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Inhibition of interleukin-8 synthesis by intraarticular methotrexate therapy in patientes with rheumatoid arthritis

Hemmung der Interleukin-8 Synthese durch intraartikuläre Methotrexat Therapie bei Patienten mit rheumatoider Arthritis

Summary 5 Patients with definite RA and knee effusions under constant doses of DMARD therapy were treated with up to 6 intraarticular injections of 10 mg methotrexate (MTX) every 3 to 7 days. A matched randomized control group who received a single i.a. injection of 40 mg triamcinolone hexacetonide (TC) was monitored according to the same protocol. The intraarticular granulocyte counts and IL-8 levels decreased in all MTX treated patients on day 10–13 and stayed low in those patients who could be re-evaluated after 13 weeks. Compared to the IL-8 levels, the other tested cytokine levels showed only minor changes on day 10–13. There was no need for re-injection in the TC group during the 13 week study phase.

We conclude that intraarticular MTX therapy results in a strong decrease of SF-granulocyte counts. This effect may be due to the impairment of IL-8 mediated chemotaxis by decreased IL-8 synthesis in synovial fluid mononuclear cells. Clinically, repeated intraarticular MTX therapy results in a worse 13 week outcome than i.a. steroid treatment measured in an intention-to-treat analysis.

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Zusammenfassung In einer Pilotstudie wurden die Effekte von intraartikulär verabreichtem Methotrexat (MTX) im Vergelich zu Corticosteroid untersucht. Voruntersuchungen anderer Arbeitsgruppen hatten gezeigt, daß steroidrefraktäre Gonarthritiden erfolgreich mit MTX i.a. behandelt werden konnten.

Patienten mit Gonarthritis bei rheumatoider Arthritis unter konstanter Basistherapie erhielten 4 bis 6 Kniepunktionen jeweils im Abstand von 3–7 Tagen mit Instillation von jeweils 10 mg MTX. Eine randomisierte Kontrollgruppe von 5 Patienten erhielt eine einzige intraartikuläre Injektion von 40 mg Triamcinolon Hexacetonid (TC) und wurde nach demselben Protokoll beobachtet. Die Granulozytenzahl in der Synovialflüssigkeit (SF) fiel am Tag 10–13 bei allen MTX-Patienten drastisch ab und blieb bei einzelnen nach 13 Wochen re-punktierten Patienten niedrig. Hierzu konkordant verhielt sich der IL-8-Spiegel in der SF. TNF-α, IL-6, IL-10 und IL-12 blieben hingegen relativ konstant. Für die TC-Patienten ergab sich keine Indikation zur Repunktion während der 13 Wochen langen Studienphase.

Daraus läßt sich folgern, daß die intraartikuläre MTX-Therapie einen Abfall der SF-Leukozyten bewirkt, der möglicherweise auf einem Abfall der IL-8 vermittelten Granulozyten-Chemotaxis durch verminderte IL-8 Synthese aus mononukleären Zellen in der Synovialflüssigkeit beruht. Klinisch scheint intraartikuläre MTX-Therapie nach 13 Wochen eine schlechtere Entzündungshemmung im Knie als eine intraartikuläre Kortikoidtherapie zu bewirken.

Key words Rheumatoid arthritis – methotrexate – intraarticular injection – IL-8

Schlüsselwörter Rheumatoide Arthritis – Methotrexat – intraartikuläre Injektion – IL-8

Introduction

Several inflammatory cytokines play a role in the pathogenesis and the clinical course of rheumatoid arthritis (RA) (1, 10, 11). Cytokine levels (TNF-α, IL-1, IL-6, IL-8) are measured in higher concentration in the synovial fluid compared to sera RA patients.

Although the antiproliferative effect of methotrexate (MTX) in doses used in tumor therapy can be explained as dihydrofolate reductase inhibition, the mode of action in low dose pulse therapy is not yet clear (13, 21, 23). A modulation of the cytokine production in the synovium is discussed (for review see 8) but no study in humans about the influence of intraarticular MTX therapy on cytokine levels has been reported.

In the clinical setting corticosteroids seem to be the most often used intraarticular medication (6). Clinical reports using MTX are rare and show controversial results (5, 12, 15, 19). The aim of this study was to further elucidate the effects of intraarticular MTX therapy on the biosynthesis of several inflammatory cytokines having pathophysiologic influence in RA.

Methods

Patient selection

10 patients with definite rheumatoid arthritis (ACR-criteria 1987 (2)) were randomly treated according to the following protocol:

- Gonarthritis for at least 3 months with clinical and sonographic finding of effusion
- radiological stage ≤2 according to Larsen (16) (to exclude severe osteoarthritis)
- DMARD therapy and constant doses of systemic steroids
- age older than 18 years
- informed consent

Excluded were all patients with anticoagulatory treatment and any bleeding condition.

A control group of 5 patients meeting the same criteria and receiving intraarticular corticosteroid treatment was recruited to be monitored according to the same protocol.

Treatment protocol

A knee effusion was aspirated to exhaustion and 10 mg MTX/2 ml volume was injected. This procedure was repeated every 3 to 7 days up to 5 times depending on the development of new effusions. A final re-evaluation was scheduled after week 13 (day 91±7) and aspiration/injection was performed if necessary.

The control group received a single intraarticular injection of 40 mg triamcinolone hexacetonide (TC).

Assessment

Synovial fluids (SF) were analyzed for leukocyte counts, differentials, glucose, protein, LDH, and cytokine levels of tumor necrosis factor-alpha (TNF-α) (detection limit 10 pg/ml), interleukin 6 (IL-6) (15.6 pg/ml), Il-8 (15.6 pg/ml), IL-10 (5 pg/ml), and IL-12 (7.8 pg/ml). (The detection limits are given in brackets). Additionally the ESR and CRP values were determined to monitor the overall disease activity. To evaluate the clinical outcome, clinical findings were recorded on day 0, day 10–13, and day 91±7.

For evaluation of systemic disease activity and its influence on the local therapeutic result, a 28-joint count (14) and morning stiffness were assessed. Local inflammatory activity of the knee was assessed by the following clinical variables: joint circumference, pain-free range of joint movement, and subjective pain in the knee (scored on a 100 mm visual-analogue-scale on which 0 indicated no pain and 100 mm (=100%) indicated hardly bearable pain). Clinical evaluation was assessed on a 5-grade scale (between "much worse" and "much better" – see Table 2) comparing to baseline evaluation both by the doctor and the patient.

Cytokine measurements

IL-6, IL-8, and IL-12 assays were purchased from R&D-Systems (Wiesbaden, FRG) and performed according to the manufacturer's manual. TNF-α and IL-10 were measured with ELISAs obtained from Immundiagnostik, (Bensheim, FRG).

Statistical analysis

Due to the small sample size no statistical comparison of clinical or humoral disease activity was planned between the MTX and the TC control group. Statview software was used for regression analysis between ESR or intraarticular leukocyte counts and each determined single cytokine level.

Results

5 Patients (2 of them rheumatoid factor-positive) were randomly recruited for intraarticular MTX treatment, all of whom were female with a median age of 60.4 years (range 52–65) and a median disease duration of 10.2 years (range 23 months–19 years). The patients were all

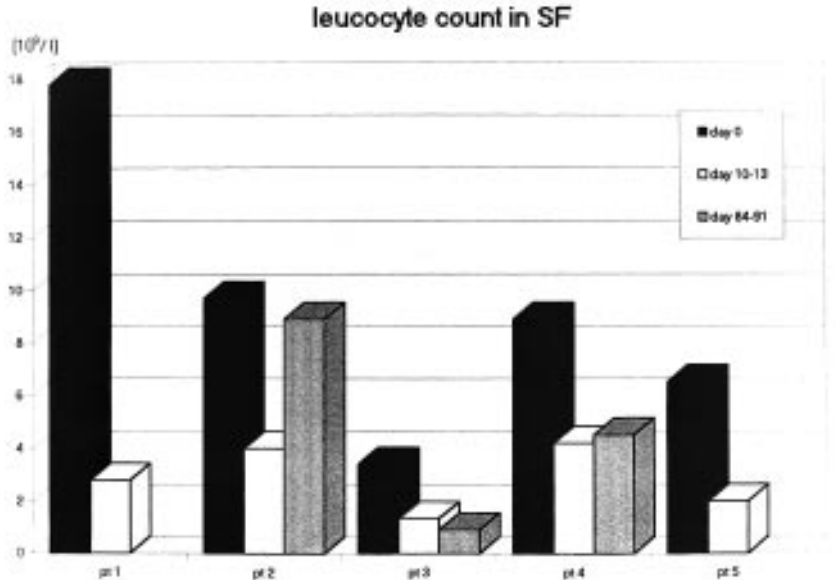

Fig. 1 Decrease of SF-leukocyte counts in 5 patients receiving repeated intraarticular MTX administration

Table 1 Synovial fluid analysis and systemic humoral inflammation activity (ESR, CRP)

Patient	Day	Leukocyte count (10^9/l)	Granulocyte (%)	Glucose (mg/dl)	Protein (g/dl)	LDH (U/l)	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)	IL-12 (pg/ml)	TNF-alpha (pg/ml)	ESR (mm/h)	CRP (mg/l)
pt 1													
	0	17.80	52	79	4.05	362	24	3852	ND	6.6	ND	7	ND
	13	2.80	8	75	2.79	137	73	316	ND	13.4	ND	3	ND
pt 2													
	0	9.70	48	75	3.70	248	516	88759	168	5.0	73	32	44.5
	10	4.00	12	92	2.76	150	435	4461	70	5.3	<1	31	30.5
	84	8.90	19	68	4.67	296	1838	20438	206	ND	235	33	43.0
pt 3													
	0	3.40	41	97	2.52	204	737	25637	93	5.9	ND	42	98.3
	10	1.35	9	99	2.16	146	761	1557	31	6.3	ND	54	89.8
	95	0.90	40	94	1.68	137	107	172	8	ND	31	12	9.6
pt 4													
	0	8.90	68	86	4.96	664	1893	96153	151	ND	79	22	49.4
	11	4.20	22				1964	56082	109	ND	76	22	29.2
	96	4.50	21				20	184	9	ND	208		
pt 5													
	0	6.50	32	103	4.49	215	1000	26282	36	ND	50	9	2.7
	13	2.00	26	85	3.82	96	530	1236	7	ND	21	5	2.5
TC 1	0	32.00	90	48	4.36	630	1621	24270	114	7.1	ND	25	28.7
TC 2	0	1.00	37	80	2.14	175	1988	18409	76	ND	345	18	20.9
TC 3	0	12.20	56	80	3.67	625	844	28512	111	5.8	69	52	108.1
TC 4	0	15.70	47	78	4.55	538	1779	79649	74	4.3	89	18	14.7
TC 5	0	19.50	51	99	4.00	526	1378	99988	102	8.7	204	36	85.0

TC = pt. received triamcinolone hexacetonide; ND = not done

treated with disease-modifying antirheumatic drugs (DMARDs): 2 with methotrexate, 2 with gold salts, and 1 with sulfasalazine. The median dose of concomitant prednisolone was 7 mg (0–10 mg). A matched control group comprised 5 females, two of them being RF-positive, with a median age of 61.7 years (range 56–69), a median disease duration of 15 years, and DMARD therapy with MTX in 2 patients. Three patients were not treated with DMARDs. The median dose of concomitant prednisolone was 7.5 mg (0–10 mg).

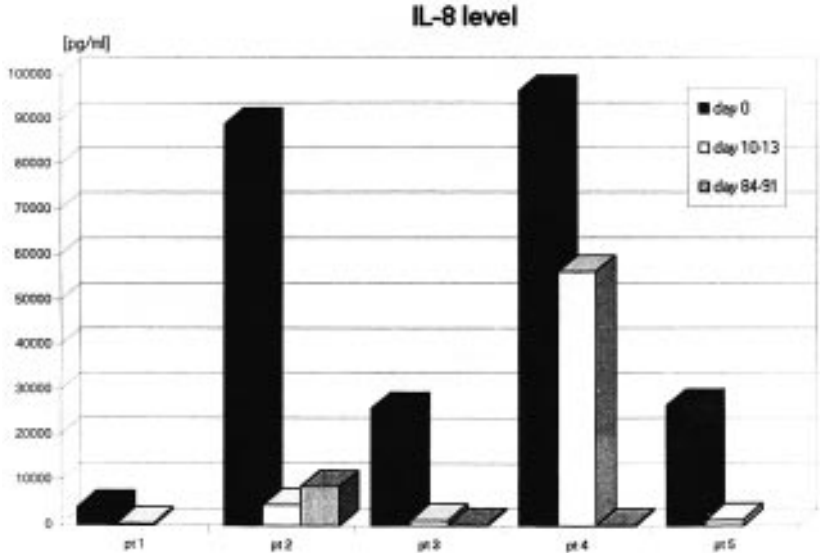

Fig. 2 Decrease of SF-IL-8 levels in 5 patients receiving repeated intraarticular MTX administration

Table 2 Clinical parameters before and during intraarticular MTX treatment

Patient	Day	Joint count	Morn stiff (min)	Knee circ (cm)	Pain free range (%)	Pain scale rest (%)	Pain scale move m (%)	Evaluation*	
								Doctor	Patient
pt 1	0	16	0	46	130	0	47		
	13	14	0	43	130	0	75	’0	’–
pt 2	0	60	120	35	130	88	88		
	10	65	180	34	110	50	50	’+	’+
	84	59	90	35	130	ND	ND	’0	’0
pt 3	0	36	20	40	120	20	50		
	10	39	15	41	120	50	80	’0	’–
	95	39	10	40	125	25	55	ND	’+
pt 4	0	33	60	39	90	50	55		
	10	23	60	38	110	15	15	’+	’0
	96	17	30	37	115	20	60	’+	’+
pt 5	0	13	0	36	110	20	50		
	13	5	0	36	125	0	20	’+	’–

ND = not done
* Evaluation: ’– – = much worse; ’– = worse; ’0 = unchanged; ’+ = better; ’++ = much better

All 5 patients were clinically and according to ultrasound studies eligible for continued intraarticular MTX treatment up to day 10–13; 3 patients continued according to the protocol and could be evaluated after 13 weeks. The matched control group of 5 patients was treated with TC only on day 0 because no further effusion appeared up to week 13. MTX related toxicity was not observed. In the MTX group, SF-leukocyte counts decreased strongly on day 10–14 (Fig. 1). This was mainly due to a decrease of granulocytes (Table 1). There were no relevant changes in SF-glucose. At least a transitory decrease of SF-protein and -LDH on day 10–14 was observed (Table 1).

The same decrease as for the granulocytes was observed for intraarticular IL-8 levels on day 10–13 (Fig. 2). The decrease was more than 90% of the initial value in 3 of 5 patients. IL-8 levels stayed low in those patients, who could be re-evaluated after 12 weeks.

The other synovial cytokines showed variable effects following treatment (Table 1). None of the levels decreased one log or more on day 10–14. IL-6 levels increased in 3 out of 5 patients during the initial period (day 0 to day 10–13). Although IL-10 levels decreased in all 4 patients tested for this parameter, only in 1 patient did it decrease to 20% of the baseline value. Intraarticular IL-12-levels were obtained in 3 patients without displaying a decrease during the initial period. TNF-α levels showed inconsistent kinetics.

Except for the drop in the CRP-level while ESR kept constant in one patient (pt. 4) from day 0 to day

10–13, both parameters showed only minor changes. The same is true for both systemic and local inflammatory clinical parameters (Table 2). Regression analysis between ESR and IL-6, ESR and IL-8, IL-6 and IL-8, IL-6 and SF-leukocyte count, and IL-8 and SF-leukocyte counts displayed no significant correlation.

Discussion

A number of clinical studies have been published to evaluate the effects of intraarticular treatment with various substances in rheumatoid arthritis (3, 7, 9, 20), while none of them report studies about cytokine kinetics. This is the first in vivo study investigating intraarticular cytokine synthesis during intraarticular methotrexate treatment.

IL-8 secretion of cultured synovial cells could not be reduced by MTX in vitro (18, 22). Principally, in vitro inhibition of IL-8 production by MTX is possible as demonstrated for peripheral blood mononuclear cells (24).

In this study, frequent intraarticular MTX injections were performed. A decrease in IL-8 levels in the synovial fluid in vivo was achieved. The properties of IL-8 as a chemotactic factor explain the simultaneous decrease in granulocyte counts although other cytokines and chemokines which were not examined may also play a role in the decrease of synovial fluid cells.

The effect of therapy was very different on the other measured cytokines, which did not show the same decreasing effects as with IL-8. In contrast to the literature, IL-8 and IL-6 levels did not change in a concordant way (4, 17). IL-10, IL-12, and TNF-α levels did not display an uniform tendency either.

Verburgh et al. (25) examined SF-levels of IL-8 in patients with RA who did not receive intraarticular treatment. He observed that IL-8 could not be related to various serological, clinical or radiological parameters but was a parameter of the activity of the local inflammatory process. Our data confirm that IL-8 is an independent parameter of serological, clinical, and radiological findings by time course observation during the intraarticular MTX-therapy. However in our study, the decrease of IL-8 levels did not seem to be associated with an improvement of the knee measured by clinical parameters.

While Hall et al. (15) reported a striking improvement in 6 out of 8 patients after intraarticular MTX treatment, a combination of 5 mg MTX plus steroid was not superior to steroid alone in a trial of 12 patients (19). Bird et al. (5) came to the same discouraging result comparing intraarticular steroid and MTX treatment. The only more recent positive evaluation of intraarticular MTX treatment in humans came from case reports by Dürk et al. (12). However, in their study group of patients with inflammatory joint disease, only one patient with RA was involved. The lack of clinical effect on synovitis is well explained by the short joint fluid elimination half life of MTX, which is relatively short comparted to the cell cycle generation time (26). In a recent study in rats, the articular clearance rate was successfully prolonged by a liposomal preparation of MTX (27). Using no special preparation, these findings may account for the apparently small clinical impact and biological effect on various cytokines which we achieved, but does not explain the drop of IL-8 levels we observed.

This study was not planned to be a clinically controlled trial, and the evaluation of clinical parameters was not an aim of this study. Since the sample size of patients in each group was too small to meet statistical standards, we only point out the continued intention to treat in all MTX patients in contrast to the TC patients over a period of 13 weeks.

References

1. Alvaro-Garcia JM, Zvaifler NJ, Brown CB, Kaushansky L, Firestein GS (1991) Cytokines in chronic inflammatory arthritis. VI. Analysis of the synovial cells involved in granulocyte-macrophage colony stimulating factor production and gene expression in rheumatoid arthritis and its regulation by IL-1 and TNF-alpha. J Immunol 146:3365–3371
2. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324
3. Bagge E, Geijer M, Tarkowski A (1996) Intra-articular administration of polyclonal immunoglobulin G in rheumatoid arthritis: A double blind, placebo-controlled pilot study. Scand J Rheumatol 25:174–176
4. Berazzolo N, Punzi L, Stefani MP, Cesaro G, Pianon M, Finco B, Todesco S (1994) Interrelationships between interleukin (IL)-1, IL-6 and IL-8 in synovial fluid of various arthropathies. Agents-Actions 41:90–92
5. Bird HA, Ring EF, Daniel R et al. (1982) Comparison of intra-articular methotrexate with intra-articular triamcinolone hexacetonide by thermography. Curr Med Res Opin 5:141
6. Caldwell JR (1996) Intra-articular corticosteroids. Guide to selection and indications for use. Drugs 52:507–514
7. Caruso I, Boccassini L, Cazzola M, Santandrea S, Montrone F, Dell'Acqua D, Salvaggio A (1992) Multiple intra-articular treatment of rheumatoid arthritis: A randomized prospective study comparing rifamycin SV with pefloxacin. J Int Med Res 20:27–39
8. Cronstein BN (1996) Molecular therapeutics. Methotrexate and its mechanism of action. Arthritis Rheum 39:1951–1960
9. Cuchacovich M, Wurgaft R, Mena MA, Valenzuela C, Gatica H, Tschernitchin AN (1991) Intraarticular progesterone inhibits (3)H-dexamethasone binding to synovial cells from patients with rheumatoid arthritis. A study by dry radioautographic technique. J Rheumatol 18:962–967

10. Dolhain RJEM, van der Haiden AN, ter Haar NT, Breedveld FC, Miltenburg AMM (1996) Shift toward T Lymphocytes with a T helper 1 Cytokine-secretion profile in the joints of patients with rheumatoid arthritis. Arthritis Rheum 39:1961–1969
11. Duff GW (1994) Cytokines and acute phase proteins in rheumatoid arthritis. Scand J Rheumatol Suppl 100:9–19
12. Dürk H, Kötter I, Saal JG (1994) Intraarticular methotrexate (MTX) therapy in corticosteroid resistant monarthritis. Arthritis Rheum 37, 9 (Suppl):554a
13. Firestein GS, Paine MM, Boyle DL (1994) Mechanism of methotrexate action in rheumatoid arthritis. Arthritis Rheum 37:193–200
14. Fuchs HA, Brooks RH, Callahan LF, Pincus T (1989) A simplified twenty-eight-joint quantitative articular index in rheumatoid arthritis. Arthritis Rheum 32:531–537
15. Hall GH, Head AC (1975) Intra-articular methotrexate (letter). Lancet 2:409
16. Larsen A, Dale K, Eek K (1977) Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol Diagn 20:481–490
17. van Leewen MA, Westr J, Limvurg PC, van Riel PL, van Rijswijk MH (1995) Interleukin-6 in relation to other proinflammatory cytokines, chemotactic activity and neutrophil activation in rheumatoid synovial fluid. Ann Rheum Dis 54:33–38
18. Loetscher P, Dewald B, Baggiolini M, Seitz M (1994) Monocyte chemoattractant protein 1 and interleukin 8 production by rheumatoid synoviocytes. Effects of antirheumatic drugs. Cytokine 6:162–170
19. Marks JS, Stewart IM, Hunter JA (1976) Intra-articular methotrexate in rheumatoid arthritis (letter). Lancet 2:857
20. Menninger H, Reinhardt S, Söndgen W (1994) Intra-articular treatment of rheumatoid knee-joint effusion with triamcinolone hexacetonide versus sodium morrhuate. Scand J Rheumatol 23:249–254
21. Olsen NJ, Murray LM (1989) Antiproliferative effects of methotrexate on peripheral blood on mononuclear cells. Arthritis Rheum 32:178–385
22. Schmidt J, Fleissner S, Heimann-Weitschat I, Lindstaedt R, Pomberg B, Werner U, Szelenyi I (1994) Effect of corticosteroids, cyclosporin A, and methotrexate on cytokine release from monocytes and T-cell subsets. Immunopharmacology 27:173–179
23. Segal R, Mozes E, Yaron M, Tartakovsky B (1989) The effects of methotrexate on the production and activity of interleukin-1. Arthritis Rheum 32:370–377
24. Seitz M, Dewald B, Ceska M, Gerber N, Baggiolini M (1992) Interleukin-8 in inflammatory rheumatic diseases: synovial fluid levels, relation to rheumatoid factors, production by mononuclear cells, and effects of gold sodium thiomalate and methotrexate. Rheumatol-Int 12:159–164
25. Verburgh CY, Hart MH, Aarden LA, Swaak AJ (1993) Interleukin-8 in synovial fluid of rheumatoid and nonrheumatoid joint effusions. Clin Rheumatol 12:494–499
26. Wigginton SM, Chu BCF, Weisman MH, Howell SB (1980) Methotrexate pharmacokinetics after intraarticular injection in patients with rheumatoid arthritis. Arthritis Rheum 23:119–122
27. Williams AS, Camilleri JP, Goodfellow RM, Williams BD (1996) A single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis. Br J Rheum 35:719–724