CONCERNING THE THERAPY OF EXPERIMENTAL BOTULINUM INTOXICATION BY MEANS OF LOW MOLECULAR POLYMER BLOOD SUBSTITUTES
(Preliminary Report)

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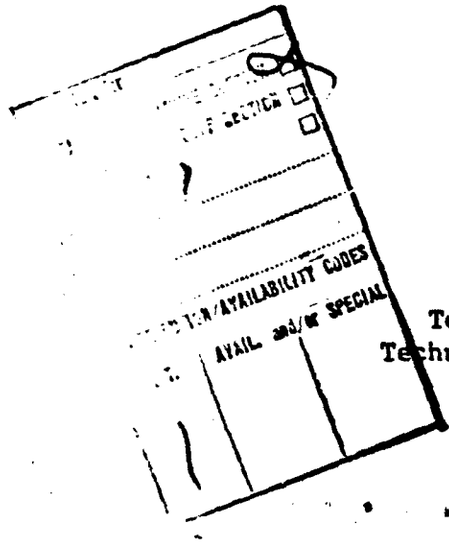

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[Following is the translation of an article by L. N. Zhuk, Military Medical Academy of the Order of Lenin imeni S. M. Kirova, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), #8, 1964, pages 26-29. The article was submitted on 16 Sep 1963. Translation performed by Sp/4 Richard M. Koplen]

In the process of development of botulinum intoxication, especially in its initial period, toxin circulates in the blood canal. Sergeyeva (1962), in experiments on rabbits, showed that during the oral and nasal introduction of toxin, it is detected in the blood in a period of 24-48 hours. So for therapeutic purposes it was fully justified, during botulinum intoxication, in using certain substances which have the capacity to absorb toxins.

In this respect special attention belongs to the disintoxication properties of low molecular polymer blood substitutes of the polyvinylpyrrolidone and polyvinyl alcohol types. Bennhold and Schubert (1943) showed that the first of these possesses the ability to absorb a number of substances which are alien for the organism. Thus, diphtherial, tetanic and botulinum toxins of types A, B and C, and also snake venoms are firmly connected with them and are removed from the organism with urine.

A therapeutic effect from the use of polyvinylpyrrolidone was obtained in an experiment and in clinical practice during tetanic intoxication, toxic diphtheria, chronic uremia, peritonitis, acute purulent otitis and radiation illness (Schubert, 1951; Sukasyan et al., 1959; From et al., 1962). On an experimental model of botulinum intoxication in guinea pigs, a therapeutic effect was obtained from the intravenous administration of polyvinylpyrrolidone (Schubert, 1951).

In recent years methods have been developed for the introduction of various chemical compounds into the structure of the indicated low molecular polymer blood substitutes (Ushakov, 1962). In experiments during botulinum intoxication, it was interesting to test various preparations of

these substances with groups having a high reactive capability included in their structure as therapeutic agents. These included acids, amines, sulfhydrils, etc. It could be assumed that during the encounter in an organism of molecules of polyvinylpyrrolidone and botulinum toxin, not only sorption occurs, but also a chemical reaction of detoxication with the groups included in the structure of the polymer.

The aim of this research was an experimental study, on a model of botulinum intoxication, of the therapeutic effect from the use of low molecular polymer blood substitutes and their copolymers -- carriers of groups with a high reactive capability (acid, amine and sulfhydryl).

We used the following preparations: Polyvinylpyrrolidone -- PVP; polyvinyl alcohol -- PVA 10,000; a copolymer of vinylpyrrolidone with crotonolic acid -- PVP (COOH); a copolymer of vinylpyrrolidone with vinylamine -- PVP (NH_2); a copolymer of vinyl alcohol with vinylmercaptan -- PVA (CH); and a copolymer of 2-methyl, 5-cinylpyridine with fumaric acid -- polyampholyte-1.

The molecular weights of the preparations constituted 10,000 -- 12,000. We prepared 2--2.5% working solutions of them in a physiological solution of sodium chloride.

From dry type A botulinum toxin we prepared three working dilutions on 1% normal rabbit serum in a physiological solution. The concentration of toxin in each subsequent dilution was decreased 1½ times. Each of the doses of toxin was administered subcutaneously in a volume of 0.5 ml to five to ten white mice weighing 18-20 grams each. In one or two hours after the injection of toxin, one of the stated preparations was introduced subcutaneously or into the caudal vein of the animals. We repeated the administration of the preparation in 24, 48 and 72 hours. The corresponding amount of physiological solution was administered to the animals of the control group at the same times and by similar methods.

Observation lasted for seven to 10 days. In the later periods the animals did not die and signs of illness were not noted among the surviving mice. We took into consideration the number of dead and surviving animals.

First of all we checked the harmlessness of all the preparations by administering them to healthy mice subcutaneously and intravenously in various volumes. In the tests we used only those doses and methods of administration of the preparations which were not accompanied by a change in the condition of the mice for a period of five to eight days.

During analysis of the therapeutic effect of the preparations, we processed the test data statistically. We determined the LD_{50} according to the method of Reed and Mench. With a positive therapeutic effect from

the use of a preparation the value of the LD_{50} logarithm was reduced. Based on the amount of difference between the logarithm of the LD_{50} in the control and in the test, it was possible to conjecture on the quantitative aspect of the therapeutic effect after the introduction of the given preparation.

For analysis of the reliability of the change of the LD_{50} logarithm under the influence of medicine, the results of the tests were processed by the method recommended by Khanin and Belgorodskaya (1963). With this aim, we calculated the difference t between the LD_{50} in the control and the test. We determined the reliability of the difference in percentages according to the table of Styudent. The difference of the values of the LD_{50} we regarded as significant if the reliability exceeded 95%.

The most expressed favorable influence on the outcome of botulinum intoxication in white mice was obtained by the use of polyvinylpyrrolidone ($t = 2.4$). A considerable, statistically reliable, effect was also noted in the use of PVA 10,000 ($t = 2.1$).

During the intravenous introduction of preparations with groups of high reactive capability (protonic acid and vinylamine) in their structure, a reduction of the LD_{50} was also noted, however the therapeutic effect in these tests turned out to be less expressed; the reliability of the difference in value of the LD_{50} in the test and the control was less than 95%, so it was not possible to consider these variations significant (see table).

During the subcutaneous introduction to white mice of the preparations under study, a noticeable therapeutic effect was not revealed ($t = 0.4$ -- 0.6).

We repeated the test on the intravenous introduction of PVA 10,000 in a somewhat different form: A single dose of toxin was administered subcutaneously to 24 mice, and of these, 16 died during a developing picture of botulism; in one to two hours after the introduction of toxin to mice of the test group we administered 0.25 ml of a 2% solution of PVA 10,000 to each, and we repeated the injections of the preparation in 24, 48 and 72 hours; the mice of the control group received intravenously, in the same periods, a corresponding amount of physiological solution. Of the 25 mice of the test group only six died. Variations of the level of lethality in the given test were significant ($t = 4.33$) with a high degree of reliability (greater than 99%).

Conclusions

1. A statistically reliable therapeutic effect in white mice was obtained from the intravenous introduction of low molecular polymer blood substitutes of the polyvinylpyrrolidone and polyvinyl alcohol types on models of botulinum intoxication. Subcutaneous introduction of these preparations proved to be ineffective.

2. Introduction of groups of high reactive capability (acids, amines, sulfhydryl) to the structure of low molecular polymer blood substitutes did not increase the therapeutic value of these preparations during botulinum intoxication.

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THERAPEUTIC EFFECTIVENESS OF THE STUDIED PREPARATIONS

Designation of preparation	Number of mice in test (numerator) and control (denominator)	Means of preparation introduction	Dosage for one administration (in ml.)	t difference	Authenticity of difference (%)
PVP and control.....	30/30	Intravenous	0.25	2.4	95-98
PVA 10,000.....	30/30	"	0.25	2.1	95
PVP (COOH).....	30/30	"	0.25	1.9	90-95
PVP (NH_2).....	30/30	"	0.25	0.9	60-70
PVA 10,000.....	15/15	Subcutaneous	0.50	0.6	40-50
PVA (CH).....	15/15	"	0.50	0.4	30
Polyampholite-1 and control.....	15/15	"	0.50	0.4	30