Eur Arch Psychiatry Clin Neurosci (1991) 240:279–281

European Archives of Psychiatry and Clinical Neuroscience

Decrease in the Carbamylcholine-Induced Chemotaxis of Monocytes in Myasthenia Gravis

Sandor Sipka[1], Peter Diószeghy[2], Ferenc Mechler[2], Katalin Lukács[1], Judit Szelényi[3], and Gyula Szegedi[1]

[1]Third Department of Internal Medicine and [2]Department of Neurology, University Medical School of Debrecen, and [3]National Institutes of Blood Transfusion, Budapest, Hungary

Received October 3, 1990

Summary. The carbamylcholine-induced chemotaxis of monocytes was decreased in patients with myasthenia gravis, whereas no change was found in the C5a-induced locomotion of these cells compared with that of the normal controls. The decrease in the chemotaxis induced by carbamylcholine correlated with the severity of clinical symptoms. The beneficial effect of thymectomy was also reflected in the improvement of chemotaxis. The method is simple, not expensive and could be used in the diagnosis of myasthenia gravis.

Key words: Carbamylcholine – Chemotaxis – Monocytes – Myasthenia gravis

Introduction

Myasthenia gravis (MG) is a disorder characterized by muscle fatigue and weakness following repetitive activation and prolonged tension of muscles. This is due to a reduction in the number of functioning acetylcholine receptors (AChRs) at the neuromuscular junctions. In most cases MG is associated with antibodies directed against the nicotinic AChRs. The antibodies are usually IgG [1, 2, 4, 5, 9, 12]. They are present in about 85% of MG patients but their titre varies greatly and does not correlate well with the clinical severity of the disease. However, intraindividually there is a good relationship between changes in titre and clinical severity, particularly during and after treatment with plasma exchange or immunosuppressive drugs.

It is known that acetylcholine (ACh) and carbamylcholine (carbachol, CCh) stimulate the chemotaxis of phagocytic cells [3, 14]. Human monocytes have AChRs [6]. In the experiments carried out for the analysis of this type of receptors, instead of ACh, CCh was used because of its stability, as CCh is not degradated by cholinesterase [15].

The present study was designed to compare the correlation between the chemotactic behaviour of monocytes of MG patients and control subjects under the influence of a CCh signal acting on the AChRs of these cells.

Offprint requests to: F. Mechler, Department of Neurology, University Medical School of Debrecen, H-4012 Debrecen, Hungary

Materials and methods

Twenty patients (27 samples) with MG representing different clinical types (1, 2A, 2B, 4, according to the classification of Osserman and Perlo [11, 13] and 25 healthy controls were included in the study. In the period of the experiments all of the patients were completely free of drugs or they had been without drugs for at least 1 week.

Human monocytes were obtained by the method of Nakagawara et al. [10]. Heparinized venous blood (15 ml) was sedimented on a Ficoll-Uromiro gradient. After washing, the cells were suspended in Parker's medium, containing 10% fetal calf serum and 20 m*M* HEPES. They were counted in a standard haemocytometer. A trypan blue exclusion test and non-specific esterase staining were performed to determine the percentage of viable (90–95%) and phagocytic cells (35–45%).

In the test of monocyte chemotaxis the chambers were divided into two compartments with a Millipore filter of 5 μm pore diameter (Sartorius Membranfilter, Goettingen, Germany). Into the upper compartments cell suspensions of 0.5 ml (0.5×10^6 monocytes in Parker's medium T 199 with 10% fetal calf serum) were transferred. In the lower compartments of the chambers 1 ml of the chemotactic factors were placed: (1) 10^{-5} *M* CCh (Sigma, St. Louis, MO, USA) or (2) zymosan (Mannozym, Budapest, Hungary) activated human serum (ZAS) containing generated C5a. In order to measure the spontaneous migration of cells, Parker medium (free of chemotactic agents) was used alone in the lower compartment of the chamber. After filling the chambers, they were kept at 37°C for 60 min, then the membranes were fixed, stained with Ehrlich's haematoxylin and the chemotaxis of cells was evaluated by the leading front method [7, 8]. By moving the micrometer screw of the microscope, the longest distance in micrometres attained in the membrane by a minimum of 2 cells/visual field (representing the cells of the leading front) during the time of incubation was measured. Ten visual fields per membrane were measured (enlargement: $63 \times 0.8 \times 12$).

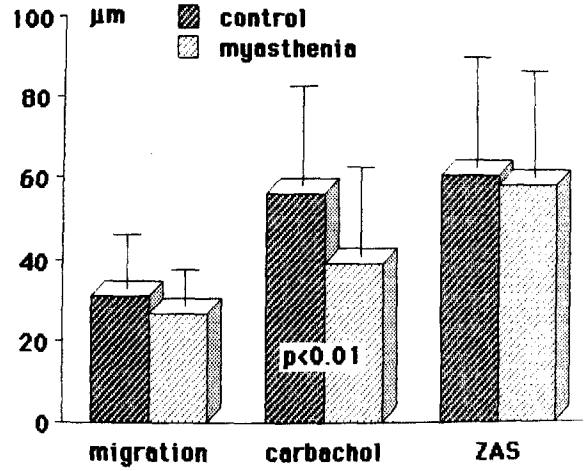

Fig. 1. Spontaneous migration, carbamylcholine-($10^{-5}M$) and ZAS-induced chemotaxis of human monocytes derived from patients with myasthenia gravis and healthy controls (mean values + SD). Number of controls: 25; number of samples from MG patients: 27; *ZAS*; zymosan-activated (C5a containing) human serum

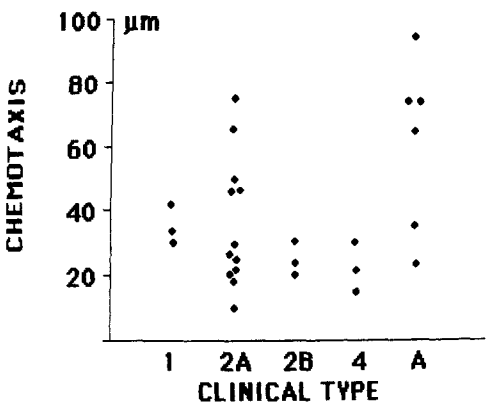

Fig. 2. The relationship between clinical state and carbamylcholine-induced chemotaxis. Clinical type *1*: ocular form; *2A*: mild generalized myasthenia gravis (MG) with ocular symptoms; *2B*: moderately severe generalized MG; *4*: late severe MG with bulbar symptoms; *A*: complete remission)

Results

CCh ($10^{-5}M$) was found to induce approximately as intensive chemotaxis of healthy monocytes as ZAS. Therefore this concentration of CCh was used throughout the experiments.

The mean value of spontaneous migration and the ZAS-induced chemotaxis of monocytes were almost the same in the patients with MG and in the controls. However, the CCh-induced chemotaxis of MG patients was significantly lower than that of controls (Fig. 1)

The decrease in CCh-induced chemotaxis was related to the clinical state of the disease. The severely ill patients (groups 2B and 4) had low values without exception, while normal results were relatively frequent in patients with complete remission (Fig. 2).

The CCh-induced chemotaxis in MG patients without thymectomy was diminished in all cases but one (Fig. 3.), while normal values were found only 1 year after thymectomy.

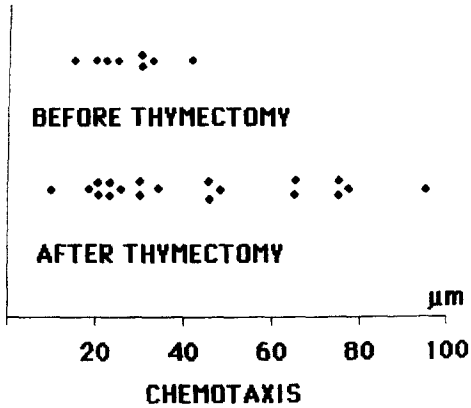

Fig. 3. The effect of thymectomy on the carbamylcholine-induced chemotaxis of monocytes

Table 1. Chemotaxis of healthy monocytes preincubated with normal and myasthenia gravis (MG) serum

Chemotactic signal	Chemotaxis of monocytes preincubated with normal serum (µm)	Chemotaxis of monocytes preincubated with MG serum (µm)
ZAS	93	75
Carbamylcholine	88	52

The preincubation of monocytes of healthy controls with the sera of MG patients resulted in a decrease of CCh-induced chemotaxis of these cells. Table 1 shows the data of such an experiment.

Discussion

The immunoprecipitation of AChRs prelabelled with ^{125}I alpha-bungarotoxin by antireceptor antibodies is a widely used method for the measurement of antibodies among the laboratory tests for MG [1, 2]. This procedure, measuring the circulating antibodies, requires a well-equipped and rather specialized laboratory. According to our present data the detection of decreased CCh-induced chemotaxis of monocytes in MG patients could be used as a simple, informative and inexpensive method in the laboratory diagnosis of MG. The critical point of this chemotaxis assay is how the subjectivity can be avoided during the microscopic evaluation. In our practice, all the samples arrive at the laboratory with code numbers and without names, so the laboratory staff do not know which sample belongs to the group of MG patients or controls. In our experimental system CCh was able to induce the chemotaxis of monocytes directly, not merely to potentiate the chemotaxis, as has been reported [3, 14].

The phenomenon of decreased CCh-induced chemotaxis is supposed to be related especially to the AChRs of MG monocytes because the ZAS-induced chemotaxis

of these cells is not impaired. Furthermore, the sera of MG patients could decrease the CCh-induced chemotaxis of healthy monocytes after preincubation, suggesting that the circulating antibodies could bind to the AChRs.

In some cases we compared the data of chemotaxis and immunoprecipitation assays. High levels of anti-ACh antibodies were found in the patients with severe MG and the CCh-induced chemotaxis of monocytes was far below the control values (unpublished data). The results of the chemotaxis test correlated with the severity of clinical symptoms and also with the clinically verified, beneficial effect of thymectomy. We suggest that the number of AChRs on the surface of MG monocytes is decreased like those on lymphocytes [16]. Consequently, these monocytes are not able to respond to CCh as actively as the monocytes of healthy persons, which have a greater number of free AChRs.

The monocyte chemotaxis assay is relatively simple and cheap. However, it requires technical skill and well-standardized circumstances throughout the procedure and evaluation. Based upon our experiments, the measurement of CCh-induced chemotaxis of monocytes may be useful in the diagnosis of MG.

References

1. Drachman DB (1981) The biology of myasthenia gravis. Ann Rev Neurosci 4:195–225
2. Drachman DB, Angus CV, Adams RN, Michelson ID, Hoffman J (1978) Myasthenic antibodies cross-link acethylcholine receptors to accelerate degradation. N Engl J Med 298:1116–1122
3. Estensen RD, Hill HR, Quie PG, Hogan N, Goldberg DN (1973) Cyclic GMP and cell movement. Nature 245:458–460
4. Lindstrom J, Seybold ME, Lennon VA, Whittingham S, Duane D (1976) Antibody to acethylcholine receptor in myasthenia gravis: prevalence, clinical correlates and diagnostic value. Neurology: 26:1054–1059
5. Lindstrom J, Shelton D, Fujii Y (1988) Myasthenia gravis. Adv Immunol 42:233–284
6. Lopker A, Abood LG, Hoss W, Lionetti FJ (1980) Stereo selective muscarinic acethylcholine and opiate receptors in human phagocytic leucocytes. Biochem Pharmacol 29:1361–1365
7. Lukács K, Frendl GY, Berényi E, Kávai M, Szabó G, Szegedi GY (1980) Monocyte chemotaxis and effect of Levamisole in Hodgkin's disease. Acta Med Acad Sci Hung 37:145–150
8. Lukács K, Kávai M, Bányai A, Sonkoly I, Végh É, Szabó GY, Szegedi GY (1984) Effects of immune complexes from SLE patients on human monocyte locomotion and Fc receptor function. Ann Rheum Dis 43:729–733
9. Morel E, Vernet-der Garabedian B, Eymard B, Raimond F, Bustarret FA, Bach JF (1988) Binding and blocking antibodies to the human acethylcholine receptor: are they selected in various myasthenia gravis forms? Immunol Res 7:212–217
10. Nakagawara A, Nathan CF, Cohn ZA (1981) Hydrogen peroxide metabolism in human monocytes during differentiation in vitro. J Clin Invest 68:1243–1251
11. Osserman KE, Genkins G (1966) Studies in myasthenia gravis. Short-term massive corticotropin therapy. JAMA 198:699–702
12. Patrick J, Lindstrom J (1973) Autoimmune reponse to acethylcholine receptor. Science 180:871–872
13. Perlo VP, Poskanzer DC, Schwab RS, Viets HR, Osserman KE, Jenkins G (1966) Myasthenia gravis: evaluation of treatment in 1355 patients. Neurology 16:431–439
14. Pham Huu T (1979) Chemotaxis of human leukocytes. II. Biomedicine 30:121
15. Richman DP, Arnason BGW (1979) Nicotinic acethylcholine receptor: evidence for a functionally distinct receptor on human lymphocytes. Proc Natl Acad Sci 76:4632–4635
16. Szelényi JG, Páldi-Haris P, Pálóczy K, Pócsik É, Horusko A, Klein M, Szobor A, Hollán S (1990) Altered function and nicotinic acetylcholine receptor (NAChR) of lymphocytes in myasthenia gravis. Int J Immunopathol Pharmacol 3:19–27
17. Vincent A, Whiting PJ, Schuelp M, Heidenreich F, Lang B, Roberts A, Willcox N, Newsom-Davis J (1987) Antibody heterogeneity and specificity in myasthenia gravis in myasthenia gravis: biology and treatment. Ann N Y Acad Sci 505:106–120