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Immediate Effects of Methylprednisolone Pulse Therapy on the Immune Response

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To study the immediate effects of high-dose intravenous methylprednisolone pulse therapy on the immune mechanisms in the absence of other immunosuppressive agents, we treated ten patients with active systemic lupus erythematosus, six with renal and three with central nervous system involvement, with three daily 1 gram intravenous doses of methylprednisolone and measured the immune response before and just after discontinuation of the drugs. After the treatment, the mean seum IgG, IgA and IgM levels were essentially unchanged. Likewise, serum C3 and C4 levels were not changed significantly. In nine of ten patients, methylprednisolone pulse therapy reduced the levels of circulating immune complexes ($p<0.05$). Thus the immediate clinical improvements with methylprednisolone pulse therapy are suggested to be the result of depression of the circulating immune complex levels.

Key Words: Methylprednisolone pulse therapy, circulating immune complexes, systemic lupus erythematosus

Corticosteroids are an important and at times lifesaving medication for the treatment of a wide range of diseases including systemic lupus erythematosus. They have many biological effects, but the pharmacologic mechanism of the action in systemic lupus erythematosus is still largely unknown.

To further increase the anti-inflammatory and immunosuppressive capabilities of corticosteroids, 'pulse' protocols have been devised where supraphar-macologic doses are given in an intermittent fashion. High-dose intravenous methylprednisolone pulse therapy has recently been advocated for the treatment of lupus nephritis (Cathcart *et al.* 1976) because of its large, favorable therapeutic effect in renal transplant rejection (Bell *et al.* 1971). Methylprednisolone pulse therapy may also be of value in life threatening nonrenal lupus erythematosus (Eyanson *et al.* 1980; Oto *et al.* 1981).

Many uncontrolled studies have suggested the effects of high-dose intravenous methylprednisolone pulse therapy on the immune mechanisms in relieving acute exacerbation of systemic lupus erythematosus (Eyanson *et al.* 1980; Isenberg *et al.* 1982). However little is known about the immediate effects of high-dose intravenuos methylprednisolone pulse therapy on the immune response in patients with systemic lupus erythematosus.

Immediate and dramatic clinical improvement in patients with systemic lupus erythematosus who were given with high-dose intravenous methylprednisolone pulse therapy prompted us to investigate patients with systemic lupus erythematosus to determine the immediate effects of high-dose intravenous methylprednisolone pulse therapy on serum immunoglobulins, complements and circulating immune complexes.

MATERIALS AND METHODS

Ten patients, one man and nine women, aged 18 to 38, were selected for study. Each patient had 4 or more of the 1982 revised American Rheumatism Association's criteria for the classification of systemic lupus erythematosus (Tan *et al.* 1982) and exhibited disease activity from 1+ to 4+ (mean activity score 2.3) (See Table 1). None of the patients had received treatment with any immunosuppressive agents other than oral glucocorticoids. Six patients had evidence of renal involvement and three patients exhibited central nervous system involvement. Patients 7 and 9

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* MPPT Methylprednisolone Palse Therapy

were given high-dose intravenous methylprednisolone pulse therapy due to severe arthritis and Raynaud's phenomenon.

Serum samples were obtained from each patient prior to and 4 hours after discontinuation of intravenous methylprednisolone, and stored at −70°C until analysed.

High-dose intravenous methylprednisolone pulse therapy was comprised of 1 gram methylprednisolone given intravenously in 200 ml normal saline over 2 hours on three successive days.

Serum IgG, IgA, IgM, C3 and C4 concentrations were measured by single radial immunodiffusion in agar using monospecific antisera (Behringwerké). Anti-DNA titrations were measured by the indirect immunofluorescent antibody technique (IFAT) using Crithidia luciliae as a substrate. Circulating immune complexes were measured by the solid phase Clq enzyme linked immunosorbent assay method (Singh *et al.* 1981).

Data were computed into means and standard deviations. The Wiolcoxon rank sum test was used for the significance testing of differences between pretreatment values and posttreatment values, and the Mann-Whitney U test for the significant testing of differences between the value of circulating immune complexs in responder and non-responder groups. The differences weer considered significant if $p<0.05$.

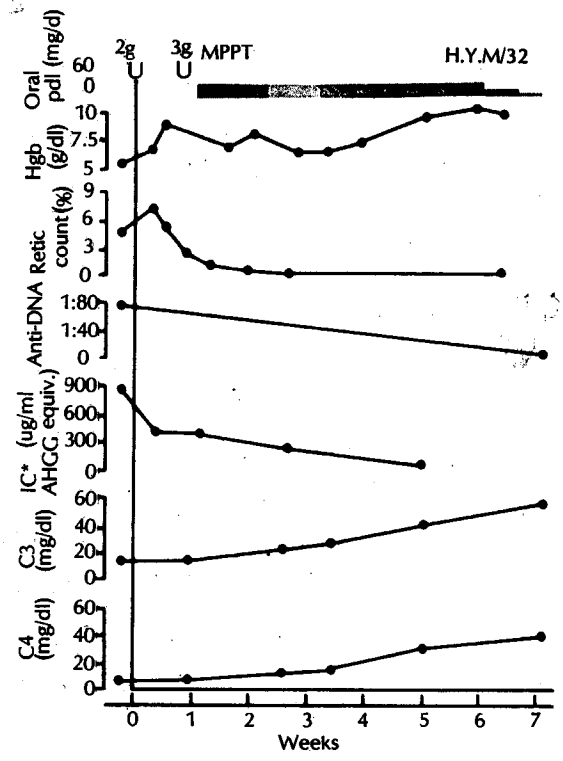

Fig. 1. Case study of a 32-year-old SLE patient given MPPT(methylprednisolone pulse therapy)
**IC = Circulating Immune Complexes*

CASE REPORT

Case 1

A 32-year-old man was admitted to the hospital with Coombs' positive hemolytic anemia, nephrotic syndrom, arthralgia, fever and erythematous skin rash. Laboratory evaluation included a Hgb of 5.4 g/dl and a reticulocyte count of 8%. Urine sediment revealed many white and red blood cells, and urinary protein excretion was 10-15 g/day. Renal biopsy showed membranous lupus nephritis. Serum C3 was below 16 mg/dl (normal 53-120 mg/dl) and C4 was below 6 mg/dl (normal 21-49 mg/dl). Anti-DNA antibody (IFAT) was detected at a titer of 1:80. Circulating immune complexes were 864 μg/ml AHGG equivalents.

The hemoglobin rose to 10 g/dl during the five weeks after high- dose intravenous methylprednisolone pulse therapy and the reticulocyte count fell rapidly from 6.6 to 1.0% on the 7th day after therapy. C3 and C4 levels began to rise slowly and were within normal limits at 6 weeks after pulse therapy. Anti-DNA antibody was detected at a titer of 1:20 on the 7th week after pulse therapy. Circulating immune complexes, initially very high, fell sharply following the pulse therapy accompanied with rapid improvement of hemolytic anemia and genreal symptoms (Fig. 1).

RESULTS

Of the 10 patients treated for active systemic lupus erythematosus, eight patients responded with immediate improvement in at least one parameter of disease activity (Table 1). Initial immunologic and laboratory profiles for all patients are illustrated in Table 2. ANA was detected at a titer of more than 1:160 in all patients. Anti-DNA was detected at a titer of more than 1:80 in 7 of 9 patients who underwent the test. Levels of circulating immune complexes were all elevated above the normal range of 25 μg/ml AHGG equivalent.

No significant changes in mean serum IgG, IgA and IgM concentrations were seen immediately after the therapy (Table 3, Figs. 2 and 3). Likewise no significant changes in mean serum C3 and C4 concentrations were seen immediately after pulse therapy. But

Table 1. Clinical activity in 10 patients with systemic lupus erythematosus

No.	Patient Sex/Age	No. of SLE criteria fulfilled	Disease severity					Therapeutic effect
			Disease* activity	Nephritis	Cerebritis	Vasculitis	Serositis	
1	M/32	8	3+	+	−	−	+	+
2	F/18	8	3+	+	+	+	+	+
3	F/26	7	4+	+	+	−	−	+
4	F/24	7	3+	−	−	−	+	+
5	F/38	4	2+	+	−	−	+	±
6	F/34	6	2+	+	−	−	−	+
7	F/34	4	1+	−	−	−	−	+
8	F/20	4	2+	−	+	−	−	+
9	F/27	5	1+	−	−	−	−	±
10	F/28	7	2+	+	−	−	−	+

* 0=inactive, 1+=minimal active, 2+=moderately active, 3+=severe, 4+=life threatneing
As used by Lahita et al. (1987)

Table 2. Immunologic and laboratory profiles of 10 patients with systemic lupus erythematosus

No.	Hgb	WBC	urine protein	ANA	AntiDNA	IgG	IgA	IgM	C3	C4	IC**
	(g/dl)	(×10/ml)	(g/24 hours)	(titer)		(mg/dl)			(mg/dl)		(ug/ml)
1	5.4	9.600	15.0	160[a]	80[b]	1.430	231	217	19.0	6.0	864
2	7.8	5.100	23.7	160	80	1.760	241	261	13.0	0.0	360
3	8.8	2.800	ND*	160	160	1.020	263	226	33.0	0.0	360
4	14.0	4.000	0.6	160	80	700	241	309	52.6	23.1	340
5	8.7	8.500	7.7	160	ND	602	171	54	42.7	15.6	78
6	7.3	4.600	1.8	160	80	651	171	109	24.6	5.0	90
7	10.8	8.100	0.2	160	80	3.590	220	319	49.2	28.6	264
8	10.3	4.700	ND	160	0	751	153	138	78.1	19.2	185
9	12.5	4.000	0.1	160	0	2.260	355	271	59.5	23.1	54
10	9.9	7.400	ND	160	160	963	210	71	56.0	39.1	300

* Not determined [a] More than 160 [b] More than 80
** Circulating immune complexes

Table 3. Effect of methylprednisolone pulse therapy on the humoral immunological values in 10 patients with systemic lupus erythematosus

Variables / Sampling time	Immunoglobulin (mg/dl, SRID) Mean±S.D.			Complement (mg/dl, SRID) Mean±S.D.		Immune complexes* (ug/ml AHGG equivalents) Mean±S.D.
	IgG	IgA	IgM	C3	C4	
Before "pulse"	1372.±948.9	217.4±52.3	197.4± 97.6	42.8±20.3	15.9±13.1	289.5±234.3
After "pulse"	1379.6±898.0	217.4±52.3	192.3±102.1	48.2±16.5	13.9± 4.1	177.0±120.3
p value (Wilcoxon rank sum test)	0.959	0.575	0.798	0.173	0.374	0.0069

SRID: Single radial immunodiffusion
AHGG: Aggregated human IgG
* Checked by solid phase Clq ELISA technique

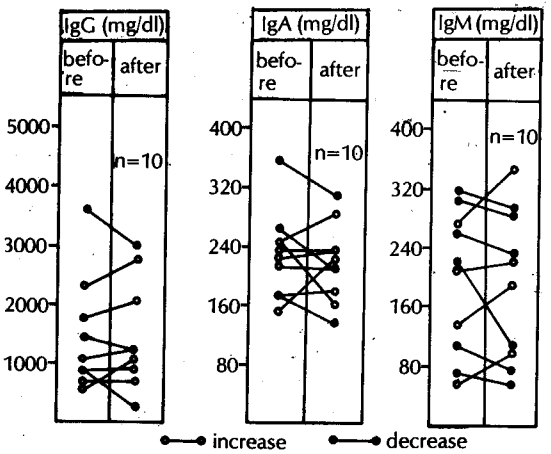

Fig. 2. Effects of methylprednisolone pulse therapy on serum immunoglobulin levels in 10 patients with SLE.

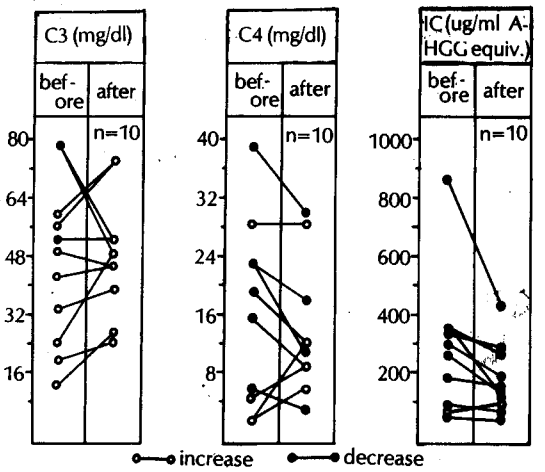

Fig. 4. Effects of methylprednisolone pulse therapy on serum complement and immune complex levels in 10 patients with SLE. AHGG : Aggregated human IgG.

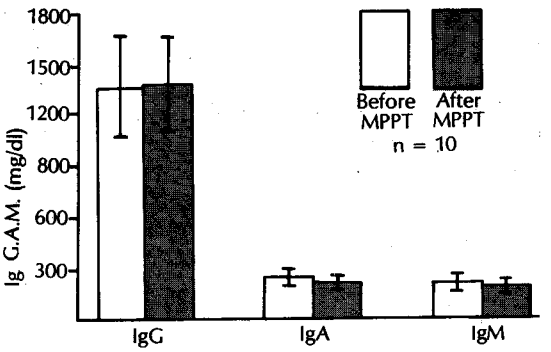

Fig. 3. Changes of immunoglobulin values (Mean±S.E.) after MPPT (methylprednisolone pulse therapy) in 10 patients with SLE

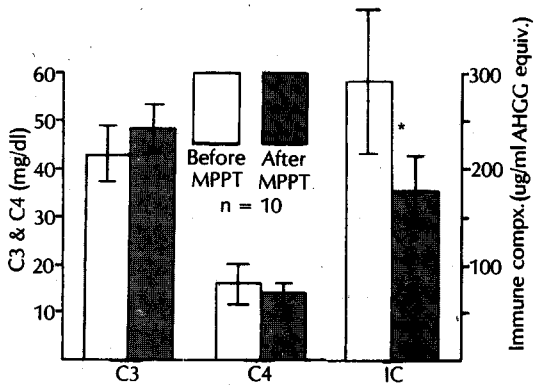

Fig. 5. Changes of complement and immune complex values (Mean±S.E.) after MPPT (methylprednisolone pulse therapy) in 10 patients with SLE
** p<0.01*

Table 4. Basal circulating immune complex levels and percent change after high-dose intravenous methylprednisolone pulse therapy

	Basal circulating immune complex (Mean±S.D.)	Percent change (Mean±S.D.)
Responders (n=8)	345.5±229.6*[a]	−36.1±18.2[b]
Nonresponders	66.0± 16.97	−6.5±30.4

* ug/ml AHGG equivalents

a; $p<0.05$ compared with values in the nonresponders

b; $p>0.05$

mean serum C3 concentration tended to be increased (+11%), and serum C3 concentration was increased in 8 or 80% of the patients treated (Table 3, Figs. 4 and 5).

Immediately after pulse therapy, there was a significant decrease in the circulating immune complex levels. The average net change in 10 patients was minus 39% and the concentrations were decreased in 9 or 90% of the patients treated (Table 3, Figs. 4 and 5). Responders were compared to non-responders with regard to pretreatment circulating immune complex levels and % change of circulating immune com-

plex. The mean level of pretreatment circulating immune complex was higher in the responder group than non-responder group ($p<0.05$), but the % change was not different significantly (Table 4).

DISCUSSION

High-dose intravenous methylprednisolone pulse therapy has been used for renal transplant rejection (Bell *et al.* 1971) and, more recently, for rapid progressive diffuse proliferative lupus nephritis (Cathcart *et al.* 1976; Cole *et al.* 1976) and for life threatening nonrenal lupus erythematosus (Eyanson *et al.* 1980). Despite the immediate efficacy of pulse therapy for active systemic lupus erythematosus, the precise mechanism of action of pulse therapy was not known well.

Three pulses of high-dose methylprednisolone did not significantly lower the serum IgG, IgA and IgM concentrations immediately after therapy. Although it has been reported that daily oral administration of corticosteroids in divided doses produced decreased serum IgG concentration, immunoglobulin levels were measured on an average of 22 days (Settipane *et al.* 1978) or 5-6 weeks (Butler and Rossen 1973) after corticosteriods were discontinued, and the decrease was only 22%. A recent study, in which six patients with rheumatoid arthritis were given one and three daily 1 gm intravenous pulses of methylprednisolone, suggested that this drug did not significantly lower the serum gammaglobulin concentration (Fan *et al.* 1978). The lack of significant change in IgG concentration might be related to the route of administration of the drug or to systemic lupus erythematosus patients having a different immunoglobulin turnover rate from that of normal subjects (Levy *et al.* 1970).

Our results showed that in 8 of 10 patients a dramatic improvement in several clinical parameters was obtained within 24 hours following the administration of methylprednisolone. The rapidity of the response suggested that the beneficial effect of methylprednisolone pulse therapy was most likely a consequence of the reduction of inflammation or its effect on the effector mechanisms of tissue damage rather than immune recognition, activation and immunoglobulin synthesis. This is supported by the observation that the circulating immune complex concentration fell rapidly, and serum C3 concentration tended to be increased after methylprednisolone pulse therapy. It is possible that methylprednisolone could affect the circulating immune complex concentration by other mechanisms other than the inhibition of antibody production. From the present study, indirect evidence about this proposition can be seen in the rapid decrease of the circulating immune complex concentration despite stable serum immunoglobulin concentration. Cairns *et al.* (1980) studied immune complex size in sequential sera from two patients with active lupus erythematosus treated with methylprednisolone pulse therapy. They found that a decrease in the levels of middle range complexes was seen by the end of the infusion and was accompanied by an increase in the levels of high molecular weight complexes. The immune complex concentration did not change in their study and this might be related to the method of assay, dependent on the inhibition of agglutination of latex IgG, which is a quantitative measure of IgG containing complexes in the test serum.

While some authors have suggested that its efficacy is the result of depression of the circulating immune complex levels (Levinsky *et al.* 1977), others have found no effect (Pussell *et al.* 1978). We have found that a good response to methylprednisolone pulse therapy was associated with a reduction in the circulating immune complex levels. In this connection it is of interest that, like Kimberly *et al.* (1981), we have found higher circulating immune complex levels in the group that responded. In conclusion, we have found that active systemic lupus erythematosus patients showed a marked improvement following the administration of three daily consecutive doses of methylprednisolone especially when their serum values of circulating immune comples were high. The immediate improvement in both renal and nonrenal manifestations is suggested to be the result of a reduction of complement fixing circulating immune complex levels.

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