SEVERE erosive rheumatoid arthritis (RA), producing progressive and serious functional impairment, remains a difficult therapeutic challenge. Cytotoxic disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate (MTX), azathioprine (AZA) and cyclophosphamide (CP), were first used to treat RA in the 1950s [1]. Their use was initially restricted to patients with refractory or life-threatening systemic disease due to concerns about toxicity. In the past decade, a new approach to treating RA has evolved. Rheumatologists now advocate earlier intervention with DMARDs, largely due to the fact that the traditional pyramidal approach to treatment has not been shown to improve clinical, functional or radiographic outcome significantly. MTX is probably the most widely prescribed DMARD in RA and its use is also increasing in other rheumatic diseases. This has been the subject of a recent review in this journal [2]. AZA is still frequently used, but perhaps more often now as a steroid-sparing agent, whereas CP is rarely used to treat uncomplicated RA.

CP and AZA are also used to treat other rheumatic diseases and in particular CP is now currently used by rheumatologists for systemic connective tissue diseases including systemic lupus erythematosus (SLE) and the systemic vasculitides, such as polyarteritis nodosa, Wegener’s granulomatosis, microscopic polyangiitis and Churg–Strauss syndrome. Most published studies on its role in arthritis treatment are from the 1960s and 1970s, but concerns about toxicity and the increasing use of other immunosuppressive agents such as MTX have led to a gradual reduction in the use of oral CP for RA with time. It tends now only to be used in extreme cases. Various protocols concerning the dose and method of administration of CP have been advocated without consensus. These range from continuous oral CP advocated by the NIH group in Wegener’s granulomatosis to pulsed oral and i.v. regimes either weekly, fortnightly or monthly (with or without i.v. steroids) and monthly i.v. CP without steroids to treat SLE. It seems likely from the available evidence that pulsed i.v. CP is as effective as continuous oral CP for producing ‘remission induction’ with possibly fewer side-effects, but a slightly higher risk of later relapse.

CP has also been used to treat vasculitis complicating RA [3, 4]. There are a few reports of its use in some of the more severe systemic complications of RA including pyoderma gangrenosum [5], Felty’s syndrome [6] and the corneal melt syndrome [7]. We have reviewed the literature relating to AZA and CP with respect to treatment in RA. We outline here the pharmacology and toxicity of these drugs, and give some advice for monitoring.

AZATHIOPRINE

Pharmacology

AZA is a cycle-specific antimetabolite which was first synthesized in 1957 and first reported to be of potential use in RA in 1964 [8]. In 1981, it was approved by the US Food and Drug Administration for use in RA. As a purine analogue, AZA derives most of its biological effects from the active ribonucleotide metabolites of its initial metabolite 6-mercaptopurine (6-MP). AZA interferes with the de novo synthesis of inosinic acid via feedback inhibition of 6-thioinosinic acid. It also inhibits the interconversion of purine bases such as inosine to adenine and guanine ribonucleotides. A small amount of 6-MP is also incorporated into RNA and DNA in the form of 6-thioguanine. Most ingested AZA is excreted in the urine in the form of thiouric acid, 10% of the drug is excreted unchanged. Following absorption, AZA is rapidly distributed throughout the body and plasma levels remain low (1 μg/ml). Determination of plasma levels is not useful in clinical practice. AZA has a plasma half-life of 3 h, is moderately protein bound (30%), and both unchanged drug and metabolic products can be dialysed. Diminished action of any of the specific enzymes required for AZA metabolism, either by competition from co-administered drugs (e.g. allopurinol-blocking xanthine oxidase) or through genetic enzyme deficiencies (e.g. Lesch Nyhan syndrome—absent hypoxanthine guanine phosphoribosyltransferase, or thiopurine methyltransferase deficiency due to genetic polymorphism) may greatly increase AZA toxicity and reduce efficacy [9–11].

Efficacy

When considering the efficacy of a DMARD, three fundamental questions need to be answered:

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- Is joint inflammation suppressed—leading to a reduction in pain and stiffness and an improvement in functional ability?
- Is radiological damage slowed or prevented?
- What is the toxicity profile?

Unfortunately, it is extremely difficult to answer many of these questions reliably when considering the efficacy of AZA due to fundamental flaws in study design. These include qualitative methodological deficiencies, inadequate sample size, short duration of follow-up, variation in drug dosage from one study to another, and the fact that the majority of studies were undertaken in patients with established, advanced or refractory disease. These facts must all be taken into consideration when critically reviewing the literature.

**Placebo-controlled studies.** Several placebo-controlled studies have demonstrated that AZA is efficacious in RA [12–18]. These studies are summarized in Table I.

Mason et al. [12] demonstrated the corticosteroid-sparing activity of AZA in a group comparative study against placebo. From an initial mean prednisolone requirement of 11.8 mg, there was a reduction in dosage of 36%, achieved without clinical deterioration, over the course of 9 months. Levy et al. [13] conducted a 12 month double-blind cross-over study of AZA 3 mg/kg/day vs placebo in 18 rheumatoid patients. Eighty-three per cent of AZA-treated patients improved (active joint count, grip strength, duration of early morning stiffness). Urowitz et al. [14] undertook a double-blind cross-over study comparing AZA against placebo in 17 patients who had been unresponsive to conventional anti-rheumatic therapy, giving each treatment for 16 weeks. AZA resulted in a statistically significant improvement in articular index, total active joint count, grip strength and total number of synovial effusions. Erythrocyte sedimentation rate (ESR) and latex titre did not change. Benefit was evident after 6 weeks of treatment; however, the drug produced further benefit between 6 and 16 weeks. Analysis of the same parameters measured after a mean period of 40 months follow-up indicated that AZA continued to exert a disease-suppressive effect [19]. Eleven out of 12 patients either maintained their initial beneficial response or showed further improvement. Four patients discontinued therapy because of lack of efficacy and one because of nausea. Goebel et al. [15] compared AZA 1.5–2.9 mg/kg/day vs placebo in a controlled clinical cross-over study for two 12 week periods. There was a 60% improvement in the joint count index in AZA-treated patients as compared with a 30% deterioration in placebo-treated patients. This was associated with a decrease in immunoglobulin levels; however, rheumatoid factor (RF) levels did not change. De Silva and Hazleman [16] switched half of a group of 32 AZA-treated patients to placebo after a mean period of 6 yr. Withdrawal of AZA had a detrimental effect on disease activity. Similar findings were reported by Cade et al. [20] who followed 16 AZA-treated patients for up to 6 yr, noting subjective improvement in all, with a striking improvement in ability to work. ESRs tended to fall, but not necessarily in parallel with systemic improvement. Positive tests for RF became negative in some patients, and others showed a fall in titre. When treatment was changed to placebo in these patients, a relapse occurred within 8 weeks. There was prompt improvement when AZA was resumed; however, several patients did not reach their previous state of well-being until after 6 or 7 months of treatment. Woodland et al. [17]

### Table I

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of study/no. of patients</th>
<th>Duration of AZA therapy (weeks/months)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mason, 1969 [12]</td>
<td>randomized double-blind 49 patients/35 completed</td>
<td>12 months</td>
<td>AZA effective in reducing corticosteroid dose by 36%; 1 AZA patient withdrawn due to adverse event, 9 placebo</td>
</tr>
<tr>
<td>Levy, 1972 [13]</td>
<td>randomized double-blind cross-over 18 patients/16 completed</td>
<td>12 months</td>
<td>Significant improvement in AZA group; 2 drop-outs due to thrombocytopenia and GI intolerance</td>
</tr>
<tr>
<td>Urowitz, 1973 [14]</td>
<td>randomized double-blind cross-over 17 patients</td>
<td>4 months</td>
<td>AZA &gt; placebo; no drop-outs due to adverse events</td>
</tr>
<tr>
<td>Goebel, 1976 [15]</td>
<td>randomized double-blind crossover 34 patients/30 completed</td>
<td>24 weeks</td>
<td>AZA &gt; placebo; 4 drop-outs due to adverse events (AZA)</td>
</tr>
<tr>
<td>De Silva, 1981 [16]</td>
<td>randomized double-blind placebo substituted for active drug drug withdrawal 32 patients</td>
<td>8 months</td>
<td>Substitution of placebo for active drug resulted in clinical deterioration; 1 drop-out due to AZA</td>
</tr>
<tr>
<td>Woodland, 1981 [17]</td>
<td>randomized double-blind half-dose AZA (1.25 mg/kg/day) vs full-dose AZA (2.5 mg/kg/day) vs placebo 42 patients/29 completed</td>
<td>6 months</td>
<td>Full dose more effective; drop-outs due to adverse events (7 full-dose AZA, 4 half-dose AZA, 2 placebo)</td>
</tr>
<tr>
<td>Kvien, 1986 [18]</td>
<td>randomized double-blind 32 patients/29 completed</td>
<td>16 weeks</td>
<td>AZA &gt; placebo leucopenia in 2 AZA patients</td>
</tr>
</tbody>
</table>

>, superior to.
compared two dosage schedules of AZA (2.5 and 1.25 mg/kg/day) vs placebo in 42 RA patients. A significantly greater response was seen in the AZA 2.5 mg group, although a greater number of patients (47 vs 29%; not statistically significant) dropped out at this dosage due to adverse effects. This suggests that a dosage of 2.5 mg/kg/day should be administered in order to achieve the maximum effect. Kvien et al. [18] conducted a comparative, double-blind, parallel 16 week clinical trial of AZA vs placebo in 32 patients with juvenile RA receiving prednisolone as a baseline therapy. The majority of disease activity measurements changed in favour of the AZA group; however, statistically significant differences were found for only two disease activity measurements: patients’ subjective evaluation of their functional capacity and their subjective total assessment. Therapy was stopped in two AZA-treated patients because of reversible leucopenia.

**What is the optimum dosage schedule?** Three studies have attempted to address this issue [17, 21, 22]. Urowitz et al. [21] conducted a random, double-blind study, with either full-dose AZA (2.0–2.5 mg/kg/day) or half-dose AZA (1.0–1.25 mg/kg/day) in 31 patients with RA resistant to corticosteroids and/or chloroquine over a 24 week time period. Both groups of patients showed statistically significant improvement in articular index, number of effusions, grip strength and 50-foot walking time. There were no significant differences between the groups in either rate or magnitude of response. Woodland et al. [17] concluded that the a dose of 2.5 mg/kg/day should be administered in order to achieve the maximum benefit, although adverse events were more frequent at higher dosage. This study has been reviewed in the previous section. Cseuz et al. [22] undertook a small, comparative, 12 week parallel, double-blind controlled study comparing daily AZA with 300 mg given only on Mondays (M), Wednesdays (W) and Fridays (F) in 30 patients with RA in order to determine whether the pulsed regimen would be as effective and less toxic than the daily regimen. There were two immediate drop-outs in the 300 mg M, W and F group due to gastrointestinal side-effects. There were no significant differences in clinical assessments or toxicity between the two groups, suggesting that intermittent oral AZA therapy may be an acceptable alternative to continuous daily treatment.

**Does AZA influence radiographic progression?** Prevention of new erosions suggests good control of disease itself rather than just relief of symptoms. Four studies have evaluated the influence of AZA on radiographic changes and provide conflicting results [23–26]. Currey et al. [23] demonstrated that both AZA and CP retarded radiographic deterioration in a randomized double-blind controlled trial of AZA vs either CP or gold over 18 months. The rate of radiological deterioration was similar in both AZA- and CP-treated groups. Hamdy et al. [24] did not demonstrate any significant difference in radiographic outcome among 42 patients randomized to receive either AZA or MTX for 52 weeks. Both groups showed evidence of progressive joint damage on radiographs taken at weeks 24 and 52, despite obvious improvement in clinical measures of disease activity. Jeuressen et al. [25] demonstrated that MTX-treated patients showed significantly less radiographic progression (new erosions, total joint score) than AZA in a study of 64 patients with active RA who either had not responded, or who experienced significant side-effects while receiving parenteral gold and/or D-penicillamine. Radiographs of hands and feet were evaluated at the start, and after 24 and 48 weeks by a blinded observer. Only 10% of AZA-treated patients stabilized radiographically compared to 29% in the MTX group. Willkens et al. [26] demonstrated a trend towards reduced radiological progression in 67 MTX-treated patients compared to AZA; however, neither multivariate nor univariate analysis demonstrated a significant difference between treatment groups.

**Comparative studies.** Table II summarizes the studies comparing AZA to a variety of other DMARDs. Seven studies have compared AZA to MTX, and concluded that either these drugs are comparable [24, 27], or that MTX has a superior benefit/adverse event effect [26, 28–31]. The efficacy of AZA has also been compared to cyclosporin (CS), D-penicillamine (D-PEN), CP, gold and chloroquine [23, 32–34, 35, 36].

Hamdy et al. [24] studied 42 patients with active synovitis who were unresponsive to one or more non-steroidal anti-inflammatory drugs (NSAIDs), gold and/or D-PEN. Patients were randomized to receive either AZA 100 mg/day or MTX 10 mg/week, orally, adjusted at pre-defined intervals, and evaluated prospectively in a double-blind comparison for 24 weeks followed by an 18 month open phase. Both treatment groups showed a significant improvement at week 24, compared with baseline status, in nine of the 10 efficacy variables. Although there were no statistical differences between the two treatment groups at week 24, there was a trend towards a more marked and rapid improvement in the MTX-treated group. By week 52 (open phase), outcome measures were not statistically different from those at week 24. Two patients withdrew from each group by week 24 due to probable drug-associated side-effects; by week 52, eight additional patients had discontinued treatment. Arnold et al. [27] randomized 53 patients previously treated with either gold or D-PEN to receive either AZA 100 mg/day or MTX 10 mg/week modified according to efficacy and laboratory parameters of toxicity. Concurrent NSAIDs and oral or intra-articular steroid were prescribed and altered according to the judgement of the supervising clinician. By week 24, there were no significant differences in clinical outcomes between the two groups, although inter-group differences were apparent with respect to increased haemoglobin (Hb) and declining ESR in MTX-treated patients. Fifty per cent of patients in both groups discontinued therapy by week 24 due
TABLE II
Comparative studies: azathioprine vs other DMARDs

<table>
<thead>
<tr>
<th>Reference</th>
<th>Drugs</th>
<th>Type of study/no. of patients</th>
<th>Duration of study (weeks)</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamdy, 1987 [24]</td>
<td>AZA vs MTX</td>
<td>randomized double-blind 42 patients/37 completed 24 weeks; 23 completed 104 weeks</td>
<td>24 78 week open</td>
<td>AZA = MTX Beneficial effect maintained for 2 years in most patients</td>
</tr>
<tr>
<td>Arnold, 1990 [27]</td>
<td>AZA vs MTX</td>
<td>open randomized 53 patients/26 completed retrospective by life-table analysis; 55 AZA or 6-mercaptopurine/38 completed; 84 MTX/67 completed</td>
<td>24</td>
<td>AZA = MTX Withdrawal rates similar</td>
</tr>
<tr>
<td>Sambrook, 1986 [28]</td>
<td>AZA/purine analogues vs MTX</td>
<td>retrospective by life-table analysis</td>
<td>52</td>
<td>MTX &gt; AZA; defined criteria of improvement seen in 61.5% of MTX patients vs 25.6% of AZA patients</td>
</tr>
<tr>
<td>Bell, 1988 [29]</td>
<td>AZA vs MTX</td>
<td>randomized double-blind 34 patients/23 completed</td>
<td>20</td>
<td>MTX &gt; AZA MTX has less severe toxicity</td>
</tr>
<tr>
<td>Jeurissen, 1991 [30]</td>
<td>AZA vs MTX</td>
<td>randomized double-blind 64 patients/37 completed</td>
<td>48</td>
<td>MTX &gt; AZA MTX has fewer serious adverse reactions</td>
</tr>
<tr>
<td>Willkens, 1992 [31]</td>
<td>AZA vs MTX vs combined AZA/MTX</td>
<td>randomized double-blind 209 patients/158 completed</td>
<td>24</td>
<td>MTX alone and AZA/MTX combination &gt; AZA</td>
</tr>
<tr>
<td>Willkens, 1995 [26]</td>
<td>AZA vs MTX vs combined AZA/MTX</td>
<td>randomized double-blind 209 patients/110 completed</td>
<td>48</td>
<td>Significant response in 45% MTX, 38% AZA/MTX and 26% AZA patients; adverse events commonest in AZA and combination arms</td>
</tr>
<tr>
<td>Forre, 1987 [32]</td>
<td>AZA vs cyclosporin A</td>
<td>open randomized 24 patients/17 completed</td>
<td>26</td>
<td>CyA &gt; AZA</td>
</tr>
<tr>
<td>Kruger, 1994 [33]</td>
<td>AZA vs cyclosporin A</td>
<td>randomized double-blind 117 patients/92 completed single-blind</td>
<td>26</td>
<td>AZA = CyA</td>
</tr>
<tr>
<td>Berry, 1976 [34]</td>
<td>AZA vs D-penicillamine</td>
<td>?</td>
<td>12</td>
<td>AZA = D-penicillamine</td>
</tr>
<tr>
<td>Paulus, 1984 [35]</td>
<td>AZA vs D-penicillamine</td>
<td>randomized double-blind 140 patients/134 completed</td>
<td>24</td>
<td>AZA = D-penicillamine AZA better tolerated</td>
</tr>
<tr>
<td>Currey, 1974 [23]</td>
<td>AZA vs i.m. gold vs oral cyclophosphamide</td>
<td>randomized double-blind 121 patients/36 completed</td>
<td>72</td>
<td>CP &gt; AZA &gt; gold; AZA and CP are steroid sparing and retard X-ray deterioration</td>
</tr>
<tr>
<td>Dwosh, 1977 [36]</td>
<td>AZA vs i.m. gold vs chloroquine</td>
<td>open randomized 33 patients/29 completed</td>
<td>24</td>
<td>AZA = gold = chloroquine</td>
</tr>
</tbody>
</table>

=, as effective as; >, superior to.

to either lack of efficacy (nine MTX, six AZA) or adverse events (five MTX, seven AZA). After 1 yr, more than half of the patients in both groups discontinued therapy because of lack of efficacy or adverse events. Adverse events were more common in MTX-treated patients; however, withdrawal rates were similar in both groups. The authors concluded that the probability of a patient continuing either therapy for >18 months was low.

Sambrook et al. [28] undertook a retrospective study of AZA and 6-MP in comparison with MTX using life-table analysis. Fifty-five patients took purine analogues, 100 mg/day (median), whilst 84 took MTX in a median dose of 7.5 mg/week. By 12 months, 29.3% of patients had ceased purine analogues due to toxicity, compared with 19.3% for MTX. Toxicity severe enough to warrant cessation of therapy was rare after 8 months for either drug. At 12 months, only 26.5% of the purine
analogue patients had achieved defined criteria for improvement compared with 61.5% of the MTX patients ($P < 0.05$). The number of patients improving on purine analogues did not increase substantially after 5 months, whereas the number improving with MTX continued to 12 months. From these data, the authors concluded that low-dose MTX was superior to low-dose purine analogues in RA. Bell et al. [29] randomized 34 patients with active RA refractory to gold, D-PEN and chloroquine in a 20 week double-blind randomized control trial of AZA (100–150 mg/day) vs MTX (10–15 mg/week). Eleven (eight AZA, three MTX) patients withdrew from the study. Six of the AZA withdrawals were due to side-effects. Efficacy of treatment, as defined by a 30% change from baseline in ESR, grip strength and number of active joints, was seen in five patients only (all MTX) at study completion, suggesting that MTX has a greater efficacy and is associated with fewer adverse events than AZA. Jeurissen et al. [30] randomized 64 patients in whom gold and/or D-PEN treatment was unsuccessful to a 48 week double-blind, randomized trial comparing AZA (100 mg daily) and MTX (7.5 mg weekly), modified after 8 weeks according to efficacy. At week 24, improvements in 12/13 clinical variables were seen in MTX-treated patients vs 6/13 in AZA-treated patients. Improvements in swollen joint count, visual analogue score (VAS) pain score, ESR, C-reactive protein, Hb and disease activity score were significantly greater at 24 weeks in MTX-treated patients by area under the curve analysis. A significant overall improvement (disease activity score) was found in 7/20 AZA-treated patients and 18/30 MTX-treated patients at 24 weeks, and in 6/12 AZA-treated patients and 19/25 MTX-treated patients by 48 weeks. The number of withdrawals due to side-effects was significantly higher in the AZA-treated group. After 48 weeks, only 12 of the AZA-treated patients (36%) compared to 25 (81%) of the MTX-treated group remained on treatment. Analysis of the radiological changes in these patients also showed more improvement in the MTX-treated group. The authors concluded that MTX was superior to AZA for treating RA with a more rapid and sustained clinical improvement sustained after 1 yr, accompanied by a lower rate of serious adverse reactions. Willkens et al. [31] compared the relative safety and efficacy of AZA, MTX and the combination of both in the treatment of active RA. Two hundred and nine patients with active RA unresponsive to injectable gold, auranofin or D-PEN were entered into a 24 week, prospective, controlled, multicentre trial and randomly assigned to one of three treatment groups. One hundred and fifty-eight patients (75%) finished 24 weeks of the study. Response rates were $> 30\%$ for all outcome measures. Combination therapy was not statistically superior to MTX therapy alone; however, both combination therapy and MTX alone were superior to AZA alone when patients were analysed by intent-to-treat analysis and with withdrawals treated as therapy failures. If only patients who continued taking the therapy were analysed, the mean improvement was greater for AZA therapy than for MTX, while the combination remained the most active. Adverse effects on the gastrointestinal tract and elevations of liver enzyme levels were the most frequent causes for discontinuation. The authors concluded that combination therapy and MTX alone were superior to therapy with AZA alone for active RA, but were not statistically different in their effect on outcome assessment. In 1995, Willkens et al. [26] reported 48 week data on 110 of these patients who remained on the initially randomly assigned regimen. The highest rate of drop-out was in the AZA group; the highest percentage of patients remaining on the initially assigned regimen was in the MTX group. Virtually every parameter studied showed at least a trend for MTX to be more effective. The combination of AZA plus MTX was thought to be neither more toxic nor more effective than MTX alone. A trend toward decreased radiological progression was seen in the MTX-treated patients. This study established that the combination of MTX and AZA was not associated with more toxicity than treatment with single agents; however, enhanced efficacy was not seen.

Forre et al. [32] randomized 24 patients with established RA to receive either CS (10 mg/kg/day) or AZA (2.5–3 mg/kg/day) for 26 weeks in an open, controlled, randomized study. Although no statistically significant difference in benefit was observed between the two groups, statistically significant improvements in 5/13 clinical parameters were observed within CS-treated patients compared to 1/13 within the AZA group, and a greater and more rapid reduction in dosage of concomitant prednisolone treatment was possible in CS-treated patients. Kruger et al. [33] undertook a 6 month, prospective, randomized double-blind multicentre study comparing AZA (1.5–2.0 mg/kg) and CS (10 mg/kg) in 117 patients with RA, and demonstrated that efficacy and tolerability were comparable in both groups. Ninety-two patients completed the study, treatment was discontinued prematurely in 12 patients in each group. Berry et al. [34] undertook a single-blind trial of AZA and D-PEN. Assessments at 3 and 12 months showed no significant differences in pain, articular index, joint swelling, grip strength or morning stiffness between the two groups. There was, however, a trend in favour of D-PEN in most of these measures and in laboratory markers of disease activity. Rises in Hb levels and falls in latex titre and ESR were greater in the D-PEN group, although many of these differences failed to reach statistical significance. Paulus et al. [35] entered 206 patients treated unsuccessfully with gold into a prospective, controlled, double-blind, multicentre trial comparing AZA with D-PEN. One hundred and thirty-four patients completed 24 weeks of therapy. Both drugs had similar efficacy; however, AZA was slightly better tolerated. Currey et al. [23] compared AZA, CP and gold under double-blind conditions for 18 months in patients with relatively early RA (50% had disease for < 2 yr). All three agents produced comparable clinical improvement; however, AZA and CP both facilitated a reduction in cortico-
steroid dosage and retarded radiological deterioration. There were fewer patient withdrawals in AZA-treated patients. Dwosh et al. [36] compared the effect of AZA with gold and chloroquine in RA patients with a disease duration of <5 yr. Eleven patients were randomly entered into each group and followed up for 24 weeks. Assessment of standard clinical and laboratory measures showed comparable and statistically significant improvements in all three groups at 12 and 24 weeks, respectively.

Combination studies Combination therapy has gained considerable popularity in recent years [37, 38]. The rationale for considering combinations of therapeutic agents stems in part from the short ‘therapeutic half-life’ of DMARDs: 50% of patients taking single DMARD therapy discontinue the medication within 2–3 yr [28, 39–41]. The pharmacodynamics of individual drugs also differ, therefore the potential for synergism or additive effects exists, resulting in an expectation of greater efficacy and/or reduced toxicity compared to the use of single agents. These studies, summarized in Table III, include AZA with sulphasalazine [SZP; 42], MTX [26, 31], hydroxychloroquine (HC)/CP [43, 44], MTX/HC [45, 46] and prednisolone [47, 48].

Waterworth [42] conducted an open study to assess the combination of AZA and SZP in 13 patients with RA who had not been well controlled by either agent alone. On combination therapy of SZP (10 patients 1 g, three patients 2 g) plus AZA (one patient 50 mg, 12 patients 100 mg) for a mean duration of 14 months, the number of swollen joints had decreased in nine, and the overall physician rating of response was good or excellent in 11 patients. Two patients had to discontinue combination treatment due to side-effects (one nausea, one leucopenia). This potential for a synergism with MTX was suggested by the fact that the inhibition of purine synthesis by MTX produces an increased concentration of phosphoribosylpyrophosphate, a cofactor in the activation of AZA. This potential was not borne out in clinical studies undertaken by Willkens et al. [26, 31], in that there was no report of any increased efficacy of combination therapy over and above MTX alone. The results of these studies were discussed in a previous section. McCarty and Carrera [43] first reported the combination of AZA, HC and CP in 17 patients with intractable RA. The initial results were impressive, namely five (29%) complete remissions with no serious complications, seven (42%) partial remissions. Four years later, in a follow-up report, Csuka et al. [44] described a low-dose combination of CY (30 ± 24 mg/day; mean ± s.d.), HC (210 ± 92 mg/day) and AZA (77 ± 44 mg/day) in 31 patients refractory to conventional therapy. After a mean duration of 43 months, the results remained favourable: 16 achieved complete remission, seven near remission, seven partial remission, one no response. Four patients developed a malignancy during therapy, three of whom died (five patients in this group had previous malignancies). Adverse events including severe infections, thrombocytopenia, stomatitis, diarrhoea and leg ulcers were common, occurring in all but six of the patients studied. The authors concluded that although this combination may have merit, it

### Table III

Combination studies which include azathioprine

<table>
<thead>
<tr>
<th>Reference</th>
<th>Drugs</th>
<th>Type of study/ no. of patients</th>
<th>Duration of study (weeks/months/years)</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterworth, 1989 [42]</td>
<td>AZA/SZP</td>
<td>open study 13 patients</td>
<td>mean 14 months</td>
<td>Effective Good response in 11 patients, 2 drop-outs</td>
</tr>
<tr>
<td>Willkens, 1992 [31]</td>
<td>AZA vs MTX vs combined AZA/MTX</td>
<td>randomized double-blind 209 patients/158 completed</td>
<td>24</td>
<td>MTX alone and AZA/MTX combination &gt; AZA</td>
</tr>
<tr>
<td>Willkens, 1995 [26]</td>
<td>AZA vs MTX vs combined AZA/MTX</td>
<td>randomized double-blind 209 patients/110 completed</td>
<td>48</td>
<td>Significant response in 45% of MTX, 38% AZA/MTX and 26% AZA patients; adverse events commonest in AZA and combination arms</td>
</tr>
<tr>
<td>McCarty, 1982 [43]</td>
<td>AZA/hydroxychloroquine/ cyclophosphamide</td>
<td>open study 17 patients</td>
<td>mean 27 ± 19 months (range 5–60)</td>
<td>Combination effective in 14 patients</td>
</tr>
<tr>
<td>Csuka, 1986 [44]</td>
<td>AZA/hydroxychloroquine/ cyclophosphamide</td>
<td>open study 31 patients</td>
<td>mean 43 months (range 12–102)</td>
<td>Effective 4 malignancies, 2 infection, 1 thrombocytopenia</td>
</tr>
<tr>
<td>McCarty, 1995 [45]</td>
<td>AZA/hydroxychloroquine/ MTX</td>
<td>open study 169 patients</td>
<td>mean 7 ± 5 years (range 1–18)</td>
<td>Complete remission in 43%</td>
</tr>
<tr>
<td>Langevitz, 1989 [46]</td>
<td>AZA/hydroxychloroquine/ MTX</td>
<td>open study 12 patients</td>
<td>range 14–18 months</td>
<td>Effective in 7 patients, 4 withdrawals after 3–4 months</td>
</tr>
<tr>
<td>Bijlsma, 1986 [48]</td>
<td>AZA/i.v. pulse methylprednisolone</td>
<td>open study 19 patients/8 completed</td>
<td>12 months</td>
<td>No significant benefit</td>
</tr>
<tr>
<td>Hantzchel, 1988 [47]</td>
<td>AZA/IM gold with i.v. pulse prednisolone hemisuccinate</td>
<td>15 in each group</td>
<td>24 weeks</td>
<td>Significant improvement in both groups; 5 AZA patients withdrawn due to toxicity/2 gold</td>
</tr>
</tbody>
</table>

=, as effective as; >, superior to.
should only be used in intractable RA when conventional management has not provided satisfactory control. They also recommended that CP should be replaced with a non-alkylating agent due to concerns regarding the absolute risk of malignancy. In 1995, McCarty et al. [45] studied the effect of combination therapies in an open sequential study of 169 patients, 69 of whom received a regimen combining pulse oral MTX, AZA and HC (MAH) in conjunction with NSAIDs, intra-articular and oral corticosteroids. The entire patient cohort showed improvement in every variable except HB at the time of the last visit ($P < 0.0004$). Disease remission was achieved in 45% of the MAH patients, near remission in 69%. On multivariate analysis, MAH patients were improved only in American Rheumatism Association functional class compared with the other groups ($P < 0.001$). Overall mortality rates did not differ significantly from that of the general population. Langevitz et al. [46] also reported the MAH combination in 12 patients with intractable RA. Seven patients responded to this combination, three patients achieving near or complete remission (swelling and tenderness of one joint or less). Hantzchel et al. [47] and Bijlsma et al. [48] both studied the effect of glucocorticoid therapy in conjunction with AZA in small numbers of patients with RA, arriving at different conclusions. Hantzchel et al. [47] concluded that prednisolone accelerates the clinical improvement afforded by AZA, but does not necessarily magnify it; Bijlsma et al. [48] did not demonstrate any benefit in AZA-treated patients in whom methyl-prednisolone pulse therapy was used in conjunction with AZA.

Toxicity

Three side-effects dominate the picture of AZA toxicity: marrow suppression, gastrointestinal intolerance and infections. The reported incidence of side-effects varies considerably from one study to another; minor side-effects are common and major side-effects including gastrointestinal upset and cytopenias occur in as many as 30% of patients. Singh et al. [49] reported on 546 AZA-treated patients surveyed prospectively and concluded that AZA presents a surprisingly benign profile, with very few serious therapeutic mishaps. The most frequently reported side-effects were nausea, vomiting and leucopenia. Gastrointestinal intolerance accounted for nearly 60% of therapy interruptions in 95 patients. AZA had to be discontinued in eight patients due to leucopenia; however, in only two cases was the leucopenia severe enough to cause major clinical problems or hospitalization. In neither of these two cases was the infection of a life-threatening nature. Of 81 hospitalizations for all causes, only eight were partly related to AZA, and no deaths were attributed to AZA therapy. No lymphomas or leukaemias were detected; the overall incidence of neoplasms was similar to that seen in RA patients receiving conventional therapy. McKendry et al. [41] treated 131 patients with either AZA ($n = 37$) or MTX ($n = 94$) for a mean time period of 38 ± 23.3 months in order to determine the nature, frequency and potential predictors of side-effects. Eleven AZA-treated patients (30%) vs 31 (33%) MTX-treated patients experienced a major side-effect; gastrointestinal symptoms and cytopenias were common in the MAH group. In addition, the majority of patients in each group (29 AZA, 79 MTX) experienced one or more minor side-effect during the follow-up period. The haematological adverse events of AZA at low dosage of 1–3 mg/kg/day are limited and include macrocytosis, leucopenia, and less commonly megaloblastic anaemia or pure red cell aplasia [50–52]. Low activity of thiopurine methyltransferase (TPMT) has occasionally been reported as a cause of AZA-related bone marrow toxicity in patients with a variety of conditions, including one with RA [53]; however, other mechanisms also contribute since similar events have also been reported in patients with normal TPMT levels [54]. Severe bone marrow suppression is rare unless associated with the simultaneous use of allopurinol [55, 56]; this usually reverses after cessation of therapy. Urowitz et al. [14] noted leucopenia in five out of 17 patients treated with AZA which required slight reduction in dosage but not cessation of therapy. In a later study of two dosage levels, Urowitz et al. [21] showed that neutropenia is not dose related, responds rapidly to treatment withdrawal and is not serious. Nausea, anorexia and vomiting are occasional side-effects which appear early in the course of treatment, usually within the first few weeks. This is not dose related and responds to treatment withdrawal, although it may occasionally preclude subsequent treatment. An increased risk of infection and accelerated nodulosis have also been reported [57, 58]. Hypersensitivity reactions to AZA are rare. Symptoms including nausea, diarrhoea, arthralgia, rash, fever, rigors, hypotension, pneumonia, pancreatitis, hepatitis, haematuria and renal insufficiency resolve on withdrawal of the drug [59–62]. These clinical features may be confused with septic shock or organ rejection in transplantation. There has been concern regarding the potential association between AZA treatment and malignant disease since early reports from the transplantation literature suggested a link [63, 64]. In RA patients, this link is less clear-cut, although malignancies have been reported in AZA-treated patients [65, 66]. Kinlen et al. [67] studied 1349 non-transplant patients who had been treated for at least 3 months with AZA, CP or chlorambucil. There was an increase in the incidence of the following malignancies in these patients: non-Hodgkin’s lymphoma (four cases as against 0.34 expected), squamous cell skin carcinoma (two cases as against 0.38 expected), all other tumours (34 cases as against 21.74 expected); however, the mortality data did not show any significant excess. This pattern of increased incidence of malignancies is similar to that seen in transplant recipients and the excess of non-Hodgkin’s lymphoma was evident soon after starting immunosuppressive therapy. Matteson et al. [68] established the RA AZA Registry (RAAR) in 1982 to examine the safety of AZA and other DMARDs in
the treatment of RA. After 7 yr, 20 malignancies had been reported in 530 DMARD-treated adult patients with RA, suggesting that there may be an increased risk of malignancy, particularly lymphoproliferative disorders. In contrast, Lewis et al. [69] reported the results of a prospective study of 311 patients with RA. Two hundred and three of this group had been treated with AZA (52 also received chlorambucil or CP). The overall death rate from neoplasia was higher than expected in the age group 45–64 yr and lower in those aged 75 and over; it should be noted that deaths were not more common in patients receiving AZA. Hazleman [70] suggested that the incidence of most of the common cancers was less in RA patients treated with AZA than in RA patients in general. Wessel et al. [71] did not demonstrate any increased incidence of neoplasia in either RA or SLE patients treated with AZA compared with a control population. AZA has also been reported to give rise to chromosomal abnormalities [72]. Hunter et al. [19] reported an increased incidence of chromosomal abnormalities (13.8% vs 6.9% in age-, sex- and duration-of-disease-matched RA controls and 4.7% in healthy controls), in a study of 12 patients taking AZA for 40 months. These observations are challenged by another long-term study in SLE [73].

Monitoring and practical management considerations. AZA should be started at an initial dose of 50 mg daily for the first week. If tolerated, the dosage should then be increased to 2.5 mg/kg/day. A full blood count and liver function tests are recommended fortnightly for the first 4 weeks and monthly thereafter. If the total white cell count falls below 3000/mm³, treatment should be stopped. Lesser falls are an indication for more frequent monitoring and dosage reduction should be considered. If it is necessary to stop treatment due to leucopenia, it may be possible to reintroduce AZA at a lower dose when the bone marrow has recovered. Nausea and gastrointestinal complications can often be reduced or eliminated by dosage reduction, patients with hypersensitivity reactions cannot be treated with AZA again. Despite reports of chromosome aberrations [72, 73], or fetal growth retardation [74] in the offspring of mothers who received AZA during pregnancy, there seems to be little evidence that AZA is teratogenic in humans. Given the nature of the severe chronic conditions for which AZA is generally used, discontinuing therapy in patients who become pregnant may not be necessary or desirable; however, it is best to avoid AZA during pregnancy when possible. In patients with renal failure and those receiving allopurinol therapy, the AZA dosage should be reduced. • There is conflicting evidence regarding the influence of AZA on radiographic progression.
• It has a slow onset of action, reaching a peak after ~4–6 months of therapy.
• A reduction of concomitant corticosteroid dosage is usually possible.
• Treatment continues to be effective for long periods of time.
• Withdrawal of therapy leads to relapse.
• A dosage of 2.0–2.5 mg/kg/day is necessary to maximize clinical effectiveness.
• Adverse events are more common at higher dosage.
• Reductions in ESR and RF titres are inconstant findings and a poor guide to clinical response.
• Combination studies using AZA have been disappointing.
• Common adverse events include gastrointestinal intolerance and marrow suppression.
• There may be a small increased risk of malignancy, particularly lymphoproliferative disorders.
• The efficacy–toxicity ratio of AZA justifies its inclusion in the list of therapeutic options in RA.

CYCLOPHOSPHAMIDE

Pharmacology

CP is an alkylating agent of the nitrogen mustard class. The phosphoester ring must be broken for it to be ‘active’ and this occurs in the liver. Active metabolites of CP (e.g. phosphoramide mustard) cross-link DNA so that it is unable to replicate. CP is cytotoxic to resting and dividing lymphocytes. In RA patients, it suppresses T-helper function, and reduces the numbers of activated T cells and B cells.

CP and its metabolites are excreted mainly in the urine. It is extensively metabolized before excretion with less than a quarter of any administered dose appearing in the urine unchanged. The urinary metabolites include carboxyphosphamide and 4-keto-phosphamide. Acrolein is a metabolite which is excreted in the urine and is thought to be responsible for bladder toxicity, which may be helped by the agent Mesna (mercaptoethanesulphonate) which detoxifies acrolein. Very small amounts of CP appear in faeces, expired air, cerebrospinal fluid, sweat, breast milk, saliva or synovial fluid. Dialysis removes almost three-quarters of any administered dose of CP so patients with renal failure need to have their drug administered after dialysis.

Efficacy

Oral CP has consistently been shown to be better than placebo both in clinical assessment and in laboratory assessment of active arthritis when used in doses of >1.5 mg/kg/day. Two studies have shown beneficial effect on bone destruction. It is clinically equal to AZA, possibly superior to i.m. gold clinically and in retarding bone destruction. Studies in general suggest that the benefits take some time to develop (4 months) with efficacy maintained for up to 25 months. After withdrawal of CP, patients usually have a relapse.
Non-articular complications such as lung disease, ulcers and neuropathy may also respond to treatment. Despite the use of CP as an immunosuppressive cytotoxic agent in malignant disease, its exact role and mode of action in RA are still uncertain. A recent review by Curtis et al. [75] showed that there was no detectable effect on patients’ immune reactivity as assessed by delayed hypersensitivity skin testing, immunoglobulin levels or in vitro lymphocyte blastogenesis and the primary immune response.

Open studies
The first report of CP’s efficacy in RA was by Fosdick et al. [76] who in 1968 described 38 patients with RA treated for 6–40 months and reported a 75% response rate. Other studies have shown good clinical effects, including detailed studies on small numbers of patients such as that by Hørslev and Petersen et al. [77] who in 1983 described three patients who had failed conventional second-line therapy who were given oral CP often with pulse methylprednisolone, and who showed quite dramatic responses. They were able to reduce the dose of CP, which was initially given daily, but later every third or fourth day. The early studies in rheumatoid vasculitis and the studies in other complications of RA were also open studies, including that of Abel et al. [3] who reported quite dramatic improvement in systemic vasculitis in five patients treated with continuous oral CP.

Comparative and controlled studies
The co-operating clinics study in 1970 reported the effect of high and very-low-dose CP in 48 patients followed for 32 weeks, showing a better response in the high-dose group [78]. A similar study by Williams et al. [79] in 1980 comparing 150 mg/day with 75 mg/day showed similar clinical efficacy in both groups.

Currey et al. [23] compared AZA, cyclophosphamide and gold, and found CP to be ‘marginally the most effective drug but prone to cause azoospermia’ and expressed concern about its long-term risk of malignancy.

Lidsky et al. [80] undertook a double-blind placebo-controlled study in 22 patients using relatively low-dose CP (0.87–1 mg/kg) and showed no difference between placebo and CP treatment. Townes et al. [81], in a double-blind cross-over study, noted significant clinical benefit with a higher dose of CP, but also expressed concern about the numbers of patients with haemorrhagic cystitis and with amenorrhoea as a consequence of CP treatment. Smyth et al. [82] in 1975 compared prednisolone plus placebo (16 patients) with prednisolone plus oral CP (13 patients) and again noted clinical benefit only in patients on high-dose CP which was also associated with increased toxicity.

Intravenous pulse CP was originally described by Scott and Bacon [4] to be effective for systemic vasculitis when compared with conventional second-line therapy including oral AZA. Intravenous pulse CP has also been used to treat inflammatory arthritis, but was found to be ineffective by Arnold et al. [83].

Combination therapy
Most of the combination therapies have been with i.v. CP and corticosteroids. This stresses the importance of interpreting the efficacy as occurring with one drug alone. In 1988, Walters and Cawley [84] compared gold with or without i.v. pulse CP and noted no difference between the two groups. In 1996, Maeyaert et al. [85] compared MTX plus monthly CP pulses vs penicillamine/Salazopyrin/Plaquenil with monthly pulses of CP and found that CP did not appear to have any additional effect (although any associated vasculitis improved).

In 1982, McCarty and Carrera [43] studied CP, AZA and hydroxychloroquine in combination in 17 patients refractory to conventional treatment studied for a mean of 27 months. There was a good effect clinically with some evidence to show a reduction in erosions. In a follow-up studied published by the same authors in 1986 [44] with increased numbers they noted that the benefit had been maintained, but four of the 31 patients had developed malignancy and the authors suggested replacing CP with a non-alkylating agent. Even with this quite prolonged long-term follow-up, they still felt that the place of this combination therapy was uncertain without controlled trials.

Toxicity
The main concern about the use of CP is in relation to its toxicity. Most of the reported studies relate to the use of continuous oral CP. In RA, the dose often has to be reduced with time due to increased sensitivity of patients’ neutrophil counts to the drug. However, marrow toxicity has not been a major problem in most studies.

The biggest cause of concern has been bladder toxicity. Early studies showed the relationship between acrolein and haemorrhagic cystitis and also bladder fibrosis [86], and it is thought that cystitis and fibrosis may be linked to an increased risk of bladder cancer, possibly exacerbated by suppression of immune surveillance [87]. Early studies in RA confirmed an increased risk of bladder cancer up to 4.1 times expected when compared with rheumatoid controls [88, 89]. Baker et al. [90] studied 119 rheumatoid patients treated with oral CP compared with 119 controls and described 37 malignancies in 29 of the CP-treated patients compared with 16 malignancies in 16 controls (P < 0.05). Bladder cancer was only seen in the rheumatoid patients (6) and there was also an increase in skin and haematological cancers. These patients were further followed by Radis et al. [91] in a 20 yr follow-up which showed that the numbers of malignancies had increased to 50 in 37 CP patients compared with 26 in 25 controls (relative risk 1.5). The numbers of bladder cancers had increased to nine, skin cancers to 19 and the important finding was that three of the bladder cancers developed 14, 16 and 17 yr after CP was stopped. A review by Jones et al. [92] looking at the malignancy risk and
mortality associated with immunosuppressive treatment in RA showed only a relatively small increase in mortality with immunosuppression, but most of the excess deaths were due to malignancy with a relative risk of 4.2 (1.7–10) and a relatively risk of immune system malignancy of 7 (0.9–56.5). In this group, the increase in malignancy associated with CP was due to two patients developing bladder cancer.

The concerns about CP-induced cystitis and bladder cancer have recently been reviewed in patients with Wegener's granulomatosis by Talar-Williams et al. [93]. Their conclusions were that 'long-term oral CP therapy is associated with substantial urotoxicity including the development of transitional cell carcinoma of the urinary bladder'. In their cohort of patients, the estimated incidence of bladder cancer after their first exposure to CP was 5% at 10 yr and 16% at 15 yr.

The only trials to compare pulsed i.v. CP with oral therapy have been undertaken in the systemic vasculitides. Adu et al. [94] randomized 54 patients with systemic vasculitis to treatment with either pulse CP and prednisolone or continuous oral prednisolone and CP. The patients on continuous CP were more likely to develop leucopenia and had slightly increased treatment-related toxicity compared with pulse CP. There was, however, little difference in terms of deaths, relapses, treatment failures, improvement in disease activity scores or renal function. Guillevin et al. [95] undertook a similar study comparing steroids and pulse CP vs steroids and oral CP in the treatment of Wegener's granulomatosis. They found pulse CP as effective as oral CP in achieving initial remission and associated with fewer side-effects and lower mortality. However, in the longer term, treatment with pulse CP was not able to maintain remission or prevent relapses as well as oral CP. Our own experience with 66 patients given pulse CP and steroids is that it is a well-tolerated, safe and effective short-term treatment for systemic vasculitis with lower toxicity, including malignancy, when compared with reported series using continuous oral CP. However, our follow-up is only for a maximum of 7 yr and longer term studies with pulse CP are needed before the picture is completely clear.

One of the intriguing findings in patients given pulse CP for systemic vasculitis complicating RA is that while there is little evidence of benefit to the joints (they are often inactive to start with), some patients develop a flare in their arthritis following i.v. treatment. We have even seen patients with systemic vasculitis with no previous arthritis develop transient synovitis a few days after pulsed therapy.

Other important toxicity factors include infertility, azoospermia and amenorrhoea. There are some data to suggest that pulsed therapy has less harmful effects on hormonal function in women. There are also data to suggest that the infertility effect increases with age. There are few figures for patients with RA, but in lupus amenorrhoea may occur in over a third of the patients [96]. The effect of CP in increasing the risk of infection is significantly complicated by the frequent combination of CP with corticosteroids. Guillevin's study showed a significant increased risk of infection in the oral CP plus steroid group compared with pulse CP and Omdal et al. [97] reported severe infections in 24% of patients treated with oral CP. Our own studies with pulse CP have shown severe bacterial infections in only 12% overall—16% of patients with vasculitis complicating RA.

Conclusions

CP is a powerful immunosuppressive agent which is clinically effective in the treatment of RA when given as continuous oral treatment. The toxicity is, however, considerable with a high risk of immunological malignancy and a particularly high risk of bladder cancer. Because of this, it is no longer used routinely. CP does have a role in the treatment of more life-threatening systemic complications of RA, particularly vasculitis, where there may be a reduction in dosage and toxicity if the CP is given i.v. Intravenous CP is ineffective for treating inflammatory arthritis.

Cyclophosphamide current issues

- Oral CP is now rarely used to treat rheumatoid synovitis.
- CP is effective in the treatment of systemic vasculitis, severe SLE and severe extra-articular manifestations of RA (especially vasculitis).
- The most worrying side-effects of continuous oral CP are haemorrhagic cystitis and bladder cancer. Previous research suggests that this is a cumulative problem which may develop decades after treatment has discontinued.
- The most effective method of delivery of CP is controversial. Some data suggest that pulsed i.v. therapy is as effective as continuous oral CP in the short term for 'remission induction', and less toxic. Late relapses may occur more commonly with pulsed i.v. treatment, although they are usually relatively mild.
- Pulsed i.v. CP is ineffective treatment for rheumatoid synovitis. It may even cause a mild flare of the synovitis.

REFERENCES


