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Increased virulence of *Mycobacterium avium* Kirschberg in thioacetamide treated rabbits

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Abstract. An attempt has been made to determine the correlation between liver damage and the virulence of mildly pathogenic *Mycobacterium avium* in thioacetamide treated rabbits. Liver damage increased the susceptibility of rabbits to infection even with a moderately virulent organism.

Keywords. Liver damage; mycobacteria; virulence; thioacetamide.

Introduction

In an earlier communication (Singh *et al.*, 1980) it was reported that a mild grade of liver damage produced in rabbits by 23 subcutaneous injections of 0.02 ml of carbon tetrachloride ($CC1_4$) increased the intensity of the disease caused by *Mycobacterium avium* Kirschberg. It was necessary to confirm the finding that liver damage really predisposes the animals to infection more acutely. This was attempted by injecting thioacetamide to produce liver damage in rabbits and challenging them with a mildly pathogenic strain of *M. avium.*

Materials and methods

Materials

Swiss albino outbred mice and rabbits were obtained from the Institute's animal colony and were maintained on standard feeding and watering conditions. The *M avium* Kirschberg (TMC 801) strain of Mycobacterium was obtained from Trudeau Inc., Sarnac Lake, New Jersey, USA with a reported history of moderate virulence for rabbits and mice.

Methods

The culture of *M. avium* was maintained on Lowenstein-Jensen medium. Thioacetamide (BDH/AR) was weighed quickly and emulsified with 10% tween 80-saline in a mortar and diluted with saline to a concentration of 50 mg/ml. To ascertain the dose of thioacetamide required to produce a mild degree of liver damage in rabbits, mice (5 per group) were administered intraperitoneal injections of 50,100,250 and 500 mg/kg of thioacetamide in 0.2 ml quantities. On the basis of

mice experiments, rabbits were injected intraperitoneally with 100 mg/kg of thioacetamide. A weekly repeat of intraperitoneal injection of 25 mg/kg was sufficient to maintain the hepatitic condition of Cameron grade I hepatitis (Cameron and Karunaratne, 1936). Three days after the first injection of thioacetamide, one group of ten rabbits were challenged intravenously with 1 mg of *M. avium* suspended in 0.2 ml of 0.07 M sodium phosphate buffer, pH 8.7. Two groups of 10 animals each were kept as controls. The first group of control rabbits received the same doses of thioacetamide but without challenging with *M. avium,* whereas the other group was kept as normal control with only *M. avium* challenge. Several parameters were recorded like general appearance of the animals, their body weights at weekly intervals and autopsy score of the lesions in the visceral organs *viz.* lungs, liver spleen and kidney. Besides these, weights and Mycobacterial viable counts of the visceral organs, impression smears of the same organs for the presence of acid fast bacilli (AFB) and the mean survival time of the animals after challenge with *M. avium* were also studied.

Results

After 72 h of the first thioacetamide exposure and challenge with *M. avium,* animals of all the three groups [(i) only thioacetamide treated; (ii) thioacetamide treated–*M. avium;* and (iii) normal–*M. avium*] were kept under strict observation. Every animal after death was necropsied to obviate the non-specific deaths. The lesions of the visceral organs provided reasonable assurance that the deaths were due to tuberculosis.

On the death of the first animal in the thioacetamide treated – *M. avium* group on day 17, two animals each from thioacetamide treated - *M. avium* and normal — *M. avium* groups were sacrificed and subjected to detailed studies including the viable counts in the visceral organs. A comparison of various parameters of study is presented in table 1. The thioacetamide-*M. avium* group of animals started deterioration in their general appearance with ruffled hair earlier than the animals of normal-*M. avium* group. Their body weights dropped suddenly while their visceral organs had more intense lesions and increased weights. The impression smears of the organs from this group had an abnormally large number of acid-fast bacteria. On the 17th day of infection, viable counts of the visceral organs in this group were also significantly higher as compared to the control group. The experiment was terminated 91 days after challenging with *M. avium* by sacrificing the surviving animals. The maximum survival time was thus 91 days. The mean survival time of thioacetamide treated-*M. avium* group was only 21.4 ± 0.6 days as compared to 80.2 ±7.5 days of the normal-*M. avium* group, which is a highly significant change (t=7.5, P<0.001) (figure 1). All the animals in the thioacetamide-treated but unchallenged group survived till the termination of the experiment. The mean weights of lungs, liver, spleen and kidney of this group of animals were 6.8 ± 0.5,47.7 ± 3.2,0.85 ± 0.2 and 9.3 ± 0.3 g respectively which are almost similar to the weights of normal animals of the same weight group (Singh *et al.*, 1980).

Table 1 Comparison of various parameters in the thioacetamide treated and untreated rabbits challenged with *M. avium* Kerschberg. (Figures in parentheses give the range).

Parameters	Thioacetamide treated-*M. avium* group				Normal-*M. avium* group			
	Lungs	Liver	Spleen	Kidney	Lungs	Liver	Spleen	Kidney
CFU/g of tissue[a]	6×10^7	2×10^7	1.8×10^8	4×10^5	8×10^5	1.4×10^6	1.6×10^6	4.0×10^5
Average AFB score[b]	3.5	3	3	2	1.5	1	2	0.5
In impression smears	(3-4)	(2-4)	(2-4)	(1-3)	(1-2)	(0-2)	(1-3)	(0-1)
Average pathological	3	2.5	3	1	1	1	1.5	0.5
score[b]	(2-4)	(2-4)	(2-4)	(0-2)	(0-2)	(0-2)	(1-2)	(0-1)
Mean organ weight g ±SE	19.9±1.4	52.1±5.3	3.1±0.3	15.6±1.5[c]	10.3±0.4	33.2±5.2	1.7±0.3	12.3±0.7[c]
MST (days)[d] ±SE		21.4±0.6					80.2±7.5	
Average body weight (Kg) ±SE								
(a) At infection		1.89±0.12					1.94±0.07	
(b) At death		1.20±0.07					1.60±0.12	

Each value is the mean ±SE of 8 individual observations.

[a] Average of 2 animals. CFU–viable counts

[b] As given in the text. Maximum score = 4.0. AFB–acid fast bacteria

[c] Weights of both kidneys

[d] MST–mean survival time

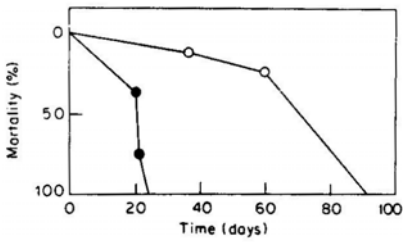

Figure 1. Percentage mortality of normal and thioacetamide-treated rabbits challenged with *M. avium* Kirschberg.

Discussion

An attempt was made to evaluate the susceptibility of the animals after mild degree of liver damage due to infection. This was first tested in carbon tetrachloride treated rabbits (Singh *etal.*,1980). There was clear indication that liver damage increased the intensity and severity of the disease. CCl_4 in many small subcutaneous doses caused essentially irreversible liver damage. Thioacetamide produce liver damage upon a single intraperitoneal injection but the effect was partially reversible (Gupta, 1956). *M. avium* Kirschberg and rabbit model was chosen with a view that this mycobacterium is mildly pathogenic for rabbits, and produces Yersin type of disease with a big lapse of time. The parameters used to study the extent and severity of pathogenesis of this infection showed that this mild pathogenic organism was severely infective in liver-damaged animals.

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