

HYALURONIDASE AND HYALURONIC ACID IN CERTAIN INFECTIOUS AND IMMUNOLOGICAL PHENOMENA

VI. EFFECT OF HYALURONIDASE AND HYALURONIC ACID ON REACTOGENIC PROPERTIES OF TETRAVACCINE AND PREVENTIVE PROPERTIES OF IMMUNE SERA

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Both native and foreign investigators have devoted deservedly great attention to studying the tissue-enzyme system, hyaluronidase-hyaluronic acid. Yet the role of this enzyme within the phenomena of immunity has remained poorly elucidated. This stimulated us to investigate the question of the effect of hyaluronidase and hyaluronic acid on the immunogenic, anaphylactogenic, reactogenic and antigenic properties and the harmlessness of the official tetravaccine, and on the preventive properties of immune sera. Selection of the tetravaccine was prompted by the well-known fact that the typhoid-paratyphoid antigens are insufficiently effective, and the dysentery antigens that go into the tetravaccine have an extremely low level of effectiveness [2,3,8,9].

Selection of hyaluronidase was dictated to us by the results of V. M. Berman's observations [1], who succeeded in experimentally increasing the effectiveness of the cholera vaccine by adding hyaluronidase to it, as well as by the works of M. V. Trius and T. N. Yashchenko [4] and V. I. Kudryavtseva [3], who accomplished the same purpose as applied to the BCG vaccine.

EXPERIMENTAL METHOD

First series of experiments. To study the question of the effect of hyaluronidase and hyaluronic acid on the immunogenic properties of the tetravaccine, we used 1675 white mice. In the first group of experiments, we studied the effect of these substances on the immunogenic properties of the vaccine's typhoid antigen (605 mice), in the second—the effect of hyaluronidase on the immunogenic properties of the paratyphoid B antigen (450 mice), and in the third and fourth groups—the effect of hyaluronidase on the immunogenic properties of the vaccine's Flexner and Sonne antigens (620 mice).

A portion of the mice were immunized with the tetravaccine (using 1125 billion microbial bodies per animal) in 0.5 ml of physiological saline. The remaining animals were immunized with the same dose of vaccine in 0.5 mg of lyophilized hyaluronidase (or hyaluronic acid). The immunization was performed in a single injection, a three-fold regime (in the study of the immunogenic properties of the typhoid-paratyphoid antigens), and a two-injection plan, at intervals of 5 days between the injections (in the study on the immunogenic properties of the dysentery antigens).

At 3, 15, and 30 days after the vaccination, some of the mice were inoculated intraperitoneally: those immunized with a single injection received 1 Dcl of the typhoid bacillus (strain Ty₂-58) type of vaccine, while those immunized with three injections received 15 and 18 Dcl of the same vaccine. At 3, 15, 30 and 60 days after the vaccination, some of the mice were inoculated with the paratyphoid B bacillus type of vaccine (strain 492), using 1 Dcl on those immunized with a single injection and 5 and 10 Dcl on those given three immunizing doses. After 85 days, some of the mice were inoculated with 1 and 2 Dcl of the Flexner (strain 337) and Sonne (strain

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5063) bacillus vaccine types. In all, a total of 1675 mice were observed over the course of 5 days, and their survival rate was computed.

Second series of experiments. A study was carried out on the character of the influence of hyaluronidase on the preventive properties of immune sera, using 6 rabbits and 420 white mice. Each of 3 control rabbits was injected intravenously with 0.5 ml of tetravaccine in 1 ml of physiological saline; each of three experimental rabbits received 0.5 ml of the same vaccine containing 2 mg of hyaluronidase. Twenty-four hours after the vaccination, blood was taken from all the rabbits, the sera from the control and experimental animals were preserved with 0.5% chloroform, and placed in a refrigerator.

These sera were used for passive intraperitoneal immunization of 260 mice: 130 mice received 0.2 ml of 50% diluted serum from the control rabbits, and 130 mice received the same dose of serum from the experimental rabbits, immunized with the tetravaccine containing hyaluronidase. Two hours after injection of the sera, the control and experimental mice were inoculated with 1, 1.5, 2, 4 and 5 Dcl of the vaccine types. Simultaneously, inoculation was carried out on 95 unvaccinated mice, in order to verify 1 and 1.5 Dcl doses.

In another part of the experiment, we determined the nature of hyaluronidase's effect on the preventive properties of rabbit sera under conditions of three-injection intravenous immunization of the animals with tetravaccine and tetravaccine combined with hyaluronidase. For this, the same six rabbits were immunized for a second and third time (with five-day intervals between the injections). The sera of these animals were obtained 60 days after the third vaccination.

A total of 160 mice underwent passive immunization: 80 mice received 0.2 ml of 50% diluted serum from rabbits immunized with the regular vaccine, and 80 mice—from rabbits immunized with the vaccine containing hyaluronidase. After 2 hours, the mice were inoculated: for the Flexner bacillus we used 5 Dcl (i.e., 5 billion microbial bodies), and for cultures of typhoid, paratyphoid B and Sonne—10 Dcl (i.e., 2 billion living specimens of typhoid and paratyphoid bacilli, and 1.5 billion Sonne bacilli). Simultaneously, 40 additional mice were inoculated (controls, receiving positively lethal doses). All of the animals were observed over a period of 5 days.

In the third series of experiments, we studied the atoxicity of lyophilized hyaluronidase (prepared in Czechoslovakia), testicular hyaluronidase (prepared by us) and hyaluronic acid (obtained by us from umbilical cords). A total of 195 mice were used. The preparations were injected subcutaneously using 1 ml, intraperitoneally also using 1 ml, and into the caudal vein using 0.5 ml.

In the fourth series of experiments, we studied the question of whether the addition of hyaluronidase to the tetravaccine leads to the development of anaphylactic properties on the part of the vaccine, preventing repeated injections of the animals with this complex. A total of 15 guinea pigs were used in the experiment. Injections of the sensitizing dose were performed subcutaneously: one group received 225 million microbial bodies of the tetravaccine, another received 0.5 mg of hyaluronidase, and a third received a mixture of the indicated doses of both preparations (5 animals in each group). After 21 days, the resolving doses were administered, using the same substances in the same order on the same animals, but injecting the material into the heart and using a ten times larger dose.

In addition, in observations on humans (18 individuals), we investigated the effect of hyaluronidase on the reactogenic and antigenic properties of the tetravaccine (using agglutinin formation as the criterion). The vaccine was injected subcutaneously, using 0.5 ml (according to the instructions in [4]) containing 1 mg of lyophilized hyaluronidase. We measured the local and systemic reaction and the intensity of formation of typhoid agglutinins in the vaccinated patients, 24 hours after the immunization.

EXPERIMENTAL RESULTS

With single-injection immunization of the mice, using regular tetravaccine and tetravaccine supplemented with hyaluronic acid, and subsequent inoculation with typhoid culture, 31.8% of the animals survived in the control (regular vaccine), while in the experimental group (vaccine plus hyaluronic acid), the figure was 24.6%. With three-injection immunization, 1.7 and 2.5% survived respectively. Thus, hyaluronic acid did not fulfill our expectations as a preparation capable of stimulating the immunogenic properties of the tetravaccine.

In the experiments using tetravaccine containing hyaluronidase, the survival rate of the mice that was immunized only once and inoculated with typhoid bacillus culture was equal to 16.6% in the control (regular vaccine) and 30% in the mice that were immunized with tetravaccine containing hyaluronidase. With three-injection immunization, the percent survival was equal to 11.7 and 66.6 respectively. The survival rate in the group of

The Effect of Hyaluronidase on the Reactogenic and Antigenic Properties of Tetravaccine
(Observations on Humans)

Temperature after vaccination		Local reaction (diam of hyperemia in cm)		Maximum titer of typhoid agglutinins		Date of vaccination against intestinal infections
after 24 hours	after 48 hours	after 24 hours	after 48 hours	before vaccination	24 hours after vaccination	
36.8°	36.8°	None	None	1:100	1:100	Not vaccinated
37.0°	36.2°	"	"	1:100	1:200	" "
36.9°	36.3°	0.4	0.3	0	1:200	" "
37.2°	36.5°	None	None	0	1:400	1954
36.6°	36.6°	5	"	0	1:400	Not vaccinated
36.2°	36.4°	None	"	0	1:200	" "
37.2°	36.7°	6.5	3.0	0	1:200	" "
36.6°	36.6°	5.5	None	1:100	1:800	1957
36.0°	36.2°	None	"	1:400	1:400	1957
36.4°	36.4°	4.5	"	0	1:100	Not vaccinated
36.6°	36.2°	None	"	0	1:200	" "
36.3°	36.5°	"	"	1:100	1:200	1955
36.8°	36.5°	"	"	0	1:400	1952
36.3°	36.3°	"	"	1:200	1:800	1959
36.4°	36.8°	0.5	0.2	1:100	1:400	1955
37.1°	36.6°	3.0	4.5	1:100	1:400	1957
36.7°	36.5°	2.5	1.5	0	1:200	Not vaccinated
36.6°	36.6°	1.5	1.0	0	1:200	Not vaccinated
Mean maximum titer of typhoid agglutinins				1:67	1:322	-

- Notes: 1—Prior to immunization, the temperature of all 18 individuals was normal.
2—The maximum temperature among the vaccinated was: after 3 hours—37.3 deg, after 6 hours—37.5 deg, after 12 hours—37.9 deg.
3—The maximum length of time for disappearance of the local reaction was 4 days.
4—Formation of paratyphoid B, Flexner and Sonne agglutinins was weaker.

mice that were immunized twice and inoculated with the culture of Flexner bacteria was equal to 11.4% in the control and 17.1% in the experimental animals. In the mice inoculated with Sonne bacilli, the survival rate was equal to 41.4% in the control and 82.9% in the experimental group.

In the study of the preventive properties of the sera from single-injection immunized rabbits, it was established that the control mice, immunized with sera from rabbits that received the regular vaccine, survived in 21.1% of the cases, while in the experimental group, 40.8% of the animals survived, i.e. hyaluronidase increased the preventive activity of the sera from single-injection immunized rabbits by 1.6 times. In this case, the preventive properties against typhoid antigen were raised by a factor of 7, and against the Sonne antigen—by many times (percent survival in the control was 0, while in the experimental group, it was 32). Hyaluronidase showed itself to be ineffective against the Flexner and paratyphoid antigens.

Three-fold immunization caused a more intense increase in the preventive activity of the sera from rabbits injected with the hyaluronidase-containing tetravaccine. In the experimental group, 82.5% of the mice survived, while in the control—41.2%, i.e. hyaluronidase increased the preventive properties of the sera from the three-fold immunized rabbits by a factor of 2; against the typhoid antigen, the increase was $2\frac{1}{2}$ times, against the paratyphoid antigen—almost 2 times, the Sonne antigen—15 times. The enzyme was only shown to be ineffective against the Flexner antigen.

Lyophilized hyaluronidase, the preparation of the penetration factor which we obtained, and hyaluronic acid were all shown to be harmless—parenteral injection of these substances into white mice was not accompanied by death of a single animal out of a total of 195 observed individuals.

The observations on the 15 guinea pigs showed that neither hyaluronidase nor tetravaccine, nor the complex of the two, causes any form of anaphylactic manifestations in the experimental animals.

The results of studying the reactogenic and antigenic properties of the tetravaccine+hyaluronidase complex are presented in the table. From the data in this table, the following general conclusion can be made: tetravaccine containing hyaluronidase does not cause either local or systemic reactions that exceed the norms established by the "Instructions" [4], and increases the intensity of typhoid agglutinin formation by almost 5 times only 24 hours after immunization of the patients with the "hyaluronidized" tetravaccine.

Summarizing the results of all the experiments, it may be noted that hyaluronidase, in the described form, raises the preventive activity of sera from rabbits that are immunized with tetravaccine in relation to the typhoid and Sonne antigens of the tetravaccine. The results of parenteral injection of hyaluronidase and hyaluronic acid into white mice testify to their complete harmlessness. The tetravaccine-hyaluronidase complex is devoid of anaphylactogenic properties, and judging from the nature of the systemic and local reactions in the vaccinated individuals, does not exceed the reactogenic properties of regular tetravaccine. Following injection of this complex into humans, early production (24 hours after immunization) of specific (typhoid) agglutinins is observed, which is not observed when the regular vaccines are used.

Lyophilized hyaluronidase increases the immunogenic properties of the typhoid antigen in the tetravaccine by 5.2 times, of the paratyphoid B antigen by 5 times, of the Flexner antigen by 1.5 times, and of the Sonne antigen by 1.7 times. On the whole, under the experimental conditions in mice, the immunogenic activity of the tetravaccine is increased by 3.4 times, which characterizes hyaluronidase as a preparation of definite interest in the problem of producing parenteral, corpuscular intestinal vaccines.

SUMMARY

Hyaluronidase increases the preventive activity of the sera of rabbits immunized with tetravaccine with respect to the typhoid, paratyphoid B and Sonne dysentery vaccine antigens. Hyaluronidase and hyaluronic acid are harmless in parenteral administration to albino mice. The tetravaccine-hyaluronidase complex is devoid of anaphylactogenic properties, and untoward reactions provoked in response to its administration (as observed on volunteers) are not greater than with the usual tetravaccine. Administration of this complex to man is accompanied by early (within 24 hours after immunization) production of typhoid agglutinins. Lyophilized hyaluronidase gives a 3-4-fold increase of tetravaccine immunogenicity as a whole; typhoid-paratyphoid antigens are activated more than dysentery antigens of tetravaccine.

LITERATURE CITED

1. V. M. Berman, in the book: Questions in Adult Immunology [in Russian]. Leningrad, No. 1 (1947). p. 9.
2. L. V. Gromashevskii, Zhurn. mikrobiol., épidemiol. i immunobiol., No. 6 (1948). p. 15.
3. V. I. Kudryavtseva, The Effect of the Dissemination Factor on the Effectiveness of BCG Vaccination. Cand. diss. Leningrad (1950).
4. M. V. Trius and T. N. Yashchenko, Probl. tub., No. 4 (1953). p. 13.
5. L. B. Kheifets et al., Zhurn. mikrobiol., épidemiol. i immunobiol., No. 10 (1958). p. 44.
6. N. Z. Yakobson, Works of the Tomsk Scientific-Research Institute of Vaccines and Sera [in Russian], Vol. 11 (1960). p. 107.