THERAPEUTIC EFFECTIVENESS OF PENICILLIN IN EXPERIMENTAL MURINE TYPHUS INFECTION IN dba MICE

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Recent experiments in these laboratories[1] have shown that penicillin strikingly inhibits the growth of murine typhus rickettsiae in the yolk sac of the developing hen's egg. We have also described[2,3] uniformly fatal murine typhus infection in brown mice of the dba strain. With the thought that therapeutic agents are most satisfactorily tested in experimental animals under conditions which produce fatal infection in the controls, we have utilized dba mice in our first tests of penicillin in murine typhus.

Material and Methods

The strain of murine typhus used had been carried in the yolk sac for eleven generations. Its virulence for mice and guinea pigs did not change during this period. The virulence of the strain for man was accidentally established by the occurrence of typhus fever in one of us during the course of these experiments. (This illness occurred in spite of the administration 2 months previously of an approved epidemic typhus yolk sac vaccine. The course of the disease was probably modified by the vaccination, since the only neurological manifestation was severe headache. Fever lasted for 2 weeks, and mild rash developed on the 7th day.)

Mice were infected in groups of 10 to 14, by the intraperitoneal injection of rickettsial doses of variable size, ranging from overwhelming doses to doses approaching the minimum necessary for uniformly fatal infection. Some experiments were conducted in an environmental temperature of 65–72°F., while others were carried out in a room with a temperature range of 75–80°F. The materials used for inoculation and the environmental temperatures were chosen in the light of information gained from previous work,[2,3] as a result of which we were able to predict quite accurately the incubation period and course of the infection in the controls in each experiment.

Half of the mice in each group were treated with penicillin. Variations in the time of initiating treatment, the daily dosage, and the route of injection of the drug are indicated in Tables I to VI. Treatment was continued until death of the treated animals, or, in case of survival, until about 24 hours after the death of the last control

[1] Greiff, D., and Pinkerton, H., *Proc. Soc. Exp. Biol. and Med.*, in press.

[2] Moragues, V., and Pinkerton, H., *J. Exp. Med.*, 1944, **79**, 35.

[3] Moragues, V., and Pinkerton, H., *J. Exp. Med.*, 1944, **79**, 41.

TABLE I

Effect of Penicillin on Murine Typhus in Mice, Experiment I
Overwhelming Dosage of Rickettsiae (Yolk Sac Suspension) Intraperitoneally

Room temperature 75–80°F.

Mouse No.	Treatment begun	Dosage of penicillin	Illness began	Survival period	Remarks
			days	*days*	
I C_1	Control		3	3½	
I C_2	Control		3	4	
I C_3	Control		3	3½	
I C_4	Control		3	4	
I C_5	Control		3	4	
I C_6	Control		3	4	
I P_1	30 hrs. after injection	640 units per day subcutaneously in divided doses at 6 a.m., 10 a.m., 1 p.m., 5 p.m., 9 p.m., and 12 mid.	3	4	No apparent effect from treatment
I P_2			3	3½	
I P_3			3	3½	
I P_4			3	3½	
I P_5			3	4	
I P_6			3	4¼	

TABLE II

Effect of Penicillin on Murine Typhus in Mice, Experiment II
Heavy Dosage of Rickettsiae (Mouse Spleen Suspension) Intraperitoneally

Room temperature 65–72°F.

Mouse No.	Treatment begun	Dosage of penicillin	Route	Illness began	Survival period	Remarks
				days	*days*	
II C_1	Control			3	3½	
II C_2	Control			4	4¾	
II C_3	Control			4	4¾	
II C_4	Control			4½	5½	
II C_5	Control			4½	5½	
II P_1	3½ hrs. after injection	864 units per day in divided doses at 9 a.m., 12 m., 3 p.m., 6 p.m., and 9 p.m.	S.C.*	5	6¼	Definite prolongation of incubation and survival periods
II P_2			S.C.	4	4½	
II P_3			I.P.*	5¼	6¼	
II P_4			I.P.	5¾	6½	
II P_5			I.P.	5	6¼	

* S.C. means subcutaneous; I.P. means intraperitoneal.

animal in the series. All penicillin injections were given under ether anesthesia, in order to minimize trauma.

Necropsy was done and Giemsa-stained smears were made from the peritoneal cavities of all mice dying spontaneously and of one mouse killed for information

TABLE III

Effect of Penicillin on Murine Typhus in Mice, Experiment III
Heavy Dosage of Rickettsiae (Mouse Spleen Suspension) Intraperitoneally

Room temperature 75–80°F.

Mouse No.	Treatment begun	Dosage of penicillin	Route	Illness began	Survival period	Remarks
				days	*days*	
III C_1	Control			4¼	4½	
III C_2	Control			4½	5¼	
III C_3	Control			5	5½	
III C_4	Control			4½	5¾	
III C_5	Control			4½	5½	
III C_6	Control			5	5¾	
III P_1	6 hrs. after injection	1100 units per day in divided doses at 9 a.m., 11 a.m., 1 p.m., 4 p.m., 7 p.m., 10 p.m., and 12 mid.	I.P.	5½	6	
III P_2			I.P.	5½	5½	Traumatic death?
III P_3			I.P.	6	6½	
III P_4			I.P.	5½		Survived
III P_5			I.P.	5½		Survived
III P_6			I.P.	6		Survived

TABLE IV

Effect of Penicillin on Murine Typhus in Mice, Experiment IV
Moderate Dosage of Rickettsiae (Mouse Brain Suspension) Intraperitoneally

Room temperature 65–72°F.

Mouse No.	Treatment begun	Dosage of penicillin	Route	Illness began	Survival period	Remarks
				days	*days*	
IV C_1	Control			5	6	
IV C_2	Control			5¼	6	
IV C_3	Control			6¼	7	
IV C_4	Control			5½	7	
IV C_5	Control			5	6¾	
IV C_6	Control			5¼	6¾	
IV P_1	24 hrs. after injection	830 units per day in divided doses at 9 a.m., 12 m., 3 p.m., 6 p.m., and 9 p.m.	S.C.	6¾	7¼	
IV P_2			S.C.	6½	7¼	
IV P_3			S.C.	6	6½	
IV P_4			I.P.	7	7½	
IV P_5			I.P.	7		Survived
IV P_6			I.P.	7		Survived

(Table VI). In about half of the animals dying, cultures were made on blood agar from heart's blood and spleen. In 4 mice, cultures were made also from lung tissue. Histological sections, including Giemsa stains, were made of the hearts, lungs, spleens, livers, kidneys, and brains of 8 animals.

TABLE V

Effect of Penicillin on Murine Typhus in Mice, Experiment V
Moderate Dosate (Mouse Brain Intraperitoneally) Approaching the Minimal Lethal Dosage

Room temperature 65–72°F.

Mouse No.	Treatment begun	Dosage of penicillin	Route	Illness began	Survival period	Remarks
				days	*days*	
V C_1	Control			7	8	
V C_2	Control			6½	7	
V C_3	Control			6½	7	
V C_4	Control			6½	7¾	
V C_5	Control			7	7½	
V C_6	Control			6½	7½	
V P_1	7 hrs. after injection	930 units per day in divided doses at 9 a.m., 11 a.m., 1 p.m., 4 p.m., 7 p.m., 9 p.m., and 12 mid.	I.P.	No illness		Survived
V P_2			I.P.	No illness		Survived
V P_3			I.P.	No illness		Survived
V P_4			I.P.	No illness		Survived
V P_5			I.P.	No illness		Survived
V P_6			I.P.	No illness		Survived

TABLE VI

Effect of Penicillin on Murine Typhus in Mice, Experiment VI
Moderate Dosage (Mouse Brain Intraperitoneally) Apparently Just below the Minimal Lethal Dosage

Room temperature 65–72°F.

Mouse No.	Treatment begun	Dosage of penicillin	Route	Illness began	Degree of illness	Survival period	Remarks
				days		*days*	
VI C_1	Control			7	Severe	8	
VI C_2	Control			7¼	Severe	7½	
VI C_3	Control			7	Severe	8½	
VI C_4	Control			7¾	Severe	9	
VI C_5	Control			8½	Severe		Survived
VI C_6	Control			7¼	Severe	8	
VI C_7	Control			7	Severe	8¾	
VI P_1	48 hrs. after injection	640 units per day in divided doses at 9 a.m., 12 m., 3 p.m., 6 p.m., and 9 p.m.	S.C.	7½	Mild	10	Aborted
VI P_2			S.C.	7	Mild		Killed
VI P_3			S.C.	8	Mild		Survived
VI P_4			S.C.	8½	Mild		Survived
VI P_5			I.P.	7½	Mild		Survived
VI P_6			I.P.	7	Mild		Survived
VI P_7			I.P.	8¼	Mild		Survived

The penicillin was furnished by Pfizer and Company, through the courtesy of the Committee on Therapeutic Agents of the National Research Council.

RESULTS

The results are shown in Tables I to VI. In Experiment I, where overwhelming doses of rickettsiae were given and treatment started 30 hours later, no therapeutic effect is apparent. In Experiment II, definite lengthening of the incubation period and survival time is apparent.

In Experiments III and IV, a survival rate of approximately 50 per cent is seen in the treated animals, while all of the controls died, as anticipated on the basis of previous observations.[2, 3]

In Experiment V, where the prolonged incubation period indicated that the dosage of rickettsiae was near the M.L.D. and treatment was begun 7 hours after injection, no illness occurred in the treated animals, while all controls died, as expected.

In Experiment VI, one moderately ill treated animal was sacrificed on the 8th day for information, and another treated animal died on the 10th day, apparently as a result of extensive postpartum hemorrhage, so that no death in the treated animals was definitely attributable to typhus. Treatment was not started in this series until 48 hours after injection with rickettsiae. The greatly prolonged incubation period and the survival of one control in this series indicate that the dosage may have been just below the M.L.D., although in previous comparable series we had found 100 per cent mortality.

Pathological and Bacteriological Studies.—A crucial point in these experiments was the determination of the presence or absence of secondary bacterial infection.

No illness or death occurred in our stock mice during the course of these experiments. Necropsies of all mice dying after the injection of rickettsiae (with the exception of mouse III P_2, Experiment III, in which hemoperitoneum was present) showed mucinous peritonitis. In every instance, including the treated mouse killed for information, rickettsiae were present in relatively large numbers, both free and within mesothelial cells and neutrophiles in the peritoneal exudate. No evidence of secondary bacterial infection was seen in the peritoneal smears of any of the mice.

The only gross finding other than the peritonitis was red consolidation of one or more lobes of the lungs. This observation was made in 8 of the 36 control mice and in 2 of the treated mice. In most instances, this condition was found in mice which had been moribund for 8 to 20 hours before death. Histological study of the lungs from these animals showed intense congestion and edema, with hemorrhage into the alveoli, and some increase in neutrophiles in the alveolar walls. The picture did not resemble that of a bacterial pneumonia.

Giemsa-stained sections showed no organisms in the lungs of these animals, except for an occasional group of rickettsiae in cells of the alveolar walls, and in pleural mesothelial cells. In three of the four instances in which such lung tissue was cultured

on blood agar, no growth occurred. In the fourth case, a large bacillus, probably a contaminant, was recovered.

As a result of these studies, the conclusion was drawn that the pulmonary lesion seen in some of the mice was not a bacterial pneumonia. It may have been a rickettsial pneumonia, or simple terminal edema and congestion in moribund animals. A point of importance was the fact that in Experiment V, where none of the treated mice showed illness, none of the controls showed the pulmonary lesion.

Cultures from the heart's blood and spleen of the control animals were uniformly sterile. Sections of the kidneys, spleens, livers, and brains of the dead mice showed no pathological lesions. Sections of heart showed a myocarditis similar to that seen in typhus infection in man.

Interpretation

On the whole it was concluded that secondary bacterial infection was not present in our mice, and that the effect observed was due to the action of penicillin on the rickettsiae. This interpretation is in harmony with the previously described inhibitory action of penicillin on rickettsial growth in the yolk sac. It is possible that the action of penicillin on rickettsiae may be exerted only on those organisms which are in the process of passing from one cell to another, but it seems more probable that penicillin affects the growth of rickettsiae within cells.

Although most of the favorable therapeutic results were obtained by the intraperitoneal injection of penicillin following the intraperitoneal injection of rickettsiae, there is some evidence, particularly in Experiment VI, that subcutaneous injection of the drug was effective. It should be noted that in most of the experiments there was a daily period of 9 hours during which the penicillin concentration of the drug was probably not maintained at effective levels.

The results obtained are of theoretical as well as practical interest. Assuming that penicillin, like the sulfa drugs, exerts its bacteriostatic action by interfering with metabolic activity, the apparent vulnerability of rickettsiae to penicillin suggests that they possess enzyme systems which enable them to carry on a certain amount of independent metabolic activity.

Obviously no prediction can be made from these experiments concerning the effect of penicillin on human typhus infection. Since the human disease is caused by the growth of rickettsiae in vascular endothelium, however, intravenous injection of penicillin would bring the drug into direct contact with the cells containing the organisms. If sufficiently high concentration of the drug can be established in the blood stream in the early stages of the disease, it would seem reasonable to expect a beneficial therapeutic effect. The results would seem to justify a thorough clinical trial of penicillin in human typhus. They suggest that treatment in the early stages of illness may be important, and that the duration and intensity of treatment should be somewhat increased over that ordinarily used in bacterial infections.

SUMMARY

The administration of penicillin in relatively large but non-toxic doses to dba mice after injection with murine typhus rickettsiae resulted in a marked reduction in mortality, particularly when the initial dosage of rickettsiae was relatively small, approaching the minimal lethal dose. No evidence of secondary bacterial infection was obtained by bacteriological and histological studies, and it seems justifiable to conclude that the greatly increased survival rate in the treated mice was caused by the action of penicillin on typhus rickettsiae.

The theoretical and practical implications of the results are discussed.