DECREASED RESISTANCE TO MYCOBACTERIAL INFECTION IN MICE FED A TRICHOTHECENE COMPOUND (T-2 TOXIN)

Koomi KANAI and Eiko KONDO

The First Department of Bacteriology and Department of Tuberculosis, National Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141

(Received January 31, 1984. Accepted March 15, 1984)

SUMMARY: The effect of T-2 toxin, a trichothecene compound, on bacterial infection was examined in mice infected intravenously with mycobacteria. T-2 toxin dissolved in olive oil was given orally in a dose of 0.1 mg, six to 12 times, at various stages of infection. The resistance-decreasing effect of the toxin was judged by two different criteria, the mouse survival period and the fate of tissue viable counts. This effect was accompanied by a decreased spleen weight. T-2 toxin was found to be a more potent immunosuppressing agent in this model than 5 mg of cortisone given intraperitoneally according to a similar schedule. In view of these observations, the potential importance of this mycotoxin was considered in relation to food hygiene.

INTRODUCTION

Trichothecene compounds known as potent mycotoxins are divided into three groups according to their chemical structures. T-2 toxin produced by several species of the genus Fusarium is one of them, and its chemical structure is 4, 15-diacetoxy-8-(3-methylbutyryloxy)-12, 13-epoxy-Δ9-trichothecene-3-ol (1). The toxin causes various pathological lesions and symptoms in experimental animals, domestic animals and men because of their cytotoxic properties (2). In fact, T-2 toxin was discovered on the basis of an epidemiological record suggesting that it is the etiologic substance of nutritional toxicosis of man and domestic animals.

More recently an immunological review summarized the data attesting that T-2 toxin has a potent immunosuppressing activity (3). This finding led us to an idea that T-2 toxin would be of practical importance not only relating to simple food poisoning but also as a resistance-lowering agent to infection in general. However, only limited information is available on this aspect. The work by Boonchuvit, Hamilton and Burmeister (4) in chicken salmonellosis may be the first example of this kind.

金井典美（国立予防衛生研究所細菌第一部）
近藤洋子（国立予防衛生研究所結核部）

— 97 —
We think that chronic infections are the preferable experimental model to acute ones for examination of any agent which might interfere with the resistance to infection, since we can easily adjust the treatment during a long period of persistent infection. With this idea in mind, an attempt was made to examine T-2 toxin for the effect on experimental mouse mycobacteriosis.

MATERIALS AND METHODS

Experimental animals were male mice of the ddY strain, weighing 18 to 20 g. Every 10 mice were housed in a metal cage floored with wood shavings and fed on pellet diet and water. In most cases, they were nourished before use to make their body weight around 30 g.

The microorganisms were a kanamycin-resistant strain of tubercle bacilli (H37RvR-KM) and Mycobacterium bovis (Ravenel strain and BCG). Bacterial suspensions were prepared by the routine method (5) from the pellicle growth of 2-week cultures on Sauton synothetic liquid medium. In every instance, injection was made intravenously in the tail vein with such suspensions.

T-2 toxin was dissolved in olive oil in a concentration of 1 mg per ml and given orally with a cannula in an amount of 0.1 ml. Generally, the schedule of toxin administration was six to 12 times starting from before or after the infection. The administration was made carefully; attention was paid to the general condition of animals and sometimes the interval between administrations was adjusted. As a reference drug for T-2 toxin, cortisone acetate was employed for comparison of the immunosuppressing activity. It was given intraperitoneally in 5-mg doses under a similar time schedule (6).

Evaluation of the effect of T-2 toxin on the infection was made by two criteria; the mouse survival period and the viable count in the tissues. Tissue viable count was made as described before (5).

RESULTS

Three groups of mice, each consisting of 14, were arranged. All the animals were infected with 0.01 mg of H37RvR-KM tubercle bacilli. The mice of one group were treated with T-2 toxin, starting from a day before infection, seven times at one-day intervals and then five times daily. Those of another group were treated with cortisone acetate in almost the same time schedule but three more times at the later stage. The remaining group was left untreated as the control group of infection only. Four, 12, 15 and 20 days after infection, three or four animals were sampled at random from each group to be subjected to autopsy, measurement of spleen weight, and viable counts.

The results are given in Figs. 1 and 2. In Fig. 1, the time course of spleen viable counts in the control group shows a typical pattern of experimental mouse tuberculosis of chronic type. After the peak in 12 days, the infection declined to some extent and then tended to persist. It should be noted that the host-parasite equilibrium as suggested here was accompanied by enlargement of the spleen (Fig. 2).

In contrast with this pattern, the fate of tubercle bacilli in the spleens
PERIOD OF T-2 TREATMENT

0.1 mg orally x 12

Fig. 1. Effect of oral administration of T-2 toxin on the fate of tubercle bacilli in the mouse spleen.

Fig. 2. Effect of oral administration of T-2 toxin on the spleen weight in experimental mouse tuberculosis.
of the T-2 group was continuous multiplication. The spleen weight became smaller in later stages of infection. These animals gradually lost their body weights from around 10 days of infection, so the last sacrifice of animals was made 2 days earlier than the other two groups. The difference in the spleen viable count between the T-2 group and the control group was a one-log level. In the cortisone-treated group, the time course of spleen viable counts and the spleen weight are mutually well correlated. In 15 days, the infection-enhancing effect of cortisone was expressed more markedly than T-2, but in 20 days the mice of this group appeared to have acquired resistance as suggested by the decline of spleen viable counts (Fig. 1). Interestingly enough, this tendency was also accompanied by spleen enlargement (Fig. 2).

In the next experiment, the effect of T-2 toxin on the vaccinated animals was examined to see the T-2 interference with the pre-established immunity to infection. The experimental method was basically the same as the previous one with the arrangement of four groups of 15 mice, in two of which the mice had been vaccinated intravenously with 1 mg of BCG 19 days before infection. The remaining two groups were left unvaccinated. In each of the groups of nonvaccination and vaccination, T-2 treatment was to be conducted daily for 15 days starting from 5 days before infection. However, it was soon found that BCG-vaccinated mice were highly sensitive to T-2 treatment showing general malaise and three cases of death. Therefore, the actual T-2 administration was performed more carefully with longer time intervals for three times before and six times after infection. In the nonvaccinated group, it was conducted three times before and eight times after infection. The periodical lung and spleen viable counts are shown in Figs. 3 and 4. These figures present good evidence to prove that the protective immunity was established in BCG-vaccinated groups suppressing completely the multiplication of the challenge inoculum, and that this immunity was so impaired in the mice treated with T-2 toxin that the challenge strain recovered showed larger viable counts than that from the untreated BCG group. A remarkable observation was concerned with the spleen weight. The spleens of the BCG-vaccinated mice were enormously enlarged weighing from 500 to 1000 mg (860 mg on the average) at the time of sacrifice. The T-2 treatment reduced this weight down to 380 mg on the average.

From the results of the preceding two experiments, the next experiment was designed to give T-2 toxin in a milder schedule, totally six times at 2-day intervals, and to assess its effect according to the modification of survival days in fatally infected mice. Four groups of 10 mice each were arranged, two of which were infected with 0.25 mg of *M. bovis* (Ravenel) and the remaining two groups left uninfected. Each group was subjected to T-2 treatment according to the above schedule from the 8th day of infection. During the course of the experiment, the mouse body weight was frequently
Fig. 3. Effect of T-2 toxin on the fate of tubercle bacilli in the lungs of normal and BCG-vaccinated mice.

Fig. 4. Effect of T-2 toxin on the fate of tubercle bacilli in the spleens of normal and BCG-vaccinated mice.
Fig. 5. Body weight change in the mice uninfected and infected with virulent *M. bovis* (Ravenel) and treated and not treated with T-2 toxin.

Fig. 6. Time course of % survival of the mice uninfected and infected with virulent *M. bovis* (Ravenel) and treated and not treated with T-2 toxin.
measured as an indicator of T-2 toxicity. The results are shown in Fig. 5. The curves of per cent survival are presented in Fig. 6. As seen in Fig. 5, the T-2 treatment alone showed little toxicity as suggested by almost the same course of body weight increase as that of the uninfected and untreated control. Even under these experimental conditions, T-2 toxin exerted a marked effect on the survival period of the infected mice. The average survival period of the infected mice was shortened from 34.9±8.5 days to 19.0±3.4 days by the treatment with T-2 toxin.

DISCUSSION

All the experiments here described offered ample evidence to show that T-2 toxin is a potent immunosuppressing agent highly active in bacterial infection. It is amazing that T-2 toxin in such a small dose as 0.1 mg given orally is much more effective in this respect than 5 mg of cortisone given intraperitoneally. Emphasis should also be placed on the fact that this toxin induces an atrophic change of the spleen, especially when it is responding to the immunologic stimuli. This suggests a possibility that the target of T-2 toxin is T cells. In agreement with this supposition, Boonchuvit et al. (4) reported that the aggravation of salmonella infection and atrophy of the spleen were well correlated in the chickens treated with T-2, but that the titers of the agglutinin formed in response to the infection were unaltered by T-2 toxin. Sato et al. (7) also found that the decreased spleen weight caused by T-2 toxin did not influence the production of HI antibodies in the chickens infected with Newcastle disease virus. Masuda et al. (8) demonstrated that the cytotoxicity of fusarenon-X, another trichothecene compound, was stronger to T cells than to B cells in tissue culture environment.

In mycobacterial infection, the role of antibodies in protective immunity has never been established. In this regard, it is also acceptable that the aggravation effect of T-2 toxin on mycobacterial infection is brought about by its effect on the T-cell system. Macrophages can possibly be another target. However, Otokawa, Sugimoto and Ueno (personal communication) observed that the morphological change of macrophages from the extended form to the round form in the presence of 12.5 ng of T-2 toxin per ml in tissue culture was not associated with their phagocytic activity. T-2 toxin enhances the sensitivity of mice to not only bacterial infection but also to the infection with Japanese encephalitis virus (Takeda, Otokawa and Ueno, personal communication).

Apart from the theoretical aspects as above, the present study revealed the potential importance of T-2 toxin in food hygiene for man and domestic animals. Especially, its resistance-lowering effect on infection could be a chronic type of hazard associated with diets. The surveillance to see to what extent this is the case in our living environment will be worthy of attempt.
ACKNOWLEDGEMENT

This study was made possible by a research grant from Science and Technology Agency of Japan. We are grateful to Dr. Y. Ueno of Tokyo University of Science who donated us T-2 toxin generously.

REFERENCES