Variation of immunological response in methotrexate-induced pneumonitis

B. Chikura¹, N. Sathi², S. Lane³ and J. K. Dawson²

Objectives. To assess the variation of peripheral blood and bronchoalveolar lavage (BAL) inflammatory cell counts and lung biopsy findings with the degree of exposure to MTX therapy.

Methods. Fifty-six (16 males; 40 females) reported cases of MTX-induced pneumonitis (MTX-P) on low-dose MTX (5–30 mg) were identified from a literature search and classified using Searles and McKendry’s criteria. The median cumulative dose was 300 mg and this was used to categorize patients into low and high MTX-exposure groups and 6 months was used to divide patients into early- and late-onset MTX-P groups.

Results. Neutrophil counts in the peripheral blood and BAL were significantly raised in the low MTX-exposure group compared with the high MTX-exposure group ($P = 0.018$ and 0.038, respectively). There were similar findings when early-onset was compared with late-onset group. Lymphocytes in BAL were significantly higher in the high MTX-exposure group compared with low-dose cumulative group ($P = 0.007$). There were 6 (11%) recorded deaths and all were in the low MTX-exposure group. Early-onset/low MTX-exposure groups had a high prevalence of lung fibrosis.

Conclusions. This is the first study to describe the variation of immunological responses in MTX-P with the degree of exposure to MTX. Our findings suggest that MTX-P can be divided into two groups: type 1 MTX-P that occurs early, predominated by neutrophils, lung fibrosis and has a high mortality; and type 2 MTX-P that occurs late, predominated by lymphocytes, has less lung fibrosis and low mortality.

KEY WORDS: Methotrexate, Pneumonitis, Lung fibrosis, Delayed hypersensitivity, Immunology, Bronchoalveolar lavage, Lung biopsy, Interstitial lung disease.

Introduction

MTX, a dihydrofolate reductase inhibitor, is the most commonly prescribed DMARD in the treatment of RA [1, 2]. MTX-induced pneumonitis (MTX-P) is a serious but rare complication of low-dose MTX treatment. Patients usually present subacutely with cough, fever, shortness of breath and other constitutional symptoms. The role of inflammatory cells in MTX-P remains obscure. Symptoms appear at variable durations of MTX therapy. This large variation in the clinical spectrum brings into question the pathophysiology of MTX-P, in particular whether the duration of MTX-P therapy and cumulative doses has an influence in the clinical expression of the disease and pathophysiological processes. The early-onset/low MTX exposure MTX-P cases may be different from the late-onset/high MTX exposure MTX-P cases pointing to different pathophysologies.

Patients and methods

Fifty-six reported cases (16 males and 40 females) [3–30] satisfying our inclusion criteria were identified from Pubmed (Medline), Proquest, Canahl and Embase databases using the following keywords and phrases: methotrexate, pneumonitis, methotrexate pneumonitis, interstitial lung disease, drug-induced pneumonitis, hypersensitivity pneumonitis and delayed hypersensitivity pneumonitis. Patients were classified using Searles and McKendry’s [25] diagnostic criteria for MTX-P. Fifty (89%) cases were classified as definite MTX-P, four (7%) probable and two (4%) possible MTX-P. Only cases that fulfilled the definite, probable and possible Searles and McKendry’s criteria for MTX-P and were on a low dose, i.e. 5–30 mg of MTX therapy, were included.

Twenty-four cases lacking essential data such as immunological data, cumulative doses and duration of MTX therapy were excluded. The degree of exposure to MTX therapy was measured by the cumulative dose and duration of MTX therapy. The median cumulative dose (300 mg) was used to divide patients into two groups: low MTX-exposure group (cumulative dose of ≤300 mg) and high MTX-exposure group (cumulative dose of >300 mg). Patients were also divided into early-onset MTX-P (<6 months) and late-onset MTX-P (>6 months). Demographic data, diagnoses, previous DMARDs, concomitant steroid use and NSAID use are shown in Table 1. On admission, 54 patients (96%) had shortness of breath, 47 (84%) had a dry cough and 41 (73%) patients were pyrexial. Only 1 (2%) patient had a productive cough. Thirty-one (55%) patients had a chest X-ray (CXR) prior to MTX therapy, of which 18 (58%) had pre-existing lung disease; abnormalities on CXR-included fibrotic changes in 17 patients and blunting of costophrenic angles in one patient. Of the 18 patients who had an abnormal pre-treatment CXR, eight had lung biopsies and only three had fibrosis on histology. All patients had a CXR done during the course of their illness and abnormalities recorded were: bilateral interstitial shadowing in 48 patients, reticular nodular shadowing and ground glass appearances in six patients, blunting of costophrenic angle in one patient and bivalveal consolidation in one patient. Three patients had high-resolution CT (HRCT) and all showed diffuse interstitial infiltrates, and none of these patients had lung fibrosis on histology. Twenty-four (43%) patients had pulmonary function tests (PFTs) done during the course of their illness. ESR was mentioned in 17 (30%) patients and the median ESR was 56 mm/h (range 40–126 mm/h). Lung biopsy was done in 24 (43%) patients and bronchoalveolar lavage (BAL) in 11 (20%) patients. There were six (11%) reported deaths, of whom one was male and five were females. Case fatality rate was comparable in the excluded cases; there were three (12.5%) deaths out of 24 cases. Female patients had a significantly longer duration of symptoms prior to hospital admission with MTX-P ($P = 0.05$) compared with male patients (median 21 days and 14 days, respectively). Forty-nine patients (88%) were treated with steroids, 33 (59%) were treated with antibiotics, 28 (50%) received both steroids and antibiotics and
compared with the late-onset group, this was not statistically significant (P = 0.071), and this could be due lack of power to detect the difference. There were no significant differences in the age distribution, clinical symptoms, pre-existing lung disease, previous DMARD use and concomitant steroid use between the two groups. Although data on PFTs are limited, patients in the low MTX-exposure group had significantly lower diffusion factor for carbon monoxide (DLCO) compared with the high MTX-P-exposure group (P = 0.015).

**Lung biopsy**

Twenty-four patients had lung biopsies done during the course of their illness (15 had transbronchial and nine had open lung biopsies). Early-onset/low MTX-exposure groups had a higher prevalence of lung fibrosis compared with the late-onset/high MTX-exposure groups (Table 4). Late-onset/high MTX-exposure groups had a higher prevalence of lymphocytic infiltration compared with the early-onset/low MTX-exposure groups. Granulomata were more prevalent in the high MTX-exposure group compared with the low MTX-exposure group.
Although there were differences between the groups, these were not statistically significant and this could be due to low power to detect the differences. Reporting of alveolitis or interstitial pneumonitis was comparable between the groups. There were no significant differences in the age distribution, clinical symptoms, pre-existing lung disease, previous DMARD use and duration of symptoms between the two groups. Of the 14 patients who had fibrosis on histological examination, three (21%) had normal CXR, six (43%) had a normal CXR and five (38%) did not have a CXR done prior to commencing MTX. Of the six patients who died, half of them had lung fibrosis on biopsy results.

Discussion

This is the first study to describe the variation of immunological responses in MTX-P with the degree of exposure to MTX. The early-onset/low MTX-exposure cases appear to be dominated by a neutrophilic response and the late-onset/high MTX-exposure by lymphocytic response. Our findings suggest that MTX-P can be divided into two groups: type 1 MTX-P that occurs early (<6 months), dominated by neutrophils, lung fibrosis, low MTX exposure and a high mortality rate; and type 2 MTX-P that occurs late (>6 months), dominated by lymphocytes, has less lung fibrosis, high MTX exposure and low mortality rate. In this study, the case fatality rate was 11% and all of the cases were in the early-onset/low MTX-exposure group. Case fatality rates in MTX-P in previous studies are higher than our finding, estimated to be between 17% and 30% [31–34]. Our finding of a low mortality could be due to case selection and reporting bias. The differences in the total and differential white cell counts are smaller in the peripheral blood compared with the striking differences in BAL differential white cell counts. This is well recognized in other conditions such as sarcoidosis [35, 36] and this is due to localization of immune responses [37].

MTX-P is described as a type IV delayed hypersensitivity pneumonitis dominated by lymphocytic proliferation and alveolitis [38] and is associated with a specific cellular immune response involving the release of cytokines [39]. Anaphylaxis, i.e., immediate hypersensitivity reaction following MTX therapy, has been reported [40]. Schnabel et al. [41] reported BAL lymphocytosis and an increase in the CD4+/CD8+ ratio in MTX-P. Other researchers have reported similar findings [39], although this is not a universal finding [42]. This alteration in the CD4+/CD8+ ratio has been reported in rheumatoid lung as well [43]. CD8 + T cells are involved in antigen recognition on the surface of alveolar cells and can trigger a cascade of an inflammatory processes culminating in interstitial pneumonitis [44].

The role of neutrophils in hypersensitivity pneumonitis is not well described. Our findings suggest that they may play a role in the early-onset/low MTX-exposure cases. Neutrophils have been implicated in the pathogenesis of lung fibrosis [45]. Crestani et al. [46] demonstrated increased levels of cytokines, which promote proliferation of type 2 alveolar cells released by neutrophils in lung fibrosis. Mild neutrophilia can occur in MTX-P [16] and neutrophilia is associated with an increased risk of developing lung fibrosis [47]. The release of neutrophil and eosinophil chemotactic activity in response to MTX by type 2 alveolar cells has been previously described [48] and this results in neutrophil and eosinophil recruitment to the site of inflammation. Neutrophils can cause lung injury by releasing gelatinases and collagenases, which results in tissue damage and lung fibrosis [49]. They also play a role in the recruitment of lymphocytes to the site of inflammation by the release of chemokines that includes IFN-γ [50, 51]. An influx of activated T lymphocytes following neutrophilic pneumonitis in animal models has been demonstrated in the past [52]. Our finding of a neutrophilic predominance in the early-onset/low MTX-exposure group may be the explanation of the higher prevalence of lung fibrosis in that group compared with the late-onset group/high MTX-exposure group. The neutrophilic response in the early-onset/low MTX-exposure group is associated with fibrotic changes and a worse outcome for the patients. This observation has not been previously described in MTX-P. Lin et al. [53] in a study of 33 patients with pulmonary sarcoidosis showed that a neutrophilic response was associated with more severe inflammatory parenchymal lung lesions and fibrotic changes. The reasons for this are obscure and need further investigations by prospective studies. We can only postulate at this stage that there could be a genetic basis for the different immunological responses. The efficacy and toxicity of MTX are determined by different metabolic pathways. Polymorphisms of the methylene tetrahydrofolate reductase (MTHFR) gene may predispose patients to side-effects [54]. Prospective studies are required to investigate this.

There were no significant differences in baseline characteristics that include pre-existing lung disease, concomitant steroid use, age distribution, previous DMARD use and clinical symptoms between the early-onset/low MTX-exposure and the late-onset/high MTX-exposure groups. A true immunological difference between the two groups is therefore most likely the cause of the immunological variations observed between the two groups. We have not found any factors that could help predict the development of either early- or late-onset MTX-P. However, the immunological data are striking and neutrophilia is a poor prognostic marker. There is no clear reason why some patients have a worse outcome than others except for the differences in neutrophil and lymphocyte counts in BAL and lung fibrosis on histology. The pre-treatment CXR abnormalities did not correlate well with histological findings on lung biopsy and this was due to the low specificity and sensitivity for a CXR to detect lung fibrosis. HRCT scanning, which is now widely available, has a higher specificity and sensitivity compared with CXR and we recommend that all patients undergo this test if interstitial lung disease is suspected.

Interestingly, only one patient was reported to be taking folic acid supplements. The role of folic acid deficiency in the pathogenesis of MTX-P has not been previously investigated. Side-effects, such as hepatotoxicity, have been attributed to folic acid deficiency [55, 56]. Folic acid deficiency could be an unrecognized risk factor; particularly in the late-onset cases. The prescribing of folic acid among rheumatologists is highly variable.

The important message to rheumatologists from this study is that early-onset MTX-P is associated with a worse outcome. Patient education before exposure to MTX is required, so that MTX-P symptoms are reported early and aggressive treatment can be commenced. Neutrophilia in peripheral blood or BAL samples, particularly in the absence of infection or corticosteroids should be considered a poor prognostic marker.

Conclusions

The peripheral blood differential white cell counts, BAL inflammatory cell counts and lung biopsy histology reports are influenced by the degree of exposure to MTX. Neutrophils predominate in the early-onset/low MTX-exposure cases and lymphocytes predominate in the late-onset/high MTX-exposure cases. These findings bring into question the pathophysiology of MTX-P, in particular whether there are different mechanisms that

### Table 4. Lung biopsy findings (n = 24)

<table>
<thead>
<tr>
<th></th>
<th>≤26 weeks</th>
<th>&gt;26 weeks</th>
<th>≤300 mg</th>
<th>&gt;300 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=16</td>
<td>n=8</td>
<td>n=16</td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>Fibrosis (%)</td>
<td>11 (65)</td>
<td>3 (43)</td>
<td>10 (63)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Lymphocytic infiltration (%)</td>
<td>4 (24)</td>
<td>5 (75)</td>
<td>4 (24)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Granulomas (%)</td>
<td>4 (25)</td>
<td>2 (25)</td>
<td>3 (19)</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Alveolitis/IP (%)</td>
<td>7 (44)</td>
<td>3 (38)</td>
<td>6 (38)</td>
<td>4 (50)</td>
</tr>
</tbody>
</table>

IP: Interstitial pneumonitis.
depend on the degree of MTX exposure. Prospective studies are required in the future to investigate this.

**Rheumatology key messages**

- Immunological responses in MTX-P vary with the degree of exposure to MTX.
- Early-onset MTX-P is associated with neutrophilia, lung fibrosis and high mortality.
- Late-onset MTX-P is associated with lymphocytosis and a low mortality.

**Disclosure statement:** N.S. has received honoraria from Procter & Gamble, Sanofi-Aventis and Servier in the past for talks to medical professionals on osteoporosis. All other authors have declared no conflicts of interest.

**References**