THERAPEUTIC STUDIES OF THE COMBINATION OF DEOXYSPERGUALIN AND PREDNISOLONE IN MRL/lpr MICE WITH ADVANCED LUPUS-LIKE DISEASE

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Female MRL/lpr mice develop lesions closely resembling human systemic lupus, and therefore can serve as models in order to examine the efficacy of immunosuppressive agents. The present study was designed to evaluate the efficacy of the combination of deoxyspergualin with prednisolone compared with each alone in 13-week-old female MRL/lpr mice. After the onset of lymphadenopathy, splenomegaly, and the elevation of plasma autoantibodies, deoxyspergualin alone or prednisolone alone was effective. An immunosuppressive regimen of deoxyspergualin combined with prednisolone was demonstrated to be superior to each single therapy concerning the amelioration of advanced disease in the MRL/lpr mice without increasing toxicity.

Autoimmune MRL/lpr mice spontaneously develop a disease resembling systemic lupus erythematosus[1]. As a result, they are routinely used as animal models in which to evaluate the efficacy of various immunosuppressive agents. Several studies have shown that immunosuppressive therapy using cyclophosphamide, cyclosporin A, methotrexate, or prednisolone is effective in treating mice developing lupus-like lesions[2~5]. We previously found that a new immunosuppressive agent, deoxyspergualin, was also effective in mice suffering advanced renal disease[6,7]. The present study was designed to determine whether a therapeutic regimen of deoxyspergualin combined with prednisolone would retard the progressive lupus-like lesions of 13-week-old female MRL/lpr mice.

Materials and Methods

Animals

Female MRL/lpr mice were purchased from Clea Japan, Tokyo, and used at the age of 13 weeks.

Immunosuppressive Agents

Deoxyspergualin (DSG)[8] was supplied by Takara Shuzo Co., Ltd., Kyoto, Japan. It was dissolved in saline and sterilized by passage through a 0.22 μM filter. Prednisolone (PSL) sodium succinate from Shionogi Pharmaceuticals Co., Ltd., Osaka, Japan, was diluted by saline as required.

Measurement of Anti-DNA Titer in Plasma

Plasma from individual mice was assayed for the presence of autoantibodies to DNA using an enzyme-linked immunosorbent assay as previously reported[6].

Measurement of Blood Urea Nitrogen (BUN)

BUN was estimated by the urease-indophenol method using a DRI-CHEM 5500 autoanalyzer (Fuji Film Co., Ltd., Tokyo).

Peripheral Blood Cell Counts

Hematological analysis of red and white blood cell counts were carried out from retro-orbital plexus bleeds of mice using a Celltak MEK-4500 hemocytometer (Nihon Koden Co., Ltd., Tokyo).

Statistical Analysis

Data were analyzed by STUDENT's *t*-test.

Results

Monotherapy of DSG and PSL in 13-week-old MRL/lpr Mice

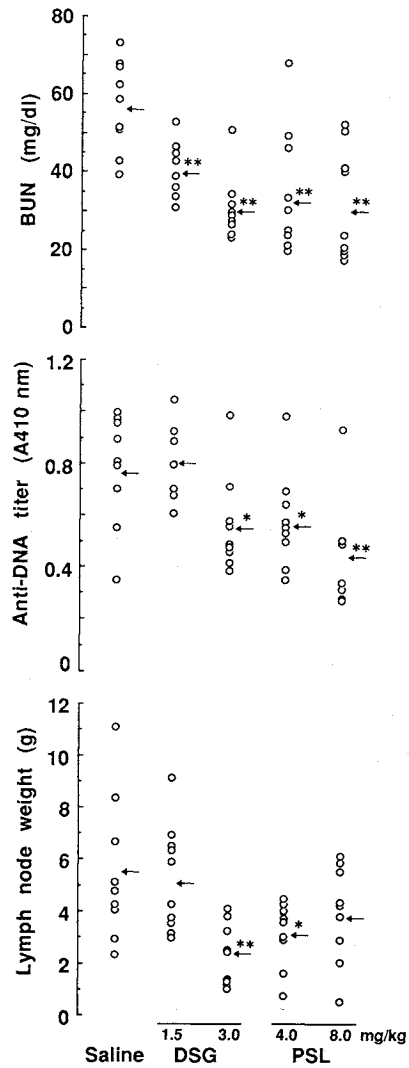
The female MRL/lpr mice we used already had lymphadenopathy, splenomegary, and an increasing anti-DNA titer at the age of 12 weeks (Table 1). Treatment with DSG or PSL was carried out from week 13 through week 20 in a regimen of five injections per week. The weight of systemic lymph nodes, plasma anti-DNA titer, and BUN values were measured (Fig. 1). The 20-week-old control mice had over 5 g of lymph nodes. The mean weight of the lymph nodes obtained from the mice treated with 3 mg/kg DSG was less than half of that of the control group. The animals receiving 4 and 8 mg/kg PSL had 58% and 68%, respectively, of the mean lymph node weight of the control group. There was a significant decrease in the plasma anti-DNA antibody level in the mice receiving 3 mg/kg DSG or 4 and 8 mg/kg PSL compared with the control group. The increase of the BUN value which accompanies the development of lupus nephritis was dose-dependently suppressed by treatment with 1.5 and 3 mg/kg DSG or 4 and 8 mg/kg PSL.

Table 1. Age-related change of lymph node and spleen weight, and plasma anti-DNA titer in female MRL/lpr mice.

Age (weeks)	*n*	Lymph node (mg)	Spleen (mg)	Anti-DNA titer (A410 nm)
8	8	100± 68[b]	189± 36	0.428±0.134
12	9	527±242	429±144	0.721±0.314
20	7	226±292	685±127	0.868±0.172

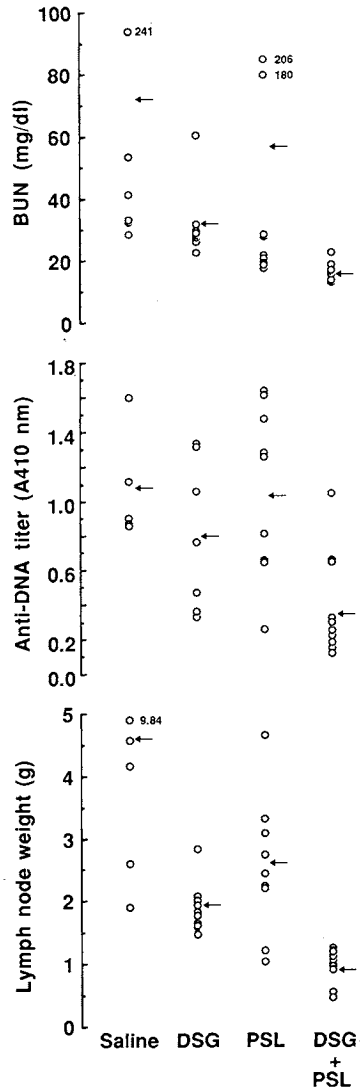
[a] Axillary and elbow lymph node weight.
[b] Mean with SD.

Fig. 1. Effect of single therapy of DSG and PSL on lymph node weight, anti-DNA titer, and BUN values in MRL/lpr mice.



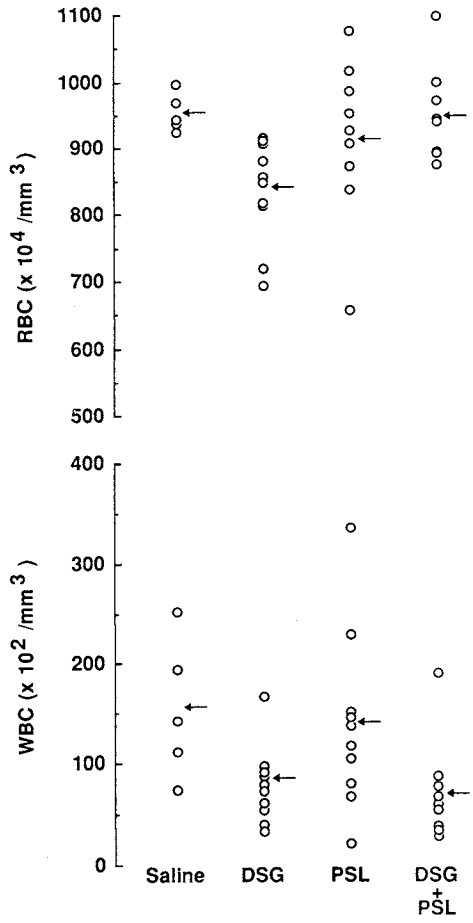
DSG and PSL were administered as 5 intravenous injections per week from week 13 through week 20. A set of control MRL/lpr mice received saline injections. The mesenteric, axillary, elbow, inguinal, submaxillary, and iliac lymph nodes were pooled individually. ←, mean values; * $P<0.05$, ** $P<0.01$ compared with the saline-treated group.

Fig. 2. Systemic lymph node weight, anti-DNA titer, and BUN values in MRL/lpr mice treated with DSG plus PSL, DSG alone, and PSL alone.



DSG (3 mg/kg), PSL (4 mg/kg), or DSG (3 mg/kg) plus PSL (4 mg/kg) were administered as 5 intravenous injections per week from week 13 through week 20. A set of control MRL/lpr mice received saline injections. The individual lymph node weight was measured as described in the legend of Fig. 1. Arrows show mean values. Statistical analysis was carried out between DSG plus PSL and each agent alone. The result for the lymph node weight was $P<0.01$ compared with each agent alone, for the anti-DNA titer, $P<0.05$ compared with DSG alone and $P<0.01$ compared with PSL alone, and for the BUN values, $P<0.01$ compared with each agent alone.

Fig. 3. Peripheral blood cell counts in MRL/lpr mice treated with DSG plus PSL, DSG alone, and PSL alone.



All experimental procedures were the same as those described in the legend of Fig. 2.

Combination Therapy of DSG and PSL in 13-week-old MRL/lpr Mice

Combined use of 3 mg/kg DSG and 4 mg/kg PSL was carried out under the same administration schedule described above. Results concerning the weight of systemic lymph nodes, the BUN values, and the circulating anti-DNA antibody levels are shown in Fig. 2. The combination therapy of DSG and PSL was more effective than either of the single therapies in suppressing the swelling of the systemic lymph nodes. The ability of the combined therapy

to reduce the increase of the anti-DNA titer was superior to that of either single agent alone. A significant decrease in the BUN value in the mice treated with DSG plus PSL was observed compared with that measured for the single agents alone. Especially, the BUN value of the combined group showed a normal value under 20 mg/dl.

As shown in Fig. 3, treatment with DSG alone resulted in a 12% decrease in peripheral red blood cell counts, but the combination of DSG plus PSL had no effect on the erythrocyte counts in comparison with that of the control group. The control mice showed a leukocytosis accompanied by the development of massive lymphadenopathy as described above. A significant decrease ($P<0.01$ as compared to the control group) in the peripheral leukocyte counts was observed in the mice receiving DSG alone; this was not observed in the group treated with PSL alone. There was no significant leukocyte count difference between the group treated with DSG alone and the combined therapy group.

Discussion

Our previous study of female MRL/lpr mice employed DSG administration starting at the age of 13 weeks when the lupus-like disease advanced, and was therapeutic in nature[7]. The present study in 13-week-old female MRL/lpr mice under the same administration regimen showed that DSG alone or PSL alone was effective. Since DSG does not have any anti-inflammatory activity, unlike glucocorticoids such as PSL, we could expect that its combined use with PSL would be more beneficial than either of the agents alone to ameliorate the advanced disease. We also showed that therapy using a combination of 3 mg/kg DSG and 4 mg/kg PSL was superior to each mototherapy. Significantly, it was very important that MRL/lpr mice receiving DSG plus PSL had normal BUN values. These results are consistent with those of SUZUKI *et al.*[9] and NEMOTO *et al.*[10] who found that a combination of DSG and corticosteroids was more effective therapy than DSG or corticosteroids alone when treatment was started at the rejection crisis in canine kidney or rat skin allotransplantation. The MRL/lpr mice receiving 3 mg/kg DSG alone showed a slight anemia, but the DSG-induced anemia never worsened in the combined use of DSG and PSL.

In conclusion, this study demonstrated that female MRL/lpr mice suffering with advanced lupus-like disease responded better to a combined therapy of DSG and PSL than to single therapy with either agent.

References

1) THEOFILOPOULOS, A. N. & F. J. DIXON: Etipathogenesis of murine SLE. Immunological Rev. 55: 179~216, 1981
2) SHIRAKI, M.; M. FUJIWARA & S. TOMURA: Long-term administration of cyclophosphamide in MRL/lpr mice. I. The effects on the development of immunological abnormalities and lupus nephritis. Clin. Exp. Immunol. 55: 333~339, 1984
3) MOUNTZ, J. D.; H. R. SMITH, R. L. WILDER, J. P. REEVES & A. D. STEINBERG: CS-A therapy in MRL-*lpr/lpr* mice: amelioration of immunopathology despite antoantibody production. J. Immunol. 138: 157~163, 1988
4) BARTLETT, R. R.; S. POPOVIV & R. X. RAISS: Development of autoimmunity in MRL/lpr mice and the effects of drugs on this murine disease. Scand. J. Rheumatol. 75 (Suppl.): 290~299, 1988
5) MIHARA, M.; A. KATSUME & Y. TAKEDA: Effect of methotrexate treatment on the onset of autoimmune kidney disease in lupus mice. Chem. Pharm. Bull. 40: 2177~2181, 1992
6) NEMOTO, K.; M. HAYASHI, Y. SUGAWARA, J. ITO, F. ABE, T. TAKITA, T. NAKAMURA & T. TAKEUCHI: Biological activities of deoxyspergualin in autoimmune disease mice. J. Antibiotics 41: 1253~1259, 1988
7) NEMOTO, K.; T. MAE, Y. SUGAWARA, M. HAYASHI, F. ABE & T. TAKEUCHI: Deoxyspergualin therapy in autoimmune MRL/lpr mice suffering advanced lupus-like disease. J. Antibiotics 43: 1590~1596, 1990
8) UMEDA, Y.; M. MORIGUCHI, H. KURODA, T. NAKAMURA, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Synthesis and antitumor activity of spergualin analogues. I. Chemical modification of 7-guanidino-3-hydroxyacyl moiety. J. Antibiotics 38: 886~898, 1985
9) SUZUKI, S.; R. HAYASHI, S. NIIYA, T. KENMOCHI, T. FUKUOKA & H. AMEMIYA: Remarkable recovery from rejection by treatment with deoxyspergualin and methylprednisolone on canine kidney allografts. Transplant. Proc. 23: 545~546, 1991

10) NEMOTO, K.; Y. SUGAWARA, T. MAE, M. HAYASHI, F. ABE, A. FUJII & T. TAKEUCHI: Therapeutic activity of deoxyspergualin in comparison with cyclosporin A, and its combined use with cyclosporin A and prednisolone in highly allogeneic skin transplantation in the rat. Agents Actions 36: 306~311, 1992