Comparison of 4,4'-Diaminodiphenylsulfone and Its Thymolated Derivative in Experimental Tuberculosis in Guinea Pigs

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Although 4,4'-diaminodiphenylsulfone (DDS) has definite antituberculosis effects in guinea pigs infected with the human type of tubercle bacilli, the toxicity of this drug has precluded its acceptance in the treatment of tuberculosis in humans. DDS and a few of its derivatives are widely used in leprosy because no other equally effective agents are available for the treatment of this disease; however, the toxicity of these drugs also limits their usefulness in this disease. Consequently, a large number of derivatives of DDS have been prepared and studied in an effort to obtain a relatively nontoxic but active antimycobacterial drug. The evidence indicates that most of these derivatives may have no advantage over DDS itself.

Of current interest is a derivative of DDS that is said to be relatively nontoxic. It is 4,4'-bis (6-thymylazo)-diphenylsulfone, which is derived from DDS by diazotization and coupling with thymol (Fig. 1). This drug will be referred to as DDS-thymol.

Therapeutic trials with DDS-thymol in tuberculous patients who failed to respond to other treatment indicated that the drug had beneficial effects and was nontoxic. It is reported to be nontoxic for guinea pigs, which is in contrast to the well-known hematotoxic effect in guinea pigs of DDS and some of its derivatives.

DDS-thymol had no therapeutic activity in experimental tuberculosis in mice. In tuberculous guinea pigs, however, the daily administration of 400 mg. of DDS-thymol increased the survival time and promoted healing of the ulcer at the site of inoculation. Also, fewer acid-fast bacilli were present in smears of various organs in comparison to the findings in untreated animals.

The following report describes a study in which it was found that administration of DDS-thymol to tuberculous guinea pigs resulted in regression and healing of lesions as determined by gross and histopathologic examination. This compound appeared to be less effective than DDS but it was less toxic.

Methods

Each of 36 mature male guinea pigs, weighing approximately 600 gm. apiece, received an intraperitoneal injection of 0.1 mg. (moist weight) of virulent human-type tubercle bacilli (H37Rv). On the 16th day after infection, six animals were killed to obtain evidence that a progressive disease had been established; these six animals were the pretreatment...
controls. On the same day, the remaining 30 animals were divided into three groups of 10 animals each as follows: (1) controls; (2) those treated with DDS,* 0.5 per cent in the diet; (3) those treated with DDS-thymol,* 1.0 per cent in the diet. All animals were fed Purina rabbit chow** to which was added 0.1 per cent of ascorbic acid.

The drugs (in powdered form) were mixed daily with the diet. The amount of DDS-thymol was selected somewhat arbitrarily. A preliminary trial showed that normal guinea pigs continued to gain weight when fed a diet containing 1.0 per cent of DDS-thymol. The amount of DDS was selected on the basis of unpublished observations showing that a diet containing 0.5 per cent of DDS, although hematotoxic, had a great therapeutic effect on tuberculosis in guinea pigs.

Six of the control animals had died by the 131st day of infection, and three of the four remaining controls were losing weight. Therefore, the experiment was discontinued on the 132nd day of infection (116 days of treatment). All surviving animals were killed. The lesions seen at necropsy were recorded schematically as shown in Figure 2. Suitable portions of tissue were preserved for histopathologic examination.

Results

Mortality.—As already indicated, six of the control animals had died by the 131st day of infection (Fig. 2). Of those given DDS-thymol, two died after only 3 and 20 days of treatment, respectively, and were considered as failures. Two other animals in this group died after 53 and 81 days of treatment, respectively. No deaths occurred in the group treated with DDS.

Gross and Histopathologic Observations.—The animals killed on the 16th day of infection (pretreatment controls) had visible lesions in the liver and spleen (Fig. 2); small tuberculous foci were seen microscopically in the lungs. It is presumed that the disease in the remaining animals, when the treatment period started, was comparable to that in the pretreatment controls. The lesions in the control animals were typical of the widespread destructive tuberculous process usually seen in experimentally infected guinea pigs.

In the group treated with DDS-thymol, the two animals considered as failures had lesions similar to those of the controls, as did also the two animals which died after 53 and 81 days of treatment. Of the six animals which received treatment for the entire 116-day period, one had a few visible lesions in the liver. This animal, as well as two others, had small but active microscopic caseous foci in the lungs. Aside from this, sections of lungs, liver and spleen of the animals given DDS-thymol contained no other microscopic evidence of actively progressing tuberculous foci. However, caseous lesions were present in the tracheobronchial, hepatic and iliac lymph nodes of each animal.

*The DDS and DDS-thymol were obtained from Dr. E. F. Roberts, Wyeth Laboratories, Philadelphia, Pennsylvania.

**Purina rabbit chow is manufactured by the Ralston Purina Company, St. Louis, Missouri.
Of the animals treated with DDS, two had multiple miliary lesions in the lungs visible at necropsy and two others had small caseous pulmonary lesions demonstrable microscopically. Peculiarly, active lesions were not seen in sections of liver or spleen of the four animals with pulmonary lesions. Sections of lungs, liver and spleen of the other animals in this group were free of active tuberculous disease. However, caseous foci were present in the tracheobronchial, hepatic and iliac lymph nodes of all the animals treated with DDS.

The index of infection, based on the extent and character of the lesions as determined histopathologically, was as follows: pretreatment controls, 55; controls, 90; those receiving DDS-thymol, 1.0 per cent in the diet, 30.0; those receiving DDS, 0.5 per cent in the diet, 15.0. The index for the animals treated with DDS-thymol includes the two which died after 53 and 81 days of treatment. If these are excluded, an index of 20 was determined for the six animals treated during the entire 116-day period.

Toxicity.—Although this study was made primarily to determine the therapeutic effect of DDS-thymol, certain limited observations suggest that this drug was well tolerated. The animals gained weight during the period of treatment. Sections of kidneys, lungs, liver and spleen showed no morbid changes that could be attributed to the drug.

The hematotoxic effects of DDS were manifested by the enlargement and the deep-purple color of the spleens of all animals in this group. Microscopically, the splenic sinusoids were greatly distended. Numerous pigment-laden macrophages lined the sinusoids and also were situated within the splenic cords. This pronounced change in the structure of the spleen was not seen in the animals given DDS-thymol.

Comment

These results show that the administration of DDS-thymol to tuberculous guinea pigs resulted in regression of the disease but give no indication

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4,4'\text{-diaminodiphenylsulfone} \\
\quad \\
4,4'\text{-bis(6-thymylazo)-diphenylsulfone}
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FIGURE 1: Structural formula of 4,4'-bis (6-thymylazo)-diphenylsulfone (molecular weight 570.7) compared to that of 4,4'-diaminodiphenylsulfone (molecular weight 248.3).
of the mode of action of the drug. There is a question as to whether DDS-thymol acts directly or owes its effect to the liberation of DDS. According to Rist and associates,9 90 per cent of the DDS-thymol given orally to mice is eliminated in the feces, which accounts for its inactivity against tuberculosis in this species. These workers considered that the reported low toxicity of DDS-thymol is due to the fact that it is stable and releases only small amounts of DDS in vivo. Furthermore, Rist's group ascribed the reported beneficial effects of DDS-thymol in tuberculosis and leprosy to liberation of small amounts of DDS over a prolonged period of therapy. In guinea pigs, on the other hand, Cotereau and associates12, 13 reported that DDS-thymol is absorbed and is present in the blood in concentrations as much as 20 times those of the DDS which is liberated. These workers thought that DDS-thymol acts directly and does not owe its effect to the release of DDS.

FIGURE 2: Schematic representation of the lesions seen at necropsy. The numbers below each animal indicate the duration of infection in days; the black bar indicates that the animal died. The oval and rectangle represent spleen and liver, respectively; the dots indicate miliary or nodular lesions, whereas complete blackening means diffuse tuberculous involvement. A dot in the arrow indicates involvement of retrosternal lymph nodes. Two animals given DDS-thymol are considered failures because they died prematurely.
In the present study, three animals died after 20, 53 and 81 days of treatment, respectively, with no evidence of any therapeutic effect from DDS-thymol. This may have been the result of poor absorption, which, however, was sufficient for a cumulative effect in animals treated for 116 days. A second possibility is that DDS-thymol does not act directly but owes its effect to liberation of DDS in amounts that are not toxic but are sufficiently great to account for the therapeutic benefit.

SUMMARY AND CONCLUSIONS

Each of 36 guinea pigs was infected intraperitoneally with virulent tubercle bacilli. After 16 days, six were killed and found to have tuberculous lesions. On the same day, the remaining animals were divided into three groups of 10 animals each, namely controls, those treated with 4,4'-diaminodiphenylsulfone (DDS), and those treated with DDS-thymol. Treatment continued for 116 days, at which time all survivors, were killed. Six controls had died by this time. All controls, including four survivors, had extensive tuberculosis.

Of those animals treated with DDS-thymol, two died prematurely; two others died after 53 and 81 days of treatment, respectively, and had extensive lesions. Of the surviving six animals, one had a few residual foci in the liver and lungs and two others had microscopically evident lesions in the lungs only. All had caseous lesions in lymph nodes. None of these animals showed any toxic effect from the drug.

None of the animals given DDS died; four had evidence of pulmonary tuberculous lesions. All had caseous foci in the lymph nodes and enlarged cyanotic spleens with dilated sinusoids and many pigment-laden macrophages.

The administration of DDS-thymol to tuberculous guinea pigs resulted in regression of the disease, but the beneficial effect was less than that of DDS. The hematotoxic effects of DDS, as determined by splenic changes, were not seen in animals given DDS-thymol. It was not determined whether DDS-thymol has a direct action or if it is active because of DDS liberated in vivo.

RESUMEN Y CONCLUSIONES

Se infectaron con bacilo tuberculoso virulento 36 cuyes, usando la vía intraperitoneal. Después de 16 días, seis se sacrificaron y se encontró que tenían lesiones tuberculosas. El mismo día los animales restantes se dividieron en tres grupos de 10 cada uno como controles, el segundo grupo se trató con 4,4'-diaminodifenilsulfona (DDS) y el otro grupo con DDS-timol. El tratamiento se prolongó por 116 días a cuyo término se sacrificaron todos los sobrevivientes.

Durante este tiempo murieron seis controles. Todos los controles incluyendo los sobrevivientes tenían tuberculosis extensa.

De los animales tratados con DDS-timol dos murieron prematuramente; otros dos murieron después de 53 y 81 días de tratamiento respectivamente y tenían lesiones extensas. De los sobrevivientes seis animales, uno tenía
un foco residual en el hígado y otros dos tenían lesiones microscópicas evidentes sólo en los pulmones. Todos tenían lesiones caseosas en los ganglios. Ninguno de estos animales mostró efecto tóxico por la droga.

Ninguno de los animales a los que se dió DDS murió; cuatro tenían evidencias de tuberculosis pulmonar. Todos tenían focos caseosos en los ganglios linfáticos y bazos crecidos y cianóticos con sinusoides ensanchados y muchos macrófagos cargados de pigmento.

La administración de DDS-thymol a los cuyes tuberculosos produjo regresión de la enfermedad pero el efecto benéfico fue menor que en los tratados por DDS según se determinó por los cambios esplénicos que no se vieron en los que recibieron DDS-timol.

No se determinó si el DDS-timol tiene una acción directa o si es activo por la liberación de DDS en vivo.

RESUME

36 cobayes furent inoculés chacun par voie intrapéritonéale avec des bacilles tuberculeux virulents. Après 16 jours, six furent tués et on constata qu'ils étaient porteurs de lésions tuberculeuses. Le même jour, les animaux restant furent divisés en trois lots de dix animaux, les témoins, ceux traités par el 4,4'-diaminodiphenyl sulfone (DDS) et ceux traités par el DDS-thymol. Le traitement fut poursuivi pendant 116 jours, au bout desquels tous les survivants furent sacrifiés. Six témoins étaient morts pendant ce temps. Tous les témoins, y compris les quatre survivants, étaient atteints de tuberculose extensive.


Aucun des animaux que avaient reçu du DDS ne mourut; quatre avaient des lésions tuberculeuses pulmonaires évidentes. Tous avaient des foyers caséeux dans les ganglions, et des rates cyanosées et volumineuses, avec des sinus dilatés et chargés de macrophages pigmentés.

L'administration de DDS-thymol aux cobayes tuberculeux a permis la régression de l'affection, mais l'effet favorable fut moindre que celui du DDS. Les effets hémato-toxiques du DDS, comme ils ont été révélés par les altérations de la rate, n'ont pas été constatés chez les animaux traités par el DDS-thymol. Il n'a pas été possible de savoir si el DDS-thymol a une action directe ou s'il est actif grâce au DDS libéré in vivo.

ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNGEN

Ein jedes von 36 Meerschweinchen wurde intraperitoneal infiziert mit virulenten Tuberkelbazillen. Nach 16 Tagen wurden 6 getötet und bei ihnen tuberkulöse Herde gefunden. Am gleichen Tag wurden die übrigen Tiere in 3 Gruppen von je 10 Tieren eingeteilt, nämlich die Kontrollen,
solche, die mit 4,4'-Diaminodiphenylsulfon (DDS) behandelt wurden, und schliesslich die mit DDS-Thymol behandelt wurden. Die Behandlung wurde 116 Tage lang fortgesetzt und zu diesem Zeitpunkt alle überlebenden Tiere getötet. 6 Kontrolltiere waren während dieser Zeit umgekommen. Alle Kontrolltiere einschl. der 4 Überlebenden hatten eine ausgedehnte Tuberkulose.


Keines der Tiere, die DDS bekamen hatten, starb. 4 hatten Anzeichen von pulmonalen tuberkulösen Herden. Alle hatten Käseherde in den Lymphknoten und eine vergrösserte cyanotische Milz mit erweiterten Sinus und zahlreichen pigmenthaltigen Makrophagen.

Die Verabreichung von DDS-Thymol an tuberkulöse Herrschweinchen hatte eine Rückbildung der Erkrankung zur Folge, jedoch war die Heilwirkung geringer als diejenige von DDS. Die haematotoxischen Wirkungen von DDS, wie sie sich in den Milzveränderungen ergaben, war nicht bei den mit DDS-Thymol behandelten Tieren zu sehen. Es wurde nicht ermittelt, ob DDS-Thymol eine direkte Wirkung hat, oder ob es aktiv ist durch in vivo frei gewordenes DDS.

REFERENCES

8 Salomon, L.: Quoted by Weiller, Pierre and Rymer, Maurice.