Inhibition of cytokine production by methotrexate. Studies in healthy volunteers and patients with rheumatoid arthritis

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Chapter 9

Summary
Rheumatoid arthritis (RA) is a chronic autoimmune disease, characterised by inflammatory reactions in the joints. In the Netherlands, 1-2% of the population suffers from RA and females are affected more often than males.

There are several cells that play a role in the inflammatory process. Synovial fibroblasts and macrophages in the joints are probably responsible for damage to these joints. Their actions can be coordinated by T-cells. The cells of the immune system communicate through interaction with molecular structures on the cell surface and by the production of cytokines. Cytokines are small proteins with a multitude of functions. Each cytokine has several functions, and several cytokines have similar functions. Some cytokines can stimulate cells to grow, to divide or to produce cytokines. Other cytokines inhibit cell growth and cell division. Predominantly pro-inflammatory cytokines are detected in the inflamed joints of RA patients.

Methorexate (MTX) is often used for treatment of RA patients. MTX was developed as an anti-cancer drug and has been used by RA patients, in low doses, since the eighties. Although clinically most patients respond positively to MTX, a lot of them discontinue treatment due to side effects. Some patients do not respond to MTX therapy.

MTX is a folate antagonist. The folate metabolism is important for many processes in the cell, such as methionine metabolism and purine and pyrimidine synthesis, necessary for synthesis of DNA and RNA. MTX's in RA patients is not completely known, although there are several theories. MTX can induce adenosine, which has an anti-inflammatory effect, especially on macrophages. Cronstein thinks the induction of adenosine is the main mechanism of MTX's working mechanism. It has been suggested that the T-cell is important for MTX to be effective, because MTX seems to induce apoptosis in T-cells, or, as others observe, halts T-cell growth.

Experiments described in this thesis were performed to increase our knowledge of the working mechanism of MTX. A better insight is important for the improvement of efficacy of MTX treatment and for the reduction of side effects.

A general introduction (chapter 1) is followed by a description of the in vitro effects of MTX in whole blood (chapter 2). MTX inhibits the production of all cytokines that are produced after stimulation of cells with anti-CD3/anti-CD28, with the exception of the production of interleukin (IL) -8. The inhibition caused by MTX can be arrested if folic acid is added. MTX will not inhibit the production of cytokine if it is stimulated with bacterial products (SAC and LPS). We will show that the usage of MTX by RA patients causes an inhibition of their cytokine production. Evidence that this inhibition is caused by MTX is based on the observation that cytokine production is continued after folic acid is added to the cell culture.

Chapter 3 studies the mechanism of inhibition of cytokine production by MTX. MTX is compared to mycophenolic acid (MPA), a medicine that is able to inhibit DNA synthesis. Like MTX, MPA inhibits the production of cytokine in whole blood after stimulation with anti-CD3/anti-CD28. MPA inhibits the production of cytokine slightly better than MTX does and, unlike MTX, it also inhibits IL-8 production. Inhibition of cytokine production by MPA is more rapid than inhibition by MTX. MTX inhibits cytokine by inducing apoptosis in the proliferating cells. MPA does not induce apoptosis, but stops the progression of the cell cycle and thus halts proliferation (and cytokine production).
As a result of these findings, we think that MTX kills proliferating T-cells and consequently, has an immunosuppressive effect. To investigate whether MTX works by increasing adenosine levels, we showed that MTX, in vitro, works differently than an adenosine analogue (cAdo) in chapter 4. Unlike MTX, cAdo inhibits IL-8 production. IL-8 is created by monocytes and probably by granulocytes, not by T-cells. cAdo also differs from MTX in its feature to inhibit cytokine production after LPS stimulation.

While comparing studies on MTX and MPA, we deduced that MPA had an effect on the production of monokines. This was further investigated in chapter 5. MPA indeed inhibits the production of monokines IL-6, IL-8 and TNF, but stimulates the production of IL-1β. A higher expression of IL-1β mRNA in the presence of MPA is not the cause of this stimulation. Because MPA decreases prolL-1β levels in the cells, it seems that MPA stimulates conversion from prolL-1β to IL-1β. If the induction of IL-1β is present in vivo, and whether this has clinical consequences, needs to be examined further.

A new method for detecting MTX and bioactive MTX in serum is described in chapter 6. This method is more sensitive than current popular methods and is easy to perform in immunological/rheumatological laboratories, without any special equipment.

For treatment of RA patients it is important to assess the efficacy of treatment at an early stage. In chapter 7 we correlated the in vitro effects of MTX with the clinical response of 34 RA patients. Blood samples were taken from these patients just before (t=0) and two hours (t=2) after they had taken MTX for the first time. The concentration of MTX was determined in t=2 samples. The t=0 samples were also used to determine the in vitro sensitivity of MTX. Cytokine production after T-cell stimulation was determined in each sample. The production of cytokine was significantly lower after usage of MTX than before. Unfortunately, in vitro sensitivity, cytokine production (before and after usage of MTX) and the plasma MTX concentration were not predictive in to the clinical response on MTX treatment. Because the clinical response was measured after 12 weeks, clinical improvement was low in this patient group. However, a negative relationship was found between the amount of inhibition of cytokine production after 2 hours and the decrease of swollen joints (SJC). This means that patients who show little inhibition of cytokine production at t=2 have a larger decrease of swollen joints. Nevertheless, this relationship is weak and possibly dependent on one single patient with a good clinical response and a higher cytokine production at t=2. More patients should be tested in order to verify this relationship. If true, we could explain such a relationship by stating that MTX also inhibits anti-inflammatory cytokines such as IL-10.

Chapter 8 of this thesis is a general discussion of the results presented in this thesis. Both MTX and MPA inhibit cytokine production of T-cells, although through different mechanisms. Unlike MTX, MPA has an effect on monocytes. We found a significant inhibition of LPS-induced and SAC-induced cytokine production and a stimulation of LPS-induced IL-1β production by MPA.

In the discussion, we have tried to establish what would be the mechanism of action of MTX when used to treat RA patients. Results of the in vitro tests suggest that the T-cell is the main target of MTX. However, this idea might not be correct since there is no relation between the in vitro and ex vivo effects of MTX on the T-cell cytokine production of RA patients and their clinical responses. Hence, we need to look for cells and cytokines that may be sensitive to MTX, as well as pharmacological-dynamic parameters that may be related to the variable clinical responses of RA patients.