Bromocriptine and Low Dose Cyclosporine in the Treatment of Experimental Autoimmune Uveitis in the Rat

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Abstract

The immunologic effects of bromocriptine and low dose cyclosporine on experimental autoimmune uveitis (EAU) induced in Lewis rats by S-antigen immunization were studied. Rats treated with a sub-optimal dose (low dose) of cyclosporine (2 mg/kg per d), bromocriptine (1.8 mg/kg per d), or both drugs were compared with untreated rats in regard to the development of EAU, lymphocyte proliferative responses, and anti-S-antigen serum antibodies. Bromocriptine alone decreased the incidence of EAU only in female rats ($P < 0.01$), did not effect the lymphocyte proliferative response, but did significantly decrease antibody titers in both males ($P < 0.004$) and females ($P < 0.0005$). Low dose cyclosporine also partially decreased the incidence of EAU in female rats, but did not decrease antibody titers or lymphocyte proliferative responses. Bromocriptine plus low-dose cyclosporine led to more marked decreases in the incidence of EAU and anti-S-antigen antibody titers as well as in the lymphocyte proliferative assay ($P < 0.01$ for males, $P < 0.0005$ for females). This study suggests that bromocriptine can enhance the immunosuppression of low dose cyclosporine.

Introduction

Recent publications have described a regulatory effect of the neuroendocrine axis on the immune system (1, 2). The anterior pituitary hormone, prolactin, has shown promise as an immune modifier. Hypophysectomy in rats results in a marked suppression of antibody production or delayed type hypersensitivity (3, 4). Treatment of hypophysectomized rats with prolactin will restore immune competence, whereas concurrent treatment with prolactin and corticosteroids will suppress the immune response (3, 4). Bromocriptine is a dopamine agonist that will suppress the secretion of prolactin by the pituitary. Several studies suggest that it has immunomodulatory properties. It has been observed that bromocriptine therapy or hypophysectomy can suppress inflammation in the adjuvant arthritis model and that this effect can be overcome with the concurrent administration of prolactin (5). In addition, pretreatment of mice with bromocriptine for 1 wk will significantly suppress the ability of their lymphocytes to respond in a mixed lymphocyte reaction (6). Bromocriptine can also diminish graft versus host reactions in the mouse (6). These studies imply that prolactin is an important trophic hormone for the immune system and that reductions in serum prolactin by hypophysectomy or by bromocriptine may profoundly suppress the immune response.

In addition, prolactin appears to compete reversibly with the binding of cyclosporine to lymphocytes (6, 7). In addition, the effects of cyclosporine in vivo can be overcome by administration of a prolactin releasing agent (Sandoz 25-240 Sandoz Pharmaceuticals, Basel, Switzerland) to animals (6). This implies that prolactin and cyclosporine may have opposing actions in the regulation of the immune system. It is therefore possible that lowering serum prolactin levels will enhance the effect of cyclosporine or permit the dose of cyclosporine to be reduced. Previous studies have demonstrated that experimental autoimmune uveitis (EAU)[1] can be inhibited in the rat by 10 mg/kg per d of cyclosporine (8). This study demonstrates the effect of prolactin suppression by bromocriptine in combination with lower dose cyclosporine (2 mg/kg per d) on EAU induced by S-antigen immunization.

Methods

Induction of experimental autoimmune uveitis. Female and male Lewis rats weighing 175–200 g were used for this series of experiments. Bovine retinal S-antigen was prepared as previously described (8). Animals were immunized by injecting into each hind footpad 0.1 cm^3 of an emulsion containing 15 μg of S-antigen in phosphate-buffered saline (PBS) mixed with an equal volume of complete Freund's adjuvant (Gibco, Grand Island, NY) augmented with H37Ra Mycobacterium tuberculosis to a concentration of 2.5 mg/ml. 14 d after immunization, the animals were anesthesized with CO_2 and blood for serum was obtained by cardiac puncture. The animals were then killed with CO_2 and the eyes and draining popliteal lymph nodes were removed.

Treatment with cyclosporine and bromocriptine. The control group consisted of 12 female and 8 male rats that were immunized as above for the development of EAU and received daily sham injections.

There were four experimental groups in which the rats were immunized with S-antigen as described above. The animals treated with bromocriptine received daily intramuscular injections of the drug dissolved in ethanol/water (1:1) beginning 5 d before S-antigen immunization and continued for 14 d after immunization. Treatment with intramuscular cyclosporine dissolved in olive oil was begun on the day of immunization and continued for 14 d. The first experimental group of 12 female and 8 male rats received low dose cyclosporine (2 mg/kg per d). The second experimental group, also 12 female and 8 male rats, received 1.8 mg/kg per d of bromocriptine. The third experimental group, 12 female and 8 male rats, received both cyclosporine and bromocriptine using the above dosage schedule. The fourth experimental group consisted of 8 female rats that received daily high dose cyclosporine (10 mg/kg per d).

Lymphocyte proliferation assay. Cultures were performed on animals from each treatment group. The cells from the popliteal lymph nodes of each animal were harvested by gentle teasing and cultured at a concentration of 1×10^6 cells/ml in 96 well culture plates (Costar, Cambridge, MA). Each well contained 200,000 cells in 0.2 ml culture medium consisting of 0.2 cm^3 of RPMI 1640 (Gibco, Grand Island, NY) supplemented

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Received for publication 27 August 1986 and in revised form 23 October 1986.

The Journal of Clinical Investigation, Inc.
Volume 79, April 1987, 1078–1081

1. *Abbreviations used in this paper:* EAU, experimental autoimmune uveitis; SI, stimulation index.

with 5% fetal calf serum (Hy-clone, Logan, UT) and 100 U/ml of penicillin and 100 μg/ml of streptomycin. Quadruplicate culture wells of cells with media, concanavalin A (Con A) 1 μg/well (used to determine that the cells were capable of proliferating) and S-antigen 5 μg/well were done on each animal. The cultures were incubated at 37°C in 5% CO_2 for 3 d and then pulsed with 1 μCi of [^{3}H]thymidine (New England Nuclear, Boston, MA) and incubated for an additional 16 h. The plates were then harvested, counted in a scintillation counter, the quadruplicate well counts were averaged and a stimulation index (SI) was calculated for each set of quadruplicates by dividing the experimental mean by the control mean.

Ocular histology. The eyes were removed and fixed in 4% glutaraldehyde, embedded in glycol methacrylate, sectioned, cleared, and then stained with hematoxylin and eosin. The presence of ocular inflammation as defined by the presence of intraocular lymphocytes and photoreceptor destruction was read by an impartial observer, using previously published criteria (9). An animal was considered to have EAU if one or both eyes had inflammation.

ELISA assay for anti-S-antigen antibody: 96 well flat bottomed plates (Costar) were coated with 50 μl of a 8 μg/ml solution of S-antigen in PBS and incubated for 1 h at 37°C. The plates were then washed three times in 1% bovine serum albumin (BSA) in PBS, incubated with BSA in PBS for 1 h at 37°C, emptied, and stored at 4°C. A dilution of 1:2500 of serum in PBS was used. 50 μl of serum were incubated at 37°C in the precoated plates for 1 h. The plates were then washed three times with 0.1% Tween (Sigma Chemical Co., St. Louis, MO) in PBS, incubated at 37°C for 50 μl of a 1:2,000 dilution of goat anti rat IgG (Kirkegaard and Perry, Gaithersburg, MD), washed three times with Tween and PBS, incubated with 50 μl each of Peroxidase Substrate Solutions A and B (Kirkegaard and Perry) and incubated for 15 min. A positive and negative standard was run on each plate and the optical density was read on a Minireader II ELISA reader (Dynatech, Alexandria, VA). The optical densities of the samples were normalized to the standard for the purposes of comparison.

Statistics. The data was analyzed utilizing the Statistical Analysis System (SAS, Cary, NC). The chi-square test was used for 2 × 3 comparisons and Fisher's Exact test was used for 2 × 2 comparisons of categorical data. The Student *t* test was used to compare continuous data between the treatment groups.

Results

Table I summarizes the incidence of ocular inflammation in the experimental groups. All untreated animals developed EAU, whereas, as previously reported (8), animals treated with 10 mg/kg of cyclosporine did not develop inflammation. The lower dose of 2 mg/kg of cyclosporine resulted in a lower incidence of disease in female rats (5/12, $P < 0.008$). Bromocriptine alone appeared to decrease the incidence of EAU in the female rats when compared with controls (6/12, $P < 0.01$), but not in the male rats. However, both female and male rats showed complete protection from EAU when treated with both bromocriptine and low dose cyclosporine ($P < 0.0005$ for females and $P < 0.0001$ for males for a 2 × 3 chi-square test with 2 degrees of freedom). The table demonstrates that the combined therapy compared with therapy of either cyclosporine or bromocriptine alone significantly diminished the occurrence of ocular inflammation (Fisher's exact test).

Figs. 1 and 2 summarize the lymphocyte proliferative responses to S-antigen in female and male rats, respectively. Both figures demonstrate a marked decrease in the lymphocyte responses of animals treated with combined bromocriptine plus low dose cyclosporine. Female rats with combined therapy had a mean SI of 1.7 compared with either control animals (mean 4.5, $P < 0.0005$; Student *t* test) or animals treated with bromocriptine alone (mean 3.6, $P < 0.0005$; Student *t* test) or cyclosporine alone (mean 5.6, $P < 0.001$; Student *t* test). Male rats with combined therapy had a mean SI of 1.1 compared to either control animals (mean 3.8, $P < 0.0005$; Student *t* test) or animals treated with bromocriptine alone (mean 2.6, $P < 0.0005$; Student *t* test) or cyclosporine alone (mean 3.4, $P < 0.001$; Student *t* test).

Table II summarizes the ELISA for S-antigen antibodies. In both male and female rats, bromocriptine alone significantly decreased circulating S-antigen antibodies ($P < 0.0005$ for female rats and $P < 0.005$ for male rats) compared with control rats. Low dose cyclosporine did not significantly decrease serum antibody. The combined cyclosporine plus bromocriptine-treated animals showed significantly decreased serum antibody, but this difference was significantly lower than the bromocriptine group in the male animals only ($P < 0.0005$).

Table I. Incidence of EAU in Rats Immunized with S-Antigen and Treated with Cyclosporine (CSA) or Bromocriptine (BR)

Group	Animals with EAU/total	Fisher's exact test Compared with CSA + BR
Female Lewis rats		
Untreated	12/12	$P < 0.0001$
CSA 10 mg/kg	0/8	not significant
CSA 2 mg/kg*§	5/12	$P < 0.04$
BR 1.8 mg/kg*‡	6/12	$P < 0.01$
CSA 2 mg/kg + BR 1.8 mg/kg*	0/12	—
* 2 × 3 chi square test; $P < 0.0005$ Fisher's exact test compared with untreated; ‡ $P < 0.008$, § $P < 0.01$		
Male Lewis rats		
Untreated	8/8	$P < 0.0002$
CSA 10 mg/kg	—	—
CSA 2 mg/kg**	5/8	$P < 0.03$
BR 1.8 mg/kg**	8/8	$P < 0.0002$
CSA 2 mg/kg + BR 1.8 mg/kg**	0/8	—
** $P < 0.0001$ using 2 × 3 chi square test		

All drugs were given daily as described in Methods.

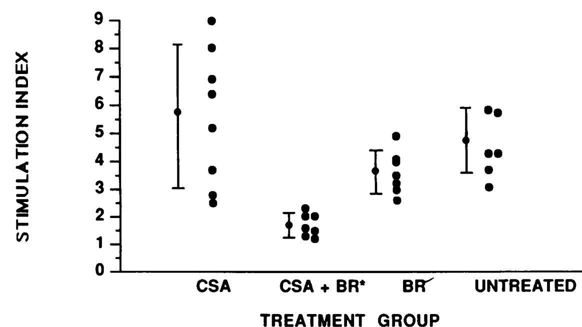

Figure 1. Lymphocyte proliferation stimulation indices in female rats treated with bromocriptine (BR) (1.8 mg/kg per d), cyclosporine (CSA) (2 mg/kg per d), combined therapy (CSA + BR) or no therapy. Mean±1 SD is shown. *$P < 0.0005$ compared with untreated group (Student *t* test).

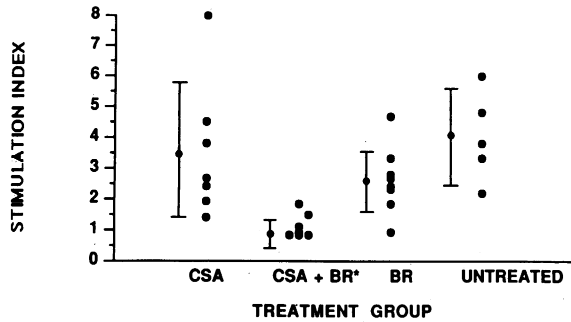

Figure 2. Lymphocyte proliferation stimulation indices in male rats treated with bromocriptine (BR) (1.8 mg/kg per d), cyclosporine (CSA) (2 mg/kg per d), combined therapy (CSA + BR) or no therapy. Mean±1 standard deviation is shown. *$P < 0.0005$ compared with untreated group (Student t test).

Prolactin was significantly suppressed by bromocriptine in both the female and male rats in this study. The serum prolactin 24 h after the last dose of bromocriptine was (female and male, respectively): untreated, 110±32 ng/ml and 19.6±4.5 ng/ml; cyclosporine only, 71±25 ng/ml and 14.3±3.5 ng/ml; bromocriptine only, 5.9±3 ng/ml and 2.5±0.5 ng/ml; cyclosporine plus bromocriptine, 7.7±3 ng/ml and 7.4±1.5 ng/ml.

Discussion

This study demonstrates that combined therapy with low dose cyclosporine plus bromocriptine suppressed the development of experimental autoimmune uveitis in Lewis rats more than either drug alone. In addition, the combined therapy resulted in lower lymphocyte proliferative responses and serum antibody production indicating that this combination is significantly more immunosuppressive than the two separately. Bromocriptine treatment alone did decrease antibody production, did not decrease lymphocyte proliferative responses and only partially decreased the incidence of EAU in female rats. Cyclosporine at the suboptimal dose used in this study did not significantly decrease serum antibody titers or lymphocyte proliferative responses, but did result in a partial decrease in the incidence of EAU.

Prolactin is a polypeptide hormone secreted by the anterior pituitary gland. Its known physiologic functions include stimulation of growth of mammary tissue and of the avian crop sac and induction of lactation. Its role in the male is less well defined. Many cells including lymphocytes express prolactin cell surface receptors (10). Prolactin is able to induce increased ornithine decarboxylase activity in lymphocytes, an enzymatic step that is integrally involved in the initiation of the immune response (10, 11). Cyclosporine has been shown to inhibit the ability of prolactin to increase ornithine decarboxylase activity in rat kidney, spleen, thymus, and other tissues (10, 11).

Cyclosporine is a fungal peptide that is immunosuppressive primarily because of its ability to interfere with the production of interleukin 2 (12). Two recent reports have documented that cyclosporine can competitively inhibit the binding of prolactin to the lymphocyte (6, 7). It has been hypothesized that cyclosporine may alter lymphocyte responsiveness by acting as a prolactin antagonist (6). The effect of cyclosporine in graft rejection can be overcome by elevating serum prolactin levels (6). Cyclosporine H, which is not immunosuppressive, does not alter the binding of prolactin to the lymphocyte (7).

Prolactin at physiologic concentrations can enhance lymphocyte proliferative to concanavalin A in vitro (13). Several investigators have shown that a decrease in serum prolactin is associated with an impairment of the immune response. Both antibody production (3) and skin test responses to a T cell antigen (4) are decreased by hypophysectomy and can be restored with replacement of prolactin alone. A parallel effect on antibody synthesis or cell mediated immunity is observed after treatment with bromocriptine (14), an ergot-derived dopamine agonist that will markedly inhibit prolactin secretion in doses such as those used in this study. Similarly, the adjuvant-induced arthritis model is significantly inhibited by hypophysectomy or bromocriptine therapy (5). Another autoimmune model, experimental allergic encephalitis, was also studied demonstrating a marginally significant decrease in the severity of this disease model following bromocriptine therapy (14). Bromocriptine has also been re-

Table II. S-Antigen Serum Antibody in Rats Immunized with S-Antigen and Treated with Cyclosporine (CSA) or Bromocriptine (BR)

Group	n	Mean*	Std Dev.	t-Test compared to Untreated	t-Test compared to CSA + BR
Female Lewis rats					
Untreated	12	1.72	0.63	—	$P < 0.001$
CSA, 10 *mg/kg*	4	0.152	0.07	$P < 0.0001$	$P < 0.04$
CSA, 2 *mg/kg*	12	1.49	0.51	$P < 0.3$	$P < 0.002$
BR, 1.8 *mg/kg*	12	1.02	0.42	$P < 0.004$	$P < 0.18$
CSA, 2 *mg/kg* + BR, 1.8 *mg/kg*	11	0.75	0.51	$P < 0.001$	—
Male Lewis rats					
Untreated	8	1.49	0.34	—	$P < 0.0005$
CSA, 10 *mg/kg*	—	—	—	—	—
CSA, 2 *mg/kg*	8	1.05	0.66	$P < 0.1$	$P < 0.008$
BR, 1.8 *mg/kg*	8	0.81	0.21	$P < 0.0005$	$P < 0.001$
CSA, 2 *mg/kg* + BR, 1.8 *mg/kg*	8	0.34	0.12	$P < 0.0005$	—

All drugs were given daily as described in Methods. * Mean optical density.

ported to decrease in vivo macrophage tumoricidal activation by lymphokines (15).

Our experimental results with bromocriptine as a sole therapy in EAU demonstrate that this drug is capable of modulating some aspects of the immune system. We can infer from the data in the literature that the mechanism of immune regulation is via the effect of bromocriptine on prolactin secretion. Our data differ from previous reports in that we found no effect on lymphocyte proliferation in vitro, whereas Hiestand et al. (6) did demonstrate an effect on the mixed lymphocyte reaction using cells removed from bromocriptine-treated animals. This may be the result of a differential sensitivity of lymphocyte subsets to prolactin. It may also reflect differences in the dose of bromocriptine used or the species and sex of the experimental animals. However, as also reported by Nagy et al. (14), bromocriptine alone did decrease antibody production in the present study. Berczi et al. showed a marked effect of bromocriptine in the adjuvant arthritis model in female rats (5), but did not investigate male rats. Our data confirm this effect in our model in the female rat, however, we did not detect a significant effect in male rats. Although prolactin was significantly decreased in both males and females treated solely with bromocriptine, the decrease was significantly more pronounced in the female animals.

The combination of low dose cyclosporine and bromocriptine may work via two distinct mechanisms. The first is the reduction of serum prolactin concentration thereby lessening its stimulation of the immune system. The second might be that the reduction in serum prolactin results in less cyclosporine being required to attain an immunosuppressive effect. Further studies are required to elucidate this mechanism. However, our data demonstrates a significant immunosuppressive interaction between the two drugs. This combined effect was clearly present in regard to the development of EAU and lymphocyte proliferative responses, but was less pronounced in the level of S-antigen antibody response because bromocriptine alone significantly reduced the titers.

Cyclosporine therapy in human eye disease has been complicated by dose-related renal toxicity (16). Lower doses of this drug will produce less toxicity, but are also less effective. A drug such as bromocriptine, which may enhance the effectiveness of lower doses of cyclosporine may potentiate the immunosuppression and produce less long-term toxicity. Many human ocular inflammatory diseases are chronic and require extended therapy; therefore, minimizing toxicity is important. Bromocriptine has been clinically utilized for many years for the treatment of prolactinoma and Parkinson's disease with minimal serious complications. A recent report described four patients with recurrent anterior uveitis that were treated with bromocriptine for one of the above medical indications and had a remission of their ocular inflammation (17). This report may be a demonstration of the effects of hormonal regulation on the immune system and several clinical studies are currently underway to test this effect.

The interaction of the endocrine and immune systems has only recently begun to be explored. In addition to prolactin, other hormones have immunomodulatory properties. Growth hormone, which is structurally similar to prolactin, has an immunostimulatory effect, whereas ACTH and corticosteroids are immunosuppressive (18). Both estrogen and testosterone affect immune function, but may stimulate or inhibit the immune response, depending on the assay that is used (2). Lastly, there is evidence that the immune responses may regulate neural and endocrine function (1, 2). These interactions may lead to the development of novel methods of regulating the immune system and treating inflammatory diseases.

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