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Effect of Colchicine on Complement Activity in Familial Mediterranean Fever*

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Abstract: The present study determined the total hemolytic activity of the complement and the activities of individual complement components, C1, C2, C3 and C4, in the blood serum of patients with FMF and assess the influence of colchicine medication on these parameters. No significant changes in the total hemolytic activity of the complement and the activities of C1, C4, C3 and C2 complement components were found between the healthy subjects and those FMF patients, who received regular colchicine treatment. On the contrary, the total hemolytic activity of the complement and C1, C2, C3 and C4 hemolytic activities in the serum of colchicine-free FMF patients were significantly higher than in the healthy subjects. In the serum of those patients, who received irregular colchicine treatment, significant changes were detected only in case of the total hemolytic activity of the complement and the hemolytic activity of C2 complement component: both parameters were higher than those of healthy subjects. Our results demonstrate that regular colchicine treatment results in suppression of the complement classical pathway functional activity in FMF patients, suggesting about possible anti-inflammatory effect of colchicine and confirm the efficiency of regular colchicine treatment.

Key words: Colchicine, complement components, complement hemolytic activity, familial Mediterranean fever

Introduction

Familial Mediterranean fever (FMF; MIM 294100) inherited as an autosomal-recessive trait, is the most prevalent hereditary periodic fever worldwide and the FMF gene (designated as MEFV) is expressed predominantly in granulocytes, activated monocytes and in early leukocyte development. FMF is a genetic autoinflammatory disorder characterized by self-limited recurrent episodes of fever and localized serosal inflammation. FMF occurs most commonly in people of non-Ashkenazi Jewish, Armenian, Arab, Greece and Turkish background. The gene found on chromosome 16, codes for a protein, pyrin. This protein is a part of regulatory pathway of inflammation and apoptosis. It was proposed that pyrin is likely normally assist in keeping inflammation under control by deactivating the immune response and that mutations in pyrin gene lead to a aberrant protein and uncontrolled inflammation. An inappropriate full-blown inflammatory reaction occurs: an attack of FMF. People

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with FMF suffer from recurring bouts of fever, most commonly with severe abdominal pain due to inflammation of abdominal cavity. Attacks can also include arthritis, chest pain from inflammation of the lung cavity and skin rashes. Some patients develop amyloidosis. (Grateau, 2005; Medlej-Hashim *et al*., 2004; Schaner and Gumucio, 2005). Since 1972 colchicine, a neutral alkaloid, has become the drug of choice for prophylaxis against FMF attacks and related inflammatory episodes and FMF-associated amyloidosis. It is well-established fact that prevention of amyloidosis is achieved through inhibiting the assembly of microtubules and mitotic spindle formation by binding beta-tubulin and making beta-tubulin-colchicine complexes. Our knowledge about the anti-inflammatory effect of colchicine is limited. Based upon the results of several studies demonstrating that colchicine is able to modulate the effects of chemokines and inhibit neutrophil and endothelial cell adhesion molecules, it is reasonable to propose that anti-inflammatory effect of colchicine is achieved through suppressive action of this drug on the inflammatory mediators (Cerquaglia *et al*., 2005).

The complement system is an essential component of the innate immunity and the major mediator of the inflammatory response. It consists of more that 20 circulating proteins and a similar number of cell-surface receptor and regulator proteins mediating a large variety of cellular and humoral interactions in the immune response. The activation of complement by the classical, alternative or lectin pathways generates opsonins, inflammatory mediators and cytolytic protein complexes that play an essential role in tissue damage and normally function to eliminate foreign pathogens and to opsonise necrotic and apoptotic cells. However, the undesirable complement activation contributes to the pathology of many human diseases by damaging tissue and promoting inflammation (Mollnes *et al*., 2002; VanOss, 1998). Deficient activity of a C5a/interleukin-8 inhibitor (Ayesh *et al*., 1993; Matzner *et al*., 2000) together with increased levels of pro-inflammatory cytokines (Akcan *et al*., 2003; Baykal *et al*., 2003; Notarnicola *et al*., 2002), circulating immune complexes (Mkrtchyan *et al*., 2002) and C-reactive protein (Korkmaz *et al*., 2002; Odabas *et al*., 2002) in FMF patients suggest that complement classical pathway activation might be involved in the uncontrolled inflammation and aberrant programmed cell death contributed to the development of FMF. However, the existing data in this field is incongruous (Ollier-Hartmann *et al*., 1981; Reimann *et al*., 1970; Schwabe *et al*., 1977; Wautier *et al*., 1981).

The present study determined the Total Hemolytic Activity of the Complement (THAC) and the activities of individual complement components, C1, C2, C3 and C4, in the blood serum of patients with FMF and assess the influence of colchicine medication on these parameters.

Materials and Methods

In the present study 32 patients with FMF were involved. All the affected subjects were Armenians born in Armenia, inpatients of Clinical Hospital N1 of the Ministry of Health of Armenia. The clinical diagnosis of FMF was based on the Tel-Hashomer criteria (Linneh *et al*., 1997). This study was conducted in the Laboratory of "Macromolecular Complexes" of the Institute of Molecular Biology of Armenian National Academy of Sciences (Yerevan, Armenia) in 2004. The affected subjects were divided into three groups. One group included the patients, who were receiving regular colchicine treatment for at least one year prior to hospitalization (n = 10; males/females 6/4; mean age±SE 27±1.4 years); the second group included those, who were receiving irregular colchicine treatment for at least one year prior to hospitalization (n = 12; males/females 9/3; mean age±SE 29±1.1 years) and the third group included those, who were not receiving colchicine treatment for at least one year prior to hospitalization (n = 10; males/females 7/3; mean age±SE 26±1.2 years). Detailed description of the affected subjects within each group is presented in Table 1. The patients were interviewed and informed that all the measurements would be performed for research purposes

Table 1: Clinical and demographic data of patients with FMF

		Number of patients		
		Regularly treated by colchicine	Irregularly treated by colchicine	Not treated by colchicine
First clinical manifestation	0-3 years	4	8	5
	3 years<14 years	3	2	4
	14 years<teenager≤18 years	2	1	-
	18 years<adult<40 years	1	1	1
Clinical signs	Fever	6	10	9
	Peritonitis	4	7	5
	Pleurisy	6	5	5
	Arthritis	2	2	1
	Renal amyloidosis	1	2	2
Heredity	Maternal	2	2	1
	Paternal	4	5	4
	Both parents	3	1	3
	N/A	1	4	2

only with no immediate therapeutic value. All FMF affected subjects entered in this study gave their informed consents to provide 5 mL of venous blood.

As a control group, 28 healthy subjects (males/females 12/16; mean age±SE 33±2.1 years) were recruited. The healthy subjects were free of any medication for at least one month prior to blood sampling. Blood samples were obtained by venipuncture at 9:00-10:00 a.m. In case of the patients sampling was performed on the second day of hospitalization. After 1h of coagulation serum was separated by centrifugation. The aliquots of collected serum samples have been stored at 30°C for further use.

A hemolytic assay was based on the standard 50% complement hemolysis test for the classical pathway of human serum complement (Whaley and North, 1997). THAC and the activities of C1, C2, C3 and C4 components of the complement were expressed in C (x) H50 units. Complement C1, C2 and C3 deficient sera were obtained by the affinity chromatography methods described earlier using human sera (Daha, 1997). Complement C4 deficient serum was obtained by the depletion of guinea pig serum using 150 mM NH4OH (Morgan, 2000).

For data analysis ordinal descriptive statistics and the Mann-Whitney U test were used. $p<0.05$ were considered significant.

Results

According to the results obtained (Table 2), the mean values of THAC as well as C1, C2, C3 and C4 hemolytic activities in the serum of FMF patients, who received no colchicine treatment, were significantly 1.7 ($p = 0.02$), 1.5 ($p = 0.05$), 1.8 ($p = 0.01$) and 1.27 ($p = 0.05$) and 1.45 ($p = 0.001$) times higher, respectively, than in the healthy subjects. On the other hand, in case of those patients, who received regular colchicine treatment, no significant difference between the affected and healthy subjects was detected in respect to the mean values of THAC ($p = 0.66$) or C1 ($p = 0.15$), C2 ($p = 0.28$), C3 ($p = 0.27$) and C4 ($p = 0.16$) hemolytic activities.

In the serum of the patients, who received irregular colchicine treatment, the mean values of THAC and C2 hemolytic activity were significantly 1.25 ($p = 0.03$) and 1.4 ($p = 0.02$) times higher, respectively, when compared with those of the healthy subjects. However, no significant changes between these patients and the healthy subjects were detected in respect to the mean values of C1 ($p = 0.75$), C3 ($p = 0.38$) or C4 ($p = 0.18$) hemolytic activities.

Table 2: Mean values (M±SE) of TCHA and haemolytic activities of C1, C4, C2 and C3 components of complement in FMF patients and healthy subjects

		Patients		
Hemolytic activity	Healthy subjects	Regularly treated by colchicine	Irregularly treated by colchicine	Not treated by colchicine
TCHA (CH50)	38.5±4.8	43.3±5.45	48.1±2.96	66.9±9.7
C1 (C1H50)	22.8±1.8	23.1±3.74	23.8±1.63	31.4±4.9
C4 (C4H50)	698.2±16.1	753.2±40.8	817.2±59.9	1014.0±75.6
C2 (C2H50)	8.7±1.6	9.5±0.56	12.1±2.75	15.4±6.62
C3 (C3H50)	133.8±17.1	136.2±8.3	163.4±31.6	169.9±25

Discussion

In the present study we assess the influence of colchicine medication on THAC and the activities of individual complement components, C1, C2, C3 and C4, in the blood serum of patients with FMF.

The results obtained suggest that regular colchicine treatment results in suppression of the increased activation of the complement system in patients with FMF and brings it to a normal level. Thus, THAC and the activities of C1, C2, C3 and C4 complement components significantly increased in the serum of those patients, who received no colchicine treatment. No significant changes in THAC and hemolytic activities of C1, C2, C3 and C4 complement components were found between healthy subjects and those FMF patients, who received regular colchicine treatment. In the serum of those patients, who received irregular colchicine treatment significant changes were detected only in case of THAC and hemolytic activity of C2 complement component: both parameters were higher than those of healthy subjects. The incongruity of our data obtained for those FMF patients, who received irregular colchicine treatment, might reflect the heterogeneity among this group patient in respect of frequency or/and duration of treatment.

Several studies have focused on the state of complement system in FMF patients. THAC and hemolytic activities of C1, C2, C3 and C4 complement components were reported to be within normal limits (Schwabe *et al*., 1977), decreased (Reimann *et al*., 1970) or increased (Ollier-Hartmann *et al*., 1981; Wautier *et al*., 1981). In the light of our present data, such discrepancy of the earlier reported data might be explained by either difference between examined patients` groups or heterogeneity of patients within each group in respect to colchicine treatment.

The results obtained suggest that complement classical pathway is quite active in FMF and that regular colchicine treatment results in suppression of the complement classical pathway functional activity in FMF patients.

The data obtained in the present study has raised a number of important questions relevant to FMF pathogenesis, especially from the point of view of immunity and once again confirms the efficiency of regular colchicine treatment. In particular, it becomes obvious that together with anti-mitotic action colchicine also possesses anti-inflammatory effect and might be considered as a prospective immuno-modulator drug. Study of the influence of colchicine on other mediators of the inflammatory immune response will provide further evidence in support of this suggestion.

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