INTRACISTERNAL INJECTION OF TETANUS ANTITOXIN IN EXPERIMENTAL TETANUS

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Experiments on albino rats with ascending and so-called descending (blood-borne) tetanus showed that injection of tetanus antitoxin into the cisterna magna in the initial stages of development of lethal tetanus and in the incubation period in ascending tetanus gives a more marked therapeutic effect than intravenous and intramuscular injection, a result attributable to the possibility of neutralizing some of the toxin entering the central nervous system when the antitoxin is injected by intracisternal route.

Like other proteins [2, 14], tetanus antitoxin does not pass through the blood-brain barrier (or does so in negligible amounts) [2, 26] and, in the existing view, it cannot neutralize toxin fixed by the brain substance. Intramuscular and intravenous injection of antitoxin for therapeutic purposes can thus neutralize only the free toxin before it has entered the central nervous system, thus limiting the effectiveness of antitoxin treatment even though it is still an essential part of the combined treatment of tetanus [4, 23]. Some results [16], including those obtained by the present authors [5], indicate that some toxin bound with its physicochemical receptor in the brain substance can, in principle, be neutralized by antitoxin (model experiments with protagon). There is thus an urgent need for the study of effective methods of bringing antitoxin to the structures of the central nervous system exposed to the action of tetanus toxin during development of the disease. Investigations [10-12, 17, 20, 21] have shown that, after injection into the ventricles or into the subarachnoid spaces of the brain, proteins can be found in adjacent neurons and glial cells.

With these results in mind, it was decided to study the effectiveness of subarachnoid injection of tetanus antitoxin and to compare it with the effectiveness of the antitoxin when injected by ordinary methods (intravenously and intramuscularly). The problem of subarachnoid injection of antitoxin has been discussed for many years in the literature (see below), but no final decision has yet been reached.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats of both sexes weighing 220-270 g. Two types of experimental tetanus were used [4]: ascending and blood-borne tetanus. The first type was produced by injecting the toxin (1 MLD) into the gastrocnemius muscle, the second by injecting the toxin (0.5 MLD) intravenously. A liquid (glycerol) form of toxin was used, made from batch No. 8 dry toxin from the I. I. Mechnikov Moscow Institute of Vaccines and Sera, containing 1200 MLD/ml for rats weighing 250 g, and a purified tetanus antiserum "Diaferm-3 IÉM, batch No. 44, manufactured by the same Institute (3000 i.u./1.9 ml). The serum was injected intramuscularly, intravenously, and suboccipitally (in the cisterna magna) in a volume of 0.2 ml and a dose of 100 i.u., corresponding to 350 i.u./kg when expressed relative to body weight; according to previous investigations in the laboratory [5], this dose gives the optimal therapeutic effect when the antitoxin is injected intramuscularly. For injection of the antitoxin in the cisterna magna the animal was lightly anesthetized with ether, and in addition, a subcutaneous injection of 0.2 ml 25% analgin solution was given 30 min beforehand to produce analgesia, to potentiate the anesthesia, and to prevent shock.

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TABLE 1. Effectiveness of Various Methods of Injection of Antitoxin in Different Stages of Development of Ascending and Blood-Borne Tetanus

Time of injection of antitoxin after injection of toxin (in h)	Method of injection of antitoxin	No. of animals in expt.	No. of animals surviving	Number of animals dying		Duration of life of dying animals (in days)	P
				during 1st 10 days	during 2nd 10 days		
	Ascending tetanus						
36	Intravenously	10	0	10	0	5.5±0.60	0.01
	Intracisternally	10	4	5	1	8.5±1.50	
48-50	Intramuscularly	11	0	11	0	4.2± 0.82	
	Intravenously	15	0	14	1	4.3±1.25	0.01
	Intracisternally	15	1	13	1	6.7±2.00	
72	Intravenously	6	0	6	0	4.7±0.43	
	Intracisternally	6	0	6	0	4.7±0.67	
Control	No antitoxin injected	13	0	13	0	4.1±0.28	
	Blood-borne tetanus						
36	Intravenously	5	1	4	0	6.2±4.19	0.01
	Intracisternally	5	3	1	1	11.5±3.5	
42-44	Intramuscularly	6	0	6	0	4.6±0.77	
	Intravenously	12	0	12	0	4.6±0.94	<0.05
	Intracisternally	12	1	9	2	7.4±1.38	
48-50	Intravenously	10	0	10	0	5.3±1.38	
	Intracisternally	10	0	10	0	5.8±1.48	
Control	No antitoxin injected	13	0	13	0	4.2±0.58	

(Control tests showed that this type of anesthesia itself has no effect on the course of tetanus toxicosis.) The injection was not preceded by withdrawal of cerebrospinal fluid.

Serum therapy was given at various times after injection of the toxin and at different stages of development of the disease: in ascending tetanus in the stage of local tetanus (36 h), in the period of initial symptoms of generalized tetanus (after 48-50 h), and in the stage of advanced, severe generalized tetanus (72 h); in the case of blood-borne tetanus, in the period of appearance of the initial manifestations of descending tetanus, i.e., rigidity of the head and neck muscles (36 h), in the stage of generalized muscle rigidity (42-44 h), and in the stage of severe general tetanus (48-50 h) with severe dyspnea, marked generalized rigidity, and so on. In addition, in a special series of experiments with ascending tetanus, the antitoxin was injected during the incubation period (6 and 12 h after injection of the toxin). The effectiveness of the methods used to inject the antitoxin was judged from the development of the disease, the survival rate, and the duration of survival of the dying animals. Since death of the animals in the late stages of the disease is associated with secondary, nonspecific pathological processes [4], death of the animals during the first and second 10-day period after injection of the toxin was considered; in the first case death of the animals can be regarded as the result of tetanus itself, in the second case as the result of secondary complications [4].

EXPERIMENTAL RESULTS

As Table 1 shows, intracisternal injection of the antitoxin gave a better therapeutic effect than intravenous and intramuscular injection. This is clear from the fact that in relatively early stages of the disease (36 h), when intravenous injection of antitoxin did not prevent death (the animals died during the first 10 days), intracisternal injection of antitoxin prevented death of 50% of the animals; the duration of survival of the dying animals receiving antitoxin intracisternally was significantly longer (by 1.5 times) than that of animals treated by intravenous injection of antitoxin.

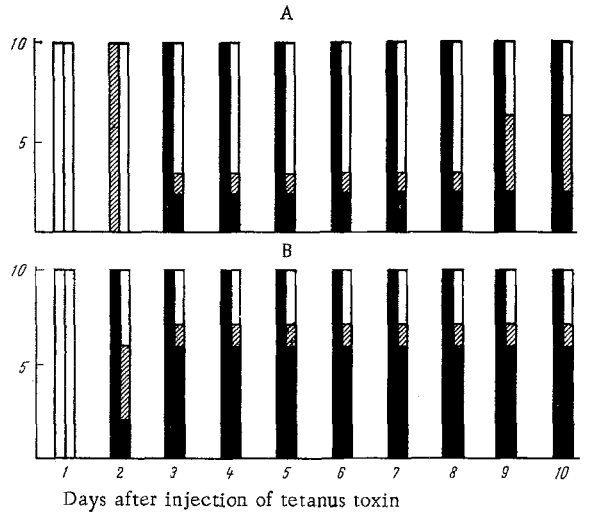

Fig. 1. Days after injection of tetanus toxin. Effect of injection of tetanus antitoxin in incubation period of ascending tetanus 6 (A) and 12 (B) h after injection of tetanus toxin. Each group of animals (left and right halves of columns) include 10 rats. Left half of columns represents intravenous injection of antitoxin, right half intracisternal injection. Unshaded parts of columns show animals not developing disease, obliquely shaded parts represent animals with slight manifestations of local tetanus (increased extensor tone), black areas denote animals with local tetanus.

In the later stages of the disease (48-50 h in the case of ascending tetanus and 42-44 h in blood-borne tetanus), neither intravenous nor intramuscular injection of the antitoxin had virtually any effect on the course of the disease (all the animals died at about the same times as the controls, not receiving antitoxin, while intracisternal injection of antitoxin, although not preventing death of the animals, considerably lengthened (by 1.5 times) their period of survival. It is a striking fact that the duration of survival of animals treated by antitoxin intracisternally at these, later stages, of toxicosis was actually slightly longer or, at least, no shorter than after intravenous injection in the early period of the disease (36 h after injection of the toxin).

It is noteworthy that the beneficial effect of intracisternal injection of antitoxin was observed in both forms of tetanus, including (and this is particularly important) in the blood-borne type of tetanus, the form of tetanus in animals which is pathogenetically close [4] to tetanus in man (descending tetanus).

It was only in a very severe form of generalized tetanus (after 72 h in the case of ascending tetanus, i.e., 24 h before death of the animals of the control group, and after 48-50 h in blood-borne tetanus) that intracisternal injection of antitoxin was just as ineffective as intravenous injection.

Intracisternal injection of antitoxin in comparatively early stages of lethal general tetanus is thus more effective than intramuscular and intravenous injection. It must be remembered that, because of the nature of the experiments on rats, intracisternal injection was associated with much greater (sometimes as much as 40-50%) loss of antitoxin, which escaped after withdrawal of the needle, so that the dose of antitoxin injected was actually much smaller than that given intravenously. Special control experiments showed that the beneficial effect of antitoxin injected intracisternally cannot be explained by additional stimulation of the central nervous system or an increase in the permeability of the blood-brain barrier resulting from injection of fluid into the subarachnoid space, for in experiments when the antitoxin was injected into the blood stream and physiological saline was injected intracisternally, the antitoxin had no beneficial effect.

The positive effect of intracisternal injection of antitoxin in the incubation period of ascending tetanus was particularly demonstrative (Fig. 1). Previous investigations [3, 4] showed that 6 h after injection of the toxin into the gastrocnemius muscle it was found along the neural pathway and could have reached the ventral roots, while after 12 h it had reached the anterior horns of the lumbar segments of the spinal cord. Intravenous injection of antitoxin at these times completely protected all the animals from general ascending tetanus, but did not prevent the development of local tetanus due to the action of toxin on structures in the anterior horns of the spinal cord [4]. Intracisternal injection of antitoxin, on the other hand, prevented the development not only of ascending tetanus, but also of local tetanus in some of the animals; in the other animals it considerably delayed the development of the disease and weakened its severity.

The mechanisms of action of antitoxin when injected intracisternally are apparently complex. Special investigations using a dye showed that after injection into the cisterna magna the dye was found in the CSF system along the whole length of the spinal cord down to the sacral segments. It is also known [7, 9] that a substance, if injected intracisternally, is disseminated with the flow of CSF in a caudal direction. Consequently, after injection into the cisterna magna, the antitoxin spreads along the spinal cord, reaching all its segments including the sacral portion. This is confirmed by the results of experiments in which

antitoxin was injected in the incubation period. Thereafter, the action of the antitoxin may be twofold. First, it may reach the region of the ventral roots in the perineural spaces communicating with the general subarachnoid space [9]. As a result it can penetrate into the neural pathway and neutralize toxin already there, or block any further advance of the toxin along the neural pathway. Second, antitoxin probably penetrates, like other proteins [10-12, 17, 20, 21] directly into the brain tissue from the CSF, neutralizing any toxin accessible to it in the brain and protecting as yet unaffected nervous structures from its action. Both these ways of entry of the antitoxin – into the neural pathways and into the brain tissue – must exist throughout the length of the spinal cord and in the medulla. For this reason, intracisternal injection of antitoxin is effective in both ascending and blood-borne tetanus. It is particularly important to note that this action of the antitoxin can be found in the bulbar region, a comparatively early victim of the action of toxin in blood-borne tetanus [4].

It is an interesting fact that when antitoxin is injected intracisternally in the incubation period, after a long period of absence of clinical manifestations of the disease they nevertheless appear, and in some animals they are observed even after very long intervals. This means that the toxin can remain for a long time in the neural pathway and can even move along it. Since antitoxin, like other proteins, is liberated from the CSF system into the blood, the blocking of the brain and central portion of the neural pathway by antitoxin weakens with time, and entry of the toxin into the spinal cord along the neural pathway in the late stages may give rise to manifestations of local tetanus. These findings show the need for further injection of antitoxin at the periphery. Such injection is particularly important in the case of bacteriogenic tetanus, when a focus of toxin production is present.

Injection of antitoxin into the CSF system of the brain for therapeutic purposes was first used by Sherrington [24] in experiments on monkeys, and it has subsequently been used in experimental and clinical practice [8, 15, 18, 19, 22] and for the treatment of livestock [1]. It was then abandoned because of the danger of complications, particularly of inflammation, cerebral edema, and other reactive changes in the central nervous system in response to the foreign protein [4, 6, 13, 25-27]. Nowadays, because it is possible to use purified preparations of antitoxin as well as other preparations with lowered reactogenicity, in conjunction with drugs controlling inflammation and edema, the problem of subarachnoid administration of antitoxin in conjunction with other therapeutic substances can once again be put forward for serious consideration.

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