Perfluorodecalin-Induced Changes in Clinical and Immunological Parameters of Experimental Allergic Encephalomyelitis

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Perftoran injected into guinea pigs for 17 days after immunization with encephalitogenic mixture reduces morbidity and/or mortality, suppresses specific inhibition of migration of peripheral blood leukocytes and skin delayed-type hypersensitivity reaction to the main myelin protein, and stimulates the production of anti-myelin antibodies.

Key Words: *experimental allergic encephalomyelitis; Perftoran; lymphocyte migration; delayed-type hypersensitivity; anti-myelin antibodies*

Therapy of multiple sclerosis and other demyelinating diseases remains an actual problem. The search for new means modifying the development of experimental allergic encephalomyelitis (EAE), which represents a model of neurological disorders and damage to nervous tissue observed in multiple sclerosis, is now in progress. In this context, considerable attention has been focused on cytochrome P-450 inductors [3,10], whose immunomodulating effects are related to functional interplay between the immune and enzyme systems [7]. Adjuvant arthritis, an autoimmune disease, is characterized by suppression of the cytochrome P-450-dependent monooxygenase system [6]. Perfluorodecalin, a cyclic perfluorocarbon, is a nonmetabolizing inductor [2]. It has been shown that the development of delayed-type hypersensitivity (DTH) suppresses the activity of the cytochrome P-450-dependent monooxygenase system in animals, while perfluorodecalin, an activator of this system, reduces local inflammatory DTH reaction [9]. Since cellular immune reactions, in particular DTH, are involved in the development of EAE [5, 11,12] and induction of EAE is inevitably accompanied by humoral immune response [13,14], the aim of the present study was to examine a possible effect of the commercial preparation Perftoran (active substance perfluorodecalin) on the development of EAE and cellular and humoral reactions to encephalitogenic antigens.

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MATERIALS AND METHODS

EAE was induced by single subcutaneous inoculation of myelin isolated from rabbit brain [8] and emulsified (1:1) in complete Freund's adjuvant. Some animals were intraperitoneally injected with Perftoran (*Perftoran* Pharmaceutic Company, Russian Academy of Medical Sciences) in a single dose of 10 ml/kg before or after inoculation. Migration activity of peripheral blood leukocytes in the presence of myelin basic protein (MBP) isolated from the brain by column chromatography [4] was assessed at the end of latent period and 3 weeks after the start of the experiment. Inhibition of leukocyte migration was evaluated by comparing zones of cell migration in the culture medium and in the presence of MBP [1]. Circulating anti-myelin complement-binding antibodies were measured and DTH was assessed by skin tests (1 ml native myelin and 0.05 mg lyophilized MBP were dissolved in 0.1 ml physiological saline and injected

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TABLE 1. Effect of Perftoran of EAE Induction

Dose of myelin in encephalitogenic mixture, mg	Time of Perftoran injection, days	Number of animals	Ill animals		Latency, days	Died	
			number	%		number	%
5	+3	17	4	23.3*	19.5±2.86*	1	5.8
	+10	18	5	27.7	15.0±1.28	2	11.0
	—	18	10	55.5	13.7±0.43	3	16.6
50	-5, +6, +17	20	11	55.0*	13.7±0.33**	7	35.0**
	—	19	16	84.2	12.7±0.19	14	73.7

Note. Here and in Tables 2-4: time of Perftoran injection is specified with respect to the day of inoculation. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with animals not treated with Perftoran.

subcutaneously). The data were processed statistically using the Student's t test.

RESULTS

Neurological symptoms of EAE such as myasthenia, motor pareses and paralyses, and dysfunction of pelvic organs appeared on day 11 after sensitization. As seen from Table 1, the lowest morbidity and maximum latency after inoculation of 5 mg myelin were observed in animals injected with Perftoran on day 3 after sensitization; both parameters significantly differed from those in the control group. A 10-fold increase in the dose of encephalitogenic agent (50 mg) led to earlier appearance of neurological disorders and increased morbidity and mortality in experimental animals. However, triple administration of Perftoran alleviated the parameters of EAE.

The data on the effect of Perftoran on the inhibition of lymphocyte migration in sensitized animals are presented in Table 2. In animals not injected with Perftoran, the inhibition of cell migration in the presence of MBP was noted on days 11 and 20 after immunization. Perftoran injected on day 3 after immunization abolished inhibition of lymphocyte migration on day 11 but not on day 20, while Perftoran injection on day 10 abolished this effect on day 20, the inhibition of leukocyte migration on day 11 being unchanged.

Skin tests were carried out on day 11 after inoculation. The reaction was evaluated by the diameter of infiltrate and erythema in the site of test antigen injection. As seen from Table 3, the number and magnitude of positive skin tests were significantly lower in Perftoran-treated animals.

Serum was tested for the presence of anti-myelin antibodies before and after the onset of the disease

TABLE 2. Effect of Perftoran on Migration of Peripheral Blood Leukocytes in Myelin-Sensitized Animals

Time of Perftoran injection, days	Zones of leukocyte migration (micrometer scale)			
	day 11		day 20	
	spontaneous	in the presence of MBP	spontaneous	in the presence of MBP
—	19.87±2.67	13.08±1.82*	21.36±2.17	15.28±1.68*
+3	23.30±2.75	19.87±2.36	24.54±2.34	18.60±1.92*
+10	21.43±2.22	15.32±2.06*	27.76±3.08	23.64±2.84

Note. *$p<0.05$ compared with the parameters of spontaneous migration of leukocytes from animals of the same group measured at the same time.

TABLE 3. Effect of Perftoran on Skin DTH in Guinea Pigs Immunized with 50 mg Myelin

Time of Perftoran injection, days	Number of animals	Reaction to myelin			Reaction to MBP		
		abs.	%	diameter, mm	abs.	%	diameter, mm
-5, +6, +17	18	14	77.7*	10.1±1.3**	4	22.2**	6.8±0.64***
— (Control)	18	18	100	14.0±0.79	11	61.1	9.4±0.49

TABLE 4. Effect of Perftoran on the Production of Anti-Myelin Antibodies in Guinea Pigs Immunized with Encephalitogenic Mixture

Time of Perftoran injection, days	Immunological parameters	Time after immunization, days					Total
		11	20	27	34	41	
+3	Presence of AB	4/17	14/16*	10/16	5/13	2/11	35/73**
		23.5%	87.5%	62.5%	38%	18.2%	48%
	AB titer	10	24±2.4	21±2.1	16±2.1	15±8.8	18.8±1.2*
+10	Presence of AB	2/18	13/16	8/16	5/15	2/15	30/80
		11.1%	81.2%	50%	33%	13.3%	37.5%
	AB titer	10	22.2±2.6	19±2.6	14±2.1	10	17.6±1.3
— (Control)	Presence of AB	1/18	9/17	7/17	3/14	1/13	21/79
		5.5%	52.9%	41%	21%	7.7%	26.6%
	AB titer	10	18±3.5	14±1.5	10	10	14.3±1.7

Note. AB: antibodies; numerator: number of animals with AB; denominator: number of examined animals.

(5 times with 5-9-day intervals). Two parameters were taken into account: the incidence and mean titers (Table 4). Much the same dynamics of these parameters was noted in animals of different groups from day 11 through day 41 after immunization. When comparing these parameters at different times postimmunization, a higher values were noted in Perftoran-treated group; although the differences were significant only after the early (on day 3) injection of Perftoran and only on day 20 postimmunization and for the integral values over the observation period.

These findings suggest that Perftoran modulates the development of EAE, reducing the morbidity and prolonging the latency of its clinical manifestations. Single injection of Perftoran produced a moderate suppressive effect, which was more pronounced when the preparation was administered in the beginning of latent period. Triple administration also decreased mortality. Suppression of EAE development was accompanied by inhibition of migration of peripheral blood leukocytes in the presence of MBP and suppression of skin DTH reactions to myelin and MBP. However, Perftoran did not inhibit generation of anti-myelin antibodies; in some cases these antibodies were detected more frequently and in higher titers. These data suggests that Perftoran is capable of suppressing EAE and cell immune reactions to myelin and MBP. This effect depends on the time of administration and the number of injections. This should be taken into account when Perftoran is used against multiple sclerosis.

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