MICROBIOLOGY AND IMMUNITY

PROTECTION OF THE CENTRAL NERVOUS SYSTEM FROM THE ACTION OF PERFRINGENS AND OEDEMATIENS IN RELATION TO THE TITER OF ANTITOXIN IN THE BLOOD

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(Received August 20, 1956. Presented by Member of the Academy AMN USSR G. V. Vygodchikov)

The necessity of an experimental study of the protection of the central nervous system by passive or active immunization against toxic infections became quite clear following the reports of a series of clinicians that with big doses of immune serum it was possible to cure serious cases of tetanus, diphtheria, gas gangrene, and botulism.

Up to that time, while the attending physicians used moderate doses of serum, the unsatisfactory results of serum therapy were ascribed to the fact that antibodies do not penetrate across the blood-brain barrier from the blood into the cerebro-spinal fluid [5, 3] and that the central nervous system is left unprotected by passive immunization (Roux and Borrel) [7, 5].

It was established by the majority of investigators that the penetration of various substances into the central nervous system depends, not on the composition of the fluid, but on the permeability of the capillaries and on the concentration of these substances in the blood [4]. In line with this, the penetration of antitoxins into the central nervous system, probably depends on the permeability of the capillaries of the brain and on the concentration of antitoxins in the blood and is completely unrelated to the presence of antibodies in the fluid.

The question arises, is the immune condition of the central nervous system altered by the intravenous injection of large doses of antitoxin, does it acquire in such cases a resistance to the action of toxins, in spite of the fact that antibodies practically do not get into the fluid?

EXPERIMENTAL METHODS

We carried out experiments with perfringens and oedematiens toxins on passively and actively immunized rabbits. We injected rabbits weighing 2.5-3 kg intravenously with various quantities of serum, then in about 4 hours, immediately after having taken blood and cerebrospinal fluid from them for determination of the titer of antitoxin, we injected the corresponding toxin into the cisterna magna.

The titer of the antiperfrigens serum was 850 AU/ cc, the titer of the anti-oedematiens serum was 100 AU/ cc. The titration of the serum and cerebrospinal fluid was carried out by the usual methods employed in bacteriological institutes, intravenously in mice.

We carried out the experiments on the actively immunized mice according to the same program that we used for the passively immunized animals. Active immunization was carried out with the toxoids which we employed for the immunization of horses to produce therepautic sera. We inoculated rabbits three times with 1-2 cc, with intervals of 20 days between the injections. About 12-15 days after the last inoculation we took blood and spinal fluid from them and injected them with the corresponding toxin.

For the experiments we used dried perfringens and oedematiens toxins obtained by precipitation with ammonium sulfate.

The minimum lethal dose (MLD) of perfringens toxin for mice injected intravenously was 0.3 mg, whereas the fatal dose for rabbits injected suboccipitally was 1.67 mg. The minimal lethal dose of oedematiens toxin for mice injected intravenously was 0.3 mg, the lethal dose for rabbits injected suboccipitally was 0.4 mg.

EXPERIMENTAL RESULTS

The results of the experiments with perfringens toxin in passively immunized rabbits are presented in Table 1. On the intravenous injection of the animals with large doses of antiperfringens serum, 1500-10,000 AU,

TABLE 1.

Effective Resistance of Passively Immunized Rabbits to Suboccipital Injection of Perfringens and Oedematiens Toxins

Name of Toxin	Experiment											Control		
	No. of Experiment	No. of Rabbits	Serum Injected (in AU)	Titer of Antitoxin (AU)		MLD Injected	Paralysis of Extremities		Died		Survived			
				In Spinal Fluid	In the blood		No. of Rabbits	On Day	No. of Rabbits	On Day		No. of Rabbits	Toxin Injected	Died
Perfringens	1	2	10 000	>0.02 <0.1	—	10	2	2	2	3—4	—	—	—	—
	2	2	1 500	<0.02	>15<20	5	—	—	2	5—6 hours	—	—	—	—
	3	8	3 000	<0.02	>30<40	3	1	19	—	—	8	2	1	2
	4	5	1 500	<0.02	>15<20	3	3	3—7	3	6—8	2	1	1	1
	5	4	1 500	<0.02	=20	2	3	4—8	3	16—17	1	1	1	1
	6	5	1 500	<0.02	>15<20	1	3	13—15	—	—	5	1	1	1
Oedematiens	1	3	5 000	<0.02	>20<40	10	—	—	3	2	—	1	1	1
	2	5	5 000	<0.02	>20<40	5	—	—	—	—	5	1	1	1
	3	7	2 500	<0.02	>10<20	5	4	2—5	2	7—9	5	1	1	1
	4	4	2 500	<0.02	>10<15	3	—	—	—	—	4	1	1	1
	5	3	1 000	<0.02	>5 <10	3	3	2—3	3	4—7	—	1	1	1

and suboccipital injection of 5-10 MLD of toxin there was produced marked dyspnea, excitement; the rabbits ran around and screamed. The state of excitement lasted about $^1/_2$-1 hour. After some hours paralysis appeared in the animals and they died from the intoxication on the 2nd-4th day after the injection of the toxin.

We further injected rabbits with moderate doses of perfringens toxin (from 1 to 3 MLD), since these doses did not produce strong excitement of the animals. Only after a dose of serum equal to 3000 AU, following the injection of 3 MLD of toxin, did all the rabbits survive.

It is quite clearly evident that the larger the dose of antitoxin, the greater proportion of the animals are protected against the injection into them of a given amount of toxin: of 8 rabbits receiving 3000 AU, after the suboccipital injection of 3 MLD of toxin all survived, and only one showed paralysis of the hind legs on the 19th day, but out of 5 rabbits receiving 1500 AU, after the suboccipital injection of the same dose of toxin only two survived, while the others showed paralysis of the extremities and died. Out of 5 rabbits receiving 1500 AU and 1 MLD of toxin, all survived, though it must be admitted that three of them showed paralysis of the extremities on the 13-15th day. Untreated control rabbits always died on the 3rd-4th day following the suboccipital injection of 1 MLD of toxin.

On the injection of 5000 AU of antioedematiens serum (Table 1) and 10 MLD oedematiens toxin rabbits also exhibited significant excitement: the animals showed dyspnea and they ran around and screamed. Such a condition passed off rather quickly; after about 10-15 minutes the rabbits calmed down, and only the dyspnea remained. All 3 rabbits died on the following day.

From our experiments carried out on rabbits passively immunized with antioedematiens serum, it is quite clearly evident that the effective resistance of rabbits on suboccipital injection of toxin, and consequently the protection of the central nervous system both depend on the size of the dose of antitoxin injected.

Thus, for example, intravenous injection of a dose of serum containing 2500 AU completely protects the central nervous system of rabbits against the suboccipital injection of 3 MLD of oedematiens toxin whereas after the injection of serum containing 1000 AU the rabbits die following the injection of 3 MLD of toxin.

Intravenous injection of 5000 AU protects the central nervous system of rabbits against 5 MLD, since all 5 rabbits were unaffected by the suboccipital injection of this dose of toxin. Following the injection of serum containing 2500 AU not all of the rabbits survived the injection of 5 MLD of toxin. In our experiments, out of 7 rabbits 2 died, but it must be noted that 4 of the seven showed significant paralysis.

The titers of perfringens and oedematiens antitoxins in the blood of all passively immunized animals were sufficiently high, whereas under the conditions of our procedure no titratable amount of antitoxin was demonstrated in the cerebrospinal fluid.

Consequently the conclusion, reached by us in previous work [1, 2] with tetanus, botulinus and diphtheria toxins, that the protection of the central nervous system in passive immunization does not depend upon the penetration of antitoxins into the cerebrospinal fluid, remains correct for these cases.

The protection of the central nervous system depends on the titer of antitoxin in the blood. The higher the titer of antitoxins in the blood, the more antibody penetrates to the brain tissue through the capillary walls, while at the same time antibody is practically absent from the cerebrospinal fluid.

Our investigations explain the conclusions of many clinicians, based on practical observations during the treatment of gas gangrene, that the larger the dose of serum injected, the higher the therapeutic effect.

Shishkov [6] writes that the high titer of antigangrene serum of the post-war series, and the small quantity of protein ballast in it, offers the possibility of significantly increasing the dose of serum. With patients in peace time he employed for gas gangrene infections serum in the first two days in doses of 120,000 to 240,000 AU per day. Single doses of antigangrene serum employed in war time were from 40,000 to 60,000. Shishkov believes that the possibility of injecting antitoxin in a dose 3-6 times larger as compared to that used in wartime without doubt significantly increases the possibility of saving patients with gas gangrene infection.

A series of experiments were carried out by us on actively immunized rabbits. It is known that rabbits, which as the result of active immunization against perfringens or oedematiens have from 5 to 10 AU of antitoxin in their blood, can tolerate known lethal doses of a culture injected subcutaneously or intravenously.

However, up to now nobody has investigated how the central nervous system of actively immunized animals would react to the injection of toxin, introduced suboccipitally into the region of the brain.

In our experiments, out of 17 rabbits actively immunized with perfringens toxoid (Table 2) seven had an antitoxin titer $> 1 < 5$ AU, six rabbits, $> 1 < 3$, four rabbits $> 3 < 5$, and only one rabbit had 0.5 AU per cc of serum. Antitoxin was practically absent from the cerebrospinal fluid. Out of 12 rabbits receiving 1 MLD of toxin by suboccipital injection, 11 were completely unaffected and only one displayed on the 5th day signs of paralysis of the extremities; this animal died on the 9th day from the intoxication. Four control rabbits, receiving 1 MLD of toxin each, died on the 3rd-4th day. After the injection of 2 and more MLD of toxin into the immune rabbits, deaths began to be produced, since the degree of immunity was evidently not that high.

In the experiments with oedematiens toxin we obtained the same pattern. After immunization of 10 rabbits with toxoid a series of 127 blood antitoxin titers fell within the limits of 1 to 5 AU, and antitoxin was not present in the cerebrospinal fluid. Out of these 10 rabbits (Table 2) 3 rabbits receiving 1 MLD each of toxin suboccipitally were unaffected; out of 3 rabbits receiving 2 MLD each one died from the intoxication with signs of paralysis of the extremities. Out of 4 rabbits each receiving 3 or 5 MLD, only one survived.

In the second series of experiments (Nos. 5, 6, 7) 10 rabbits were immunized with toxoid of series 129. The titer of antitoxin in the blood was within the limits of 5 to 20 AU, and antitoxin was absent from the cerebrospinal fluid. All 6 rabbits which were injected with 2 or 5 MLD oedematiens toxin survived; it is true, however, that those which received 5 MLD each showed signs of paralysis of the hind legs. Out of 4 rabbits, each of which was injected with 10 MLD of toxin, only one was unaffected. After the injection of the toxin there was observed

TABLE 2

Effective Resistance of Actively Immunized Rabbits on Suboccipital Injection of Perfringens and Oedematiens Toxins.

Type of Toxin	Experiment									Control		
	No. of Experiment	No. of Rabbits	Titer of Antitoxin (AU)		MLD Toxin Injected	Paralysis of Extremities		Died	Survived	No. of Rabbits	Toxin Injected	Died
			In the cerebrospinal fluid	In the blood		No. of Rabbits	On Day					
Perfringens	1	12	<0.02	>0.5<5	1	1	5	1	11	2	1	2
	2	3	<0.02	>1<5	2	3	2—3	3	—	1	1	1
	3	2	<0.02	>1<5	5	2	—	2	—	1	1	1
Oedematiens	1	3	<0.02	> 1< 5	1	—	—	—	3	1	1	1
	2	3	<0.02	> 1< 5	2	1	4	1	2	1	1	1
	3	2	< 0.02	> 1< 5	3	1	3	1	1	—	—	—
	4	2	<0 02	> 1< 5	5	2	2—3	2	—	1	1	1
	5	2	<0 02	>10<15	2	—	—	—	2	—	—	—
	6	4	<0.02	>10<15	5	4	6—10	—	4	2	1	2
	7	4	<0.02	>10<15 =20	10	4	2—5	3	1	—	—	—

marked dyspnea and excitement, the animals screamed and threshed about. The antitoxin titer in the unaffected animal was 20 AU. In our experiments control untreated rabbits into which we injected 1 MLD of toxin each, always died on the 3rd-4th day.

Therefore, the protection of the central nervous system of actively immunized animals depends on the antitoxin titer in the blood. On the injection into the central nervous system of known lethal doses of toxins, only rabbits with a high titer of antitoxin in the blood survive.

SUMMARY

Experiments on passively and actively immunized rabbits with perfringens and oedematiens toxins showed that only the animals with a high blood antitoxin titer survive the introduction of lethal doses into the central nervous system.

These investigations throw light on the conclusion, reached by certain clinicians on the basis of practical experience in treating gas gangrene that large doses of serum produce better therapeutic results.

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