

THE EFFECTS OF 4-4'-DIAMINODIPHENYL SELENIDE,
DIPHENYL SULFONE, AND 4-4'-DIAMINODIPHENYL IN THE
TREATMENT OF MURINE LEPROSY

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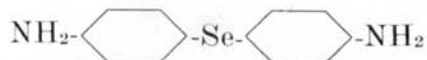
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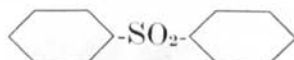
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In previous reports, by Hadler, Carvalho and Mauri (²) and Hadler and Ziti (³), certain compounds structurally related to 4-4'-diaminodiphenyl sulfone (DDS) were studied with respect to their activity on murine leprosy. The present report concerns an investigation of other substances, also chemically related to DDS, in a further attempt to test the structure-activity relationship. The following three substances were used:¹

- (1) 4-4'-diaminodiphenyl selenide:



- (2) diphenyl sulfone:



- (3) 4-4'-diaminodiphenyl (benzidine):



The chemical analogy between these substances and DDS,



The activity of these compounds has been investigated on the basis of the survival rate, and of the histologic structure of the lesions, of rats inoculated with *M. leprae murium* and treated by these drugs, as compared with inoculated but untreated control animals. The effects have also been compared with the known effects of treatment with DDS reported by Mauri, Hadler and Carvalho (⁴) in animals inoculated and kept under the same conditions.

¹These substances were prepared by the Laboratory of Chemotherapy of the Instituto Butantan, São Paulo, Brazil.

MATERIAL AND METHODS

Wistar rats of both sexes, weighing 120-140 gm., were inoculated, the same *M. leprae murium* suspension being used for all. A dose of approximately 5.5 mgm. of bacilli was injected intraperitoneally into each animal. The treatment was started 7 days after the inoculation, and was continued until the animals died or were killed. All of the substances were administered orally, added to the food.

The numbers of rats used in each treatment, the dosages used, and the length of treatment with each drug, together with the results as expressed in means of survival and coefficient of regression, are indicated in Table 1. The dosages were chosen on the basis of preliminary studies of toxicity; high but apparently not toxic doses were used. The weight of each animal was taken every 10 days throughout the experiment, as a means of disclosing any toxic effects of the drugs. The toxic effects were also evaluated in uninoculated animals which had received the same drugs in the same dosages.

Most of the treated animals were retained until they died as a consequence of murine leprosy; these animals were used in determining the survival time. Other animals were killed periodically, providing material for the pathologic study of the disease; pieces of different organs were taken, fixed in a 10 per cent formalin solution, and paraffin sections were stained by the hematoxylin-eosin and the Ziehl-Neelsen methods.

The survival time was statistically estimated on the basis of the survival mean and of the regression of the number of dead animals in time, both calculated for each treatment and for the control group. The analysis of variance was used to test the significance of the differences among the means.

RESULTS

Based on the analysis of variance of the survival means, the results shown in Table 1 indicate that only the animals treated with DDS showed a longer survival time when compared with animals of the control group which received no drug. Treatment with 4-4-diaminodiphenyl selenide, or with diphenyl sulfone, does not produce any modification as far as the survival is concerned. The benzidine treatment, on the other hand, exerts some accelerative effect on the evolutive rate of the infection, since in the rats so treated the survival time was significantly shorter.

The pathologic studies carried out during the experiment show that only DDS treatment modifies the normal evolution and the microscopic aspect of the leprosy lesions. Only in this instance were regres-

TABLE 1.—Numbers of animals, doses of drugs, and length of treatment, and means of survival and coefficients of regression.

Drug	Number of animals	Doses (mgm/day)	Length of treatment (days)	Mean of survival (days)	Coefficient of regression
4-4-diaminodiphenyl selenide	20	6.5	238	160.8	-0.090
Diphenyl sulfone	20	30.9	204	151.7	-0.239
Benzidine	50	1.1	185	69.8	-0.420
DDS	20	40.0	802	427.4	-0.015
Control	20	—	277	187.3	-0.166

sive lesions seen. In the rats treated with 4-4'-diaminodiphenyl selenide, diphenyl sulfone and benzidine the lesions did not present any structural alteration, as compared with those of the control animals. These results indicate that these substances are not effective in producing involutive alterations of the lesions.

However, with respect to the appearance and the development of the lesions, the animals treated with benzidine differ from those of the control. In the benzidine-treated group the degree of development and the evolutive rate of the lesions were both increased. Rats inoculated with *M. leprae murium* and treated with benzidine show lesions, at the 110th-120th day after inoculation, which are comparable to those of the control animals on the 6th-7th month after inoculation, as far as the degree of development is concerned. This fact suggests that benzidine exerts an accelerating effect upon the evolutive rate of murine leprosy.

Benzidine and 4-4'-diaminodiphenyl selenide, in the doses used, showed a slight toxic effect as evidenced by the weight patterns of the animals which received them. However, the results obtained in a group of 20 rats treated with benzidine but not inoculated, indicate that benzidine alone decreases the survival of the animals.

DISCUSSION

The present investigation demonstrates that certain changes made in the DDS molecule, such as the replacement of the $-SO_2-$ group by selenium, or the removal of the SO_2 or of the NH_2 groups, abolishes its activity on murine leprosy. These results are in agreement with those previously reported (^{2,3}), according to which DDS lost its antileprosy activity when the SO_2 group was replaced by $-SO-$, $-S-$, $-CO-$ and $-NH-$ groups.

On the other hand, the present work shows a peculiar effect of benzidine, this substance accelerating the evolutive rate of murine leprosy. Benzidine exerts some toxic effects in the dose used, acting on the weight pattern and especially on the survival rate of the animals. These toxic effects, however, cannot account for the increased development of the leprosy lesions in the benzidine-treated animals; but that is regarded as the result of an aggravative effect of this drug. This statement is in agreement with the higher degree of development of the leprosy lesions in benzidine-treated rats which had died early.

The results indicate that the organic selenium compound employed is not effective against murine leprosy. This is in disagreement with the conclusion of Berny and Chabaud (¹) concerning rats inoculated with *M. leprae murium* and treated with organic selenium compounds, since they reported a decrease in the evolutive rate of the disease. However, the method employed in their experiment does not allow the correct evaluation of the effect of drugs on murine leprosy.

SUMMARY

In an attempt to study the activity of certain compounds structurally related to DDS on murine leprosy, the eventual effect of 4,4'-diaminodiphenyl selenide, diphenyl sulfone and 4,4'-diaminodiphenyl was investigated in rats inoculated peritoneally with *M. leprae murium*. The results were based on the survival rates and the histologic structure of the lesions of treated animals as compared with those which received no treatment.

4,4'-diaminodiphenyl selenide and diphenyl sulfone did not exhibit any alterations, either on the survival rate or on the structural aspects of the lesions. They did not show any therapeutic effect.

4,4'-diaminodiphenyl (benzidine) is also ineffective. On the other hand, it decreases the survival time of the animals, by increasing the evolutive rate of the lesions. This effect seems to be independent of the toxic action of this drug.

RESUMEN

A fin de estudiar la actividad de compuestos estructuralmente relacionados con el DDS, en la lepra murina, se investigó el efecto eventual de 4,4'-diaminodifenilselenuro, de la difenilsulfona e del 4,4'-diaminodifenila (bencidina) en ratones "Wistar" inoculados con *M. leprae murium*. Los resultados se basaron en las curvas de supervivencia y en la estructura de las lesiones leprosas.

Fué demostrado que el 4,4'-diaminodifenilselenuro y la difenilsulfona no producen modificaciones en la supervivencia ni en la estructura de las lesiones leprosas, en comparación con animales testigos no tratados. El 4,4'-diaminodifenila tampoco presenta efecto terapéutico, notándose, incluso, una reducción del tiempo de supervivencia de los animales tratados y una evolución más rápida de las lesiones leprosas. Este efecto parece ser independiente de la acción tóxica de la droga.

RESUMÉ

Etudiant l'activité dans la lèpre murine de certains composés dotés d'une structure voisine de la D.D.S., les auteurs ont recherché l'action du 4,4'-diamino diphenyl seleniure, de la diphenyl sulfone et du 4,4'-diamino diphenyl chez des rats inoculés péritonéalement par *M. leprae murium*. Les résultats ont été établis d'après le taux de survie et la structure histologique des lésions chez les animaux traités et chez ceux qui n'étaient pas traités.

Le 4,4'-diamino diphenyl seleniure et la diphenyl sulfone n'ont entraîné aucune modification du taux de survie ni de la structure des lésions. Ils n'ont témoigné d'aucune activité thérapeutique.

La 4,4'-diamino diphenyl (benzidine) est également dépourvue d'action. Par contre, ce produit, en accélérant le développement des lésions, réduit le temps de survie des animaux. Cette action semble indépendante de l'effet toxique du produit.

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