Project D52-18-20-09

501

160 Copies

ANTIBACTERIAL ACTION OF A REACTION PRODUCT OF CYSTEINE AND IRON

III. *IN VIVO* ACTION ON PNEUMOCOCCUS INFECTION IN MICE

By N. A. Hinton, Jack Konowalchuk, and G. B. Reed

DSIS circ: CD/C, Med.

Dist:

1 - SW

1 - SES

1 - DRRL

1 - Ref. File

1 - S/L Library

Reprint

DSIS circ: CD/C, Med.

Dist Copy 2 - SW
3 - SES
4 - DRRL
5 - Reference File
6 - S/L Library
(gone)

Reprinted from
CANADIAN JOURNAL OF MICROBIOLOGY
1 : 211-215. 1954

Reprinted from Canadian Journal of Microbiology, 1 : 211-215. 1954

ANTIBACTERIAL ACTION OF A REACTION PRODUCT OF CYSTEINE AND IRON

III. *IN VIVO* ACTION ON PNEUMOCOCCUS INFECTION IN MICE[1]

By N. A. Hinton[2], Jack Konowalchuk[2], and G. B. Reed[3]

Abstract

A colloidal sulphur preparation, formed by autoclaving a dilute solution of cysteine and ferric ammonium citrate, was shown to have no toxicity for mice after eleven 1-mgm. intravenous doses on alternate days. Single doses up to 2 mgm. subcutaneously were not toxic but larger doses by this route produced necrosis. A single 1-mgm. dose of the preparation given to mice intravenously afforded no protection against a lethal dose of *Diplococcus pneumoniae* Type III when given simultaneously. However, groups of mice given a 1-mgm. intravenous dose of the complex and challenged with a lethal dose of pneumococcus at intervals up to 168 hr. after the therapy show no protection for the first 24 hr. following the therapy, increasing protection from 24 to 78 hr., complete protection at 78 hr., and decreasing protection from 78 to 168 hr.

As shown by Konowalchuk *et al.* (1), when cysteine hydrochloride and ferric ammonium citrate are mixed in dilute solutions and autoclaved for extended periods an insoluble precipitate is formed. This precipitated substance was initially considered to be a cysteine–iron complex, later it was demonstrated to be a form of colloidal sulphur. The substance was shown to be highly bacteriostatic *in vitro*, especially for Gram positive species of bacteria.

This paper describes *in vivo* action of the complex in pneumococcus infections in mice.

Toxicity of Sulphur Preparation for Mice

Ten mice were given 1 mgm. of the sulphur preparation, suspended in 0.5 ml. of saline, intravenously every other day for 22 days, i.e. a total of 11 mgm. The animals exhibited no signs of illness or discomfort following the injection of this milky suspension. Three weeks after the last dose they were killed by air embolism. No gross pathological changes were noted and sections of lungs, kidney, liver, and spleen gave no evidence of tissue damage.

Single doses of the complex up to 2 mgm. given subcutaneously had no local effect. Doses of 5 mgm. caused wide areas of necrosis in two to three days with extensive sloughing. Five daily doses of 8 mgm. given orally by passing a catheter into the stomach failed to produce evidence of injury.

Disposition of the Colloidal Sulphur in the Animal Body

As previously shown (1), the complex consists of rounded aggregates 1 to 2 μ in diameter which form a semistable colloidal suspension in water or physiological saline. When a dilute suspension of the complex is brought into

[1] *Manuscript received July 8, 1954.*
Contribution from the Defence Research Board, Kingston Laboratory, Kingston, Ont.
[2] *Scientific Research Officer.*
[3] *Superintendent.*

contact with phagocytic cells, in a microscopic preparation, the particles rapidly disappear. It is assumed that they are taken up by the phagocytes. However, up to the present time no method has been found to demonstrate the particles in the cell cytoplasm. When the complex is injected into the animal, especially by intravenous or intraperitoneal routes, it appears to be taken up by reticulo-endothelial and other phagocytic cells. This is a matter of considerable interest from the point of view of chemotherapy, particularly in tuberculosis since the tubercle bacilli are known to survive for long periods in mononuclear phagocytic cells, Lurie (2), Sabin and Doan (5).

Markham and Florey (3) have shown that India ink, injected into tuberculous animals, is taken up by macrophages at the margin of older spreading lesions so that tuberculous areas were surrounded by carbon-filled mononuclear cells. In the case of younger lesions carbon-filled cells were present in the deeper layers. Markham *et al.* (4), Saunders *et al.* (6), and Su (7) found a similar wide distribution of the insoluble particulate antibiotic micrococcin. The authors discuss the probable significance of this distribution in the therapy of tuberculosis. However, the trials with micrococcin, Su (7), were disappointing, possibly owing to the very low solubility of micrococcin relative to its inhibiting potential for tubercle bacilli.

The experiments described below approach the subject indirectly by using a chemotherapeutic agent which is apparently taken up rapidly by phagocytic cells and a microorganism, the pneumococcus, which multiplies in the body largely in the extracellular fluids.

Pneumococcus Culture

When 1 ml. of a 1–100,000 dilution of a laboratory strain of *Diplococcus pneumoniae* Type III grown in heart infusion broth for 18 hr. was injected into 20-gm. white mice, the animals died in 48 hr. with little variation in the time of death. With smaller doses the results were irregular.

Simultaneous Infection and Therapy

A group of white mice of similar age and weight were given 1 ml. of 1–100,000 dilution of an 18-hr. culture of the above strain of pneumococcus intraperitoneally. Immediately after infection half the group of mice were given intravenously 1 mgm. cysteine–iron complex suspended in 0.5-ml. physiological saline, a single dose. The treated and untreated mice died in an average of 48 hr.

Advanced Therapy

A group of 120 white mice of uniform age and weight were given intravenously 1 mgm. of cysteine–iron complex suspended in 0.5 ml. of saline. The treated animals were divided into 12 lots of 10 each. The first lot of 10 was challenged, immediately after treatment, with 1 ml. of a 1–100,000 dilution of an 18-hr. culture of the above strain of pneumococcus. Other

groups were similarly challenged at intervals of 3 to 168 hr. after the single intravenous injection of cysteine–iron complex. The number of mice surviving in each group is shown in Fig. 1. It is apparent from these data that the single injection of the complex exerted no protective action in the first 24 hr. within the animal. Between 48 and 78 hr. within the animal there was a progressive increase in protective action to complete protection at 78 hr. From 78 to 168 hr. there was a progressive decline in protective action.

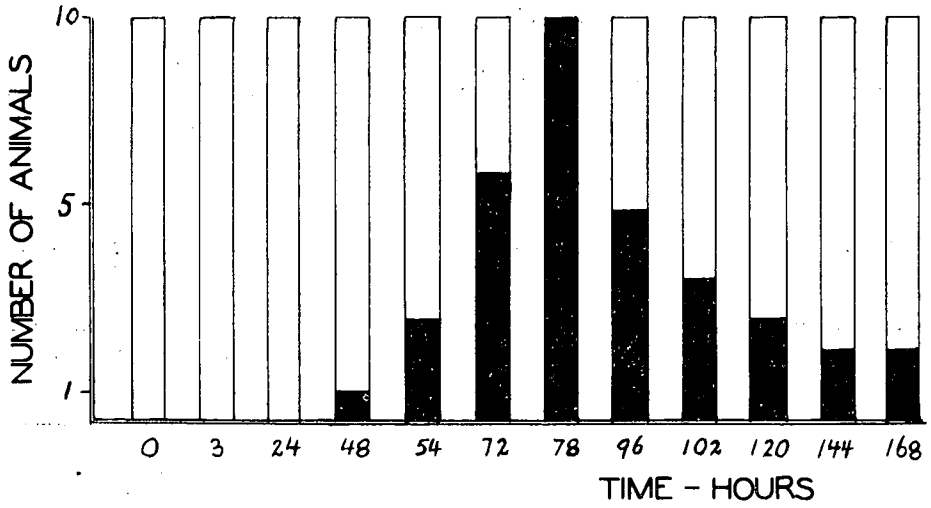

FIG. 1. Mice were given a single intravenous injection of the complex and challenged at intervals of 0 to 168 hr. later with a lethal dose of pneumococcus. The figure shows in white the number of animals which died and in black the number which survived.

Varying Doses of the Colloidal Sulphur Preparation

Similar results were obtained when the dose of the complex was varied. Groups of mice were injected intravenously with a single dose of cysteine–iron complex from 0.1 to 1 mgm., in each case suspended in 0.5 ml. of saline. At intervals of 24 to 96 hr. after the intravenous injection of complex the animals, in groups of 10, were challenged with 1 ml. of a 1–100,000 dilution of an 18-hr. culture of pneumococcus. As indicated in Table I, regardless of the size of

TABLE I

MICE GIVEN A SINGLE INTRAVENOUS INJECTION OF 0 TO 1 MGM. OF THE COMPLEX WERE CHALLENGED, IN GROUPS OF 10, WITH A LETHAL DOSE OF PNEUMOCOCCUS AT INTERVALS OF 24 TO 96 HR. AFTER THERAPY

Interval, therapy to challenge, in hours	Number of mice surviving when challenged after treatment with:				
	0 mgm.	0.1 mgm.	0.25 mgm.	0.5 mgm.	1.0 mgm.
24	0/10	0/10	0/10	0/10	0/10
48	0/10	0/10	0/10	3/10	2/10
78	0/10	3/10	4/10	8/10	10/10
96	0/10	1/10	2/10	4/10	5/10

the therapeutic dose, there was no apparent protective action at 24 hr. after therapy, slight protection with the larger doses at 48 hr., high level protection at 78 hr., and decreasing protection at 96 hr. As in the previous experiment, it is apparent that a period of approximately 78 hr. must elapse following injection of the complex before maximum protective action develops in the animals.

Daily Injections of the Complex

When daily injections of the complex were given for seven days and the animals, in groups of 10, challenged, as before, with a lethal dose of pneumococcus at intervals of 24 to 96 hr. after the last injection as shown in Table II, the highest level of protection was at 24 hr. and there was progressively less protection up to 96 hr.

TABLE II

MICE GIVEN DAILY INTRAVENOUS INJECTIONS OF 1 MGM. OF THE COMPLEX WERE CHALLENGED, IN GROUPS OF 10, WITH A LETHAL DOSE OF PNEUMOCOCCUS AT INTERVALS OF 24 TO 96 HR. AFTER THE LAST THERAPEUTIC INJECTION

Interval from last injection to challenge, in hours	No. of mice surviving after seven daily injections of:	
	0.1 mgm.	0.25 mgm.
24	5/10	8/10
48	3/10	6/10
78	3/10	5/10
96	0/10	1/10

Routes of Injection

A comparison was made of three routes of introduction of the complex. Since it was known from preliminary trials that subcutaneous and oral routes were less effective than intravenous, the dose was graded. Groups of mice were given single doses of 0.5 mgm. intravenously, 1 mgm. subcutaneously, and 10 mgm. orally. At intervals of 24 hr. to 96 hr. the mice were challenged, in groups of 10, with a lethal dose of pneumococcus. As indicated in Table III, the highest protection resulted from the small intravenous injection and, as

TABLE III

MICE GIVEN A SINGLE DOSE OF THE COMPLEX BY INTRAVENOUS, SUBCUTANEOUS, OR ORAL ROUTE WERE CHALLENGED, IN GROUPS OF 10, WITH A LETHAL DOSE OF PNEUMOCOCCUS AT INTERVALS OF 24 TO 96 HR. AFTER THERAPY

Interval from therapy to challenge, in hours	Number of mice surviving after:		
	Intravenous, 0.5 mgm.	Subcutaneous, 1 mgm.	Oral, 10 mgm.
24	0/10	0/10	0/10
48	3/10	0/10	0/10
78	8/10	4/10	0/10
96	4/10	2/10	0/10

shown in the previous experiments, the maximum protection was at 78 hr. after the therapeutic injection. Though the subcutaneous injection of twice the intravenous dose gave a much lower level of protection the maximum was again at 78 hr. after therapy. Oral therapy gave no evidence of protection even with 20 times the intravenous dose.

Discussion

The reason for the lag of some 78 hr. between the injection of the complex and the development of protection against pneumococcus infection is far from obvious. The most likely explanation is associated with phagocytosis of the injected particulate matter. If, as seems likely from the data shown in the earlier paragraphs, the complex is rapidly taken up by phagocytic cells, the pneumococcus is free to multiply in the extracellular fluids without interference by the bacteriostatic agent.

If this is correct, the complex must leave the cells in order to exert the observed delayed effect in pneumococcal infection. If the complex is liberated in particulate form it should be again taken up by phagocytic cells as in the first instance. It is suggested that the intracellular complex slowly dissolves, or is converted by metabolic activity, to an active product, and diffuses into extracellular fluid. The concentration of complex reached in the fluids will depend upon the rate of solution and diffusion of the drug as against the rate of its destruction or elimination. By this mechanism an accumulation of dissolved complex may be visualized as reaching a peak protective level in three days, after which the level declines with exhaustion of the intracellular depots of the drug.

References

1. KONOWALCHUK, J., CLUNIE, J. C., HINTON, N. A., and REED, G. B. Antibacterial action of a reaction product of cysteine and iron. II. Preparation and properties of the substance. Can. J. Microbiol. 1 : 182-189. 1954.
2. LURIE, M. B. The correlation between the histological changes and the fate of living tubercle bacilli in the organs of tuberculous rabbits. J. Exptl. Med. 55 : 31-54. 1932.
3. MARKHAM, N. P. and FLOREY, H. W. The effects on experimental tuberculosis of the intravenous injection of insoluble substance: experiments with carbon. Brit. J. Exptl. Pathol. 32 : 25-33. 1951.
4. MARKHAM, N. P., HEATLEY, N. G., SAUNDERS, A. G., and FLOREY, H. W. Behaviour *in vitro* of particulate micrococcin. Brit. J. Exptl. Pathol. 32 : 136-145. 1951.
5. SABIN, F. R. and DOAN, C. A. The relation of monocytes and clasmatocytes to early infection in rabbits with tubercle bacilli. J. Exptl. Med. 46 : 627-644. 1927.
6. SAUNDERS, A. G., FLOREY, H. W., and WELLS, A. Q. The behaviour of intravenously injected particles of carbon and micrococcin in normal and tuberculous tissue. Brit. J. Exptl. Pathol. 32 : 452-457. 1951.
7. SU, T. L. Micrococcin. An antibacterial substance formed from *Micrococcus*. Brit. J. Exptl. Pathol. 29 : 473-481. 1948.