THE IMMUNOSUPPRESSIVE EFFECT OF BROMOCRIPTINE IN RATS

MUSTAFA AKAYDIN
GÜLSEN ÖNER
TUNCER KARPUZOGLU

SUMMARY: In order to investigate the role of bromocriptine (BCR) on immune response we have undertaken an experimental study and its results were compared with those of another immunosuppressive agent, Cyclosporine A (CsA). 63 mole Swiss albino rats were divided into 4 groups. Twenty two of those were used as a control and received intraperitoneal saline for 4 days. The other groups were treated with 3 mg/Kg CsA, 200 μg/Kg of BCR and the combination of these two drugs for the same period. CsA treatment caused significant decrease in T cell proliferation and macrophage phagocytic activity, BCR showed similar depression both in the response of T cell and phagocytic activity. However the combination of these two drugs showed no additive effect on immune system. As a conclusion, a PRL secretion inhibitor BCR has been found to be a potent immunosuppressive agent which is comparable to CsA.

Key Words: Immunosuppression, bromocriptine, cyclosporine A.

INTRODUCTION

The presence of prolactin receptors on lymphocytes may imply its role in the physiologic regulation of the immune response (13,14).

It has been suggested that cyclosporine A (CsA) and prolactin (PRL) compete for a common binding site on T cell membrane (8,9,13) and the reduction of circulating PRL levels by bromocriptine (BCR) leads to a decrease of lymphocyte responsiveness to mitogenic stimulation in *in vitro* studies (8, 9) as well as graft survival times in *in vitro* experiments (7–9).

Taken together, these evidences suggest that suppression of circulating prolacting levels by BCR, decreases the dose of CsA necessary for effective immunosuppression. However, the results of several authors who studied this hypothesis demonstrated some supportive evidences (6,12) as well as the opposites (4, 5).

Ferrero *et al.* obtained additive effect with the combination of CsA and BCR in pancreatic allografts of rats (6). On the other hand Dijkmans *et al.* who studied the effect of this combination in *in vitro* system, found an equal immunosuppressive response to the expected effects of the separate drugs instead of additive (4).

The results of Russell *et al.* who showed the competition of CsA for the PRL receptors which exist on lymphocyte membrane (13) were not supported by the findings of Varma and Ebner (14) who suggest that CsA does not exert its action by interfering with the binding of PRL to its receptor.

The existence of immunosuppressive effect of BCR both *in vivo* and *in vitro* system may imply that BCR exerts its action on immune system at least in two ways. One of them is the inhibition of pituitary PRL secretion which was shown by several investigators (6–9). The second way may be explained by the direct action of BCR on immune system. The conflictions in the literature may come from these direct and indirect actions of BCR on immune system.

In view of this knowledge our present study was undertaken to investigate the possible direct effect of BCR on immune system and compare it with CsA.

MATERIALS AND METHODS

In these experiments 53 male Swiss Albino rats (weighing 164.45±12.80 g) were used. All animals were fed with a standard rat diet and tap water *ad libitum* during the experimental period.

From Department of General Surgery,
From Department of Physiology, Akdeniz University, Faculty of Medicine, Antalya, Kepez, Türkiye.

Twenty two of the animals were used as a control group and received 1 ml/100 g body weight of saline intraperitoneally (i.p.) for 14 days.

Fifteen animals in the second group were treated with BCR (20 μg/ml/100 g b.wt., i.p.) for the same period. The third group composed of 12 rats, received 300 μg/ml/100 g b.wt. of CsA i.p. for 14 days. The combination of the same doses of CsA and BCR (300 μg and 20 μg respectively) was given to 14 animals in the fourth group.

On the 15th day, after 16 hours fasting all animals were anesthetized with light ether and their peritoneal macrophages were obtained by the method of Brunda *et al.* using phosphate buffered Krebs solution (1).

Through the midline incision abdomens of the rats were opened, their mesenteric lymph nodes were dissected and used for T cell isolation by nylon wool columns prepared as described by Julius *et al.* (10).

Isolated T cells were incubated in 1 ml of M 199 at 37°C for 72 hours. The response to mitogen (PHA 1:10) (Difco 0528-57-5) in the three treated groups was expressed as a percent of control group at 72 hours.

The peritoneal macrophages were incubated at 37°C with 1% of carbon particles for 60 minutes. The number of particles phagocytized by 100 macrophages was counted under light microscope and accepted as a mean phagocytic activity of each rat.

The statistical evaluation of the results was made by using student's "t" test.

RESULTS

The change of body weight

The mean increase of body weight in 14 days was 33.91±2.40% in the control group and 38.8±2.63% in BCR treated animals. CsA treatment for 14 days caused a significant reduction in weight gain (19.95 ± 3.38%, $p<0.01$). Whereas the addition of BCR to CsA treatment prevented this reduction significantly (37.37 ± 4.58%, $p<0.01$) (Table 1, Figure 1).

The measurement of hematocrits showed no significant difference in all groups.

The phagocytic activity of the macrophages

The mean particles phagocytized by peritoneal macrophages was 10.07 ± 0.03 in the control group. In the BCR treated rats the mean value declined to 6.14±0.21 particles/cell. The mean phagocytic activity of rats treated with CsA alone and CsA+BCR combination was 5.75 ± 0.07 and 5.53 ± 0.08 particles/cell respectively (Table 1, Figure 1).

The decreases of phagocytic activities seen in all the treated groups differed significantly from those of control rats ($p<0.01$).

Mitogenic responses

The response of T cells to mitogenic stimulation at 72 hours was accepted as 100% in the control group and the T cell responses of the other three groups were expressed as percentage of the control values.

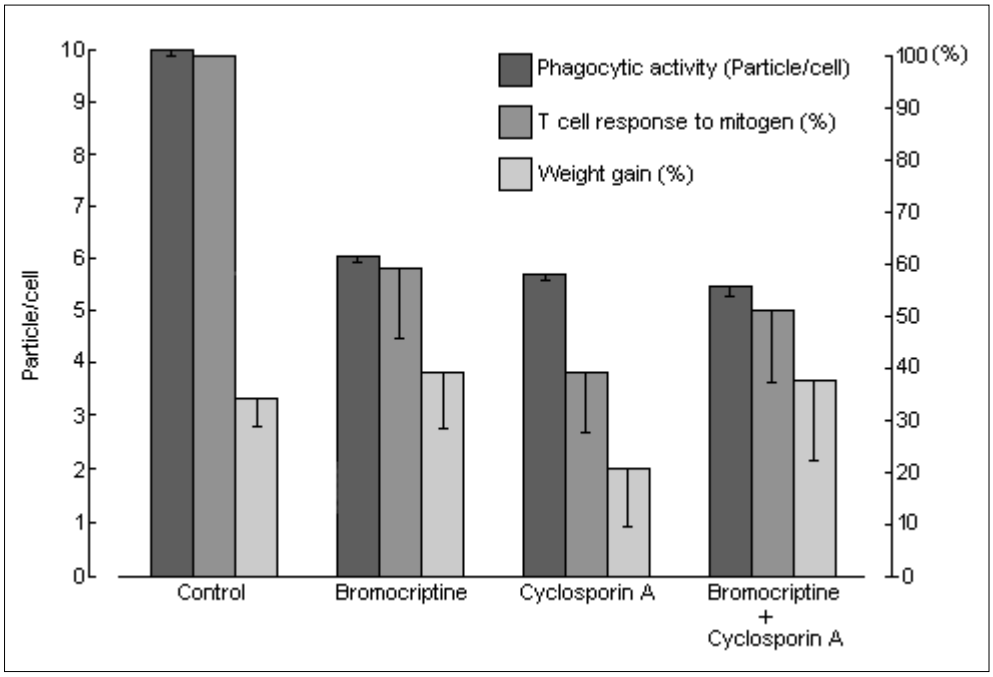
The mitogenic response in the BCR treated group was 59.30±4.84%. This depression seen in T cell proliferation was statistically significant ($p<0.01$). CsA alone caused a marked inhibition of T cell response. The mean mitogenic response was found to be 38.95±2.6% in this group ($p<0.01$). CsA and BCR combination had 50.91 4.57% of T cell response ($p<0.01$) (Table 1, Figure 1).

Table 1: The effect of immunosuppressive agents on the immune response (Mean ± SE)

Group of rats	Hematocrit levels (%)	Phagocytic activity (Particle/cell)	T cell response to mitogen (Percent of controls)	Weight gain (%)
Control (1 ml SF x 14 days)	40.55 ± 3.45 (n=6)	10.7 ± 0.03 (n=20)	100 (n=21)	33.91 ± 2.49 (n=6)
Bromocriptine (200 μg/kg x 14 days)	41.54 ± 2.62 (n=15)	6.14 ± 0.21* (n=14)	59.3 ± 4.84* (n=8)	38.80 ± 2.63 (n=15)
Cyclosporin A (3 mg/kg x 14 days)	44.18 ± 2.06 (n=11)	5.75 ± 0.07* (n=13)	38.95 ± 2.60* (n=9)	19.95 ± 3.38* (n=12)
Cyclosporin A + Bromocriptin	45.54 ± 2.81 (n=13)	5.53 ± 0.08* (n=13)	50.91 ± 4.57* (n=9)	37.37 ± 4.58* (n=14)

*Difference from controls ($p<0.01$)

Figure 1: Phagocytic activities, T cell response, % weight gain of the treated rats in comparison with the controls (Mean±SD).



DISCUSSION

The results of this experiment show that body weight gain is lower in CsA treated rats than those of other groups. This finding is in a well accord with the results of Devarajan *et al.* (3) who showed 10% less food intake in CsA treated rats in their experiments. The reason of lower body weight can not be explained simply by less food intake because hypovolemia with normal hematocrit levels is another effect of CsA treatment in rats (10).

A significant decrease in phagocytic activities seen in treated groups indicates that either alone or in combination, CsA and BCR effect on macrophages. Most authors (4,14) have reported that CsA is a potent immunosuppressive agent preferentially active against proliferating T cells. It shows its action by blocking the transcription of IL-2 gene (11) and inhibits calmodulin dependent cellular events (14). However different opinions appeared recently in the literature. One of them belongs to Colombani and Hess (2) who state that CsA effect both T independent B cells and macrophage responses as well as the other organ systems. Our results which show depressed phagocytic activity are supported by the cited paper above. However there is no direct evidence showing the change in the phagocytic activity due to CsA treatment. The unexcepted effect of BCR on phagocytic cells, may be a dopaminergic receptor mediated direct effect as well as lack of prolactin in the circulation.

Our results as well as others have showed that T cell proliferative response to mitogen is depressed significantly in CsA treated group. However the mechanism by which BCR decreases proliferation rate of T cell is unclear. This depression can be explained in party by the reduction of circulating prolactin levels. Since was measured the response of pretreated T cells to mitogen, neither the blocking of PRL receptors by CsA nor lower PRL levels can explain completely these *in vitro* lack of responses.

The combination of CsA with BCR did not produce an additive suppression on T cell proliferation and phagocytic activity, whereas these drugs were found more effective when used separately.

As a result our findings suggest that BCR, a potent dopaminergic against has a strong immunosuppressive effect which is comparable to that to of CsA.

ACKNOWLEDGEMENT

This research was supported by University Research Foundation (87-01-0103-03) and Sandoz.

REFERENCES

1. Brunda MJ, Taramelli D, Holden HT, Varesio L : Suppression of in vitro Maintenance and Interferon Mediated Augmentation of Natural Killer Cell Activity by Adherent Peritoneal Cells

from Normal Mice. J Immunol, 130:1974-1979, 1983.

2. Colombani PM, Hess AD : T Lymphocyte Inhibition by Cyclosporine (Potential Mechanisms of Action). Biochem Pharmacol, 36:3789-3793, 1987.

3. Devarajan P, Kaskel FJ, Arbeit LA, Moore lC : Cyclosporine Nephrotoxicity: Blood Volume, Sodium Conservation and Renal Hemodynamics. Am J Physiol, 256:71-78, 1989.

4. Dijkmans BAC, Vries E, de Vreede TM, Cats A : Effects of Anti-Rheumatic Drugs on in Vitro Mitogenic Stimulation of Peripheral Blood Mononuclear Cells. Transplant Proc, 20:253-258, 1988.

5. Dougados M, Duchesne L, Amor B : Bromocriptine and Cyclosporine A Combination Therapy in Rheumatoid Arthritis. Arthritis Rheum, 31:1333-1334, 1988.

6. Ferrero ME, Marni A, Corbetta G, Gaja G : Survival of Pancreas Allografts in Rats Treated with Cyclosporine and Bromocriptine. Transplant Proc, 19:3923-3926, 1987.

7. Hiestand PC, Gale JM, Mekler P : Soft Immunosuppression by inhibition of Prolactin Release: Synergism with Cyclosporine in Kidney Allograft Survival and in the Localized Graft-Versus Host Reaction. Transplant Proc, 18:870-872, 1986.

8. Hiestand PC, Mekler P : Cyclosporin and Prolactin Mediated Control of Immunity. Prog Allergy, 38:239-246, 1986.

9. Hiestand PC, Mekler P, Nordmann R, Grieder A, Permmongkol C : Prolactin as a Modulator of Lymphocyte Responsiveness Provides a Possible Mechanism of Action for Cyclosporine. Proc Natl Acad Sci, 83:2599-2603, 1986.

10. Julius MM, Simpson E, Herzenberg LA : A Rapid Method for the isolation of Functional Thymus-Derived Murine Lymphocytes. Eur J Immunol, 3:645-649,1972.

11. Kumagai N, Benedict SH, Mills GB, Gelfand EW : Cyclosporine A Inhibits Initiation but not Progression of Human T Cell Proliferation Triggered by Phorbol Esters and Calcium lonophores. J Immunol, 141:3747-3752, 1988.

12. Palestine AG, Muellenberg-Coulombre CG, Kim MK, Gelato MC, Nussenblatt RB : Bromocriptine and Low dose Cyclosporine in the Treatment of Experimental Autoimmuno Uveitis in the Rat. J Clin Invest, 79:1078-1081, 1987.

13. Russel DH, Kibler R, Matrisian L, Larson DF, Poulos B, Magun BE : Prolactin Receptors on Human T and B Lymphocytes: Antagonism of Prolactin by Cyclosporine. J Immunol 134:3027-3031, 1985.

14. Varma S, Ebner KE : The Effect of Cyclosporine A on the Growth and Prolactin Binding to Nb-2 Rat Lymphoma Cells. Biochem Biophys Res Commun, 156:233-239, 1988.

Correspondence:
Mustafa Akaydin
Department of General Surgery,
Faculty of Medicine,
Akdeniz University,
Kepez, Antalya,
TÜRKIYE.