

Inhibitory Effect of Cyclosporin A on Histamine Release from Human Leukocytes and Rat Mast Cells

C. PEDERSEN, H. PERMIN, P. STAHL SKOV, S. NORN, M. SVENSON,
H. MOSBECH and K. BENDTZEN

Laboratory of Immunology, University Clinic for Infectious Diseases M and Medical Dept. TA, Rigshospital and
Dept. of Pharmacology, University of Copenhagen, Copenhagen, Denmark

The influence of cyclosporin A (CyA) on human basophil histamine release induced *in vitro* by specific antigen, anti-IgE, calcium ionophore A23187, or concanavalin A (Con A) was studied. CyA inhibited the release induced by these four stimulators. It is suggested that the drug acts directly on the target cell, since similar effect was obtained with isolated peritoneal rat mast cells. The basophil histamine release was not changed by a non-immunosuppressive cyclosporin derivate.

Key words: anti-allergic; antigen; anti-IgE; basophils; calcium ionophore; Con A; cyclosporin A; histamine release; house dust mite allergy; mast cell.

Accepted for publication 22 August 1984

Cyclosporin A (CyA) is a fungal metabolite produced by the strain *Tolypocladium inflatum* Gams (8). The agent has potent immunosuppressive properties. It is effective in preventing rejection in patients receiving renal allografts, and it prevents graft versus host disease after allogeneic bone marrow transplantation (5, 6). CyA has been found to inhibit *in vitro* the activation of T-lymphocytes by lectins and the calcium ionophore A23187 (4). The mode of action of the immunosuppressive effect of CyA is believed to be due to inhibition of an early step in the activation of T-lymphocytes, probably at the level of interleukin 1 function (1, 8).

A role in the release of allergic mediators has not been elucidated. In this study we have therefore investigated the influence of CyA on the release of histamine from human basophil leukocytes and isolated rat mast cells.

MATERIAL AND METHODS

Patients

The investigation included nine healthy individuals and eight asthmatic patients with a well-documented history of symptoms of house dust mite allergy. All subjects showed a positive response to *Dermatophagoides pteronyssinus* extract by RAST, basophil histamine release, bronchial provocation, and skin prick test.

Material

Cyclosporin A and the immunologically inactive cyclosporin, 38-262n, were kindly donated by Sandoz (Basle, Switzerland), house dust mite extract (Pharmalgen *Dermatophagoides pteronyssinus*) was from Pharmacia AB,

Sweden, calcium ionophore A23187 from Calbiochem AG, Switzerland, rabbit-antihuman IgE (ϵ -chain) from Behringwerke AG, Germany, and concanavalin A was purchased from Pharmacia AB, Sweden. Horse serum was complement-inactivated by heating to 56°C for 30 min; it was dialyzed against 0.9% NaCl prior to use.

Preparation of leukocyte suspensions

Blood samples (10 ml) were collected by venipuncture in plastic tubes containing 0.5 ml 0.2 M EDTA (pH 7.2). 20 ml of 0.9% NaCl was added to the samples and the leukocytes were isolated by Ficoll-Hypaque gradient centrifugation, as earlier described (3). The interphase consisting of mononuclear cells containing approx. 2% basophils was washed twice with Tris-AMC (Tris-(hydroxymethyl)-amino-methane 25 mM at pH 7.6, NaCl 0.12 M, KCl 5 mM, CaCl₂ 0.6 mM, MgCl₂ 1.1 mM, human serum albumin 0.3 mg/ml, and glucose 1 mg/ml). The cells were finally resuspended in 2.5 ml Tris-AMC.

Basophil histamine release

80 μ l leukocyte suspension containing 2000–3000 basophils was incubated in plastic tubes at 37°C for 30 min with 10 μ l of Tris-AMC or

cyclosporins. Cyclosporin A and cyclosporin 38-262n were examined in final concentrations of 100 and 1000 ng/ml, if not otherwise stated. The concentrations of free cyclosporin A in the buffer was, however, only 20% of the added amount, since 80% is adsorbed to the plastic tubes, as demonstrated in these cultures by radioactive-labelled cyclosporin A (results not shown). Histamine release was induced by adding 10 μ l of *Dermatophagoides pteronyssinus* extract, anti-IgE, calcium ionophore A23187 or Con A to the samples, and the incubation was continued for 40 min. In a few experiments (Table 1) the leukocytes were washed twice to remove free CyA before incubation with the stimulators. With each wash the cells were transferred to new tubes. The final concentration of the stimulators constituted 100 BU/ml $10^{-3} \times 400,000$ IU/ml, 1×10^{-6} M, and 400 μ g/ml, respectively. Cyclosporins and stimulators were omitted for the determination of spontaneous histamine release and the total histamine content; in the latter case the samples were left at 4°C. 3 ml of ice-cold Tris-AMC was added to the cell samples and they were centrifuged at 2000 g for 10 min. Besides histamine the supernatants also contained protein and other substances which might have interfered with the histamine analysis. It was therefore discarded and the residual histamine was determined in the cell sediment according to the method of Stahl Skov & Norn (7). By addition of cyclosporins to the cell samples after 35 min incubation with the stimulators we found that the cyclosporins *per se* did not interfere with the spectrofluorometrical assay. The release of histamine was expressed as a percentage of the total histamine content of the sample. The spontaneous histamine release amounted to 1–10%.

Sensitized rats

Adult male Wistar rats weighing 200–250 g were sensitized to horse serum. 1 ml of the serum was given by one subcutaneous injection mixed with 1.0 ml (10^{10} bacilli) pertussis vaccine as adjuvant. The rats were used 3–6 weeks after sensitization.

Table 1

Influence of cell wash on the effect of cyclosporin A

	Inhibition (%) of histamine release by cyclosporin A	
	Washed cells ¹	No wash ¹
Anti-IgE	84 $\pm$ 10	91 $\pm$ 5
Calcium ionophore	72 $\pm$ 4	77 $\pm$ 7
Concanavalin A	73 $\pm$ 13	69 $\pm$ 21

Human leukocytes were incubated with CyA (1000 ng/ml) and then washed twice to remove unbound agent. Thereafter histamine release was induced by the stimulators. Mean of four experiments $\pm$ SEM.

¹ No difference between washed and unwashed cells ($P > 0.05$).

Isolation of rat mast cells

Mast cells from the peritoneal and thoracic cavities were isolated by centrifugation on a Ficoll density gradient by the method of Thon & Uvnäs (9). The mast cells collected from the gradient 95% purified were washed three times with Tris-AMC. Finally, the mast cells were suspended to a concentration of 2,500–7,500 cells/ml.

Histamine release from isolated rat mast cells

The mast cells were incubated at 37°C for 15 min with Tris-AMC or cyclosporins. Cyclosporin A and cyclosporin 38-262n were examined in final concentrations of 10, 100, 250, 1000, and 2000 ng/ml. Thereafter, the cells were washed twice with Tris-AMC by transferring the cells to new tubes after each wash. Histamine release was induced by incubating 90 µl of the pretreated mast cells with 10 µl of 10% solution of horse serum at 37°C for 15 min. 3 ml ice-cold Tris-AMC was added to each tube and they were centrifuged at 2000 g for 10 min, and the histamine release was estimated as mentioned above.

Statistical evaluation

The Wilcoxon test for paired differences was used. Mean $\pm$ SEM is given.

RESULTS

The influence of cyclosporin A (CyA) on basophil histamine release was examined in leukocyte suspensions from nine normal individuals (Fig. 1). The release of histamine was induced by anti-IgE, calcium ionophore, and Con A. CyA, in concentrations of 100 and 1000 ng/ml, reduced the release caused by all three stimulants. A 70–80% inhibition was obtained in anti-IgE- and calcium ionophore-induced histamine release, while in the case of Con A the inhibition was 30%. In all cases the inhibition was found to be statistically significant ($P < 0.05$). Similar experiments with the biologically inert cyclosporin 38-262n did not cause significant inhi-

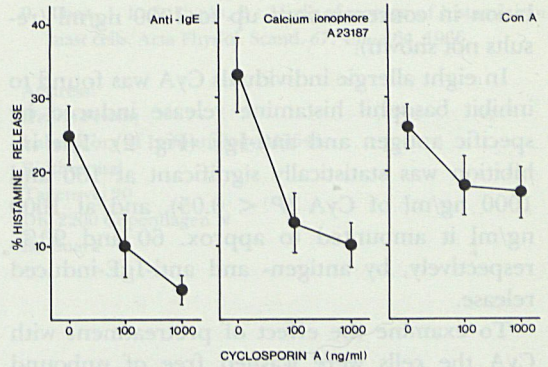


Fig. 1. Inhibition of histamine release by cyclosporin A in leukocyte suspensions from normal individuals. The release was caused by anti-IgE, calcium ionophore, and Con A. Each experiment included nine persons. Mean $\pm$ SEM.

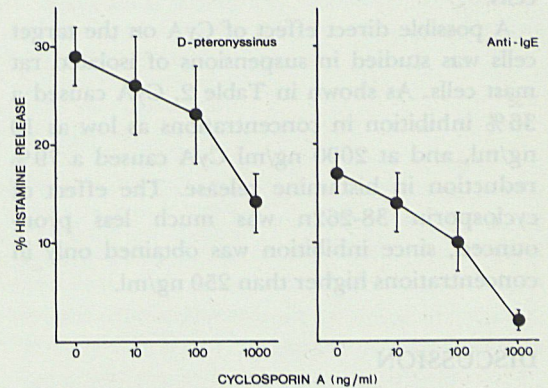


Fig. 2. Inhibition of histamine release by cyclosporin A in leukocyte suspensions from allergic individuals. The release was caused by specific antigen and anti-IgE. Each experiment included eight persons. Mean $\pm$ SEM.

Table 2

Inhibition of histamine release from rat mast cells by cyclosporin A and cyclosporin 38-262n

Concentration (ng/ml)	Inhibition (%)	
	Cyclosporin A	Cyclosporin 38-262n
10	36 $\pm$ 14	1 $\pm$ 1
100	65 $\pm$ 6	12 $\pm$ 6
250	—	15 $\pm$ 6
1000	64 $\pm$ 5	50 $\pm$ 5
2000	79 $\pm$ 7	60 $\pm$ 8

Histamine release was induced by specific antigen. The experiments included five rats. Mean $\pm$ SEM.

bition in concentrations up to 1000 ng/ml (results not shown).

In eight allergic individuals CyA was found to inhibit basophil histamine release induced by specific antigen and anti-IgE (Fig. 2). The inhibition was statistically significant at 100 and 1000 ng/ml of CyA ($P < 0.05$), and at 1000 ng/ml it amounted to approx. 60 and 90%, respectively, by antigen- and anti-IgE-induced release.

To examine the effect of pretreatment with CyA the cells were washed free of unbound CyA after the pretreatment and thereafter incubated with stimulators. Table 1 shows that the inhibitory effect of CyA on histamine release induced by anti-IgE, calcium ionophore and Con A was similar in washed and unwashed cells.

A possible direct effect of CyA on the target cells was studied in suspensions of isolated rat mast cells. As shown in Table 2, CyA caused a 36% inhibition in concentrations as low as 10 ng/ml, and at 2000 ng/ml CyA caused a 79% reduction in histamine release. The effect of cyclosporin 38-262n was much less pronounced, since inhibition was obtained only in concentrations higher than 250 ng/ml.

DISCUSSION

We found CyA to be a potent inhibitor of histamine release from human basophil leukocytes *in vitro*. The inhibitory effect was demonstrated in type I allergic patients suffering from house dust mite allergy. Significant inhibition of antigen-induced release was obtained with 100 and 1000 ng/ml CyA. The concentration of CyA in the medium was, however, only about 20% of the added amount, since approx. 80% was adsorbed to the plastic tubes. An inhibitory effect might therefore be expected *in vivo*, since therapeutic, immunosuppressive doses in man correspond to serum concentrations in the range of 100–400 ng/ml (2). The immunologically inactive cyclosporin, 38-262n, did not change the release of histamine from human basophil leukocytes.

We suggest that CyA acts directly on the target cells, since inhibition of histamine release

was also found in isolated rat mast cells. The effect of pretreatment with the drug persisted during a two-fold washing procedure of the cells and the following incubation period (40 min) with stimulators. Therefore, CyA might act via high affinity binding sites or, alternatively, biochemical events which are of long-lasting nature.

To investigate the mechanism of the inhibitory effect of the drug on histamine release we used different types of stimulators. Antigen and anti-IgE trigger the mediator release by opening receptor-operated calcium channels, whereas the calcium ionophore is thought to act as a calcium carrier, which bypasses these channels. Since the release induced by these three types of stimulators was inhibited by the drug, we suggest that CyA acts at different sites and/or at a common subsequent or at a modulating step.

Drugs of the cyclosporin type might be a new approach to the treatment of type I allergic diseases.

ACKNOWLEDGEMENTS

The study was supported by Sygekassernes Helsefond, Fortsættelsessygekassen Danmarks Sundhedsfond, Nationalforeningen til Bekæmpelse af Tuberkulose og Sygdomme i Åndedrætsorganerne, J.L.-Fondet, Thorvald Madsens Legat, Købmand Sven Hansen og hustru Ina Hansens Fond, and Forskningsfonden for Storstrøms Amt.

REFERENCES

1. Bendtzen, K., Petersen, J. & Sjøberg, B.: Effects of cyclosporin A and methylprednisolon on the immune response. *Acta Pathol. Microbiol. Scand. (C)* **91**, 159–167, 1983.
2. Britton, S. & Palacios, R.: Cyclosporin A – usefulness, risks and mechanism of action. *Immunological Rev.* **65**, 5–22, 1982.
3. Jensen, C., Stahl Skov P. & Norn, S.: Inhibitory effect of calcium antagonists on histamine release from human leukocytes. *Allergy* **38**, 233–237, 1983.
4. Kay, J. F., Benzie, C. R. & Borghetti, A. F.: Effect of cyclosporin A on lymphocyte activation by the calcium ionophore A23187. *Immunology* **50**, 441–446, 1983.
5. Merion, R. M., White, D. J. G., Thiru, S., Evans, D. B. & Calne, R. Y.: Cyclosporin: five years' experience in cadaveric renal transplantation. *N. Engl. J. Med.* **310**, 148–154, 1984.

6. Speck, B., Gratwohl, A., Osterwalder, B., et al.: Allogeneic bone marrow transplantation: The Basel trial with cyclosporine. *Transplant. Proc.* 15, Suppl. 1, 2617-2619, 1983.
7. Stahl Skov, P. & Norn, S.: A simplified method for measuring basophil histamine release and blocking antibodies in hay fever patients. Basophil histamine content and cell preservation. *Acta Allergol.* 32, 170-182, 1977.
8. Thomson, A. W.: Immunobiology of cyclosporin A - a review. *Aust. J. Exp. Biol. Med. Sci.* 61, 147-172, 1983.
9. Thon, I. L. & Uvnäs, B.: Mode of storage of histamine in mast cells. *Acta Physiol. Scand.* 67, 455-464, 1966.

Address:

Henrik Permin

Laboratory of Immunology M7641

Rigshospital

Tagensvej 20

DK-2200 Copenhagen N

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.