Pharmacological Reports
2006, 58, 473–492
ISSN 1734-1140

Review

Methotrexate in rheumatoid arthritis

Jerzy Świerkot, Jacek Szechiński

Department of Rheumatology, Medical University of Wrocław, Wiśniowa 36, PL 51-137 Wrocław, Poland

Correspondence: Jerzy Świerkot, e-mail: jurekswierkot0@poczta.onet.pl

Abstract:
A variety of disease-modifying antirheumatic drugs (DMARDs) are available to control the clinical activity of rheumatoid arthritis (RA). Methotrexate (MTX), an analogue of folic acid and of aminopterin, is the most commonly used DMARD and is now prescribed worldwide to at least 500,000 patients with RA. The mechanism by which MTX used at a low dose modulates inflammation in RA is still unknown. Monitoring of the therapy in terms of MTX concentration in patients with RA seems not to have a significant influence on the effectiveness of the treatment. Two meta-analyses showed that MTX has one of the best efficacy/toxicity ratios. It should be the first DMARD used in the majority of patients with RA at this time. However, a significant number of patients treated only with MTX fail to achieve optimal disease control, so there are many combinations of DMARD regimes. It is hoped that more aggressive use of conventional DMARDs and biological agents will result in less disability and a higher proportion of patients achieving remission. The therapy of RA is a dynamic process and requires maintaining a delicate balance between benefits and risks. Even with the newer biological agents, MTX continues to serve as a reference point and there is still a role for MTX in the treatment of RA patients.

Key words:
rheumatoid arthritis, methotrexate

Abbreviations: ACR – American College of Rheumatology, ADA – adenosine deaminase, AICAR – 5-aminoimidazole-4-carboxamide ribonucleotide, ALT – alanine aminotransferase, AMP – adenosine 5-monophosphate, AST – aspartate aminotransferase, AZA – azathioprine, COX – cyclooxygenase, CSA – cyclosporin A, CYC – cyclophosphamide, DHFR – dihydrofolate reductase, DMARD – disease-modifying antirheumatic drug, D-Pen – D-penicillamine, GAR – glycinamide ribonucleotide, GSTM – intramuscular gold, HAQ – Health Assessment Questionnaire Disability Index, HCQ – hydroxychloroquine, ICAM -1 – intracellular adhesion molecule-1, IFN – interferon, Ig – immunoglobulin, IL – interleukin, IL-1ra – interleukin1 receptor antagonist, LEF – leflunomid, LTB_4 – leucotriene B4, MTX – methotrexate, 7-OH-MTX – 7-hydroxymethotrexate, PBLs – peripheral blood lymphocytes, PBMCs – peripheral blood mononuclear cells, PHA – phytohemagglutinin, RA – rheumatoid arthritis, RF – rheumatoid factor, RT-PCR – reverse transcriptase-polymerase chain reaction, SSZ – sulfasalazine, TNF – tumor necrosis factor, VCAM-1 – vascular cell adhesion molecule-1

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology, principally affecting smaller synovial joints in a symmetrical fashion, leading, in most cases, to joint destruction. Extra-articular manifestations are common and a variety of immunological abnormalities that lead to disability are evident. Its frequency is high; in Poland about 1% of the population suffers from RA. RA is associated with pain, deformity, decreased quality of life, and disability, which in turn affect patients' ability to work. Growing evidence suggests that rheumatoid arthritis should no longer be considered a benign disease. Considerable data suggest that this disease is associated with diminished long-term survival, and bone damage can occur

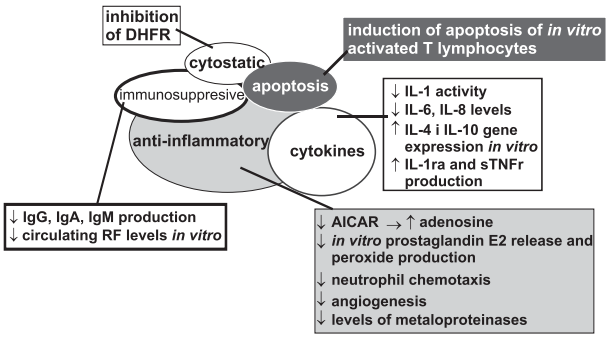

Fig. 1. Scheme of the mechanism of MTX action in RA: RA – rheumatoid arthritis, DHFR – dihydrofolate reductase, Ig – immunoglobulin, RF – rheumatoid factor, IL – interleukin, TNF – tumour necrosis factor, IL-1ra – interleukin 1 receptor antagonist, AICAR – 5-aminoimidazole-4-carboxamide ribonucleotide

very early in the course of the disease. A variety of disease-modifying antirheumatic drugs (DMARDs) are available to control the disease process in RA. The goal of treatment is to improve patients' quality of life and prevent joint destruction. Rheumatologists have completely remodelled the traditional "therapeutic pyramid" and now treat RA more aggressively and when the disease is at a less active degree than ever before. In 1989, a step-down bridge model was proposed: rapid-acting and slow-acting antirheumatic drugs should be used to achieve early, sustained control of inflammation and to prevent joint destruction. Methotrexate (MTX) is prescribed worldwide to at least 500,000 patients with RA and is the most commonly used DMARD.

MTX mechanism of action

Methotrexate (4-amino-N10-methylpteroyl glutamic acid) is an analogue of folic acid and of aminopterin (4-amino-pteroyl glutamic acid) that is also a folic acid antagonist. It was first introduced in 1948 to treat acute leukemia. Many pharmacological mechanisms of MTX action have been suggested, including inhibition of purine synthesis, promotion of adenosine release, inhibition of production of proinflammatory cytokines, suppression of lymphocyte proliferation, neutrophil chemotaxis and adherence, and reduction of serum immunoglobulin. However, the mechanism by which MTX at a low dose modulates inflammation in RA is still unknown. Rapid clinical remission and fast flare of the disease after MTX discontinuation suggest that the anti-inflammatory elements of the mechanisms of MTX action play a much larger part in RA treatment than the antiproliferative ones (Fig. 1, Tab. 1). Studies to date indicate that the most important actions of low-dose MTX are its effects in increasing adenosine level and reducing the pro-inflammatory while increasing the anti-inflammatory cytokine levels.

Effects on dihydrofolate reductase

MTX with high affinity binds and inactivates the dihydrofolate reductase (DHFR), resulting in the depletion of metabolically active intracellular folates with subsequent inhibition of the synthesis of thymidylate and inosinic acid. Inhibition of DHFR causes cessation of the synthesis of purine metabolites which are important for cell proliferation. In RA patients, this is rather not the main element of action because the doses required for MTX's antiproliferative effect are considerably higher.

Effects on adenosine

The intracellular 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) formylotransferase also plays an important role in the purine metabolism of the cell. Its inhibition by low-dose MTX decreases the conversion of AICAR to formyl-AICAR.

- Accumulation AICAR inhibits the degradation of adenosine 5-P and adenosine by adenosine 5-monophosphate (AMP) deaminase and adenosine deaminase (ADA).
- As concentrations of adenosine and adenosine-5-P rise intracellularly, they are more likely to appear in the extracellular milieu.
- In the extracellular space, adenosine 5-P is converted to adenosine, which binds predominantly to A2αreceptors.
- After binding to the A2 receptor, the intracellular cyclic adenosine monophosphate (cAMP) level increases.
- Higher levels of cAMP produce a range of anti-inflammatory effects, such as decreased secretion of tumour necrosis factor (TNF), interferon (IFN)-γ interleukin (IL)-12, IL-6, and inhibition of phagocytosis [19].

Thus adenosine-mediated anti-inflammatory effects may play a central role in producing the anti-inflammatory actions of MTX, as was demonstrated recently by Riksen et al. in patients with RA [17, 91].

Tab. 1. Mechanism of MTX action in RA – experimental data

Point of MTX action		MTX action
in vitro	adenosine	increases adenosine release from fibroblasts and endothelial cells [17]
	cytokines	inhibits IL-1 activity with no effect on IL-1 production* [59] increases IL-2 synthesis by mononuclear cells [73] decreases IL-2 gene expression in peripheral blood mononuclear cells reduces IL-8 synthesis by mononuclear cells [99, 100] increases IL-4 and IL-10 gene expression of peripheral blood mononuclear cells [16] increases Th2 and decreases Th1 cytokine production [16]
	lymphocytes	enhances or suppresses proliferation [59]
	apoptosis	induces apoptosis of mitogen-activated $CD4^+$ and $CD8^+$ lymphocytes, but not resting T cells [34] good response of RA patients to MTX treatment is not always accompanied by a PBMC response to MTX [108]
	eicosanoids	inhibits IL-1β-stimulated production of prostaglandin E2, but no effect on cyclooxygenase (COX)-1 nor COX-2 mRNA expression [112].
	neutrophil	inhibits neutrophil adherence
	synovial fibroblast	inhibits proliferation [59]
	adhesion molecules	decreases expression in synovial tissues [18, 99]
	blood vessels	inhibits neovascularization [99, 100]
	rheumatoid factor	decreases production [16]
in vivo	adenosine	inhibits deamination of adenosine and potentiates adenosine-induced vasodilatation [91]
	cytokines	no effect on IL-1 production [59] no effect on IL-4 production [16] minor (reduce) or no effects on TNF-α production by blood mononuclear cells [100]**
	collagenolytic proteases	decreases of the metalloproteinase-1(MMP-1)/tissue inhibitor of metalloproteinase-1(TIMP-1) ratio in synovial tissue[19]
	blood vessels	inhibits neovascularization [99, 100]
	rheumatoid factor	decreases production [59]
ex vivo	adenosine accumulation	increases adenosine release from fibroblasts and endothelial cells [17]
	cytokine	inhibits activity IL-1,IL-6,IL-8 [59] enhanced IL-2 production [59] minor or no effects of TNF-α production by blood mononuclear cells** [99] stimulates soluble TNF receptor p75 and IL-1ra release [100]
	eicosanoids	decreases or increases production of LTB_4 by neutrophils and total plasma LTB_4 concentration [42, 67] no effect or inhibition of leukotrienes [59]
	apoptosis	peripheral blood lymphocytes (PBLs) from MTX-treated RA patients underwent apoptosis upon *ex vivo* activation [34].
	neutrophil function	inhibits chemotaxis[59]
	rheumatoid factor	no effect or decreases production [59, 98]
	Lymphocyte, mononuclear cell populations	Controversial: no effect [3, 81] increases T cells [118] lower T suppressor cells and absolute lymphocyte counts [115]
animal model	adenosine	increases adenosine release from fibroblasts and endothelial cells [17]
	cytokine	inhibits activity of IL-1* [59] decreases the synovial fluid TNF-α concentration [16]
	eicosanoid production	no effect on prostaglandins or leucotriens [5]
	blood vessels	inhibits neovascularization [5, 99, 100]
	macrophage	inhibits activation [98]

* There is a 60% homology in amino-acid sequence between dihydrofolate reductase and IL-1β, and MTX decreases the binding of IL-1β to the interleukin 1 receptor on peripheral blood cells [74]. The inhibitory effect of IL-1 is abolished by addition of folinic acid. **Perhaps MTX decrease TNF-α production locally in synovium, so we do not see an effect of MTX therapy on TNF-α concentration in the peripheral blood of patients with RA

Effects on cytokines

MTX reduces the production of proinflammatory cytokines, decreases the gene expressions of TH1cytokines, and increases those of anti-inflammatory TH2 cytokines (Tab. 1). The inhibition of the monocytic and lymphocytic pro-inflammatory cytokines involved in rheumatoid synovitis seems to play an important role in the anti-inflammatory action of low-dose MTX.

MTX effects on immunoglobulin

Variable effects of MTX treatment on immunoglobulin M (IgM) rheumatoid factor production were shown. MTX's influence on B-cell function in RA is probably not a major target of its action, and it is difficult to conclude that the effects of MTX therapy on RF levels are related to the beneficial effects of this drug in the therapy of RA [3, 98].

Effect on T cells

The effect of MTX on T cells is likely to be minor at the doses used in RA. The immunosuppressive effect with low-dose MTX is controversial (12 weeks of therapy diminished the number of circulating T and B cells, while long-term MTX therapy led to an increase in the percentage of CD3 and CD4 cells in the peripheral blood) [115, 118].

MTX effects on cyclo- and lipooxygenase

An anti-inflammatory effect of MTX has been suggested by its rapid onset of action (4–6 weeks after therapy begin) and the equally rapid flare after drug discontinuation [116]. Its effect on the generation of leucotriene remains somewhat controversial and is unlikely to contribute on its own in a major way to the efficacy of MTX therapy [42, 71]. MTX applied to RA synoviocyte cultures *in vitro* inhibited the IL-1β-stimulated production of prostaglandin E2, whereas neither cyclooxygenase (COX)-1 nor COX-2 mRNA expression was affected. This suggested that MTX could have an anti-inflammatory action by decreasing prostaglandin E2 release [112]. COX-2 activity was found to be reduced in the plasma of RA patients treated with MTX compared with healthy controls [71]. However, a specific COX-2 inhibitor, celecoxib, had no significant effect on MTX pharmacokinetics in patients with RA [50]. In conclusion, the effect on cyclo- and lipooxygenase seems to be indirect.

MTX effects on apoptosis

Apoptosis is important in the down-regulation of the immune responses after the activation and proliferation of T and B cells. In recent years, an association between apoptosis and autoimmune diseases, including RA, has been reported. It has been considered that the process of apoptosis may play an important role in RA by limiting synovial tissue hyperplasia [55, 77].

- MTX could induce *in vitro* apoptosis of mitogen-activated $CD4^+$ and $CD8^+$ lymphocytes, but not resting T cells [34].
- peripheral blood lymphocytes (PBLs) from MTX-treated RA patients underwent apoptosis upon *ex vivo* activation [34].
- MTX-induced apoptosis of mitogen-activated cells occurred through a CD95-independent pathway [33].
- good response of RA patients to MTX treatment is not always accompanied by a peripheral blood mononuclear cell (PBMC) response to MTX *in vitro* [108].

It seems that either apoptosis of the cells in the tissue directly involved in the inflammatory process is more important than that observed in peripheral blood lymphocytes or another mechanism of the MTX action may be responsible for the clinical improvement in patients treated with low doses of MTX.

Other effects of MTX

Low-dose MTX in RA treatment seems to exert its anti-inflammatory effects by acting at different levels of the pathophysiological cascade [19, 20, 30, 56, 57]:

- it decreases the recruitment of inflammatory cells in joints,
- it has a significant suppressive effect on neutrophil chemotaxis,
- it reduces the numbers of macrophages and inflammatory cells in synovial tissue,
- it reduces intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1(VCAM-1) in synovial tissue,
- it decreases metalloproteinase-1 production, which is probably caused by direct cytokine regulation by MTX (down-regulation of IL-1),
- it inhibits neovascularization (controversial)

– it suppresses TNF-α-induced expression of ICAM-1 and VCAM-1 by vascular endothelial cells [113]

– its inhibition of angiogenesis does not significantly contribute to the anti-arthritic effect of MTX seen in patients and animal models for RA [29].

Pharmacokinetics

MTX is usually given orally in patients with RA.

Bioavailability of low-dose oral MTX:

• Bioavailability is relatively high, but there is individual variability among patients.
• MTX is mainly absorbed in the proximal jejunum [104].
• The absorption of MTX administered orally at 7.5 mg/week is roughly equivalent to that of parenterally administered drug, but the absorption of oral MTX drops off by as much as 30% when the weekly dose is 15 mg or greater [41].
• Absorption may be reduced in a setting of intestinal pathology, such as inflammatory bowel disease, shortened bowel, or other malabsorption syndrome.
• Absorption is not reduced by concomitant food intake.
• The bioavailability of intramuscular (*im*) and subcutaneous (*sc*) MTX is similar to that of intravenous (*iv*) MTX.

MTX distribution:

• After absorption, MTX is 35 to 50% albumin-bound, while 7-hydroxymethotrexate, the principal metabolite, is 91–95% albumin-bound.
• Transport of MTX and 7-hydroxymethotrexate (7-OH-MTX) into cells occurs both passively and actively (by folate receptors FR-α and FR-β), and by facilitated diffusion [49, 102].
• MTX reaches its greatest concentrations in the kidney, liver, gall bladder, spleen, skin, and red blood cells.
• MTX concentration in red blood cells may reflect its toxic effects on bone marrow cells and the level of accumulation in hepatocytes.
• MTX distributes to the synovial fluid, with a ratio of synovial fluid to plasma concentration of approximately 1 [120]. Four hours after *iv* MTX administration, the synovial fluid concentration equals serum levels. Intra-articular injection, may therefore, not yield any advantages over systemic therapy.
• MTX accumulates in the extravascular pool, so it must be used with extreme caution in patients with pleural effusion or ascites.

Metabolism of MTX:

• When the dose is less than 50 mg/m^2, as in the treatment of RA, most of the drug is excreted unaltered in the urine.
• Less than 10% of the dose of MTX is oxidized to 7-OH-MTX.
• A portion of intracellular MTX and 7-OH-MTX is metabolized to polyglutamates. The polyglutamate metabolites are stored in the liver and in erythrocytes for long periods. Slow release of polyglutamated MTX from cells may contribute to the prolongation of the third phase of elimination.

MTX clearance:

• 65–80% of the drug is excreted by the kidneys (the major part in the first 12 h after administration) and 20–35% is secreted with the bile and metabolized or transferred to other compartments.
• In renal elimination, glomerular filtration plays the major role, while those of tubular secretion and reabsorption are less important.
• Active biliary secretion is a minor pathway for MTX excretion, but it becomes more important in patients with renal insufficiency. MTX excreted in the bile is converted to 2,4-diamino-N10-methylpteroic acid (DAMPA) by carboxypeptidase in gut flora [21].
• Patients with impaired renal function will have reduced clearance of the drug from plasma and will thus be at a greater risk of toxicity.
• Hemodialysis and peritoneal dialysis resulted in only transient decreases in MTX concentrations because MTX is a drug with low-to-medium protein-binding and high tissue distribution.

The usual terminal serum half-life of MTX is approximately 7–10 h, but some patients have prolonged elimination half-lives (of about 26 h). MTX clearance in patients with RA is 80–90 ml/min/m^2 [81]. The concentration of MTX in red blood cells remained stable over a 9-day period, whereas its concentration in the serum fell below the limit of detection 52 h after the dose [61]. Individual clearance of MTX could be determined by examining only two plasma samples (at 0.5 and 2.0 h after administration). Plasma MTX measurements are not helpful in defining an optimal treatment regimen [63]. In our study,

we determined the concentration of MTX in 49 patients with RA who received 10 mg of the drug orally once a week. No correlations among concentrations, pharmacokinetic parameters of MTX, duration of the disease, and disease activity were found. There was no significant differentiation observed in MTX pharmacokinetic parameters in relation to the time of therapy. There were also no differences in drug concentration between patients receiving prednisone and those who did not and between groups receiving diclophenac, naproxen, and ketoprofen [120].

The MTX dose should be decreased in the elderly (> 65 years) and in patients with renal impairment [9].

Drug dosage

Methotrexate may be given orally (in tablet or liquid form) or parenterally, by *sc* or *im* injection. Studies have demonstrated less toxicity with weekly administration of MTX than administration over five to seven days. Pandya et al. did a pilot study to see if MTX twice weekly is superior to MTX once weekly, because the half-life of MTX active compound, the polyglutamate MTX, is three days. At 8 and 16 weeks there was no significant difference in ACR 20% and ACR 50% responses. This study suggests that MTX twice weekly has no advantage over once weekly regarding efficacy [83]. The following dose schedules are commonly used:

1. single weekly oral or *im* low dose
2. doses divided into two or three weekly doses at consecutive 12-h intervals

If significant improvement is not noted, the dosage may be gradually increased. The usual starting dose in RA is 7.5–10 mg per week. If a positive response has not occurred within 4 to 8 weeks after MTX initiation and there has been no toxicity, the dose should be increased (by 2.5–5 mg/week each month) to 20–25 mg per week before considering the treatment a failure. To improve the efficacy of MTX at dosages of 20–25 mg weekly or more, a change to parenteral administration (*sc*) should be considered [41, 44, 64]. All schedules should be continually adjusted to clinical response and adverse reactions of the individual patients. Patients responding to MTX therapy generally maintain the improvement as long as the therapy is continued. Flare is observed within weeks after cessation. If remission is obtained (complete remission is uncommon) the dosage should be reduced or a weekly pulse regimen could be changed to a fortnightly one [109].

Drug interactions (Tab. 2)

Folate supplementation during MTX therapy

Folic acid and folinic acid reduce some adverse events (gastrointestinal intolerance, stomatitis, hepatotoxicity, hyperhomocysteinaemia, alopecia) associated with MTX, so most of rheumatologists recommended all patients taking MTX to take them as well even though this may be accompanied by a slight reduction in efficacy. A very important report on folic acid supplementation was published by Morgan et al. [76]. The patients received placebo or 5 or 27.5 mg of folic acid weekly. This study demonstrated that folate intake leads to a reduction in side effects without reduced efficacy. In a 48-week trial, Hoekstra et al. analyzed the toxicity of MTX in patients who received either folic acid or placebo. The addition of folate was strongly associated with a lack of hepatotoxicity [43]. Van Ede et al. demonstrated in a randomized controlled trial that treatment with either folate regimen resulted in a reduction in the incidence of liver enzyme elevations [111]. Patients receiving folate were less likely to discontinue MTX than patients in the placebo group, but folate supplementation was associated with higher mean doses of MTX. Another reason to use folic acid in MTX-treated patients is for its effectiveness in reducing plasma homocysteine levels. Hyperhomocysteinemia is an independent risk factor for coronary artery disease, and premature mortality in patients with RA is caused by accelerated atherosclerosis. Slot demonstrated in a small study that P-homocysteine concentrations negatively correlated with erythrocyte folate after four weeks. P-homocysteine and erythrocyte folate were measured before the start of MTX treatment, after four weeks of MTX treatment, and after further four weeks of treatment with MTX supplemented with folic acid (15 mg per week). The author concluded that treatment with MTX induced a significant rise in P-homocysteine that was neutralized by folic acid supplementation [103].

After a meta-analysis, Whittle at al. proposed that folic acid supplements be prescribed routinely to all patients receiving MTX for the treatment of RA. They

Tab. 2. MTX interactions with other drugs

Drug	Effect of interaction	Comments
Neomycin Nystatin Vancomycin	decrease in MTX absorption by 30–50%	↓ MTX efficacity
Kanamycin	increase in MTX gastrointestinal absorption	↑ MTX efficacity
Cholestyramine	increase in MTX elimination	↓ serum MTX levels
Salicylates p-Aminohipurate	reduce MTX elimination in urine	drugs secreted by the organic acid transport system alike as is MTX ↑ MTX efficacity and toxicity
NSAIDs**	decrease glomerular filtration of MTX, secondary to NSAID-induced renal capillary constriction alter protein binding of MTX and 7-OH-MTX impairment of the hepatic metabolism of MTX	Salicylates decrease MTX renal clearance by 35–47% Toxicity increased by: reduced renal function, hepatic failure, advanced age, high dosing of MTX (>15 mg/week)
Probenecid *	inhibits MTX renal tubular secretion inhibits MTX biliary excretion	↑ MTX toxicity ↓ MTX clearance by up to 60% ↑ mean serum MTX concentration
Aminoglycosides, Amphotericin B, Cyclosporine	decrease in MTX elimination	Patients with decreased renal clearance are at risk of more frequent or more severe toxicity due to MTX
Trimethoprim- Sulfamethoxazole*	inhibition of the same enzyme as MTX decrease MTX clearance due to inhibition of tubular secretion alter binding of MTX to plasma proteins	These drugs are also antifolates and their use with MTX is associated with severe bone marrow suppression and pancytopenia
Cephalosporin	inhibition of MTX renal excretion	probably by competition for tubular secretion
Folic acid	block MTX reabsorption at the distal tubule	↓ toxicity ↓ or no effect on MTX efficacity
Corticosteroids (long-term treatment)	hinder MTX absorption or increase in MTX metabolism	20% decrease in MTX clearance
Hydroxychloroquine	reduction in MTX clearance or increase in the active tubular reabsorption	Increases the area under the curve of MTX serum concentrations in time by 65%
Theophylline	MTX may decrease the clearance of theophylline	Theophylline level should be monitored when used with MTX

* Probenecid and cotrimoxazole should be avoided in MTX-treated patients. ** Aspirin seems to decrease total clearance and renal clearance of MTX at the same time it increases the AUC of MTX. The kinetic interaction between aspirin and MTX is greater than for most other NSAIDs, but the clinical importance of this is not clear [70, 92]. Most of the reports on severe adverse effects resulting from co-administration of NSAIDs and salicylates with MTX are associated with high dosages of MTX. The toxicity of MTX plus NSAIDs is clinically rare. Whenever NSAIDs are added or changed in the regimens of patients receiving a stable dose of MTX, serum creatinine levels should be re-examined

recommended 5 mg of oral folic acid given in the morning following the day of MTX administration. The folic acid dose can be increased if the side effects continue. Folate supplements do not appear to significantly reduce the effectiveness of MTX in the treatment of RA, so the benefits outweighs the risk [119]. In patients in whom folic acid is not adequate, one can try using folinic acid (initial dose: 5 mg/week) administrated 24 h after MTX.

Discussions about the necessity of folic acid supplementation were resumed after the publication of a *post hoc* analysis of two randomized, controlled studies by Khanna et al. Nine to 21% fewer MTX-treated RA patients taking folic acid had American College of Rheumatology (ACR) 20%, 50%, or 70% improvement at 52 weeks compared with those who did not receive folic acid, so some rheumatologists believe

that prophylactic prescription of folic or folinic acid to all RA patients receiving MTX is not required [26, 52].

Contraindications

Patients with alcoholism, ongoing chronic liver disease, renal insufficiency, untreated folate deficiency, leucopenia, thrombocytopenia, significant anemia, immunodeficiency syndromes, or those being treated with trimethoprim should not receive methotrexate. Women of childbearing age should practice appropriate contraception before using MTX. MTX is not recommended for nursing mothers. Women who wish to conceive should discontinue MTX for at least one menstrual cycle, but better is for at least 3–6 months. Males should discontinue MTX at least three months before conception is attempted.

The use of methotrexate in the perioperative period

MTX did not increase the risk of infections or other postoperative complications in patients with RA, and treatment should not be stopped in patients whose disease is controlled by the drug before operation. Grennan et al. formulated such an opinion after a prospective, randomized study involving 388 patients with RA (160 of whom were taking MTX) followed for one year after elective orthopedic surgery [38]. Similar findings in a randomized, prospective study involving 64 patients were reported by Sany et al. [94].

Monitoring (Tab. 3)

Clinical use

MTX monotherapy

In 1972, Hoffmeister reported the results of MTX *im* therapy in 29 patients with rheumatoid arthritis receiving a dosage of 10–15 mg/week. Fourteen patients showed marked improvement and 11 patients experienced moderate improvement [45]. Six randomized, controlled trials of MTX were initiated in the early 1980s. The largest of these studies (189 patients) compared low-dose MTX (7.5–15 mg/week) with placebo. MTX produced a significant improvement in all efficacy variables measured [121]. Clinical improvement usually started after three weeks of therapy, and maximum response occurred at two to three months. Most investigators observed flare within a month of cessation of therapy. Statistically signifi-

Tab. 3. Monitoring during MTX treatment in RA

Baseline studies	complete blood cell count with platelet count, serum creatinine level, liver blood tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, bilirubin], serum albumin and serology for hepatitis B and C, and chest X-ray
Every 4–8 weeks studies*	complete blood cell count, platelet count, AST, ALT, serum albumin and creatinine levels.
Pretreatment liver biopsy	a) prior excessive alcohol consumption, b) persistently abnormal baseline AST values, c) chronic hepatitis B or C infection
Liver biopsy during treatment with MTX**	a) five of nine determinations of AST within a given 12-month interval (6 of 12 if tests are performed monthly) are abnormal (defined as an elevation above the upper limit of normal) b) there is a decrease in serum albumin below the normal range (in the setting of well-controlled rheumatoid arthritis)

* MTX dosage should be reduced or discontinued if the white cell or platelet counts fall significantly or if there is significant or persistent AST, ALT elevation. MTX appears to be seldom associated with clinically significant hepatic side effects, so some rheumatologists now consider less frequent AST, ALT monitoring [114]. ** Discontinue MTX in a patient with persistent liver test abnormalities who refuses liver biopsy and if results of biopsy are grades IIIB or IV [60]

cant linear dose improvement was noted with 10 mg/m^2/week versus 5 mg/m^2/week versus placebo. MTX was approved by the United States Food and Drug Administration in 1988 as a therapy for RA arthritis.

MTX has been compared with other DMARDs (Tab. 4).

MTX and intramuscular gold (GSTM)

In three double-blind controlled studies comparing MTX and GSTM, no significant differences in effectiveness and in the progression of radiographic evidence of erosion were seen, but MTX was better tolerated [75, 89, 107]. Menninger demonstrated in a three-year study comparing MTX and GSTM in 174 patients with erosive RA that withdrawal from the study because of toxicity was significantly higher in the GSTM group (53% *vs*. 16% for MTX) [72]. Rau et al. demonstrated in a three-year study that MTX and GSTM were able to reduce the slope of radiographic progression during three years of follow-up. There was some advantage of parenteral gold, but no significant intergroup difference [90]. Spanish authors demonstrated after one year of treatment with disease-modifying antirheumatic drugs (GSTM or MTX) that, although a substantial reduction in disease activity was observed during the one year of follow-up, radiographic progression was observed in up to 36% [93]. In a comparative study, GSTM and low-dose MTX showed equivalent efficacy, but toxicity was more common in patients treated with GSTM (withdrawals for toxicity: 43% GSTM and 19% MTX). GSTM, although more toxic, remains a useful alternative for patients in whom MTX is contraindicated.

Tab. 4. Comparison of MTX with other DMARDs in treating RA

Drug	Efficacy	Adverse events
Auranofin	MTX > auranofin	MTX < auranofin
Intramuscular gold	MTX = intramuscular gold	MTX < intramuscular gold
D-penicillamine	MTX > D-penicillamine	MTX < D-penicillamine
Sulfasalazine	MTX > sulfasalazine	MTX ≥ sulfasalazine
Azathioprine	MTX ≥ azathioprine	MTX ≤ azathioprine
Cyclosporin A	MTX = cyclosporin A	MTX < cyclosporin A
Hydroxychloroqiune	MTX > hydroxychloroqiune	MTX ≥ hydroxychloroqiune
Leflunomid	MTX = leflunomid	MTX = leflunomid
Etanercept	MTX < etanercept	MTX = etanercept
Adalimumab	MTX < adalimumab	MTX = adalimumab

MTX – methotrexate

MTX and azathioprine (AZA)

In a 24-week double-blind study of 42 patients, Hamdy et al. compared MTX (5–15 mg/week) and AZA (50–150 mg/day). The MTX-treated group exhibited a trend toward more rapid and marked improvement. After 24 weeks and after one year, radiological evidence of progressive joint damage was similar in both treatment groups. The authors concluded that AZA and MTX were similarly effective in the treatment of RA [40]. Interesting results were shown by Willkens et al. after a 48-week, prospective, multi-centre, controlled trial. Two hundred nine patients with RA were randomized to receive MTX (5–15 mg/week), AZA (50–150 mg/day), or their combination (5 mg MTX/week plus 50 mg AZA/day to 7.5 mg MTX/week plus 100 mg AZA/day). Forty-five percent of the patients in the MTX-only group had at least 30% improvement in at least three of four variables compared with 38% and 26% in the combination and AZA-only groups, respectively [122]. In another 48-week, randomized, controlled trial, the number of withdrawals caused by adverse effects was significantly higher among patients receiving AZA than MTX and efficacy was better in the MTX group [48]. These authors concluded that MTX was superior to AZA in treating RA.

MTX and cyclosporin A (CSA)

MTX was compared with CSA in a 34-week, double-blind, randomized study of 264 patients with RA. MTX (7.5–15 mg/week) was superior to CSA (2.5–5.0 mg/kg/day) in improving disease activity. Both MTX and CSA combined with prednisolone were effective in patients with RA in an open randomized trial. About a hundred (102) RA patients were treated with either CSA (3 mg/kg/day) or MTX (0.15 mg/kg /week). Statistically non-significant differences between the two groups in efficacy and radiographic progression were demonstrated [1].

MTX and leflunomide (LEF)

In a large 12-month, randomized, double-blind, placebo-controlled trial with over 400 patients with active RA, MTX was compared with LEF. Thirty-five percent of patients receiving MTX and 41% receiving LEF had successful response, and there were no statistically significant differences. The mean time to initial response was 9.5 weeks for patients treated with MTX compared with 8.4 weeks for patients receiving LEF. Strand et al. concluded that both drugs were comparable in efficacy [105]. Cohen et al. demonstrated in a group of 235 patients that 67% of MTX-treated and 79% of LEF-treated patients achieved an ACR improvement response of 20% or greater ($p = 0.049$), but LEF was statistically significantly superior to MTX in improving physical function as measured by the Health Assessment Questionnaire Disability Index (HAQ) [13]. Also, Chinese authors showed in a large study (566 RA patients) that the efficacy of LEF 20 mg once daily was similar to that of MTX 15 mg once weekly. The incidence of adverse events and the rate of withdrawal due to adverse events were lower in the LEF group than in the MTX group (16.84% *vs*. 28.17%) [4]. Aletaha et al. obtained a slightly different result. They studied the survival and clinical effectiveness of LEF compared with MTX and sulfasalazine (SSZ) in 1088 patients with RA. LEF courses were stopped earlier for adverse events ($p < 0.001$) than MTX courses. MTX mean survival was 28 months, LEF 20 months, and SSZ 23 months. They concluded that at present, MTX continues to be the most effective drug in clinical practice [2]. In a two-year European study, MTX was compared with LEF in 999 patients with active RA. After one year the subjects could choose to continue in the study for a second year of double-blind treatment. Improvements seen in the MTX-treated group were significantly greater after 52 weeks, but in the second year the distinction between the two treatment groups in the number of tender joints and patient global assessment was lost. After two years, radiographic progression was significantly less marked in the patients who had received MTX [25].

Pincus et al. demonstrated that 57% RA patients were still on MTX after five years compared with 18–25% taking other agents (GSTM, hydroxychloroquine (HCQ), D-penicillamine (D-Pen), AZA) [86]. Other authors found the median time at discontinuation of HCQ, GSTM, and D-Pen treatment was two years or less, compared with 4.25 years for MTX [123]. In 1992, Felson et al. published a meta-analysis of trials investigating DMARDs. MTX was among the most efficacious drugs (together with SSZ, GSTM, and D-Pen) and had the least toxicity [27]. Furst compared seven DMARDs on the results of controlled trials. In effectiveness, the results were: MTX = GSTM ≥ D-Pen = AZA ≥ HCQ = SSZ = auranofin. However, the results on toxicity were: HCQ = AZA = MTX ≤ auranofin = SSZ ≤ D-Pen ≤ GSTM [32]. The two meta-analyses showed that MTX had one of the best efficacy/toxicity ratios. Also, from a study of 428 RA patients that compared HCQ (200–400 mg/day), D-Pen (500– mg/day), SSZ (2–3 g/day), auranofin (6 mg/day), GSTM (50 mg/week), MTX (0.15 mg/kg /week, per os), CSA (3 mg/kg/day), AZA (2–3 mg/kg /day), and cyclophosphamide (CYC) (1–2 mg/kg /day), it appeared that MTX had the longest survival time. The main reasons for discontinuation of treatment were drug inefficacy (HCQ), followed by adverse drug reactions (D-Pen) [84]. After examining data collected over a 20-year period on 1160 patients treated with MTX or HCQ or GSTM, Hurst et al. concluded that MTX was the most effective DMARD of these three because of the length of the therapeutic period. A second trial of the same drug was far less effective than the first course [46].

The long-term survival of MTX treatment was also demonstrated in the RA DMARD Intervention/Utilization Study (Radius). This was a prospective, six-month study on 2202 patients. Ninety-one percent remained on MTX, 79% on LEF, 84% on HCQ, and 75% on SSZ for six months [97]. In addition, Kinder et al. have shown that MTX is well tolerated in clinical practice in the medium to long term. Between 1986 and 1999, 673 patients were treated with MTX. The probability of patients remaining on treatment for five years after starting MTX was 0.74. Life-threatening side-effects were identified in 12 patients (1.8%) [53].

After many studies done in the past years, we know that early and aggressive therapy of RA close to remission is not achieved. After an observational dataset comprising 3342 DMARD courses, Aletaha et al. demonstrated evidence of a change in DMARD patterns of effectiveness. We must use DMARDs immediately after diagnosis, use the most effective DMARD, and switch regimens rapidly if the level of disease activity in newly diagnosed RA patients requires a higher prescription rate of more aggressive drugs such as MTX, as well as to decrease the lag time until MTX is instituted in RA patients over the

Tab. 5. Comparison of MTX in monotherapy *versus* MTX in combination therapy in treating RA

Combination therapy	Comparison of efficacy	Adverse events
MTX + CSA	MTX < MTX + CSA	MTX < MTX + CSA
MTX + HCQ	MTX < MTX + HCQ	MTX ≥ MTX + HCQ
MTX + SSZ + HCQ	MTX < MTX + SSZ + HCQ	MTX ≤ MTX + SSZ + HCQ
MTX + LEF	MTX < MTX + LEF	MTX < MTX + LEF
MTX + etanercept	MTX < MTX + etanercept	MTX ≤ MTX + etanercept
MTX + infliximab	MTX < MTX + infliximab	MTX ≤ MTX + infliximab
MTX + adalimumab	MTX < MTX + adalimumab	MTX ≤ MTX + adalimumab
MTX + anakinra	MTX < MTX + anakinra	MTX < MTX + anakinra
MTX + SSZ	MTX ≤ MTX + SSZ	MTX ≤ MTX + SSZ
MTX + GSTM	MTX = MTX + GSTM	MTX < MTX + GSTM
MTX + AZA	MTX = MTX + AZA	MTX < MTX + AZA

MTX – methotrexate, RA – rheumatoid arthritis, CSA – cyclosporin A, HCQ – hydroxychloroquine, SSZ – sulfasalazine, LEF – luflunomid, GSTM – intramuscular gold, AZA – azathioprine

years [1]. Although results of many studies have shown the efficacy of MTX in RA, it is very important to know its effect on mortality in patients with the disease. To answer this question, Choi et al. estimated mortality in a cohort including 1240 patients with RA seen at the Wichita Arthritis Center from 1981 through 1999 (588 were treated with MTX). The mortality hazard ratio for MTX use compared with no MTX use was 0.4 (95% *CI*: 0.2–0.8). The hazard ratio of MTX use for cardiovascular death was 0.3 (0.2–0.7), whereas that for non-cardiovascular deaths was 0.6 (0.2–1.2). These data indicate that MTX may provide a substantial survival benefit, largely by reducing cardiovascular mortality [12].

Combination therapy

A significant number of patients treated only with MTX fail to achieve optimal disease control, so there are many DMARD combination regimes (Tab. 5). The ideal outcome of combination DMARD therapeutic strategies is one that is synergistic for efficacy and lacking any additive effects of toxicity. Early randomized controlled trials of combination therapy generally failed to demonstrate an advantage of these regimens over MTX alone [85, 87]. Substantial changes in study design, including more careful selection of the study population based on an incomplete response to MTX, have led to a better understanding of the advantages of combination therapy.

1) MTX + CSA

CSA may block oxidation of MTX to its relatively inactive metabolite 7-OH-MTX, thereby potentiating MTX efficacy. Fox et al. demonstrated in a study of 30 RA patients that co-administration of CSA and MTX led to a 26% increase in mean peak plasma MTX concentration, an 18% increase in the mean plasma MTX concentration area under the curve (AUC), and an 80% decrease in plasma 7-OH-MTX AUC. In 13 patients receiving a 10-mg MTX dose, CSA reduced urinary 7-OH-MTX excretion by 87% without altering MTX excretion. MTX did not alter the pharmacokinetics of CSA or its metabolites [31]. Tugwell et al. compared adding CSA (2.5–5 mg/kg) or placebo to patients who had a partial response to MTX at the maximum tolerated dose. In that trial, 48% of patients who received MTX + CSA and 16% who received MTX + placebo met ACR 20% criteria. However, serum creatinine levels were increased in the CSA group [110]. Gerards et al. compared the efficacy and toxicity of CSA monotherapy with CSA plus MTX combination therapy in patients with early RA. The median Larsen score increased to 10 points in the monotherapy group and to 4 points in the combination therapy group. Forty-seven percent of patients in the monotherapy group versus 57% of those in the combination therapy group had reached ACR20 at week 48, 25% versus 48% of patients had reached an ACR50 response, and 12% *vs.* 20% of patients had reached an ACR70 response. There was only a tendency towards more toxicity in the combination therapy group. They concluded that combination therapy was probably better in improving clinical disease activity, and definitely better in slowing radiological progression [37]. In a 12-month study of 105 patients, the authors demonstrated that CSA + MTX was more effective than CSA + HCQ or CSA alone in improving clinical data and inhibiting radiographic progression, although the differences were not significant in this relatively small study.

However, the difference was significant in favor of CSA + MTX regarding ACR 50% response [96].

2) MTX + SSZ

The effectiveness of MTX does not appear to be really enhanced by combining it with SSZ. Maillefert et al. demonstrated in a five-year, multicentre, prospective, randomized trial comparing MTX monotherapy with the combination therapy MTX and SSZ in 205 patients with early RA no differences in the two groups in disease activity score, health assessment questionnaire, or radiological changes [68]. In another open-design study of 40 RA patients, after 24 weeks there was a greater decrease in mean disease activity score in the combination-treatment group (MTX and SSZ) than the MTX-only treatment group, without an increase in toxicity [39]. Dougados et al. demonstrated no evident beneficial effect of a combination of MTX plus SSZ or either MTX or SSZ in 209 RA patients after a double-blind, randomized, 52-week trial. There was a trend in favor of a lower progression rate in the combination group (but not statistically significant). Adverse events occurred significantly more often in the combination group, but discontinuations caused by adverse events were comparable [22].

3) MTX + HCQ

In a randomized cross-over study with 10 healthy subjects, Carmichael et al. demonstrated that the mean AUC for MTX increased and the maximum MTX concentration (C_{max}) decreased when MTX was co-administered with HCQ compared with MTX administered alone. The time to reach C_{max} for MTX administration also increased during co-administration with HCQ. These results may explain the increased potency of the MTX + HCQ combination over MTX as a single agent and also the sustained effects of MTX when administered with HCQ. In addition, the reduced C_{max} of MTX observed during co-administration may explain the diminution of acute liver-adverse effects [11].

4) MTX + GSTM, MTX + AZA

No advantage was shown when MTX was added to GSTM or AZA. Until now there has been only one study which provides evidence that in RA patients with suboptimal response to MTX therapy, adding GSTM results in significant clinical improvement [66].

5) MTX + doxycycline

Another combination therapy was presented by the Rheumatoid Arthritis Investigational Network group. Sixty-six patients were treated for two years with doxycycline 20 mg or 100 mg plus MTX compared with MTX alone. Doxycycline is not only an antibiotic, but also a metalloproteinase inhibitor, and previous studies with minocycline had suggested a modest benefit in RA. Both doxycycline groups demonstrated statistically superior improvement over MTX monotherapy. It is amazing that the response to MTX monotherapy was lower than that reported in other trials (ACR20: 33%, ACR50: 12%, ACR70: 8%). We, therefore, need more clinical observations regarding this therapy [24].

6) Triple DMARD therapy

a) There are also interesting results of the study comparing monotherapy and triple DMARD therapy. The superiority of the combination of MTX, SSZ, HCQ, and prednisolone compared with monotherapy was demonstrated in an open, two-year randomized trial with 195 RA patients. Patients were treated either with a combination of MTX 7.5 to 15 mg/week plus SSZ 1 to 2 g/d plus HCQ 300 mg/d plus prednisolone 5–10 mg/d, or therapy with a single DMARD combined with oral prednisolone up to 10 mg/d. After one and two years of treatment, significantly greater clinical improvement and significantly less radiographic progression occurred in the combination group [78].
b) O'Dell et al. compared treatment of RA with MTX alone, SSZ and HCQ, and a combination of all three medications in a two-year study (102 patients with RA) [79]. The rate of discontinuation of therapy for drug toxicity was higher in the MTX group than for either of the other two groups. They demonstrated a 50% improvement in symptoms of arthritis without evidence of toxicity in 33% of patients treated with MTX alone, 40% of patients treated with SSZ and HCQ, and 77% of patients treated with all three drugs. In addition, it was found that patients with shared epitope positivity were more likely to achieve 50% improvement if treated with the triple therapy, but those negative for the shared epitope responded just as well to MTX alone as to the combination of MTX

+ SSZ + HCQ. These authors continued to establish the efficacy and toxicity of the combination therapy in another study. One hundred seventy-one RA patients who had not previously been treated with combinations of the study medications were randomized to receive one of the three treatment combinations in this two-year, double-blind, placebo-controlled protocol. HCQ was given at a dosage of 200 mg twice a day, MTX was accelerated from 7.5 mg/week to 17.5 mg/week, and SSZ was escalated from 500 mg twice a day to 1g twice a day in patients who were not in remission. Patients receiving the triple combination responded the best, with 78% achieving an ACR 20% response at two years compared with 60% of those treated with MTX and HCQ ($p = 0.05$) and 49% of those treated with MTX and SSZ ($p = 0.002$). Similar trends were seen for the ACR 50% response. All combination treatments were well tolerated. The authors concluded that the triple combination of MTX, SSZ, and HCQ was well tolerated and that its efficacy was superior to that of the double combination of MTX and SSZ and marginally superior to that of the double combination of MTX and HCQ [80]. Calguneri et al. compared monotherapy with MTX, SSZ, or HCQ with double therapy (MTX + SSZ or MTX + HCQ) and triple therapy (MTX + SSZ + HCQ) in 180 patients with early RA in an open, randomized, two-year study. A 50% or more improvement was shown in 88% patients after triple therapy, 73% after double, and 49% after monotherapy. Radiographic scores were improved or unchanged in 69% of patients receiving triple therapy, 64% receiving double, and 25% those on single therapy. No difference in the number of adverse events among the treatment groups was observed [10].

c) An interesting analysis of improvements and toxicity in RA patients treated with a step-up combination therapy (MTX, CSA, SSZ) or monotherapy for three years was done by Ferraccioli et al. MTX (group 1), CSA (group 2), or SSZ (group 3) was used for six months. Then a combination of two drugs (CSA and MTX) was employed in groups 1 and 2. SSZ was added after 12 months if improvement was less than ACR50 with the combination. Group 3 continued with SSZ alone. At the 18-month follow-up, 90% of group 1 and 88% of group 2, but only 24% of group 3 had reached ACR50. Side effects occurred in 62% of group 1, 60% of group 2, and 48% of group 3. MTX appears to be the fastest-acting agent. A step-up approach with MTX plus CSA plus SSZ led to a 50% improvement according to the ACR criteria in most patients [28].

d) The Combinatietherapie Bij Reumatoide Artritis (COBRA) trial demonstrated that a step-down combination therapy with prednisolone, MTX, and SSZ was superior to SSZ monotherapy for suppressing disease activity and radiological progression of RA. Also, after five years there were benefits of the combination therapy versus SSZ alone in radiographic score [65].

e) After a five-year trial (after two years the drug treatment strategy was no longer restricted) of 195 patients with RA, Finnish authors concluded that the cumulative duration of work disability per patient-observation year was significantly lower in those randomized to a combination therapy (SSZ, MTX, HCQ plus prednisolone) than in those randomized to a single therapy (with or without prednisolone): a median of 12.4 days versus 32.2 days. This was mainly due to differences in sick leaves [88].

After a 10-year study of 4253 patients, Ortendahl et al. came to the conclusion that MTX treatment of RA in practice differs substantially from common perception and appears suboptimal by being too little, too late, and too long to treatment change [82]. Zeidler et al. suggested that combination therapy should be started if active disease is still present after three months of treatment with a single standard DMARD, mostly MTX plus low-dose prednisolone, and that combination DMARD therapy should be used before TNF blocking agents [124].

7) MTX + biological agents

Because of radiographic evidence of progressive bone loss and the inability to eliminate synovial proliferation with MTX, it became apparent that therapy for RA needed further advancement. In the past years, new disease-modifying antirheumatic drugs have been approved. Each of these agents has demonstrated efficacy compared with placebo in randomized, controlled studies. Because MTX had a dominant therapeutic role, the new drugs were also studied in combination with it. The introduction of TNF-α antagonists in 1998 has had a significant impact on the treatment of RA. Infrequent adverse events, including serious infections, particularly tuberculosis, which may be atypical in presentation, development of a systemic lupus erythematosus-like syndrome, additional cases of congestive heart failure, demyelinating syndromes, and increased risk of lymphoma, have been reported. Additional post-marketing studies are necessary to determine the true risk of TNF-α antagonists [47].

a) MTX + etanercept. Bathon et al. compared MTX (20 mg/week) and the TNF antagonist etanercept (10 mg or 25 mg subcutaneously, twice weekly) in 632 patients with early RA. Patients who received the 25-mg dose of etanercept had more rapid improvement and significantly less progression in radiographic disease than patients treated with MTX. Fewer patients in the etanercept group than in the MTX group experienced adverse events or discontinued treatment because of adverse events. The authors concluded that etanercept as a monotherapy was safe and superior to MTX in reducing disease activity, arresting structural damage, and decreasing disability over two years in patients with early, aggressive RA [6, 35]. After the TEMPO study, it is known that the combination of etanercept and MTX is significantly better in reducing disease activity, improving functional disability, and retarding radiographic progression compared with MTX or etanercept alone. This was a double-blind, randomized, clinical study in 686 patients with active RA. These patients received etanercept 25 mg (subcutaneously twice a week), oral MTX (up to 20 mg every week), or the combination. The number of patients reporting infections or adverse events was similar in all groups [54].

b) MTX + infliximab. A total of 428 subjects with active RA despite therapy with MTX were enrolled in a randomized, double-blind trial (ATTRACT: Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy) evaluating the clinical efficacy and safety of infliximab therapy. In patients who received infliximab with MTX there were statistically significant improvements in clinical benefit and radiographic progression. The most benefit was gained when dosages were higher than 3 mg/kg or were given more frequently: every four weeks instead of eight [106]. Maini et al. demonstrated in a two-year study that infliximab plus MTX provided significant, clinically relevant improvement in physical function and quality of life, accompanied by inhibition of progressive joint damage and sustained improvement in the signs and symptoms of RA among patients who had previously incomplete response to MTX alone. Median changes from baseline to week 102 in the total radiographic score were 4.25 for patients who received the MTX-only regimen and 0.50 for patients who received the infliximab plus MTX regimen. The proportion of patients achieving an ACR20 response at week 102 varied from 40% to 48% for the infliximab plus MTX groups compared with 16% for the MTX-only group [69].

c) MTX + adalimumab. The ARMADA trial evaluated the efficacy and safety of adalimumab, a fully human monoclonal TNF-α antibody, in combination with MTX in RA patients with active disease despite treatment with MTX. Adalimumab was safe and well tolerated; comparable numbers of adalimumab-treated patients and placebo-treated patients reported adverse events. The addition of adalimumab at a dosage of 20 mg, 40 mg, or 80 mg administered *sc* every other week to long-term MTX therapy in patients with active RA provided significant, rapid, and sustained improvement in disease activity over 24 weeks compared with MTX plus placebo [117]. The efficacy of adalimumab in RA patients who had an inadequate response to MTX was also demonstrated in the 52-week, double-blind, placebo-controlled study. Adalimumab was more effective than placebo in inhibiting the progression of structural joint damage, reducing the signs and symptoms, and improving physical function in patients with active RA. The rate of all adverse events was comparable in the adalimumab and placebo groups, although the proportion of patients reporting serious infections was higher in patients receiving adalimumab (3.8% *vs*. 0.5%) [51].

d) MTX + anakinra. To evaluate the efficacy and safety of anakinra, a recombinant human IL-1 receptor antagonist, in combination with MTX in patients with active RA, Cohen et al. carried out a 24-week study in 419 patients. In patients with persistently active RA, the combination of anakinra and MTX was safe and well tolerated and provided significantly greater clinical benefit than MTX alone [14]. These results were confirmed in the next double-blind, randomized, placebo-controlled trial (506 RA patients). Significantly greater proportions of anakinra-treated patients achieved ACR20 (38% *vs*. 22%), ACR50 (17% *vs*. 8%), and ACR70 (6% *vs*. 2%) responses. Anakinra was well tolerated, with a safety profile similar to that of placebo with one exception: mild to moderate injection site reactions were more common with anakinra than with placebo (65% *vs*. 24%) [15]. Genovesa et al. demonstrated that combination therapy with etanercept plus anakinra was not better than etanercept alone, but was associated with an increased safety risk. The incidence of serious infections, injection-site reactions, and neutropenia was increased with combination therapy. The authors concluded that combination therapy with etanercept and anakinra is not recommended for the treatment of patients with RA [36].

e) MTX + abatacept. MTX was also used in a combination with abatacept (CTLA4Ig), a fusion protein that consists of the external domain of human CTLA4 and the heavy-chain constant region of human IgG1. Abatacept binds to CD80 and CD86 on antigen-presenting cells, blocking the engagement of CD28 on T cells and preventing T-cell activation. After six months, patients treated with 10 mg of abatacept per kilogram were more likely to have an ACR 20, 50, or 70% responses than patients in the placebo group.

The treatment with abatacept was well tolerated, and the patients on abatacept also had improved health-related quality of life. CTLA4Ig was well tolerated, with an overall safety profile similar to that of placebo [62].

f) MTX + rituximab. Another very interesting combination is that of MTX and rituximab. Rituximab is a chimeric anti-CD20 monoclonal antibody that causes selective and transient depletion of the $CD20^+$ B-cell subpopulation. Edwards et al. showed in a randomized, double-blind, controlled 48-week study that a single course of two infusions of rituximab, alone or in combination with either CYC or continued MTX, provided significant improvement in disease symptoms at both 24 and 48 weeks. During the 48-week follow-up period, serious infections occurred in one patient (2.5 percent) in the control group and in four patients (3.3 percent) in the rituximab groups. The risk of infection with rituximab requires further evaluation in controlled clinical trials [23].

In studies comparing MTX directly with biological agents, the biological agents have greater efficacy in patients with very severe disease, but the best results are seen in patients who take a combination of MTX and biological agents. These data have established that MTX should probably be the first DMARD used in the majority of patients with RA at this time. Based on the results of prospective observational studies and on the known pharmacological properties of MTX (a large interindividual variation in bioavailability), Seitz et al. have concluded that any novel disease-modifying antirheumatic drug/biological agent has to be compared with MTX given parenterally and in maximum weekly doses (up to 25 mg) [101].

The goal of treatment is to improve both general health and health-related quality of life (QOL). MTX, LEF, CSA, glucocorticoids, etanercept, infliximab, and adalimumab clinically and statistically significantly improved QOL in patients with RA [8].

Conclusions

The therapy of RA is a dynamic process and requires maintaining a delicate balance of benefits and risks. Experience and familiarity with the currently available agents and knowledge of the nature of the disease are necessary in order to make better therapeutic decisions. MTX has excellent efficacy and an acceptable toxicity profile. However, a number of patients do not tolerate MTX and an alternative DMARD should be chosen. The choice of an alternative DMARD should be made after careful consideration, including concomitant diseases, existing medication, and drug compliance. In patients with RA who are unable to tolerate MTX, the alternatives are parenteral administration (*sc*) of MTX or HCQ, SSZ for mild-to-moderate disease and CSA or LEF for severe disease, given in combination with low-dose oral corticosteroids. Biological anticytokine agents, etanercept, infliximab, adalimumab, and anakinra are now available for use in RA. It is hoped that more aggressive use of conventional DMARDs and biological agents will result in less disability and a higher proportion of patients achieving remission [17, 58, 95]. The new agents are expensive, but the annual costs must be weighed against the personal and social expense of joint arthroplasty, hospitalization, disability, and diminished quality of life that accompanies poorly controlled RA. We need further studies to allow efficacious and cost-effective drugs to be used to prevent the long-term complications of uncontrolled RA. Even with the newer biological agents, MTX continues to serve as a reference point and, in some cases, adjuvant therapy. There is still a role for MTX in the treatment of RA patients.

References:

1. Aletaha D, Smolen JS: DMARD use in early rheumatoid arthritis. Lessons from observations in patients with established disease. Clin Exp Rheumatol, 2003, 21, Suppl 31, 169–173.
2. Aletaha D, Stamm T, Kapral T, Eberl G, Grisar J, Machold KP, Smolen JS: Survival and effectiveness of leflunomide compared with methotrexate and sulfasalazine in rheumatoid arthritis: a matched observational study. Ann Rheum Dis, 2003, 10, 944–951.
3. Andersen PA, West SG, O'Dell JR, Via CS, Claypool RG, Kotzin BL: Weekly pulse methotrexate in rheuma-

toid arthritis. Clinical and immunologic effects in a randomized, double-blind study. Ann Intern Med, 1985, 103, 489–496.
4. Bao C, Chen S, Gu Y, Lao Z, Ni L, Yu Q, Xu J et al.: Leflunomide, a new disease-modifying drug for treating active rheumatoid arthritis in methotrexate-controlled phase II clinical trial. Chin Med J, 2003, 8, 1228–1234.
5. Bannwarth B, Labat L, Moride Y, Schaeverbeke T: Methotrexate in rheumatoid arthritis. An update. Drugs, 1994, 47, 25–50.
6. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, Genovese MC et al.: A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med, 2000, 343, 1586–1593.
7. Battistone MJ, Wiliams HJ: Disease-modyfying antirheumatic drugs 3: methotrexate. In: Rheumaology. 3rd edn. Ed. Hochberg MC, Silman A, Smolen J, Weinblatt ME, Weisman M, Mosby, Elsevier Limited; 2003, 417–429.
8. Blumenauer B, Cranney A, Clinch J, Tugwell P: Quality of life in patients with rheumatoid arthritis: which drugs might make a difference? Pharmacoeconomics, 2003, 13, 927–940.
9. Bressolle F, Bologna C, Kinowski JM, Arcos B, Sany J, Combe B: Total and free methotrexate pharmacokinetics in elderly patients with rheumatoid arthritis. A comparison with young patients. J Rheumatol, 1997, 24, 1903–1909.
10. Calguneri M, Pay S, Caliskaner Z, Apras S, Kiraz S, Ertenli I, Cobankara V: Combination therapy versus monotherapy for the treatment of patients with rheumatoid arthritis. Clin Exp Rheum, 1999, 17, 699–704.
11. Carmichael SJ, Beal J, Day RO, Tett SE: Combination therapy with methotrexate and hydroxychloroquine for rheumatoid arthritis increases exposure to methotrexate. J Rheumatol, 2002, 10, 2077–2083.
12. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F: Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet, 2002, 6, 1173–1177.
13. Cohen S, Cannon GW, Schiff M, Weaver A, Fox R, Olsen N, Furst D et al: Two-year, blinded, randomized, controlled trial of treatment of active rheumatoid arthritis with leflunomid compared with methotrexate. Arthritis Rheum, 2001, 44, 1984–1992.
14. Cohen S, Hurd E, Cush J, Schiff M, Weinblatt ME, Moreland LW, Kremer J et al.: Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum, 2002, 3, 614–624.
15. Cohen SB, Moreland LW, Cush JJ, Greenwald MW, Block S, Shergy WJ, Hanrahan PS et al.: A multicenter double-blind randomized placebo-controlled trial of Kineret® (anakinra), a recombinant interleukin 1 receptor antagonist, in patients with rheumatoid arthritis treated with background methotrexate therapy. Ann Rheum Dis, 2004, 63, 1062–1068.
16. Constantin A, Loubet-Lescoulié P, Lambert N, Yassine-Diab B, Abbal M, Mazieres B, de Preval C et al.: Antiinflammatory and immunoregulatory action of methotrexate in the treatment of rheumatoid arthritis. Arthritis Rheum, 1998, 41, 48–57.
17. Cronstein BN: Going with the flow: methotrexate, adenosine, and blood flow. Ann Rheum Dis, 2006, 65, 421–422.
18. Cutolo M, Bisso A, Sulli A, Felli L, Briata M, Pizzorni C, Villaggio B: Antiproliferative and antiinflammatory effects of methotrexate on cultured differentiating myeloid monocytic cells (THP-1) but not on synovial macrophages from patients with rheumatoid arthritis. J Rheumatol, 2000, 27, 2551–2557.
19. Cutolo M, Sulli A, Pizzorni C, Seriolo B, Straub RH: Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis. Ann Rheum Dis, 2001, 60, 729–735.
20. Dolhain RJ, Tak PP, Dijkmans BA, De Kuiper P, Breedveld FC, Miltenburg AM: Methotrexate reduces inflammatory cell numbers, expression of monokines and of adhesion molecules in synovial tissue of patients with rheumatoid arthritis. Br J Rheumatol, 1998, 37, 502–508.
21. Donehower RC, Hande KR, Drake JC, Chabner BA: Presence of 2,4-diamino-N10-methylpteroic acid after high-dose methotrexate. Clin Pharmacol Ther, 1979, 26, 63–72.
22. Dougados M, Combe B, Cantagrel A, Goupille P, Olive P, Schattenkirchner M, Meusser S et al.: Combination therapy in early rheumatoid arthritis: a randomised, controlled, double blind 52 week clinical trial of sulphasalazine and methotrexate compared with the single components. Ann Rheum Dis, 1999, 58, 220–225.
23. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T: Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med, 2004, 17, 2572–2581.
24. Elliott JR, Paulsen GA, Mallek JA, Mikuls TR, Weaver CA, Glickstein S et al.: Treatment of early sero-positive rheumatoid arthritis: doxycycline with methotrexate versus methotrexate alone. RA: Randomized Clinical Trials. Program and Abstracts of the American College of Rheumatology 67th Annual Scientific Meeting; October 23–28, 2003, Orlando, Florida. Abstract S1708.
25. Emery P, Breedveld FC, Lemmel EM, Kaltwasser JP, Dawes PT, Gomor B, Van Den Bosch F et al.: A comparison of the efficacy and safety of leflunomid and methotrexate for the treatment of rheumatoid arthritis. Rheumatology, 2000, 39, 655–665.
26. Endresen GK, Husby G: Folate supplementation during methotrexate treatment of patients with rheumatoid arthritis. An update and proposals for guidelines. Scand J Rheumatol, 2001, 303, 129–134.
27. Felson DT, Anderson JJ, Meenan RF: Use of short-term efficacy/toxicity tradeoffs to select second-line drugs in rheumatoid arthritis. A metaanalysis of published clinical trials. Arthritis Rheum, 1992, 35, 1117–1125.
28. Ferraccioli GF, Gremese E, Tomietto P, Favret G, Damato R, Di Poi E: Analysis of improvements, full responses, remission and toxicity in rheumatoid patients treated with step-up combination therapy (methotrexate, cyclosporin A, sulphasalazine) or monotherapy for three years. Rheumatology, 2002, 8, 892–898.

29. Fiehn C, Wunder A, Krienke S, Max R, Ho AD, Moehler T: Lack of evidence for inhibition of angiogenesis as a central mechanism of the antiarthritic effect of methotrexate. Rheumatol Int, 2005, 25, 108–113.
30. Firestein GS, Paine MM, Boyle DL: Mechanisms of methotrexate action in rheumatoid arthritis. Selective decrease in synovial collagenase gene expression. Arthritis Rheum, 1994, 37, 193–200.
31. Fox RI, Morgan SL, Smith HT, Robbins BA, Choc MG, Baggott JE: Combined oral cyclosporine and methotrexate therapy in patients with rheumatoid arthritis elevates methotrexate levels and reduces 7-hydroxymethotrexate levels when compared with methotrexate alone. Rheumatology, 2003, 8, 989–994.
32. Furst DE: Rational use of disease-modifying antirheumatic drugs. Drugs, 1990, 39, 19–37.
33. Genestier L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP: Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. J Clin Invest, 1998, 102, 322–328.
34. Genestier L, Paillot R, Quemeneur L, Izeradjene K, Revillard JP: Mechanisms of action of methotrexate. Immunopharmacology, 2000, 47, 247–257.
35. Genovese MC, Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC et al.: Etanercept versus methotrexate in patients with early rheumatoid arthritis: two-year radiographic and clinical outcomes. Arthritis Rheum, 2002, 6, 1443–1450.
36. Genovese MC, Cohen S, Moreland L, Lium D, Robbins S, Newmark R, Bekker P: Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. Arthritis Rheum, 2004, 5, 1412–1419.
37. Gerards AH, Landewe RB, Prins AP, Bruijn GA, Goei The HS, Laan RF, Dijkmans BA: Cyclosporin A monotherapy versus cyclosporin A and methotrexate combination therapy in patients with early rheumatoid arthritis: a double blind randomised placebo controlled trial. Ann Rheum Dis, 2003, 4, 291–296.
38. Grennan DM, Gray J, Loudon J, Fear S: Methotrexate and early postoperative complications in patients with rheumatoid arthritis undergoing elective orthopedic surgery. Ann Rheum Dis, 2001, 60, 214–217.
39. Haagsma DJ, Vanriel PLC, Vandeputte LBA: Combining sulphasalasine and methotrexate in rheumatoid arthritis: early clinical impressions. Br J Rheumatol, 1995, 34, S2, 104–108.
40. Hamdy H, McKendry RJR, Mierins E, Liver JA: Low dose methotrexate compared with azathioprine in the treatment of rheumatoid arthritis. A twenty-four-week controlled clinical trial. Arthritis Rheum, 1987, 30, 361–368.
41. Hamilton RA, Kremer JM: Why intramuscular methotrexate works better than oral drug in patients with rheumatoid arthritis. Br J Rheumatol, 1997, 36, 86–90.
42. Hawkes JS, Cleland LG, Proudman SM, James MJ: The effect of methotrexate on ex vivo lipooxygenase metabolism in neutrophils from patients with rheumatoid arthritis. J Rheumatol, 1994, 21, 55–58.
43. Hoekstra M, van Ede AE, Haagsma CJ, van de Laar MA, Huizinga TW, Kruijsen MW, Laan RF: Factors associated with toxicity, final dose, and efficacy of methotrexate in patients with rheumatoid arthritis. Ann Rheum Dis, 2003, 62, 423–426.
44. Hoekstra M, Haagsma C, Neef C, Proost J, Knuif A, van de Laar M: Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. J Rheumatol, 2004, 31, 645–648.
45. Hoffmeister RT: Methotrexate therapy in rheumatoid arthritis, abstracted. Arthritis Rheum, 1972, 15, 114.
46. Hurst S, Kallan MJ, Wolfe FJ, Fries JF, Albert DA: Methotrexate, hydroxychloroquine, and intramuscular gold in rheumatoid arthritis: relative area under the curve effectiveness and sequence effects. J Rheumatol, 2002, 8, 1639–1645.
47. Imperato AK, Smiles S, Abramson SB: Long-term risks associated with biologic response modifiers used in rheumatic diseases. Curr Opin Rheumatol, 2004, 3, 199–205.
48. Jeurissen MEC, Boerbooms AMT, van de Putte LBA, Doesburg WH, Mulder J, Rasker JJ, Kruijsen MW et al.: Methotrexate versus azathioprine in treatment of rheumatoid arthritis: a forty-eight week randomized, double-blind trial. Arthritis Rheum, 1991, 34, 961–972.
49. Kamen BA, Wang MT, Streckfuss AJ, Peryea X, Anderson RG: Delivery of folates to the cytoplasm of MA104 cells is mediated by a surface receptor that recycles. J Biol Chem, 1988, 263, 13602–13609.
50. Karim A, Tolbert DS, Hunt TL, Hubbard RC, Harper KM, Geis GS: Celecoxib, a specific COX-2 inhibitor, has no significant effect on methotrexate pharmacokinetics in patients with rheumatoid arthritis. J Rheumatol, 1999, 26, 2539–2543.
51. Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS, Fischkoff SA et al.: Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. Arthritis Rheum, 2004, 5, 1400–1411.
52. Khanna D, Park GS, Paulus HE, Simpson KM, Elashoff D, Cohen SB, Emery P et al.: Reduction of the efficacy of methotrexate by the use of folic acid: *post hoc* analysis from two randomized controlled studies. Arthritis Rheum, 2005, 52, 3030–3038.
53. Kinder AJ, Hassell AB, Brand J, Brownfield A, Grove M, Shadforth MF: The treatment of inflammatory arthritis with methotrexate in clinical practice: treatment duration and incidence of adverse drug reactions. Rheumatology, 2005, 44, 61–66.
54. Klareskog L, van der Heijde D, de Jager JP, Gough A, Kalden J, Malaise M, Martin Mola E et al.: Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet, 2004, 28, 675–681.
55. Kobayashi T, Okamoto K, Kobata T, Hasumana T, Nishioka K: Apomodulation as a novel therapeutic concept for the regulation of apoptosis in rheumatoid synoviocytes. Cur Opin Rheumatol, 1999, 11, 188–193.
56. Kraan MC, de Koster BM, Elferink JG, Post WJ, Breedveld FC, Tak PP: Inhibition of neutrophil migration soon

after initiation of treatment with leflunomide or methotrexate in patients with rheumatoid arthritis: findings in a prospective, randomized, double-blind clinical trial in fifteen patients. Arthritis Rheum, 2000, 43, 1488–1495.
57. Kraan MC, Reece RJ, Barg EC, Smeets TJ, Farnell J, Rosenburg R, Veale DJ et al.: Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis. Findings in a prospective, randomized, double-blind, parallel-design clinical trial in thirty-nine patients at two centers. Arthritis Rheum, 2000, 43, 1820–1830.
58. Kremer JM: Rational use of new and existing disease-modifying agents in rheumatoid arthritis. Ann Intern Med, 2001, 134, 695–706.
59. Kremer JM: The mechanism of action of methotrexate in rheumatoid arthritis: the search continues. J Rheumatol, 1994, 21, 1–5.
60. Kremer JM, Alarcon GS, Lightfoot RW Jr, Willkens RF, Furst DE, Williams HJ, Dent PB et al.: Methotrexate for rheumatoid arthritis. Suggested guidelines for monitoring liver toxicity. Arthritis Rheum, 1994, 37, 316–328.
61. Kremer JM, Galivan J, Streckfuss A, Kamen B: Methotrexate metabolism analysis in blood and liver of rheumatoid arthritis patients: association with hepatic folate deficiency and formation of pyroglutamate. Arthritis Rheum, 1986, 29, 832–835.
62. Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, Russell A et al.: Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med, 2003, 13, 349, 1907–1915.
63. Lafforgue P, Monjanel-Mouterde S, Durand A, Catalin J, Acquaviva PC: Lack of correlation between pharmacokinetics and efficacy of low dose methotrexate in patients with rheumatoid arthritis. J Rheumatol, 1995, 5, 844–849.
64. Lambert CM, Sandhu S, Lochhead A, Hurst NP, McRorie E, Dhillon V: Dose escalation of parenteral methotrexate in active rheumatoid arthritis that has been unresponsive to conventional doses of methotrexate: a randomized, controlled trial. Arthritis Rheum, 2004, 2, 364–371.
65. Landewe RB, Boers M, Verhoeven AC, Westhovens R, van de Laar MA, Markusse HM, van Denderen JC et al.: COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis Rheum, 2002, 2, 347–356.
66. Lehman AJ, Esdaile JM, Klinkhoff AV, Grant E, Fitzgerald A, Canvin J, METGO Study Group: A 48-week, randomized, double-blind, double-observer, placebo-controlled multicenter trial of combination methotrexate and intramuscular gold therapy in rheumatoid arthritis: results of the METGO study. Arthritis Rheum, 2005, 52, 1360–1370.
67. Leroux JL, Damon M, Chavis C, Crastes de Paulet A, Blotman F: Effects of a single dose of methotrexate on 5- and 12-lipoxygenase products in patients with rheumatoid arthritis. J Rheumatol, 1992, 19, 863–866.
68. Maillefert JF, Combe B, Goupille P, Cantagrel A, Dougados M. Long term structural effects of combination therapy in patients with early rheumatoid arthritis: five year follow up of a prospective double blind controlled study. Ann Rheum Dis, 2003, 62, 764–766.
69. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Furst D, Weisman MH, St Clair EW: Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. Arthritis Rheum, 2004, 4,1051–1065.
70. Mandel MA: The synergistic effect of salicylates on methotrexate toxicity. Plast Reconstr Surg, 1976, 57, 733–777.
71. Mello SB, Barros DM, Silva ASF, Laurindo IM, Novaes GS: Methotrexate as a preferential cyclooxygenase 2 inhibitor in whole blood of patients with rheumatoid arthritis. Rheumatology, 2000, 39, 533–536.
72. Menninger H, Herborn G, Sander O, Blechschmidt J, Rau R: A 36 month comparative trial of methotrexate and gold sodium thiomalate in the treatment of early active and erosive rheumatoid arthritis. Br J Rheumatol, 1998, 37, 1060–1068.
73. Miller LC, Dempsey C, Schaller JG, Dinerello CA: Methotrexate increases production of interleukin 2. Cytokine Res, 1988, 7, 735.
74. Miller LC, Dinerello CA: Interleukin 1β is structurally related to dihydrofolate reductase: Effects of methotrexate on IL-1. Cytokine Res, 1988, 7, 1–49.
75. Morassut P, Goldstein R, Cyr M, Karsh J, McKendry RJR: Gold sodium thiomalate compared to low dose methotrexate in the treatment of rheumatoid arthritis. A randomized, double blind 26-week trial. J Rheumatol, 1989, 16, 302–306.
76. Morgan SI, Baggot JE, Vaughn WH, Austin JS, Veitch TA, Lee JY, Koopman WJ et al.: Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis: a double-blind, placebo-controlled trial. Ann Intern Med, 1994, 191, 833–841.
77. Mountz JD, Zhou T, Su X, Wu J, Cheng J: The role of programmed cell death as an emerging new concept for the pathogenesis of autoimmune diseases. Clin Immunol Immunopathol, 1996, 80, 2–14.
78. Mottonen T, Hannonen P, Leirisalo-Repo M, Nissila M, Kautiainen H, Korpela M, Laasonen L et al.: Comparison of combination therapy with single-drug therapy in early rheumatoid arthritis: a randomised trial. FIN-RACo trial group. Lancet, 1999, 353, 1568–1573.
79. O'Dell JR, Haire CE, Erikson N, Drymalski W, Palmer W, Eckhoff PJ, Garwood V et al.: Treatment of rheumatoid arthritis with methotrexate alone, sulfasalazine and hydroxychloroquine, or a combination of all three medications. N Engl J Med, 1996, 334, 1287–1291.
80. O'Dell JR, Leff R, Paulsen G, Haire C, Mallek J, Eckhoff PJ, Fernandez A et al.: Treatment of rheumatoid arthritis with methotrexate and hydroxychloroquine, methotrexate and sulfasalazine, or a combination of the three medications: results of a two-year, randomized, double-blind, placebo-controlled trial. Arthritis Rheum, 2002, 5, 1164–1170.
81. Olsen EA, Durham MD: The pharmacology of methotrexate. J Am Acad Dermatol, 1991, 25, 306–318.

82. Ortendahl M, Holmes T, Schettler JD, Fries JF: The methotrexate therapeutic response in rheumatoid arthritis. J Rheumatol, 2002, 10, 2084–2091.
83. Pandya S, Aggarwal A, Misra R: Methotrexate twice weekly vs once weekly in rheumatoid arthritis: a pilot double-blind, controlled study. Rheumatol Int, 2002, 22, 1–4.
84. Papadopoulos NG, Alamanos Y, Papadopoulos IA, Tsifetaki N, Voulgari PV, Drosos AA: Disease modifying antirheumatic drugs in early rheumatoid arthritis: a longterm observational study. J Rheumatol, 2002, 2, 261–266.
85. Paulus HE: Protocol development for combination therapy with disease-modifying antirheumatic drugs. Semin Arthritis Rheum, 1993, 23, S1, 19–25.
86. Pincus T, Brooks RH, Kim YW et al.: Methotrexate continued over 5 years significantly longer than other second-line drugs in 536 private practice rheumatoid arthritis patients. Arthritis Rheum, 1990, 33, S9, 40.
87. Proudman SM, Conaghan PG, Richardson C, Griffiths B, Green MJ, McGonagle D, Wakefield RJ et al.: Treatment of poor-prognosis early rheumatoid arthritis: a randomized study of treatment with methotrexate, cyclosporin A, and intraarticular corticosteroids compared with sulfasalazine alone. Arthritis Rheum, 2000, 43, 1809–1819.
88. Puolakka K, Kautiainen H, Mottonen T, Hannonen P, Korpela M, Julkunen H, Luukkainen R: Impact of initial aggressive drug treatment with a combination of disease-modifying antirheumatic drugs on the development of work disability in early rheumatoid arthritis: a five-year randomized followup trial. Arthritis Rheum, 2004, 1, 55–62.
89. Rau R, Herborn G, Menninger H, Sangha O: Progression in early erosive rheumatoid arthritis: 12-month results from a randomized controlled trial comparing methotrexate and gold sodium thiomalate. Br J Rheumatol, 1998, 37, 1220–1226.
90. Rau R, Herborn G, Menninger H, Sangha O: Radiographic outcome after three years of patients with early erosive rheumatoid arthritis treated with intramuscular methotrexate or parenteral gold. Extension of a one-year double-blind study in 174 patients. Rheumatology, 2002, 41, 196–204.
91. Riksen NP, Barrera P, van den Broek PH, van Riel PL, Smits P, Rongen GA: Methotrexate modulates the kinetics of adenosine in humans *in vivo*. Ann Rheum Dis, 2006, 65, 465–470.
92. Ronney RW, Furst DE, Koehnke R, Burmeister L.: Aspirin is not associated with more toxicity than other NSAID in patients with RA treated with methotrexate. J Rheumatol, 1993, 20, 1297–1302.
93. Sanmarti R, Gomez A, Ercilla G, Gratacos J, Larrosa M, Suris X, Vinas O et al.: Radiological progression in early rheumatoid arthritis after DMARDS: a one-year followup study in a clinical setting. Rheumatology, 2003, 9, 1044–1049.
94. Sany J, Anaya JM, Canovas F, Combe B, Jorgensen C, Saker S, Thaury MN et al.: Influence of methotrexate on the frequency of postoperative infectious complications in patients with rheumatoid arthritis. J Rheumatol, 1993, 20, 1129–1132.
95. Saravanan V, Hamilton J: Advances in the treatment of rheumatoid arthritis: old versus new therapies. Expert Opin Pharmacother, 2002, 7, 845–856.
96. Sarzi-Puttini P, D'Ingianna E, Fumagalli M, Scarpellini M, Fiorini T, Cherie-Ligniere EL, Panni B et al.: An open, randomized comparison study of cyclosporine A, cyclosporine A + methotrexate and cyclosporine A + hydroxychloroquine in the treatment of early severe rheumatoid arthritis. Rheumatol Int, 2005, 25, 15–22.
97. Schiff M, Keystone E, Cush J, McCarthy M, Kaye H, Frundlich B et al.: Discontinuation of DMARD therapy in patients with rheumatoid arthritis enrolled in RADIUS 1-rates, reason, and predictors of early drug withdrawal. RA-Treatment II. Program and Abstracts of the American College of Rheumatology 67th Annual Scientific Meeting; October 23–28, 2003, Orlando, Florida. Abstract S1708.
98. Segal R, Yaron M, Tartakovsky B: Methotrexate: mechanism of action in rheumatoid arthritis. Semin Arthritis Rheum, 1990, 20, 190–199.
99. Seitz M: Molecular and cellular effects of methotrexate. Curr Opin Rheumatol, 1999, 11, 226–232.
100. Seitz M, Loetscher P, Dewald B, Towbin H, Rordorf C, Gallati H, Baggiolini M, Gerber NJ: Methotrexate action in rheumatoid arthritis: stimulation of cytokine inhibitor and inhibition of chemokine production by peripheral blood mononuclear cells. Br J Rheumatol, 1995, 34, 602–609.
101. Seitz M, Villinger PM: Dosage and mode of administration of methotrexate in clinical trials: comment on the article by Genovese et al. Arthritis Rheum, 2003, 48, 863.
102. Sierra EE, Briggle KE, Spinella MJ, Goldman ID: pH dependence of methotrexate transport by the reduced folate carrier and the folate receptor in L1210 leukemia cells. Further evidence for a third route mediated at low pH. Biochem Pharmacol, 1997, 53, 223–231.
103. Slot O: Changes in plasma homocysteine in arthritis patients starting treatment with low-dose methotrexate subsequently supplemented with folic acid. Scand J Rheumatol, 2001, 30, 305–307.
104. Songsiridej N, Furst DE: Methotrexate – the rapidly acting drug. Bailliere's Clin Rheumatol, 1990, 4, 575–593.
105. Strand V, Cohen S, Schiff M, Weaver A, Fleischmann R, Cannon G, Fox R et al.: Treatment of active rheumatoid arthritis with leflunomid compared with placebo and methotrexate. Arch Intern Med, 1999, 159, 2542–2550.
106. St Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible T, Kavanaugh A, Keystone EC: The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum, 2002, 46, 1451–1459.
107. Suarez-Almazor ME, Russell AS: Parenteral methotrexate or gold for rheumatoid arthritis: a follow up. Clin Exp Rheumatol, 1990, 8, 163–166.
108. Swierkot J, Miedzybrodzki R, Szymaniec S, Szechinski J: Activation dependent apoptosis of peripheral blood mononuclear cells from patients with rheumatoid arthritis treated with methotrexate. Ann Rheum Dis, 2004, 63, 599–600.
109. Tishler M, Caspi D, Yaron M. Methotrexate treatment of rheumatoid arthritis: is a fortnightly maintenance schedule enough? Ann Rheum Dis, 1992, 51, 1330–1331.

110. Tugwell P, Pincus T, Yocum D, Stein M, Gluck O, Kraag G, McKendry R et al.: Combination therapy with cyclosporine and methotrexate in severe rheumatoid arthritis N Engl J Med, 1995, 333, 137–142.
111. Van Ede AE, Laan RFJM, Rood MJ, Huizinga TW, van de Laar MA, van Denderen CJ, Westgeest TA et al.: Effect of folic or folinic acid supplementation on the toxicity and efficacy of methotrexate in rheumatoid arthritis: a forty-eight week, multicenter, randomized, double-blind, placebo-controlled study. Arthritis Rheum, 2001, 44, 1515–1524.
112. Vergne P, Liagre B, Bertin P: Methotrexate and cyclooxygenase metabolism in cultured human rheumatoid synoviocytes J Rheumatol, 1998, 25, 433–440.
113. Yamasaki E, Soma Y, Kawa Y, Mizoguchi M: Methotrexate inhibits proliferation and regulation of the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 by cultured human umbilical vein endothelial cells. Br J Dermatol, 2003, 149, 30–38.
114. Yazici Y, Erkan D, Harrison MJ, Nikolov NP, Paget SA: Methotrexate use in rheumatoid arthritis is associated with few clinically significant liver function test abnormalities. Clin Exp Rheumatol, 2005, 23, 517–520.
115. Wascher TC, Hermann J, Brezinschek HP, Brezinschek R, Wilders-Truschnig M, Reiner F, Krejs GJ: Cell-type specific response of peripheral blood lymphocytes to methotrexate in the treatment of rheumatoid arthritis. Clin Invest, 1994, 72, 535–540.
116. Weinblatt ME: Methotrexate. In: Kelley's Textbook of Rheumatology, Eds. Ruddy, Harris, Sledge. 6 th Ed.W.B. Saunders Company, Philadelphia, 2002, 841–852.
117. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, Teoh LA: Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. Arthritis Rheum, 2003, 1, 35–45.
118. Weinblatt ME, Trentham DE, Fraser PA, Holdsworth DE, Falchuk KR, Weissman BN, Coblyn JS: Long-term prospective trial of low-dose methotrexate in rheumatoid arthritis. Arthritis Rheum, 1988, 31, 167–175.
119. Whittle SL, Hughes RA: Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review. Rheumatology, 2004, 3, 267–271.
120. Wiela-Hojenska A. Orzechowska-Juzwenko K, Swierkot J, Wiland P, Hurkacz M, Szechinski J: Monitoring methotrexate therapy in patients with rheumatoid arthritis. Int J Clin Pharmacol Ther, 2004, 42, 434–441.
121. Williams HJ, Willkens RF, Samuelson CO, Alarcon GS, Guttadauria M, Yarboro C, Polisson RP et al.: Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis. A controlled clinical trial. Arthritis Rheum, 1985, 28, 721–730.
122. Willkens RF, Sharp JT, Stablein D, Marks C, Wortmann R: Comparison of azatioprine and methotrexate, and the combination of the two in the treatment of rheumatoid arthritis. A forty-eight-week controlled clinical trial with radiologic outcome assessment. Arthritis Rheum, 1995, 12, 1799–1806.
123. Wolfe F, Hawley DJ, Cathey MA: Termination of slow acting antirheumatic therapy in rheumatoid arthritis: A 14-yaer prospective evaluation of 1017 consecutive starts. J Rheumatol, 1990, 17, 994–1002.
124. Zeidler H, Wollenhaupt J: Combined basic therapeutic drugs. From individual hope to targeted use. Z Rheumatol, 2001, 60, 477–480.

Received:
June 2, 2005; in revised form: May 19, 2006.