

Pharmacological and Clinical Basis of Treatment of Familial Mediterranean Fever (FMF) with Colchicine or Analogues: An Update

C. Cerquaglia, M. Diaco, G. Nucera, M. La Regina, M. Montalto and R. Manna*

Department of Internal Medicine, Catholic University, Largo F. Vito 1, 00168 Rome, Italy

Abstract: Familial Mediterranean Fever (FMF), an autosomal recessive disorder, is characterised by recurrent attacks of fever and serositis, lasting 24-72 hours.

Since 1972 colchicine has become the drug of choice for prophylaxis against FMF attacks and amyloidosis FMF-associated.

Colchicine, an alkaloid neutral, is absorbed in the jejunum and ileum. It is metabolised by liver and only small amounts are recovered unchanged in the urine. Really plasma half-life is prolonged in patients with liver or renal failure.

Colchicine is able to prevent activation of neutrophils, binding β -tubulin and making β -tubulin-colchicine complexes; this way inhibits assembly of microtubules and mitotic spindle formation; moreover its mode of action includes modulation of chemokines, prostanoids production, inhibition of neutrophil and endothelial cell adhesion molecules.

The minimal daily dose in adults is 1.0 mg/die, but in children there is not a definite dose.

Since *in vitro* high dosages of colchicine stop mitosis, this drug might interfere with male and female fertility and with children growth, but, according to current guidelines and because of rare side effects of the drug, FMF patients are recommended to take colchicine.

Since colchicine treatment is often complicated by frequent gastrointestinal side effects, by our experience, in order to improve colchicine tolerance we recommend: lactose-free diet and treatment of intestinal bacterial overgrowth and/or Hp-infection, assessed by breath tests.

Since our data showed that 10-15% of FMF patients seem are non-responders or intolerant to colchicine, today we are working in the design of colchicine analogues which may have lesser toxicities and a larger therapeutic window.

Keywords: Familial Mediterranean Fever, colchicine, analogues of colchicine.

INTRODUCTION

Familial Mediterranean Fever (FMF) is an autosomal recessive disorder due to a genetically determined inflammatory unbalance. Traditionally, it is common among populations living around Southern and Eastern coasts of Mediterranean Sea: Armenians, Turks, Middle-Eastern Moslems, non-Ashkenazi Jews, but, because of the migrations during the centuries, FMF is no longer a rare disease in European countries like Italy, France and Greece [1].

Clinically, FMF is characterised by recurrent, acute, self-limiting attacks of fever and serositis, lasting on average 24-72 hours; its inflammatory burst affects serosal membranes, so FMF can cause symptoms and signs of peritonitis and/or pleuritis and/or pericarditis and/or sinovitis and/or orchitis.

Amyloidosis is the main complication of the disease; it develops in a small percentage of patients according to genotype (M694V mutation increase the risk of development) and maybe ancestry. The kidneys are the main target of amyloidosis, resulting in chronic renal failure and renal transplant.

It was Goldfinger who, as first, has introduced colchicine to treat FMF and since 1972 this drug has been the golden standard to prevent FMF attacks and reduce the risk of amyloidosis.

The use of colchicine in the previous years has been revised by E. Ben-Chetrit and Y. Molad [2,3].

Colchicine, an ancient anti-inflammatory drug, is an alkaloid extracted from a plant, *Colchicum Autumnale*, very common in South-Central Europe and in North-Central Italy (Fig. 1).

It has been used for centuries in acute gout arthritis, but its efficacy has been provided also in other diseases: Familial Mediterranean Fever (FMF), Primary Biliary Cirrhosis [4,5], Psoriasis [6], Palmo-Plantar Pustulosis [7], Necrotizing Vasculitis [8], Behçet's syndrome [9], Sweet's syndrome [10], Scleroderma [11], Sarcoidosis [12], Amyloidosis [13] and Idiopathic Pulmonary Fibrosis [14].

The name "*colchicine*" has a greek origin: it comes from the name of the ancient district on the eastern shore of the Black Sea where this plant grew. Padanius Dioscorides, a Greek surgeon in the Roman Army during the rule of Nero (AD 54-68), first described the meadow saffron in his *De Materia Medica*, a pharmacopeia which systematically described about

600 plants. Already at Dioscoride's time, the toxicity of colchicine was known, but, only after VI century AD, extracts of colchicine were used to relief joint pain. Alexander of Tralles (5.500 AD) mentioned as first the use of *Colchico* bulbs (named *hermadactyl* or *finger of Hermes*) as a remedy for acute gouty arthritis in the "Therapeutica"; another citation is in the Ebers papyrus (1.500 AD) [15].



Fig. (1). *Colchicum autumnale*.

Colchicine was introduced as a drug for acute gout by Baron von Storch, the physician of the Empress Maria Teresa, in 1763, but it was Benjamin Franklin, affected himself by gout, that spread this drug as the most effective therapy for gout.

Pelletier and Caventou extracted the alkaloid from the bulbs in 1820, then it was used for the treatment of several inflammatory diseases [2]. Since 1972, when Goldfinger noticed that a patient affected by gout and FMF got an improvement of both the clinical conditions, colchicine has become the drug of choice for prophylaxis against FMF [16].

Structure and Pharmacokinetics

Colchicine consists of pale yellow scales or powder, which become dark when it is exposed to air or light. It is a neutral alkaloid which can be extracted from two plant of the lily family: *Colchicum autumnale* (meadow saffron) and *Gloriosa superba* (glory lily).

*Address correspondence to this author at the Department of Internal Medicine, Periodic Fevers Research Centre, Catholic University, Largo F. Vito 1, I-00168 Rome, Italy; Tel: ++39 (06) 30155173 - 30154335; Fax: ++39 (06) 35502775; E-mail: rmanna@rm.unicatt.it

The absolute configuration was determined by Corrodi and Hardegger in 1955. Colchicine is a tricyclic alkaloid, the main features of which include a trimethoxyphenyl ring (A ring), a seven membered ring (B ring) with an acetamide at the seven position, and a tropolonic ring (C ring) (Fig. 2).

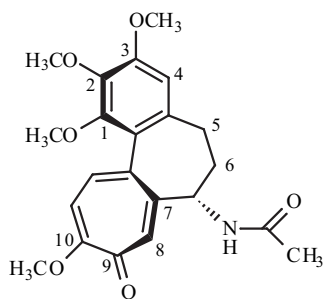


Fig. (2). Structure of colchicine.

Few pharmacokinetic studies on colchicine have been fulfilled, and their results are somewhat contradictory because of the different methods used to measure colchicine and because of the heterogeneity of the groups tested [3].

It is well known that colchicine is a neutral and slightly soluble in water and in ether, freely soluble in ethanol and chloroform therefore it is highly lipid soluble compound at physiologic pH (pKa 12,8, MW 398), which enables its rapid passage into body tissues.

After i.v. administration of 2 mg of colchicine, a rapid rise and drop of its plasma levels occurred within the first 10 minutes, followed by a logarithmic decline. The half-life is $19,3 \pm 7,5$ minutes [17].

After oral administration, colchicine is absorbed in the jejunum and ileum with a single zero-order rate process, with a bioavailability ranged from 25 to 50% [2,3]. It shows a low-affinity binding to albumin and a large steady-state distribution volume [18]. Peak plasma levels, after oral administration, are reached within 2 hours and its half-life is about of 4 hours, although it can be detected in leukocytes for up to 10 days after administration [2].

As colchicine disappears very quickly from plasma and it continues to be excreted next days after ingestion, it has been hypothesised that this drug is trapped in body tissues for long time.

In 1996 Ferron *et al.* noticed that, after colchicine ingestion, there is a second plasma peak within 6 hours, probably due to a second absorption site or to enterohepatic re-circulation; however large differences were observed respecting the time to peak and the peak concentration among subjects [18]. This could be due to gastro-intestinal mucosal variability, pH at the absorption site or the rate of colchicine release from binding compounds in the bowel.

Colchicine is mainly metabolised by liver and only 10-25% of an oral dose is recovered unchanged in the urine [2]. Hepatic metabolism involves demethylation by the cytochrome P450 system (CYP 450) and, in particular, by the isoform CYP 3A4 with production of 3-demethylcolchicine and 2-demethylcolchicine [19].

The biological effects of metabolites of colchicine are unclear, meanwhile the presence of both metabolites in the bile and intestinal secretion suggest an enterohepatic re-circulation [20].

Urinary excretion accounts for 20% of the i.v. dose at 2 hours and 30% after 24 hours; small but measurable amounts were found in the urine after 7 and 10 days [21]. For this reason plasma half-life is prolonged in patients with renal failure, especially when renal and hepatic diseases are both present [22]. Half-life time ($T_{1/2}$) of colchicine is prolonged twice or 3 times in patients with renal failure in comparison with healthy subjects, meanwhile $T_{1/2}$ is prolonged over 10 times in presence of cirrhosis and renal failure. Thus, patients with renal or liver disease who are on colchicine should be carefully monitored for possible toxic effects of the drug [22].

A recent study has shown the presence of little amounts of colchicine in an umbilical cord blood sample suggesting the transplacental passage of the drug [23]. Moreover, colchicine is present in the breast milk where it can bind fat acids and proteins.

Pharmacodynamics

Colchicine produces its therapeutic effect by interfering with the inflammatory process. It reduces inflammatory response occurring in gout

Biochemistry, Vol. 32, No. 49, 1993

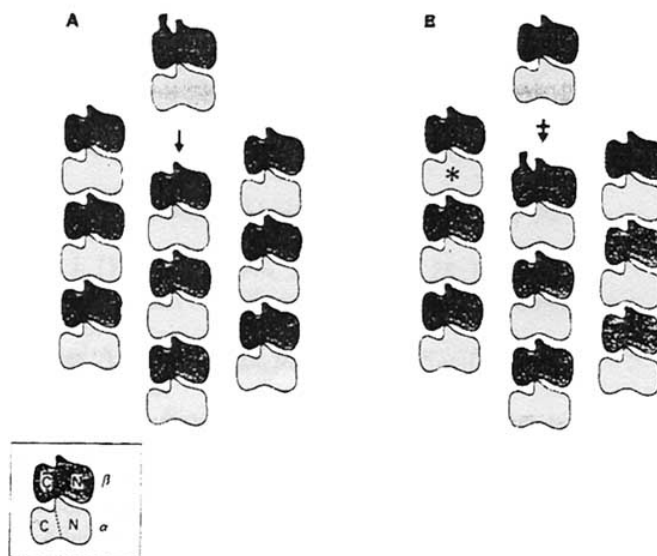


Fig. (3). Interaction between Colchicine and Tubulin [26].

A portion of the microtubule lattice is shown in schematic form.

Each of three protofilaments shown is composed of $\alpha\beta$ -dimers. As shown in the inset, the α -subunits are white and the β -subunits are shaded. The contact between αN and βC in the isolated dimer is known from cross-linking data, as is the interdimer contact between βN and αC , observed between dimers in the polymer.

In panel A, a tubulin-colchicine complex is depicted about to add to the growing polymer. The βC domain of the tubulin-colchicine complex is shown to contain a loop not present in the normal dimer. This represents the area around Arg-390 that is unfolded by colchicine and cleaved by trypsin and chymotrypsin. The contact area between this dimer and the polymer involves the unaltered α -subunit of the colchicine dimer and the surfaces of the β -subunit exposed at the end of the polymer. Since all of these surfaces are normal, the dimer can add despite the altered β -subunit conformation. Once added, however (panel B), the altered dimer impedes the addition of a further dimer longitudinally due to the unfolded loop region of βC , here shown on the distal surface of the polymer. Alternatively or in addition, the altered βC region might protrude to the side, and interfere with the addition of a dimer lateral to it, i.e., the position marked with an asterisk. Blocking addition to either position or both would impede extension of one or both protofilaments and poison the helical growth of the microtubule [26].

and FMF, because it is able to prevent activation, degranulation and migration of neutrophils, binding β -tubulin and making β -tubulin-colchicine complexes; this way inhibits assembly of microtubules and mitotic spindle formation [24].

Now, it is widely known that its action depends on A and C rings that bind tubulin [25]. So topolone methyl ester, an analogue of C ring, and mescaline, an analogue of A ring, are able to bind tubulin and inhibit polymerization of microtubules.

The bond between colchicine and tubulin, as shown by Sackett D.L. *et al.* on *Biochemistry* [26], induces a conformational change of both the molecules resulting in a tight complex with modified polymerisation properties.

The study of tubulin proteolysis by tripsin and chymotrypsin, in presence or absence of colchicine, has demonstrated that colchicine changes the clivage pattern by both the enzymes because it shows two new binding-sites for these enzymes.

Tubulin is an heterodimer consisting of 2 subunits, α and β , bound together. The contact between α N and β C in the isolated dimer is due to cross-linking while the interdimer contact is due to surface β N and α C (Fig. 3).

Colchicine binds domain C of β -subunit changing its conformation and so it prevents the addition of further dimers either longitudinally and laterally. In any case, inhibitory concentrations of tubulin-colchicine complexes are lower than free tubulin concentrations.

As colchicine is able to bind tubulin, it disrupts the mitotic spindle determining dissolution of microtubules in granulocytes and other cells; this function can explain its anti-inflammatory action.

Colchicine induce anti-inflammatory effects by:

- ✓ inhibition of the synthesis of Tumor Necrosis Factor α (TNF α) by macrophages and down-regulation of surface expression of TNF α -receptor on macrophages and endothelial cells; thus it interferes with the priming effect of TNF α on neutrophils before their activation by monosodium-urate crystals [27,28];
- ✓ inhibition of leukotriene B4 synthesis, powerful chemotactic agent [29,30];
- ✓ block of Cyclooxygenase-2 (COX-2) activity, prostaglandin E2 and thromboxane A2 synthesis of mononuclear phagocytes with subsequent reduction of swelling and pain in gout and FMF [31];
- ✓ reduced adhesion of neutrophils to endothelium inhibiting P- and E-selectin-mediated endothelial and L-selectin-mediated neutrophilic adhesiveness. Colchicine effects on E-selectin-mediated endothelial adhesiveness is microtubule-dependent and occurs at concentrations that are over a thousand-fold lower than those required to affect neutrophil function. The prophylactic effect on gout and FMF attacks could just be due to this mechanism [32,33,34,35];
- ✓ tyrosine phosphorylation and superoxide anion production inhibition [36];
- ✓ arachidonate release and 5-lipoxygenase inhibition in alveolar macrophages [37,38];
- ✓ suppression of delayed hypersensitivity reactions [39], histamine [40], insulin [41] and parathormone release [42].

In summary, the most important effect of prophylactic doses of colchicine is to prevent chemotaxis of neutrophils; at higher concentrations colchicine reduces the surface expression of L-selectin on leukocytes, and interferes with the rolling of neutrophils to the inflammation site; finally at higher concentrations it blocks phospholipase A2 activation [43], lysosomal enzymes release and phagocytosis; however, its most strong effect also to inhibit chemotaxis of neutrophils at low doses (1×10^{-8} mol/L). It seems that this effect is related to high colchicine concentration in leukocytes, although we do not know why colchicine has a special affinity for these cells.

Some study showed that, conditions being equal, granulocytes are able to contain higher concentrations of colchicine compared to lymphomonocytes [44]; in patients with FMF, colchicine concentration in granulocytes is on average threefold that in lymphomonocytes [45]. This fact is due to the different activity in these two cellular populations of a P-glycoprotein efflux pump, encoded by the multiple drug resistance gene 1 (MDR 1), which can bind colchicine and expels the drug from cells.

Klimecki *et al.* investigated the P-glycoprotein pump expression in circulating blood cells and found that granulocytes, opposite to lymphomonocytes, have small amount of this pump and it is not primarily localized in their membrane [45]. This could be the reason why colchicine is mainly amassed in the granulocytes.

Nobody until now has investigated if FMF patients non-responders to colchicine could have an higher activity of P-glycoprotein pump in their granulocytes compared to responders.

Colchicine Drug Interactions

Colchicine alters absorption of nutrients and other drugs from bowel [46]. Oral administration of colchicine induces vitamin B12 malabsorption, possibly by reducing intrinsic factor receptors on intestinal mucosa, and lactose intolerance [47,48]. It produces mucosal injury characterised by a hyperplastic crypt-villous atrophy pattern and increased mitotic rate [49].

Colchicine interferes with other drugs because of its metabolism by CYP 450 (isoform CYP 3A4) [50] (Table 1).

Inhibitors of CYP 450 (as cimetidine) and CYP 450 3A4 (as diltiazem, grapefruit juice, erythromycin) induce reduction of colchicine metabolism and consequently increase colchicine blood levels, whereas inducers of CYP 450 (as rifampicin, phenobarbital, phenytoin) work in opposite way.

Substrates of CYP 450 3A4 (as lovastatin, cyclosporin, verapamil, oestrogen, steroids) are affected by co-administration of colchicine.

Subscriptions

Colchicine is currently indicated for the treatment of gouty arthritis acute attacks [51], for prophylaxis during intercritical phases and in the late chronic tophaceous phase as well for prophylaxis of attacks and prevention of AA-amyloidosis in FMF.

It is also used in Behçet's disease and Primary Biliary Cirrhosis, while it is not clear its effect on Scleroderma, Sarcoidosis, Necrotizing Vasculitis, Palmo-Plantar Pustulosis, Sweet's syndrome and Psoriatic arthritis.

The drug can be given orally and intravenously, which has lower toxic gastrointestinal effects but the risk of serious and even fatal toxic effects related to this administration route have encouraged the removal of the

Table 1. Inhibitors and Substrates of CYP 450 [3]

Inhibitors of CYP 450	Inhibitors of CYP 3A4	Substrates of CYP 3A4		Inducers of CYP 3A4
Cimetidine	Diltiazem	Lovastatin	Colchicine	Rifampicin
	Gestodene	Midazolam	Oestrogen	Phenobarbital
	Grapefruit Juice	Cyclosporin	Steroids	Phenytoin
	Ketoconazole	Terfenadine	Dapsone	
	Toleandomycin	Testosterone	Diltiazem	
	Erythromycin	Nifedipine	Erythromycin	
		Verapamil	Lidocaine	

intravenous preparation from the pharmacopoeia. In Italy, only tablets are available.

In case of intravenous colchicine, the following guidelines and resections are firmly recommended [52]:

- ✓ intravenous and oral colchicine should not be given concomitantly. If the patient has not responded to colchicine or he is already taking maintenance doses, an alternative drug should be used in place of further colchicine;
- ✓ the doses must be reduced by at least 50% in patients with renal or hepatic disease and in elderly patients. The drug should not be used at all at creatinine clearance below 10 ml/min. It must be emphasized that colchicine is not removable by dialysis or exchange transfusion;
- ✓ colchicine should not be given to patients with combined liver and kidney disease;
- ✓ the intravenous dose should be given cautiously and slowly to minimize the risk of extravasation;
- ✓ single intravenous dose should be no greater than 2-3 mg, and no more than a cumulative total dose of 4 mg/die.

Based on these guidelines, colchicine may be given at an initial dose of 2.0 mg following by 2 additional doses of 1 mg every 6 hours for a maximal total dose of 4 mg/die.

Dosage and Efficacy

For preventing FMF attacks, the minimal daily dose in adults is 1.0 mg/die. In children there is not definite dose, but it must be adjusted according to their body weight and surface area.

A recent study has determined the "effective colchicine dose" for children in terms of body weight and surface area: for children less than 5 years of age is 0.07 mg/kg/day or 1.9 mg/m²/day, while for the others it is 0.03±0.02 mg/kg/day or 1.16±0.45 mg/m²/day. [53]. Following plasma Serum Amyloid Protein (SAA) concentrations may guide us in monitoring the dosage of the drug, as well as the clinical feature and serum C-reactive protein (CRP) levels [54].

When the attacks are not controlled, the dose may be increased to 2.0 mg daily, meanwhile a higher dose should be given only for short period to avoid toxicity.

In patients with renal failure, general dosing guidelines [20] are shown in Table 2.

Table 2. Renal Failure: Recommendations for Dosage of Colchicine [20]

GFR mL/min/m ²	Recommendations
> 60	✓ Use normal dose
20-60	✓ Use 65-75% of normal dose ✓ Avoid chronic administration
≤ 20	✓ Use 50% of normal dose ✓ Avoid chronic administration ✓ Do not use I.V. preparation

The literature data show that 60% of FMF patients treated with colchicine experiment complete remission, 30% significant improvement while 15% are non responders [3], but our data point out that only 5% of FMF patients have complete remission, 80% strong improvement with reduction of number and severity of attacks and 10-15% are non-responders or intolerant.

Drug discontinuation results in relapse in most patients within a few days.

Since colchicine is poorly able to control joint involvement, non-steroidal anti-inflammatory drugs are useful to this aim.

At last, we remark that as FMF attacks sometimes are related to menstruations, colchicine could be less effective and increased dosage could be necessary at this time.

The reasons of this observed relationship are still unknown, but it has been proposed that oestrogens, which decrease during menstruation, could play a protective effect in the women in free periods by menses. Actually, it seems that these hormones may mimic colchicine effect. They can inhibit tubulin assembly using a binding site analogous to colchicine and can lower intercellular adhesion molecules expression. Also, as colchicine

and oestrogens are substrates of the same cytochrome in the liver, when oestrogens levels decrease, more enzymes are available for colchicine metabolism with subsequent reduction of its blood levels [55].

Colchicine and Male Fertility

Since *in vitro* high dosages of colchicine stop mitosis inhibiting assembly of microtubules many serious questions raised regarding chromosomal and gonadal aberrations.

Sporadic reports have signalled azoospermia in patients with gout after chronic treatment with colchicine.

In consideration of young age of most FMF patients, the concern about fertility is more relevant.

Cohen *et al.* [56] performed a cytogenetic evaluation in FMF patients receiving long-term colchicine. No significant differences were found between the patients group and controls. In another controlled study, 6 FMF patients receiving long-term colchicine, no effect on fertility, on levels of spermatocytes, testosterone follicle-stimulating hormone, luteinizing hormone and prolactin showed [57], while a study performed on 16 patients affected by FMF on colchicine reported four cases of infertility: 1 azoospermia, and 3 with normal spermogram but a pathological hamster zona-free ova penetration test. It was supposed that colchicine may affect microtubular function of spermatocytes [58].

For this reason, some studies aimed to evaluate colchicine effects on sperm motility, were performed *in vitro*. The results showed that sperm motility is significantly inhibited only after incubation with a minimal concentration of 10 µg/mL for at least 18 hours. [59]. As plasmatic colchicine levels on therapeutic dosage is about 3 ng/mL, the amount of colchicine able to affect *in vitro* sperm motility is 3000 folds higher. It appears unlikely that standard colchicine treatment may inhibit sperm motility *in vivo* unless the drug has a very high and special affinity for spermatozoa.

The literature data show that the frequency of oligo or azoospermia due to colchicine depends on underlying diseases. In a study of Bremner and Paulson [60], 6 healthy volunteers received colchicine for 4-6 month and they don't show any effect on spermatogenesis; a study by Ben Chetrit and Levy showed that only 2/150 FMF patients on colchicine have oligo or azoospermia [3]; finally in a study enrolling 62 Turkish men with Behçet's disease treated with colchicine oligospermia was evident in 23 patients and azoospermia in 2 patients [61].

The current guidelines recommend males FMF patients to take colchicine as it could affect sperm motility and production but at therapeutic doses this complication is really rare.

So data suggest that infertility and disturbed spermatogenesis not only result from colchicine use but also depend on other factors such as genetic background or other underlying diseases (i.e. amyloidosis testicular in FMF patients).

Colchicine and Female Fertility and Pregnancy

We investigated retrospectively by questionnaire the reproductive history of our patients in fertile-life [62]. We selected 45 patients, 30 females and 15 males, aged from 27 to 76 years, suffering from FMF-related symptoms for 27 years on average (range 5-55 yrs). The frequency of attacks in this group was 17.75 per annum (range 1-48 per annum). Almost all our patients have attacks characterised by fever and peritonitis; 23 of them, before the diagnosis, underwent to at least 1 surgical operation: appendectomy, ovariectomy, cholecistectomy and explorative laparotomy.

Repeated peritonitis and surgery exposed these patients to the risk of peritoneal adhesions that can affect reproduction by tubal obstructions. Moreover, failure to ovulate, insufficient or ineffective sperm, amyloidosis of sexual glands and recurrent unavailability to coitus due to the acute attacks are the main factors limiting reproduction in FMF.

Our patients had 2 children on average (11 of them are not married and have never conceived). 2 patients, 1 male and 1 female, reported difficulties to conceive (in the male a transient oligospermia was documented). 1 patient reported 6 abortions and, then, a pre-term delivery (1,150 kg). A pre-term birth (29 weeks of pregnancy), occurred also in another patient. Another one reported 2 intrauterine deaths (39 weeks of pregnancy): the reason was not ascertained. No one of the children has malformations; cariotype was not done in anyone.

According to these findings, FMF does not seem to reduce markedly fertility in the affected (reproductive-related problems only in 1%).

Theoretically colchicine could have teratogenic effects as it inhibits mitosis, but as the diagnosis and the administration of colchicine in our patients were subsequent to the conception, we could not value the effects of colchicine on reproductive function [61,62].

The literature data based on surveys larger than ours, suggest that colchicine not only does not reduce the rate of female fertility, but may control FMF attacks in many women who present exacerbation in pregnancy and may reduce peritoneal adhesions preventing mechanical causes of infertility in FMF women [3].

The actual guidelines recommend to take colchicine during pregnancy and perform an amniocentesis if it is indicated.

Colchicine and Children

Literature data suggest that breast feeding is safe as daily amount of colchicine ingested by the nursing baby is less than 1/10 of the therapeutic dose per kilogram given to the adult [3] and it does not interfere with child growth.

So, current guidelines recommend not to stop administration of colchicine during breast feeding and to begin treatment in children patients as soon as possible.

Colchicine and Amyloidosis

Although FMF is a benign, self-limiting disease, it can be complicated by amyloidosis becoming a serious disease.

Amyloidosis secondary to FMF is characterised by polymerization of serum amyloid A (SAA), an acute phase protein, into amyloid fibrils and by their deposition in multiple organs, primarily in the kidney leading to chronic renal failure, but also in liver, spleen, heart and testes.

Colchicine is the drug of choice to prevent amyloidosis and, for its effects, it is also used to prevent fibrosis in cirrhotic livers as well as in sclerodermatous skin.

This drug slows down the rate of renal function's deterioration and reduces proteinuria in cases of renal failure due to amyloidosis. It has been supposed that it interferes with amyloidosis process through 2 mechanisms: indirect and direct. Really colchicine reduces indirectly SAA levels by controlling FMF attacks and it blocks directly the organization and deposition of amyloid fibers [65].

Colchicine and Tolerance

Colchicine treatment is complicated by frequent gastrointestinal adverse effects such as diarrhoea and enhanced intestinal permeability with lactose malabsorption. Colchicine could affect gastrointestinal mucosa with 2 different mechanisms:

- inhibition of Na⁺K⁺ exchange pump which regulates water and electrolytes transport;
- direct mucosal damage of small and large bowel [66].

For this reason we advise our patients to follow a lactose-free diet as lactose could worsen the diarrhoea colchicine-induced.

Moreover as an eventual small intestine bacterial overgrowth could determine severe gastrointestinal disorders which sometimes oblige patients to discontinue colchicine, recently we studied if treatment of eventual small intestinal bacterial overgrowth can improve gastrointestinal colchicine tolerance (unpublished data). We studied 25 patients with FMF (7 m, 18 f, mean age 32.3, range 18-63) who, after starting colchicine (1-3 mg/die), experienced the onset or worsening of diarrhoea unresponsive to lactose-free diet. We scored diarrhoea by a validated diarrhoea score based on number and consistency and imperiousness of stools. All patients underwent to H₂ glucose-lactulose breath-test for detection of small intestinal bacterial overgrowth. In presence of bacterial overgrowth, we treated the patients with rifaximin or paramomycin 1 week per month.

After three months patients were re-evaluated by H₂ glucose-lactulose breath-test and diarrhoea score. The breath tests were positive in 17/25 patients. In patients with bacterial overgrowth, basal diarrhoea score was 3.94±1.9; instead, after eradication of bacterial overgrowth the score became 0.41±0.7, with statistical difference (p<0.001). In all patients treated with antibiotic therapy, H₂ glucose-lactulose breath-test was negative.

Our experience suggests that an evaluation for small intestinal bacterial overgrowth is useful in patients experimenting colchicine gastrointestinal intolerance, because antibiotic therapy can improve colchicine-induced diarrhoea and so patient's compliance. In these

patients, we recommend to perform H₂ glucose-lactulose breath-test because it is quick, easy, non-invasive and efficient.

According to the well known influence of simultaneous Helicobacter Pylori (Hp) infection on severity of gastritis and ulcers in patients on long-term therapy with aspirin or non-steroidal-anti-inflammatory drugs (NSAIDs) and the beneficial effects of eradication of Hp in aspirin and NSAIDs users, we usually perform Urea breath test prior to start colchicine therapy. Our findings suggest that Hp eradication is useful to improve colchicine tolerance [66].

In summary, in order to increase compliance to colchicine we recommend:

- lactose-free diet;
- treatment of intestinal bacterial overgrowth and/or Hp-infection, assessed by breath tests.

Colchicine and Toxicity

Therapeutic oral doses in patients without hepatic or renal failure have few side effects excepted gastrointestinal ones: diarrhoea, vomiting, cramping abdominal pain and hyperperistalsis.

Rarely, at high doses bone marrow failure, skin eruptions, nettle-rash, purple, stomatitis, intestinal bleeding can occur, meanwhile long-term administration can result in ovarian and testicular dysfunction, steatorrhea, and Lyell syndrome [3].

Neuromuscular toxicity was reported in patients with renal failure; it usually recovers after drug withdrawal.

The fatal dose of acute colchicine poisoning is estimated at about 0,9 mg/kg.

A multi-organ failure characterized by cholera-like syndrome, dehydration, shock, leukocytosis, alopecia, acute renal and hepatic failure, bone marrow failure, Adult Respiratory Distress Syndrome (ARDS), arrhythmias and heart failure, fever, coagulopathy, neuromuscular involvement, epileptic seizures, coma and death can occur.

Toxic manifestations by colchicine can be divided into 3 sequential and overlapping stages (Table 3).

Table 3. Colchicine Toxicity: Stages [3]

Stage I	Stage II	Stage III
Abdominal pain	Renal failure	Leukocytosis
Nausea	Respiratory failure	Alopecia
Vomiting	Cardiac failure	
Diarrhea	Pancytopenia	
Dehydration	Metabolic acidosis	
	Electrolyte disturbances	
	DIC	
	Convulsions	
	Coma	

In the first stage, who begin within 24 hours from drug administration, there are gastrointestinal symptoms with significant fluid loss (abdominal pain, nausea, vomiting, diarrhoea, dehydration), volume depletion and leukocytosis.

In the second stage, 24-48 hours after drug administration, multiorgan failure occurs (renal, respiratory and cardiac failure, pancytopenia, metabolic acidosis, electrolyte disturbances, Disseminated Intravascular Coagulation, convulsion, coma).

If the patient survives, in the third stage there is a *restitutio ad integrum* of bone marrow with rebound leukocytosis, recovery of multiorgan injury and appearance of alopecia.

Clinical management of colchicine toxicity can be difficult because of widespread involvement of various vital organs. Since hemodialysis and hemoperfusion are not effective measures because of the high volume of distribution, an aggressive primary decontamination with gastric lavage and activated charcoal is required as early as possible. Therapy is

basically supportive: it should begin with shock-preventing measures. Symptomatic and supportive treatment may include atropine and morphine for abdominal pain relief, and artificial respiration with oxygen to combat respiratory distress. A recent paper reports a successful treatment by Fab fragments of antibodies anti-colchicine. However, these antibodies have been used only experimentally [68].

Precautionary Measures

Colchicine treatment could be performed with caution, especially in the following cases:

- ✓ severe hepatic failure and cholestasis because of reduced metabolism;
- ✓ chronic renal failure (the reduced excretion can increase the risk of side effects especially the haematological ones);
- ✓ elderly;
- ✓ myasthenia gravis (possible worsening of the disease);
- ✓ glucose-6-phosphate dehydrogenase (colchicine can trigger an hemolytic attack).

In all these cases patients must be carefully evaluated by physicians in order to prevent side effects.

The Future: the Analogues of Colchicine

Present and future work in the design of colchicine analogues and other agents that inhibit tubulin polymerization will attempt to make agents with reduced toxicities and a larger therapeutic window [69].

These analogues may more successfully treat diseases in which colchicine is presently used, such as familial Mediterranean fever (FMF) [70], chronic constipation [71], immunosuppression, and several other pathophysiological processes.

Another major goal is to determine the precise interaction between colchicine and tubulin dimers. Knowledge of the tubulin-colchicine interactions at the atomic level may lead to the design of better drugs, with a more favourable therapeutic index.

One insight into the molecular action of colchicine has been the determination of the biologically active conformation [72,73]. (-)-Colchicine has only one stereogenic center: carbon -7.

The designation of this carbon is S, according to the common Cahn-Ingold-Prelog rules. However, colchicine is also asymmetric due to axial chirality. The single bond between the A and C rings is rotationally restricted, in a similar manner to certain substituted biphenyls (Fig. 4).

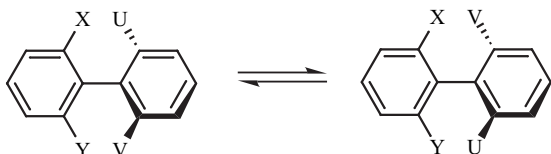


Fig. (4). Chiral substituted biphenyl.

This restriction adds a degree of asymmetry to the molecule.

In 1933, Kuhn designated this type of stereoisomerism as atropisomerism (from Greek "a" meaning not; "tropos" meaning turn) [74]. The designation of this asymmetry is "aS or aR," according to the rules of molecular asymmetry, in which the "a" stands for axial chirality [75]. In colchicine, the C-C bond between the A and C rings is the chiral axis (Fig. 5).

In light of this molecular asymmetry, colchicine has four stereoisomers, as shown in Fig. 5. Each pair has either the R or S configuration at C-7.

(-)-(aS,7S)-Colchicine, the natural isomer, can interconvert between the two conformational isomers aR and aS, given enough energy. The energy barrier of rotation in colchicine, approximately 22-24 kcal/mol [73], is large enough to allow the synthesis and isolation of the conformations as stereoisomers.

The research of many medicinal chemists, in particular Arnold Brossi, has led to the conclusion that the counterclockwise aS conformation is that of the naturally occurring alkaloid [72,73,76-79].

Shown in Fig. 6 are energy-minimized models of the atropisomers of (7S)-colchicine (Constructed using Sybyl 6.4, Tripos, Inc). Note the very different arrangement of the acetamide group in the two conformations.

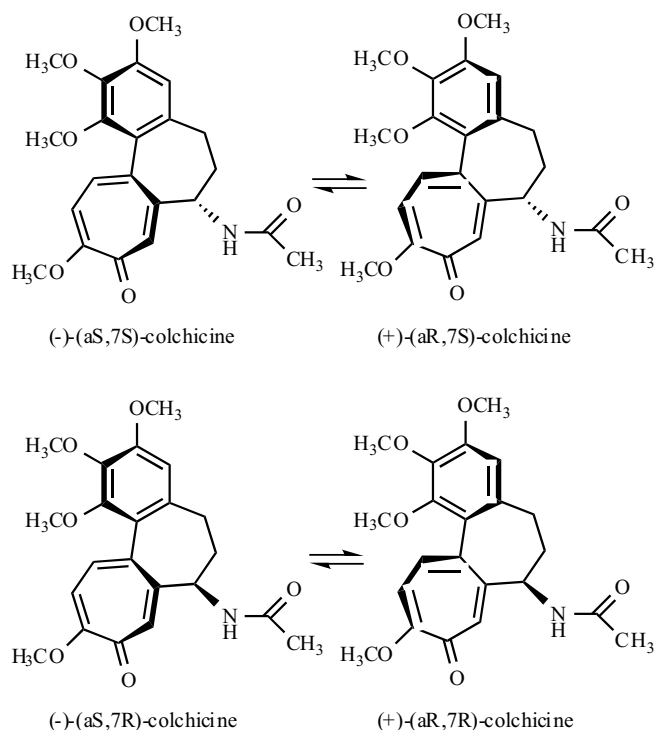


Fig. (5). Stereoisomers of Colchicine.

In the search for more effective agents than natural colchicine, medicinal chemists have synthesized hundreds of analogues of colchicine and colchicine-like compounds. Analysis of these data has yielded information about the optimal structural requirements for binding to tubulin and inhibiting tubulin polymerization. The basic, although not comprehensive, structure-activity relationships (SARs) are summarized in Fig. 7, adapted from Boye and Brossi [73].

(+)-(aR,7R)-Colchicine (Fig. 6), the unnatural enantiomer of (-)-colchicine, is devoid of tubulin binding activity. The appropriate torsion angle (about 53 degrees) between rings A and C is required for tubulin binding ability. Removal or demethylation of the methoxy groups decreases potency.

On the B ring, the acetamide can be replaced by other alkyl amides with retention of potency; however, the free amine has decreased antitubulin activity. The acetamide can be eliminated altogether, and activity is retained. On the C ring, demethylation to the 10-OH (i.e. colchicine) destroys activity.

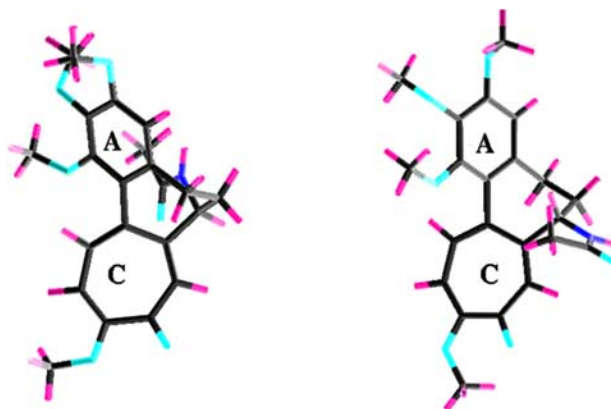


Fig. (6). (aS, 7S)-Colchicine (left) and (aR, 7S)-Colchicine (right).

Replacement of the 10-methoxy with SCH₃ or NR₂ leads to increased potency. Reversal of the oxygen pattern (i.e. 9-methoxy and 10-keto) produces isocolchicine, which is inactive. It is apparent that the tropolonic

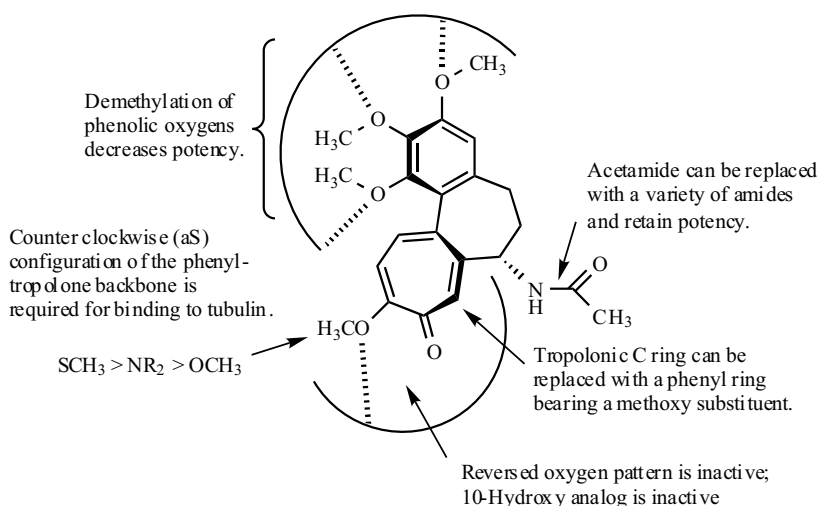


Fig. (7). Structure-activity relationship of colchicine analogues.

functionality contributes to activity. This seven membered C ring can, however, be replaced with an anisole ring, producing a bridged biphenyl, which retains tubulin binding activity, as long as the torsion angle between the rings A and C is acceptable (Fig. 7).

In our Department of Biochemistry we are testing *in vitro* the potency of colchicine analogues by inhibiting of the chemiluminescence of neutrophils of FMF patients [80]; our protocol in working process aims to test gut toxicity and abnormalities of tight-junctions of enterocytes by measuring the ZO-1 protein (Zonula Occludens) on immunofluorescence in immortalized intestinal cells (HT-29).

CONCLUSIONS

Familial Mediterranean Fever is a benign disease when treated by colchicine. A long-life colchicine therapy can determine a reduction of number of FMF attacks, of their severity, preventing amyloidosis; in this way a real improvement of the quality of life of the patients can be achieved.

Colchicine is able to prevent activation of neutrophils, binding β -tubulin and making β -tubulin-colchicine complexes; this way inhibits assembly of microtubules and mitotic spindle formation; moreover its mode of action includes modulation of chemokines, prostanoids production, inhibition of neutrophil and endothelial cell adhesion molecules.

The drug is metabolised by liver and only small amounts are recovered unchanged in the urine; so plasma half-life is prolonged only in patients with liver or renal failure with an increase of side effects.

To improve colchicine tolerance and in order to prevent discontinuation of treatment we recommend to exclude other intestinal co-factors which can complicate therapy: as a small intestine bacterial overgrowth assessed by H₂-glucose-lactulose breath test and Helicobacter pylori infection assessed by Urea-breath test. These tests might be performed before starting therapy and if positive an antibiotic therapy will be done; moreover a lactose free-diet can be followed to prevent diarrhoea.

Biochemical studies on colchicine analogues are in progress in order to obtain drugs with few side effects and better efficacy.

ABBREVIATIONS

ARDS	=	Adult Respiratory Distress Syndrome
COX-2	=	Cyclooxygenase 2
CRP	=	C-reactive protein
CYP 450	=	Cytochrome P450
FMF	=	Familial Mediterranean Fever
GFR	=	Glomerular filtration rate
Hp	=	Helicobacter Pylori
MDR 1	=	Multiple drug resistance gene 1
NSAIDs	=	Non-steroidal-anti-inflammatory drugs
SAA	=	Serum Amyloid Protein

TNF α	=	Tumor Necrosis Factor α
ZO-1	=	Zonula Occludens protein

REFERENCES

- Manna, R.; La Regina, M.; Nucera, G.; Diaco, M.; Procopio, A.; Gasbarrini, G.; Notarnicola, C.; Kone-paut, I.; Touitou, I. *Eur. J. Hum. Genet.*, **2003**, *11* (1), 50-56.
- Molad, Y.M.D. *Curr. Rheumatol. Rep.*, **2000**, *4* (3), 252-6.
- Ben-Chetrit, E.; Levy, M. *Semin. Arthr. Rheum.*, **1998**, *28*, 48-59.
- Kershenovich, D.; Varga, F.; Garcia, Tao.; Tamayo, R.P.; Gent, M.; Rojkind, M. *N. Engl. J. Med.*, **1988**, *318*, 1709-1713.
- Ikeda, T.; Tozuka, S.; Noguchi, O.; Kobayashi, F.; Sakamoto, S.; Marumo, F. *J. Hepatol.*, **1996**, *24*, 88-94.
- McKendry, R.J.; Siegel, S.; Al-Awadhi, A. *Ann. Rheum. Dis.*, **1993**, *52*, 826-828.
- Takigawa, M.; Myachi, M.; Tagami, H. *Arch. Dermatol.*, **1982**, *118*, 458-460.
- Hazen, P.G.; Michel, B. *Arch. Dermatol.*, **1979**, *115*, 1303-1306.
- Sander, H.M.; Randle, H.W. *Cutis*, **1986**, *37*, 344-386.
- Su, W.P.; Fet, D.C.; Gibson, L.E. *Semin. Dermatol.*, **1995**, *14*, 173-178.
- Torres, M.D.; Furst, D.E. *Rheum. Dis. Clin. North Am.*, **1990**, *16*, 217-241.
- Kaplan, H. *N. Engl. J. Med.*, **1960**, *213*, 774-781.
- Cohen, A.S.; Rubinow, A.; Anderson, J.J.; Skinner, M.; Mason, J.H.; Libbey, C. *Am. J. Med.*, **1987**, *82*, 1182-1190.
- Douglas, W.W.; Ryu, J.H.; Swensen, S.J.; Offord, K.P.; Schroeder, D.R.; Caron, G.M.; Doremee, R.A. *Am. J. Respir. Crit. Care Med.*, **1998**, *158* (1), 220-225.
- Hartung, E.F. *Ann. Rheum. Dis.*, **1954**, *13*, 190-199.
- Goldfinger, S.E. *N. Engl. J. Med.*, **1972**, *287*, 1302.
- Wallace, S.L.; Omokoku, B.; Ertel, N.H. *Am. J. Med.*, **1970**, *48*, 443-448.
- Ferron, G.M.; Rochdi, M.; Jusko, W.J.; Scherrmann, J.M. *J. Clin. Pharmacol.*, **1996**, *36*, 874-883.
- Tateiski, T.; Soucek, S.; Caraco, Y.; Guengrich, F.V.; Wood, A.J. *Biochem. Pharmacol.*, **1997**, *10*, 111-116.
- Levy, M.; Spino, M.; Read, S.E. *Pharmacother.*, **1991**, *11*, 196-211.
- Ertel, N.H.; Wallace, S.L. *Clin. Res.*, **1971**, *19*, 348.
- Ben-Chetrit, E.; Scherrmann, J.M.; Zylber-Katz, E.; Levy, M. *J. Rheumatol.*, **1994**, *21*, 710-713.
- Zahir, A.; Scherrmann, S.M.; Wechsler, B.; Zerah, X.; Goodeau, P. *J. Rheumatol.*, **1994**, *21*, 383.
- Vandecandelaere, A.; Martin, S.R.; Engelborghs, Y. *Biochem. J.*, **1997**, *323*, 189-196.
- Andreu, J.M.; Timasheff, S.N. *Biochemistry*, **1982**, *21*, 534-543.
- Sackett, D.L.; Varna, J.K. *Biochemistry*, **1993**, *32* (49), 13560-13565.
- Li, Z.; Davis, G.S.; Mohr, C.; Naim, M.; Gemsa, D. *Immunobiol.*, **1996**, *195*, 624-639.
- Ding, A.H.; Porteu, F.; Sanchez, E.; Nathan, C.F. *J. Exp. Med.*, **1990**, *171*, 715-727.
- Serhen, C.N.; Lundberg, U.; Weissmann, G.; Samuelsson, B. *Prostaglandins*, **1984**, *27*, 503-581.
- Reibman, J.; Haines, K.A.; Rich, A.M.; Cristello, P.; Giedd, K.M.; Weissmann, G. *J. Immunol.*, **1986**, *136* (3), 1027-1932.
- Pouliot, M.; James, M.J.; McCall, S.R.; Naccache, P.H.; Cleland, L.G. *Blood*, **1998**, *91*, 1768-1776.

- [32] Fordham, J.N.; Kirwan, J.; Cason, H.L.F. *Ann. Rheum. Dis.*, **1981**, *40*, 605-608.
- [33] Asako, H.; Kubes, P.; Baethge, B.A.; Wolf, R.E.; Granger, D.N. *Inflammation*, **1992**, *16* (1), 45-56.
- [34] Cronstein, B.N.; Wiessmann, G. *Arthritis Rheum.*, **1993**, *36*, 147-157.
- [35] Cronstein, B.N.; Molad, Y.; Reibman, J.; Balakhane, E.; Levin, R.I.; Weissmann, G. *J. Clin. Invest.*, **1995**, *96*, 994-1002.
- [36] Roberge, C.J.; Gaudry, M.; Gilbert, C.; Malawista, S.E.; De Medicis, R.; Lussier, A.; Poubelle, P.E.; Naccache, P.H. *J. Leukoc. Biol.*, **1996**, *59*, 864-871.
- [37] Peters-Golden, M.; McNish, R.W.; Davis, J.A.; Blackwood, R.A.; Brock, T.G. *Am. J. Physiol.*, **1996**, *271*, 1004-1013.
- [38] Zurier, R.B.; Hoffstein, S.; Wiessman, G. *J. Cell Biol.*, **1973**, *58*, 27-41.
- [39] Mekori, Y.A.; Baram, D.; Goldberg, A.; Klajman, A. *Cell Immunol.*, **1989**, *120*, 330-340.
- [40] Gillespie, E.; Levine, R.J.; Malawista, S.E. *J. Pharmacol. Exper. Therap.*, **1968**, *164*, 158-165.
- [41] Malaisse, W.J.; Malaisse-Lagae, F.; Van Obbergen, E.; Somers, G.; Devis, G.; Ravazzola, M.; Onci, L. *Ann. N.Y. Sci.*, **1975**, *253*, 630.
- [42] Reaven, E.P.; Reaven, G.M. *J. Clin. Invest.*, **1975**, *56*, 49.
- [43] Paya, M.; Terencio, M.C.; Ferrandiz, M.L.; Alcaraz, M.J. *Br. J. Pharmacol.*, **1996**, *117*, 1773-1779.
- [44] Chappay, O.N.; Niel, E.; Waiter, J.L.; Hung, P.P.; Dervichian, M.; Cattani, D. *Clin. Pharmacol. Ther.*, **1993**, *54*, 360-362.
- [45] Klimecki, W.T.; Futscher, B.W.; Grogan, T.M.; Dalton, W.S. *Blood*, **1994**, *84*, 2451-2458.
- [46] Venho, V.M.K.; Koivuniemi, A. *Acta Pharmacol. Toxicol.*, **1978**, *43*, 251-259.
- [47] Webb, D.I.; Chodos, R.B.; Mahar, C.Q.; Faloon, W.W. *N. Engl. J. Med.*, **1968**, *279*, 845-850.
- [48] Fradkin, A.; Yahav, J.; Zemer, D.; Jonas, A. *Isr. J. Med. Sci.*, **1995**, *31*, 616-620.
- [49] Hart, J.; Lewin, K.J.; Peters, R.S.; Scher, A.D. *Dig. Dis. Sci.*, **1993**, *38*, 207-2021.
- [50] Desmond, P.V.; Patwardhan, R.V.; Parker, R.; Schenker, S.; Speeg, K.V. *Life Sci.*, **1989**, *26*, 1261-1268.
- [51] Emmerson, B.T. *N. Engl. J. Med.*, **1996**, *334* (7), 445-451.
- [52] Wallace, S.L.; Singer, J.Z. *J. Rheumatol.*, **1998**, *15*, 495-499.
- [53] Özkaya, N.; Yalçınkaya, F. *Clin. Rheum.*, **2003**, *22*, 314-317.
- [54] Seza, O. *Eur. J. Pediatr.*, **2003**, *162* (7-8), 449-454.
- [55] Ben-Chetrit, E.; Levy, M. *Ann. Rheum. Dis.*, **2002**, *62*, 916-919.
- [56] Cohen, M.M.; Levy, M.; Eliakim, M. *Am. J. Med. Sci.*, **1977**, *274*, 147-152.
- [57] Levy, M.; Yaffe, C. *Fertil. Steril.*, **1978**, *29*, 662-668.
- [58] Ehrenfeld, M.; Levy, M.; Margalioth, E.J.; Eliakim, M. *Andrologia*, **1986**, *13*, 420-426.
- [59] Ben-Chetrit, A.; Ben-Chetrit, E.; Niztan, R.; Ron, M. *Int. J. Fertil.*, **1993**, *38*, 301-304.
- [60] Bremner, W.J.; Paulson, C.A. *N. Engl. J. Med.*, **1976**, *294*, 1384-1385.
- [61] Sarica, K.; Suzer, O.; Gurler, A.; Baltaci, S.; Ozdiler, E.; Dincel, C. *Eur. J. Mol.*, **1995**, *22*, 39-42.
- [62] Diaco, M.; La Regina, M.; Nucera, G.; Mancarella, L.; Manna, R.; Gasbarrini, G. *Clin. Experim. Rheum.*, **2002**, *20* (Suppl 26), 106.
- [63] Tanchev, S.; Papov, I.; Tomov, S. *Akush. Gynecol. Sofiia.*, **1993**, *32*, 41-42.
- [64] Cousin, C.; Palaric, J.C.; Jacquemand, F.; Lucas, S.; Girard, J.R. *J. Gynecol. Obstet. Biol. Reprod. Paris*, **1991**, *20*, 554-561.
- [65] Ben-Chetrit, E. *J. Nephrol.*, **2003**, *16*, 431-434.
- [66] Ratnaik, R.N.; Jones, T.E. *Drugs Aging*, **1998**, *13* (3), 245-253.
- [67] La Regina, M.; Nucera, G.; Diaco, M.; Montalto, M.; Manna, R.; Gasbarrini, G. *Clin. Experim. Rheum.*, **2002**, *20* (Suppl 26), 105.
- [68] Baud, F.J.; Sabouraud, A.; Vicaut, E.; Taboulet, P.; Lang, J.; Bismuth, C.; Rouzioux, J.M.; Schermann, J.M. *N. Engl. J. Med.*, **1995**, *332* (10), 642-645.
- [69] Chen, K.; Kuo, S.C.; Hsieh, M.C.; Mauger, A.; Lin, C.M.; Hamel, E.; Lee, K.H. *J. Med. Chem.*, **1997**, *40*, 3049-3056.
- [70] Buskila, D.; Zaks, N.; Neumann, L.; Livneh, A.; Greenberg, S.; Pras, M.; Langevitz, P. *Clin. Exp. Rheumatol.*, **1997**, *15*, 355-360.
- [71] Verne, G.N.; Eaker, E.Y.; Davis, R.H.; Sninsky, C.A. *Dig. Dis. Sci.*, **1997**, *42*, 1959-1963.
- [72] Capraro, H.G.; Brossi, A. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, **1984**; Vol. 23, pp. 1-70.
- [73] Boye, O.; Brossi, A. In *The Alkaloids*; Brossi, A., Cordell, G.A., Ed.; Academic Press: New York, **1992**, Vol. 41, pp. 125-178.
- [74] Kuhn, R. In *"Molekulare Asymmetrie" in Stereochemie*; Freudenberg, K., Ed.; Franz Deuticke, **1933**, pp. 803.
- [75] Eliel, E.L.; Wilen, S.H. In *Stereochemistry of Organic Compounds*; John Wiley and Sons, Inc.: New York, **1994**, pp. 1119-1122.
- [76] Brossi, A. *J. Med. Chem.* **1990**, *33*, 2311-2319.
- [77] Wildman, W.C. In *The Alkaloids*; Manske, R.H.F.; Holmes, H.L., Ed.; Academic Press: New York, **1968**, Vol. 11, pp. 407-456.
- [78] Cook, J.W.; Loudon, J.D. In *The Alkaloids*; Manske, R.H.F.; Holmes, H.L., Ed.; Academic Press: New York, **1952**; Vol. 2, pp. 261-330.
- [79] Wildman, W.C. In *The Alkaloids*; Manske, R.H.F., Ed.; Academic Press: New York, **1960**; Vol. 6, pp. 220-246.
- [80] Anton, P.A.; Targan, S.R.; Vigna, S.R.; Durham, M.; Schwabe, A.D.; Shanahan, F. *J. Clin. Immunol.*, **1988**, *8* (2), 148-156.