Observation on serum anti-double stranded DNA antibodies of tripterine in systemic lupus erythematosus of (NZB×W)F1 mice

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Tripterine is one of the major active components isolated from the traditional Chinese herb *Tripterygium wilfordii* Hook. f. Previous studies have shown that tripterine inhibits not only humoral and cellular immune responses but also the inflammatory response.[1]

This study aimed at exploring the inhibitory effects of tripterine on lupus nephritis. We studied the effect of tripterine in (NZB×W)F1 mice, who spontaneously develop autoimmune disease characterised by the production of dsDNA antibodies and the development of a severe immune complex glomerulonephritis, like in human lupus nephritis.[2 3] The dsDNA antibodies are thought to have a role in the pathogenesis of mouse lupus-like nephritis.[4] Raised levels of circulating anti-dsDNA antibodies often precede the development of nephritis.[5 6]

(NZB×W)F1 female mice, 2 months of age at the start of the experiment, were used in these studies. All animals were housed at constant temperature and fed a standard diet. The mice were evaluated for proteinuria every two weeks using the Coomassie blue G dye binding assay.[7] After starting treatment all mice were housed in metabolic cages, and 24 hour urinary protein was collected for determination of basal urinary protein excretion levels. Levels >3 mg/day during the subsequent follow up were considered abnormal.[8] Blood was collected from the ophthalmic venous plexa for determination of the packed cell volume, and the serum was stored at –20°C; serum anti-dsDNA antibody levels were measured by enzyme linked immunosorbent assay (ELISA)[9] before treatment and then every two weeks. Twenty four hour urine samples were collected for determination of basal urinary protein excretion. The (NZB×W)F1 mice were randomly allocated to one of three groups to evaluate the therapeutic effect of tripterine on survival of the animals. Group A animals were untreated; groups B and C were given weekly intraperitoneal injections of tripterine 3 mg/kg wt and 6 mg/kg wt, respectively. The experiment was continued for 20 weeks.

We found that tripterine reduced the urinary protein excretion. Before treatment, animals in both groups B and C had similar concentrations of 24 hour urine protein. Untreated lupus mice (group A) at 6 weeks of age had an increased concentration of 24 hour urine protein. The mean (SD) urinary protein excretion was significantly reduced in tripterine treated groups (B and C) in comparison with the control group A (mean (SD) 0.43 (0.09) and 0.38 (0.13) *v* 14.89 (5.11) μg/min, $p<0.001$). There was no significant difference in proteinuria between groups B and C after eight weeks (0.53 (0.15) *v* 0.50 (0.19) μg/min).

The levels of serum anti-dsDNA autoantibodies were evaluated every two weeks during the study (table 1). Different doses of tripterine suppressed the serum level of anti-dsDNA antibodies at different stages.

In untreated animals a rise in the level of serum dsDNA antibodies preceded the change of proteinuria at 2–4 weeks.

Our preliminary study indicates that tripterine greatly reduces the amount of urine protein excretion, suppressing the formation of serum anti-dsDNA antibodies. It ameliorates the clinical symptoms of the (WZB×) F1 mice, improves their survival rate, and has a definite protective effect on lupus nephritis. These results suggest that clinical studies should be carried out to explore the exciting possibility that tripterine might be used in the treatment of human lupus.

Table 1 Serum level of anti-dsDNA antibodies at different stages in groups given different doses of tripterine in comparison with the control group (OD, mean (SD))

Weeks	A group	B group	C group
0	0.30 (0.07)	0.22 (0.15)	0.14 (0.09)
2	0.60 (0.17)	0.18 (0.14)**	0.18 (0.12)**
4	0.43 (0.05)	0.37 (0.26)	0.25 (0.09)
6	0.99 (0.28)	0.48 (0.16)**	0.31 (0.18)***
8	0.87 (0.17)	0.42 (0.22)**	0.23 (0.11)***
10	0.82 (0.42)	0.24 (0.16)**	0.33 (0.24)**
12	0.74 (0.41)	0.32 (0.15)**	0.28 (0.12)**
14	1.18 (0.48)	0.32 (0.13)***	0.38 (0.16)***
16	0.88 (0.16)	0.33 (0.10)**	0.22 (0.09)***
18	1.58 (0.35)	0.31 (0.11)***	0.23 (0.10)***
20		0.30 (0.07)	0.25 (0.09)

Note: In the groups treated with tripterine (B and C groups) in comparison with the control group (A). **p<0.01; ***p<0.001.

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Clodronate induced uveitis

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We are reporting on a 68 year old woman, treated for postmenopausal osteoporosis with clodronate, a non-nitrogen, halogen containing bisphosphonate (BP), who developed a bilateral, drug related, anterior acute uveitis. BPs are generally well tolerated. Uveitis is an ocular adverse effect hitherto described only for nitrogen containing bisphosphonates (N-BPs).[1 2] To our knowledge, this is the first report of uveitis induced by a non-N-BP.

CASE REPORT

In April 2001 the patient presented with a three month history of spinal pain, following an accidental fall. Spine and pelvis *x* ray examination showed osteoporotic fractures in T12, L1, and L2 vertebral bodies. Bone densitometry dual energy *x* ray absorptiometry evaluation showed remarkably reduced mineral density in both vertebral and femoral neck sites (T score –4.3 and –4.08, respectively). Because the patient had had reflux oesophagitis, we treated her with 100 mg once a week of intramuscular (IM) clodronate, the only parenteral BP available for outpatients in our country. The treatment progressively reduced the spinal pain.

In August 2001 the patient started to have the first mild symptoms of a bilateral iritis, such as transient tearing, photophobia, and ocular redness, attributed by her ophthalmologist to viral infection. Thus, the clodronate treatment was continued. The patient was treated with topical corticosteroids, and the ocular problems promptly resolved. However, thereafter they regularly recurred in the 24–72 hours after each IM clodronate administration. Because the intensity and persistence of her ocular symptoms from time to time progressively worsened, in September 2001 the patient returned for our consultation. Ocular examination disclosed marked bilateral perikeratic hyperaemia, and anterior uveitis was diagnosed. Routine biochemical and inflammatory measurements were normal. Autoantibodies were negative, as well as HLA typing for the B27 antigen. The patient was treated for seven days with topical corticosteroids and cycloplegic drugs, and recovered completely. The clodronate treatment was discontinued and the ocular symptoms did not recur.

In January 2002, we rechallenged with the drug, because the patient asked for another course of treatment, but after the first IM clodronate administration, the ocular complaints started again. The patient was not taking any other drug. Thus, a bilateral anterior uveitis related to clodronate was diagnosed and the drug was permanently suspended. Thereafter, the patient was consistently symptom-free.

DISCUSSION

Ocular adverse effects have been hitherto reported only for the N-BPs risedronate,[1] pamidronate,[1] and alendronate.[2] Interestingly, a patient previously tolerant to the non-nitrogen derivative etidronate shortly developed anterior uveitis after both oral risedronate and intravenous pamidronate,[1] suggesting that the chemical structure may play a part in the pathogenesis of the eye disease. The ocular side effects have been interpreted as a consequence either of an allergic reaction or an acute inflammatory response.[3] N-BPs are known to cause transient pyrexia, a flu-like syndrome, and serological changes resembling a typical acute phase response, and also to stimulate the release of proinflammatory cytokines, such as tumour necrosis factor α, interleukin 1, and interleukin 6.[4] Thus, they may act as adjuvants in an immune reaction, which might have the uvea as a target organ. Instead, clodronate inhibits proinflammatory cytokine and nitric oxide secretion from activated macrophages, especially when delivered into cells by liposomes.[5] In our case, the bilateral anterior uveitis, correlated well with the parenteral clodronate administration, and might be related to an idiosyncratic reaction rather than to a cytokine mediated process.

To our knowledge, ocular adverse manifestations have not hitherto been described for the non-N-BP clodronate. Because the BPs are successfully used in an increasingly broad range of diseases, we wish to report this observation, which suggests the need for a careful evaluation of ocular symptoms developing during treatment with any BP, independently of its chemical structure.

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