

The pathogenicity of *Mycobacterium tuberculosis* during chemotherapy

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ABSTRACT: We used the guinea pig as an experimental model to investigate the pathogenicity of *Mycobacterium tuberculosis*. Sputum samples were injected subcutaneously into guinea pigs and the animals were killed and an autopsy performed after eight weeks. The likelihood of the sputum samples producing tuberculosis in the guinea pig was related to culture positivity rather than to duration of chemotherapy. This study does not support the belief that a change in pathogenicity occurs during treatment of pulmonary tuberculosis.

Eur Respir J., 1990, 3, 399-402.

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Keywords: AIDS; guinea pig inoculation; *Mycobacterium tuberculosis*; pathogenicity; tuberculosis chemotherapy.

Received: November 1988; accepted after revision October 17, 1989.

Inhalation of aerosolised sputum from a patient with pulmonary tuberculosis is by far the most common method of transmission of tuberculosis today [1]. Anti-tuberculosis chemotherapy is highly effective in preventing the transmission of the disease from a patient to a susceptible or non-immune host, and infectiousness for intimate household contacts seems to diminish quickly following the introduction of chemotherapy [2]. Nevertheless the time at which a patient becomes non-infectious has never been established. It has, however, become accepted that after two weeks chemotherapy a patient does not represent a serious infectious risk [3]. The most important result of anti-tuberculosis drugs is probably the reduction in the number of bacilli in the sputum and the reduction in cough [3-5]. Patients are regarded as non-infectious, even if their sputum remains positive on smear and culture, which suggests that anti-tuberculous chemotherapy brings about a change in pathogenicity. We have used the guinea pig as an animal model to test this hypothesis.

Methods

Twenty-nine sputum samples from 21 patients with direct smear positive pulmonary tuberculosis were taken and prepared in a standard manner. All patients were receiving supervised triple therapy with Rifampicin, Isoniazid and Ethambutol and had infection with fully sensitive *Mycobacterium tuberculosis*. 2 ml of each sputum sample was treated with 4% NaOH for 15 min; then 16 ml of distilled water was added and the preparation was centrifuged at 3000 rpm for 15 min. The deposit was resuspended in 1 ml of water, divided into

two aliquots one of which was cultured on Lowenstein-Jensen slopes and the other injected subcutaneously into the thigh of a guinea pig.

The guinea pigs were killed after 8 weeks and an autopsy was performed. The presence and extent of disease in the guinea pigs was classified as follows: no evidence of tuberculosis (Grade 0); evidence of local disease with or without regional lymph node involvement (Grade 1); disseminated tuberculosis with minimal involvement of the spleen and no lesions in the peritoneal cavity (Grade 2); generalised disease including involvement of spleen and peritoneal cavity (Grade 3).

Direct staining of sputum was performed using an immunofluorescence technique and was graded as follows:

Microscopy:	> 10 AFB per oil emersion field	+++
	1 - 10 AFB per oil emersion field	++
	10 - 99 AFB per 100 oil emersion fields	+
	1 - 9 AFB per 100 oil emersion fields	±
	No AFB per 100 oil emersion fields	None seen

Culture results on Lowenstein-Jensen medium were graded as follows:

No growth	0
< 20 colonies	±
50-100 colonies	+
>100 colonies	++

We used multiple regression analysis (MRA) as described by ARMITAGE and BERRY [6] to analyse the relationship between the extent of guinea pig lesions and the three parameters - duration of chemotherapy, direct smear positivity and culture positivity.

Results

Figures 1, 2 and 3 show the relationship between direct smear, Lowenstein-Jensen culture and duration of treatment, respectively, and the extent of disease produced in the guinea pigs by aliquots from the same specimens. There were 25 specimens of sputum which were positive for acid fast bacilli on direct staining and of these 17 produced tuberculous lesions in the guinea pigs. There were 21 positive sputum cultures and 16 of these produced guinea pig tuberculous lesions. There were four positive cultures of sputum, the aliquots of which did not produce lesions in the guinea pigs. There were, however, no more than 4 colonies on any of these cultures. Whilst there was a relationship between the duration of treatment and the absence of tuberculous lesions in inoculated guinea pigs, the ability to produce lesions in the guinea pig was associated with the degree of positivity of the sputum and not the duration of therapy alone. MRA showed that the correlation between severity of the guinea pig lesions was strongest with culture positivity ($r=0.78$), weaker with sputum positivity on direct smear ($r=0.77$) and weakest with duration of chemotherapy ($r=-0.21$).

Extent of guinea pig lesions

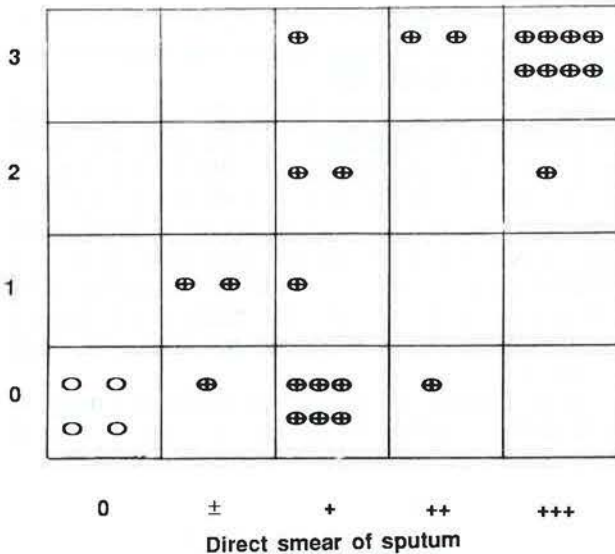


Fig. 1. - The extent of tuberculous lesions in relation to direct smear positivity. ⊕: Specimens of sputum with positive direct smear; ⊙: Specimens of sputum with negative direct smear.

Culture of guinea pig tissue was carried out on four animals. In two cases the sputum had been positive both on direct staining and on culture, there were extensive lesions present in the guinea pig and in both cases guinea pig tissue culture grew *Mycobacterium tuberculosis*. In a third case the sputum was weakly positive on direct staining and on culture, produced minimal lesions in the guinea pig but no mycobacteria were grown on tissue culture. In the fourth case the sputum was positive on direct smear but negative on culture, there were no lesions present in the guinea pig and the tissue culture was negative for *Mycobacterium tuberculosis*.

Extent of guinea pig lesions

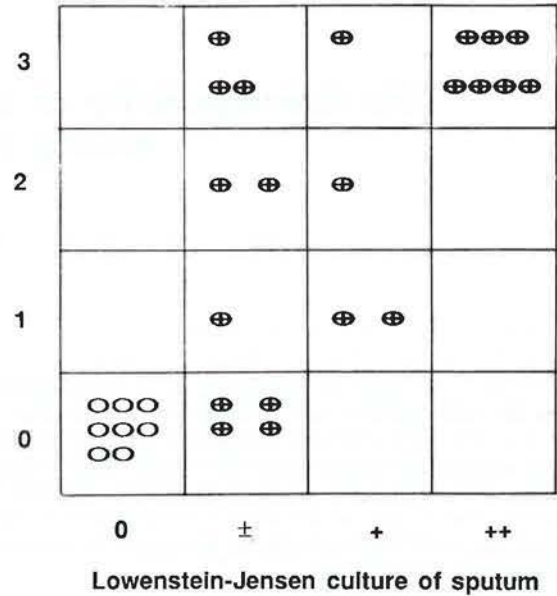


Fig. 2. - The extent of tuberculous lesions in relation to culture positivity. ⊕: Specimens of sputum with positive culture; ⊙: Specimens of sputum with negative culture.

Extent of guinea pig lesions

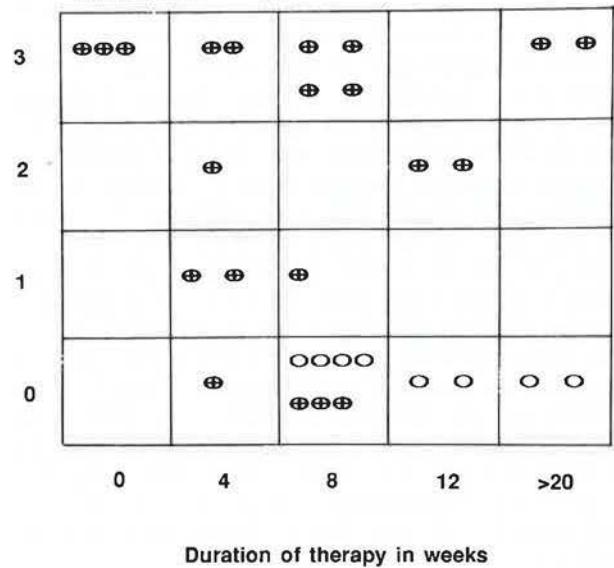


Fig. 3. - The extent of tuberculous lesions in relation to duration of therapy. ⊕: Specimens of sputum with positive culture; ⊙: Specimens of sputum with negative culture.

Discussion

Our primary aim was to determine whether tubercle bacilli from patients who were receiving chemotherapy remained pathogenic despite treatment, provided that the bacilli were still present in the sputum and that this was independent of the duration of chemotherapy. If pathogenicity was dependent on the duration of therapy alone it would become obvious in the protocol used and would be independent of the route of infection. The limitations

of an animal model raises the question as to whether such an approach is any more significant than culture in an artificial medium. It has long been accepted that sputum may remain culture positive for several weeks following the initiation even of modern anti-tuberculosis therapy. In our experience it takes four months therapy on standard doses of Rifampicin, Isoniazid and Ethambutol for 96% of patients to become culture negative [7]. Based largely on the Madras experience and a number of other indirect studies there has been a tendency to assume that these organisms are non-pathogenic [2, 8-11]. Chemotherapeutic agents in sputum will tend to inhibit bacillary growth whereas artificial culture media tend to encourage growth. It is difficult therefore to predict the effect of aerosolised sputum, from tuberculous patients on chemotherapy, in susceptible humans. In these circumstances it is argued that an organism which has been exposed to chemotherapy might become of such low virulence that it was unable to cause disease even though it may grow in an artificial medium.

The best model, therefore, would have been normal man, but of course this would be unacceptable. It was with that background that the use of an animal model was considered of greater significance than a positive culture in an artificial medium.

We are aware of the problem of extrapolating from guinea pig models to man. Furthermore the route of infection (subcutaneous injection), is artificial and does not mimic natural infection in man. Nevertheless since an animal model is essential for this study the guinea pig is suitable. It is difficult to be sure of the relative susceptibility to infection of guinea pigs compared to man. It is known that the susceptibility of guinea pigs is equivalent to that of anthropoids and monkeys for the human bacillus and slightly greater for bovine strains [12]. The subcutaneous route of experimental infection is used because it is reliable and known to be effective. The alternative route of aerosolization of sputum might seem nearer to the human situation but although the guinea pig is susceptible to tuberculosis they rarely contract the disease naturally and while they may excrete the tubercle bacillus in urine and faeces, natural infection among cage mates is uncommon and the lungs are seldom prominently involved [13].

Our study does not offer conclusive proof that bacilli from patients on anti-tuberculous drugs causes disease in man. However, consideration of the evidence supporting claims that non-infectivity could be assumed at two or three weeks or even two months shows that it is based on inadequate data and is not supported by our study. In fact there is no direct evidence and some of the indirect evidence is poor [14].

Modern chemotherapy rapidly and dramatically reduces the number of bacilli in the sputum and is effective in reducing cough, and by these mechanisms greatly and quickly reduces the risk of spreading infection. We know from epidemiological studies that the risk of infection in close family contacts, once chemotherapy has been established, is negligible but to extrapolate that this is due to chemotherapy is unwarranted. The index case is most infectious before initiation of therapy. If transmis-

sion of infection to close family contacts has not occurred in the pre-treatment phase it will not occur after therapy has begun. What this study shows is that if the patient continues to cough and remains sputum positive, particularly culture positive, he produces bacilli which are pathogenic. It therefore re-opens the question as to the possibility of transmitting infection from patients on chemotherapy to non-immune new contacts. It also raises the possibility of infecting patients already immunocompromised, either by co-existing disease or iatrogenically or by AIDS. The position with regard to the discharge of patients with tuberculosis on chemotherapy to their own homes is clear [2, 5]. We suggest, however, that patients who have sputum positive tuberculosis and are in hospital despite being on effective chemotherapy cannot be regarded as non-infectious. The organisms from such patients retain the ability to cause disease in guinea pigs and there is no direct evidence that their pathogenicity for susceptible or hypersusceptible humans is different.

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Caractère pathogène de Mycobacterium tuberculosis au cours du traitement. L.J. Clancy, P. Kelly, L. O'Reilly, C. Byrne, E. Costello.

RÉSUMÉ: Pour expérimenter le caractère pathogène de *Mycobacterium tuberculosis*, nous avons étudié un modèle expéri-

mental chez le cobaye. Des échantillons d'expectorations ont été injectés par voie sous-cutanée aux cobayes, et les animaux ont été tués après huit semaines, et une autopsie a été pratiquée. Les risques qu'avaient les échantillons d'expectorations de produire une tuberculose du cobaye, furent en relation avec la positivité à la culture plutôt qu'avec la durée de la chimiothérapie. Cette étude ne renforce pas l'hypothèse selon la quelle modification du caractère pathogène des mycobactéries apparaît au cours du traitement de la tuberculose pulmonaire.

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