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Synergism between long-acting bromocryptine microcapsules and cyclosporine A in the prevention of various autoimmune diseases in rats

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Abstract. Pre-treatment of male Sprague-Dawley rats with long-acting bromocryptine microcapsules (CBLA) significantly inhibited the arthritic response to Freund's complete adjuvant and reduced weight loss, thymolysis, splenomegaly and leukocytosis. In the prevention of adjuvant arthritis (AA), the combination of CBLA plus sub-optimal doses of cyclosporine A (CsA) was more efficient than either of the drugs alone. Sub-optimal doses of CsA were 0.1 and 1.0 mg/kg/day s.c. for 5 days. Furthermore, CBLA alone did not decrease the incidence of experimental allergic uveitis (EAU) in the male Lewis rats. Low-dose CsA reduced the incidence of uveitis by 50%, and with the addition of CBLA, 100% of rats were protected. Low-dose CsA was 2 mg/kg/day i.m. for 14 days. Long-term treatment of male Sprague-Dawley rats with CBLA alone reduced the incidence and severity of spontaneous autoimmune periarteritis nodosa (PN) in a dose-dependent manner; CsA was less potent than CBLA, and only additive effects were obtained. Finally, for the prevention of spontaneous autoimmune insulin-dependent diabetes (DM), the administration of CBLA did not improve the effect of a low-dose CsA in male BB rats. Nevertheless, a delay in onset of DM could be achieved. A sequential therapy using CsA plus CBLA clearly showed beneficial effects. The dose of CsA was 10 mg/kg p.o. 6 days/week for 21 weeks. Compared with Sprague-Dawley or Lewis male rats, BB male rats showed only weak prolactin suppression after the same doses of CBLA. It is suggested that the use of CBLA may be particularly beneficial in autoimmune disorders. The effectiveness of the combination therapy CBLA plus CsA, however, was dependent on the model considered. Various factors could play a role: (1) the different ways of administering CsA (s.c. in AA, i.m. in EAU and PN, oral in DM); (2) strain-dependency in the capacity of CBLA to suppress Prl secretion; and (3) at least in the BB rats, the transient increase of CsA bioavailibility which was possibly induced by CBLA.
Key words. Adjuvant arthritis; experimental allergic uveitis; periarteritis nodosa; diabetes mellitus; prolactin; bromocryptine; cyclosporine A.

Freund's complete adjuvant (FCA) triggers the development of an autoimmune disease in rats, the so-called adjuvant arthritis [1]. An extensive network of neuroendocrine processes may play an important role in the pathogenesis of AA [2]. This includes an early enhancement of prolactin (Prl) mRNA synthesis [3] and increase of Prl secretion [2, 3]. Daily injections of bromocryptine mesylate (CB-154) – an ergot derivative that inhibits Prl secretion – reduce the severity and incidence of AA in rats, as does hypophysectomy [4]. The effect of CB-154 is reversed by administration of ovine Prl. We show here that CB-154 injected in the morning allows an escape from the suppression of Prl release during the night. This could contribute to incomplete immunosuppression. The i.m. injection of encapsulated bromocryptine (CBLA, Parlodel LA), however, has a long-acting effect. CBLA blocks the development of AA and suppresses FCA-induced increased ornithine decarboxylase (ODC) activity in the lymphoid tissues [5]. ODC is an enzyme involved in cell growth, proliferation and differentiation [6].

Furthermore, injection of FCA containing bovine retinal S-antigen into the hind footpad produces experimental uveitis (EAU) in the rat [7]. Cyclosporine A (CsA) – a well-known immunosuppressive agent [8] – inhibits the development of both AA and EAU [7, 8]. In female rats, CB-154 enhanced the effect of low-dose CsA in the model of EAU [9]. In humans, CB-154 alone was found to be effective against chronic recurrent uveitis [10] and psoriasis [11].

Periarteritis nodosa (PN) is a rare autoimmune disease in humans which affects small- and medium-sized arteries and occurs predominantly in males. A condition similar, if not identical, to the human disease occurs spontaneously in rats [12]. PN in rats is an age-related autoimmune process, in which endocrine dysregulations might play a role. Thus, PN is induced by chronic administration of oestrogen [13], while in female rats, the disease is prevented by long-term treatment with CB-154 [14].

Finally, the BB rat is accepted as the most useful animal model of human type I (insulin-dependent) diabetes. BB rats spontaneously develop diabetes mellitus leading to death unless insulin injections are given. Humoral autoimmunity, like production of islet cell-, insulin- and thyroglobulin autoantibodies, as well as cellular immune

reactions, have been described. It has been reported that general immunosuppression by administration of high-dose CsA inhibits the manifestation of diabetes in BB rats. The administration of CB-154, however, does not improve or prolong the protective effect of short-term CsA therapy [15].

Most previous reported work was in females. The aim of this work was to investigate the effect of CBLA alone or CBLA plus CsA on various autoimmune diseases in male rats.

Materials and methods

Induction of adjuvant arthritis. Ten male Sprague-Dawley rats (100–150 g) per group were pair-housed in a temperature- (24 °C) and light-controlled (12 h/day) room. Food and water were given ad libitum. AA was induced by a single i.d. injection of FCA (at the base of the tail), containing heat-killed *Mycobacterium butyricum* (0.1 to 1.0 mg/rat on day 0; Difco, Detroit, IL). The hind limb thickness was measured at three different positions with a linear gauge coupled to an electronic readout (Mitutoyo, Tokyo). Percent of reduction of the arthritic disease was defined as follows:

$$1 - \frac{\text{hind limb thickness of the group X growth controls}}{\text{positive controls} - \text{growth controls}} \times 100$$

Treatment of AA rats. Suspensions of CBLA or placebo microcapsules (Sandoz, Basel) were prepared in the dextran vehicle provided with the drug. Given 3 days before FCA, CBLA or placebo microcapsules were injected i.m. while the rats were under ether anaesthesia. Doses of 1.0–10.0 mg/kg of CBLA were injected in a volume of 0.1 ml (i.e. 0.05 ml in each thigh).

For comparison, suspensions of CB-154 were prepared in isotonic saline. CB-154 was injected s.c. while the rats were under ether anaesthesia. A dose of 5 mg/kg was injected daily for five days (beginning on day 0).

CsA (Sandimmune, Sandoz, Basel) was dissolved in ethanol (25 g/ml) and diluted to 50 mg/ml with isotonic saline. The drug was administrated s.c. at sub-optimal doses of 0.1 and 1.0 mg/kg for 5 days after immune stimulation. Ten mg/kg of CsA was necessary to completely inhibit AA in preliminary studies. Cardiovascular and renal functions were determined in the laboratory of Dr H. Siegl, Sandoz, Basel.

Blood and tissue collection. Between days 3 and 4 after FCA, four rats per group were killed by decapitation every 2 h for 24 h (beginning at 08.00 on day 3). Trunk blood was taken. Serum and thymus were kept at −20 °C and −80 °C, respectively, until examination. On day 22 after FCA, the rats were killed. Trunk blood was taken, leukocytes counted, and thymus and spleen weight were noted.

Thymus ornithine decarboxylase activity. The thymus ODC was determined as reported elsewhere [5]. Briefly, tissues were homogenised and centrifuged, and the supernatant fractions were incubated for 30 min at 37 °C in glass tubes—fitted with a rubber stopper and center wells containing filter paper—in the presence of [1-^{14}C]ornithine hydrochloride (Amersham, Arlington Heights, IL). The $^{14}CO_2$ liberated from the enzymatic reaction was collected on the filter papers, and radioactivity was counted in Toluol/Omnifluor scintillant (New England Nuclear, Boston, MA). Protein concentrations in homogenates were measured spectrophotometrically at 595 nm using Bio-Rad Dye Reagents (Bio-Rad, Richmond, CA).

Induction of experimental allergic uveitis. EAU was induced in Lewis rats (n = 8 or 12 females and 8 males per group) weighing 174–203 g. Bovine retinal S-antigen was prepared as described by Nussenblatt et al. [7]. Animals were stimulated by injecting into each hind footpad 0.1 ml of an emulsion containing 15 mg of S-antigen in phosphate buffered saline (PBS) mixed with an equal volume of FCA containing 1 mg/ml *M. butyricum*. Fourteen days after immunisation, the animals were killed with CO_2, and the eyes were removed, fixed in 4% glutaraldhehyde bedded in glycol methacrylate, sectioned, cleared and then stained with haematoxylin and eosin (all from Fluka, Buchs, Switzerland). The presence of occular inflammation, defined by the presence of intraoccular lymphocytes and photoreceptor destruction, was read by an impartial observer, using previously published criteria [7]. An animal was considered to have EAU if inflammation was present in one or both eyes. Control groups received an injection of vehicle (PBS + paraffin oil) given intramuscularly.

Treatment of EAU rats. There were five groups in which the rats were immunised with S-antigen. The groups that were pre-treated with CBLA 10 mg/kg (i.m.) received the drug 3 days before immunisation. Treatment with i.m. CsA (2 or 10 mg/kg/day) dissolved in olive oil was begun on the day of immunisation (day 0) and continued for 14 days.

Periarteritis nodosa. The development of PN was followed in 589 Sprague-Dawley rats (eight weeks old), equally divided into 1 control and 11 treated groups. The rats were kept in a pathogen-free, temperature- and light-controlled room. A full necropsy was carried out on all animals dying spontaneously as well as those surviving until the end of the study (two years). The latter were killed by CO_2 inhalation. Tissues were fixed in 4% buffered formaldehyde, trimmed, embedded in paraplast, cut at 5 μm and routinely stained with haematoxylin and eosin (Fluka). In male rats, the testis is the most suitable organ for microscopic evaluation of PN, because the arteries of this organ are commonly affected in this species. The vessels of the testis were

therefore used to grade the lesion according to their severity as follows:

0 Normal
1 Occasional and/or slight perivascular inflammatory cell infiltration, and/or slight medial hyperplasia
2 Generalised slight perivascular inflammatory cell infiltration, slight medial hyperplasia and single vessels with fibroid material in the wall
3 Up to one-third of the vessels with fibroid material in the wall, perivascular inflammatory cell infiltration and/or moderate proliferation causing some stenosis of the vessels
4 As grade 3, with up to one-half of the vessels involved
5 As grade 4, with over half the vessels involved

A mean 'vessel score' for each treatment group was obtained using the following formula:

$$\frac{(5n_5+4n_4+3n_4+2n_2+n_1)}{N}$$

where n_5 is the number of animals with grade 5 lesions, n_4 is the number of animals with grade 4 lesions and so forth, and N is the total number of animals examined in that treatment group.

Treatment of PN rats. CBLA (0.1 to 10 mg/kg, given monthly) and/or CsA (2 or 10 mg/kg/day for five days, every two weeks) were injected intramuscularly.

Type I diabetes mellitus. Male BB/W/D rats were derived from a colony kindly supplied by Dr A. A. Like, University of Massachusetts, Worcester (USA). The criterion for diabetes manifestation was persistent hyperglycaemia with blood glucose values above 250 mg/dl for five days. Blood was obtained by cutting the tail tip during the experiment and by decapitation at the end.

Treatment of BB rats. CBLA (10 mg/kg) was given i.m. biweekly. Empty microcapsules served as control. Injection sites were changed each time from one leg to another.

CsA was the drinking solution for humans (concentration 100 mg/ml) diluted with Miglyol 812 (a neutral oil of Dynamit Nobel, Castrop-Rauxel, Germany). One milliliter of the final concentration was applied per kilogram of body weight. CsA or Miglyol 812 was given six days per week.

A group of 24 male BB rats constituted the control group, which received CsA vehicle (Miglyol 812) from day 50 up to 150 and placebo microcapsules in dextran vehicle biweekly. Group 2 was treated with CsA (4 mg/kg/day) plus empty microcapsules from 50 to 150 days of age. A third group in addition to CsA received CBLA every 14 days. Group 4 received CsA (10 mg/kg/day) and placebo microcapsules from 50 to 63 days of age. Group 5 received CsA between days 50 and 63 and CBLA between days 64 and 150.

Serum levels of prolactin, bromocryptine and cyclosporine A. In general, the rats were killed between 10.30 and 11.30 (with the exception of those in the study of circadian rhythm of Prl secretion upon FCA). After centrifugation at 2000*g*, the serum was stored at −20 °C. The serum level of prolactin, bromocryptine and/or CsA was determined by radioimmunoassays at Sandoz Ltd., Basel (laboratories of Dr P. Marbach and P. C. Hiestand), using iodine-radiolabelled compounds and an LKB Ria-gamma counter (Pharmacia, Dübendorf, Switzerland).

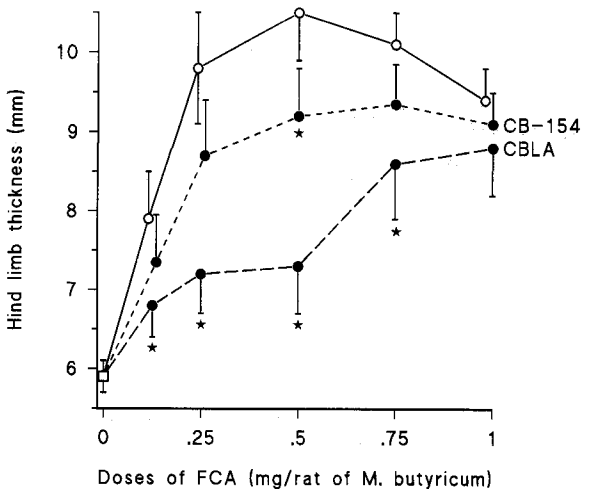

Figure 1. Inhibition of AA in male rats by bromocryptine microcapsules is dependent on the dose of injected heat-killed mycobacteria. Ten male rats per group were pre-treated with 1.0 mg/kg of bromocryptine microcapsules (CBLA, i.m.) or placebo microcapsules three days before administration of different doses of FCA (0.1, 0.5 and 1.0 mg/rat heat-killed *M. butyricum*, i.d.). For comparison, a dose of 5 mg/kg of CB-154 was injected daily for five days (beginning at time of immunisation with 0.5 mg/rat of *M. butyricum*, i.d.). The hind limb thickness was measured on day 22 after FCA. □, Growth controls; ○, FCA plus placebo microcapsules; ●, FCA plus bromocryptine (CBLA or CB-154); ★, Significant inhibition of hind limb swelling compared with positive controls.

Statistics. Results of experiment 1 (AA) were analysed by one-way analysis of variance, with $p < 0.05$ as significant level. The results of experiments 2 (EAU) and 3 (PN) were examined statistically using the two-way Fisher-Yates test. In experiment 4 (BB rats), the chi-square test allowed the observation of significant differences at the $p < 0.05$ level.

Results

AA in male Sprague-Dawley rats. The concentration of mycobacteria in the suspending vehicle influenced the incidence of arthritis and the efficiency of CBLA (fig. 1). The hind limb swelling produced by a low dose of FCA (0.1 mg/rat of *M. butyricum*) was significantly inhibited (by 52%) with a dose of 1 mg/kg of CBLA. The AA induced by an optimal dose of FCA (0.5 mg/rat) was inhibited by 70% both in severity (fig. 1, table 1) and incidence (data not shown), while the disease produced by higher doses of FCA (1.0 mg/rat)

Table 1. Prevention of arthritis induced by Freund's complete adjuvant (0.5 mg/rat of *M. butyricum*, i.d., on day 0) in male rats. The hind limb thickness is given for day 22 after stimulation. Synergism between long-acting bromocryptine microcapsules (CBLA, i.m., on day −3) and sub-optimal doses of cyclosporine A (s.c., for five days beginning on day 0).

	Cyclosporine A (mg/kg/day)[a]		
	0	0.1	1.0
Hind limb thickness in mm (mean ± SEM)[b]			
Positive controls	10.8 ± 0.7	9.4 ± 0.6	8.3 ± 0.4*§
CBLA 0.1 mg/kg	7.7 ± 0.8§	7.1 ± 0.7§	6.7 ± 0.5*§
CBLA 1.0 mg/kg	7.4 ± 0.6§	6.9 ± 0.4§	6.3 ± 0.3*§
CBLA 10.0 mg/kg	7.9 ± 0.5§	6.6 ± 0.5§	6.4 ± 0.2*§

[a]Ten mg/kg of CsA is necessary to inhibit adjuvant arthritis completely.
[b]The growth controls have a hind limb thickness of 5.9 ± 0.4 mm.
*Significant inhibition compared with positive controls without daily treatment with cyclosporine A (analysis of variance < 0.05).
§Significant inhibition of hind limb swelling compared with positive controls without pre-treatment with CBLA.

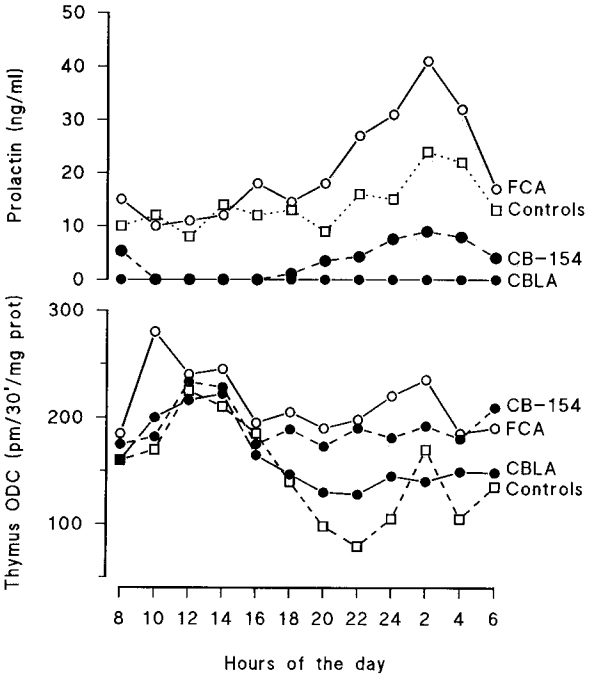

Figure 2. Circadian rhythms of serum prolactin levels and thymus ornithine decarboxylase activity (medians), 3–4 days after administration of FCA (0.5 mg/rat of heat-killed *M. butyricum*, i.d.). Effect of bromocryptine microcapsules (CBLA, 1 mg/kg, i.m.) and bromocryptine mesylate (CB-154, 5 mg/kg/5 days, s.c., at 9.00). □, Untreated controls; ○, FCA plus placebo microcapsules; ●, FCA plus bromocryptine (CBLA or CB-154).

was not significantly reduced by pre-treatment with CBLA. Additional pathophysiological alterations occurred after inoculation of supra-optimal doses of mycobacteria, e.g. heart rate at rest > 450 per minute, proteinuria, and decreased water and sodium excretion. The animals lost weight and had fever. CBLA reduced the weight loss and transient high blood pressure, but did not affect high heart rates, alterations of renal functions or fever (data not shown).

Measured 22 days after FCA, the degree of thymolysis and splenomegaly positively correlated with the hind limb thickness ($r = 0.95$ and 0.78, $p < 0.001$ and 0.005, respectively). Pre-treatment with 1 mg/kg of CBLA reduced both splenomegaly (by 15%) and thymolysis (by 52%) induced by an optimal dose of FCA (0.5 mg/rat of mycobacteria). The major alterations induced by FCA in the peripheral blood included an increase in the total leukocyte count (150% over placebo-treated controls). Inhibition of FCA-induced leukocytosis with CBLA reached 45% (data not shown).

The maximal effect of CBLA occurred with the lowest dose tested (0.1 mg/kg) (table 1). CB-154 was less potent, the maximal reduction of AA being 33% (fig. 1). One mg/kg CBLA continuously suppressed Prl release for 15 days (data not shown). Figure 2 shows that, 3–4 days after FCA, an injection of CB-154 in the morning, but not the pre-treatment with CBLA, allows an escape of Prl secretion during the night. This is accompanied by an incomplete suppression of the FCA-induced enhancement of thymus ODC activity.

CsA inhibited AA in a dose-dependent manner, with a plateau near 100% efficiency (data not shown). Thus, 10 mg/kg of CsA are necessary to inhibit AA completely. A sub-optimal dose of CsA (0.1 mg/kg/day) reduced the severity and incidence of AA only by 30% (table 1) and 40%, respectively. Additional pre-treatment with CBLA reduced the severity of the arthritic disease by 90% and the incidence by 80%. Compared with the difference between growth controls and positive controls, the mean loss of body weight was inhibited by 32–44% with either CBLA or CsA alone (0.1 mg/kg/day) and by 56–79% with the combination of CBLA plus the same low dose of CsA ($p < 0.001$) (data not shown). Thus, such a combination was more efficient than either CBLA or a sub-optimal dose of CsA alone.

EAU in Lewis rats. Table 2 illustrates that CBLA alone decreases the incidence of EAU in the female Lewis rat. Low-dose CsA (2 mg/kg/day i.m. for 14 days) reduces the incidence of uveitis by 50%, and with addition of CBLA 100% of the rats were protected. In male rats, CBLA alone did not block the development of EAU. However, low-dose CsA also reduced the incidence of uveitis by about 50%, and with the addition of CBLA all animals were protected.

PN in male Sprague-Dawley rats. Long-term treatment of male rats with CBLA reduced the incidence and severity of PN in a dose-dependent manner (table 3). CsA alone only weakly suppressed the development of PN, while CBLA seems the most potent drug. Combination of both drugs produced only additive effects.

Type I DM in male BB rats. The percentage of male BB rats with diabetes (i.e. blood glucose > 250 mg/dl for five days) among the five groups is given in table 4. The

Table 2. Incidence of experimental allergic uveitis in male and female Lewis rats immunised with retinal S-antigen (day 0) and treated with cyclosporine A (i.m., for 14 days beginning on day 0) and/or bromocryptine microcapsules (CBLA, i.m., on day −3). The presence of ocular inflammation as defined by the presence of intraocular lymphocytes and photoreceptor destruction was estimated on day 15 after immunisation.

	Cyclosporine A (mg/kg/day)		
	0	2	10
A. Female rats			
Controls	12/12	5/12	0/8
CBLA 10 mg/kg	6/12*	0/12*	nd
B. Male rats			
Controls	8/8	5/8	0/8
CBLA 10 mg/kg	8/8	0/8*	nd

*Significantly decreased incidence compared with positive controls receiving CsA or not (Fisher-Yates test, $p < 0.05$).
nd = not determined.

Table 3. Incidence of macroscopic periarteritis nodosa in male rats (over 2 years): influence of cyclosporine A (i.m., for 5 days every two weeks) and/or bromocryptine microcapsules (CBLA, i.m., given monthly) on lesion severity as assessed by histological examination.

	Cyclosporine A (mg/kg/day)		
	0	2	10
A. Mean 'vessel score' of all rats[a]			
Controls	2.96 (50)	2.82 (49)	2.37 (49)*
CBLA 0.1 mg/kg	2.79 (49)	2.61 (49)	2.24 (49)*§
CBLA 1.0 mg/kg	2.20 (49)*	2.00 (49)*+	1.55 (49) *§+
CBLA 10.0 mg/kg	1.67 (49)*	1.88 (49)*+	1.18 (49)*+
B. Mean 'vessel score' of rats dying spontaneously[a]			
Controls	2.85 (33)	2.51 (30)	2.12 (30)*
CBLA 0.1 mg/kg	2.60 (25)	2.45 (22)	1.86 (22)*§
CBLA 1.0 mg/kg	1.70 (17)*	1.65 (17)*+	1.45 (20) *§+
CBLA 10.0 mg/kg	1.41 (17)*	1.44 (16)*+	1.26 (19)*+
C. Mean 'vessel score' of 2-year-old rats[a]			
Controls	3.18 (17)	2.89 (19)	2.42 (19)*
CBLA 0.1 mg/kg	3.00 (24)	2.89 (27)	2.56 (27)*
CBLA 1.0 mg/kg	2.46 (32)*	2.19 (32)*+	1.62 (29)*§+
CBLA 10.0 mg/kg	1.81 (32)*	1.64 (33)*+	1.13 (30)*§+

[a]Number of animals is in parentheses.
*Significant decreased score compared with untreated controls (Fisher-Yates test, $p < 0.05$).
§Significant decreased score compared with rats treated with CBLA alone.
+Significant decreased score compared with rats treated with cyclosporine A alone.

percentage of BB rats that develop diabetes in this colony was between 50 and 80%; therefore, there must be more than 20 rats per group to achieve statistical significance.

The rats received either combination therapy or sequential therapy. Treatment with low-dose CsA (4 mg/kg p.o.) increased the incidence of diabetes (18/24 = 75%) when compared with the sham-treated control group (13/24 = 54%). In the group with combination therapy, injection of CBLA (10 mg/kg i.m.) did not affect the diabetes rate induced by the low dose of CsA (17/24 = 71%).

At various time points there was considerable variation in plasma concentration of active compound (e.g. in BB rats receiving either CBLA alone or CsA alone: 7–17, 5–7 and 3–11 ng/ml bromocryptine, or 11–4100, 4–450 and 10–86 ng/ml CsA, 4 h, 1 day and 10 days after beginning, respectively). Figure 3 shows that during the first two weeks of combination therapy CBLA may allow higher bioavailability of CsA. This period (days 80–100) was associated with a delay in the development of DM (table 4). Thereafter, there was no difference in rats receiving CsA alone, including the steady increase in bioavailability during the next two weeks. In addition, the large variation in measured Prl levels (on day 150) leaves open whether CBLA administration caused a decrease of secretion in BB rats: controls, 5.4 ± 2.2 ng/ml; CsA (4 mg/kg) 4.0 ± 2.4 ng/ml; CsA plus CBLA 2.4 ± 0.4 ng/ml. Ten times less CBLA per kilogram completely inhibited Prl secretion for 12–15 days in Sprague-Dawley and Lewis rats.

Table 4. Incidence (%) of spontaneous autoimmune diabetes mellitus in male BB rats (24 animals per group). Effect of combination therapy low-dose cyclosporine A (CsA, 4 or 10 mg/kg per os, 6 days/week) plus bromocryptine microcapsules (CBLA, 10 mg/kg i.m., biweekly) or of sequential therapy short-term high-dose CsA plus CBLA.

	Age (days)								
	70	80	90	100	110	120	130	140	150
A. Untreated controls	0%	0%	12%	21%	25%	42%	46%	50%	54%
B. Combination therapy (days 50–150)									
CsA (4 mg/kg)	0	8	12	17	29	42	54	67	75*
CsA + CBLA	0	0	0	0	12	42	62	67	71*
C. Sequential therapy (CsA, days 50–63; CBLA, days 64–150)									
CsA (10 mg/kg)	0	0	0	0	0*	12*	25*	42	42
CsA + CBLA	0	0	0	0	0*	12*	17*	17*§	17*§

*Significant differences with the control group (chi-square test, $p < 0.05$).
§Significant decreased incidence compared with the respective CsA-treated group.

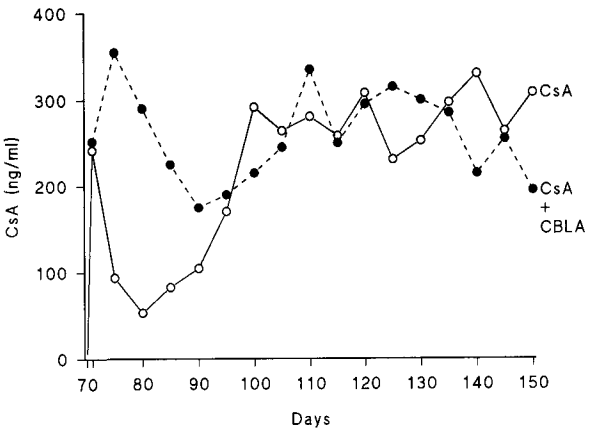

Figure 3. Whole blood CsA levels in BB male rats given CsA alone (○, 4 mg/kg p.o., six days per week) or combination of CsA plus bromocryptine microcapsules (●, CsA + CBLA, 10 mg/kg, i.m.) (n = 24 rats/group). CsA level was estimated by radioimmunoassay every five days after administration of the drug. The medians are presented because of the very high inter-individual variability. The bioavailability of CsA is increased during the first two weeks of combination therapy.

In the group with sequential therapy, short-term treatment with a higher dose of CsA (10 mg/kg) reduced the frequency of diabetes (9/24 = 37%, compared with 54% in the control group). This difference was not significant ($p = 0.11$), but the delay of diabetes onset supports an effect of the drug. Administration of CBLA starting after discontinuation of CsA further reduced diabetes rate (4/24 = 17%, $p < 0.05$). On day 150, serum Prl was not significantly different between the groups: CsA (10 mg/kg for the first 14 days), 3.8 ± 1.1 ng/ml; CsA plus conversion to CBLA, 3.0 ± 0.8 ng/ml. Again, this observation raises the question whether BB rats are less sensitive to bromocryptine than other strains.

In rats developing DM, the increased blood glucose as measured on days 145–150 was not significantly different between the control and treated groups (data not shown).

Discussion

Pre-treatment of male Sprague-Dawley rats with CBLA significantly inhibited the arthritic response to FCA, and reduced thymolysis, splenomegaly and leukocytosis. Thymolysis probably occurs after chronic elevation of serum corticosterone at onset of hind limb swelling [3]. The fact that CBLA efficiently suppresses the arthritic response induced by an optimal dose of FCA (i.e. 0.5 mg/rat of mycobacteria), but not by higher doses, supports the hypothesis that Prl amplifies the response of an ongoing autoimmune reaction [3, 4]. Interestingly, in a time-course study, two of five rats that received CBLA developed joint lesions on day 24, i.e. approximately nine days after the exhaustion of the microcapsule. No beneficial effect was observed when bromocryptine treatment was initiated after the establishment of the disease in this model (unpubl. observations).

It seems sufficient to block the peak of Prl secretion during the early latency period after inoculation of heat-killed mycobacteria to observe an inhibition of the arthritis response. In the suppression of FCA-induced thymus ODC activity and of the subsequent arthritic disease, CB-154 is less potent than the encapsulated form (CBLA). This could be due to the incomplete inhibition of the FCA-induced Prl secretion by CB-154. The difference becomes apparent during the dark phase (20.00 to 02.00).

In the prevention of AA, the combination of CBLA plus low dose of CsA was more efficient than either of the drugs alone. On the one hand, CBLA is an immunosuppressive drug acting via inhibition of Prl secretion and is efficient during the latency period, i.e. before the onset of arthritis (days 1–9). On the other hand, CsA has both immunosuppressive and anti-inflammatory properties; i.e., it is also efficient during the active inflammatory period (days 10–21).

Furthermore, CBLA alone decreased the incidence of EAU in the female but not in the male Lewis rats. This difference may be due to a higher expression of Prl receptors in females. Low-dose CsA reduced the incidence of uveitis by 50%, and with the addition of CBLA 100% of rats were protected. These results with CBLA are similar to those reported by Palestine et al. [9] using CB-154. In humans, bromocryptine seems to have a prophylactic effect on anterior uveitis [16]. The administration of CB-154 and CsA, either alone or in combination, results in down regulation of the serum levels of antinuclear autoantibody [17]. Interestingly, in systemic lupus erythematosus and probably other connective tissue disorders, the increased level of Prl correlates with the presence of autoantibodies, e.g. anti-dsDNA and anti-cardiolipin autoantibodies [18].

In female rats, a reduction in the incidence and severity of PN and a decreased incidence of spontaneous mammary tumours were obtained with CB-154 [14]. In our study, long-term treatment of male Sprague-Dawley rats with CBLA alone reduced the incidence and severity of PN in a dose-dependent manner; CsA alone was less potent than CBLA alone, and only additive effects were obtained.

Finally, compared with Sprague-Dawley or Lewis male rats, BB male rats showed only weak Prl suppression after the same doses of CBLA. This may be responsible for the low efficiency of CB-154 [15] or CBLA alone in this model. Similarly, CB-154 is unable to suppress Prl secretion in the nonobese diabetic mouse [19]. In this model, nevertheless, the incidence of diabetes was significantly lower in female mice receiving CB-154 injections.

In BB rats, low dose of CsA induces a striking increased incidence of type I DM, while a higher dose has beneficial effects. CsA aborts lymphocyte activation at an early stage in vitro. It is therefore widely assumed that this also occurs in vivo. However, in certain animal

models, it has been suggested that T cells (and perhaps B cells) can become primed and even proliferate in the presence of CsA, and it has been postulated that the drug has a different mode of action in vivo and in vitro [20].

The co-administration of CBLA seems unable to modify the effect of low-dose CsA. A delay in onset could occur, but in the long term the increased incidence after low-dose CsA is unchanged. Similar disappointing results are reported in humans [21]. On the other hand, the sequential therapy of CsA plus CBLA clearly showed beneficial effects. The reason for this synergy needs to be explored. There are many interactions between CsA and Prl. Thus, CsA not only interferes with Prl binding to its receptors on lymphocytes [22], but also inhibits Prl induction of ornithine decarboxylase in various rat tissues [23] and increases the action of Prl on gonadal functions [24]. In addition, CsA stimulates Prl production [25] that can subsequently be inhibited by bromocryptine.

Beside suppression of Prl secretion, two other mechanisms for the beneficial effect of bromocryptine must be considered: (1) a direct effect at the level of leukocytes or other cells participating in the inflammatory process, and/or (2) a change in CsA bioavailability.

Direct treatment of T lymphocytes with CB-154 causes inhibition of IL-2-, antigen- or allogen-induced proliferation [26]. However, this inhibition is observed only at concentrations that are 50- to 100-fold greater than those found in vivo (unpubl. observations).

More important is the possibility that CBLA could induce a transient change in CsA bioavailability. Our data suggest an association between the delay in DM-onset observed with the combination therapy (CsA plus CBLA) and a transient increase in CsA bioavailability. Acute increase of CsA in blood has been reported after co-administration with ketoconazole, erythromycin or cimetidine, which all interfere with the hepatic microsomal cytochrome P-450 activity [27, 28]. In humans, both bromocryptine and CsA compete as substrates of cytochrome P-450 3A [29]. This could explain the higher CsA levels measured in BB rats during the first weeks of combination therapy. In addition, a long-term influence of CBLA is not excluded. As previously reported with higher doses of CsA [30], there is a steady increase in bioavailability of the drug over time. The plateau is reached after about one month. The suggested mechanism is an induction of gut receptors for the drug [31]. Whether or not CBLA interferes in this system is unknown.

The use of CBLA may be particularly beneficial in some autoimmune disorders and offers an alternative to the current regimen, such as azathioprine and steroids. Suppression of circulating Prl by bromocryptine appears to improve the immunosuppressive effect of low-dose CsA [9]. Alternatively, CBLA may be used to decrease the dose of CsA, and thereby reduce the risk associated with long-term CsA therapy. The low dose of CsA as opposed to the high dose must be defined for each model. The comparison is difficult because of (1) the wide variability in CsA pharmacokinetics that depends e.g. on method of administration, strain, age and disease state; (2) the possible modification in CsA bioavailability induced by CBLA; and (3) the strain-dependency of CBLA efficiency in suppressing Prl secretion. However, in most models, it can be concluded that Prl suppression plays an essential role in the beneficial effect of bromocryptine.

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