NS		NS		NS		NS		NS		NS		NS		NS	
LUNG	500.0	500.0	150.0	350.0	19.5	43.0	0.5	1.5	500.0	350.0	150.0	150.0	21.5	15.0	-	-
	350.0	350.0	75.0	112.5	2.0	2.5	-	-	350.0	500.0	150.0	350.0	28.0	37.0	-	0.5
	350.0	350.0	150.0	75.0	7.5	2.0	-	-	500.0	350.0	350.0	65.5	150.0	4.0	18.0	0.5
	500.0	350.0	150.0	150.0	5.0	12.0	-	-	350.0	500.0	150.0	350.0	10.0	75.0	-	2.0
	350.0	350.0	112.5	75.0	5.5	4.5	-	-	500.0	500.0	350.0	350.0	150.0	75.0	10.0	15.5
STATISTICAL DECISION	NS		NS		NS		NS		NS		NS		NS		NS	

*A: treated with Trichlorfon

**B: control

† -: none CFU

†† NS: statistically not significant (Mann-Whitney test, $\alpha = 0.05$)

Table 3

Post infection survival time (in days) of hamsters inoculated with *Mycobacterium bovis* (strain AN5) according to the experimental groups and animal identifications. São Paulo, 1992.

EXPERIMENTAL GROUPS ANIMAL IDENTIFICATIONS	A*	B**
1	56	63
2	56	49
3	51	49
4	56	57
5	44	49
6	35	49
7	35	63
8	43	49
9	49	39
10	50	39
MEAN	47.5	50.6
STATISTICAL DECISION	NS†	

*A: treated with Trichlorfon; **B: control
†† NS: statistically not significant (Student "t" test, $\alpha = 0.05$)

treated with Trichlorfon, an evidence of a possible interference of this product on the immune system. Nevertheless, these results could be related to infectious dose, site of inoculation, virulence and pathogenicity of strain AN5.

The results of the work corroborate the observations of Hermanowicz; Kossmann[5] (1984) that found a decrease of neutrophil chemotaxis in human beings occupationally exposed to organophosphorus compounds, including Trichlorfon, without an increase of frequency of infections localized out of the high respiratory tract.

Many authors verified that administration of Trichlorfon decreases some parameters used for measurement of the immune response status[4,5,12], but according to results of this experiment and based on the observations of Hermanowicz; Kossmann[5] (1984), Trichlorfon does not interfere on the host susceptibility or, if so, it seems to be very mild.

RESUMO

A evolução da doença causada pela inoculação intraperitoneal de *Mycobacterium bovis* (cepa AN5) foi avaliada em hamsters (*Mesocricetus auratus*) tratados de segundas a sextas-feiras, com doses subcutâneas de 30 mg/kg de Triclorfon, por um período de 8 semanas consecutivas. Os critérios adotados para essa avaliação foram o tempo de sobrevivência após a inoculação e a quantificação do agente no baço, fígado, rim e pulmão colhidos aos 1, 15, 29 e 45 dias após a inoculação. Nessas oportunidades também foram realizados exames histopatológicos. Os animais do grupo tratado com Triclorfon e do grupo controle desenvolveram tuberculose progressiva. Os resultados da quantificação do agente nos órgãos examinados, e do tempo de sobrevivência após a inoculação, não mostraram qualquer influência da administração do Triclorfon.

UNITERMOS: Hamsters; *Mycobacterium bovis*; Triclorfon.

REFERENCES

1-BERMUDEZ, L.E.; PETROFSKY, M.; KOLONOSKY, P.; YOUNG, L.S. An animal model of *Mycobacterium avium* complex disseminated infection after colonization of the intestinal tract. **Journal of Infectious Diseases**, v.165, p.75-9, 1992.

2-ELLMAN, G.L.; COURTNEY, D.; ANDRES JUNIOR, V.; FEATHERSTONE, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. **Biochemical Pharmacology**, v.7, p.88-95, 1961.

3-FERREIRA NETO, J.S.; PINHEIRO, S.R.; MORAIS, Z.M.; SINHORINI, I.L.; ITO, F.H.; VASCONCELOS, S.A. Avaliação quantitativa da concentração de micobactérias em órgãos e humores de hamsters experimentalmente infectados com *Mycobacterium bovis*, estirpe AN5. **Brazilian Journal of Veterinary Research and Animal Science**, v.31, n.2, p.131-9, 1994.

4-GARCIA CASTANO, C.H. **Influência do inseticida organofosforado no desenvolvimento do granuloma induzido por BCG e na resposta à prova de tuberculina, em hamster**. São Paulo, 1991. 64p. Dissertação (Mestrado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo.

5-HERMANOWICZ, A.; KOSSMANN, S. Neutrophil function and infectious disease in workers occupationally exposed to phosphoorganic pesticides: role of mononuclear-derived chemostatic factor for neutrophils. **Clinical Immunology and Immunopathology**, v.33, p.13-22, 1984.

6-KRUSTEV, E.; KONSTANTINOV, P.; VASILEV, V. Effect of subtoxic doses of Dipterex (Trichlorfon) on guinea pigs. **Veterinarnomeditsinski Nauki**, v.13, p.48-52, 1976.

7-MARIANO, M. The experimental granuloma. Hypothesis to explain the persistence of the lesion. **Revista do Instituto de Medicina Tropical**, v.37, n.2, p.161-76, 1995.

8-PINHEIRO, S.R.; VASCONCELOS, S.A.; ITO, F.H.; FERREIRA NETO, J.S.; MORAIS, Z.M. Influência da matéria orgânica na atividade micobactericida de cinco desinfetantes químicos de uso pecuário. **Brazilian Journal of Veterinary Research and Animal Science**, v.29, n.1, p.51-60, 1992.

9-REISS, C.S.; HERRMAN, J.M.; HOPKINS II, R.E. Effect of anthelminthic treatment on the immune response of mice. **Laboratory Animal Science**, v.37, p.773-5, 1987.

10-SIEGEL, S. **Estatística não paramétrica**. São Paulo, McGraw-Hill, 1981.

11-VIEIRA, S.; HOFFMANN, R. **Estatística experimental**. São Paulo, Atlas, 1989.

12-WORLD HEALTH ORGANIZATION. **Organophosphorus inseticides: a general introduction**. Geneva, 1986 (Environmental Health Criteria, 63).

Recebido para publicação: 03/08/94
Aprovado para publicação: 08/11/95