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The long-acting dopamine agonist bromocriptine mesylate as additive immunosuppressive drug after kidney transplantation

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Abstract

Background. Acute rejection is an important risk factor for kidney graft loss. As evidence suggests that prolactin has important immunostimulatory properties, we conducted a randomized, prospective open trial in which bromocriptine, a drug suppressing prolactin secretion, was administered as an additive immunosuppressive drug after first cadaver kidney transplantation.

Methods. In the treatment group bromocriptine was given intramuscularly to 22 patients after their first kidney transplantation along with conventional immunosuppression (cyclosporin A, glucocorticoids). Twenty-three patients receiving only conventional immunosuppression served as control subjects. The incidence of acute graft rejections, graft losses, and infections was evaluated.

Results. Serum prolactin concentrations were slightly elevated above normal values before transplantation (32 ± 5.3 ng/ml) and decreased to values between 13 and 16 ng/ml in the control group and were totally suppressed in the bromocriptine group. After 6 months of follow-up overall patient and allograft survival was 97.7% and 91% respectively. Acute rejection episodes occurred in 31 patients (77.5%): 15 in the bromocriptine group *vs* 20 in the control group (n.s.). In each group eight patients experienced a cytomegalovirus infection. The incidence of severe bacterial infections (i.e. pneumonia and sepsis) was five and six respectively. The necessity of haemodialysis after transplantation was 3 1% in the patients on bromocriptine and 23% in those without.

Conclusions. Suppression of circulating prolactin concentration by bromocriptine did not improve the clinical outcome of patients after kidney transplantation receiving cyclosporin and prednisolone.

Key words: acute rejection; bromocriptine; immune system; kidney transplantation; prolactin

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Introduction

A link between the neuroendocrine and immune systems has long been postulated. The first report of an immunoenhancing effect of prolactin (PRL), a 24-kDa single-chain hormone secreted by the anterior pituitary, dates from 1983 [1]. Prolactin receptors were demonstrated on human T and B lymphocytes [2,3] and antibodies against pituitary prolactin have been shown to potently inhibit human lymphocyte proliferation in response to T and B cell rnitogens [4]. In addition, a pathogenic role of prolactin in adjuvant arthritis [5], iridocyclitis [6], and rejection episodes after heart transplantation [7] has been postulated. These observations suggested that PRL may be involved in the regulation of humoral and cell-mediated immunity, and led to the assumption that suppression of prolactin might result in suppression of the immune system [8].

Indeed, further studies demonstrated that treatment with bromocriptine, a dopamine receptor agonist suppressing pituitary prolactin secretion, inhibited the development of experimental uveitis in rats [9], as well as endogenous iridocyclitis [6], rheumatoid arthritis [10], experimental allergic encephalitis [11], and experimental SLE [12]. In 1988 a reduced incidence of acute rejection episodes through inhibition of prolactin release by dopamine and bromocriptine as adjuvant to conventional immunsuppressive therapy in human heart transplantation [13] as well as prolonged mouse skin graft survival [14] were reported.

These clinical findings correlate well with *in vitro* studies using human lymphocytes where bromocriptine suppressed mixed lymphocyte reactions in a dose-dependent fashion. Furthermore, combined treatment of bromocriptine and cyclosporin resulted in an additive suppressive effect on T-cell proliferation and IL -2 receptor expression [15].

The aim of the present study was to determine the value of adding the dopamine agonist bromocriptine mesylate to standard immunosuppressive therapy (cyclosporin A, glucocorticoids) with the objective of decreasing the incidence of acute rejection episodes after kidney transplantation.

Subjects and methods

Patients

The study design was randomized, prospective, and open. The study population consisted of 45 recipients of first cadaver kidney transplants, who had $<5\%$ preformed cytotoxic HLA antibodies. Patients were randomly assigned by chart numbers to one of two groups. Details on the study population show a well-balanced distribution of patients following randomization (Table 1).

Group 1 included 23 patients who received conventional immunosuppression with cyclosporin and prednisolone. Twenty-two patients were assigned to group 2 with the following protocol: oral administration of bromocriptine, 5 mg/day until anticoagulant therapy or haemodialysis were stopped, and then intramuscular injection of 100 mg of the long-acting dopamine agonist bromocriptine mesylate (Parlodel LAR®, Sandoz, Basel, Switzerland) monthly, along with standard immunosuppression as in group 1.

In both groups cyclosporin (Sandoz, Basel, Switzerland) was administered intravenously (3 mg/kg body-weight per day) for the first 72 h after transplantation followed by oral administration twice daily. True plasma levels were monitored daily during hospitalization, and during every outpatient visit thereafter, and were maintained between 120 and 140 ng/ml during the first postoperative month and between 100 and 120 ng/ml thereafter. Cyclosporin levels and doses did not differ between the two groups throughout the study period (Table 2).

Prednisolone was started on the day of transplantation at a dose of 200 mg/day and tapered by 40 mg/day until a dose of 20 mg/day was reached. The dose was reduced further to a maintenance dose of 5–10 mg/day as soon as possible, but in general after 3–6months. Azathioprine was added to standard therapy at a dose of 1 mg/kg/day after the first rejection episode.

Plasma prolactin concentrations were measured preoperatively, on days 1, 3 and 5 postoperatively, and at monthly intervals thereafter.

Table 1. Patient characteristics

	Bromocriptine	Controls	*P*
Male : female	12 : 10	16 : 07	NS
Age (mean ± SD)	49.6 ± 13.2	52.0 ± 14.8	NS
Duration of dialysis (mo) prior to NTX (mean ± SD)	18.2 ± 9.6	22.3 ± 10.8	NS
Donor age (mean ± SD)	50.0 ± 12.4	46.2 ± 13.4	NS
Donor sex (% male)	63	63	
Preformed cytotoxic HLA antibodies (%)	0.75	0.78	NS
HLA mismatches			
HLA-A	0.77 ± 0.52	0.78 ± 0.63	NS
HLA-B	0.77 ± 0.52	0.74 ± 0.54	NS
HLA-DR	0.59 ± 0.49	0.39 ± 0.5	NS
Cold ischaemia (h)	21.5 ± 7.9	19.4 ± 5.4	NS
Necessity of haemodialysis after NTX	31%	23%	NS

Laboratory investigations

Duplicate determinations of circulating prolactin were performed by an enzyme-linked immunoadsorbent assay (Boehringer Mannheim, Mannheim, Germany). Plasma cyclosporin concentrations were measured by a monoclonal fluorescence polarization immunoassay (Abott Chicago, USA).

Follow-up

After discharge, patients were seen once a week for 1 month, every 14 days thereafter for an additional 4 weeks, and at least once a month thereafter.

Acute allograft failure and acute allograft rejection

Acute postoperative allograft failure was defined by the necessity of haemodialysis within the first week after engraftment. If allograft function did not improve, a percutaneous needle biopsy was performed after 4–6 days and every 5–7 days thereafter if necessary. Allograft rejection was suspected clinically if serum creatinine concentration rose or urinary volume decreased in the absence of clinical signs of dehydration or elevated cyclosporin levels. In patients with laboratory evidence of cyclosporin toxicity the dose of the drug was reduced and rejection was suspected if allograft function did not improve within 3 days. In all patients a renal ultrasonography, including the measurement of a resistence index, was performed on a regular basis. The diagnosis of rejection was followed by a 3-days pulse therapy with dexamethasone in a dosage of 100 mg i.v. per day. In 26 patients diagnosis of rejection was confirmed histologically by the Banff criteria [27]. In case of steroid resistance and in patients with severe histological signs of rejection, treatment was performed either with ATG (Thymoglobuline, Pasteur–Merrieux France) or with OKT 3 (Orthoclone OKT 3, Cilag, Switzerland).

Cytomegalovirus (CMV) infection was diagnosed serologically by determination of pp 65 antigenaemia twice a week, by weekly screening for seroconversion or by virus isolation from urine. Treatment with gancyclovir was initiated if at least one of the above-mentioned tests gave a positive result and one clinical sign compatible with CMV infection was found. Ganciclovir was administered i.v. for at least 10 days

Table 2. Cyclosporin concentrations and doses (mean ± SD)

Days	30	60	90	120	150	180
Bromocriptine						
CsA-serum concentration (ng/ml)	135.8 ± 6.9	138.0 ± 9.3	128.0 ± 11.4	136.1 ± 9.4	127.4 ± 10.5	115.7 ± 10.2
CsA dose (mg/kg/day)	3.21 ± 0.35	2.75 ± 0.32	3.13 ± 0.31	3.01 ± 0.27	2.86 ± 0.27	2.63 ± 0.27
Controls						
CsA serum concentration (ng/ml)	129.8 ± 6.2	137.5 ± 12.3	134.6 ± 10.3	129.8 ± 6.7	121.6 ± 10.9	119.4 ± 10.6
CsA dose (mg/kg/day)	3.33 ± 0.32	3.12 ± 0.32	3.01 ± 0.26	2.85 ± 0.24	2.51 ± 0.66	2.52 ± 0.23

in a dose adjusted to excretory allograft function. All patients on anti-rejection treatment, including a mono- or polyclonal antibody, additionally received an anti-CMV treatment prophylactically.

The study was approved by the Human Ethics Committee of the University of Vienna Medical School, and all patients were asked to give their informed consent.

Statistical analysis

To test for a significant difference between the treated and untreated patient groups Fisher's exact test was performed for rejections and HLA classification, and Spearman rank correlation was calculated for creatinine and cyclosporin A levels and doses.

Results

Patient and allograft outcome

Overall patient survival was 97.7% after 6 months, one patient in the control group died of fungal septicaemia 4 months after engraftment. Allograft survival at the end of the 6 months follow-up period was 91%. In the control group one patient lost his graft from irreversible rejection after 10 days, as did two patients receiving bromocriptine after 10 and 14 days respectively. In the bromocriptine group one additional patient lost his graft after 5 months. Histologically, chronic allograft rejection was the prominent feature. In the remaining patients serum creatinine levels did not differ significantly between the two groups throughout the study period (Table 3).

Serum prolactin concentrations and numbers of allograft rejection episodes

Before transplantation prolactin serum concentrations were slightly above the normal range due to renal insufficiency in both groups (33.0 ± 5.4 and 32.4 ± 5.7 ng/ml respectively; normal range 2.5–20 ng/ml). Serum prolactin was completely suppressed in patients receiving bromocriptine mesylate during the entire follow-up period. In the control group prolactin levels decreased to stable values between 13 and 16 ng/ml.

Rejection episodes ($n = 35$) occurred in 31 patients (77.5%) during the 6 months follow-up period without any statistical difference in frequency between the two groups as far as the frequency of rejections per patient (odds ratio: 1.47, 95% confidence interval: 0.37–5.95) and histological severity according to the Banff criteria [27] was concerned (Table 4). No difference was seen between groups in the cumulative dose of antirejection treatment with dexamethasone and the percentage of patients who needed mono- or polyclonal antibody treatment. The cumulative prednisolone dose over the 6 months follow-up was 2970.3 ± 85.5 and 2947.5 ± 100.7 mg respectively.

In each group eight patients experienced a CMV infection, while severe bacterial infections (i.e. pneumonia and sepsis) were seen in six of the control group and in five of those patients receiving bromocriptine.

Adverse effects

No major complications occurred after bromocriptine administration, and side-effects were minimal, consisting mainly of dizziness and nausea. Therefore no patient was withdrawn from the study.

Discussion

Acute rejection is an important risk factor for graft loss after cadaveric kidney transplantation [16–19]. Although graft rejections are treated with increasing success by new drugs, any antirejection therapy is accompanied by negative effects such as adverse drug reactions, hospitalization, and considerable costs. Due to its prolactin-suppressing effect the dopamine agonist bromocriptine has long been used in the treatment of hyperprolactinaemia and its safety has been proven in

Table 3. Creatinine serum concentrations (mean $\pm$ SD)

Days	30	60	90	120	150	180
Bromocriptine group: creatinine concentration (mg/100 ml)	1.68 ± 0.84	1.51 ± 0.31	1.43 ± 0.30	1.49 ± 0.30	1.51 ± 0.36	1.46 ± 0.30
Control group: creatinine concentration (mg/100 ml)	1.70 ± 0.96	1.45 ± 0.41	1.37 ± 0.39	1.39 ± 0.40	1.44 ± 0.42	1.39 ± 0.37

Table 4. Rejection episodes, Banff criteria and antirejection treatment

	Month 1 1./2. rejection	Month 2–3 1./2. rejection	Month 3–6 1./2. rejection	Banff criteria or clinical diagnosis Grade I/Grade II/ clinical diagnosis	Cumulative prednisolone dose (mg/6 months)	mono- or polyclonal antibodies
Bromocriptine ($n = 19$)	13/0	0/2	0	5/7/3	2970.3 ± 85.5	10
Controls ($n = 21$)	16/0	2/2	0	4/10/6	2947.5 ± 100.7	9

long-term studies over many years. Evidence from *in vitro* and *in vivo* studies in animals and men [1–15], suggesting that prolactin has immunostimulatory properties, prompted the present study.

Prolactin receptor, a member of the growth factor receptor superfamily, has been identified on human T-and B- lymphocytes [2]. Furthermore prolactin was found to induce IL-2 receptors on splenocytes, hence increasing lymphocyte response to this cytokine [20], and to enhance PBMC proliferation in response to IL-2 and PHA [21]. In addition, prolactin-translocation into the nucleus of T cells is necessary for IL-2-stimulated proliferation of T cells [22]. Furthermore, decreased IFN γ production was measured in hypoprolactinaemic animals [8] and anti-PRL antibodies have been shown consistently to inhibit mitogen-induced stimulation of human PMNC. Both effects could be restored by the addition of human prolactin [4]. It has also been demonstrated that prolactin antagonizes competitively the action of cyclosporin A at the receptor level in mixed populations of human peripheral blood lymphocytes in a dose-dependent manner [2]. Already physiological PRL concentrations result in a significant reduction of specific binding of cyclosporin in human peripheral blood mononuclear cells *in vitro*. In addition, recent studies demonstrated an immunosuppressive activity by bromocriptine itself on human B lymphocyte function *in vitro*, and on T-cell proliferation through blocking of IL-2 production by T cells [15,24]. Furthermore combined treatment of bromocriptine and cyclosporin has been demonstrated to result in an additive immunosuppressive effect, compared to cyclosporin alone, as has been shown in animal studies and in experiments with human lymphocytes *in vitro* [15,26].

As T-lymphocyte activation and production of cytokines such as IL-2, IFN γ and IL 4 are of prominent importance in acute kidney allograft rejection, we examined the influence of bromocriptine, a dopamine receptor agonist suppressing prolactin secretion, on the outcome after kidney transplantation, by evaluating the incidence of rejection episodes and infections. Although serum prolactin concentrations were effectively suppressed by monthly intramuscular bromocriptine administration, we failed to demonstrate a positive effect on acute rejection episodes and graft survival in the bromocriptine-treated patients in comparison to the control group in this 6-month open study. Also the cyclosporin concentrations and the total amount of glucocorticoids administered in the whole 6-month period were similar in both groups. Thus, differences in the dosage of standard immunosuppressive therapy cannot account for the lack of *in vivo* immunosuppressive activity of additional prolactin suppression in humans. Furthermore serum creatinine concentrations and the incidence of infections were similar in both groups. These findings are in contrast to previous studies which reported prolonged cardiac and kidney graft survival after treatment with dopamine agonists in combination with low-dose cyclosporin schedules in comparison to low-dose cyclosporin alone in rats [25,26]. The fact that in the present study bromocriptine was added to standard cyclosporin therapy without differences in cyclosporin dosage and serum levels in both groups could possibly explain the data: The potent immunosuppressive effect of cyclosporin in standard dosage alone might not be augmented by suppression of prolactin.

In conclusion, suppression of circulating pituitary derived prolactin levels with bromocriptine does not improve the immunosuppressive effect of cyclosporin at standard dosage nor increase the incidence of infections after first cadaver kidney transplantation. As clinical use of cyclosporin as immunosuppressive agent to prevent graft loss is limited by its dose-related side-effects, the combination of low-dose cyclosporin in addition to the immunosuppressive effect of bromocriptine-induced prolactin suppression could be an interesting alternative. Further studies will be necessary to validate the effectiveness of combining bromocriptine with low-dose cyclosporin in kidney transplantation.

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References

1. Nagy E, Berzi I, Friesen HC. Regulation of immunity in rats by lactogenic and growth hormones. *Acta Endocrinol* 1983; 102: 351–357
2. Russel JH, Kibler R, Matrisian L, Larson DF, Poulus B, Magun BE. Prolactin receptors on human T and B lymphocytes: antagonism of prolactin binding by cyclosporine. *J Immunol* 1985; 134: 3027–3031
3. Russel DH, Matrisian L, Kibler R, Larson DF, Poulus B, Magun BE. Prolactin receptors on human lymphocytes and their modulation by cyclosporine. *Biochem Biophys Res Commun* 1984; 121(3): 899–906
4. Hartmann DP, Holaday JW, Bernton EW. Inhibition of lymphocyte proliferation by antibodies to prolactin. *FASEB J* 1989.3: 2194–2202
5. Berczi I, Nagy E, Asa SL, Kovacs K. The influence of pituitary hormones on adjuvant arthritis. *Arthritis Rheum* 1984; 27: 682–688
6. Hedner LP, Bynke G. Endogenous Iridocyclitis relieved during treatment with bromocriptine. *Am J Ophthalmol* 1985; 100: 618–619
7. Carrier M, Emery RW, Wild-Mobley J, Perrotta NJ, Russel DH, Copeland JG. Prolactin as a marker of rejection in human transplantation. *Transplant Proc* 1987; 3442–3443
8. Bernton EW, Meltzer MS, Holaday JW. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. *Science* 1988; 239: 401–404
9. Palestine AG, Muellenberg-Coulombre CG, Kim MK, Gelato MC, Nussenblatt RB. Bromocriptine and low dose cyclosporine in the treatment of experimental autoimmune uveitis in the rat. *J Clin Invest* 1987; 79: 1078–1081
10. Dougados M, Duchesne L, Amor B. Bromocriptine and cyclosporin A combination therapy in rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 1333–1335
11. Dijkstra CD, Rouppe van der voort E, De Groot CJA *et al.* Therapeutic effect of the D2-dopamine agonist bromocriptine on acute and relapsing experimental allergic encephalomyelitis. *Psychoneuroendocrinology* 1994; 19: 135–142
12. Blank M, Krause I, Buskila D *et al.* Bromocriptine immunomodulation of experimental SLE via induction of nonspecific T suppressor cells. *Cell Immunol* 1995; 162: 114–122
13. Carrier M, Wild J, Pelletier LC, Copeland JG. Bromocriptine as an adjuvant to cyclosporine immunsuppression after heart transplantation. *Ann Thorac Surg* 1990; 49: 129–132

14. Compton CC, Rizk I, Regauer S, Burd E, Holaday J, Kenner J. The effect of bromocryptine induced hypoprolactinemia on xenogeneic and allogeneic skin graft survival in a mouse model. *J Burn Care Rehabil* 1994; 15: 393–400
15. Morikawa K, Oseko F, Morikawa S Immunosuppressive activity of bromocryptine on human T lymphocyte function *in vitro*. *Clin Exp Immunol* 1994; 95: 514–518
16. Lindholm A, Ohlman S, Albrechtsen D, Tufveson G, Persson H, Persson NH. The impact of acute rejection episodes on longterm graft frinction and outcome in 1347 primary renal transplants treated by cyclosporine regimens. *Transplantation* 1993; 56: 307–315
17. Gulanikar AC, MacDonald AS, Sungurtekin U, Belitsky P. The incidence and impact of early rejection episodes on graft outcome in recipients of first cadaver kidney transplantation. *Transplantation* 1992; 53: 323–328
18. Basadonne GP, Matas AJ, Gillingham KJ *et al.* Early versus late acute renal allograft rejection: impact on chronic rejection. *Transplantation* 1993, 55: 993–995
19. Tesi RJ, Elkhammas EA, Henry ML, Davies EA, Salazar A, Ferguson RM. Acute rejection episodes: best predictor of long term primary cadaveric renal transplant survival. *Transplant Proc* 1993; 25: 901–902
20. Mukherjee P, Mastro AM, Hymer WC. Prolactin induction of interleukin 2 receptor on rat splenic lymphocytes. *Endocrinology* 1990; 126: 88–94
21. Athreya BG, Pletcher J, Zulian F, Weiner DB, William WV. Subset-specific effects of sex hormones and pituitary gonadotropins on human lymphocyte proliferation *in vitro. Clin Immunol Immunopathol* 1993; 66: 201–211
22. Clevenger CV, Altmann SW, Prystowsky MB. Requirement of nuclear prolactin for interleukin-2-stimulated proliferation of T lymphocytes. *Science* 1991; 253: 77–79
23. Varma S, Ebner KE. The effect of cyclosporin A on the growth and prolactin binding to Nb-2 rat lymphoma cells. *Biochem Biophys Res Commun* 1988; 1: 233–239
24. Morikawa K, Oseko F, Morikawa S. Immunosuppressive property of bromocryptine on human B lymphocyte function *in vitro*. *Clin Exp Immunol* 1993; 93: 200–205
25. Hiestand PC, Gale JM, Mekler P. Soft immunsuppression by inhibtion of prolactin release: synergism with cyclosporine in kidney allograft survival and in the localized graft-versus host reaction. *Transplant Proc* 1986; 18 (4): 870–872
26. Wilner ML, Etteneger RB, Koyle MA, Rose JT. The effect of hypoprolactinemia alone and in combination with cyclosporine on allograft rejection. *Transplantation* 1990; 49: 264–267
27. Solez K, Axelsen RA, Benediktson H *et al.* International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. *Kidney Int* 1993; 44: 411–422

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