Illinois Academy of Science Transactions, Vol. 46, 1953

SURVIVAL FOLLOWING TREATMENT WITH SULFONES IN EXPERIMENTAL TUBERCULOSIS IN GUINEA PIGS

BEN C. SHER AND JOHN M. KLOECK
Municipal Tuberculosis Sanitarium, Chicago

From the time that Robert Koch isolated and successfully cultured *Mycobacterium tuberculosis hominis,* the bacillus causing tuberculosis, the world has waited hopefully for an effective means for combating this disease. The chief hope seemed, from the beginning, to lie in bacteriological and vaccine approaches, and since that time there has been constant activity and much progress in these directions. Meanwhile, advances were made in the use of X-rays, testing for early diagnosis, and subsequent sanitarium care where indicated.

Extensive studies were made in the use of drugs and antimicrobial agents in experimental and clinical tuberculosis. The encouraging results led to their use as adjuncts to the older and proved methods of treatment. And now the antimicrobial agents, among them, para-amino salicylic acid, streptomycin, dihydrostreptomycin, and isonicotinic acid hydrazid have become of prime importance and have opened up new potentialities in this field. It is hoped that these advances are only the forerunners of progress to come in the constant search for new and effective agents and methods for the treatment and prevention of this disease.

In order to select new substances more expeditiously, they are usually screened in-vitro so as to establish a table of relative tuberculostatic values. The use of these values presupposes: (1) that the compounds are sufficiently soluble in the culturing media to give an effective concentration, and (2) that some of the compounds which might be effective in animal tests may have been ruled out prematurely. But since it is impractical to establish in-vivo ratings on every compound made available for this purpose, a method is needed even though it has limitations. On this basis it seemed logical to select compounds from a table of correlations between in-vivo and in-vitro ratings. Promin (Sodium P,P′-Diaminodiphenylsulfone-N,N′Didextrosesulfonate) and Sulfone 1048 (Disodium salt of N,N′Bis-(B-carboxy-B-acetamido-ethylthiamethyl)-4,4′-diaminodiphenylsulfone) appeared to be the most promising from the results of these studies. They also demonstrated further promise in some exploratory studies against experimental tuberculosis, previously reported (1), and were, therefore, selected for these studies.

METHOD

Previous work demonstrated that under optimum conditions Sulphone 1048 retarded or inhibited the tuberculous process to such a degree that

there were no macroscopic post mortem findings. The duration of these experiments was 45 days. It would have been of interest had we been able to prolong these experiments indefinitely to determine if positive findings would develop. Consequently in designing the experiments for this investigation the purpose was not so much to determine how effective these drugs were but rather to determine what the prolonged effect would be following a comparatively short period of treatment.

Animals and controls. — Thirty young female guinea pigs were adjusted to the routine of the animal room for nine weeks. At the end of this time their weights ranged from 610 to 730 grams. The left groin of each pig was inoculated with 0.0005 mg. of H37 RV tubercle bacilli which was cultivated for one month on Saenz media. The infecting emulsion was prepared by mechanically suspending a weighed quantity of the tubercle bacilli in a heavy-walled glass tube equipped with a pestle ground to fit (2). The bacilli were first ground in a mechanical grinder and gradually brought to the required concentration by diluting the suspension with physiological salt solution.

Drug therapy. — Treatment was started two days after infection and continued daily for 58 days. The drug was administered by mixing it with chopped green vegetables in sufficient quantity so that the Promin treated group received 400 mg. per pig per day, and the Sulfone 1048 treated group received 300 mg. per pig per day. The addition of 40 percent sucrose syrup made this mixture more acceptable to the guinea pigs. For purposes of control each animal in the control group received the same quantity of chopped vegetables and sucrose syrup daily. In previous work we and other workers have consistently obtained uniform results with this technique. After 60 days the animals were given only routine care, and autopsy findings were recorded after death.

Complications.—During the course of this experiment three animals died of pneumonia. There was the possible contributing factor of a protracted heat spell, for it was during such a period, 64 to 67 days after the experiment started, that these animals died. Three animals, one from each group, were sacrificed one month after the experiment started to observe the progress of the disease. These findings are discussed below.

Results

Ordinarily, comparative survival times are calculated from the date of infection of the experimental animals. On this basis the average survival time of the treated animals was more than three times as long as that of the untreated control animals; 68 days was the average survival time of the controls as compared with 211 days for the Promin treated group and 227 days for those treated with Sulfone 1048. These comparisons significantly indicate the effectiveness of these drugs against experimental tuberculosis in guinea pigs. They show that Sulfone 1048 is somewhat more effective than Promin, a trend consistently obtained in our in-vivo and in-vitro experiments.

For this discussion, however, it is more instructive to compare the survival times for those periods during which no treatment was given. Therefore, inasmuch as treatment was stopped 60 days after the infecting inoculation was administered, 60 days are subtracted from the above survival period of the treated groups. This leaves 151 days for the Promin treated group and 167 days for the Sulfone 1048 group, or more than twice as long in each series as the average life span of the control group.

Importance is attached to this observation because under comparable circumstances it should be anticipated that animals starting with a considerable amount of tuberculosis would die sooner than a freshly infected group of animals; instead, the reverse of this was true. That there was a formidable amount of tuberculosis at around 60 days is established by evidence that was obtained from the post-mortem findings of a limited number of animals sacrificed during this period, and from the findings in those animals dying meanwhile of other causes. The post-mortem ratings at 32 days for Promin treated animals was 68; for the Sulfone treated animals it was 16; and for the control animals at 26 days it was 68. At 64 and 67 days the treated animals showed ratings of 68 and 90, while the rating in the control animals was 100 at 37 days and beyond. Also, it may be established, on the basis of presumptive evidence from comparable experiments, that while the animals were under treatment the disease progressively increased with time. This evidence is derived from a series of 113 guinea pigs similarly treated with the above and similar sulfones. Periodically groups of these animals were sacrificed within a period of 26 to 100 days. A composite summary of the post-mortem ratings is shown in table 1.

Table 1 shows that the treated animals developed tuberculosis at a slower rate than the control animals did. However, in less than 4 months the amount of involvement was equal to or greater than the lethal amounts found in the control groups. After 4 months, although this is not indicated in the table, the disease continued to spread to such an extent that post-mortem ratings no longer served as a measure of the extent of the disease. Here there is an appar-

TABLE 1.—POST-MORTEM RATINGS

Treated Groups		Untreated Groups	
Duration in days	Post-mortem ratings*	Duration in days	Post-mortem ratings*
30	0-10%	26	48%
45-66	42-57%	45	87%
88-100	72-100%	59	88%-100%
116 days and beyond	100%	64 days and beyond	100%

* In order to estimate the post-mortem rating or gross involvement a sum of 100 was set for maximum involvement, and the individual organs were assigned the following numbers to indicate the maximum gross involvement in that organ:

Lymph nodes 8
Spleen 24
Liver 28
Lungs 40

This arbitrary choice is based on the hypothesis that the organs which are most readily involved should be given lower values. Also, since each number is divisible by 4, the common procedure of designating the amount of involvement of each organ by +, ++, +++, ++++ is readily transferred into this system of evaluation.

ent paradox in that more tuberculosis is found in animals receiving beneficial treatment than in untreated groups. This could only come about because the animals developing the disease under the protection of chemotherapy survived for a longer time, thus permitting fuller development of the lesions. The animals in this present series living longer than 4 months lived from 149 to 390 days, and the longer the survival period the more massive the involvement appeared to be.

Obviously the lethal nature of the tuberculosis found here was greatly modified and moderated because of the administration of these drugs. This is all the more significant because there was no localizing of the lesions nor was there any chemical sterilization or signs of regressions. Thus the animal body tolerated large quantities of active tuberculosis over a long period of time.

The suggestion presents itself then that an immunity has been produced, despite the progressive tuberculous process. This may correspond to some clinical observations made in the earlier use of isonicotinic acid hydrazid, used then primarily in cases which did not respond to para-amino salicylic acid or streptomycin therapy. It was reported in some instances that patients under this treatment had a feeling of well being and showed improvement without the corresponding roentenological improvements being found. Also, in chronic cases of tuberculosis there may be extensive involvement, which in other individuals would be sufficient to cause death. Here we have a difference in the response to the organism, probably due to immunilogical differences.

This leads to the provoking question of how antimicrobial agents may affect the course of disease. In clinical tuberculosis the organisms are present when therapy is begun. Some immunity may have been established by this time, owing to growth of the tubercle bacilli in the host, yet the immunity is insufficient to enable the host to overwhelm the disease without assistance. The question then arises whether the antimicrobial agent produces an added immunization or similar effect, causing further improvement.

That this might be so may be inferred from the preceeding experiments, in which survival time increased for the most part after treatment was stopped. It might also be inferred from some previous studies on the combined action of diamino diphenyl sulfone and immunization (vaccination). In these studies it was determined, among other things, that the combined action of vaccination and the sulfone was more effective than vaccination alone.

Summary and Conclusions

(1) Sulfone 1048 and Promin were tested against experimental tuberculosis. Both were effective and increased survival time more than three times as compared to the controls.

(2) Sulfone 1048 was more effective than Promin, which corroborates the in-vitro findings.

(3) Although the drugs were administered for only 58 days, the effectiveness remained after treatment stopped, and the animals survived without drugs twice as long as the control animals. Probably some immunological response was stimulated by the action of these drugs.

REFERENCES CITED

1. Derivatives of P,P′-diaminodiphenylsulfone and sulfanilamide in experimental tuberculosis, Henry C. Sweany, Ben C. Sher, and John M. Kloeck: Amer. Rev. of Tuberculosis, vol. 53, no. 3, March 1946.

2. The combined action of P,P′-diaminodiphenylsulfone and immunization in experimental tuberculosis, Ben C. Sher and John M. Kloeck: Amer. Rev. of Tuberculosis, vol. 53, no. 3, March 1946.