Methotrexate: from its introduction to non-oncologic therapeutics to anti-TNF-α

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ABSTRACT

The history of the rheumatologic use of methotrexate until the 1990s will be reviewed, beginning with its pharmacology, with the focus on rheumatoid arthritis (RA). The insufficient availability of cortisone in the 1950s as well as the early recognition of its potential toxicity stimulated searches for alternative anti-inflammatory drugs. Two related derivatives of folic acid, aminopterin and amethopterin (MTX,) were found to give rapid symptomatic relief in cases of psoriasis vulgaris and psoriatic arthritis. For several years MTX was used primarily to treat psoriasis, and the dermatologic treatment protocols came to be used by rheumatologists. Giving MTX weekly rather than daily was found to diminish the risk of toxic effects. MTX became favoured over cyclophosphamide because of its lack of carcinogenicity, and although azathioprine lacked the hepatotoxicity of MTX, its anti-rheumatic effects were considered to be somewhat weaker. Although trials of MTX for the treatment of severe RA began in the 1960s, the first placebo-controlled study of MTX in RA was reported in 1985 and a comparison with Myochrysine in 1987. MTX has replaced gold compounds because it has been found to be more rapidly effective and better tolerated. The mechanisms of its anti-rheumatic effects remain incompletely explained, as are explanations of instances of its failure. Its recent use in combination with anti-TNFα agents appears to be another therapeutic advance.

Introduction

Methotrexate® (Lederle, MTX) entered clinical medicine as an innovative anti-neoplastic drug in 1948. This article traces its adaptation first to non-neoplastic proliferative dermatoses, mainly psoriasis, and to its competitive introduction into rheumatologic therapeutics. Although MTX has come to be used in the treatment of many of these diseases, this review is limited to rheumatoid arthritis (RA): How did it become favoured over other agents in the treatment of progressive RA, how has the risk-benefit ratio of MTX been evaluated, and how did the enthusiasm for the use of MTX as the principal drug in the treatment of progressive RA wane in favour of its combination with other categories of powerful immunosuppressive drugs.

Pharmacology of methotrexate

Pteroyl-glutamic acid was isolated in 1941 from leafy vegetables and named folic (foliage) acid. It has different co-enzymatic functions depending on the position on the pteroyl ring at which a particular radical is attached. The pharmaceutical pteroyl-glutamic acid is not an active co-enzyme. To become metabolically active, folic acid is reduced to tetra-hydro folic acid by dihydrofolate reductase. Methotrexate (MTX) differs structurally from folic acid at two sites: an amino is replaced by a hydroxyl at one site and a methyl group replaces an amino at another. Its effects result from inhibition of the reductase. At higher oncologic dosages, MTX also inhibits thymidylate synthetase, and this indirectly interferes with DNA synthesis. MTX is oxidised to 7-hydroxy-MTX by aldehyde reductase and primarily has renal excretion. Thus, the action of MTX is prolonged by renal failure (1). About 40% of tritium-labelled MTX is excreted unchanged in the urine within 48 hours after intravenous administration. Thereafter, one to two percent per day is excreted for several weeks, largely as cleavage products of MTX (2). A fluorimetric method to determine MTX concentrations in blood was published in 1958 (3). Two hours after administration, the MTX concentration in blood is the same whether it has been taken oral-

ly or by intra-muscular injection and is usually immeasurable after 24 hours.(4) A more sensitive radio-immunoassay became available in 1975 (5).

It is likely that the early research on the mechanism of the MTX effect, focused on epidermal cell metabolism in psoriasis, has little relevance to the explanation of its anti-rheumatic effects (6). In addition to retarding the rate of epidermal cell division, MTX inhibits granulocyte activity, depresses B-lymphocytes, but not T-lymphocytes; it does not inhibit the synthesis or secretion of the pro-inflammatory interleukin-1 (IL-1), although it may inhibit some parameters of IL-1 activity (7). Alarcón *et al*. (1990, Birmingham, AL) reported the interesting observation that MTX reduces the concentration of both IgA and IgM rheumatoid factor, while clinical improvement correlates only with reduction of IgM RF (8). The precise mechanisms of its anti-rheumatic effects remain uncertain. Intra-articular injection of MTX is relatively ineffective compared to corticosteroids in acutely counteracting synovitis (9, 10).

Circumstances of the introduction of methotrexate in non-oncologic therapeutics

In December 1950, Philip S. Hench (1894-1965) in his Nobel Prize lecture stated referring to corticosteroids: "But how these hormones accomplish, for example, their anti-rheumatic effect is still quite unknown…. Current opinion is that the hormones act at the tissue, or cell, level. But this tentative conclusion must be the subject of much further study" (11). A Swiss symposium "On the Influence of the Hypophysis and the Adrenal Cortex on Biological Reactions" in 1951 contained 31 papers, of which only one pertained to "rheumatism," and this not to cortisone (12). Thus the immediate demand for synthetic cortisone to treat a plethora of conditions that had in common only inflammation and/or pain had no basis in patho-physiologic understanding. In the early 1950s the supply of cortisone still was insufficient to meet the demand, and its toxic effects were beginning to be recognised. Several gold compounds that were recognised to act slowly and have a substantial potential toxicity had been standard therapy for progressive rheumatoid arthritis (RA) since the 1930s. Thus, there were stimuli to search for potential alternative anti-inflammatory medications.

Jimenez Diaz *et al*. (1951, Madrid) suspected that nitrogen mustard "might well be useful" in the treatment of RA based on the tenuous analogy that corticotrophin, cortisone and nitrogen mustard all cause lymphopenia. It was administered in four 6mg doses to nine patients, of whom five "improved extraordinarily" (13). This admittedly preliminary report stimulated a flurry of interest. Whether the varied short-term results should be attributed to different treatment courses is unclear. By far the largest experience with nitrogen mustard therapy of RA was that of Arthur L. Scherbel at the Cleveland Clinic (14). He employed it as IV solo therapy in 1951 for 17 patients, but then used it in combination with ACTH in the next five years. "In 88 per cent of 263 patients there was rapid and complete or almost complete relief of joint manifestations, toxicity and fever." Despite Scherbel's enthusiasm, this therapeutic approach had vanished by 1960.

However, in the hope of more durable effects, derivatives of nitrogen mustard came to be employed in connective tissue disease therapy. Even before the introduction of these "alkylating agents" into oncologic therapy, aminopterin, an anti-metabolic analogue of the vitamin, folic acid, began in 1948 to be used experimentally in the treatment of childhood leukaemia. One of its effects is to interfere with the proliferation of connective tissue. Therein, although by a different mechanism, it resembles the action of corticosteroid drugs. Based on this consideration, Richard Gubner, a New York cardiologist, in 1951 administered aminopterin to several patients with psoriasis, psoriatic arthritis, and RA. Improvement in both cutaneous and articular symptoms occurred, usually by the second week of therapy, although complicated by various signs of toxicity (15, 16). Therefore, this drug was not soon evaluated further as an anti-rheumatic agent. Seven years later Edmondson and Guy (Pittsburgh dermatologists) treated 32 psoriatic patients with aminopterin, and several subsequently with MTX (17). Response to both drugs was favourable and similar.

The largest early (1963) comparative trial included 91 psoriasis patients treated with aminopterin and 36 with MTX. Aminopterin was more effective, but elicited more frequent, mainly gastro-intestinal, signs of toxicity. No hepatic function tests were reported (18).

Corticosteroid research was continuing simultaneously. Triamcinolone, orally or topically, was shown in 1958 to be superior to previous non-fluorinated steroids in affecting psoriatic lesions (19). However, also in 1958, the possibility of using an anti-metabolite was revived at the National Institutes of Health (NIH) with a clinical study of MTX in cases of psoriatic arthropathy and RA. Patients with psoriatic arthropathy had a more favourable response of their arthritis than those with RA (20). This investigation then was modified into a double-blind study of 21 cases of psoriatic arthropathy (20 rheumatoid factors negative). Cutaneous and articular improvement persisted throughout treatment, regardless of its duration, but relapses occurred within weeks of discontinuance (21). The 1964 report of this investigation appears to have been particularly influential in stimulating interest in MTX for the treatment of psoriatic arthropathy, and equivocally of RA. However, interest in MTX therapy developed much more rapidly among dermatologists than rheumatologists, perhaps because its dermatologic action could be better defined.

In the 1960s MTX was administered to treat psoriasis either with 2.5mg or 5.0mg per day, usually for 5 consecutive days per week, or a weekly oral or I.M. dose of 25mg. or 50mg (22). In 1971 Weinstein (Miami, FL) introduced a weekly oral schedule of three doses in 24 hours once per week, beginning with 2.5mg per dose and gradually increasing to 5mg per dose (23). The rationale for this was to obtain a maximum effect on the accelerated cell cycle of psoriatic lesions and to diminish toxicity. It became standard practice.

Methotrexate for rheumatoid arthritis

When immunosuppressive drugs became more widely tried in non-neoplastic diseases they were conceived of as primary therapy for psoriasis and other proliferative dermatoses, but in rheumatology rather to permit lower, less toxic doses of corticosteroids. Gross *et al*. (Zurich) gave this as the reason to search for drugs that will permit such dosages to be reduced or eliminated (24). They were encouraged to undertake a trial with RA patients by personal communications with Peter A. Miescher (then in Lausanne, Switzerland), who had treated SLE patients with 6-mercapto-purine, azathioprine, or MTX, first published in 1965 (25). The initial treatment was 50mg IV weekly for six weeks. Response frequently was considered insufficient, so that some patients received MTX for 20 weeks. The number of the 35 patients who also received oral 6-MP or azathioprine is unclear. One leukopenic MTX patient died of systemic candidiasis. This patient had received four 50mg injections followed by several injections of 6-MP before thirteen 25mg injections of MTX. The longest MTX treatment lasted 187 days, with a total dose of 925mg. Anorexia was more common with MTX, and stomatitis occurred only with MTX. The trial was considered successful because corticosteroid dosage could be reduced or discontinued in 27 patients. Azathioprine appeared to be less effective than MTX in permitting reduction of corticosteroid dosage.

The chief competitors of MTX were azathioprine and cyclophosphamide, and in Europe, chlorambucil. (26) These, optimistically, came to be added to gold compounds and penicillamine as "disease modifying drugs." Mason *et al*. (1969, London) confirmed the better tolerance of MTX over azathioprine (27). Urowitz *et al*. (1973, Toronto), in a placebo controlled cross-over investigation found that the incidence of intolerance to azathioprine was similar to that of MTX, but that it spares the liver (28). They agreed that it is somewhat less effective. Fosdick *et al*. (1968, Tuscon, AZ), introduced cyclophos-phamide therapy of RA after experience with 38 patients who had received the drug for at least half a year (29). It also enabled reduction of the dose of other ant-rheumatic drugs, but clinical benefit in most cases was associated with a potentially dangerous leukopenia. The first controlled study of cyclophosphamide, while it demonstrated symptomatic benefits, showed that 90% of patients who received 150mg per day suffered toxic symptoms (30). In 1973 Hurd (Dallas, TX) reviewed experimental data pertaining to the anti-inflammatory and immunosuppressive properties of azathioprine, cyclophosphamide and MTX, and concluded that cyclophosphamide is the most potent immuno-suppressant and should be therapeutically superior to the other two drugs, which he considered to be equivalent (31). Evidence of carcinogenic effects, especially on the bladder, accumulated in the late 1980s and led to its abandonment in RA therapeutics (32).

Although MTX has usually been prescribed orally, a trial of intravenous administration of up to 50 mg every two to four weeks was reported in 1982 (33), and the first trial with I.M. administration was reported in 1984 (34). However, MTX regimens for RA patients largely employed Weinstein's psoriasis protocol. Willkens *et al*. (Seattle) in 1971 began to treat patients whose RA had been unresponsive to other medications with 2.5mg MTX for three doses 12 hours apart once per week. If there was no clinical response in four weeks, then the dosage was doubled. Twenty out of 32 patients responded favourably within three months (35). Instead of divided doses, 7.5mg weekly in a single dose, if necessary increased to 10mg or 15mg. subsequently also came to be used. But the question of the risk/benefit proportion remained of concern.

A trial of 500mg/m^2 of MTX IV, followed 24 hours later by Leucovorin (folinic acid, N-formyl-tetrahydrofolic acid) 50mg/m^2 in divided oral doses, was begun at the NIH in 1987. Clinical improvement was obtained without toxic manifestations after four infusions at two week intervals (36). This was still well tolerated when extended to six months, although most improvement occurred during the first three months. RA flared by three months after cessation of this treatment. Subsequently, a 52-week study was carried out in Montreal in which Leucovorin was added double blind to 7.5mg weekly MTX p.o. Side effects were diminished in the Leucovorin cohort without diminished MTX efficacy (37).

Tishler *et al*. (1988, Tel Aviv) had shown that while Leucovorin successfully counteracts symptoms of MTX toxicity, it also blocks its anti-inflammatory action in RA (38). The disparity from Tishler's finding was explained in that Leucovorin should be taken in a smaller dosage and a day after the MTX. Morgan *et al*. (1989, Birmingham, AL) concluded that a sub-normal serum folate concentration predicts the development of MTX toxicity, and they showed that taking as little as one mg of folic acid per day reduces this risk with less cost than Leucovorin (39).

In 1987 the American College of Physicians recommended the dosage scheme that Weinstein had proposed in 1971: 2.5mg q12h for three doses once per week, to be doubled after six weeks if response was unsatisfactory; intra-muscular dosage may be increased from 5mg to 25mg per week. Blood counts and hepatic function tests should be done monthly (40).

Hoffmeister (Spokane, WA, 1983) reported that among his 78 patient cohort (41), 35% were withdrawn because of toxic effects, 69% of these occurring during the first year. None had received more than 15mg MTX per week. Nausea and stomatitis were the most frequent signs of toxicity, but most patients were relieved by a dose reduction.

Furst *et al*. (1989, New Brunswick, NJ) found no clear dose to toxicity relationship between weekly doses of 7.5–10 mg, 15–22.5 mg and 27.5–35 mg, but the findings did suggest better responses with larger doses (42). As was true of many trials, the cohorts were too small to arrive at reliable conclusions.

In 1990 Scully *et al*. (Salt Lake City) published their uniquely designed study of 124 RA patients (41% male).(43) All had begun to take MTX no later than

1983 and were followed for up to five years from the inception of this medication, on which 31% remained after five years. 16% were discontinued because of lack of response to 7.5mg/week for 12 weeks, rather than increasing the dose. This is similar to the 18% ineffectiveness rate Hoffmeister had observed.

Clinical comparisons of anti-rheumatic drugs

As the number of potent immunosuppressive drugs increased, so did the clinical comparisons. The first of these pertained to azathioprine, cyclophosphamide and sodium aurothiomalate (Myochrysine®) over 18 months (44). It appeared in 1974 and found little difference in efficacy between any of these drugs, except that both "showed advantages (over gold) in reducing the rate of joint deterioration as seen radiologically and in steroid sparing" when they were administered in early RA.

Williams *et al*. (Salt Lake City) stated in 1985 that seven investigations of the efficacy of MTX in RA had been published, but none with a control group (45). Their placebo-controlled study of MTX permitted a stable dose of prednisone, or an NSAID to be continued. Therapeutic response was favourable compared to placebo, but this was only a 12-week protocol.

A comparative efficacy study of Myochrysine and MTX was not undertaken before 1985. In this Canadian double blind 26-week study of 40 RA patients, either 50mg. of Myochrysine or 10mg MTX was injected at weekly intervals (46). Of the 20 MTX patients, five had a adverse reaction, resulting in withdrawal of one patient, versus 15 adverse reactions in 11 patients, resulting in seven withdrawals of Myochrysine patients.

While several studies that compared the effects of azathioprine with other drugs followed, the first study that included MTX appeared in1987. Hamdy *et al*. (Ottawa, ONT) compared the efficacy of 100mg. azathioprine per day with 10mg. MTX, orally per week for one year (47). The MTX cohort tended to more rapid clinical improvement, but overall the response to both agents was similar. A Dutch 48-week double blind comparison of 100mg. azathioprine and 7.5mg MTX gave results more strongly in favour of MTX in regard to response of clinical and laboratory parameters and toxicity (48).

Methotrexate toxicity and its evaluation

Since MTX primarily affects rapidly dividing cells, such as in psoriatic lesions, one characteristic of toxicity pertains to tissues in which cell division normally is relatively rapid. Consequently, oral ulcerations and dyspepsia typically are the most frequent early symptoms, and may occur within weeks of initiating therapy. Early dermatologic reports were concerned mainly with possible bone marrow injury and did not test for changes in hepatic function. Kremer and Phelps (Albany, NY) found in an RA cohort that had taken MTX for between 79 and 107 months, adverse reactions continued to occur at a rather constant individually unpredictable rate: for instance, leukopenia after seven years. No adverse occurrence required removal from the cohort, although dosage might be reduced. Neither MTX efficacy nor toxicity could be correlated with HLA haplotypes (49).

Fries *et al*. in a multicentre study collected the most recent determination of SGOT and SGPT from a large number of RA patients who were taking any of 12 relevant drugs.(50) Mean SGOT and SGPT values were highest and essentially the same in those who were taking either aspirin or MTX, but not both. However, in those patients who were taking both drugs SGOT increased by 50% and SGOT more than doubled (15.2%, 16.7% of cases, respectively). Consequently, MTX might be discontinued when it is not the offending drug. Gispen *et al*. (Birmingham, AL, 1986) showed that free MTX blood levels are higher with concomitant NSAID therapy due to displacement of MTX from albumin binding sites (51). It has repeatedly been shown that the predictive value of moderately abnormal hepatic enzyme tests for the presence of hepatic fibrosis is unreliable (22).

The first report of biopsy proven liver injury in a non-leukemic adult was published in 1964 and pertained to a woman who received MTX for psoriatic arthropathy. A liver biopsy nine months after she had been receiving 25–50 mg IM per week showed periportal fibrosis (52). In 1968 three patients (2 psoriasis, 1 psoriatic arthropathy) who had been treated with MTX for 11–40 months had developed cirrhosis.(53) Weinstein *et al*. (Miami, 1970) found fatty metamorphosis in 21 MTX treated psoriatic patients, but they were reluctant to attribute this to MTX rather than chronic alcohol consumption (22). Dahl *et al*. (Newcastle upon Tyne, 1972) performed liver biopsies on 44 patients who did not consume significant amounts of alcohol and were treated with MTX for psoriasis. Seventeen (38%) had hepatic cirrhosis or fibrosis (54). They made the important observation that hepatic injury is more likely when small doses of MTX are taken frequently (2.5mg. several days per week p.o.) than 10–25 mg. p.o. or I.M. q 1–4 weeks. Tolman *et al*. (Salt Lake City, 1985) presumed that the 3%–5% cirrhosis that had been reported in psoriasis was attributable to daily dosage of MTX (55).

Brick *et al*. (Morgantown, WV) found no case of cirrhosis either in seven reports that included liver biopsies of 233 RA patients who had not received MTX, or in 13 studies of liver biopsies in 694 patients performed after varying lengths of MTX therapy (56). However, some degree of hepatic fibrosis has been found in biopsies of 17% of 859 MTX treated RA patients (42). The greatest exposure to MTX has been reported by Aponte and Petrelli (Cleveland), who performed liver biopsies on 26 RA patients who had been taking weekly MTX for a mean of 12.6 years and had a mean cumulative dose in excess of 4.6 gm. No cirrhosis was found even in these patients, although some fatty infiltration was common (57).

Pulmonary fibrosis resulting from interstitial pneumonitis associated with MTX therapy was first reported in diseases other than acute lymphatic leukaemia in 1971, in a case of psoriasis (58), but in RA not until 1983 (59). Weinblatt (Boston) reviewed nine such cases in 1985 (60). All had been taking a weekly oral dose of MTX; in seven

the cumulative dose at pulmonary diagnosis was less than 400mg.

Despite its rapid cellular activity, effects on bone marrow have been rare. According to McKinnon *et al*. (Seattle), as of 1985 only seven instances of leukopenia and three of pancytopenia had been reported, to which they added six cases of pancytopenia, two being fatal.(61) Bone marrow injury appears to be related to renal failure.

Methorexate combination therapy and the introduction of "biologic" agents

Could therapeutic efficacy be improved more safely by combining MTX with another drug than by increasing its dosage? The possibility that combinations of immunosuppressive drugs, in analogy with oncologic therapeutics, might be effective against recalcitrant RA was suggested by McCarty and Carrera (Milwaukee, WI) in 1982 in the first of several articles. This was based on their favourable experience, beginning in 1975, with a combination of cyclophosphamide, azathioprine and hydroxy-chloroquine (62). Trials of MTX and Auranofin® by Williams *et al*.(63), or MTX and azathioprine by Willkens *et al*. both reported in 1992 (64), did not substantiate the superiority of supplementing MTX with these drugs. However, the combination of MTX with cyclosporine has shown therapeutic superiority after six months, without increased toxicity (65).

The introduction of several categories of inhibitors of specific molecular components of inflammation began about a decade after MTX became a principal anti-rheumatic drug. Their development will only be alluded to briefly. The first trial of such an agent was reported from Switzerland in 1987: a monoclonal antibody against CD4 cells (66). The first of a series of monoclonal antibodies against tumour necrosis factor alpha (TNF-α) was reported from London in 1993 (67). In an eight week trial of infliximab (Remicade®, Centocor) 10 RA patients received two infusions and another 10 received four infusions, with identical, favourable immunological and symptomatic results. In 1999 the addition of etanercept (Enbrel®, Pfizer), another TNF-α inhibitor, to MTX therapy was reported (68). Subsequently, it has become common practice in RA patients who have had an unsatisfactory response to MTX to add one of the new synthesised antibody agents to MTX (69).

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