

TETANUS IMMUNIZATION WITH A SINGLE DOSE OF VACCINE. IMPORTANCE AND POSSIBILITIES FOR MASS-IMMUNIZATION PRO- GRAMS. PRELIMINARY REPORT ON EXPERIMENTAL STUDIES

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SUMMARY

Humans and animals experiments had already demonstrated the possibilities of protection against Tetanus with a single dose of toxoid. The Authors studied different preparations of vaccines and concluded that the more potent one was the mixture of a potent natural anatoxin plus the adjuvant 65. This vaccine was tested in animals and humans and showed to be more potent than the classical toxoids. The method of injection under pression (jet-injection) was considered important to accomplish the immunization because it allows wider contact of the antigen with elements able to produce antitoxins besides others advantages such as rapidity, economy of people and materials of injection.

The single-dose-scheme of protection against Tetanus was advised to countries that cannot afford enough money to fight the disease and intend to decrease the high index of mortality among their inhabitants.

INTRODUCTION

The classical schemes for Tetanus immunization includes 3 or, at least, 2 dosis of toxoid and the accomplishment of such schemes does not carry too much problems when we are dealing with well developed countries. But, when we are drawing a mass-immunization program to cover millions of individuals living in vast areas of under-developed countries, things change tremendously and all kinds of difficulties appear to obstruct the accomplishment of such a plan. Besides the lack of a preventive medicine mentality among these populations, the governments, for a lot of reasons, do not include in their budgets enough amounts of money to fight preventable diseases and millions of people pay, every year, with their own lives, the toll for such a situation.

Considering all these difficulties and as an effort to contribute to make simpler the schemes of immunization against Tetanus, we carried out some experiments to prove that it is possible to afford full protection against the disease with a single dose of an adequate vaccine.

Many experiments had already shown that a single dose of toxoid confer to animals a full protection against Tetanus and that they may survive the challenging with many lethal dosis of toxin^{1, 2}. SMITH⁷ demonstrated that a single dose of toxoid is enough to protect mice or guinea-pigs when challenged with tetanic spores, 10 or 12 days later. He also showed that a single dose of toxoid is enough to determine, in humans, the appear-

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rence of the minimum protective level of antitoxins at the end of the fourth week.

The experiments of NEWELL et al.⁵ on the prevention of Tetanus neonatorum showed that a single dose of aluminum phosphate adsorbed tetanus toxoid was able to determine an immediate and marked difference in the incidence of Tetanus neonatorum among the babies born from mothers vaccinated during the pregnancy. Based on the experiments of NEWELL et al.⁵ we are authorized to admit that a much better protection should be afforded by actively immunized individual, since the humoral immunity gained by the babies through the placenta is less effective than the cellular immunity conferred by the toxoid to the mother. We believe that, if a single dose of toxoid is able to markedly diminish the incidence of Tetanus neonatorum, we have strong reasons to admit that the same single dose of vaccine should be more efficient in diminishing the incidence of others forms of Tetanus which, very probably, are less severe than neonatorum Tetanus, in the vast majority of instances.

Another important fact to discuss, when we are going to admit the possibility of immunization with a single dose of toxoid is *the necessity or not* of the presence of the minimum title of antitoxin in the blood to testify the grade of protection attained by the animal actively immunized with one dose of toxoid. HEINIG³ demonstrated that a single dose of toxoid was enough to full protect 15 horses and 38 sheeps when challenged with up to 120 M.L.D. of tetanus toxin, *despite the absence of measurable antitoxin in the blood of the animals*. The experiments of WOLTERS and DEHMEL (cit. 2) demonstrated that the title of 0.01 unit, considered as the minimum protective level of antitoxin in the blood, is much above the "real" protective level, which can be determined only by challenging the animal with toxin. Titers as low as 0.005 units were able to full protect WOLTERS and DEHMEL when they were inoculated with many lethal dosis of toxin. This two researchers were protected by a high degree of cellular immunity, acquired years before through the injection of Tetanus toxoid.

The grade of protection afforded by a single dose of toxoid is much higher than indicated by the level of antitoxins in the blood

Under the influence of such a knowledge we tried to obtain a potent antitetanic vaccine which, in a single dose, could determine full protection against the disease, in a short period of time and the level of antitoxin produced could be high enough to merit the confidence of those who evaluate the potence of the vaccines only by the classical standards that includes, necessarily, the determination of the amounts of antitoxin elaborated by the body under toxoid stimulus.

MATERIAL AND METHODS

With preliminary experiments we tested different adjuvants which, joined to the anatoxin, could determine the higher and faster production of antitoxin in guinea-pigs. Seventeen groups (each one with 10 animals) of guinea-pigs weighing \pm 350 g were submitted to the experiment. The following preparations were inoculated in a single dose of 0.5 or 1 ml: natural anatoxin; natural anatoxin plus peanut oil (1:1); natural lyophilized anatoxin plus peanut oil (1:1); natural anatoxin plus adjuvant 65 (1:1); aluminum precipitated anatoxin plus peanut oil (1:1); aluminum precipitated anatoxin plus adjuvant 65 (1:1); lyophilized natural anatoxin; aluminum precipitated anatoxin; natural anatoxin plus peanut oil and 0.1 ml of Tween 1% (1:1).

The adjuvant 65 was prepared according to our citations 6 and 11.

Titration of antitoxins were carried out at 30, 60 and 120 days from the injection of the different preparations and the results were compared.

The potence of our natural anatoxin was tested in guinea-pigs and showed the following results: 1 ml of the natural anatoxin was able to protect 100% of the animals (10 animals) when challenged against 10 M.L.D. of toxin at the end of the sixth week. Another test of protection showed that 100% of the animals were protect against 2 M.L.D. of toxin at the end of the 15th day; with the same dose of natural anatoxin 90% of the animals were protected

against 5 and 10 M.L.D. at the end of the 15th day. However, when challenged with 5 and 10 M.L.D. of toxin 50% of the animals showed symptoms of Tetanus (1 to 2 +) which disappeared at the end of 1 week and no deaths occurred among the 30 animals submitted to this trial of earlier protection. The title of antitoxin at the 15th day was of 0.01 unit per ml of serum.

The four more potent preparations were separated according to the antigenic power demonstrated through the titrations of antitoxins and the results are shown in the Fig. 1.

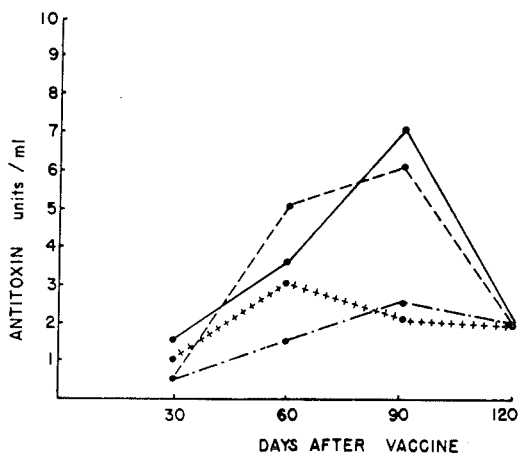


Fig. 1 — The antigenic power of the four more potent vaccines are represented here. The preparations were inoculated in guinea-pigs, in a single dose. The four groups were chosen among those showing the most satisfactory results in our experiments which included 17 groups of animals.

- Alum. ppt. anat. + peanut oil. Dose: 2 ml
- + + + + Nat. anat. + adjuv. 65. Dose: 2 ml
- Alum. ppt. anat. + adjuv. 65. Dose: 2 ml

Proportion of components: 1:1

RESULTS

We can see in the Fig. 1 that the more potent preparation was the mixture of a potent natural anatoxin plus the adjuvant 65. This preparation should be our vaccine of choice for further experiments in humans.

Our preparation of choice showed to be, in a single dose, 10 to 15 times more potent than the single dose of precipitated anatoxin used by D'ANTONA¹ in his experiments in animals and more efficient than the toxoid used by SMITH⁷, also in a single dose, in humans.

Our experiment in animals showed that a single dose of an adequate vaccine is able to sensitize the body to elaborate antitoxins and, at the same time, to work as a booster for a period of 2 or 3 months. This booster effect is clearly demonstrated by following the curve of antitoxins at 1 to 4 months from the injection of the vaccine.

Further experiments were carried out in humans in order to confirm the results we obtained in animals. Some alterations were done in these humans experiments:

a) The proportion of the components changed to 2 parts of natural anatoxin to 1 part of adjuvant 65. This had to be done because the proportion of 1:1 rendered the preparation too thick and very difficult to pass through the orifices of the jet-gun.

b) Instead of injection with the classical needle and syringe we used the jet-injector apparatus because we were interested to know how this apparatus works when using thick toxoids and to study the reactions of the body to the jet inoculations. Despite rendered the preparations less thick we still have doubts on the exact proportion of the components in each injection.

The first human experiment we carried out in ten volunteers of the military police of the State of São Paulo. Seven of the men were injected with 0.5 ml of the vaccine using the jet-injector apparatus. Three others men were injected with 1 ml of the same vaccine but using the classical needle and syringe. The route of inoculation was intramuscular. The results of the experiment are shown in Table I.

As we see in Table I, 100% of the individuals of the jet-injector group showed serological conversions at the end of 30 or 60 days. Surprisingly, only one individual, among the three that received 1 ml of the vaccine through the needle, showed conver-

TABLE I

Ident.	Units/ml before	Units/ml 30 days	Units/ml 60 days
1	-0.01	+0.01 -0.05	0.05
2	-0.01	-0.01	+0.05 -0.1
3	-0.01	—	+0.01
4	-0.01	-0.01	0.05
5	-0.01	—	0.01
6	-0.01	0.01	+0.01 -0.1
7	-0.01	—	0.05
8	-0.01	-0.01	-0.01
9	-0.01	-0.01	0.01
10	-0.01	-0.01	-0.01

Note — Individuals numbers 1 to 7 were inoculated with the jet-injector and numbers 8 to 10 with the classical needle.

sion at the end of 60 days. One explanation for this phenomenon would be that the jet-system allows the formation of millions of particles of the vaccine and the antigenic stimulus under this condition is greater. We already know that small amounts of smallpox vaccine diluted to 1:100 or 1:50 are enough to determine serological conversion to 90 to 100% of the vaccinées, when we use the jet-injector appareil^{4, 10}. Like we observed in animals, the vaccine showed to be still active after 1 month from the injection. No untoward effects were observed among the vaccinated individuals.

Despite convincing the results of this first human trial, we thought that better results

could be obtained with 1 ml of the vaccine instead of 0.5 ml. The amount of 1 ml of vaccine contains 0.66 ml of natural anatoxin and, obviously, should determine a greater production of antitoxin and a better grade of protection. So, ten children at the age of 2 to 5 years were selected in one nursery among those not previously vaccinated against Tetanus and 1 ml of the vaccine was injected with the jet-injector appareil. Samples of blood were taken before and at the end of the 30th day and 60th day after the vaccination with a single dose. No untowards effects were observed among the children and the results are represented in Table II.

TABLE II

Ident.	Units/ml before	Units/ml 30 days	Units/ml 60 days
1	0.01	0.5	0.7
2	+0.01 -0.1	0.1	0.05
3	-0.01	+0.01 -0.1	+0.01 -0.05
4	-0.01	+0.05	+0.5 -0.7
5	+0.1	+5	+12
6	-0.01	+0.01 -0.1	0.05
7	-0.01	0.1	0.1
8	-0.01	+0.1 -1	0.15
9	-0.01	+1	+1 -1.5
10	0.01	+5	+12

Results of antitoxin titrations in the blood of children vaccinated with 1 ml of the vaccine showed that this should be the dose of choice, since 100% of serological con-

versions were obtained at the 30th day after the vaccination as we can see at the Table II. The level of antitoxins are, also, higher with this dose (compare with Table I).

DISCUSSION

The results obtained with this vaccine allow us to state that a single dose of an adequate vaccine is able to confer a high grade of protection against Tetanus and millions of individuals should afford the benefit of it in countries where it is very difficult to inject, in such a large population, 2 or 3 dosis of toxoid, in a short period of time. We believe that the single-dose-scheme accomplished with the jet-injectors of multiple dosis (2,500 injections per hour) is the more rapid method of protection that could be carried out to decrease the tremendous index of mortality by Tetanus in many countries all over the world. We agree, on the other hand, that the single-dose-scheme is important only for countries where there is no conditions to plan a mass immunization program which includes two or three dosis of toxoid. We are convinced also, that there is much more scientific reasons to believe on the efficacy of a single dose of vaccine in the protection against Tetanus than on the world-wide belief in the protection afforded by the use of antitetanic serum with the classical dosis of 1.500 or even 3.000 I.U.⁹.

With the expenses required for the production and use of A.T.S. we can easily offer, to millions of people, a single dose of vaccine which is much safer, not hazardous and long-lasting.

The animals experiments showed also that a single dose of an adequate toxoid, even when affording only a partial immunity, this is enough to, significantly, diminish the lethality (deaths are very unusual when the animals are challenged against many lethal dosis of tetanus toxin).

All in all, we strongly believe that a "bath" of mass-immunization with a single dose of an adequate Tetanus vaccine would be the only plan which could be offered to many countries to fight the disease and rapidly recover the time they lost in preventing the deaths by Tetanus which should be prevented since 1924, when RAMON discovered the anatoxin. People immunized with a single-dose must be advised to use a booster dose of toxoid whenever necessary and possible. With this precautions we be-

lieve that a full protection is afforded and, surely, the index of mortality and lethality should decrease significantly.

RESUMO

Imunização contra o tétano com dose única de vacina. Importância e possibilidade em programas de imunização em massa. Nota preliminar sobre estudos experimentais.

Experimentações humanas e em animais já haviam demonstrado as possibilidades de proteção contra o tétano com dose única de toxóide. Os Autores estudaram diferentes preparações de vacina e concluíram que a preparação mais potente era constituída de uma mistura de potente anatoxina mais o adjuvante 65. Esta vacina quando testada em animais e seres humanos demonstrou ser mais potente que os clássicos toxóides. O método de injeção sob pressão (a jacto) foi considerado importante nesse esquema de imunização porque êle permite contato mais amplo entre o antígeno e os elementos produtores de antitoxinas, além de outras vantagens, tais como rapidez, economia de pessoal e de material de injeção.

O esquema de dose única foi aconselhado para países que não possuem verbas suficientes para combater a doença e pretendem diminuir os altos índices de mortalidade entre os seus habitantes.

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